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## Ameliorative effects of *Phyllanthus niruri* on Haematological and Serum biochemical profile of Guinea fowls raised with aflatoxin contaminated feed

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**Abstract**

This study was conducted to investigate the effect of aflatoxin and protective role of *Phyllanthus niruri* herb against the effect of aflatoxin on haematological (Haemoglobin, Packed Cell volume and Differential count of blood cells) and serum biochemical profile (Total Protein, Serum albumin, Serum Cholesterol and Serum Uric acid) in Guinea fowls. A total of 270 day old keets were divided into six groups (45 chicks each) as following: The T1 Control (without Aflatoxin), T2 (diet with 1 ppm aflatoxin B1), T3 (diet with 2 ppm aflatoxin B1), T4 (diet with 1% herb powder alone), T5 (1 ppm aflatoxin B1 and 1% herb powder) and T6 (2 ppm aflatoxin B1 and 1% herb powder). The results revealed that haemoglobin content and lymphocyte count in 2 ppm aflatoxin B1 fed group were significantly ( $P < 0.05$ ) depressed and heterophils count was significantly ( $P < 0.05$ ) elevated than control at 12<sup>th</sup> week of age. Serum concentrations of total protein and albumin were significantly ( $P < 0.01$ ) depressed whereas uric acid level in serum was significantly ( $P < 0.01$ ) elevated than control at 12<sup>th</sup> week of age. Aflatoxin B1 (1 ppm) caused no significant changes in haematology and biochemical parameters at 6<sup>th</sup> and 12<sup>th</sup> weeks of age. Supplementation of 1 percent *Phyllanthus niruri* herb powder to toxin free diet caused no significant changes in haematological and biochemical parameters compared to control. Decreased level of haemoglobin content, lymphocyte count, serum total proteins and albumin due to aflatoxin B1 (2 ppm) feeding were numerically increased by the supplementation of *Phyllanthus niruri* herb powder but the improvement was statistically non-significant.

**Keywords:** Guinea fowl, Aflatoxin, *Phyllanthus niruri*, Haematological and Biochemical profile

**Introduction**

Guinea fowl (*Numida meleagris*) is the best alternative of poultry species for the poor rural people. It is known for its hardiness, adaptability and sporty meat flavour. The meat of Guinea fowl is superior to the meat of other poultry species as it has higher protein percentage, less carcass fat and more iron and vitamin [1]. Guinea fowl rearing is gaining importance in developing countries including India. Blood parameters (physiological norms) manifest the internal environment of the living body.

One of the most important problems in Indian conditions that occurs as a result of unconditioned storage of food and foodstuff is mycotoxins [2]. Mycotoxins are toxic metabolic by-products, which are produced by fungi. Naturally fungi on crops produce mycotoxins in the fields, during harvest and in storage. Contamination of food or feedstuffs and their consumption can result in mycotoxicosis in poultry. In these mycotoxins, aflatoxins are the mostly seen and aflatoxin B1 is the most harmful one [3, 4]. Synthesis of aflatoxins in feeds are increased at temperature above 27° C (80 F), humidity levels greater than 62%, and moisture levels in the feed above 14% [5]. All animal species are susceptible to aflatoxicosis, but Guinea fowls are less susceptible to the heat stress and aflatoxin [1].

Aflatoxicosis causes several defects in organs and tissues, decrease in growth rate, increase in death rate, immunosuppression, anaemia, and increase in coagulation time and deteriorates lipid, carbohydrate, and protein metabolism [2, 6]. As a result of toxic effect of aflatoxin, biochemical and haematological parameters have been reported to be changed importantly. In chronic and subclinical aflatoxicosis case, changes in biochemical and haematological parameters occur before clinical symptoms develop [7, 8].

Significant changes in serum biochemical and haematological parameters are seen in aflatoxicosis cases, and these can assist in the diagnosis of toxicities [9, 10]. Extensive research has been conducted to counter mycotoxicosis by nutritional, chemical, physical, or biological strategies [11, 12, 13]. In recent days, various herbs also focussed to counteract the ill effects caused by mycotoxins in poultry. The objective of this study was to evaluate the toxic effects of aflatoxin on haematological and serum biochemical parameters on Guinea fowls as well as to determine the ameliorative effects of *Phyllanthus niruri* herb powder on aflatoxin B1 in Guinea fowls.

## Materials and Methods

### Production of Aflatoxin

*Aspergillus parasiticus* NRRL 2999 strain procured from National Centre for Agricultural Utilization Research, Microbial Genomics and Bioprocessing Unit, 1815N University Street, Peiria, Illinois 61604, USA was used to produce aflatoxin. The fungus was maintained by sub culturing it on potato dextrose agar at 10 days interval and aflatoxin for experimental induction was produced on rice [14]. The aflatoxin content of cultured rice powder was quantified by using Thin Layer Chromatography [15] at Pharmacovigilance Laboratory for Animal Feed and Food Safety (PLAFFS), Madhavaram Milk Colony, Chennai.

### Preparation of experimental diet

The feed ingredients free of any toxins were only used for the preparation of experimental diets. Guinea fowl mash feed were procured from Central Feed Technology Unit (CFTU), Kattupakkam and analysed to ensure the absence of aflatoxin. Weighed amount of powdered culture material containing known amount of aflatoxin was added to the herb free control diet (T1) to prepare two more experimental diets containing aflatoxin at 1 (T2) and 2 ppm (T3) levels. One percent of Deoiled rice bran was replaced by *Phyllanthus niruri* herb powder to prepare T4 diet. Weighed amount of powdered culture material containing known amount of aflatoxin was added to T4 diet to prepare two more experimental diets containing both herb powder (1%) and aflatoxin at either 1 (T5) or 2 ppm (T6) level.

### Biological Experiment

A biological trial was conducted in the University Research Farm, Madhavaram Milk Colony, TANUVAS, Chennai with 270-day old keets purchased from SRS Hatcheries Ltd., from Palladam, Tamil Nadu. The experiment was conducted for a period of twelve weeks duration. The keets were reared under cage system and good management practices were followed during the experiment and were provided with respective treatment feed and water Ad Libidum throughout the experimental period. No vaccination or antibiotic administration was done during the experimental period.

### Experimental design

Two hundred and seventy keets were used in this experiment. The keets were wing banded, weighed individually and randomly distributed into six treatment groups with three replicates each consisting of 15 keets.

Experimental groups can be classified as follows:

T1 – Control diet

T2 - 1 ppm aflatoxin B1 added to control diet

T3 - 2 ppm aflatoxin B1 added to control diet

T4 – 1 percent *Phyllanthus niruri* herb powder added to control diet

T5 - 1 ppm Aflatoxin B1 plus 1 percent *Phyllanthus niruri* herb powder added to control diet

T6 - 2 ppm Aflatoxin B1 plus 1 percent *Phyllanthus niruri* herb powder added to control diet

### Collection of blood samples

Two millilitres of blood was collected from wing vein of six keets in each treatment at 6<sup>th</sup> and 12<sup>th</sup> weeks of age using a 2 ml syringe for estimation of haematological and serum biochemical parameters. A sample of 0.5 ml blood was taken in EDTA (Ethylene Diamine Tetra Acetic Acid) vacutainer tube and remaining blood was collected in serum collection vacutainer tubes and kept undisturbed for serum separation.

### Haematological parameters

Haematological studies included the determination of haemoglobin by Sahli's acid haematin method [16], PCV by Micro haematocrit method [17] and differential count by using Wright's stain [18].

### Serum biochemical parameters

Samples of blood collected in serum collection vacutainer tubes were allowed to clot and centrifuged at 2500 rpm for 5 min to separate the sera.

- Serum Total Proteins were estimated spectrophotometrically using Modified Biuret and Dumas method utilizing the kit supplied by Robotnik (India) Pvt. Ltd., Navi Mumbai, India.
- Serum albumin was estimated spectrophotometrically by using Bromocresol green method utilizing the kit supplied by Robotnik (India) Pvt. Ltd., Navi Mumbai, India.
- Serum total Cholesterol was estimated spectrophotometrically by Cholesterol dehydrogenase/ peroxidase (CHOD/ POD) method utilizing the kit supplied by Robotnik (India) Pvt. Ltd., Navi Mumbai, India.
- Serum uric acid was estimated spectrophotometrically method by using the kit supplied by Robotnik (India) Pvt. Ltd., Navi Mumbai, India.

### Statistical analysis

All the statistical analysis was performed by using SPSS software (version 20.0) as per [19]. The means were compared by One-way ANOVA for significant differences among treatments.

## Results

### Haematological parameters

The mean haematological values of Guinea fowl at sixth and twelfth week of age as influenced by feeding *Phyllanthus niruri* herb powder and graded levels of aflatoxin alone or in combination are presented in Table1. Statistical analysis of the data revealed significant ( $P \leq 0.05$ ) differences among different treatments in haemoglobin, heterophils and lymphocyte values at twelfth week of age. At sixth week of age, there were no significant differences existed between treatment groups for different parameters studied.

At twelfth week of age, haemoglobin values of birds fed with 2 ppm Aflatoxin B1 without or with 1 % *Phyllanthus niruri* herb powder was significantly ( $P \leq 0.05$ ) lower than that of control. The values of all the other treatment groups fell in

between and revealed no significant difference among themselves and with other groups. Heterophils count was significantly ( $P \leq 0.05$ ) higher in 2 ppm Aflatoxin B1 (2 ppm) fed groups compared to all the other treatment groups except 2 ppm aflatoxin B1 and 1% herb powder fed group which revealed an intermediary value. Lymphocyte count was

significantly ( $P \leq 0.05$ ) lower in 2 ppm aflatoxin B1 alone fed group compared to control, 1 ppm aflatoxin B1 and 1% herb powder alone fed groups which showed statistically similar values among themselves. Aflatoxin B1 (1 ppm and 2 ppm) fed groups had intermediary values which were statistically like all other groups.

**Table 1 :** Haematological values and differential count of Guinea fowls influenced by Aflatoxin B1 and *Phyllanthus niruri* herb powder supplementation in diet (n=6)

Treatment	Hb (g %)		PCV (%)		Heterophils (%)		Lymphocyte (%)		Monocyte (%)		Eosinophil (%)	
	6 <sup>th</sup> week	12 <sup>th</sup> week	6 <sup>th</sup> week	12 <sup>th</sup> week	6 <sup>th</sup> week	12 <sup>th</sup> week	6 <sup>th</sup> week	12 <sup>th</sup> week	6 <sup>th</sup> week	12 <sup>th</sup> week	6 <sup>th</sup> week	12 <sup>th</sup> week
T1 Control diet	11.17 ±0.477	10.50 <sup>a</sup> ±0.56	33.33 ±0.62	39.67 ±1.56	46.83 ±2.92	47.83 <sup>b</sup> ±2.46	47.83±2.44	46.83±2.54	3.50 ±0.56	3.33 ±0.61	1.83 ±0.48	2.00 ±0.26
T2 AFB1 (1 ppm)	11.00 ±0.26	9.50 <sup>ab</sup> ±0.43	34.33 ±0.33	38.83 ±1.92	47.67 ±1.23	48.83 <sup>b</sup> ±2.88	47.00±1.44	45.50±2.67	3.33 ±0.49	4.33 ±0.95	2.00 ±0.63	1.33 ±0.33
T3 AFB1 (2 ppm)	11.33 ±0.88	8.83 <sup>b</sup> ±0.31	36.17 ±1.28	36.83 ±2.66	47.17 ±1.28	56.67 <sup>a</sup> ±1.33	46.50±1.18	38.67±1.05	3.83 ±0.31	3.00 ±0.73	2.50 ±0.43	1.67 ±0.42
T4 Herb powder (1%)	11.50 ±0.56	9.83 <sup>ab</sup> ±0.31	35.67 ±0.72	39.67 ±1.33	47.83 ±2.37	48.50 <sup>b</sup> ±1.89	46.83±2.15	45.33±1.98	3.50 ±0.56	3.67 ±0.80	1.83 ±0.31	2.50 ±0.43
T5 Herb powder (1%) plus AFB1 (1 ppm)	10.83 ±0.65	9.48 <sup>ab</sup> ±0.31	35.67 ±1.15	40.17 ±1.28	50.00 ±1.79	50.33 <sup>b</sup> ±1.63	44.67±2.09	43.33±1.76	3.17 ±0.40	4.17 ±0.54	2.17 ±0.60	2.17 ±0.4
T6 Herb powder (1%) plus AFB1 (2 ppm)	11.10 ±0.52	9.00 <sup>b</sup> ±0.26	35.83 ±1.17	41.00 ±1.53	46.17 ±1.58	52.17 <sup>ab</sup> ±1.72	48.00±1.69	42.50±2.03	3.33 ±0.33	3.33 ±0.42	2.50 ±0.22	2.00 ±0.73
Overall mean	10.97 ±0.24	9.42 ±0.18	35.17 ±0.39	39.36 ±0.71	47.61 ±0.77	50.72 ±0.93	46.81±0.72	43.69 ±0.90	3.44 ±0.18	3.64 ±0.28	2.14 ±0.18	1.94 ±0.18
F Value	0.81 <sup>NS</sup>	3.12 <sup>*</sup>	1.36 <sup>NS</sup>	0.65 <sup>NS</sup>	0.45 <sup>NS</sup>	12.58 <sup>*</sup>	0.41 <sup>NS</sup>	2.76 <sup>*</sup>	0.25 <sup>NS</sup>	0.55 <sup>NS</sup>	0.43 <sup>NS</sup>	0.79 <sup>NS</sup>

<sup>NS</sup> - Non significant, <sup>\*</sup> - Significant ( $P \leq 0.05$ ).

Means bearing different superscripts within the column differ significantly ( $P \leq 0.05$ ).

### Serum biochemical parameters

The mean values of serum total proteins, albumin, uric acid and cholesterol levels in Guinea fowl estimated at the end of sixth and twelfth weeks of age as influenced by different dietary treatments are presented in Table 2. Serum total proteins and uric acid level at sixth and twelfth weeks of age revealed significant ( $P \leq 0.01$ ) differences among treatments; whereas serum albumin revealed significant ( $P \leq 0.01$ ) difference among treatments only at twelfth week of age. Serum cholesterol level was not altered by feeding either aflatoxin B1 or herb powder or in combination.

At sixth week of age, the serum total proteins level in control and herb powder alone fed groups were significantly ( $P \leq 0.05$ ) higher than herb powder plus aflatoxin B1 fed groups (1 ppm and 2 ppm), whereas 1 ppm aflatoxin B1 fed group

maintained a value in between herb powder plus aflatoxin fed groups (1 ppm and 2 ppm) and 2 ppm aflatoxin B1 alone fed group and statistically similar to both the treatments. Among all treatments, significantly ( $P \leq 0.05$ ) lowest serum total proteins level was noticed in 2 ppm aflatoxin B1 fed group.

At twelfth week of age, compared to control, significantly ( $P \leq 0.05$ ) lower serum total proteins level was observed in all other treatment group. The serum albumin level in 2 ppm aflatoxin B1 fed birds was significantly ( $P \leq 0.05$ ) lower than all other treatments at twelfth week of age. The birds fed herb powder alone had significantly ( $P \leq 0.05$ ) highest serum albumin level than all other treatment groups except control and 1 ppm aflatoxin plus herb powder fed group where it is non-significant.

**Table 2:** Serum biochemical values of Guinea fowls influenced by Aflatoxin B1 and *Phyllanthus niruri* herb powder supplementation in diet (n=6)

Treatment	Serum total protein (g/dL)		Serum albumin (g/dL)		Serum uric acid (mg/dL)		Serum cholesterol (mg/dL)	
	6 <sup>th</sup> week	12 <sup>th</sup> week	6 <sup>th</sup> week	12 <sup>th</sup> week	6 <sup>th</sup> week	12 <sup>th</sup> week	6 <sup>th</sup> week	12 <sup>th</sup> week
T1 Control diet	3.43 <sup>a</sup> ±0.19	4.02 <sup>a</sup> ±0.28	1.44±0.07	1.24 <sup>ab</sup> ±0.05	5.91 <sup>b</sup> ±0.24	5.09 <sup>bc</sup> ±0.5	144.02±9.52	116.63±4.67
T2 AFB1(1 ppm)	2.74 <sup>bc</sup> ±0.10	2.84 <sup>bc</sup> ±0.1	1.19±0.09	1.07 <sup>bc</sup> ±0.07	6.22 <sup>b</sup> ±0.37	5.62 <sup>b</sup> ±0.21	159.84±8.00	116.71±10.21
T3 AFB1(2 ppm)	2.34 <sup>c</sup> ±0.25	2.39 <sup>c</sup> ±0.14	1.39±0.19	1.03 <sup>c</sup> ±0.05	8.40 <sup>a</sup> ±0.77	6.97 <sup>a</sup> ±0.38	150.64±13.23	112.31±1.81
T4 Herb powder (1%)	3.62 <sup>a</sup> ±0.17	3.18 <sup>b</sup> ±0.15	1.43±0.11	1.33 <sup>a</sup> ±0.01	5.49 <sup>b</sup> ±0.28	4.38 <sup>c</sup> ±0.26	136.89±5.19	101.68±2.92
T5 Herb powder (1%) plus AFB1 (1 ppm)	2.90 <sup>b</sup> ±0.12	3.08 <sup>b</sup> ±0.11	1.46±0.15	1.16 <sup>abc</sup> ±0.04	6.87 <sup>b</sup> ±0.46	4.95 <sup>bc</sup> ±0.13	175.17±14.02	110.31±6.89
T6 Herb powder (1%) plus AFB1 (2 ppm)	2.94 <sup>b</sup> ±0.08	3.01 <sup>b</sup> ±0.11	1.65±0.23	1.09 <sup>bc</sup> ±0.08	8.27 <sup>a</sup> ±0.31	5.14 <sup>bc</sup> ±0.27	175.47±24.93	101.44±3.22
Overall mean	3.00±0.09	2.95±0.11	1.43±0.06	1.15±0.03	7.08±0.30	5.36±0.18	157.01±5.84	109.85±2.41
F Value	8.38 <sup>**</sup>	13.28 <sup>**</sup>	0.94 <sup>NS</sup>	4.51 <sup>**</sup>	12.84 <sup>**</sup>	7.90 <sup>**</sup>	1.32 <sup>NS</sup>	1.45 <sup>NS</sup>

<sup>NS</sup> - Nonsignificant, <sup>\*\*</sup> - Significant ( $P \leq 0.01$ ).

Means bearing different superscripts within the column differ significantly ( $P \leq 0.05$ ).

### Discussion

#### Haematological parameters

The results of this study revealed that the haemoglobin level

and lymphocyte count were significantly reduced and heterophils count at 12 weeks of age was significantly increased due to feeding of 2 ppm Aflatoxin B1 contaminated

feed in guinea fowls. The depression in haemoglobin level in aflatoxicosis was well in broilers [20], Japanese quails [21] and ducks [22]. The reduction in haemoglobin level might be due to depressing effect of aflatoxin on haematopoietic tissue and may be related to the inhibition of protein synthesis by aflatoxin.

Like the present study, the reduction in lymphocyte count at various concentration of dietary aflatoxin in various poultry species was reported by many researchers [9, 23]. The reduction of lymphocyte count may be due to the depletion of lymphoid cells in the bursa of Fabricius, thymus and spleen by aflatoxin. The increased heterophils in aflatoxin treated Guinea fowls found in the present study was reported in broilers [20, 23]. The increase in heterophils counts may be due to elicitation of inflammatory response by aflatoxin [24].

The supplementation of *Phyllanthus niruri* herb powder has not counteracted the deleterious effects of aflatoxin B1 (1 and 2 ppm) feeding in the present study. This was in correspondence with the findings of Sundaresan, 2007 [25]. The reduced lymphocyte and increased heterophils counts observed in this study were restored to normal with the supplementation of 1 percent *Phyllanthus niruri* herb powder in 2 ppm aflatoxin B1 contaminated feed at 12 weeks of age. The available literature on *Phyllanthus niruri* aflatoxin interactions in restoration of haematological parameters is scanty to make significant elucidations.

### Serum biochemical parameters

The present study revealed that aflatoxin B1 at 1 ppm level caused significant reduction in serum total proteins. But at 2 ppm level, significant reduction was observed in serum concentration of total proteins and uric acid at sixth and twelfth weeks of age and albumin at twelfth week of age. However, serum cholesterol concentration was not affected by aflatoxin B1 feeding in the diet of guinea fowls. The depression in serum total proteins during aflatoxicosis was well recognized by various authors at varying levels of aflatoxin in broilers [20, 29], Japanese quails [4, 33] and ducks [22, 26]. The reduction in serum total protein was attributed to be failure in digestion and absorption of proteins in gastrointestinal tract [27] and decline in protein synthesis by forming adduct with DNA, RNA and protein, inhibition of RNA synthesis and inhibition of DNA dependent RNA polymerase activity as well as due to degranulation of endoplasmic reticulum [28].

The reduction in serum albumin concentration at 2 ppm dietary aflatoxin B1 level was also reported in broilers [29]. The depressing effect of various levels of aflatoxin on serum albumin in broilers was reported by El-Ghany, 2013 [30]. The reduction in albumin level in toxin fed birds could be due to degradation of endoplasmic reticulum in hepatocytes and covalent binding of AF metabolites to template RNA causing inhibition of protein synthesis.

Significant elevation in serum uric acid level in broiler chicken fed 2.5 mg/ kg AFB1 contaminated feed [30]. On the contrary, in ducks, significant reduction in serum uric acid level by feeding lower doses of 0.5 and 0.8 mg/ kg aflatoxin B1 contaminated diet [31]. This significant increase in uric acid levels might be due to higher catabolism of protein during aflatoxin and renal tissue damage which in turn disrupts excretion of waste metabolites.

Similar to the present study, no change was observed in serum cholesterol concentration in broilers fed with 2.5 ppm level of aflatoxin from three to six weeks of age. In disparity to the

present report, several authors reported decreased serum cholesterol concentration due to aflatoxin in broilers [9, 32] and Japanese quails [33].

In the present study, all the serum biochemical parameters studied (total protein, albumin, cholesterol and uric acid) except serum total protein at twelfth week of age remained unaltered due to the inclusion of 1 percent *Phyllanthus niruri* herb powder in the aflatoxin free diet. However, the literature citations regarding the influence of *Phyllanthus niruri* herb powder alone on serum biochemistry in poultry are scanty.

The reduced concentrations of serum total proteins at six weeks and serum uric acid at twelve weeks of age due to aflatoxin feeding showed significant improvement when supplemented with herb powder. The decreased albumin and increased concentration of serum uric acid levels observed in this study due to 2 ppm aflatoxin feeding were partially restored by the supplementation of herb powder. Similar to the present findings, Sundaresan, 2007 [25] also reported significant improvement in serum total protein and uric acid levels after supplementing the aflatoxin treated birds with *Phyllanthus niruri* herb powder.

### Conclusion

In the present study, it is concluded that the dietary inclusion of 2 ppm Aflatoxin B1 caused significant reduction in haemoglobin, lymphocyte count, serum total protein, Albumin and Uric acid also caused increase in heterophils count. Feeding *Phyllanthus niruri* in toxin free diet showed no change in haematological and serum biochemical parameters. Supplementation of *Phyllanthus niruri* to 2 ppm aflatoxin B1 fed group restored the lymphocyte and heterophils count, serum total protein, albumin and uric acid to normal, but there is no improvement in haemoglobin level at 12<sup>th</sup> week of age.

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