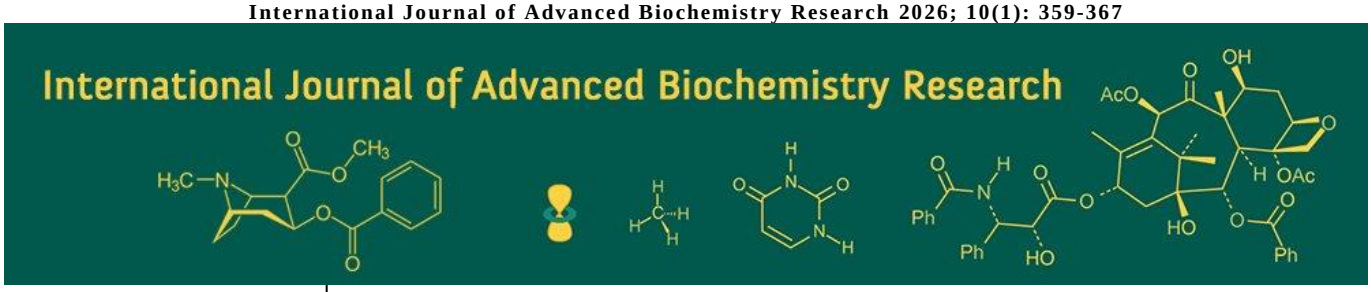

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Evaluation of Toxicopathological changes in broilers with experimental aflatoxicosis and the therapeutic potential of a Polyherbal Intervention

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Abstract
Aflatoxin contamination in poultry feed continues to threaten broiler health, growth efficiency and overall production sustainability. Its pronounced hepatotoxic, immunosuppressive and pro-oxidative properties result in marked alterations in hematology, serum biochemical profiles, antioxidant enzyme activities and characteristic gross and histopathological lesions. Currently, cost-effective and practical methods to prevent AF induced toxicity in chickens are limited. One of the approaches to overcome mycotoxicosis in poultry is using herbal products. Polyherbal nutraceutical containing *Tephrosia purpurea* aerial part, *Punica granatum* fruit rind, *Acacia nilotica* bark, *Tamarindus indica* seed coat was used in this study as mitigating agents to know the therapeutic potential in counteracting the aflatoxicosis in broilers. A study was conducted to evaluate the toxicopathology of experimental aflatoxicosis in broiler chickens and its mitigation using a novel polyherbal nutraceutical. Day-old Cobb broiler chicks were randomly allotted to six treatments (N=15) for 42 days: T_1 (control), T_2 (1 ppm AF), T_3 (AF + binder 500 g/ton), T_4 (AF + PHN 500 g/ton + binder 1000g/ton), T_5 (AF + binder 500 g/ton + PHN 500 g/ton) and T_6 (AF + binder 500 g/ton + PHN 1000 g/ton). Aflatoxin-fed birds showed significantly elevated serum AST, ALT, ALP, creatinine, BUN and significant reductions in haemoglobin, packed cell volume, total protein and albumin. Antioxidant enzymes (SOD, CAT) were decreased and hepatic lipid peroxidation increased. Gross lesions included pale to yellowish colour, enlarged liver and kidneys with lymphoid depletion of the bursa, spleen and thymus. Microscopically, aflatoxin treated birds revealed severe hepatic vacuolar degeneration, bile-duct hyperplasia, periportal necrosis and renal tubular degeneration. Supplementation with PHN and binder (T_6) significantly restored haematology, serum biochemical, antioxidant profiles and reduced histopathological lesions. Based on these results, we conclude that supplementation of PHN enhanced recovery, though without statistically significant synergism and their supplementation can reduce oxidative stress thus restoring the liver damage caused by AF in broilers chicken.

Keywords: Aflatoxin, Polyherbal nutraceutical, apoptosis, binder, broilers

Introduction
The poultry industry is among the fastest-growing sectors of global livestock production. While overall agricultural growth remains at 1.5-2% annually, poultry production-particularly eggs and broiler meat-has consistently achieved growth rates of 8-10% (APEDA, Government of India). India plays a major role in this expansion, ranking third in global egg production and eighth in broiler meat production (FAOSTAT, 2022). The 20th Livestock Census reports India's poultry population at 851.81 million, reflecting a 16.81% increase over the previous census (Department of Animal Husbandry and Dairying, 2019). The sector contributes nearly 1% to national GDP and about 15% to livestock GDP, underscoring its economic and nutritional importance. Feed quality is a critical determinant of poultry health and productivity; however, contamination of feed ingredients remains a major challenge. Among feed contaminants, mycotoxins-particularly aflatoxins-pose a serious threat to poultry production, especially in tropical and subtropical regions such as India. *Aspergillus flavus*, a soil-borne fungus, proliferates under high moisture and moderate temperatures (25-35°C), conditions that favor aflatoxin biosynthesis and contamination of feedstuffs such as maize and groundnut (Shabeer *et al*., 2022; Norlia *et al*., 2020) [51, 34].

Improper drying and prolonged storage further exacerbate fungal growth and toxin synthesis (Sarma *et al*., 2017) [50]. Aflatoxins are difuranocoumarin compounds mainly produced by *A. flavus* and *A. parasiticus*, with AFB1, AFB2, AFG1 and AFG2 as the principal forms. Among these, aflatoxin B1 (AFB1) is the most prevalent and toxic in poultry feed (De Ruyck *et al*., 2015; Kumar *et al*., 2017) [9, 20]. The FAO estimates that approximately 25% of global cereals and animal feedstuffs are contaminated with mycotoxins (Pandya & Arade, 2016) [37]. Consumption of aflatoxin-contaminated feed in poultry leads to hepatotoxicity, nephrotoxicity, intestinal hemorrhage, immunosuppression, reduced growth performance and increased mortality (Roze *et al*., 2013; Pauletto *et al*., 2023) [47, 39]. AFB1 adversely affects productivity and may result in toxin residues in meat and eggs, posing food safety concerns (Kassaw *et al*., 2022; Niu *et al*., 2025) [17, 33]. Aflatoxins are highly stable and not completely eliminated during feed processing. In hepatocytes, AFB1 is metabolized by cytochrome P450 enzymes into a reactive 8,9-epoxide that binds to DNA and proteins, inducing oxidative stress, lipid peroxidation and mutagenic GC→TA transversions (Liew & Mohd-Redzwan, 2018; Fouad *et al*., 2019) [23, 11]. Histopathological alterations include hepatic lipidosis, necrosis, bile duct hyperplasia and fibrosis (Mendieta *et al*., 2018) [26]. Due to its potent carcinogenicity, AFB1 is classified as a Group 1 carcinogen by the International Agency for Research on Cancer (Li *et al*., 2021) [22]. Several physical, chemical and biological detoxification strategies have been explored, with feed adsorbents being the most widely adopted. Adsorbents such as bentonite, zeolite, hydrated sodium calcium aluminosilicate, yeast products and activated charcoal can bind aflatoxins in the gastrointestinal tract and reduce absorption (Ramos & Hernandez, 1996; Miazzo *et al*., 2000) [41, 28]. However, potential interference with nutrient and mineral bioavailability limits their long-term use. In recent years, particularly following the COVID-19 pandemic, there has been growing interest in natural feed additives. Polyherbal nutraceuticals rich in bioactive phytochemicals have gained attention due to their antioxidant, hepatoprotective and immunomodulatory properties. A formulation containing *Tephrosia purpurea*, *Punica granatum*, *Acacia nilotica* and *Tamarindus indica* represents a promising natural strategy to mitigate aflatoxicosis. These plants are rich in flavonoids, tannins and polyphenols known to support hepatic function. Considering the limitations of conventional adsorbents, evaluation of such polyherbal interventions offers a sustainable approach for alleviating aflatoxin-induced toxicity in broiler chickens.

Materials Methods

- **Procurement and quantification of Aflatoxin:** Aflatoxin B_1 powder was procured from the Department of Livestock Production and Management, Veterinary College, Hebbal, Bengaluru, and stored in airtight containers at 2-8 °C until use. Both control and aflatoxin-contaminated diets were quantified for aflatoxin levels by HPLC at the Pharmacovigilance Laboratory for Animal Feed and Food Safety, TANUVAS, Chennai. Aflatoxin levels were detected only in traces in the control diet, while the contaminated diet contained 983 ppb.
- **Experimental birds and biological feeding trial:** The experiment was conducted in a randomized completely block design with six treatments, each having six replicates of 15 Ross broiler chicks (total=540), housed in 36 independent pens under uniform management in an open-sided deep-litter house. Brooding was provided up to three weeks using incandescent bulbs, with continuous lighting, and standard feeders and drinkers in each pen. Birds were fed ad libitum with a basal diet formulated as per NRC (1994), with slight modification according to Ven Cobb recommendations, comprising pre-starter (1-14 d), starter (15-28 d), and finisher (29-42 d) diets. Required levels of aflatoxin and polyherbal nutraceutical were incorporated into the basal diet, and experimental diets were analysed to confirm aflatoxin content. Toxin-mixed feed was offered from day 1 to 21, while nutraceutical supplementation continued until 42 days. The study was approved by the Institutional Animal Ethics Committee (VCS/IAEC/SA-125/2025-26). Experimental chicks were vaccinated against ND with F1 strain on seventh day and booster dose with Lasota strain on twenty first day through intra ocular route and IBD with intermediate strain on fourteenth day and on twenty eighth day through intra ocularroute.
  Description of experimental design: The experiment comprised six dietary treatments (T_1-T_6). T_1 received a basal diet, while T_2 was fed 1 ppm aflatoxin. T_3 received 1 ppm aflatoxin with a toxin binder (1000 g/ton), whereas T_4-T_6 received 1 ppm aflatoxin with graded levels of toxin binder (1000, 750 and 500 g/ton) and polyherbal nutraceutical (500, 750 and 1000 g/ton), respectively. The polyherbal nutraceutical contained *Tephrosia purpurea* aerial parts, *Punica granatum* fruit rind, *Acacia nilotica* bark, and *Tamarindus indica* seed coat.
- **Clinical signs:** All the chicks in different groups were observed daily for clinical signs and was recorded daily till the end of experimental trial i.e., 42days.
- **Hematobiochemical analysis:** On the day 21 and 42 of the experiment, blood samples were drawn from wing vein or as per CCSEA guidelines for hematological and biochemical analyses. Hematological parameters including TEC, TLC, Hb and PCV were carried out using conventional methods. Hb was estimated by Acid haematin method using Sahli's instrument. Packed cell volume (PCV) was estimated according to Jain (1986) [15]. Total erythrocyte count (TEC) and Total leucocyte count (TLC) were done as per the method described by Nambiar (1960) [31] using diluting fluid recommended by Natt and Herrick (1954) [32]. For biochemical analysis, blood collected in serum vacutainers was centrifuged at 3000 rpm for 15 minutes, and the separated serum stored at -20°C was analysed for ALT, AST, ALP, total protein, albumin, BUN and creatinine using the Meryl® COMPACT biochemical analyser (Version, 1.007) in Navi Mumbai, India. The reagents were manufactured from Erba Manheim®, Pvt. Ltd., Mumbai, M.S, India were used.
- **Estimation of antioxidant enzymes:** The representative tissue samples from liver were dissected and washed with normal saline to remove any tissue debris and blood clots. The collected liver samples were homogenized in a solution of ice-cold 0.1M Phosphate buffered saline (pH 7.4) at 4 ºC and centrifuged for 10

minutes at 15,000 rpm. The supernatants were stored at -80ºC for analysis of superoxide dismutase, catalase and thiobarbituric acid reactive substances. SOD activity was estimated following Madesh and Balasubramanian (1998) [25]. Catalase activity was estimated using the method of Aebi (1984) [3] by monitoring the decomposition of H_2O_2 at 240 nm. The peroxidative damage of vital organ liver tissue was evaluated in terms of lipid peroxidation (LPO). Lipid peroxidation in tissue samples prepared was measured as thiobarbituric acid reactive substance (TBARS) called malondialdehyde (MDA) formed per 'g' of tissue according to Paula *et al*. (2005) [38].

- **Histopathological analysis:** Liver tissue from birds of all the groups was subjected to histopathological studies. The tissue was fixed using 10 per cent Neutral Buffered Formalin solution and sections of 5-6 (µ) thickness was cut and stained with routine H&E (Luna, 1968) [24]. Similarly, the standard procedure of Masson's trichome staining was employed to stain required histopathological sections (Suvarna *et al*., 2018) [54].

Statistical Analysis

The values obtained from the various experiments were expressed as Mean±Standard Error (SE). The data obtained from the present study was analysed using One-way ANOVA followed by post-hoc test (Turkey's test), (Snecdecor and Cochran, 1994) using the software SPSS, version-16.0.

Results

- **Clinical signs:** Clinical signs appeared one week after aflatoxin administration in the aflatoxin group T_2, characterized by dullness, depression, reduced feed intake and body weight, stunted growth, ruffled feathers, mild lameness and diarrhoea. Polyherbal nutraceutical supplemented groups showed only mild dullness, inappetence, and slight weight reduction, while chicks in the control group remained active and apparently healthy.
- **Haematology:** The mean hematological values (±SEM) on days 21 and 42 are presented in Table 1. There was a significant ($p \leq 0.05$) decrease in RBC, WBC, Hb and PCV in the T_2 when compared with T_1 and the other groups on day 21. Groups T_3 to T_6 showed significantly higher ($p \leq 0.05$) RBC and WBC values than T_2, with no significant difference among themselves. On the other side, there was significant ($p \leq 0.05$) increase in Hb and PCV concentration in group T_6 compared with aflatoxin treated group T_2. By day 42, T_2 showed a non-significant reduction in RBC and PCV, while Hb and WBC remained significantly ($p \leq 0.05$) lower than T_1. No significant differences were observed among the remaining treatment groups.
- **Serum biochemistry:** The mean serum biochemical values (±SEM) on days 21 and 42 are presented in Table 2 and 3 respectively. On day 21 there was a significant ($p \leq 0.05$) increase in the ALT, AST and ALP activities in the T_2 (aflatoxin positive) when compared with control group. On the other hand, there was a significant ($p \leq 0.05$) decrease in the AST activity in the T_4 and T_6 when compared with the T_2 group and non-significant increase compared to T_1 control. Meanwhile, ALT and ALP activities were significantly ($p \leq 0.05$) decreased in T_4, T_5 and T_6 when compared with the T_2 group. Regarding the results of total protein and albumin there were significant ($p \leq 0.05$) decrease in T_2 when compared with the T_1. On the other side, there was non-significant increase in the total protein and albumin in T_4, T_5 and T_6 when compared with the T_2 (aflatoxin positive), but there was no significant differences among the groups. Regarding the results of the kidney function, the concentrations of creatinine and BUN were significantly ($p \leq 0.05$) increased in T_2 when compared T_1. On the other side, the creatinine and BUN concentration was significantly ($p \leq 0.05$) decreased in T_5 and T_6 when compared to T_2 (aflatoxin positive) but was non-significant when compared among the other groups. By day 42 there was significant increase in AST, ALT, creatinine and BUN levels in T_2 compared to T_1, but was nonsignificant when T_2 was compared with other groups.
- **Antioxidant enzymes:** The mean values of Catalase and Superoxide dismutase enzyme activity of liver tissue with standard error of mean on day 21 and 42 of the experiment have been presented in Table 4.
- **Superoxide dismutase (SOD):** On day 21 and 42 there was a significant ($p < 0.05$) decrease in superoxide dismutase enzyme activity of group T_2 birds in comparison to group T_1 birds. The mean values of group T_3 to T_6 were non-significantly ($p > 0.05$) increased in comparison to group T_2 birds and were comparable to that of group T_1 birds. SOD activity on both intervals (day 21 and day 42) showed there is no significant differences among other treatment groups (T_3-T_6).
- **Catalase:** On day 21 and 42 there was a significant ($p < 0.05$) decrease in catalase enzyme activity of group T_2 birds in comparison to group T_1 birds. The CAT activity in the liver on day 21 was significantly ($p < 0.05$) higher in group T_4 and T_6 compared to T_2 (aflatoxin group) but non-significant difference compared to T_1. Whereas on day 42 no significant difference was observed among other treatment groups.
- **Lipid peroxidation (LPO):** The mean values of liver tissue Thiobarbituric acid reactive substances (TBARS) in nmol Malondialdehyde (MDA)/g of tissue levels with standard error of mean on day 21 and 42 of the experiment have been presented in Table 5. There was significant ($p < 0.05$) increase in MDA levels in T_2 group birds compared with group T_1 birds on both days. But there was significant decrease in group T_5 and T_6 compared to group T_2, non-significant increase compared to group T_1 on day 21. Whereas on day 42 no significant difference was observed among other treatment groups (T_3-T_6).

Histopathology

It was observed that the broiler chicks group that was fed basal diet showed normal histology in which the normal hepatocytes (H) and around the portal area (PA) consisted of the bile duct, portal vein, and hepatic arteriole branch (Fig 1 and 2). Conversely, the addition of aflatoxin had an adverse effect on liver showing congestion of vessels and sinusoidal spaces, congregation of hepatocytes giving glandular appearance with prominent basement membrane particularly around central vein, multifocal haemorrhages, focal hepatitis

with bullous vacuolar degeneration. There were multifocal areas of inflammation, portal hepatic necrosis, mild degree of fibrous connective tissue proliferation in the portal region, with bile duct hyperplasia and accumulation of inflammatory cells around bile duct and central vein (Fig 3 to 8). Multifocal areas of inflammation in portal traid were also noticed. These microscopic lesions were almost of same degree on day 42 of the experiment. The lesions in liver showed persistence of bile duct hyperplasia with cholangitis and dilatation of central vein.

Polyherbal nutraceutical treated groups (Groups T_4, T_5 and T_6) showed mild granular degenerative changes in few cells while other hepatocytes appearing apparently normal with well-defined hepatic cords and proliferation of connective tissue between cords when compared to group T_2 on day 21(Fig 9 to 12). On day 42 these lesions severity was reduced compared to group T_2 and there were mild degenerative changes with maintenance of normal architecture of hepatocytes and hepatic cords.

Table 1: The Mean (±SE) values of hematological parameters in different treatment groups at different interval of time

Treatment group (s)	21st day				42nd day			
	RBC	WBC	Hb	PCV	RBC	WBC	Hb	PCV
T1	2.45±0.27^{a}	20.97±0.42^{a}	9.45±0.27^{a}	28.35±0.18^{a}	2.04±0.27	19.95±0.31^{a}	9.42±0.30^{a}	28.16±0.58
T2	1.40±0.20^{c}	17.96±0.18^{c}	8.01±0.2^{b}	22.86±0.49^{c}	1.81±0.21	17.75±0.28^{c}	7.72±0.20^{b}	26.09±0.46
T3	1.96±0.14^{b}	19.21±0.43^{b}	8.81±0.22^{ab}	25.53±0.29^{b}	1.93±0.12	19.34±0.42^{b}	8.26±0.19^{ab}	26.14±0.29
T4	1.98±0.19^{b}	20.13±0.16^{ab}	8.98±0.19^{a}	25.07±0.63^{b}	2.10±0.24	20.26±0.39^{ab}	8.24±0.47^{ab}	26.94±0.92
T5	1.87±0.23^{b}	19.58±0.21^{b}	8.72±0.16^{ab}	26.58±0.49^{ab}	2.35±0.26	19.57±0.52^{b}	8.43±0.5^{ab}	27.18±0.78
T6	2.02±0.14^{b}	20.27±0.17^{ab}	9.02±0.13^{a}	25.42±0.31^{b}	2.10±0.25	20.82±0.17^{ab}	8.33±0.45^{ab}	28.15±0.81

Note: Data were analyzed by One-way ANOVA followed by Turkey's *post hoc* multiple comparison test and mean values with different superscript differ significantly.
Values are statistically significant at $p \leq 0.05$.

Table 2: The Mean (±SE) values of biochemical parameters in different treatment groups on day-21

Treatment group(s)	AST	ALT	ALP	Creatinine	BUN	Total Protein	Albumin
T_1	157.04±15.87^{c}	9.51±0.4^{d}	652.38±41.69^{c}	0.66±0.05^{c}	7.55±0.55^{c}	3.57±0.3^{a}	1.9±0.17^{a}
T_2	240.68±14.13^{a}	18.64±1.11^{a}	861.39±40.71^{a}	2.12±0.08^{a}	15.72±0.67^{a}	2.14±0.23^{b}	1.06±0.1^{b}
T_3	210.56±10.46^{ab}	15.09±0.61^{b}	815.96±27.13^{ab}	1.76±0.17^{ab}	12.11±0.85^{b}	2.86±0.17^{ab}	1.53±0.15^{ab}
T_4	185.28±12.44^{bc}	13.59±0.39^{bc}	713.3±25.09^{bc}	1.73±0.16^{ab}	11.77±0.43^{b}	2.48±0.18^{b}	1.26±0.12^{b}
T_5	199.81±14.40^{ab}	11.51±0.47^{cd}	706.43±29.4^{bc}	1.49±0.09^{b}	10.41±0.33^{b}	2.68±0.2^{ab}	1.43±0.05^{ab}
T_6	182.8±11.38^{bc}	12.22±0.61^{cd}	685.3±35.98^{bc}	1.59±0.12^{b}	10.62±0.59^{b}	2.40±0.22^{b}	1.33±0.08^{b}

Note: Data were analyzed by One-way ANOVA followed by Turkey's *post hoc* multiple comparison test and mean values with different superscript differ significantly.
Values are statistically significant at $p \leq 0.05$.

Table 3: The Mean (±SE) values of biochemical parameters in different treatment groups on day-42

Treatment group (s)	AST	ALT	ALP	Creatinine	BUN	Total Protein	Albumin
T_1	177.83±9.21^{b}	9.29±0.39^{b}	730.35±63.89	0.38±0.05^{b}	6.99±0.42^{bc}	3.64±0.26	1.65±0.14
T_2	226.31±10.48^{a}	12.04±0.74^{a}	833.21±31.91	1.01±0.09^{a}	8.35±0.25^{ab}	2.35±0.32	1.49±0.16
T_3	205.12±18.33^{ab}	11.19±0.49^{ab}	795.55±54.78	0.9±0.07^{a}	9.47±0.29^{a}	3.33±0.37	1.52±0.21
T_4	188.41±12.74^{ab}	10.82±0.58^{ab}	763.66±27.14	0.86±0.09^{a}	7.32±0.41^{bc}	3.10±0.28	1.33±0.11
T_5	185.18±8.99^{ab}	10.31±0.55^{ab}	729.41±58.61	0.87±0.06^{a}	7.05±0.7^{bc}	3.39±0.63	1.49±0.14
T_6	181.31±7.26^{ab}	9.85±0.35^{ab}	769.41±55.68	0.83±0.11^{a}	6.33±0.31^{c}	3.48±0.36	1.63±0.17

Note: Data were analyzed by One-way ANOVA followed by Turkey's *post hoc* multiple comparison test and mean values with different superscript differ significantly.
Values are statistically significant at $p \leq 0.05$.

Table 4: The Mean (±SE) of Superoxide dismutase SOD activity (U/mg protein) and Catalase (CAT) activity ('mmol' H_2O_2utilized / min/mg protein) of liver in treatment groups at different intervals of time

Treatment group (s)	SOD		CAT	
	Day 21	Day 42	Day 21	Day 42
T_1	77.03±5.68^{a}	70.81±6.83^{a}	161.06±16.83^{a}	130.82±15.3^{a}
T_2	48.29±5.64^{b}	40.15±5.49^{b}	64.45±8.28^{c}	70.32±6.27^{b}
T_3	58.55±7.03^{ab}	47.62±7.11^{ab}	102.32±7.98^{bc}	98.15±6.55^{ab}
T_4	49.84±6.14^{b}	54.49±5.12^{ab}	123.46±15.31^{ab}	114.44±13.58^{ab}
T_5	56.26±4.01^{ab}	52.06±6.28^{ab}	109.09±11.07^{bc}	103.52±11.56^{ab}
T_6	61.45±8.12^{ab}	57.4±5.75^{ab}	139.14±4.92^{ab}	121.51±7.28^{a}

Note: Data were analyzed by One-way ANOVA followed by Turkey's *post hoc* multiple comparison test and mean values with different superscripts differ significantly. Values are statistically significant at $p \leq 0.05$.

Table 5: The Mean (±SE) of Malondialdehyde (MDA) levels (nmol/g) formed in liver in treatment groups at different intervals of time

Treatment group (s)	MDA	
	Day 21	Day 42
T_1	80.33±14.86^{b}	90.46±7.81^{b}
T_2	161.41±18.48^{a}	146.57±20.36^{a}
T_3	117.17±15.56^{ab}	115.84±9.76^{ab}
T_4	118.74±7.17^{ab}	118.25±12.82^{ab}
T_5	91.96±14.33^{b}	109.54±7.91^{ab}
T_6	99.15±7.99^{b}	101.9±6.28^{ab}

Note: Data were analyzed by One-way ANOVA followed by Turkey's *post hoc* multiple comparison test and mean values with different superscripts differ significantly. Values are statistically significant at $p \leq 0.05$.

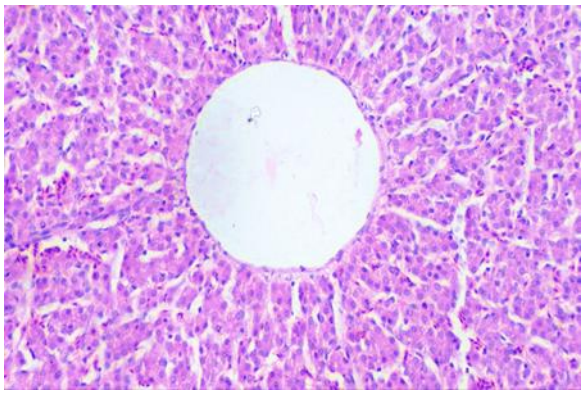


Fig 1: Section of liver from control bird (T_1) showing normal architecture with central vein and sinusoids (H&E X 200)

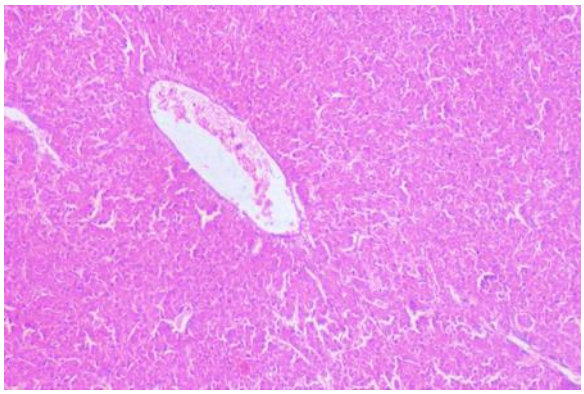


Fig 2: Section of liver from control bird (T_1) showing normal architecture of hepatic tissue (H&E X 100

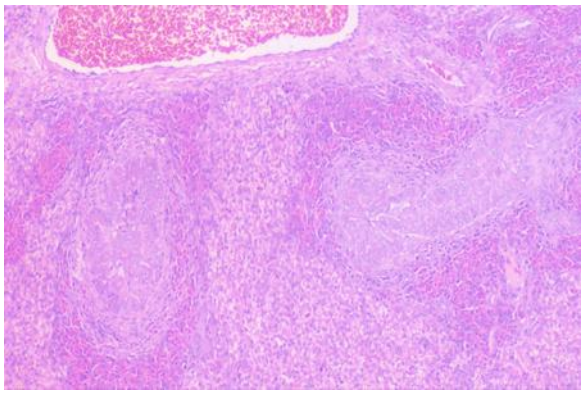


Fig 3: Section of liver from 1 ppm AF fed bird (T_2) showing periportal hemorrhage, fibrosis, portal venular congestion and mild vacuolar degeneration of hepatocytes (H&E X 100)

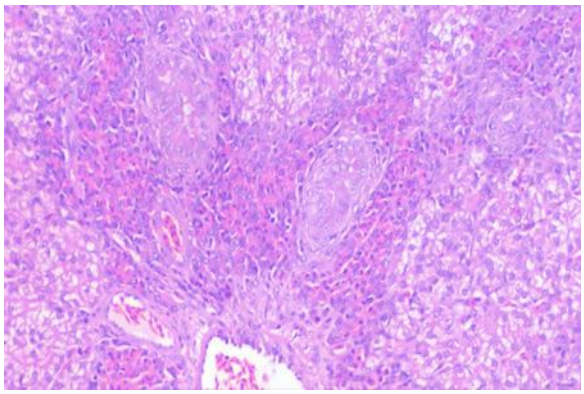


Fig 4: Section of liver from AF fed bird (T_2) showing periportal hemorrhage, multiple bile duct with epithelial hyperplasia and mild cholangitis (H&E X 200)

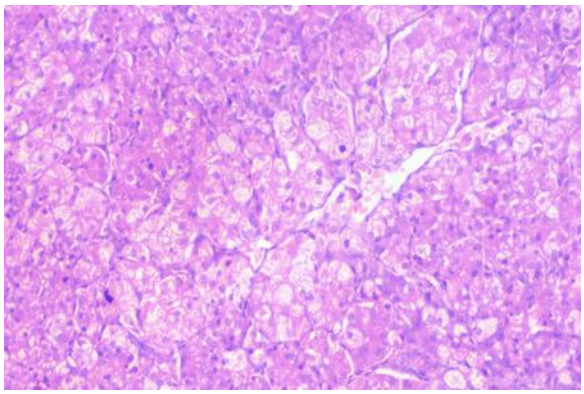


Fig 5: Section of liver from AF fed bird (T_2) showing prominent bullous vacuolar degeneration with fibrotic pattern around hepatocytes and prominent fibrotic pattern around hepatocytes and prominent

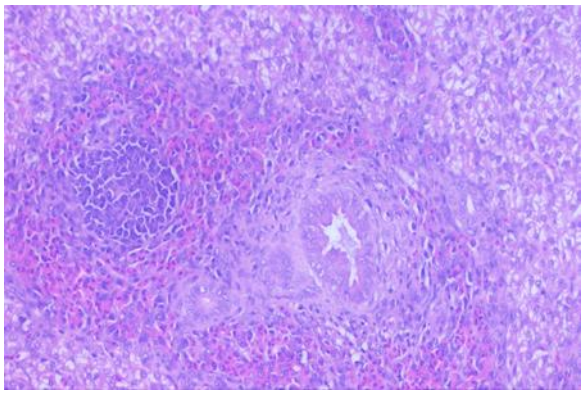


Fig 6: Section of liver from AF fed bird (T_2) showing bile duct with hyperplastic change, multifocal hemorrhage, focal congregation of inflammatory cells, vacuolar changes in hepatic parenchyma and cells, vacuolar changes in hepatic parenchyma and

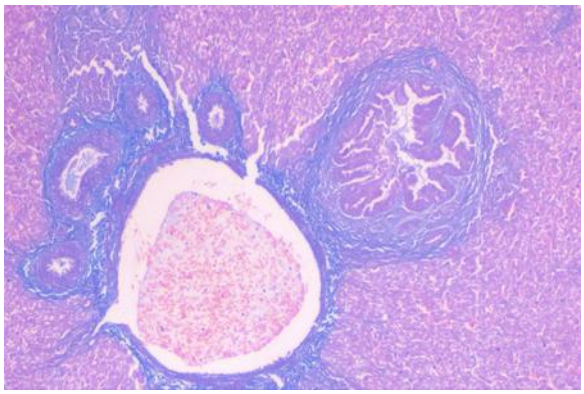
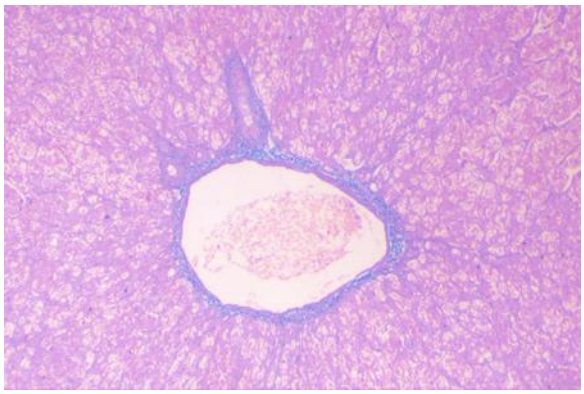


Fig 7: Section of liver from AF fed bird (T_2) showing portal triad with portal vein congestion and dilatation, multiple bile duct with one of them showing bile duct epithelial hyperplasia causing folds of lining epithelial epithelial hyperplasia causing folds of lining epithelial cells. Entire area surrounded by fibrous connective tissue and inflammatory cells (MTC X 200)



iver from AF fed bird (T_2) showing thin but distinct fibrous around the portal area indicating periportal fibrosis. Also, mild duct epithelia present within the portal triad. (MTC X 200)

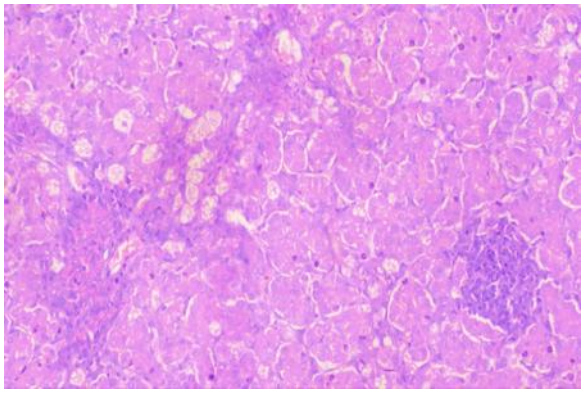


Fig 9: Section of liver from group (T_4) showing mild granular degeneration of hepatocytes, few cells showing hydropic change and focal areas of inflammation (H&E X 200)

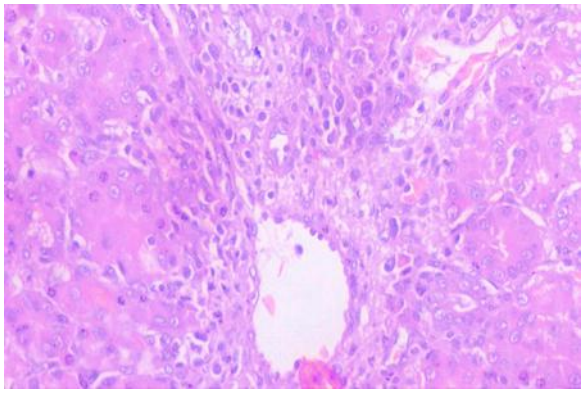


Fig 10. Section of liver from group (T_5) showing periportal hepatocytes appearing normal although there is portal hepatitis & mild fibrosis (H&E X 200)

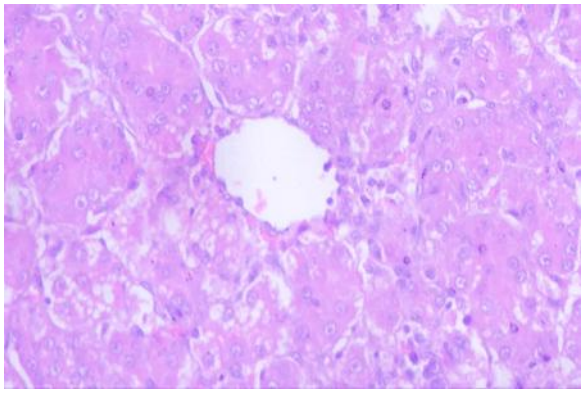


Fig 11: Section of liver from group (T_6) showing mild vacuolar change in few cells while other hepatocytes appearing apparently normal with well-defined hepatic cords (H&E X 400)

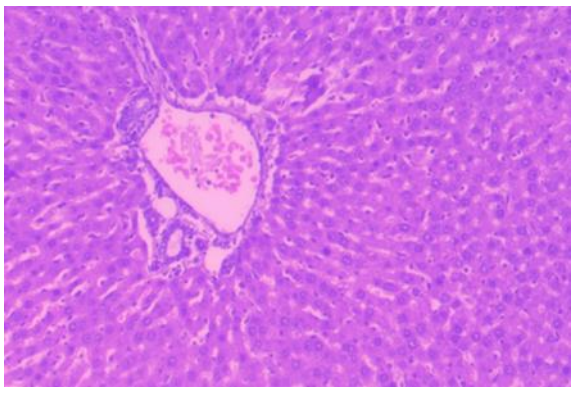


Fig 12: Section of liver from group (T_6) showing central vein with radiating plates of hepatocytes, hepatic cords and sinusoids are well-arranged, normal portal triad indicating apparently normal architecture (H&E X 200)

Discussion

In the present study, sub-acute exposure to aflatoxin (1 ppm) induced dullness, depression, reduced feed intake and body weight, stunted growth, slow feather growth, ruffled feathers and mild diarrhoea. Similar clinical signs have been reported in chickens exposed to aflatoxin levels (125-250 ppm) by earlier workers (Mourad *et al*., 2020; Ashry *et al*., 2022) [29, 6]. In contrast, birds in T_4, T_5 and T_6 groups showed no marked clinical signs, except mild depression and inappetence. The reduced severity of symptoms and improved feather quality indicate the protective effect of the polyherbal nutraceutical.

The present study demonstrated marked hematotoxic effects of aflatoxin B1 in broilers, evidenced by significant reductions in RBC, WBC count, haemoglobin and PCV in the aflatoxin-fed group (T_2), confirming its anaemic and leukopenic effects (Arafat and Khan, 2017; Ashry *et al*., 2022; Badmos *et al*., 2025) [5, 6, 7]. The anaemia may be due to bone marrow suppression, impaired protein synthesis due to hepatic damage, reduced nutrient absorption and oxidative damage to erythrocyte membranes leading to

hemolysis (Abdel-Wahhab *et al*., 2002) [2]. Reduced TLC indicated immunosuppression resulting from impaired lymphopoiesis, granulopoiesis and lymphoid organ atrophy (Rathod *et al*., 2017) [43], although variable leukocyte responses have been reported elsewhere. Supplementation with toxin binders and polyherbal nutraceuticals (T_3-T_6) significantly ameliorated these alterations, restoring hematological values towards normal. This improvement may be attributed to reduced intestinal absorption of aflatoxin by binders (Mgbeahuruike *et al*., 2018) [27] and the antioxidant, membrane-stabilizing and immunomodulatory effects of phytochemicals (Gowda *et al*., 2008; Verma *et al*., 2017) [13, 55]. However, leukocyte recovery was comparatively slower, suggesting prolonged immunotoxic effects of aflatoxin.

Serum biochemical analysis revealed significant elevations in AST, ALT and ALP activities in aflatoxin-fed birds, indicating hepatocellular injury and hepatobiliary dysfunction. These findings corroborate earlier reports on aflatoxin-induced liver damage in poultry (Kilany *et al*., 2020; Ashry *et al*., 2022) [19, 6]. The increased serum enzyme activities are attributed to hepatocyte membrane damage, lipid peroxidation and leakage of cytosolic enzymes into circulation (Singh *et al*., 2015) [52]. Aflatoxin exposure also resulted in significantly elevated serum creatinine and BUN levels, indicating impaired renal function and reduced glomerular filtration, consistent with previous studies (Mourad *et al*., 2020) [29]. Additionally, significant reductions in serum total protein and albumin levels were observed in aflatoxin-fed birds, reflecting compromised hepatic synthetic function and possible renal protein loss. Hypoproteinemia and hypoalbuminemia have been attributed to inhibition of protein synthesis through aflatoxin-macromolecule adduct formation and impaired transcriptional activity in hepatocytes (Rotimi *et al*., 2017) [45]. Dietary supplementation with toxin binders alone partially ameliorated these biochemical disturbances, while combined supplementation with polyherbal nutraceuticals (T_4-T_6) significantly improved liver enzyme activities, renal biomarkers and protein profiles. The hepatoprotective and nephroprotective effects are likely mediated by the antioxidant, anti-inflammatory and membrane-stabilizing properties of flavonoids, tannins and polyphenols present in the polyherbal nutraceutical (Lansky and Newman, 2007; Ali *et al*., 2012; Gupte *et al*., 2022) [21, 4, 14]. Although partial recovery was evident following toxin withdrawal, persistent alterations in some parameters indicate the cumulative and slowly reversible nature of aflatoxin toxicity.

In the present study, a significant elevation in liver MDA levels accompanied by a marked reduction in CAT and SOD enzyme activities was observed, indicating increased lipid peroxidation and oxidative stress in birds. Similar suppression of antioxidant enzymes under aflatoxicosis has been reported earlier (Priya *et al*., 2019; Sang *et al*., 2023; Oloruntola *et al*., 2025) [40, 49, 36]. The decline in enzymatic activity may be attributed to excessive reactive oxygen species (ROS) generated during cytochrome P450-mediated bioactivation of aflatoxin B_1, leading to oxidative overload and enzyme exhaustion (Abdel-Wahhab *et al*., 2010) [1]. Additionally, lipid peroxidation products such as malondialdehyde (MDA) can directly inactivate antioxidant enzymes, while aflatoxin-induced disturbances in essential trace minerals (Cu, Zn, Mn) further impair SOD functionality (Gora *et al*., 2014) [12]. Dietary supplementation with bentonite and polyherbal nutraceuticals partially restored antioxidant status in aflatoxin-exposed broilers, as indicated by increased SOD and CAT activities and reduced hepatic MDA levels compared to group T_2. The effect was more pronounced in combined and higher-dose polyherbal treatments, suggesting a synergistic, dose-dependent protection, though complete normalization was not achieved. Similar partial recovery of antioxidant enzymes and attenuation of lipid peroxidation with toxin binders and herbal antioxidants have been reported earlier (El-Nekeety *et al*., 2017; Gora *et al*., 2014; Chen *et al*., 2024) [10, 12, 8]. These protective effects are attributed to the antioxidant, free radical-scavenging, membrane-stabilizing and hepatoprotective properties of flavonoids, tannins and phenolics present in *Tephrosia purpurea*, *Punica granatum*, *Acacia nilotica* and *Tamarindus indica* (Ali *et al*., 2012; Khatri *et al*., 2009; Gupte *et al*., 2022; Nabi *et al*., 2022) [4, 14, 30]. Overall, partial normalization of antioxidant indices highlights the role of bentonite and polyherbal formulations in mitigating, though not fully reversing, aflatoxin-induced oxidative stress in broilers.

Histopathologically, liver of group T_2 birds showed severe hepatic lesions such as vacuolar degeneration, congestion, haemorrhages, inflammatory cell infiltration, bile duct hyperplasia and periportal fibrosis, corroborating earlier reports in aflatoxin-intoxicated chickens (Rashidi *et al*., 2020; Ashry *et al*., 2022; Sadiek *et al*., 2022) [42, 6, 48]. These alterations are attributed to disrupted lipid and fatty acid metabolism, oxidative stress, inflammation and hepatocyte apoptosis induced by AFB1 (Singh *et al*., 2015; Rotimi *et al*., 2019) [52, 46]. On the day 21 of the experiment, a marked increase in biliary hyperplasia was evident in the liver tissue. According to Rensburg *et al*. (2006) [44], this phenomenon likely reflects a compensatory response aimed at regenerating the hepatic parenchyma, particularly when the parenchymal cells have become incapable of self-repair. In contrast, polyherbal nutraceutical and binder supplementation (T_4-T_6) showed reduced severity of hepatic lesions, indicating hepatoprotective and antioxidant effects against aflatoxicosis. Similar protective effects have been reported with polyherbal supplements (Kalorey *et al*., 2005) [16] and curcumin supplementation in broilers (Zhang *et al*., 2024) [56].

Conclusion

Feeding aflatoxin-contaminated diets to broiler chickens induced pronounced biochemical, antioxidant, hematological and histopathological alterations, clearly reflecting hepatic and renal dysfunction, oxidative stress and tissue injury. Elevated serum enzymes (AST, ALT, ALP), BUN and creatinine, along with reduced total protein and albumin confirmed organ toxicity, while suppression of antioxidant enzymes and increased lipid peroxidation further substantiated aflatoxin-induced oxidative damage. Dietary supplementation with the polyherbal nutraceutical at 1000 g/ton combined with toxin binder at 500 g/ton (T_6) partially ameliorated aflatoxin-induced adverse effects, as evidenced by partial restoration of serum biochemical, antioxidant and hematological profiles and noticeable reduction in severity of histopathological lesions in the liver. Overall, the findings demonstrate that inclusion of the polyherbal nutraceutical with toxin binder confers substantial protection against aflatoxicosis in broiler chickens, enhancing health status and

resilience to toxin-induced damage. Further studies involving larger sample sizes, multiple tissue targets and comprehensive apoptotic gene profiling are warranted to elucidate the molecular mechanisms underlying hepatocellular degeneration and protection.

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