

Ameliorative Effect of Adhatoda Vasica Against Aflatoxicosis in Broiler Chicken

Sakthi Priya Muthusamy

sakthipriyatanuvas2012@gmail.com

Tamil Nadu Veterinary and Animal Sciences University

Jagadeeswaran Appusamy

Tamil Nadu Veterinary and Animal Sciences University

Natarajan Amirthalingam

Tamil Nadu Veterinary and Animal Sciences University

Research Article

Keywords: Aspergillus parasiticus, Aflatoxin B1 (AFB1), Broilers, Growth performance, Bone morphometry, Serum biochemistry, Histopathology

Posted Date: August 24th, 2023

DOI: <https://doi.org/10.21203/rs.3.rs-3255365/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Background : The effect of aflatoxin in broilers is well known. However dietary supplementation of crude leaf powder of *Adhatoda vasica* was found to decrease the negative effect of aflatoxin B1 because of bio-adsorbing feature of the active ingredient and the detoxifying nature of the herbal material. The main goal of this study was to ascertain how *Adhatoda vasica* crude leaf powder along with aflatoxin contaminated diet ameliorated the toxic effects of aflatoxin B1 on zootechnic performance indicators, bone morphometric characters, serum biochemistry, antioxidant profile, organ weights, gross pathology and histopathology of liver and kidney. Broiler chicks of one day old were divided into six groups. The G1 group was given a formulated basal diet without any supplements. The G2 group was fed with balanced diet with *Adhatoda vasica* crude leaf powder at 0.5 % inclusion. The G3 group was fed with balanced diet with aflatoxin B1 (50 µg/kg). The G4 group was fed with balanced diet with aflatoxin B1 (50 µg/kg) and Silymarin @1000mg/kg feed. The G5 was fed with balanced diet with aflatoxin B1 (50 µg/kg) and *Adhatoda vasica* crude leaf powder at 0.1 % and G6 was fed with balanced diet with aflatoxin B1 (50 µg/kg) and *Adhatoda vasica* crude leaf powder at 0.5 % inclusion from day 1 to day 35 of age.

Results: According to the results of the study, Aflatoxin supplementation in the feed had a detrimental impact on zootechnical performance, bone morphometric characters, serum biochemical parameters, gross and histopathology. Aflatoxin supplementation led to liver injury that was indicted by serum biochemistry and histopathology in the liver and kidney tissues. Supplementation of the crude leaf powder of *Adhatoda vasica* exhibited significant detoxifying effect as evidenced by improved growth performance and bone morphometric characters and ameliorating changes in the levels of serum biochemical parameters and the antioxidant enzymes. The effect of the herb in reducing the pathological lesions were also similar to the standard drug.

Conclusion: Crude leaf powder of the plant *Adhatoda vasica* could be used as a suitable herbal alternative to mitigate aflatoxicosis in broiler chicken.

Background

Among the livestock sectors, poultry is one of the most important and fastest-growing sectors and is better organized and industrialised and is progressing towards modernization. Egg and broiler meat provides protein and maintains the nutrition of the consumer. Thus poultry meat has become a major source of nutrition in India and its share accounts for 28 per cent of the other meat sources. However, there are some threatening factors associated with the poultry meat sector which include disease outbreaks, competition in the international market, feed cost, indiscriminate use of antibiotics, lack of proper biosecurity standards and presence of *Salmonella sp.*, infection in the meat [1].

Various infectious pathogens which impair the production include bacteria, viruses, parasites, and fungi [2]. Among these, fungal contamination is viewed as a serious condition as it is often challenging [3]. These fungi produce low-molecular-weight compounds in the substrates known as secondary metabolites, with confirmed toxic properties referred to as mycotoxins [4]. Among these, aflatoxins (AF) which include B1

(AFB1), B2 (AFB2), G1 (AFG1) and G2 (AFG2) are highly toxic, mutagenic, teratogenic, and carcinogenic compounds that are produced as secondary metabolites by fungi belonging to several *Aspergillus* species, mainly *A. flavus* and *A. parasiticus* [5–9]. The toxic effects of AF are dependent on the dose and exposure time which determine the acute or chronic poisoning. Acute toxic syndrome occurs by the ingestion of feed contaminated with high concentrations of AF and the effects are observed in a short period of time. It is mainly characterized by rapid deterioration of the affected birds with loss of appetite, acute hepatitis, jaundice, hemorrhage and death. The liver is the main organ affected with lesions of hemorrhagic necrosis, centrilobular congestion, bile duct hyperplasia and fatty infiltration of hepatocytes [10] and thus demands much toxicological and mycological research over other mycotoxins for finding out solutions to the problem that has really threatened and caused a negative impact on the scope of animal product exports to many tropical countries [11].

As these toxins are extremely durable, prevention is not always possible and detoxification by various physical, chemical and biological methods have been often followed [12]. In the physical methods *viz.*, pressure cooking and roasting, certain nutrients will be lost [13]. Chemical methods employed using ammoniation, treatment with formaldehyde and calcium hydroxide, and sodium bisulfate is restricted for safety issues [14]. Detoxification by microorganisms [15] leads to release of undesirable compounds by the microbes. Use of toxin binders with mycotoxin-contaminated feeds, bind the toxins and inhibit their absorption from the gastrointestinal tract, thereby minimizing the toxic effects to livestock and the carryover of these fungal metabolites into animal products [16] and also lead to impairment of nutrient utilization. To overcome these issues related to detoxification, in recent years, detoxification methods based on medicinal plants have become attractive because of economic viability and eco-friendly nature. Several organic extracts and essential oils from medicinal and aromatic plants have proven effective against mycotoxigenic moulds or mycotoxin biosynthesis. Natural phytochemicals like ethanolic turmeric extract [17], resveratrol [18] and *Urtica dioica* seeds [19] and an alkaloid vasicine from *Adhatoda vasica* [20] may be an alternative to synthetic chemicals for detoxification of mycotoxin.

Therefore, the hypothesis of this study is that *Adhatoda vasica* crude leaf powder with numerous pharmacological properties would mitigate aflatoxicosis in broiler chicken. In this study, we would focus on *in vivo* studies to assess zoo technic performance indicators, bone morphometry, serum biochemical profile, antioxidant status, gross and histopathology of vital organs. This research plays a crucial role in explaining the mitigating effect of vasicine under two different levels of exposure and exploring the potential effects of the plant on the health of the birds.

Results

Symptoms associated with aflatoxicosis in birds

From the beginning of the trial, clinical symptoms of the aflatoxicosis broilers were observed. The observed clinical symptoms included stunted growth of chicks, leg paralysis, weakness and incoordination and ruffled feathers. The histopathology lesions of the sacrificed birds revealed the enlargement of liver

and kidney with haematoma in the tissues. The birds of the group 2, 4,5 and 6 on sacrifice revealed normal appearance of liver and kidney with no pathognomonic lesions.

Effect of plant on growth / zootechnic performance

The effect of addition of *Adhatoda vasica* crude leaf powder at 0.1% and 0.5% inclusion to the aflatoxin contaminated basal diet against the toxic effects of aflatoxin on growth performance in broilers fed for 35 days has been shown in Tables 1, 2 and 3 and Figs. 1, 2, 3 and 4. The mean values of the final body weight, body weight gain and feed intake in the aflatoxin fed group (G3) were significantly ($p < 0.05$) decreased than that of the control group (G1). Dietary supplementation of *Adhatoda vasica* crude leaf powder lowered the negative effect of aflatoxin on the final body weight, body weight gain and feed intake of broilers similar to the SLM treated group (G3). As shown in the table, the inclusion of *Adhatoda vasica* crude leaf powder in the aflatoxin contaminated diet in was able to mitigate the effect similar to the SLM treated group with the better effect at the highest level of inclusion. Moreover, the crude leaf powder of the plant fed as such at 0.5% also did not exhibit any toxic effects in birds.

Table 1

Effect of *A. vasica* on weekly body weight (g / bird / week, Mean $\pm$ S.E) in AFB1 fed broilers (n = 36 with three replicates)

Group	Particulars	0 day body weight	1st week	2nd week	3rd week	4th week	5th week
I	Basal feed	46.89 $\pm$ 0.02	167.58 ^d $\pm$ 0.83	410.03 ^e $\pm$ 3.86	908.03 ^h $\pm$ 3.62	1421.83 ^j $\pm$ 10.90	1854.86 ⁱ $\pm$ 36.36
II	Basal feed + <i>A. vasica</i> crude powder alone @ 0.5%	48.36 $\pm$ 0.01	161.44 ^d $\pm$ 0.96	398.43 ^e $\pm$ 2.50	898.44 ^h $\pm$ 6.45	1402.44 ^j $\pm$ 5.38	1844.61 ⁱ $\pm$ 21.27
III	Basal feed + AFB1@ 50 μ g / kg	48.02 $\pm$ 0.01	129.72 ^a $\pm$ 0.38	331.47 ^a $\pm$ 11.29	671.53 ^a $\pm$ 11.97	970.61 ^a $\pm$ 8.69	1244.47 ^b $\pm$ 30.15
IV	Basal feed + AFB1 @ 50 μ g / kg + SLM @1000 mg / kg	46.59 $\pm$ 0.01	155.75 ^c $\pm$ 1.57	354.48 ^{cd} $\pm$ 2.42	875.83 ^g $\pm$ 1.75	1239.42 ^f $\pm$ 19.29	1529.14 ^c $\pm$ 25.62
V	Basal feed + AFB1 @ 50 μ g / kg + <i>A. vasica</i> crude powder @ 0.1%	47.79 $\pm$ 0.02	154.97 ^c $\pm$ 2.62	344.20 ^b $\pm$ 3.64	831.97 ^e $\pm$ 6.14	1331.06 ⁱ $\pm$ 8.55	1647.61 ^f $\pm$ 8.55
VI	Basal feed + AFB1 @ 50 μ g / kg + <i>A. vasica</i> crude powder @ 0.5%	47.22 $\pm$ 0.01	144.92 ^b $\pm$ 1.76	352.75 ^c $\pm$ 2.70	844.11 ^f $\pm$ 3.47	1294.31 ^g $\pm$ 13.38	1706.81 ^h $\pm$ 17.90
overall mean bearing different superscripts between rows differ significantly at 5% ($p < 0.05$) level							

Table 2

Effect of *A. vasica* on weekly body weight gain (g/bird/week, Mean $\pm$ S.E) in AFB1 fed broilers (n = 36 with three replicates)

Group	Particulars	1st week	2nd week	3rd week	4th week	5th week
I	Basal feed	120.69 ^e $\pm$ 2.20	242.45 ^d $\pm$ 4.21	498.00 ^f $\pm$ 8.17	513.80 ^j $\pm$ 9.14	433.03 ^g $\pm$ 12.59
II	Basal feed + <i>A. vasica</i> crude powder alone @ 0.5%	113.08 ^d $\pm$ 2.28	236.99 ^d $\pm$ 7.18	500.01 ^f $\pm$ 8.50	504.00 ^{ij} $\pm$ 8.75	442.17 ^g $\pm$ 19.03
III	Basal feed + AFB1@ 50 μ g / kg	81.70 ^a $\pm$ 2.20	201.75 ^b $\pm$ 6.50	340.06 ^a $\pm$ 6.90	299.08 ^b $\pm$ 5.19	273.86 ^b $\pm$ 13.34
IV	Basal feed + AFB1 @ 50 μ g / kg + SLM @1000 mg / kg	109.16 ^c $\pm$ 2.18	198.73 ^{ab} $\pm$ 5.55	521.35 ^g $\pm$ 7.13	363.59 ^d $\pm$ 8.62	289.72 ^b $\pm$ 15.53
V	Basal feed + AFB1 @ 50 μ g / kg + <i>A. vasica</i> crude powder @ 0.1%	107.18 ^c $\pm$ 2.86	189.23 ^a $\pm$ 5.81	487.77 ^e $\pm$ 9.76	499.09 ⁱ $\pm$ 9.98	316.56 ^c $\pm$ 17.74
VI	Basal feed + AFB1 @ 50 μ g / kg + <i>A. vasica</i> crude powder @ 0.5%	97.70 ^b $\pm$ 2.12	207.83 ^b $\pm$ 7.01	491.36 ^e $\pm$ 8.57	450.20 ^g $\pm$ 9.43	412.50 ^f $\pm$ 14.16
Overall mean bearing different superscripts between rows differ significantly at 5% (p < 0.05) level						

Table 3

Effect of *A. vasica* on weekly feed consumption (g/bird/week, Mean $\pm$ S.E) and feed conversion efficiency (FCR) in AFB1 fed broilers (n = 36 with three replicates)

Group	Particulars	1st week	2nd week	3rd week	4th week	5th week	Cumulative feed consumed	FCR
I	Basal feed	174.33 ^b $\pm$ 3.08	345.33 ^f $\pm$ 2.28	540.33 ^e $\pm$ 0.88	854.67 ^e $\pm$ 2.28	1020.03 ^h $\pm$ 1.00	2934.69 ^h $\pm$ 1.88	1.62
II	Basal feed + <i>A. vasica</i> crude powder alone @ 0.5%	168.33 ^b $\pm$ 2.02	332.00 ^d $\pm$ 2.89	537.00 ^e $\pm$ 2.7	850.67 ^e $\pm$ 1.37	1017.67 ^h $\pm$ 1.98	2905.67 ^h $\pm$ 1.22	1.62
III	Basal feed + AFB1 @ 50 μ g / kg	162.33 ^a $\pm$ 2.33	316.67 ^c $\pm$ 2.94	464.67 ^a $\pm$ 1.88	735.67 ^a $\pm$ 2.99	967.67 ^d $\pm$ 1.34	2647.01 ^b $\pm$ 0.98	2.21
IV	Basal feed + AFB1 @ 50 μ g / kg + SLM @ 1000 mg / kg	169.00 ^b $\pm$ 1.24	319.46 ^c $\pm$ 2.60	474.24 ^b $\pm$ 1.56	737.04 ^a $\pm$ 1.49	968.00 ^d $\pm$ 2.04	2667.74 ^c $\pm$ 1.27	1.80
V	Basal feed + AFB1 @ 50 μ g / kg + <i>A. vasica</i> crude powder @ 0.1%	171.00 ^b $\pm$ 3.62	320.67 ^c $\pm$ 2.45	496.78 ^c $\pm$ 2.18	797.00 ^d $\pm$ 1.44	980.00 ^f $\pm$ 1.93	2765.45 ^f $\pm$ 2.17	1.73
VI	Basal feed + AFB1 @ 50 μ g / kg + <i>A. vasica</i> crude powder @ 0.5%	163.67 ^a $\pm$ 2.89	320.33 ^{ab} $\pm$ 1.94	485.33 ^b $\pm$ 2.18	795.33 ^d $\pm$ 1.81	976.67 ^e $\pm$ 1.94	2741.33 ^e $\pm$ 2.43	1.65
Overall mean bearing different superscripts between rows differ significantly at 5% (p < 0.05) level								

Feed Conversion Ratio (FCR) was calculated for all the groups at the end of the fifth week. Maximum FCR was noticed in AFB1 fed group. However, treatment with SLM and *A. vasica* was able to improve the feed conversion efficiency. It was better with *A. vasica* at 0.5 per cent for AFB1 contaminated group.

Effect of the plant on Bone morphometric characteristics

The effect of addition of *Adhatoda vasica* crude leaf powder at 0.1% and 0.5% inclusion to the aflatoxin contaminated basal diet against the toxic effects of aflatoxin on bone morphometric characters in broilers fed for 35 days has been shown in Tables 4,5,6 and 7 and Figs. 5,6,7 and 8. The toxin was influencing the growth of bones as evidenced by measurement of weight, height and diameter which was significantly ($p < 0.05$) reduced in the aflatoxin fed group (G3). As given in the table, *A. vasica* treated group was found to be similar to the SLM treated group as noticed by significant ($p < 0.05$) improvement in the bone morphometric characters.

Table 4
Effect of *A. vasica* on shank height (Mean $\pm$ S.E) in AFB1 fed broilers (n = 8)

Group	Particulars	Shank height (cm)
I	Basal feed	7.48 ^e $\pm$ 0.07
II	Basal feed + <i>A. vasica</i> crude powder alone @ 0.5%	7.24 ^{de} $\pm$ 0.08
III	Basal feed + AFB1 @ 50 μ g / kg	6.24 ^a $\pm$ 0.09
IV	Basal feed + AFB1 @ 50 μ g / kg + SLM @1000 mg / kg	6.97 ^{bc} $\pm$ 0.12
V	Basal feed + AFB1 @ 50 μ g / kg + <i>A. vasica</i> crude powder @ 0.1%	7.19 ^{cde} $\pm$ 0.11
VI	Basal feed + AFB1 @ 50 μ g / kg + <i>A. vasica</i> crude powder @ 0.5%	7.08 ^{bcd} $\pm$ 0.13
Overall mean bearing different superscripts between rows differ significantly at 5% ($p < 0.05$) level		

Table 5
Effect of *A. vasica* on bone weight (Mean $\pm$ S.E) in AFB1 fed broilers (n = 8)

Group	Particulars	Femur (g)	Tibiotarsus (g)	Tarsometatarsus (g)
I	Basal feed	5.75 ^e $\pm$ 0.45	12.75 ^e $\pm$ 1.19	6.25 ^{bcd} $\pm$ 0.59
II	Basal feed + <i>A. vasica</i> crude powder alone @ 0.5%	5.50 ^{de} $\pm$ 0.33	12.00 ^e $\pm$ 0.53	6.38 ^d $\pm$ 0.18
III	Basal feed + AFB1@ 50 μ g / kg	4.00 ^{abc} $\pm$ 0.00	6.00 ^a $\pm$ 0.38	5.00 ^{ab} $\pm$ 0.38
IV	Basal feed + AFB1 @ 50 μ g / kg + SLM @1000 mg / kg	5.25 ^{de} $\pm$ 0.37	7.75 ^{abc} $\pm$ 0.59	6.25 ^{bcd} $\pm$ 0.25
V	Basal feed + AFB1 @ 50 μ g / kg + <i>A. vasica</i> crude powder @ 0.1%	4.75 ^{bcde} $\pm$ 0.37	7.50 ^{abc} $\pm$ 0.33	5.25 ^{ab} $\pm$ 0.37
VI	Basal feed + AFB1 @ 50 μ g / kg + <i>A. vasica</i> crude powder @ 0.5%	5.50 ^{de} $\pm$ 0.50	8.50 ^{bc} $\pm$ 0.50	6.13 ^{bc} $\pm$ 0.30
Overall mean bearing different superscripts between rows differ significantly at 5% (p < 0.05) level				

Table 6
Effect of *A. vasica* on bone height (Mean $\pm$ S.E) in AFB1 fed broilers (n = 8)

Group	Particulars	Femur (cm)	Tibiotarsus (cm)	Tarsometatarsus (cm)
I	Basal feed	6.00 ^c $\pm$ 0.00	8.00 $\pm$ 0.0	6.00 ^e $\pm$ 0.00
II	Basal feed + <i>A. vasica</i> crude powder alone @ 0.5%	6.00 ^c $\pm$ 0.00	8.00 $\pm$ 0.0	6.00 ^e $\pm$ 0.00
III	Basal feed + AFB1@ 50 μ g / kg	5.75 ^b $\pm$ 0.16	8.00 $\pm$ 0.0	5.00 ^b $\pm$ 0.00
IV	Basal feed + AFB1 @ 50 μ g / kg + SLM @1000 mg / kg	6.00 ^c $\pm$ 0.00	8.00 $\pm$ 0.0	6.00 ^e $\pm$ 0.00
V	Basal feed + AFB1 @ 50 μ g / kg + <i>A. vasica</i> crude powder @ 0.1%	6.00 ^c $\pm$ 0.00	8.00 $\pm$ 0.0	6.00 ^e $\pm$ 0.00
VI	Basal feed + AFB1 @ 50 μ g / kg + <i>A. vasica</i> crude powder @ 0.5%	6.00 ^c $\pm$ 0.00	8.00 $\pm$ 0.0	6.00 ^e $\pm$ 0.00
Overall mean bearing different superscripts between rows differ significantly at 5% (p < 0.05) level				

Table 7
Effect of *A. vasica* on bone diameter (Mean $\pm$ S.E) in AFB1 fed broilers (n = 8)

Group	Particulars	Femur (cm)	Tibiotarsus (cm)	Tarsometatarsus (cm)
I	Basal feed	1.00 ^b $\pm$ 0.00	0.72 ^e $\pm$ 0.00	0.69 ^d $\pm$ 0.00
II	Basal feed + <i>A. vasica</i> crude powder alone @ 0.5%	1.00 ^b $\pm$ 0.00	0.71 ^{cde} $\pm$ 0.00	0.68 ^{cd} $\pm$ 0.00
III	Basal feed + AFB1@ 50 μ g / kg	0.98 ^a $\pm$ 0.02	0.60 ^a $\pm$ 0.04	0.53 ^a $\pm$ 0.13
IV	Basal feed + AFB1 @ 50 μ g / kg + SLM @1000 mg / kg	1.00 ^b $\pm$ 0.00	0.69 ^c $\pm$ 0.00	0.66 ^c $\pm$ 0.00
V	Basal feed + AFB1 @ 50 μ g / kg + <i>A. vasica</i> crude powder @ 0.1%	1.00 ^b $\pm$ 0.00	0.69 ^c $\pm$ 0.00	0.69 ^d $\pm$ 0.00
VI	Basal feed + AFB1 @ 50 μ g / kg + <i>A. vasica</i> crude powder @ 0.5%	1.00 ^b $\pm$ 0.00	0.71 ^{cde} $\pm$ 0.00	0.68 ^{cd} $\pm$ 0.00
Overall mean bearing different superscripts between rows differ significantly at 5% (p < 0.05) level				

3.2.3 Effect of vasicine on serum biochemical profile

The effect of the crude leaf powder supplementation of *Adhatoda vasica* to the aflatoxin contaminated basal diet against the toxic effects of aflatoxin on serum biochemical parameters has been shown in the Tables 8, 9, 10, 11 and 12 and Figs. 9, 10, 11, 12 and 13. *A. vasica* treatment significantly (p < 0.05) reduced the serum biochemical enzymes and increased the antioxidant enzymes. The results indicate a reduction in severity of lesions associated with aflatoxicosis similar to the SLM treatment group.

Table 8
Effect of *A. vasica* on serum total protein, albumin, globulin, albumin and globulin ratio (Mean $\pm$ S.E) in AFB1 fed broilers (n = 8)

Group	Particulars	Total protein (g / dl)	Albumin (g / dl)	Globulin (g / dl)	A:G ratio
I	Basal feed	3.63 ^h $\pm$ 0.18	2.05 ^{ab} $\pm$ 0.16	1.58 ^d $\pm$ 0.18	1.30 ^a $\pm$ 0.20
II	Basal feed + <i>A. vasica</i> crude powder alone @ 0.5%	3.38 ^g $\pm$ 0.18	2.00 ^{ad} $\pm$ 0.0	1.38 ^d $\pm$ 0.16	1.45 ^a $\pm$ 0.20
III	Basal feed + AFB1@ 50 μ g / kg	2.25 ^{ab} $\pm$ 0.25	2.20 ^{ab} $\pm$ 0.16	0.25 ^a $\pm$ 0.13	8.00 ⁱ $\pm$ 0.18
IV	Basal feed + AFB1 @ 50 μ g / kg + SLM @1000 mg / kg	2.60 ^c $\pm$ 0.13	2.05 ^{ab} $\pm$ 0.18	0.55 ^{ab} $\pm$ 0.19	3.73 ^g $\pm$ 0.25
V	Basal feed + AFB1 @ 50 μ g / kg + <i>A. vasica</i> crude powder @ 0.1%	3.00 ^d $\pm$ 0.18	2.29 ^{abc} $\pm$ 0.13	0.71 ^{abc} $\pm$ 0.23	3.23 ^e $\pm$ 0.20
VI	Basal feed + AFB1 @ 50 μ g / kg + <i>A. vasica</i> crude powder @ 0.5%	3.62 ^h $\pm$ 0.18	2.63 ^{bcd} $\pm$ 0.20	0.99 ^d $\pm$ 0.25	2.66 ^c $\pm$ 0.20
Overall mean bearing different superscripts between rows differ significantly at 5% (p < 0.05) level					

Table 9
Effect of *A. vasica* on serum ALT, AST and GGT (Mean $\pm$ S.E) in AFB1 fed broilers (n = 8)

Group	Particulars	ALT (SGPT) (Units / L)	AST (SGOT) (Units / L)	GGT (Units / L)
I	Basal feed	39.88 ^{bcd} $\pm$ 3.03	152.25 ^a $\pm$ 13.99	9.63 ^a $\pm$ 0.53
II	Basal feed + <i>A. vasica</i> crude powder alone @ 0.5%	45.38 ^d $\pm$ 3.42	164.38 ^a $\pm$ 6.32	11.88 ^{ab} $\pm$ 1.89
III	Basal feed + AFB1@ 50 μ g / kg	106.50 ^e $\pm$ 4.17	337.60 ^c $\pm$ 10.06	27.63 ^d $\pm$ 1.25
IV	Basal feed + AFB1 @ 50 μ g / kg + SLM @1000 mg / kg	37.88 ^{abcd} $\pm$ 2.00	205.38 ^b $\pm$ 18.42	17.25 ^{abcd} $\pm$ 1.40
V	Basal feed + AFB1 @ 50 μ g / kg + <i>A. vasica</i> crude powder @ 0.1%	33.25 ^{abc} $\pm$ 2.18	257.59 ^b $\pm$ 13.62	16.38 ^{abcd} $\pm$ 1.89
VI	Basal feed + AFB1 @ 50 μ g / kg + <i>A. vasica</i> crude powder @ 0.5%	33.75 ^{abc} $\pm$ 2.72	224.58 ^b $\pm$ 16.59	13.00 ^{abc} $\pm$ 1.82
Overall mean bearing different superscripts between rows differ significantly at 5% (p < 0.05) level				

Table 10
Effect of *A. vasica* on serum ALP and LDH (Mean $\pm$ S.E) in AFB1 fed broilers (n = 8)

Group	Particulars	ALP (Units / L)	LDH (Units / L)
I	Basal feed	3120.00 ^a $\pm$ 380.40	1264.50 ^a $\pm$ 92.57
II	Basal feed + <i>A. vasica</i> crude powder alone @ 0.5%	3271.25 ^a $\pm$ 296.41	1580.00 ^a $\pm$ 94.33
III	Basal feed + AFB1@ 50 μ g / kg	14192.50 ^d $\pm$ 1170.20	5381.25 ^d $\pm$ 92.77
IV	Basal feed + AFB1 @ 50 μ g / kg + SLM @1000 mg / kg	3774.91 ^a $\pm$ 1334.63	2351.25 ^{bc} $\pm$ 96.31
V	Basal feed + AFB1 @ 50 μ g / kg + <i>A. vasica</i> crude powder @ 0.1%	6598.75 ^{bc} $\pm$ 913.79	2820.00 ^{bc} $\pm$ 55.57
VI	Basal feed + AFB1 @ 50 μ g / kg + <i>A. vasica</i> crude powder @ 0.5%	4101.25 ^{ab} $\pm$ 987.78	2433.88 ^{bc} $\pm$ 10.03
Overall mean bearing different superscripts between rows differ significantly at 5% (p < 0.05) level			

Table 11
Effect of *A. vasica* on serum uric acid and creatinine (Mean $\pm$ S.E) in AFB1 fed broilers (n = 8)

Group	Particulars	Uric acid (mg / dl)	Creatinine (mg / dl)
I	Basal feed	6.50 ^a $\pm$ 0.20	0.43 ^a $\pm$ 0.02
II	Basal feed + <i>A. vasica</i> crude powder alone @ 0.5%	6.60 ^a $\pm$ 0.19	0.42 ^a $\pm$ 0.01
III	Basal feed + AFB1@ 50 μ g / kg	16.56 ^e $\pm$ 0.33	0.71 ^{ef} $\pm$ 0.01
IV	Basal feed + AFB1 @ 50 μ g / kg + SLM @1000 mg / kg	11.43 ^b $\pm$ 0.26	0.49 ^b $\pm$ 0.02
V	Basal feed + AFB1 @ 50 μ g / kg + <i>A. vasica</i> crude powder @ 0.1%	12.22 ^c $\pm$ 0.35	0.48 ^b $\pm$ 0.03
VI	Basal feed + AFB1 @ 50 μ g / kg + <i>A. vasica</i> crude powder @ 0.5%	12.56 ^c $\pm$ 0.29	0.55 ^c $\pm$ 0.03
Overall mean bearing different superscripts between rows differ significantly at 5% (p < 0.05) level			

Table 12
Effect of *A. vasica* on serum antioxidant profile (Mean $\pm$ S.E) in AFB1 fed broilers (n = 8)

Group	Particulars	Superoxide dismutase (mg / g of protein)	Glutathione peroxidase (mg / g of protein)	Catalase (mg / g of protein)
I	Basal feed	37.82 ^f $\pm$ 1.34	105.41 ^e $\pm$ 1.55	11.32 ^e $\pm$ 0.50
II	Basal feed + <i>A. vasica</i> crude powder alone @ 0.5%	36.51 ^f $\pm$ 3.26	103.88 ^e $\pm$ 1.00	12.22 ^e $\pm$ 0.71
III	Basal feed + AFB1@ 50 μ g / kg	20.45 ^b $\pm$ 3.34	67.97 ^b $\pm$ 0.47	6.61 ^b $\pm$ 0.65
IV	Basal feed + AFB1 @ 50 μ g / kg + SLM @1000 mg / kg	28.04 ^{cde} $\pm$ 1.91	86.10 ^d $\pm$ 2.35	7.54 ^{bc} $\pm$ 0.09
V	Basal feed + AFB1 @ 50 μ g / kg + <i>A. vasica</i> crude powder @ 0.1%	23.95 ^{bc} $\pm$ 0.70	78.40 ^c $\pm$ 1.61	7.62 ^{bc} $\pm$ 0.56
VI	Basal feed + AFB1 @ 50 μ g / kg + <i>A. vasica</i> crude powder @ 0.5%	26.82 ^{cd} $\pm$ 0.85	82.84 ^{cd} $\pm$ 1.50	8.12 ^{cd} $\pm$ 0.38
Overall mean bearing different superscripts between rows differ significantly at 5% (p < 0.05) level				

3.2.4 Effect of vasicine on Hepatotoxicity and Nephrotoxicity

The mean weight of the organs among the groups are presented in Table 13 and Fig. 14.

Table 13
Effect of *A. vasica* on relative organ weight (Mean $\pm$ S.E) in AFB1 fed broilers (n = 8)

Group	Particulars	Liver with gall bladder (g / 100 g)	Kidney (g / 100 g)
I	Basal feed	9.70 ^a $\pm$ 0.25	1.74 ^a $\pm$ 0.15
II	Basal feed + <i>A. vasica</i> crude powder alone @ 0.5%	9.76 ^a $\pm$ 0.36	1.74 ^a $\pm$ 0.17
III	Basal feed + AFB1@ 50 μ g / kg	20.09 ^d $\pm$ 0.51	1.91 ^c $\pm$ 0.16
IV	Basal feed + AFB1 @ 50 μ g / kg + SLM @1000 mg / kg	11.77 ^b $\pm$ 0.25	1.84 ^b $\pm$ 0.17
V	Basal feed + AFB1 @ 50 μ g / kg + <i>A. vasica</i> crude powder @ 0.1%	11.53 ^b $\pm$ 0.07	1.86 ^b $\pm$ 0.18
VI	Basal feed + AFB1 @ 50 μ g / kg + <i>A. vasica</i> crude powder @ 0.5%	13.48 ^c $\pm$ 0.07	1.84 ^b $\pm$ 0.15
Overall mean bearing different superscripts between rows differ significantly at 5% (p < 0.05) level			

Liver from the aflatoxin treated group was enlarged, pale or yellow, soft and friable whereas, these changes were mitigated in the *A. vasica* treated group similar to the basal feed group and SLM treated group. Histopathological examination revealed varying degrees of changes in the hepatocytes like fatty degeneration, pyknosis and karyorrhexis of nucleus, sinusoidal congestion, necrosis of cells around the portal triad, microvesicle formation, kupffer cell proliferation and glandular transformation in the toxin fed group and these changes were mitigated in the *A. vasica* treated group similar to the basal feed group and SLM treated group. The kidneys were enlarged slightly with histopathological evidences of revealed varying degrees of changes like degeneration and desquamation of the tubular epithelial cells, marked intertubular congestion, infiltration of inflammatory cells in the inter tubular area, necrosis and thickening of the basement membrane of the glomeruli in the toxin fed group and these changes were mitigated in the *A. vasica* treated group similar to the basal feed group and SLM treated group.

Histopathological examination

The effect of crude leaf powder of *A. vasica* on gross and histopathology has been presented in Figs. 15, 16, 17 and 18. Liver in the control and the *A. vasica* and SLM treated group was normal and in toxin induced group, it was enlarged, pale or yellow, soft and friable. Also the toxin induced group revealed varying degrees of changes in the hepatocytes like fatty degeneration, pyknosis and karyorrhexis of nucleus, sinusoidal congestion, necrosis of cells around the portal triad, microvesicle formation, kupffer cell proliferation and glandular transformation and these changes were mitigated in the vasicine treated group with the maximum effect at 0.5% similar to SLM treated group.

Discussion

Mycotoxins are the secondary metabolites produced by various toxigenic fungi belonging to three genera viz., *Aspergillus*, *Fusarium* and *Penicillium* sp. Because of the ubiquitous occurrence, heat stability and lipophilic nature of these metabolites, the toxic syndromes are termed as mycotoxicoses which are frequently encountered in animals and poultry consuming toxin contaminated feeds. To overcome the toxic effects of these fungal metabolites, various *in vivo* and *in vitro* strategies have been tried to ameliorate / detoxify / remove the toxins from the feed and raw materials used in birds. In recent days, herbs are being used in many studies to mitigate the toxic effect of mycotoxins.

On subjecting the *A. vasica* crude leaf powder to *in vivo* studies, the reduction in body weights in broiler birds due to dietary concentration of 50 µg / kg of AFB1 was found to be 32.91% in absolute terms. The results are in agreement with the findings of previous reports. Presence of AFB1 in feed in poultry caused a poor growth rate [21] and significantly reduced the body weight or body weight gain [22–24]. In this study, supplementation of the leaf powder of *A. vasica* was able to improve the body weight by 21.73% at 0.1 per cent level and 24.93% at 0.5 per cent level in AFB1 fed group which were numerically higher than the improvement (15.35%) shown by SLM treated group. Thus *A. vasica* was able to produce better improvement in weight gain.

With reference to the feed consumption, AFB1 at the concentration of 50 µg / kg reduced the feed consumption by 9.80% thereby reducing the efficiency and impairing the production performance in birds and increasing the cost of production which may certainly be an economic impact imposed on the farmers. Decreased feed consumption was reported in birds with AFB1 [25] present in the diet of broiler chicks. Significant reductions in feed efficiency at varying levels of dietary AF have also been reported [26–29] on supplementation. In broilers the adverse effect of AFB1 on feed conversion ratio at the end of first week was negligible, second week was medium and third to six weeks was very large [30]. The results of our study are in accordance with the above findings. Hence, it can be stated that AFB1 at dietary concentrations of 50 µg / kg either caused significant depressions in feed intake and feed conversion efficiency which were the results of the lowered body weight gain observed in this study.

Research works done on a few mitigating natural plant supplements are available. curcuminoids caused a significant improvement in feed efficiency in toxin fed birds [31]. In this study, the feed consumption in the birds treated with the leaf powder of *A. vasica* at the concentration of 0.1 and 0.5 per cent had been found to improve feed consumption by 4.04% and 3.21% in AFB1 fed group whereas it was observed to be very low in SLM treated group (0.71%). As far as feed conversion efficiency was concerned, 36.42%, 35.80% and 41.36% reduction was noticed in AFB1. *A. vasica* supplementation in the groups of AFB1 showed 29.63% and 34.57% improvement in FCR at 0.1 per cent and 0.5 percent level of inclusion respectively, which were far higher than the value of improvement of 25.31% recorded by SLM treated group. Thus the results suggest the potential use of the plant [32] for the purpose of improving the performance of broiler birds impacted by the presence of AFB1 in the feed.

In this study, femur weight was found to reduce to an extent of 30.43%, tibiotarsus weight was reduced to the extent of 52.94% and tarsometatarsus weight was reduced to the extent of 20.00% in toxin fed group. As per the earlier reports, young chicken fed with graded levels of AF and OT affected the material properties of bone like breaking strength, displacement and diameter [33]. Reduction in serum Ca and P concentrations upon AF exposure [34–36], interfered vitamin D metabolism are the reasons suggested for the adverse effects of AFB1 on bone integrity like reduced bone strength and leg weakness in broilers [21]. In ducks also, AF caused deformity in bone structures [37].

In our study, treatment with *A. vasica* crude leaf powder at 0.5 per cent level was found to overcome deformity in femur weight by 26.09% in toxin fed group almost comparable to 21.74% improvements by SLM supplementation. Femur height was also increased by 4.17%. As far as tibiotarsus weight is concerned, treatment with *A. vasica* crude leaf powder at 0.5 per cent level was found to improve by 19.61%, almost comparable to the 13.73% in SLM treated group. With regard to the tarsometatarsus weight, treatment with *A. vasica* crude leaf powder at 0.5 per cent level was found to overcome the deformity by 18.08% and numerically higher than the 20.00% of SLM group. Tarsometatarsus height was increased by 16.67% comparable to the 16.67% in SLM group. On treatment with *A. vasica* at the concentration of 0.1 and 0.5 per cent, the deformity in bones were found to be reduced with better effect at the highest level of inclusion thereby suggesting the use of the plant for the purpose.

In the present study, there was reduction of serum proteins, albumin and globulin in the toxin fed group. Serum proteins level is considered to be the most sensitive indicator of aflatoxicosis, with different classes of proteins being affected differently. The marked sensitivity of serum protein levels reflects the principal mechanism of AF toxicity, wherein the AF was reported to bind to DNA and caused disruption to transcription [38]. Chicks given AFB1 alone showed a significant decrease in the levels of serum total proteins from day 14 onwards [22]. Thus, the results of our findings are supportive of the above discussions. It can be stated that AFB1 at dietary concentrations of 50 µg / kg inhibited protein synthesis and thereby reduced the levels of albumin and globulin and altered the albumin globulin ratio in the circulating blood. Hence, AFB1 is a potential inhibitor of protein synthesis leading to impaired performance which may be attributed to the immune suppression that might occur owing to protein depletion. Thus the weakened immune system might have led to the growth of secondary bacteria ultimately affecting the growth performance in birds. The enzymes are found to be elevated in the serum of AFB1 fed group. ALT is liver specific enzyme, whereas AST derived from most organs such as heart, skeletal muscles, kidney, brain, pancreas, lungs, leukocytes and erythrocytes other than liver. AFB1 generally increased the activity of AST, ALT, ALP, LDH and GGT [39]. Increased levels of serum creatinine [40] and subsequent accumulation of uric acid [21] has been linked to damages in kidney tissues [41] and decreased the activity of SOD, catalase and GPx enzymes. AF [42].

A. vasica supplementation reversed serum protein level partially by 37.74% numerically higher than 9.64% of SLM treated. Feeding of the leaf powder of *A. vasica* at the concentration of 0.1 and 0.5 per cent was able to reduce the degenerative changes and protect the cell integrity of liver and kidney and thereby reducing the enzyme leakage from the cell especially at the highest level of inclusion. Few studies conducted with plant sources was interesting to observe that supplementation of curcuminoids [35],

resveratrol [18], the active principle curcumin [43–45] and *A. vasica* to the AFB1 diet significantly improved the serum values of total protein, album [32] in and globulin by reducing the detrimental adduct formation and also reduced the liver and kidney injury in toxin induced study and protected the antioxidant system and enzyme levels. *A. vasica* at the concentration of 0.1 and 0.5 per cent was able to reduce the degenerative changes and protect the cell integrity and can be recommended to use *A. vasica* in birds to mitigate aflatoxicosis.

In this study, there was a marked increase in the relative weight of liver with enlargement, paleness and friable. Lethal aflatoxicosis caused yellow colouration of liver due to congestion or fat accumulation and similar gross changes in liver [46–48] and histopathological changes [49–50] were recorded in birds induced with mycotoxins. Also, kidney was found to be enlarged [51 & 21] with varying degrees of degeneration in toxin fed broilers.

A. vasica treated groups showed significant mitigating effects of AFB1 with reduction in relative organ weight of liver and kidney in birds and offered hepato-renal protection as reported on supplementation of curcumin, resveratrol [52] and *Silybum marianum* [47]. Thus *A. vasica* had produced substantial reversal of organ toxicity.

The better binding of the alkaloid vasicine present in the plant *Adhatoda vasica* to the aflatoxin B1 as revealed by docking studies [20] also strongly suggest that the toxin mitigating potential of the plant.

Thus, inclusion of *A. vasica* in the feed at the rate of 0.1 per cent and 0.5 per cent resulted in better body weight gain and improved the feed conversion efficiency and ameliorated the toxic effects produced in liver and kidney. Hence, it may be recommended to farmers to include *A. vasica* leaf powder in feed at 0.1 per cent level for economic reasons so as to achieve better results in terms of production performance and on other biochemical and antioxidant profiles and to mitigate the overall toxic effects produced by aflatoxin in birds.

Conclusion

Feeding of *A. vasica* crude powder as such to the birds at 0.5% level was found to be safe with no toxicity of the major organs involved. The toxin mitigating potential of the plant was evident through their antioxidant activity, serum biochemical markers, through their effect on the zoo technic performance indicators and also through the gross and histopathology. The principal alkaloid vasicine in the leaf of the plant could be the possible mediator for their potent toxin mitigating activity since their binding efficiency was proved by *in silico* docking studies with promising dock score and their pharmacological activity like antioxidant, antidepressant, anticancer effect has been proved.

Methods

Reagents and Chemicals

All the solvents and reagents of analytical grade were purchased from M/s. Sigma Aldrich Chemicals India Pvt. Ltd., and used without further purification. Water purified by millipore purified system was used for the entire analysis. Reference standards of AFB1 were supplied *as gratis* by the Animal Feed Analytical and Quality Assurance Laboratory (AFAQAL), Veterinary College and Research Institute, Namakkal.

In vivo experimental design

Day old broiler straight run chicks (Vencobb strain) obtained from a commercial hatchery in Namakkal was used for the experimental study. The trial was conducted after obtaining approval from the Institutional Animal Ethical Committee (IAEC) with the approval No: IAEC / 10 / VCRI - NKL / 2019.

AFB1 was produced using the pure culture of *Aspergillus parasiticus* MTCC No. 2797. on rice as per the method of [53] and estimated as per the in house method of AFAQAL (modified method of [54] for estimation of AF) at Animal Feed Analytical and Quality Assurance Laboratory, Veterinary College and Research Institute, Namakkal. The powdered substrate containing toxin was mixed in the broiler feed to arrive at a final concentration of 50 µg of AFB1 for every kg of feed (µg / kg) and analyzed for confirmation before initiation of the feeding trial.

Plant material used

A. vasica leaves were collected from the herbal garden of the Department of Veterinary Pharmacology and Toxicology, Veterinary College and Research Institute, Namakkal, shade dried, powdered and stored until further use. The crude powder of *A. vasica* was mixed in the feed at 0.1% and 0.5% and fed to the respective groups.

Reference drug used

SLM (Trade name: Silybon) tablets, composed of 140 mg each of the active principle were purchased from M/s. Microlabs India Ltd., Bengaluru, Karnataka and were used for the study. SLM tablets were ground using a mechanical blender to fine powder and was included in the feed at the dose rate of 1000 mg / kg of feed.

Experimental design

The day old broiler chicks on arrival were weighed and tagged with wing band for identification. They were randomly distributed in to fourteen different treatment groups (three replicates of 36 chicks per dietary treatment) as shown below.

EXPERIMENTAL DESIGN

n = 36 (Each treatment with 3 replicates)

Group	Treatment	Particulars
I	T ₁	: Basal feed
II	T ₂	: Basal feed + <i>A. vasica</i> crude powder alone @ 0.5 mg/kg of feed
III	T ₃	: Basal feed + AFB1 @ 50 µg / kg alone
IV	T ₄	: Basal feed + AFB1 @ 50 µg / kg + SLM @1000 mg / kg
V	T ₅	: Basal feed + AFB1 @ 50 µg / kg + <i>A. vasica</i> crude powder @ 0.1 mg/kg of feed
VI	T ₆	: Basal feed + AFB1 @ 50 µg / kg + <i>A. vasica</i> crude powder @ 0.5 mg/kg of feed

All the birds were reared in deep litter system under standard and uniform managerial conditions. Vaccination was followed as per the standard schedule.

Assessment of zootechnic performance, bone morphometry, biochemical and antioxidant profile

The body weight, weight gain, feed consumption and feed conversion efficiency was calculated at weekly intervals. The shank height and the weight, height and diameter of the bones were measured [55]. The serum samples were analyzed for total protein, albumin, globulin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and gamma-glutamyl transferase (GGT), uric acid and creatinine using M/s. Daksha Diagnostic Kits in Biosystems 415 Random Access Auto Analyzer according to the recommendations of the manufacturer at Centralised Clinical Laboratory, Veterinary College and Research Institute, Namakkal. The activities of the antioxidant enzymes in the serum *viz.*, Superoxide dismutase (SOD) was estimated by the method of [56], Catalase (CAT) by the method of [57] and Glutathione peroxidase activity (GPx) as per the method of [58].

Gross and Histopathological examination

Gross lesions and histopathology lesions were recorded in liver and kidney [59].

Statistical analysis

Completely randomized design was followed for the experiments [60] and the data collected on various parameters were analyzed statistically by one - way ANOVA using SPSS software (Version 20). Post - hoc analysis was done by Duncan's significance difference test. Results are expressed as Mean ± SE.

Declarations

Acknowledgements

The authors are highly thankful to the mute creatures involved in the study.

Guidelines

All methods were carried out in accordance with the relevant guidelines and regulations.

ARRIVE Guidelines

The authors confirm that the study was carried out in compliance with the ARRIVE guidelines.

Authors contributions

Conceptualization and designing – Sakthi Priya Muthusamy and Jagadeeswaran Appusamy

Investigations – Jagadeeswaran Appusamy and Natarajan Amirthalingam

Methodology - Jagadeeswaran Appusamy and Natarajan Amirthalingam

Writing, Review and editing - Sakthi Priya Muthusamy, Jagadeeswaran Appusamy and Natarajan Amirthalingam

All authors have read and agreed to the published version of the manuscript.

Funding

There is no external funding for the study.

Informed consent statement

Not applicable

Data availability statement

Data will be made available on request.

Ethics approval and consent to participate

The current study was approved by the Institutional Animal Ethical Committee (IAEC) of Veterinary College and Research Institute, Namakkal under Tamil Nadu Veterinary and Animal Sciences University with the approval No: IAEC / 10 / VCRI - NKL / 2019.

Consent for publication

Not applicable

Declaration of competing Interest

The authors declare that they have no financial conflicts of interest.

Author details

¹Assistant Professor, Department of Veterinary Pharmacology and Toxicology, Veterinary College and Research Institute, Namakkal, Tamil Nadu, India

²Professor and Head, Department of Veterinary Pharmacology and Toxicology, Veterinary College and Research Institute, Namakkal, Tamil Nadu, India

³Professor and Head, Animal Feed Analytical and Quality Assurance Laboratory, Veterinary College and Research Institute, Namakkal, Tamil Nadu, India

References

1. Sharma G. "Indian Poultry Industry: Challenges, Problems and Opportunities". poultry punch online magazine. 2020; pp: 1.
2. Hafez MH and Attia YA. "Challenges to the Poultry Industry: Current Perspectives and Strategic Future after the COVID – 19 Outbreak". *Frontiers in Veterinary Science*. 2020. 7:516. <https://doi.org/10.3389/fvets.2020.00516>
3. Munkvold GP. "Cultural and genetic approaches to managing mycotoxins in maize". *Annual review of phytopathology*. 2003a;**41**: 99–116.
4. Bhat R, Rai RV and Karim AA. "Mycotoxins in Food and Feed: Present status and future concerns". *Comprehensive Review in Food Science and Food Safety*. 2010; **9**: 57–81.
5. Groopman JD, Cain LG and Kensler TW. "Aflatoxin exposure in human populations: measurements and relationship to cancer". *Critical Reviews in Toxicology*. 1988; **19**: 113–146.
6. Massey TE, Stewart RK, Daniels JM and Liu L. "Biochemical and molecular aspects of mammalian susceptibility to aflatoxin B1 carcinogenicity". *Proceedings of the Society for Experimental Biology and Medicine*. 1995; **208**: 213–227.
7. Romagnoli B, Menna V, Gruppioni N and Bergamini C. "Aflatoxins in spices, aromatic herbs, herb-teas and medicinal plants marketed in Italy". *Food Control*. 2007; **18**: 697–701.
8. O'Riordan MJ and Wilkinson MG. "A survey of the incidence and level of aflatoxin contamination in a range of imported spice preparations on the Irish retail market". *Food Chemistry*. 2008; **107**: 1429–1435.
9. Wilkinson JR, Kale SP, Bhatnagar D, Yu J, Ehrlich KC. Expression Profiling of Non-Aflatoxigenic *Aspergillus parasiticus* Mutants Obtained by 5-Azacytosine Treatment or Serial Mycelial Transfer. *Toxins (Basel)*. 2011; 3(8): 932–948. doi: 10.3390/toxins3080932
10. Bennet JW and Klich M. Mycotoxins. *Clinical Microbiology Review*. 2003; 16 (3):497–516.
11. Jand SK, Kaur P and Sharma NS. "Mycoses and mycotoxicosis in poultry: A review". *Indian Journal of Animal Science*. 2005; **75** (4): 465–476.
12. Oluwafemi, F and. Taiwo VO. "Reversal of toxigenic effects of aflatoxin B1 on cockerels by alcoholic extract of African nutmeg, *Monodora myristica*". *Journal of the Science of Food and Agriculture*. 2004; **84**: 333–340.

13. Park JW and Kim YB. "Effect of pressure cooking on aflatoxin B1 in rice". *Journal of Agricultural and Food Chemistry*. 2006; **54** : 2431–2435.
14. Piva FP, Galvano RD, Pietri AP and Piva RD. "Detoxification methods of aflatoxins". *Nutrition Research*. 1995; **15**: 767–776
15. Shantha, T. "Fungal degradation of aflatoxin B1". *Natural Toxins*. 1999; **7**: 175–178.
16. Ramos AJ and Hernandez E. "Prevention of aflatoxicosis in farm animals by means of hydrated sodium calcium aluminosilicate addition to feed stuffs: A review". *Animal Feed Science and Technology*. 1997; **65**: 197–206.
17. Rangasaz N and Ahangaran MG. "Evaluation of turmeric extract on performance indices impressed by induced aflatoxicosis in broiler chickens". *Toxicology and Industrial Health*. 2011; **27**(10): 956–960.
18. Sridhar M, Suganthi RU and Thammiah V. Effect of dietary resveratrol in ameliorating aflatoxin B1 induced changes in broiler birds, *Journal of Animal Physiology and Animal Nutrition*. 2015; **99** (6) : 1094–1104.
19. Uyar A, Yener Z and Dogan A. "Protective effects of *Urtica dioica* seed extract in aflatoxicosis: histopathological and biochemical findings". *British Poultry Science*. 2016; **57** (2): 235–245.
20. Priya MS, Jagadeeswaran A, Raja MJ, Natarajan A, Srinivasan P and Ranganathan V. Phytochemical analysis of *Adhatoda vasica* and identification of an isolated alkaloid vasicine using HPTLC. *The Pharma Innovation*. 2021; 10 (9):1370–1377.
21. Hamilton PB, Huff WE, Harris JR and Wyatt RD. "Natural occurrences of ochratoxicosis in poultry". *Poultry Science*. 1982; **61**(9): 1832–1841.
22. Jindal N, Mahial SK and Mahajan NK. "Toxicity of aflatoxin B 1 in broiler chicks and its reduction by activated charcoal". *Research in Veterinary Science*. 1994; **56**: 37–40.
23. Elaroussi MA, Mohamed FR, El Barkouky EM, Atta AM, Abdou AM and Hatab MH. "Experimental ochratoxicosis in broiler chickens". *Avian Pathology*. 2006; **35** (4): 263–269.
24. Duarte SC, Lino CM and Pena A. "Ochratoxin A in feed of food producing animals: an undesirable mycotoxin with health and performance effects". *Veterinary microbiology*. 2011; **154**: 1–13.
25. Beura CK, Johri TS, Sadagopan VR and Panda BK. Interaction of dietary protein level on dose - response relationship during aflatoxicosis in commercial broilers, Physical responses livability and nutrient retention". *Indian Journal of Poultry Science*. 1993; **28**: 170–177
26. Reddy PS, Reddy CV, Reddy VR and Rao PV. "Occurrence of aflatoxin in some feed ingredients in three geographical regions of Andhra Pradesh". *Indian Journal of Animal Science*. 1984; **54**: 235–238.
27. Smith JW and Hamilton PB. Aflatoxicosis in broiler chickens. *Poultry Science*. 1970; **49**: 207–215.
28. Johri TS, Agrawal R and Sadagopan VR. 1988. "Aflatoxin occurrence in feedstuffs and its effect on poultry production". Proceedings of XII National conference and symposium of IPSA, CARI, Izatnagar, U.P., India.
29. Verma J, Beura CK and Johri TS. 1992. "Faecal fat excretion by the chicks as an indicator of aflatoxicosis". Proceedings - XIV Annual poultry conference and symposium, Pantnagar, India, pp. 38.

30. Suganthi RU, Suresh KP and Parvatham R. "Effect of aflatoxin on feed conversion ratio in broilers: A meta-analysis". *Asian Australian Journal of Animal Science*. 2011; **24** (12): 1757–1762.
31. Gowda NKS, Ledoux DR, Rottinghaus GE, Bermudez AJ and Chen YC. "Efficacy of turmeric (*Curcuma longa*): containing a known level of curcumin, and a hydrated sodium calcium aluminosilicate to ameliorate the adverse effects of aflatoxin in broiler chicks". *Poultry Science*. 2008; **87**: 1125–1130.
32. Kannan K, Mathivanan R, Sudha MG, Sokkalingam B, Balagopal R and Velazhahan R. "Effect of *Adhatoda vasica* leaf powder as herbal feed additive on growth performance and biochemical parameters of serum and liver of broilers". *Biochemical and Cellular Archives*. 2017; **17**(1): 407–413.
33. Huff WE and Doerr JA. "Synergism between aflatoxin and ochratoxin A in broiler chickens". *Poultry Science*. 1980; **60**: 550–555.
34. Ledoux, DR, Rottinghaus GE, Bermudez AK and Debolt MA. "Efficacy of a hydrated sodium calcium aluminosilicate to ameliorate the toxic effects of aflatoxin in broiler chicks". *Poultry Science*. 1999; **78**: 204–210.
35. Gowda KSN, Ledoux DR, Rottinghaus GE, Bermudez J and Chen YC. "Antioxidant efficacy of curcuminoids from turmeric (*Curcuma longa* L.) powder in broiler chickens fed diets containing aflatoxin B1". *British Journal of Nutrition*. 2009; **102**: 1629–1634.
36. Zhao J, Shirley RB, Dibner JD, Uraizee F, Officer M, Kitchell M, Anon MV and Knight CD. "Comparison of hydrated sodium calcium aluminosilicate and yeast cell wall on counteracting aflatoxicosis in broiler chicks". *Poultry Science*. 2010; **89**: 2147–2156.
37. Khajareru J and Khajareru S. 1999. "Protective effects of mycosorb against aflatoxicosis in ducklings and broilers". Oster presentation of Alltech's 15th Annual symposium on Biotechnology in the feed industry, Lexington, KY.
38. Clifford JI and Rees K.R. "The action of aflatoxin B1 on the rat liver". *Biochemical Journal*. 1967; **102**: 65–75
39. Brinda R, Selvaraj V, Uma D, Malathi D, Paranidharan V and Velazhahan R. "Role of *Adhatoda vasica* (L.) Nees leaf extract in the prevention of aflatoxin-induced toxicity in wistar rats". *Journal of the Science of Food and Agriculture*. 2013; **93**: 2743–2748
40. Agawane SB and Lonkar PS. "Effect of probiotic containing *Saccharomyces boulardii* on experimental ochratoxicosis in broilers: Hematobiochemical studies". *Journal of Veterinary Science*. 2004; **5** (4): 359–367.
41. Riahi I, Marquis V, Ramos AJ, Brufau J, Garcia EE and Vendrell AMP. "Effects of Deoxynivalenol-contaminated diets on productive, morphological, and physiological indicators in broiler chickens". *Animals*. 2020; **10** (10): 1795–1806.
42. Eraslan G, Akdogan M, Yaran E, Findokuyucu F, Efcit D and Altintas D. "The effects of aflatoxins on oxidative stress in broiler chickens". *Turkish Journal of Veterinary and Animal Sciences*. 2005; **29**: 701–707.
43. Cheng Y, Li HL, Wang HF, Sun HF, Liu YF, Peng SX, Liu KX and Guo ZY. Inhibition of nicotine - DNA adduct formation in mice by six dietary constituents". *Food and Chemical Toxicology*. 2003; **41**(7): 1045–1050.

44. El Bahr SM.. "Effect of curcumin on hepatic antioxidant enzyme activities and gene expression in rats intoxicated with aflatoxin B1". *Phytotherapy Research*. 2015; **29** (1): 134–140.
45. Zhang NY, Qi M, Zhao L, Zhu MK, Guo J, Liu J, Gu CQ, Rajput SA, Krumm CS and Qi DS. Curcumin prevents aflatoxin B1 hepatotoxicity by inhibition of cytochrome P450 isozymes in chick liver. *Toxins*. 2016; **8** (11): 327–337.
46. Slowik J, Graczyk S and Madej JA. The effect of single dose of AFB1 on the value of nuclear index of blood lymphocytes and on histopathological changes in the liver, bursa of fabricius, suprarenal glands and spleen in ducklings. *Folia. Histochemica et Cytobiologica*. 1985; **3**: 71–80.
47. Stoev SD, Anguelov, G, Ivanov I and Pavlov D. Influence of ochratoxin A and an extract of artichoke on the vaccinal immunity and health in broiler chicks. *Experimental and Toxicologic Pathology*. 2000; **52**: 43–55.
48. Swaminathan B, 2002. Individual and combined effects of ochratoxin A and citrinin toxicity in broilers. M.V.Sc. thesis submitted to Tamil Nadu Veterinary and Animal Sciences University, Chennai.
49. Kumar R and Balachandran, C. "Histopathological changes in broiler chickens fed aflatoxin and cyclopiazonic acid". *Veterinarski arhiv*. **2009**: **79**: 31–40.
50. Hassan ZU. Khan MZ, Khan A, Javed I, Sadique U and Hameed MR. Effect of ochratoxin A contaminated feed on several health and economic parameters in white leghorn cockerels. *Pakistan Veterinary Journal*. 2012; **32** (1): 35–40.
51. Tung HT, Wyatt RD, Thaxton P and Hamilton PB. "Impairment of kidney function during aflatoxicosis". *Poultry Science*. 1973; **52**: 873–878.
52. El Agamy DS. Comparative effects of curcumin and resveratrol on aflatoxin B1 - induced liver injury in rats. *Archives of toxicology*. 2010; **84** (5): 389–396.
53. Shotwell OL, Hesseltine CW, Stabblefield RD and Sorenson GW. "Production of aflatoxin on rice". *Applied Microbiology*. 1966; **14**: 425–428.
54. Romer TR. "Screening methods for the detection of aflatoxins in mixed feed and other agricultural commodities with subsequent confirmation and quantitative measurement of aflatoxin in positive samples". *Journal – Association of Official Analytical Chemists*. 1975; **58**: 500–506.
55. Zhang B and Coon CN. "The relationships of various tibia bone measurements in hens". *Poultry Science*. 1997; **76**: 1698–1701.
56. Marklund S and Marklund G. Involvement of the superoxide anion radical in the autooxidation of pyrogallol and a convenient assay for superoxide dismutase. *European Journal of Biochemistry*. 1974; **47**: 469–474.
57. Claiborne AL. 1985. CAT activity. In: R. A. Greenwald (ed.), CRC Handbook of methods in oxygen radical research. CRC Press, Boca Raton, 283–284.
58. Moron MS, Depierre JW and Mannervik B. "Levels of glutathione, glutathione reductase and glutathione S transferase in rat lung and liver". *Biochimica et Biophysica Acta*, 1979; **582**: 67–78
59. Bancroft JD and Gamble M. 2008. "Theory and practice of histological techniques". 6th Edition, Churchill Livingstone, Elsevier, China.

Tables

Tables 1 to 13 are available in the Supplementary Files section

Figures

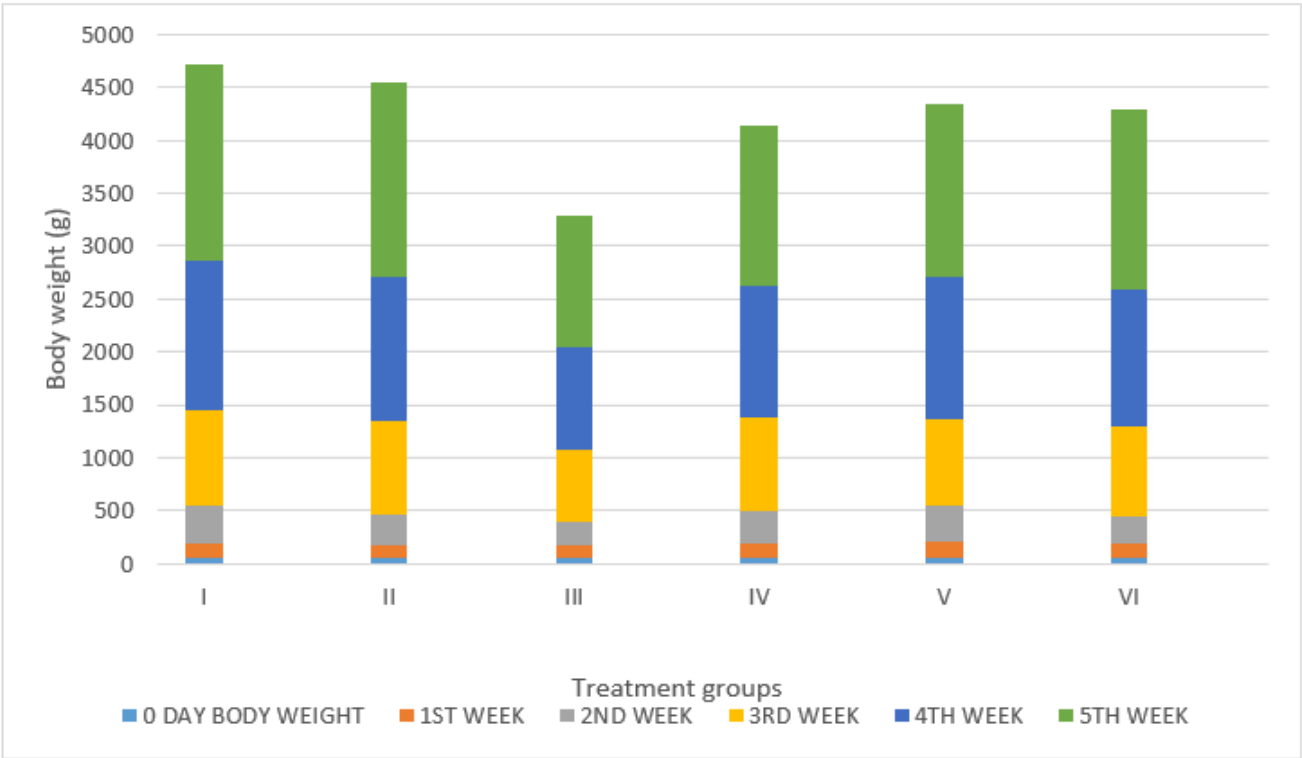


Figure 1

Effect of *A. vasica* on weekly body weight (g/bird/week, MEAN±S.E) in AFB1 fed broilers (n=36 with three replicates)

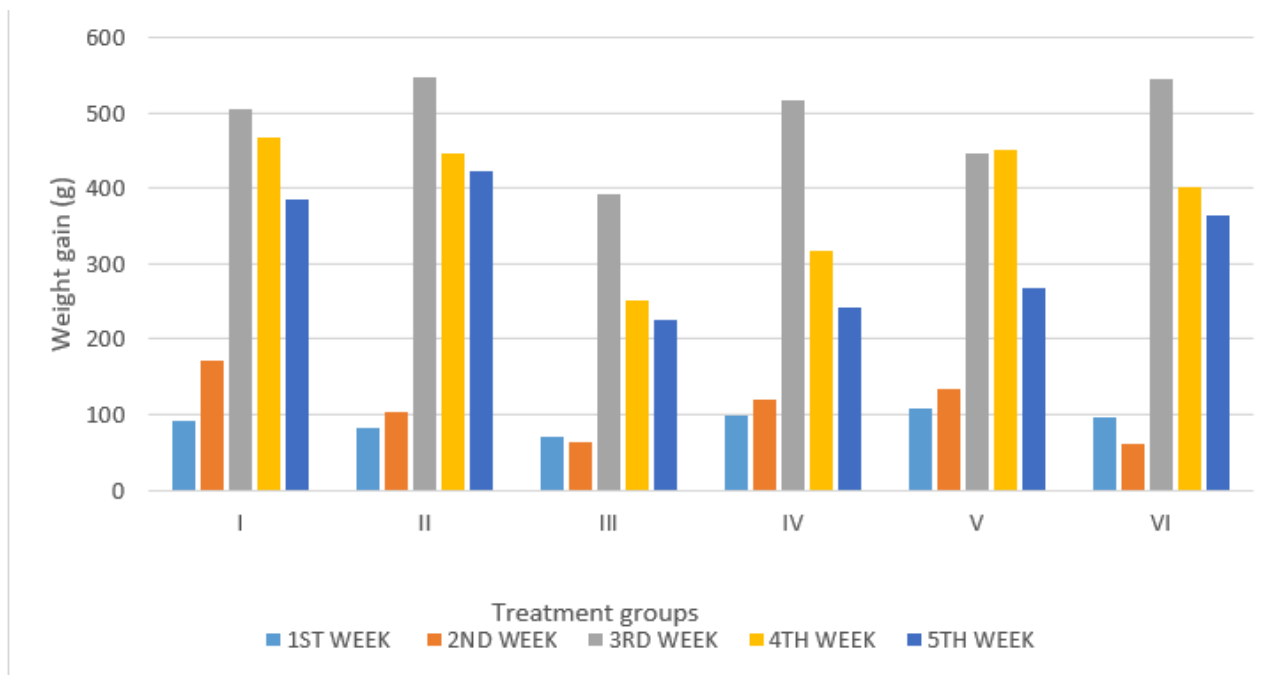


Figure 2

Effect of *A. Vasica* on weekly body weight gain (g/bird/week, Mean $\pm$ S.E) in AFB1 fed broilers (n=36 with three replicates)

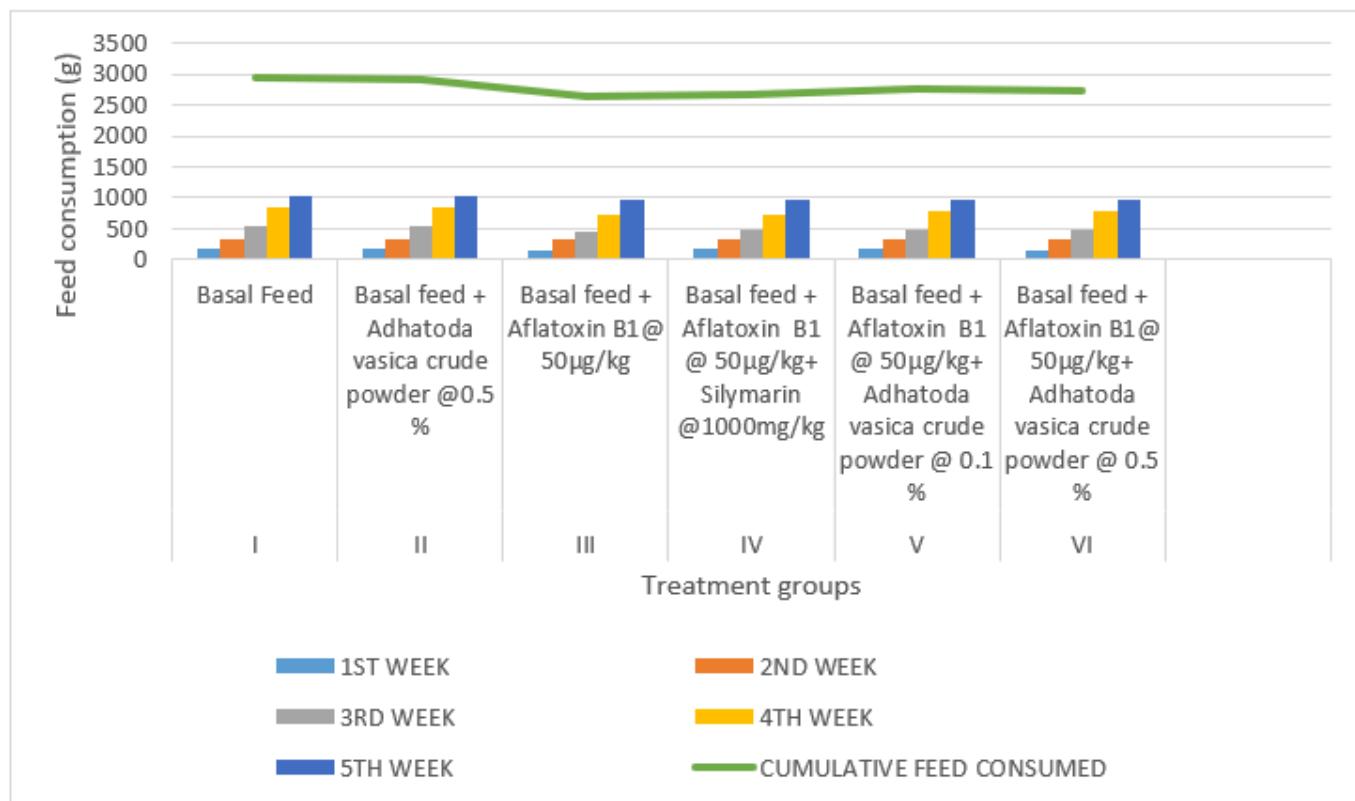


Figure 3

Effect of *A. Vasica* on weekly feed consumption (g/bird/week) and feed conversion efficiency (FCR) in AFB1 fed broilers (n=36 with three replicates)

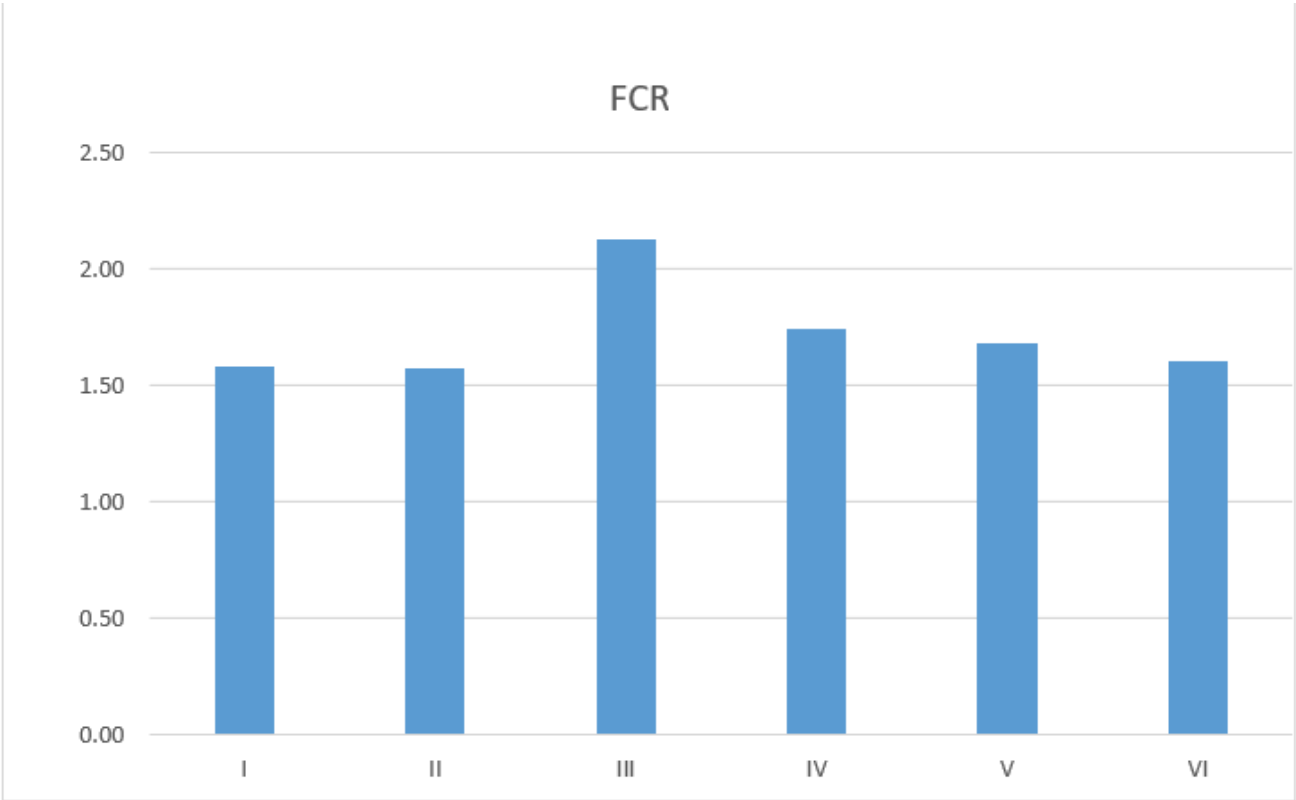


Figure 4

Effect of *A. Vasica* on feed conversion efficiency (FCR) in AFB1 fed broilers (n=36 with three replicates)

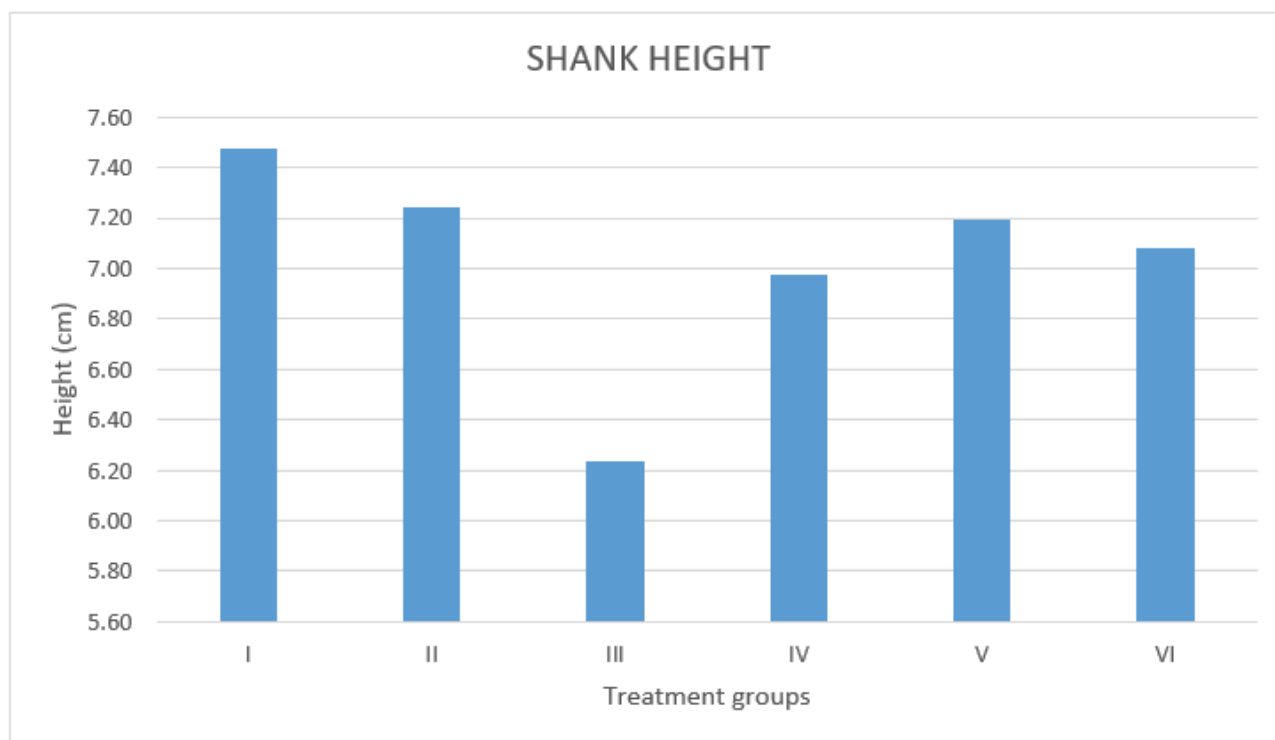


Figure 5

Effect of *A. Vasica* on shank height (cm) (MEAN±S.E) IN AFB1 AND OTA fed broilers (n=8 with three replicates)

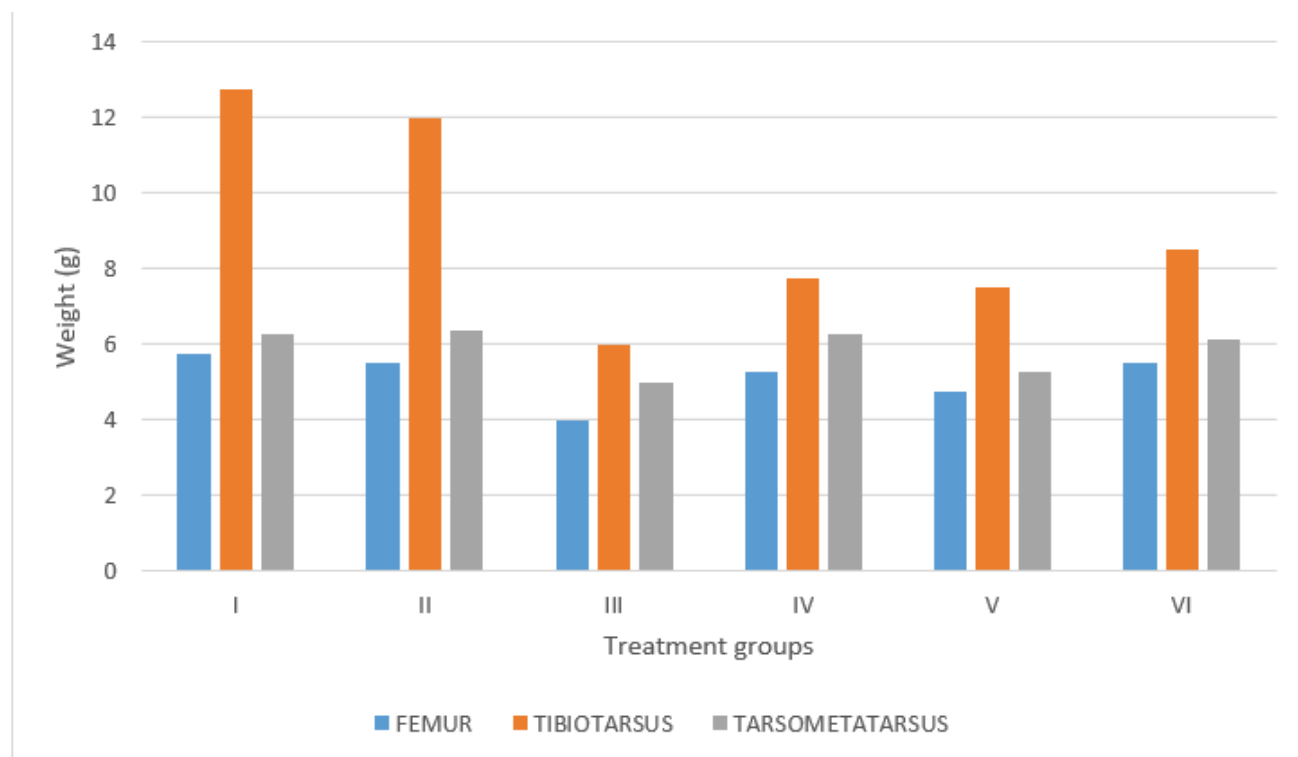


Figure 6

Effect of *A. Vasica* on bone weight (g) (Mean $\pm$ S.E) in AFB1 fed broilers (n=8 with three replicates)

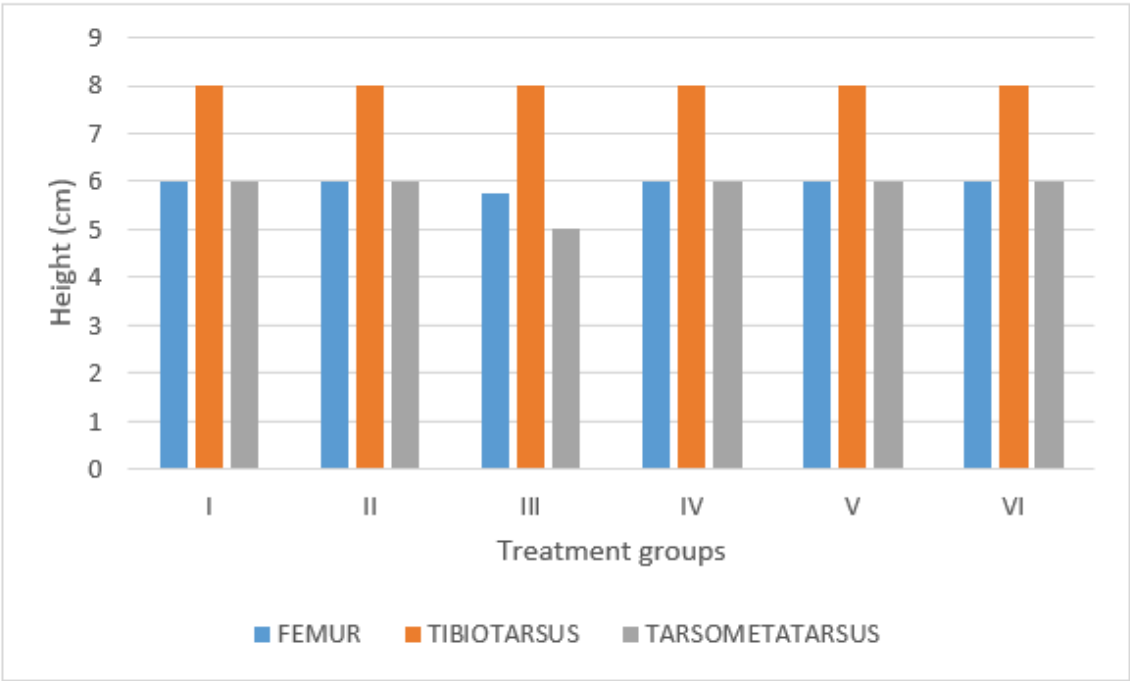


Figure 7

Effect of *A. Vasica* on bone height (cm) (Mean $\pm$ S.E) in AFB1 fed broilers (n=8 with three replicates)

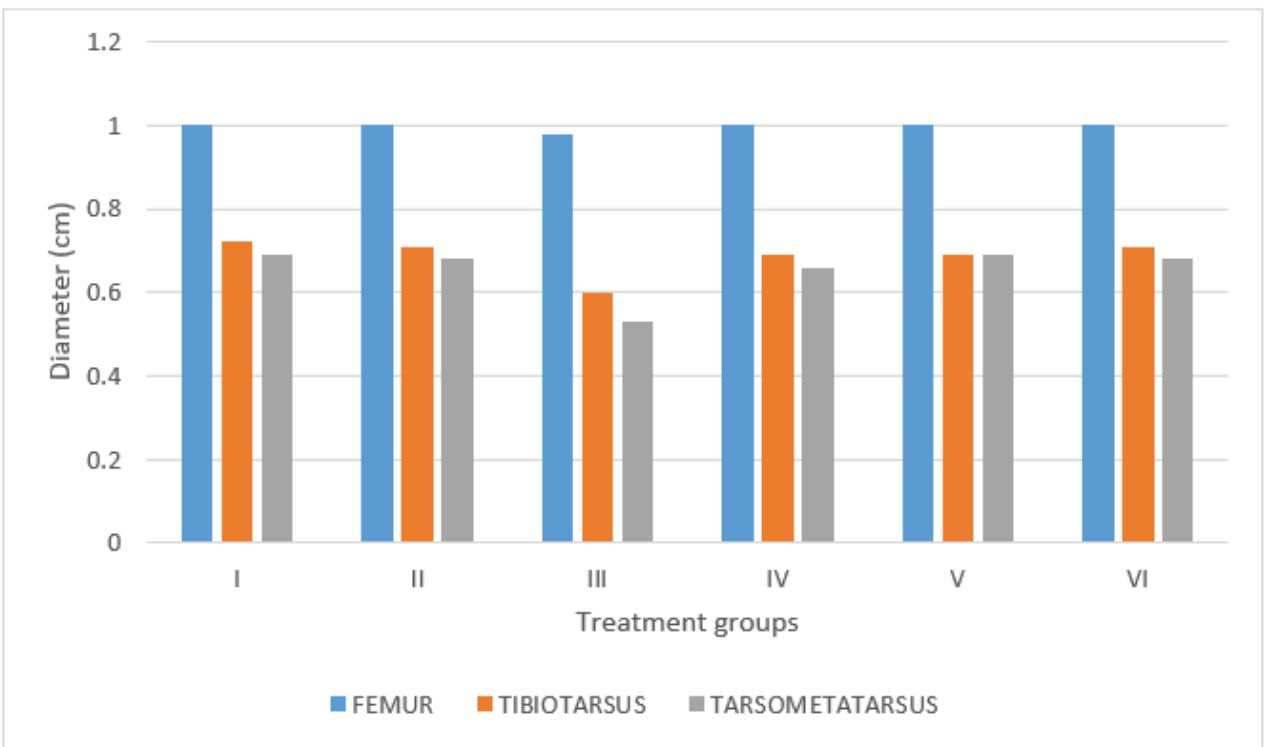


Figure 8

Effect of *A. Vasica* on bone diameter (cm) (Mean $\pm$ S.E) in AFB1 fed broilers (n=8 with three replicates)

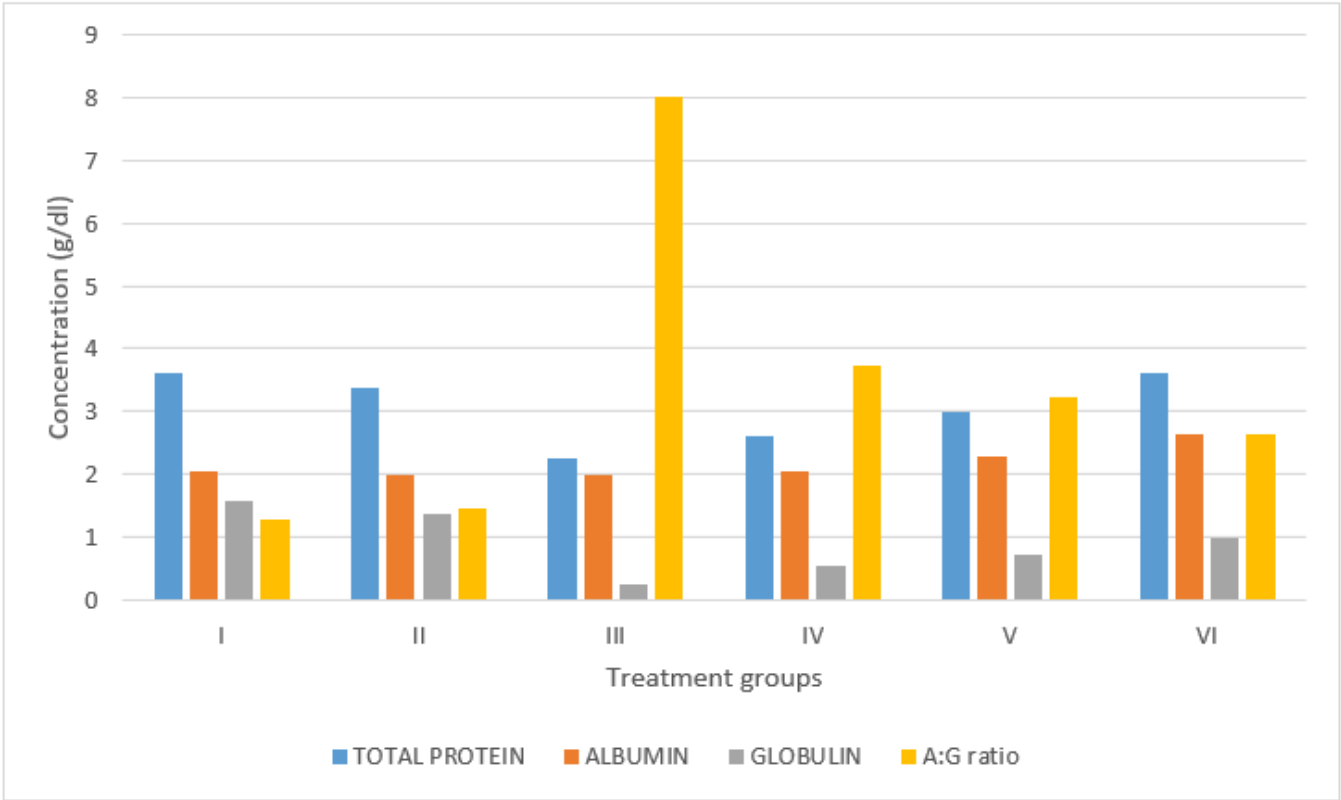


Figure 9

Effect of *A. Vasica* on total protein, albumin and globulin (g/dl) (Mean $\pm$ S.E) in AFB1 fed broilers (n=8 with three replicates)

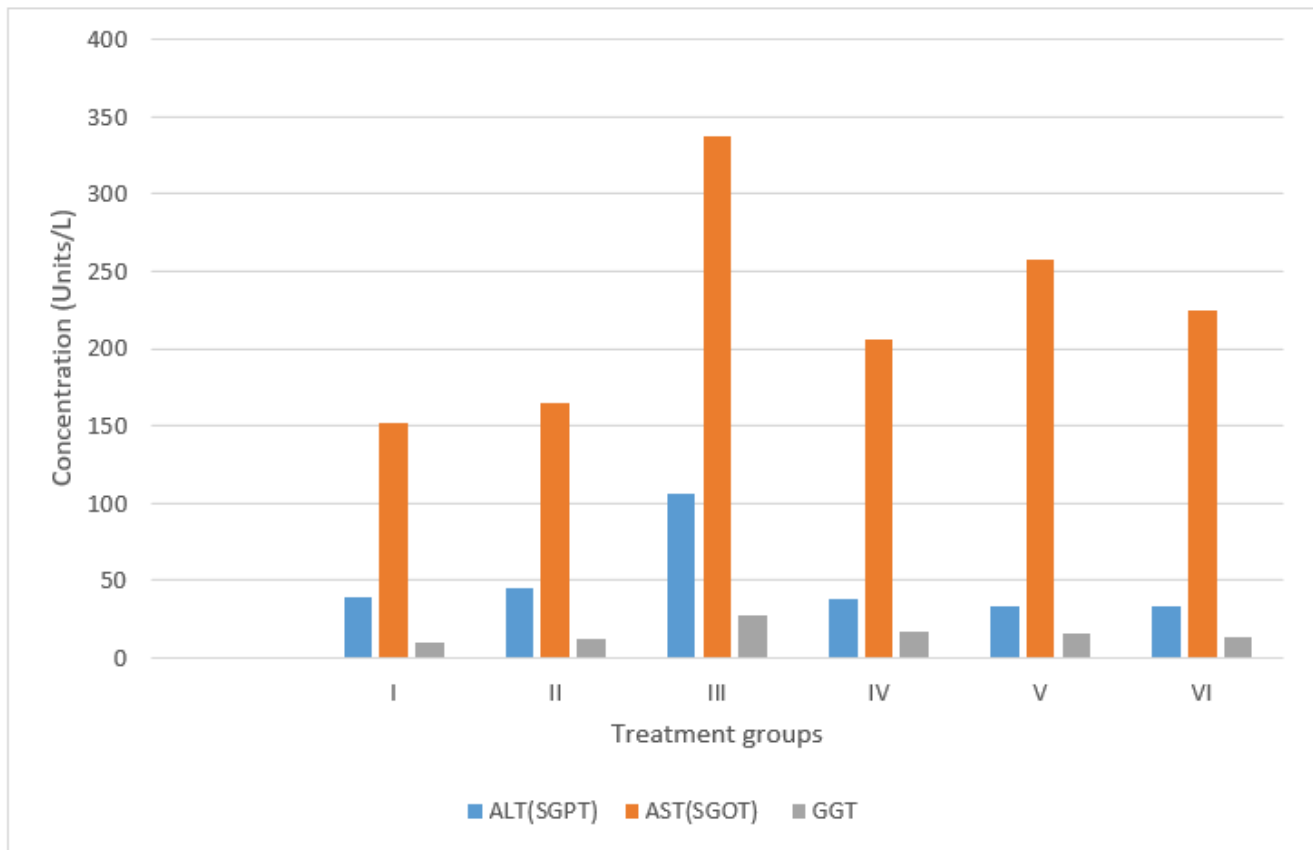


Figure 10

Effect of *A. Vasica* on ALT, AST AND GGT (U/L) (Mean $\pm$ S.E) in AFB1 fed broilers (n=8 with three replicates)

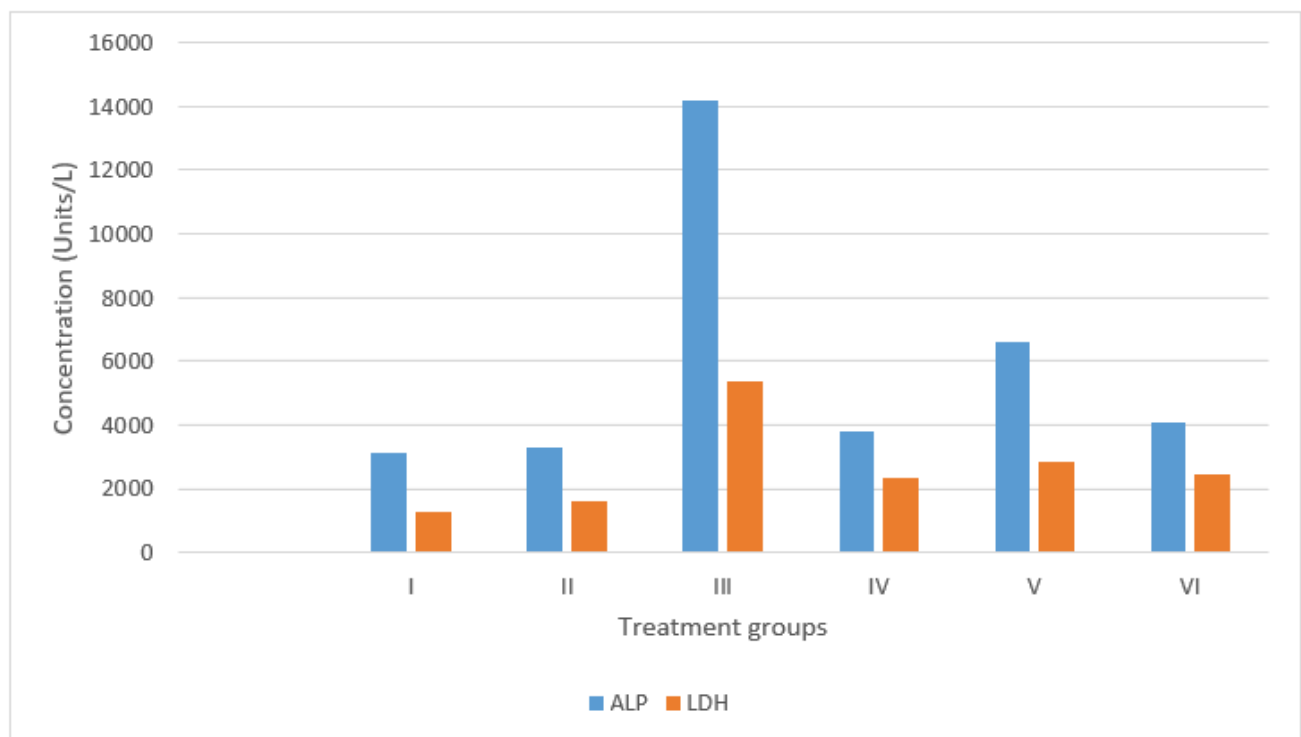


Figure 11

Effect of *A. Vasica* on ALP AND LDH (U/L) (Mean $\pm$ S.E) in AFB1 fed broilers (n=8 with three replicates)

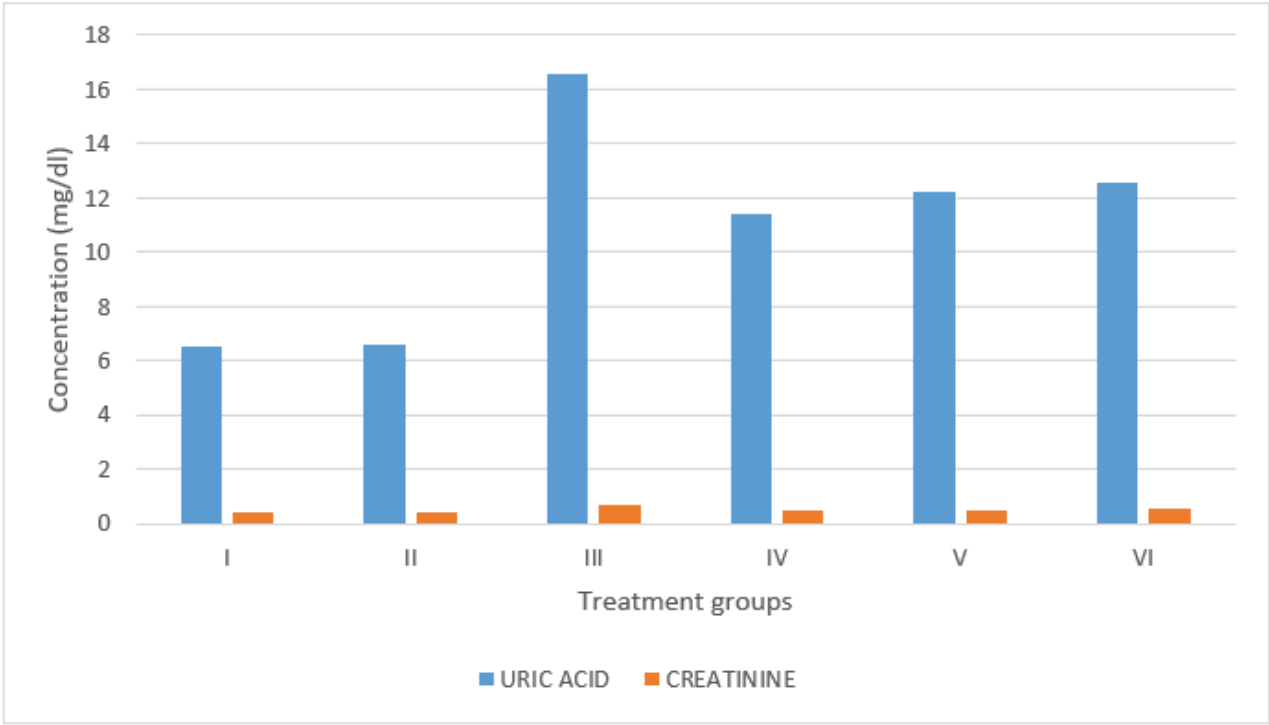


Figure 12

Effect of *A. Vasica* on serum uric acid and creatinine (Mean $\pm$ S.E) in AFB1 fed broilers (n=8 with three replicates)

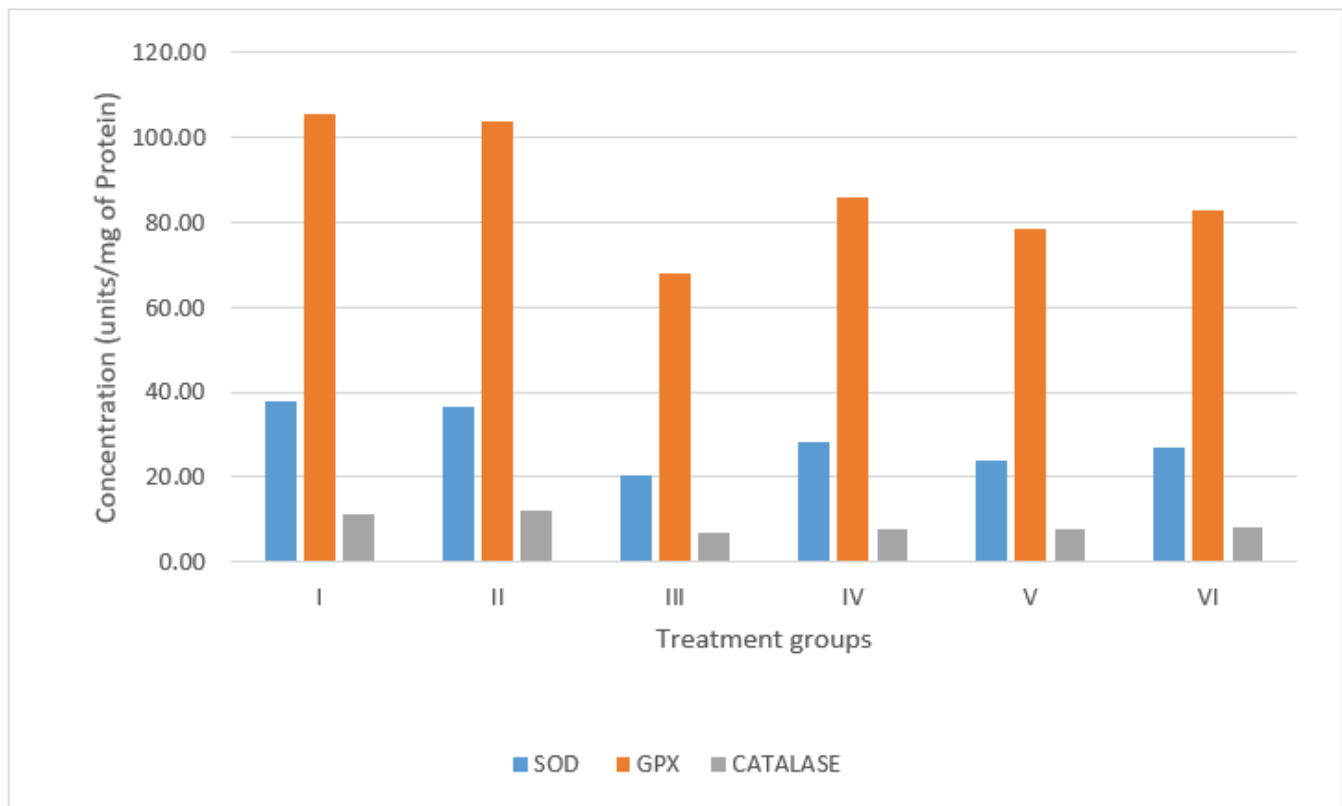


Figure 13

Effect of *A. Vasica* on antioxidant profile (mg/g of protein) (Mean $\pm$ S.E) in AFB1 fed broilers (n=8 with three replicates)

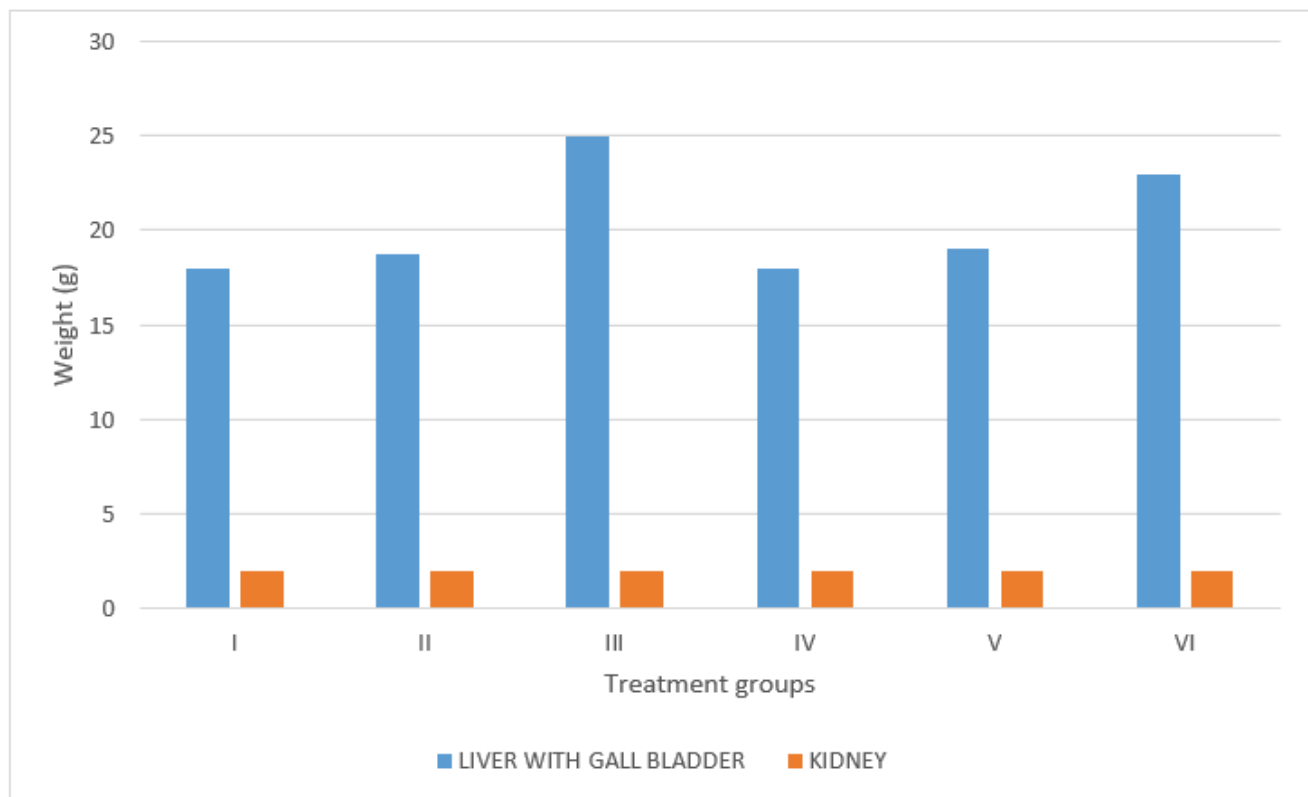


Figure 14

Effect of *A. Vasica* on organ weight (g) (Mean $\pm$ S.E) in AFB1 fed broilers (n=8 with three replicates)

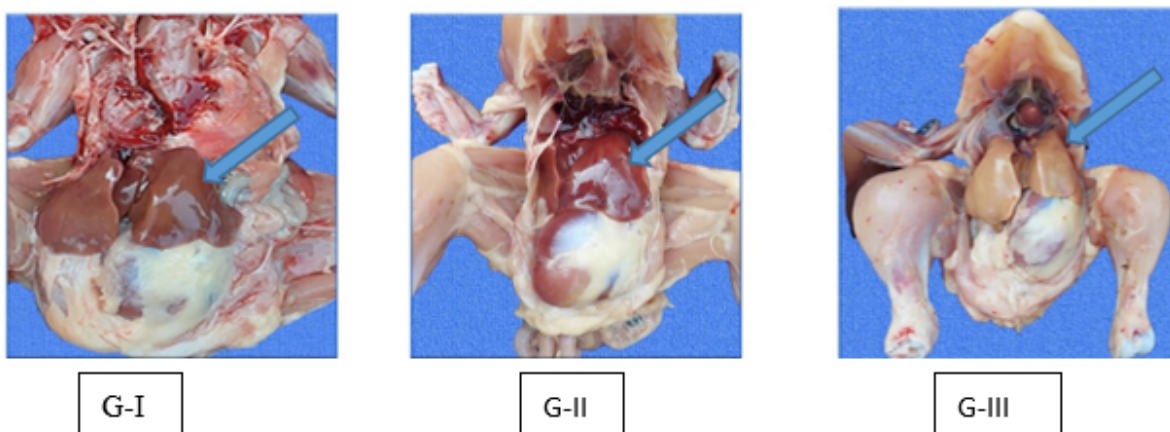


Figure 15

Effect of *A. Vasica* on gross pathology of liver in AFB1 fed broilers

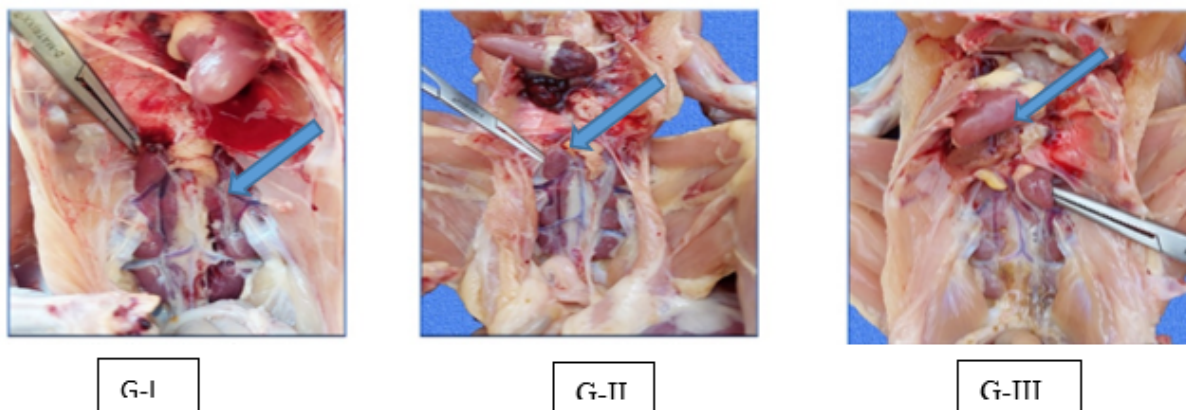


Figure 16

Effect of *A. Vasica* on gross pathology of kidney in AFB1 fed broilers

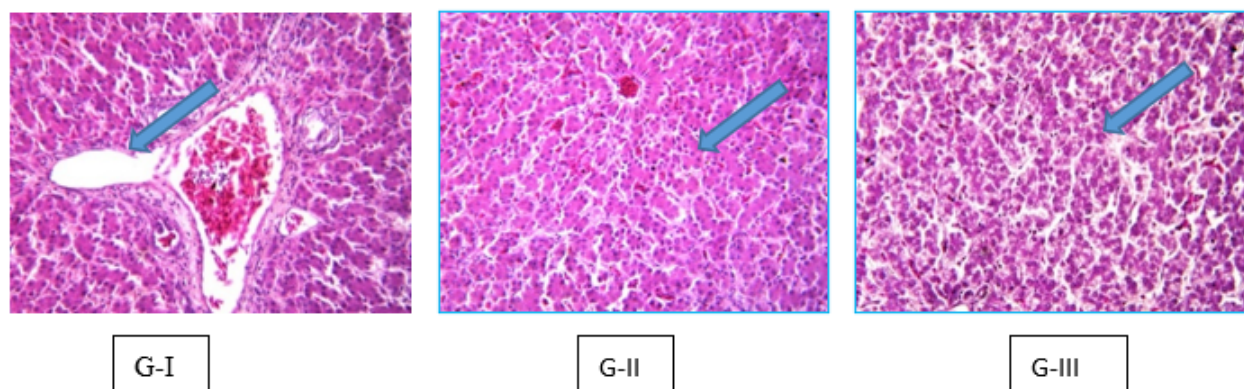


Figure 17

Effect of *A. Vasica* on histo pathology of liver in AFB1 fed broilers

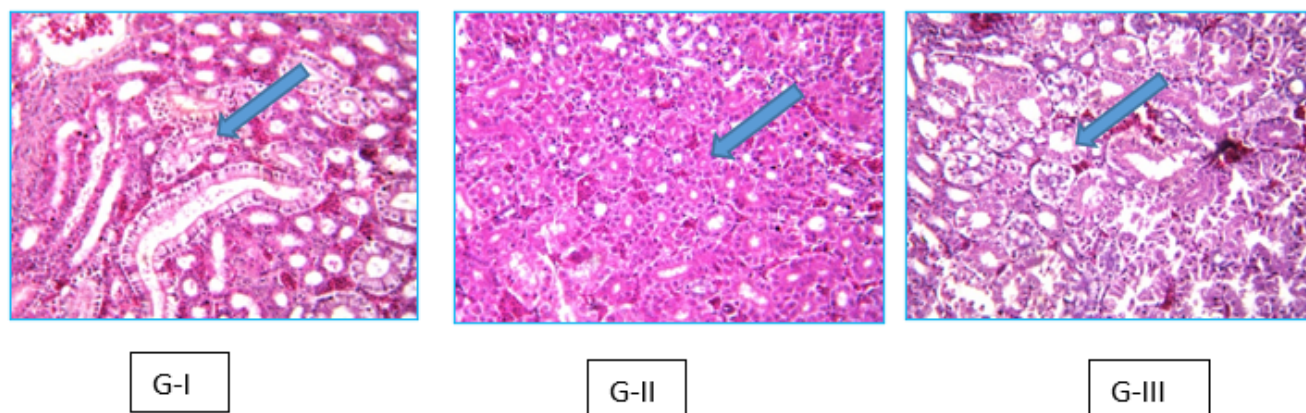


Figure 18

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [TableFinal.docx](#)