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AMELIORATIVE AND PROTECTIVE EFFECTS OF A MIXTURE OF *Aframomum melegueta*, *Syzygium aromaticum*, AND *Xylopia aethiopica* ON AFLATOXICOSIS IN BROILER CHICKEN

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ABSTRACT

Spices have been used since time immemorial for various purposes. This study investigated the biochemical and histological effect of a mixture of three spices (*Aframomum melegueta*, *Syzygium aromaticum,* and *Xylopia aethiopica*) commonly employed for various therapeutic activities on biochemical and histological derangements brought about by feeding broiler chickens with aflatoxin-contaminated diet. Twenty-five 6-week-old chickens were randomly placed in five groups containing five birds each. Birds in Groups I, II, and III were the normal, negative, and positive controls while groups IV and V were treated groups. The Chickens were sacrificed on the 29th day. Tissues and organs were excised for biochemical and histological analyses. The results showed that consumption of an Aflatoxin B1-contaminated diet resulted in decrease in the concentrations and activities of bilirubin, creatinine, uric acid, plasma ALT and AST, SOD, GPx, CAT, TC, TAG, VLDL, and LDL and decrease of liver ALT and AST, kidney ALP and GSH in the tissues and organs with respect to the control group. Histological analyses of the liver and kidney showed that Aflatoxin B1 caused architectural disruptions in the organs and tissues. However, all the metabolic derangements as well as structural alterations in livers and kidneys were ameliorated/restored in both pre-treated and post-treated groups without causing any apparent distress to the birds. It is suggested that the inclusion of these spices in the diet of chickens has the potential for the prevention and management of aflatoxicosis.

Keywords: Aflatoxin, *Aframomum melegueta,* Spices, *Syzygium aromaticum, Xylopia aethiopica*.

INTRODUCTION

A spice is a substance of plant origin of which no principle, volatile oil, or flavour, has been removed and is cultivated specifically for seasoning food rather than nutrition. It can be found in any part of a plant or herb (bark, dry seed, flower, root, or fruit) and is used in minute quantities to add flavour, aroma and colour or as a preserving agent (Rastogi *et al.,* 2017; Freedman 2020). Medicinally, spices are used in the folklore treatment and management of many ailments, and researchers have also revealed that a number of them possess and demonstrated a variety of biological activities such as antioxidant, anti-inflammatory, antitumor, antibacterial, anti-microbial as well as immune-modulatory activities (Gupta, 2010: Liu *et al.,* 2011).

Aflatoxins have been demonstrated to have toxic effects on poultry and other animals. This includes decreased appetite, weight loss, lowering of immune response, reproductive dysfunctions such as abortion and teratogenic tendencies as well as premature death (Shuaib *et al*., 2010; Datsugwai *et al.,* 2013, Marchese *et al*., 2018, Rushing and Selim, 2019). It is reported that Aflatoxin - B1 elicits its toxic effects through its biotransformed intermediates such as aflatoxin-B1-8,9-epoxide (Bailey *et al.,* 1996; Guengerich *et al.,* 1998; Tola and Kebede, 2016). Many times, aflatoxins contaminate chicken feed due to poor storage facilities and the use of contaminated materials (especially corn) in feed preparation. This contamination is a major cause of death in poultry, with attendant economic implications. The study examines the effects of the mixture of spices on biochemical parameters of plasma, liver and kidney and histoarchitecture of organs of birds fed with aflatoxin-contaminated diets. This is intending to use the mixture in the management of aflatoxicosis.

MATERIALS AND METHODS

Aframomum melegueta (Roscoe) K. Schum (seeds), *Syzygium aromaticum (L.) Merr. & L. M. O. Perry* (flower buds), and *Xylopia aethiopica* (Dunal) A. Rich. (seeds) were purchased at the New Market, Ile-Ife. They were identified and authenticated at the IFE Herbarium, The Department of Botany, Obafemi Awolowo University.

All reagents used for this study were of analytical grade.

Broiler chicks (n = 30) were reared for six (6) weeks for this study. They were maintained on broiler starter feed for the first two weeks and grower feed for the remaining four weeks. The broiler chicken used for this study were kept and sacrificed according to the regulations and approval of the Health Research Ethics Committee, The Institute of Public Health, Obafemi Awolowo University, Ile-Ife, Nigeria with registration number IPH/OAU/12/2388.

Experimental Design

Preparation of Spices: 100 g each of *A. melegueta* (powdered seeds), *S. aromaticum* (powdered seeds), and *X. aethiopica* (powdered seeds) was weighed separately and mixed manually to make 300 g of a mixture of spices.

Preparation of Poultry Diets: Two poultry diets were compounded from a standard diet by adding aflatoxin and the mixture of spices separately to form aflatoxin B1-contaminated and spices-supplemented diets respectively according to the procedure of AboSaleh *et al.,* 2019 as follows:

(a) Aflatoxin B1-contaminated diet (AFB1-CD; 0.3 mg aflatoxin- B1/kg standard diet); and

(b) Spices-supplemented diet (SSD; 20 g spice mixture/kg diet).

Grouping and Treatment of Broiler Chickens

Broiler chickens (25), five birds each in five groups, were treated as follows:

Group 1(normal group): given standard diet and water for twenty-eight (28) days,

Group II (Aflatoxin group): given an aflatoxin B1-contaminated diet (AFB1-CD) for fourteen (14) days,

Group III (Spice-supplemented group): given a spices-supplemented diet (SSD) for fourteen (14) days,

Group IV (Post-treated group): given AFB1-CD for fourteen (14) days followed by SSD for another fourteen (14) days, and

Group V (Pre-treated group): given SSD for fourteen (14) days followed by AFB1-CD for another fourteen (14) days.

Sacrifice of Animals/Collection of Tissues and Organs

The birds were fasted overnight on the 29th day and euthanized with diethyl ether. Blood was collected, through the brachial wing vein puncture, into heparinized bottles. Whole blood was collected into plain vials for haemoglobin concentration determination while another blood sample was taken for preparation of plasma. The liver and kidney tissues of the birds were excised aseptically, rinsed in normal saline, blotted between tissue paper to remove blood stains and stored inside a deep freezer at -2°C.

Preparation of Blood Plasma and Tissue Homogenates

Blood tissue was centrifuged at 3000 rpm for 10 min. The supernatant was collected as plasma into EDTA bottles and kept in a deep freezer at -2°C. Liver and kidney homogenates (10% (w/v)) were prepared with 0.1 M sodium phosphate buffer (pH 7.4), placed in vial bottles and stored at -2°C.

Biochemical Assays

Protein: The concentration of proteins in the plasma, kidney and liver were determined following the method described by Schacterk and Pollack (1973). Bovine serum albumin (BSA) was used as standard.

Haemoglobin: Blood (whole, 50 µl) was mixed with 4.5 ml haemoglobin reagent (sodium cyanide (5 mg), 50 mg potassium hexacyanoferrate (III) $K_3(FeCN)_6$ and 100 mg $NaHCO_3$ dissolved in 50 ml of distilled water and made up to 100 ml with distilled water) in triplicate, mixed and incubated for 10 min at room temperature. The absorbance of the reacting mixture was measured at 540 nm against a reagent blank (Mattenheimer, 1970, Oyedapo & Araba, 2001).

Haemoglobin concentration (mg/dL blood) =

$$\frac{\Delta \text{ absorbance } (540)}{\varepsilon} \times MW \times 0.1 \times \frac{Tv}{Sv}$$

where: ε (molar extinction coefficient) = 11000 $M^{-1}cm^{-1}$; Sv (sample volume) = 50 µl; Tv (total volume) = 4.5mL; MW (molecular weight) = 16114 g/mol

Evaluation of Kidney Markers

(a): Creatinine: The concentration of creatinine was estimated by following the procedure described by Bartels and Bohmer (1972) using Randox creatinine diagnostic kit by Randox Laboratories Ltd. The assay mixture contained 100 mL plasma and 1 mL reagent solution, shaken together, and incubated at room temperature for 15 minutes. The absorbance of the mixture was read at 520 nm against a reagent blank. The concentration of creatinine was calculated thus:

The concentration of creatinine (mg/dL) =

$$\frac{\Delta \text{ in Absorbance of sample}}{\Delta \text{ in Absorbance of standard}} \text{ x}$$

concentration of standard $\left(\frac{\text{mg}}{\text{dL}}\right)$

Where: Δ in Absorbance = absorbance at 120 s – absorbance at 30 s

(b): Uric Acid: The level of plasma uric acid was determined in accordance to the method of Fossati *et al.* (1980) with the use of a Randox uric acid diagnostic kit by Randox Laboratories Ltd The assay mixture contained plasma 20 µL and 1 mL reagent mixture. Standard uric acid (20 µl) was also added to 1 mL sample reagent. The mixtures were incubated for 15 minutes at room temperature. At 520 nm, the absorbance was read against a reagent blank. The concentration of uric acid was calculated using the expression:

Uric acid concentration $\left(\frac{mg}{dL}\right) =$

$$\frac{Absorbance\ of\ sasmple}{Absorbance\ of\ standard} \text{ x}$$

concentration of standard $\left(\frac{\text{mg}}{\text{dL}}\right)$

Evaluation of Liver Markers

(a): **Assay of Alanine and Aspartate Aminotransferase Activities**: The activity of the aminotransferases in the plasma and liver homogenates was assayed in accordance to the standard procedure described by Reitman and Frankel (1957). Alanine and aspartate aminotransferase catalyse the transamination reaction of α-oxoglutarate with L-alanine and L-aspartate. The intermediate products (pyruvate and oxaloacetate) formed react with 2,4-dinitrophenylhydrazine to form pyruvate hydrazone and oxaloacetate hydrazone whose absorbances are taken at 546 nm. The enzyme activities were extrapolated from the standard calibration curve.

(b): Alkaline Phosphatase Activity Assay: The procedure described by Saini and Van Etten (1978) as modified and described by Oyedapo (1996) was employed. P-nitrophenyl phosphate (Na/K salt) in the presence of alkaline phosphatase is hydrolysed to p-nitrophenol and inorganic phosphate in the presence of alkaline phosphatase. The p-nitrophenol liberated absorbs maximally at 410 nm in an alkaline medium.

Activity (unimole p-nitrophenol/mL) =

$$\frac{\Delta \text{ Abs}_{410\,\text{nm}}}{\varepsilon \text{ x d}} \text{ x } \frac{1}{t} \text{ x } 10^6 \text{ x } \frac{\text{Tv}}{\text{Sv}}$$

Where: $Abs_{410\,nm}$ = absorbance read at 410 nm; ε (molar extinction coefficient) = 1.88 x 10^3 $M^{-1}cm^{-1}$; t (reaction time, 15 min); Sv (sample volume, 0.05 mL); Tv (total assay volume, 3.05 mL)

(c): Bilirubin: the total Plasma bilirubin was evaluated spectrophotometrically based on the procedure of Jendrassik and Grof (1938). The concentration of total plasma bilirubin was calculated as:

The concentration of total bilirubin = 108 x $Abs_{578\,nm}$

Where; 10.8 = molar absorptivity constant of bilirubin at 578 nm.

Evaluation of Antioxidant Activities

(a): **Glutathione peroxidase (GPx) activities**: The activities of glutathione peroxidase (GPx) in the liver and kidney homogenates were carried out in accordance to the procedure described by Rotruck *et al.* (1973). The activity was calculated as follows;

GPx activity (µmol GSH/mg protein) =

$$\frac{\text{Abs}_{412} \times \text{Tv} \times \text{d.f}}{\varepsilon\,(6.22 \times 10^3) \times \text{ev}}$$

Where: Abs = Absorbance at 412; df = dilution factor; Ɛ = extinction coefficient of DTNB; Tv = Total assay volume and ev = enzyme volume.

(b): C**atalase (CAT) activities:** A procedure described by Sinha (1972) was employed to assay the activities of CAT. The activity is expressed thus

Catalase activity (U/min/mg protein) =

$$\frac{\Delta A \times \partial.f \times sv}{tv \times \varepsilon\,(0.0436)}$$

Where: ΔA (change in absorbance); ∂f (dilution factor); sv (sample volume); tv (total assay volume); and Ɛ (the molar extinction coefficient of hydrogen peroxide).

(c) S**uperoxide dismutase (SOD) activities**: The activity of SOD was assayed in accordance to the procedure of Maklund and Maklund (1974) as modified (Gavali *et al.,* 2013). The SOD activity was evaluated as follows:

SOD activity (Unit/min /mg protein) =

$$\frac{\text{percentage inhibition}}{\text{50 x concentration of tissue protein}}$$

Where: Percentage inhibition =

$$\frac{\text{Average change in absorbance of substrate}}{\text{Average change in absorbance of blank}} \times 100$$

Average absorbance per min = $\frac{A_3 - A_0}{2.5}$

Where: A_0 (absorbance at 30 s) and A_3 (absorbance at 180 s).

(d): **Reduced glutathione GSH) concentration**: The method described by Moron *et al.* (1979) using 10 mg/L glutathione as standard was used in the determination of GSH concentration.

Evaluation of Lipid Profile

The evaluation of lipid profile (triacylglycerols (TG) (Bucolo and David, 1973), total cholesterol (TC) (Allain *et al.*, 1974 as reported by Third Report of the NCEP, 2001), high-density lipoprotein-cholesterol (HDL-c) (Lopes-Virella *et al.* 1977, as reported by Third Report of the NCEP, 2001); very low-density lipoprotein-cholesterol (VLDL-c) and low-density lipoprotein-cholesterol (LDL-c)) (Friedewald *et al.*, 1972 as reported by Solnica *et al.,* 2024).

Histological Investigations

A part of the right lobe of the liver and a cross-section of the kidney from each group were fixed in 10 % (w/v) formal saline for the histological study. The fixed tissues were processed and sectioned. Each section was dipped in hematoxylin and eosin dye (Prophet *et al.,* 1992). The micro-photograph of slides was carried out on the samples using Leica ICC50HD.

Statistical Analysis

The data obtained was expressed as Mean ± SEM, n=5 except stated otherwise. GraphPad Prism 5 was used in the analysis (One-Way Analysis of Variance, ANOVA) of values obtained. The differences in values were considered significant at $P < 0.05$.

RESULTS

Haemoglobin concentration was not significantly ($P < 0.05$) affected by consumption of aflatoxin B1-contaminated diet and spice-supplemented diet (Figure 1).

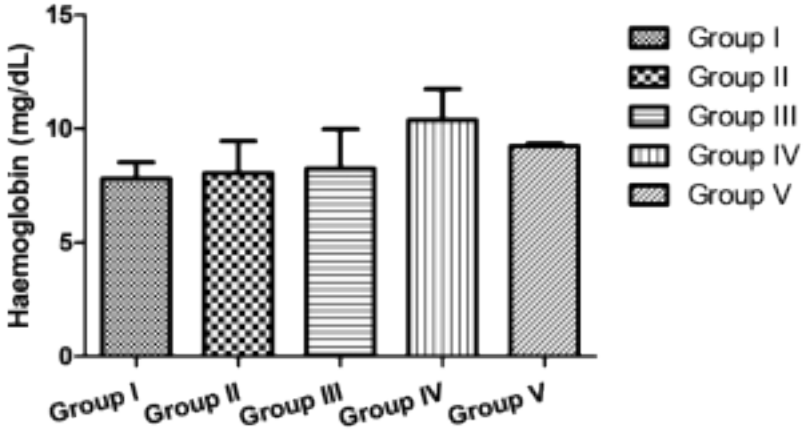


Figure 1: Effect of AfB1-contaminated and Spice-supplemented diet on concentrations of haemoglobin. a = P < 0.05 differ significantly in contrast to control (group I), and b = P < 0.05 differ significantly in comparison to group II. Group I = Control Birds (normal feed + water); Group II = AFB1-CD group; Group III = SSD group; Group IV = post-treated group; and Group V = Pre-treated group.

AFB1-CD had a significant negative impact on kidney function as shown in Figures 3 & 4. Concentrations of creatinine (Figure 3) and uric acid (Figure 4) were increased significantly (P < 0.05) in birds treated with AFB1-CD only (group II). However, the pre-treated and post-treated groups (IV and V) had levels comparable to those of the normal group (I).

Consumption of AFB1-CD significantly increased the concentration of bilirubin (Figure 2) in group II (0.32 ± 0.02mg/dL) when compared to other groups (I: 0.19 ± 0.01 mg/dL, III: 0.18 ± 0.01 mg/dL, IV: 0.22 ± 0.01 mg/dL and V: 0.25 ± 0.01 mg/dL.

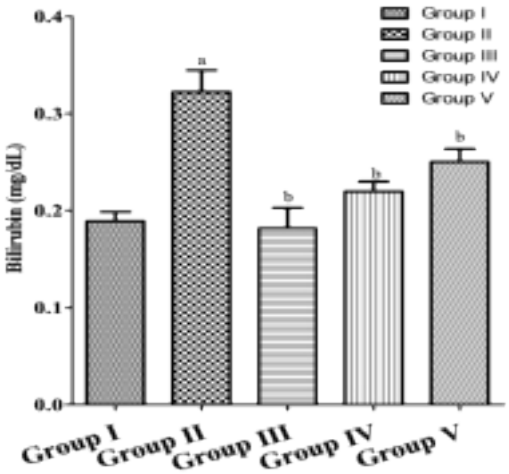


Figure 2: Effect of AfB1-contaminated and Spice-supplemented diet on the concentrations of bilirubin. a = P < 0.05 differ significantly in contrastto control (group I); and b = P < 0.05 differ significantly in comparison to group II. Group I = Control Birds (normal feed + water); Group II = AFB1-CD group; Group III = SSD group; Group IV = post-treated group; and Group V = Pre-treated group.

The concentration of creatinine (Figure 3) in the group fed with an AfB1-contaminated diet only was significantly (P < 0.05) elevated in contrast to the groups of birds that were fed with the standard broiler diet (group I; 0.83 ± 0.05 mg/dL) and SSD only (group III; 0.90 ± 0.02 mg/dL).

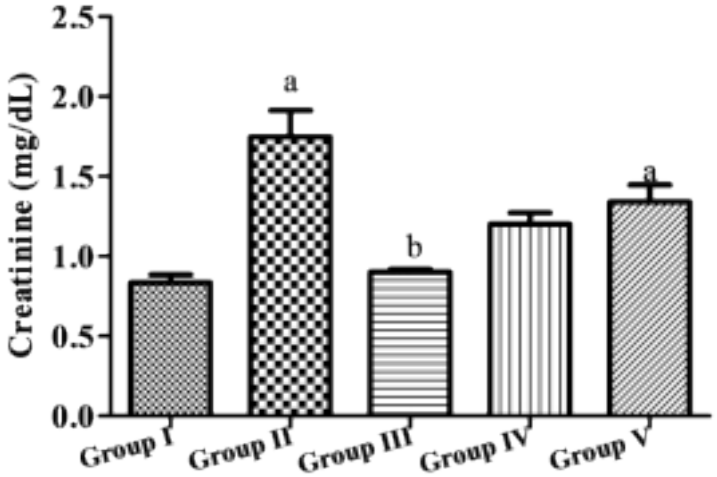


Figure 3: Effect of AfB1-contaminated and Spice-supplemented diet on the concentrations of creatinine. a = P < 0.05 differ significantly in contrast to control (group I); and b = P < 0.05 differ significantly in comparison to group II. Group I = Control Birds (normal feed + water); Group II = AFB1-CD group; Group III = SSD group; Group IV = post-treated group; and Group V = Pre-treated group.

The level of uric acid in group II (7.09 ± 0.29 mg/dL) was significantly elevated in comparison to all the other groups. (Figure 4)

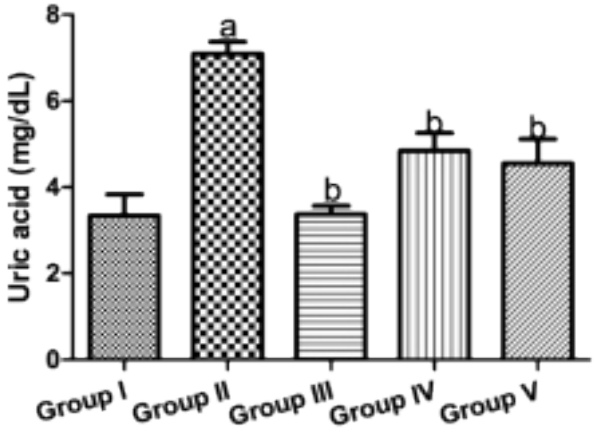


Figure 4: Effect of AfB1-contaminated and Spice-supplemented diet on the concentrations of uric acid. a = P < 0.05 differ significantly in contrastto control (group I); and b = P < 0.05 differ significantly in comparison to group II. Group I = Control Birds (normal feed + water); Group II = AFB1-CD group; Group III = SSD group; Group IV = post-treated group; and Group V = Pre-treated group.

The function of the liver was markedly affected (P < 0.05) in the birds fed with AFB1-CD. The activity of alanine aminotransferase (Figure 5) was considerably higher (P < 0.05) in plasma of birds fed with AFB1-CD while it was reduced in the livers. SSD does not have any considerable effect (P < 0.05) on the activity of the enzyme but brought about a considerable increase (P < 0.05) in the activity of ALT in birds when used as either a preventive or curative means.

There was a consequential increase (P < 0.05) in the activity of AST (Figure 6) in the plasma and a decrease in livers AST of birds fed with AFB1-CD in comparison to the normal group. SSD had no considerable effect on the plasma and liver AST. SSD significantly lowered the activity of the plasma enzyme when used in pre-treating or post-treating the birds when compared to group II. Nonetheless, no marked change (P < 0.05) was observed in the activity of AST in the livers of birds in all groups.

The activity of ALPase (Figure 7) in the kidneys of

the bird is not significantly ($P < 0.05$) affected by AFB1-CD, SSD or both diets. The same can be said for AST activity in the livers of birds fed with AFB1-CD only. However, there was a marked increase ($P < 0.05$) in ALPase activity in group fed with SSD only and the post-treated group.

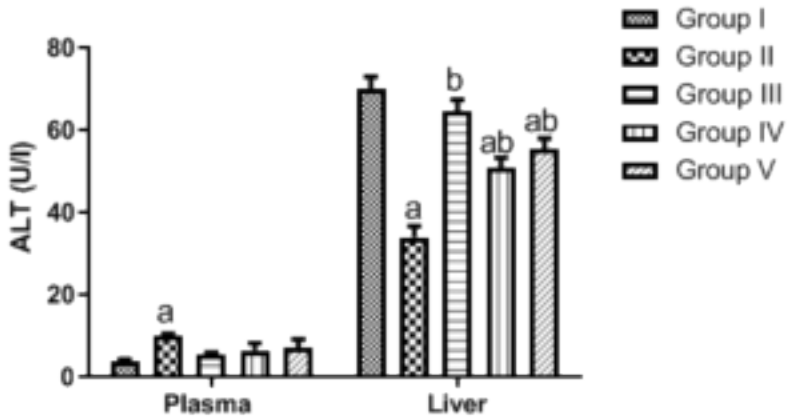


Figure 5: The Effect of AfB1-contaminated and Spice-supplemented diet on the activity of Alanine aminotransferase (ALT) in plasma and liver.
a = $P < 0.05$ differ significantly in contrast to control (group I); and b = $P < 0.05$ differ significantly in comparison to group II. Group I = Control Birds (normal feed + water); Group II = AFB1-CD group; Group III = SSD group; Group IV = post-treated group; and Group V = Pre-treated group.

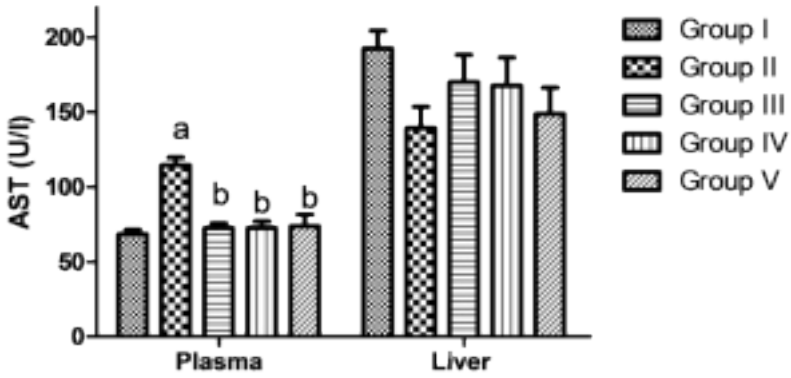


Figure 6: Effect of AfB1-contaminated and Spice-supplemented diet on the activity of Aspartate aminotransferase (AST) in plasma and liver.
a = $P < 0.05$ differ significantly in contrast to control (group I); and b = $P < 0.05$ differ significantly in comparison to group II. Group I = Control Birds (normal feed + water); Group II = AFB1-CD group; Group III = SSD group; Group IV = post-treated group; and Group V = Pre-treated group.

There was no marked difference in plasma ALPase activity across the groups. However, the liver ALPase activity in group II (169.81 ± 8.49 unimole p-nitrophenol /ml) was considerably lower ($P < 0.05$) than in group III (311.85 ± 46.18 unimole p-nitrophenol /ml) and group IV (332.58 ± 6.68 unimole p-nitrophenol /ml). (Figure 7).

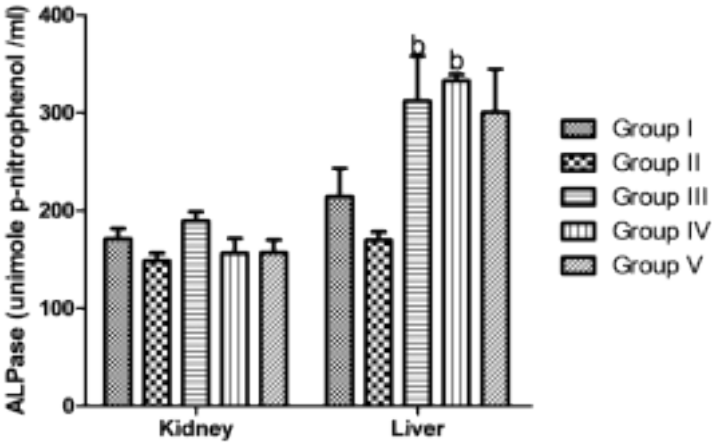


Figure 7: Effect of AfB1-contaminated and Spice-supplemented diet on the activity of alkaline phosphatase (ALPase) in kidney and liver.
a = P < 0.05 differ significantly in contrast to control (group I); and b = P < 0.05 differ significantly in comparison to group II. Group I = Control Birds (normal feed + water); Group II = AFB1-CD group; Group III = SSD group; Group IV = post-treated group; and Group V = Pre-treated group.

The liver and kidney antioxidant system activities had a marked increase (P < 0.05) in the antioxidant enzymes' activities and a significant reduction was detected in all groups fed with AFB1-CD (Figure 8 A-D). SSD reduced the effects of AFB1-CD on the pre-treated and post-treated groups significantly (P < 0.05) on the antioxidant enzymes' activities while significantly increasing (P < 0.05) the concentration of the antioxidant molecule.

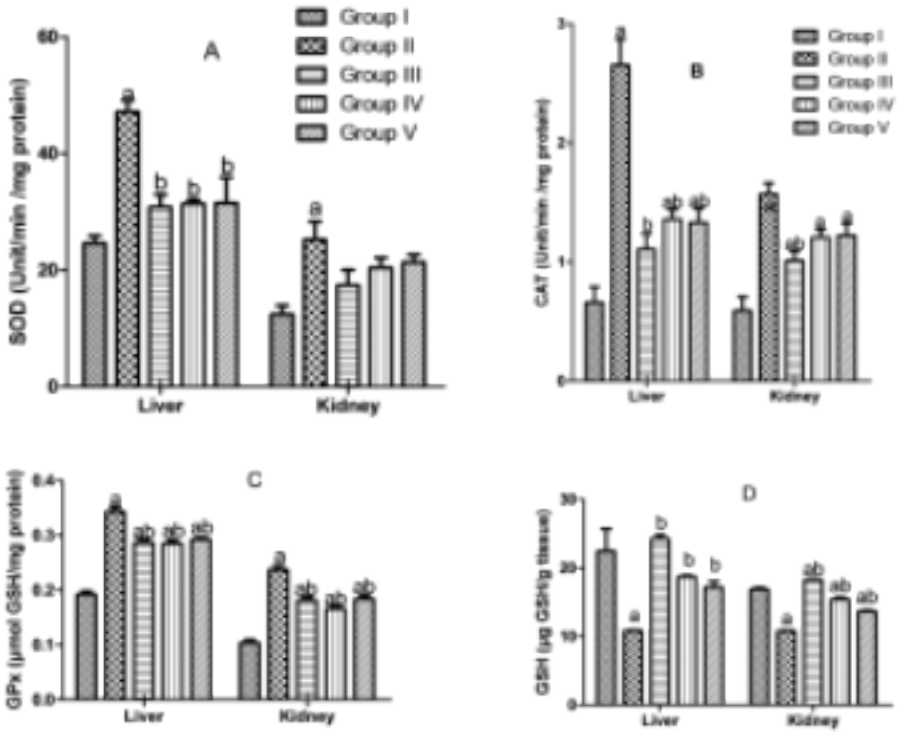


Figure 8: (A – D): Effects of AfB1-contaminated and Spice-supplemented diet on the antioxidant system.
a = P < 0.05 differ significantly in contrast to control (group I); and b = P < 0.05 differ significantly in comparison to group II. Group I = Control Birds (normal feed + water); Group II = AFB1-CD group; Group III = SSD group; Group IV = post-treated group; and Group V = Pre-treated group.

The concentrations of TC, TAG, LDL-c and VLDL-c (Figure 9) in groups fed with AFB1-CD were markedly increased ($P < 0.05$), HDL-c concentrations were markedly lowered ($P < 0.05$) while all the cholesterol levels were not affected by the consumption of SSD only. However, SSD was able to minimise the effects of AFB1-CD on the concentrations of triacylglycerol while VLDL-c concentration was markedly reduced ($P < 0.05$) in both pre-treated and post-treated groups.

The concentration of triacylglycerols (Figure 9) in group II (90.43 ± 3.22 mg/dL) was considerably elevated ($P < 0.05$) in contrast to group I (53.97 3.94 mg/dL), group III (61.01 ± 2.41 mg/dL), group IV (65.10 ± 2.16 mg/dL) and group V (66.19 ± 3.16 mg/dL) while group III, IV and V were not statistically different from group I.

The concentration of total cholesterol in group II (112.35 ± 3.55 mg/dL) was markedly elevated ($P < 0.05$) in relation to all other groups.

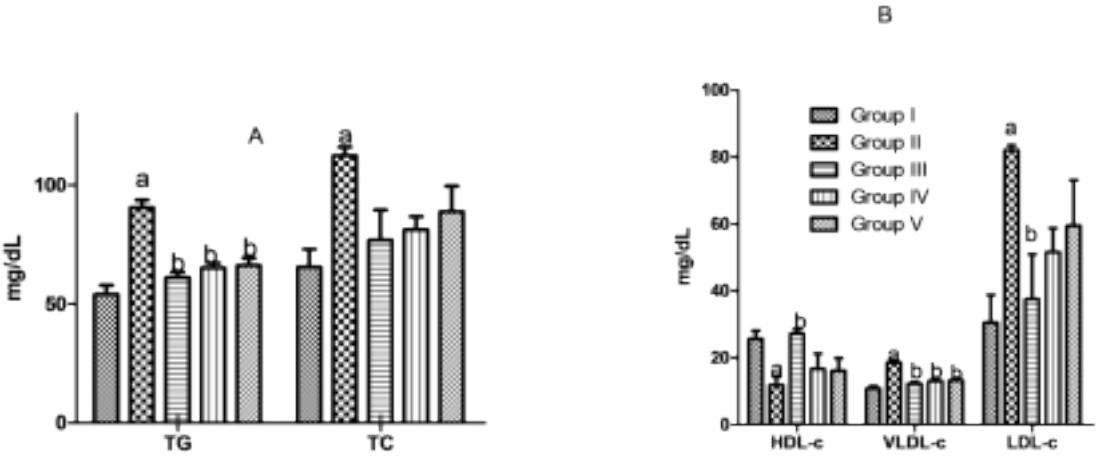
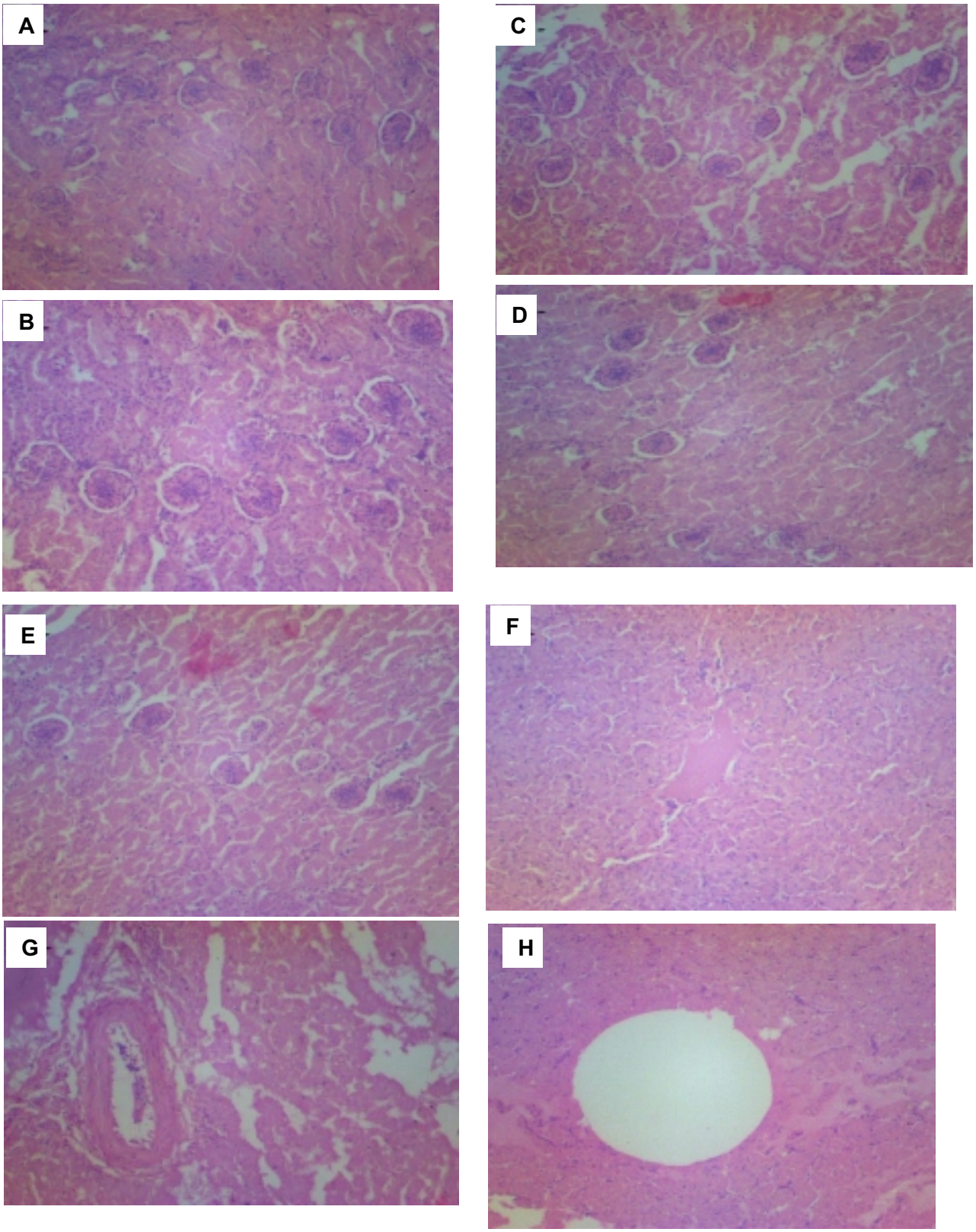


Figure 9: Effect of AfB1-contaminated and spices-supplemented diets on lipid profile.
a = $P < 0.05$ differ significantly in contrast to control (group I); and b = $P < 0.05$ differ significantly in comparison to group II. Group I = Control Birds (normal feed + water); Group II = AFB1-CD group; Group III = SSD group; Group IV = post-treated group; and Group V = Pre-treated group.

The histological observation of the kidney was graded based on the structures of the glomeruli, interstitium, filtration spaces, urinary poles and inflammation of the tissues as presented in plate 1. Kidney histology of the birds in group I (Plate 1A) appeared normal. The interstitium was adequate, glomeruli were normal and appeared healthy. Filtration spaces were well defined with vascular poles well distinguishable from the urinary poles. In groups IV (Plate 1D) and V (Plate 1E), there was a reduction in glomeruli diameter and a reduction in the quantity of glomerulus. The tubules were well identified within the interstitium with a clear lumen. However, in Group IV, the glomeruli were larger in number and slightly larger when compared to Group V. Group II (Plate 1B) showed conspicuously enlarged glomeruli with preserved filtration spaces. The majority of the glomeruli showed no sign of inflammation but some of them showed signs of glomerulonephritis, closing up of the filtration spaces. The interstitium was normal but most of the tubules were clogged. No sign of tubulorrhexis was observed. In group III (Plate 1C) however, the kidney tubules appeared normal and unclogged. The glomeruli were normal as observed in the control. The interstitium was adequate, and the vascular and urinary poles of the glomeruli were distinct. The histoarchitecture was well preserved.

The histology of the liver was graded on the presence of distinct boundaries of cells, central veins, sinusoids and portal triad system (Plate 1). Group I (Plate 1F) had normal histoarchitecture with distinct sinusoids. Group II (Plate 1G)

showed a disrupted histoarchitecture showing no structural distinction except for bounded vessels by thick tunics. Group IV (Plate 1I) and V (Plate 1J) showed similar architecture as group II except for the presence of central veins and portal triad system. The histology of the liver of group III (Plate 1H) was similar to that of control but the sinusoids were clogged and not so distinct. There was also the presence of visible vacuoles.



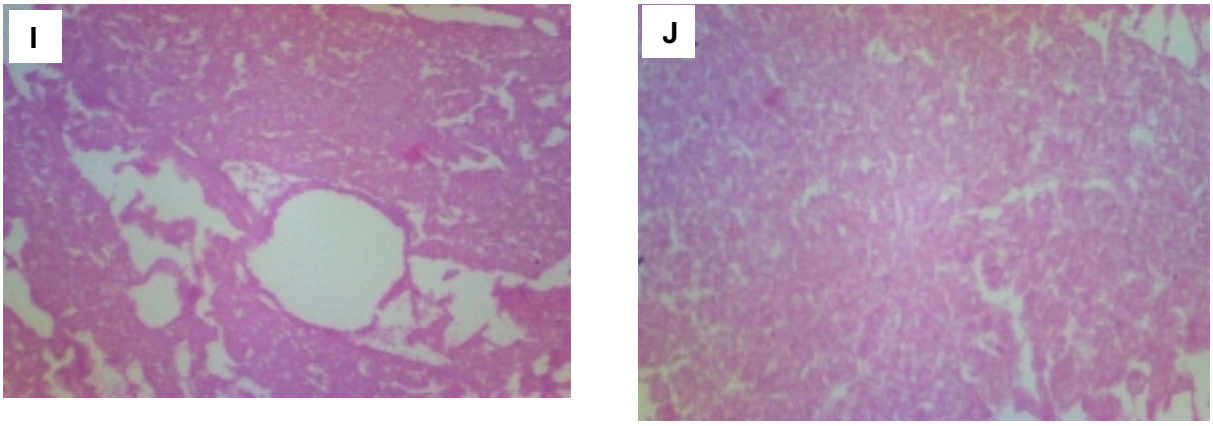


Plate 1: Microscopic sections showing effects of AfB1-Contaminated and Spices-Supplemented diets on Kidney and Liver Tissues (H & E, Magnification X400)
Where; A = Kidney (Group I; Control Birds); B = Kidney (Group II; AFB1-CD group); C = Kidney (Group III; SSD group); D = Kidney (Group IV; Post-treated group); E = Kidney (Group V; Pre-treated group); F = Liver (Group I; Control Birds); G = Liver (Group II; AFB1-CD group); H = Liver (Group III; SSD group); I = Liver (Group IV; Post-treated group) and J = Liver (Group V; Pre-treated group)

DISCUSSION Aflatoxins are a group of mycotoxins majorly synthesized by fungi of the *Aspergillus* family (Saini and Kaur, 2012). Out of about seventeen (17) species which have been so far isolated, four species (B1, B2, GI and G2) are well-known and extensively studied toxicologically (WHO, 1979), and Aflatoxin B1 (AFBI) is the most acutely toxic of aflatoxins to various species (humans and animals) (Thajuddin *et al.,* 2011). Consumption of food exposed to AFB1 or indirect contamination with this mycotoxin has been reported to cause encumbered growth, suppression of the immune system, cancer, and damage to genes and foetus (Ali *et al.,* 2021, Ates *et al.,* 2022).

Chicken feed is often exposed to contamination by aflatoxin as a result of poor storage facilities and the use of contaminated materials in feed preparation (Tola and Kebede, 2016, Ferrari et al., 2022). Aflatoxicosis (aflatoxin poisoning) has resulted in severe economic loss in poultry - quail, broilers, layers, ducklings, and turkeys (CAST 1989, Winter and Pereg, 2019). It affects important parameters such as weight, feed consumption efficiency (intake and conversion), egg production as well as reproductive performance. Moreover, aflatoxin induces stress and increased mortality in the course of infection and has accounted for the death of hundreds of poultry birds. Specifically, it is postulated that AFB1 and its metabolites cause nephrotoxicity as well as liver dysfunction in poultry birds (Calnek *et al.* 1997*;* Kurniasih and Prakoso., 2019*).*

Studies previously conducted have revealed that spices are rich in biomolecules that elicit antimicrobial, anti-inflammatory, antipyretic, antifertility analgesic, antioxidant, antioxidant, antithrombotic, antiproliferative, anti-anaphylactic, molluscicidal, organ-protective, cytotoxic, antiallergic, sexual enhancing, antiarthritic, anti-ischemic activities (Umukoro and Aladeokin, 2011; Dohnal *et al.*, 2014; Erhirhie and Moke, 2014; Mbaveng and Kuete, 2017). This study was conceived to evaluate both the protective and enhancing potential of a mixture of three commonly used spices (*Aframomum melegueta*, *Syzygium aromaticum*, and *Xylopia aethiopica*) on the biochemical parameters of AFBI-exposed broiler chickens.

The consumption of AFB1- contaminated diet did not elicit a negative impact on the concentration of haemoglobin when compared with Group 1 although there was a slight increment in the concentrations of haemoglobin in groups pre-treated and post-treated with diet containing spices. Wilujeng (2020) had earlier reported that AFBI did not elicit any form of metabolic derangement in the blood components and parameters of the exposed animals.

There was a noticeable increase in concentrations of uric acid and creatinine in groups fed with AFB1-CD when juxtaposed with the control group. Earlier studies conducted by Gunduz *et al.* (2021) reported elevation of creatinine in animals exposed to AFBI. Tung *et al.* (1975) and Saki *et al.* (2018) discovered and reported a similar elevation in uric acid concentration in exposed animals to AFBI. However, consumption of spices containing diets in (Groups IV, and V) caused a marked reduction in the concentrations of these metabolites, indicating that the spices exerted a protective effect and also reduced the deleterious effects of metabolites generated from AFB1-CD on the kidneys of the birds. Other ameliorative agents which have been employed in the diet of animals to mitigate against the deleterious effect of AFB1 on the kidney include the leaf of Neem (*Azadirachta indica*) meal (Ubua *et al.*, 2018) as well as cassava-based diets supplemented with African Yam bean concentrate (Anya *et al.*, 2018). Our results suggest that the mixture of spices in the SSD could exert a similar nephroprotective effect.

There was a marked elevation in the level of bilirubin in the group fed with AFB1-CD when compared with the normal group (Figure 2). AFB1-CD also perturbed the activity of ALT in groups fed with the diet while those fed SSD had normal ALT values (Figure 5). Furthermore, the activity of AST was also higher in the plasma of groups fed with AFB1-CD while there was a reduction in the activity of the enzyme in the liver majorly due to damage to the integrity of hepatocytes thereby leaking the enzyme into the bloodstream (Figure 6). However, the mixture of spices in SSD was able to reduce and remediate the metabolic derangement in the membrane of the liver resulting from the consumption of aflatoxin B1. The perturbation in the activity of ALT may be a result of hepatocellular injury (Amacher, 2002; Ozer *et al.,* 2008 and Aulbach and Amuzie, 2017) while the rise in AST activity may be a result of derangement, not specific to the liver but also kidney, heart and skeletal muscles (Giffen *et al.*, 2002; York, 2017). The feeding of the birds with the mixture of spices (SSD) did not affect the activity of the enzyme in the birds. Moreover, the activities of AST in the plasma of chickens pre-treated and post-treated with spices were similar to that of the Group I. This indicates that the mixture of spices mitigated the derangement of AST.

There was no statistical difference in the activity of kidney alkaline phosphatase (ALP of birds across the groups, although there was a slight decrease in the activity of liver ALPase of birds in birds fed with AFB1-CD (Figure 7). A decline in the activity of ALPase in the liver is specific for metabolic derangement or damage to the hepatocyte most especially the hepatobiliary system (Aulbach and Amuzie, 2017). This could be an indication that there was potential damage to the liver.

Activities of antioxidant enzymes (SOD, CAT and GPx) were highly increased in birds fed with AFB1-CD (Figure 8 A-C). This could be a result of the need for the cells to quench oxidative stress as a consequence of the consumption of aflatoxin B1. This elevation agreed with the report of Arbona *et al.* (2003) and Irato and Santovito (2021) that activities of antioxidant enzymes are increased in the presence of aflatoxin-B1. SSD was able to diminish the effects of aflatoxin-B1in groups IV and V by reducing and mitigating the elevation of antioxidant enzymes. Although there was an elevation in the activities of the antioxidant enzymes in the birds fed with only a spice-supplemented diet, their activities were markedly lower than those obtained in group II. The elevation observed in group III may be a consequential effect of the various biological activities of spices elicited in the body. The non-enzymatic antioxidant, glutathione, was significantly depleted in groups fed with AFB1-CD (Figure 8D). The consumption of the spice-supplemented diet markedly mitigated against the depletion of this molecule (Moussa et al., 2019).

The consumption of aflatoxin - BI caused abnormalities in lipid metabolism. There were increased levels of TG, TC, LDL-c; and VLDL-c concentrations while there was a decrease in the concentration of HDL-c. These abnormalities observed aligned with the observations of Abdel-Wahab *et al.* (2010) and Brinda *et al.*, (2013). Dyslipidaemia in birds may be a result of hepatic lesions, chronic renal failure, nephrotic syndrome and disruption of cholesterol metabolism by liver cells (Edrington *et al.*, 1995; Abdel-Wahab *et al.*

2010). These various metabolic disorders were remediated in both groups IV and V as a result of the consumption of the mixture of spices in SSD. The mixture of spices did not elicit any negative effect on the lipid profile of the birds.

Histological studies indicate that Aflatoxin B1 caused damage to the glomeruli of the kidney, with signs of glomerulonephritis, closing up of filtration spaces and clogging of tubules. This finding is in agreement with the findings of (El-Mahalaway, 2015). The consumption of SSD was able to ameliorate these signs while not causing any significant damage to the kidney in the process. Damages were also observed in the liver of birds as a result of consumption of aflatoxin B1 where the structure of the liver was disrupted (Saleem *et al.,* 2021). The disruption was reduced in groups IV and V as a result of the effects of spices in the SSD.19

CONCLUSION

This study observed that *S. aromaticum, X. aethiopica* and *A. melegueta* possess bioactive molecules which have potential for the prevention and management of metabolic derangement and structural disruptions that arise as a result of the consumption of aflatoxin B1.

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CONFLICT OF INTEREST

The authors hereby declare no conflict of interest

AUTHORS CONTRIBUTIONS

BSO - data curation, methodology, formal analysis, investigation, visualization and writing (original draft), funding acquisition; OOO – conceptualization, methodology, resources, supervision, writing (review and editing) and funding acquisition; EBO - project administration, writing (review and editing); MAE – methodology, writing (review and editing); FAO – visualization, writing (original draft) and formal analysis

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