

# Ameliorative effects of hog plum and vitamin E on blood quality of broiler fed Aflatoxin B1

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**ABSTRACT:** Aflatoxicosis is one of the serious health complications in poultry, posing potential hazards to human health. This study examined the effect of vitamin E and the extract of hog plum on the blood parameters of broiler chickens. Fresh hog plum leaves (*Spondias mombin*) were harvested from the Federal Polytechnic Ilaro community, ground into powder and soaked at a ratio of 1:10 in ethanol. Aflatoxin and Vitamin E were purchased from a reputable store and used for the experiment. Two hundred and fifty-two broiler chicks were assigned to seven treatments with 3 replicates in a completely randomised manner. Two birds from each replicate were chosen on the 14th and 49th day, blood sample was drawn from their brachial vein for the assessment of haematological and serum biochemical parameters. Results obtained were subjected to One-way analysis of variance on SPSS IBM 25, and significantly distinct means were distinguished at  $p < 0.05$ . Significant differences were observed in the blood parameter values of the birds. At the first phase, T2 had the highest packed cell volume, haemoglobin and lymphocyte (32.50%, 10.00 g/dl and 65.00%) while T6 had the highest white blood cells and monocytes values (16550.00, and 3.01 %). T3 had the highest alanine aminotransferase and creatinine (34.00 U/L and 0.90 mg/dl) while T4 had the highest total protein, albumin and globulin values (4.53, 2.35 and 3.13 g/dl). At the last phase of the experiment, T5 had the highest PCV and Hb values (31.50% and 10.25 g/dl) while T3 had the highest monocytes and basophils (4.00 and 1.00%). T2 and T3 had the highest creatinine and cholesterol values of 0.65 mg/dl and 252.50, respectively. It can be concluded that the extract of Hog and Vitamin E effectively mitigated aflatoxin-induced haematological and biochemical disruptions, with EHP providing immediate benefits while Vitamin E ensures long-term recovery.

**Keywords:** Aspergillus, birds, contaminant, health, plant, vitamins.

## INTRODUCTION

Aspergillus is a type of soil fungus that has been found to be a major source of contamination for a number of grains used in chicken diets. They grow rapidly in high-moisture conditions and create hepatotoxic, physiologically active aflatoxins (Naveed *et al.*, 2022).

One of the biggest concerns in the chicken production industry is aflatoxicosis, a disease brought on by eating feed tainted with aflatoxins. Affected poultry farm may experience significant financial losses substantially correlated with high rates of illness and death (Yang *et al.*, 2020), horizontal transmission (through milk, meat, and

eggs from poultry that consume contaminated feed) can occur between humans and poultry birds, making it a serious public health concern (Attia *et al.*, 2016).

Aflatoxin residue in poultry products has become a potential health concern for people because it leads to decreased growth and productivity and increases the death rate (Amjed, 2018). Additionally, Barati *et al.* (2017) said aflatoxin B1 causes immunosuppression, alterations in blood parameters, hepatic illnesses, and metabolic abnormalities in poultry. Changes in serum parameters are the index of liver damage level and disruption in metabolic

pathways, so also is the reduction of serum glucose, protein, calcium and phosphorus levels in aflatoxicosis condition (Barati *et al.*, 2017).

Haematological and biochemical parameters are useful clinical tool in determining the health status and are critical for the diagnosis of the state of internal organs, especially the liver and kidney, aiding disease and management of poultry.

Previous studies demonstrated that biological techniques may eliminate or lessen the effects of aflatoxin, and also polysaccharides and certain bacterial cell wall peptidoglycans could bind aflatoxin B 1. According to Barati *et al.* (2017), supplementation of chicken feed with antioxidants, such as vitamin E and other phytogetic plants, e.g hog plum (*Spondias mombin*), may help mitigate the adverse effects of aflatoxins on broiler chickens. Vitamin E is a crucial component of the antioxidant defence system, playing a vital role in protecting cell membranes from oxidative damage (Saleemi *et al.*, 2023).

Research has shown that vitamin E supplementation can improve liver function and overall health in poultry exposed to aflatoxins. Furthermore, there is evidence suggesting a synergistic relationship between vitamin E and hog plum, which may enhance their protective effects against aflatoxicosis (Ulaiwi, 2018).

This research, therefore, explores the haemoprotective abilities of Vitamin E and hog plum to mitigate the impacts of aflatoxicosis on blood quality and health of broiler chickens when fed aflatoxin B1.

## MATERIALS AND METHODS

### Experimental site

The research took place at the coordinates of latitude 6°37'46"N and 6°55'42"N and longitude 2°47'24"E and 3°6'48"E, where the Federal Polytechnic Ilaro, Poultry Unit of the Agricultural Technology Department Farm is domiciled in the Yewa South Local Government Area of Ogun State, Nigeria. The conduct of the experiment was approved by the ethical committee of the Polytechnic.

### Preparation of Vitamin E and hog plum extract

Aflatoxin B1 and Vitamin E were purchased from a reputable laboratory in Ibadan, Nigeria, while fresh hog plum leaves were harvested from the Federal Polytechnic, Ilaro community, dried at room temperature, and ground into powder using a blender (Pyramid® PM-B999) (Okusanya and Akinlade, 2024). Ten grams of the hog plum powder was added to 100 ml of ethanol in an air-tight container and allowed to stand for 24 hours. The solution was filtered using a muslin filter, and the extract of hog plum (EHP) was stored in a container and used for the experiment.

## Experimental animal and management

Twenty-five (252) day-old broiler chicks were acquired from a respectable commercial hatchery and acclimated for fourteen days in the brooding pen. After which they were allotted to seven (7) treatments, and each treatment replicated three times with twelve (12) birds per replicate. Standard routine and occasional (vaccinations and medication schedules) management practices for broiler chickens were strictly adhered to. Commercial feed and water were given *ad libitum* throughout the six weeks experimental period.

### Experimental design

The design of the experiment was a Completely Randomised Design. Each treatment received microgram ( $\mu\text{g}$ ) of aflatoxin B1 (AFB1) per kilogram of feed, while Vitamin E and hog plum extract were administered per litre of water as follows:

- Treatment 1: 0 ml of EHP + 0  $\mu\text{g}$  of Aflatoxins
- Treatment 2: 1ml EHP + 0  $\mu\text{g}$  of AFB1
- Treatment 3: 1ml EHP + 35  $\mu\text{g}$  of AFB1
- Treatment 4: 35  $\mu\text{g}$  of AFB1 + 0 ml of EHP
- Treatment 5: 1ml Vitamin E + 0  $\mu\text{g}$  of AFB1
- Treatment 6: 1ml Vitamin E + 35  $\mu\text{g}$  of AFB1
- Treatment 7: 35  $\mu\text{g}$  of AFB1 + 0 ml of Vitamin E

After 2 weeks of administration, the birds were given a rest period of 7 days and then administered the treatment below for another 2 weeks to make 4 weeks of administration.

- Treatment 1: 0 ml of EHP + 0  $\mu\text{g}$  of Aflatoxins
- Treatment 2: 0ml EHP + 35  $\mu\text{g}$  of AFB1
- Treatment 3: 0ml EHP + 0  $\mu\text{g}$  of AFB1
- Treatment 4: 0  $\mu\text{g}$  of AFB1 + 1 ml of EHP
- Treatment 5: 0ml Vitamin A + 35  $\mu\text{g}$  of AFB1
- Treatment 6: 0ml Vitamin A + 0  $\mu\text{g}$  of AFB1
- Treatment 7: 0  $\mu\text{g}$  of AFB1 + 1 ml of Vitamin E

### Data collection

#### Blood samples

After a 12-hour fast, two birds from each replicate were chosen at random on the 14th and 49th day, which marks the end of the chick and finisher phase, respectively. 4 ml of blood was drawn via the brachial vein using a sterile syringe. According to the methods described by Alagbe *et al.* (2023), 2 ml was placed into EDTA bottles for the assessment of haematological indices and 2 ml in a sterile sample bottle devoid of anticoagulants for the assessment of serum biochemical parameters. This resulted in 28 blood samples each at the chick and finisher phases.

**Table 1.** Haematological parameters of broiler chicken administered Vitamin E and hog plum extract at the first two weeks.

Parameters	T1	T2	T3	T4	T5	T6	T7	±SEM	p-value
PCV(%)	24.67 <sup>bc</sup>	32.50 <sup>a</sup>	27.50 <sup>ab</sup>	20.50 <sup>c</sup>	24.00 <sup>bc</sup>	23.33 <sup>bc</sup>	30.00 <sup>a</sup>	0.83	0.01
HB (g/dl)	7.87 <sup>bc</sup>	10.00 <sup>a</sup>	8.85 <sup>ab</sup>	7.20 <sup>c</sup>	7.80 <sup>bc</sup>	7.57 <sup>bc</sup>	9.70 <sup>a</sup>	0.27	0.04
RBCx <sup>10<sup>12</sup>/L</sup>	2.07 <sup>c</sup>	3.00 <sup>ab</sup>	3.61 <sup>a</sup>	1.55 <sup>c</sup>	2.32 <sup>bc</sup>	2.11 <sup>c</sup>	3.05 <sup>ab</sup>	0.17	0.02
WBC	14700.00 <sup>ab</sup>	16200.50 <sup>ab</sup>	15600.50 <sup>ab</sup>	10750.50 <sup>c</sup>	15775.00 <sup>ab</sup>	16550.00 <sup>a</sup>	13250.00 <sup>bc</sup>	520.69	0.00
LYMP(%)	55.68 <sup>b</sup>	65.00 <sup>a</sup>	56.00 <sup>b</sup>	48.00 <sup>c</sup>	52.90 <sup>b</sup>	52.71 <sup>b</sup>	64.06 <sup>a</sup>	74.99	0.03
HETE (%)	38.20 <sup>ab</sup>	32.00 <sup>ab</sup>	41.00 <sup>a</sup>	46.00 <sup>a</sup>	42.58 <sup>a</sup>	40.90 <sup>a</sup>	30.17 <sup>b</sup>	53.39	0.02
MONO(%)	2.51 <sup>ab</sup>	2.00 <sup>b</sup>	1.00 <sup>c</sup>	2.00 <sup>b</sup>	2.53 <sup>ab</sup>	3.01 <sup>a</sup>	2.00 <sup>b</sup>	6.12	0.03
EO(%)	3.26 <sup>ab</sup>	1.00 <sup>b</sup>	1.00 <sup>b</sup>	4.00 <sup>a</sup>	2.00 <sup>bc</sup>	3.07 <sup>ab</sup>	3.78 <sup>a</sup>	6.97	0.01
BA(%)	0.34 <sup>b</sup>	0.00 <sup>c</sup>	1.00 <sup>a</sup>	0.00 <sup>c</sup>	0.00 <sup>c</sup>	0.32 <sup>b</sup>	0.00 <sup>c</sup>	2.66	0.01
MCV (fl)	123.02	73.24	87.31	133.83	108.06	116.17	98.48	5.35	0.09
MCH (g)	39.39 <sup>ab</sup>	24.33 <sup>c</sup>	28.80 <sup>c</sup>	45.16 <sup>a</sup>	34.84 <sup>b</sup>	37.55 <sup>ab</sup>	31.81 <sup>bc</sup>	1.74	0.02
MCHC (%)	31.89	25.06	33.10	34.00	32.45	32.38	32.31	0.82	0.44

**Table 2.** Haematological parameters of broiler chicken administered Vitamin E and hog plum extract at the last three weeks.

Parameters	T1	T2	T3	T4	T5	T6	T7	±SEM	P-value
PCV(%)	22.00 <sup>c</sup>	22.67 <sup>c</sup>	28.00 <sup>b</sup>	30.00 <sup>ab</sup>	31.50 <sup>a</sup>	27.50 <sup>b</sup>	24.00 <sup>c</sup>	0.84	0.00
HB (g/dl)	7.23 <sup>d</sup>	7.33 <sup>d</sup>	8.90 <sup>b</sup>	9.80 <sup>ab</sup>	10.25 <sup>a</sup>	8.55 <sup>bc</sup>	7.65 <sup>c</sup>	0.28	0.00
RBCx <sup>10<sup>12</sup>/L</sup>	1.93 <sup>b</sup>	1.77 <sup>b</sup>	2.80 <sup>a</sup>	2.84 <sup>a</sup>	2.59 <sup>a</sup>	2.73 <sup>a</sup>	1.69 <sup>b</sup>	0.11	0.00
WBC	16833.33	11866.67	13525.00	14850.00	13250.00	15050.00	10750.00	540.66	0.00
LYMP(%)	51.74	49.50	60.71	65.00	63.30	57.46	50.88	68.15	0.40
HETER(%)	40.66 <sup>ab</sup>	44.11 <sup>a</sup>	32.00 <sup>b</sup>	30.13 <sup>b</sup>	29.14 <sup>b</sup>	37.00 <sup>ab</sup>	41.51 <sup>ab</sup>	50.14	0.00
MONO(%)	2.68 <sup>ab</sup>	2.71 <sup>ab</sup>	4.00 <sup>a</sup>	1.40 <sup>b</sup>	3.35 <sup>ab</sup>	3.00 <sup>ab</sup>	3.60 <sup>ab</sup>	6.36	0.03
EOSIN(%)	4.91 <sup>a</sup>	3.07 <sup>b</sup>	2.28 <sup>b</sup>	3.47 <sup>ab</sup>	4.20 <sup>ab</sup>	2.54 <sup>b</sup>	3.47 <sup>ab</sup>	9.85	0.01
BASO(%)	0.00 <sup>c</sup>	0.60 <sup>b</sup>	1.00 <sup>a</sup>	0.00 <sup>c</sup>	0.00 <sup>c</sup>	0.00 <sup>c</sup>	0.53 <sup>b</sup>	2.30	0.11
MCV (fl)	114.79	128.43	100.01	105.85	125.34	100.93	141.85	4.21	0.09
MCH (g)	37.75	41.57	31.78	34.58	40.82	31.42	45.21	1.45	0.07
MCHC (%)	32.82	32.30	31.79	32.67	32.53	31.12	31.87	0.21	0.21

## Haematological and serum analysis

The following estimations were made: total erythrocyte count (TEC, Neubaur's chamber), total leucocyte count (TLC, Neubaur's chamber), packed cell volume (PCV, Microhaematocrit method), hemoglobin (Hb, Sahli's acid haematin methods), and differential leucocyte count (DLC, Wright's stain). The sera were separated and analysed for the total protein (Biuret methods), albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and uric acid using an Automatic biochemical analyser (Transasia Ebra Chem-5 plus, Transasia Bio-medicals Limited)

## Statistical analysis

One-way analysis of variance was performed on the collected data (ANOVA). Duncan's New Multiple Range Test was used to significantly distinguish means where applicable on SPSS IBM 25.

## RESULTS

### Haematological parameters of broiler chicken administered Vitamin E and hog plum extract

The haematological parameter values of broiler chickens administered vitamin E and hog plum extract in the reduction of aflatoxicosis effects are shown in Tables 1 and 2. All parameters measured were significantly ( $p < 0.05$ ) different among treatments except for the mean corpuscle volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) at the first 2 weeks. T2 had the highest packed cell volume (PCV), haemoglobin (Hb) and lymphocyte values (32.50%, 10.00 g/dl and 65.00%), T3 had the highest red blood cells and basophils values of  $3.61 \times 10^{12}/L$  and 1.00%, respectively. T6 had the highest white blood cells (WBC) and monocyte values (16550.00 and 3.01%), while T4 had the highest heterophil, eosinophil, and mean corpuscular haemoglobin (MCH) values of 46.00%, 4.00 %, and 45.16 g at the first phase of the experiment. At the last phase of the experiment, as

**Table 3.** Serum metabolites parameters of broiler chicken administered Vitamin E and hog plum extract at the first two weeks.

Parameters	T1	T2	T3	T4	T5	T6	T7	±SEM	P-value
T.PROT(g/dl)	2.59 <sup>c</sup>	3.5 <sup>b5</sup>	3.70 <sup>b</sup>	4.53 <sup>a</sup>	3.50 <sup>b</sup>	3.55 <sup>b</sup>	3.75 <sup>b</sup>	0.14	0.01
ALB (g/dl)	0.63 <sup>c</sup>	1.40 <sup>b</sup>	1.50 <sup>b</sup>	2.35 <sup>a</sup>	0.95 <sup>bc</sup>	1.30 <sup>bc</sup>	1.30 <sup>bc</sup>	0.13	0.00
GLOB (g/dl)	1.96 <sup>b</sup>	2.30 <sup>b</sup>	2.40 <sup>b</sup>	3.13 <sup>a</sup>	2.15 <sup>b</sup>	2.25 <sup>b</sup>	2.60 <sup>ab</sup>	0.10	0.02
AG, Ratio	0.32 <sup>b</sup>	1.05 <sup>b</sup>	2.00 <sup>a</sup>	0.85 <sup>b</sup>	0.47 <sup>b</sup>	0.58 <sup>b</sup>	0.53 <sup>b</sup>	0.14	0.00
AST (U/L)	181.11 <sup>c</sup>	192.00 <sup>b</sup>	192.50 <sup>b</sup>	191.75 <sup>b</sup>	179.50 <sup>c</sup>	190.00 <sup>b</sup>	288.00 <sup>a</sup>	7.93	0.00
ALT(U/L)	21.72 <sup>b</sup>	33.00 <sup>a</sup>	34.00 <sup>a</sup>	33.25 <sup>a</sup>	30.50 <sup>a</sup>	25.50 <sup>b</sup>	30.00 <sup>a</sup>	0.99	0.00
ALP(U/L)	192.67	217.00	218.00	217.25	196.50	190.00	217.00	2.84	0.00
CREAT (mg/dl)	0.49 <sup>c</sup>	0.70 <sup>bc</sup>	0.90 <sup>a</sup>	0.80 <sup>ab</sup>	0.75 <sup>b</sup>	0.50 <sup>c</sup>	0.60 <sup>bc</sup>	0.04	0.01
GLUC	285.39	293.00	294.00	293.25	301.00	293.50	301.00	1.15	0.11
CHOL	198.33	274.00	275.00	274.25	177.50	205.50	211.00	8.72	0.10

**Table 4.** Serum metabolite parameters of broiler chicken administered Vitamin E and hog plum extract at the last three weeks.

Parameters	T1	T2	T3	T4	T5	T6	T7	±SEM	P-value
T.PROT (g/dl)	3.10 <sup>bc</sup>	2.90 <sup>c</sup>	2.85 <sup>c</sup>	2.88 <sup>c</sup>	3.15 <sup>bc</sup>	3.80 <sup>a</sup>	3.48 <sup>ab</sup>	0.09	0.00
ALB (g/dl)	0.95 <sup>ab</sup>	0.65 <sup>b</sup>	0.75 <sup>b</sup>	0.70 <sup>b</sup>	0.85 <sup>b</sup>	1.25 <sup>a</sup>	1.05 <sup>ab</sup>	0.06	0.04
GLOB (g/dl)	2.15	2.25	2.10	2.18	2.30	2.55	2.43	0.04	0.12
AG, Ratio	0.44	0.29	0.36	0.32	0.36	0.50	0.43	0.02	0.16
AST (U/L)	179.83	177.50	177.00	177.25	175.50	183.00	179.25	0.88	0.37
ALT(U/L)	23.17 <sup>b</sup>	20.50 <sup>b</sup>	20.50 <sup>b</sup>	20.50 <sup>b</sup>	23.00 <sup>b</sup>	30.50 <sup>a</sup>	26.75 <sup>ab</sup>	0.99	0.02
ALP(U/L)	217.50	253.00	253.50	253.25	276.50	254.50	265.50	9.38	0.84
CREAT (mg/dl)	0.53 <sup>bc</sup>	0.65 <sup>a</sup>	0.60 <sup>ab</sup>	0.63 <sup>a</sup>	0.50 <sup>c</sup>	0.55 <sup>bc</sup>	0.53 <sup>c</sup>	0.01	0.00
GLUC	290.50	279.00	288.00	283.50	288.00	292.00	290.00	2.27	0.81
CHOL	194.00 <sup>b</sup>	195.00 <sup>b</sup>	252.50 <sup>a</sup>	223.75 <sup>ab</sup>	196.50 <sup>b</sup>	209.50 <sup>b</sup>	203.00 <sup>b</sup>	5.59	0.02

shown in Table 2, T1 had the highest WBC and eosinophil values (16833.33 and 4.91%). T2 had the highest heterophil value of 44.11% while T5 had the highest PCV and Hb values (31.50 % and 10.25 g/dl). T3 had the highest monocytes and basophils (4.00 and 1.00 %) values.

#### Serum metabolite parameters of broiler chicken administered Vitamin E and hog plum extract

The serum metabolite values of broiler chickens administered vitamin E and hog plum extract in the reduction of aflatoxicosis effects are shown in Tables 3 and 4. All parameters measured were significantly ( $p < 0.05$ ) different among treatments except for the ALP, glucose and cholesterol at the initial phase and globulin, Aspartate transferase (AST), Alkaline phosphatase (ALP) and cholesterol at the final phase. T3 had the highest alanine aminotransferase (ALT) and creatinine value of 34.00 U/L and 0.90 mg/dl. Significance difference ( $p < 0.05$ ) also existed between the total protein, albumin and globulin, where T4 had the highest values (4.53, 2.35 and 3.13 g/dl), respectively, at the initial phase as shown in Table 3. In Table 4, which signifies the last phase of the experiment,

T2 and T3 had the highest creatinine and cholesterol values of 0.65 mg/dl and 252.50, respectively, whereas T6 had the highest total protein, albumin and ALT values of 3.80g/dl, 1.25 g/dl and 30.50 U/L, respectively.

#### DISCUSSION

Haematological and biochemical parameters represent pivotal indicators of physiological and metabolic status in broilers, of good health, nutritional status, and response to stressors such as toxins or disease (Khan *et al.*, 2022). The following key parameters of the blood system are used extensively for measuring oxygen transportation ability, immune system function, and general health - packed cell volume (PCV), hemoglobin (Hb), red blood cell (RBC) count, white blood cell (WBC) count (Sharma *et al.*, 2022). Changes in these parameters may indicate a particular disorder, such as anaemia, inflammation, or infection. The report shows that low PCV and Hb usually denote nutritional inadequacy or toxic stress, and elevated WBCs suggest immune activation from infection or inflammation (El-komy *et al.*, 2024).

The highest PCV (32.50%), Hb (10.00 g/dL), and lymphocyte count (65.00%) were observed in T2 (EHP

alone), and this demonstrates that *Spondias mombin* extracts can improve oxygen-carrying capacity and immune function. This is consistent with the investigations of Bello *et al.* (2024) that *Spondias mombin* leaf extract supplementation improved PCV and Hb level significantly in broiler chickens because of its haematinic and immunomodulatory activities. In addition, *Spondias mombin* supplementation reinforced haematological factors and immune response in poultry, which is related to its bioactive compounds.

The group that received aflatoxin B<sub>1</sub> and EHP concurrently had the highest red blood cell count (RBC: signifying that *Spondias mombin* extract (EHP) can efficiently mitigate the impact of AFB<sub>1</sub> in inducing anaemia and promote basophil-mediated immune response. These observations support the research of Makinde *et al.* (2020), which revealed that plant phytochemicals extracted from plant materials such as *Spondias mombin* can be beneficial for the treatment of myelosuppression and modulate the innate immunity of poultry. This is in agreement with Ojo *et al.* (2022), who found that *Spondias mombin* supplementation significantly improved hematoprotection against aflatoxicosis in broilers by bringing back RBC parameters and amplifying leukocyte profiles.

The substantially elevated white blood cell (WBC 16,550.00 cell/ $\mu$ l) and differential leukocyte number (heterophils 40.90%, monocytes 3.01%, eosinophils 3.07%) in Vitamin E-supplemented broilers were attributable to a marked increase of innate immunity most probably helping to combat aflatoxin B<sub>1</sub> (AFB<sub>1</sub>)-induced immunosuppression by promoting leukocyte mobilization. These results are in agreement with Alaba *et al.* (2024), who showed immunorestorative effects of Vitamin E. The highest mean corpuscular haemoglobin (MCH) in T4 is probably the compensatory effect for AFB<sub>1</sub> induced oxidative stress damage to RBCs, causing anaemia as seen in this group which corroborates the findings of Mahrose *et al.* (2021).

At the second phase of the experiment, T1 had the highest WBC and eosinophil values (16833.33 and 4.91%). T2 had the highest heterophil value of 44.11% while T5 had the highest PCV and Hb values (31.50% and 10.25 g/dl). T3 had the highest monocyte and basophil values (4.00 and 1.00%). The values of T1 parameters interpreted as the most important in terms of sustainability in the immune status of birds point to the idea that birds in this group did not come into contact with aflatoxin throughout the experiment. The increased WBC and heterophil numbers are in agreement with the studies demonstrating AFB<sub>1</sub>-free conditions maintain leukocytes (Ekpo *et al.*, 2022).

The highest PCV (31.50%) and Hb (10.25 g/dl) in T5 suggest Vitamin E's regenerative efficacy in reversing AFB<sub>1</sub> induced anaemia. Improved RBC indices in T5 indicate that AFB<sub>1</sub> induced hemotoxicity has been completely overcome. AFB<sub>1</sub> has been reported to inhibit

erythropoiesis via interfering with nutrient uptake (e.g., iron) and protein synthesis, leading to anaemia (Ekpo *et al.*, 2022). Vitamin E in T5 probably decreased AFB<sub>1</sub> bioavailability, which safeguarded erythrocyte integrity.

Vitamin E is a powerful antioxidant, whose therapeutic potential is of great interest concerning the counteraction of the haematologic adverse effects of aflatoxin B<sub>1</sub>. Mishra and Swain (2022) found that young chickens exposed to aflatoxin B<sub>1</sub>, with a dietary supplement with vitamin E and selenium, had better red blood cell count, haemoglobin value, hematocrit, and white blood cell threshold in the chicken.

The prolonged elevation in monocytes (4.00%) and basophils (1.00%) in the EHP treated group (T3) implies long-term immunomodulatory effects that *Spondias mombin* extract caused prior to the study, which supports reports of immunomodulatory effects of *Spondias mombin* on poultry birds from other scientists. Oladeji *et al.* (2022) reported that *S. mombin* tannins result in persistent (28-day) monocyte booster, in broilers through IL-1 $\beta$ /CSF-1 signalling pathway. Akinmoladun *et al.* (2021) also reported that about 40% of basophil elevation was seen for 3 weeks post-treatment in chickens, and this was attributed to quercetin compounds.

Biochemical parameters give information about the work of various organs in the body and metabolic pathways. Liver enzymes like alanine aminotransferase (ALT), aspartate aminotransferase (AST) are biomarkers of liver function, and also, serum albumin, total protein as indicating protein metabolism, and the nitrogen status (Abdel Rahman *et al.*, 2021). Other variables like blood glucose, cholesterol and triglycerides indicate energy turnover and lipid balance (Saha *et al.*, 2024).

In this study, T4 (AFB<sub>1</sub> alone group) had the highest total protein (4.53 g/dl) and albumin (2.35 g/dl), which could be due to hepatic acute reaction to AFB<sub>1</sub> toxicity, increased protein synthesis as oxidative stress before chronic damage (Abdel Rahman *et al.*, 2021). Globulin increase (3.13 mg/dl) indicates chronic inflammation (Surai *et al.*, 2018).

The AST level of T7 supported severe AFB<sub>1</sub> mediated hepatocellular necrosis, while the AST (192.5 U/L) level in T3 (EHP+AFB<sub>1</sub>) remained normal, due to the *Spondias mombin*'s hepatoprotective flavonoids preserving the membrane integrity (Akinmoladun *et al.*, 2021). T6 (Vit E+AFB<sub>1</sub>) ranked between the others (190 U/L), in line with its radical scavenging capacity for  $\alpha$ -tocopherol (Surai, 2002).

At the last phase of the experiment, the total protein (3.80 g/dl), albumin (1.25 g/dl) and ALT (30.50 U/L) on T6 had the highest value. Elevated protein levels show Vitamin E's regenerative effects, while the fact that ALT is increased indicates that there is ongoing stress on the liver to release the liver's 'defective' cells arising out of prior AFB<sub>1</sub> insult for Vitamin E supplementation, partial normalization has been observed to require approximately greater than 4 weeks post-exposure (Chen *et al.*, 2024).

Higher creatinine (0.65 mg/dl) and cholesterol (252.50 mg/dl) in T2 and T3 relate to a possible strain on the kidneys and cholesterol dysregulation, showing that EHP possibly does not reverse chronic aflatoxicosis effects. AFB<sub>1</sub> leads to oxidative damage of liver cells, a decrease in protein synthesis and death of lipid metabolism. Chronic exposure results in increased liver enzymes ALT and lowered albumin are measured in untreated groups like T4.

Vitamin E has an antioxidant effect on oxidative stress by stimulation of antioxidant enzymes (e.g. glutathione peroxidase). Its capacity to improve protein synthesising capability and decrease liver enzyme activity makes it good for the long-term recovery of aflatoxicosis. In spite of the fact that hog plum extract is characterised by polyphenols, whose antioxidant properties are recognised, its effectiveness in adjusting chronic metabolic disorders is inferior to that of Vitamin E. Elevated cholesterol and creatinine values in T3 indicate that EHP may need to be dosed higher or maybe combined for best protection.

## Conclusion

It can be concluded that both the extract of hog plum and Vitamin E effectively mitigated AFB<sub>1</sub>-induced haematological and biochemical disruptions, with EHP providing immediate immune benefits and Vitamin E ensuring long-term recovery. The study recommends the supplementation of extracts of hog plum at 1 mL/L in broiler diets. These findings align with recent research on AFB<sub>1</sub> toxicity and antioxidant interventions, reinforcing the potential of phytogenic and vitamin-based strategies in poultry health management. The major limitation of the study was the scarcity and cost of purchasing aflatoxin and vitamin E. Extended or prolonged trials are needed to refine the dosages used in this study and to reaffirm the efficacy of the test ingredients.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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