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RS Khetmalis
PhD. Scholar, Division of Pathology, ICAR- IVRI, Izatngar, Bareilly, Uttar Pradesh, India

BK More
Department of Veterinary Pathology, K. N. P. College of Veterinary Science, Shirwal, Satara, Maharashtra, India

CS Mote
Department of Veterinary Pathology, K. N. P. College of Veterinary Science, Shirwal, Satara, Maharashtra, India

SN Jadhav
Department of Veterinary Biochemistry, K. N. P. College of Veterinary Science, Shirwal, Satara, Maharashtra, India

GN Aderao
PhD Scholar, Division of Animal Nutrition, ICAR- IVRI, Izatngar, Bareilly, Uttar Pradesh, India

Correspondence
RS Khetmalis
PhD. Scholar, Division of Pathology, ICAR- IVRI, Izatngar, Bareilly, Uttar Pradesh, India

Effect of induced aflatoxicosis on haemato-biochemical attributes in broilers and its amelioration by using *Emblica officinalis*

RS Khetmalis, BK More, CS Mote, SN Jadhav and GN Aderao

Abstract
An experiment was conducted to evaluate the effect of induced aflatoxicosis on haemato-biochemical attributes in broilers and its amelioration by using *Emblica officinalis*. Day old broiler chicks were divided into four groups of ten each and were fed on same basal feed (BF) from 8th day to 42nd day with some addition on feed. Chicks of group T0 were fed on BF only, group T1 was fed on BF with aflatoxin B1 (AFB1) @150 ppb, group T2 was fed on BF with AFB1 @150 ppb and *E. officinalis* @5 g/kg of feed and group T3 was fed on BF mixed with *E. officinalis* @5 g/kg of feed. Haemato-biochemical attributes on 42nd day revealed significant ($P<0.05$) reduction of Hb, TEC and TLC in T1 (8.52, 2.05 and 18.1) and T2 (8.73, 2.20 and 19.8) as compared to T0 (9.05, 2.37 and 21.2) and T3 (9.5, 2.55 and 22.8). AST and ALT of T1 (281, 17.7) was significantly ($P<0.05$) higher than T0, T2 and T3. Serum TP, albumin and globulin was significantly ($P<0.05$) lower in T1 (2.74, 1.45 and 1.29) than other groups. The serum cholesterol (mg/dL) and triglycerides (mg/dL) in T1 (78.4, 118) and T2 (28.0, 123) were significantly lower than T0 (109, 131) and T3 (120, 140). Supplementation of *E. officinalis* alone or with AFB1 in feed showed improvement in aflatoxin fed chicks. Therefore, it is concluded that supplementation of *E. officinalis* is effective for protection of AFB1 toxicity in broilers and could be used for prevention of harmful effects caused by aflatoxicosis in broilers.

Keywords: Aflatoxicosis, aflatoxinB1, *E. officinalis*, haemato-biochemical and broiler

Introduction
Aflatoxin (AF) is major mycotoxin produced by fungus *Aspergillus flavus* and *A. parasiticus* [1]. Mycotoxins are heat stable, low molecular weight secondary metabolites produced by fungi. Fungi responsible for toxin production are ubiquitous in naturethat grows on the different cereal crops responsible for contamination of human and animal foods [2]. These fungi affect crops viz., wheat, walnut, corn, cotton, peanut and tree nutsand its 20 ppb permissible limitrecommended by the U.S. Food and Drug administration for domestic foods [3]. Mycotoxins have several harmful biological effect like carcinogenesis, mutagenesis, malformations, hepatotoxicity, renal and skin toxicities and immunosuppression. Also, they are reported to have negative effects oncarbohydrates, lipid metabolism and protein, nucleic acid synthesis [4]. Aflatoxin B1 when ingested is metabolized in liver to AFB1- 8, 9-endo and exo-epoxide residues. These residues react with several macromolecules such as mitochondria, nuclear nucleic acids and nucleoproteins [5] leading to various toxicological, mutagenic and carcinogenic effects [6, 7]. Mycotoxin toxicity have been reported to produce oxidative stress due to generation of free radicals [8-10]. AFB1 @ 150 ppb produced hepatic parenchymal derangement, vacuolar cytoplasmic changes and cellular swelling with degenerative changes in a kidney of broilers [11].

In recent years herbal supplementation as phytogenic feed additives, immune protectant and growth enhancer is on rise in animal and poultry nutrition for prevention of various residual effect of chemical and drugs in chicken. Among these *E. officinalis* (Amla) is one of the most important and effective herbal supplement. It has been identified that amla has immune-modulatory role in poultry and shown to have several positive effects including protection against oxidative damages as it contain ascorbic acid, gallic acid and tannic acids [12] that have antioxidant effect [13]. The present experiment was planned to investigate the adverse effect of AFB1 @ 150 ppb/ kg feed and preventive role of dietary *E. officinalis* supplementation @ 5g/kg feed on haemato-biochemical attributes in broilers.

2. Materials and Methods

The experiment was conducted in department of veterinary pathology, KNP College of Veterinary Science, Shirwal, Satara in the month of June 2014 on broiler chicks to study the effect of aflatoxin B1 on haemato-biochemical alteration with its amelioration by feeding *Emblica officinalis.*

2.1 Aflatoxin B1 and *Emblica officinalis* (Amla) powder

The pure crystalline AFB1 was procured from HiMedia, India. AFB1 was dissolved in chloroform (1mg/10mL) with thorough mixing of solution in ground feed to obtain premix as suggested by [14, 15]. The premix feed was kept overnight at room temperature for evaporation of the solvent. Premix was then mixed with the basal diet to obtain desired level of 150 ppb AFB1/kg feed. *E. officinalis* powder was purchased from local market andwas mixed to obtain feed having 5g *E. officinalis /*kg feed

2.2 Diet and management of chicken

Forty day-old Ven-Cobb-400 broiler chicks were randomly distributedin four groups of ten birds in each. Standard managemental practices were followed during the experimental period. The diets of birds were free of contaminants that would interfere with the study objectives. The ration formulated was as per BIS- 2007 specification for broiler chicken.

2.3 Treatment of Chicks

Experimental design consist of four dietary treatments (1) control groupT0: birds were fed on a basal diet throughout experimental period; (2) group T1 birds were fed basal diet supplemented with AFB1 @150 ppb per kg of feed; (3) group T2 birds were fed basal diet supplemented with *E. officinalis* @5 g/kg feed +AFB1 @150 ppb/kg of feed and (4) group T3 birds were fed basal diet supplemented with *E. officinalis* @5 g/kg of feed. Feeding of respective diets was commenced from 8 day of age and was continued till the end of experiment.

2.4 Haematological attributes

Blood was collected in anticoagulant (EDTA) containing vials at the time of slaughter *i.e.* 42 day. Estimation of different haematological attributes viz. Haemoglobin (Hb), Packed Cell Volume (PCV), Total Leucocyte Count (TLC) and Total Erythrocyte Count (TEC) was done as per methods described by Sastry [16].

2.5 Serum biochemistry

Blood was collected at 42 day in plain vials and it was kept at room temperature for separation of serum. These serum samples were used for biochemical estimation of AST [17], ALP [17], total protein by Biuret method [17], ALT [18], albumin by BCG dye method [19], globulin by subtracting the serum albumin levels from that of serum total protein (TP) level, cholesterol by CHOD-PAP method [20] triglycerides by GPO-Trinder method and creatinine [21]. Analysis of these attributes was donewithin 24 hours of collection. Diagnostic kits used were of Erba Diagnostics, USA and Biochemical Auto analyzer used was Falcon 260.

2.6 Statistical Analysis

Data obtained from the experiment were subjected to one way analysis of variance (ANOVA) using the standard procedures of Snedecor and Cochran [22]. Significance between the treatment groups was declared at $P<0.05$.

3. Results

3.1 Serum Haematological Parameters

Hb levels (g/dL) were significantly higher ($P<0.05$) in T3 (9.5) as compared to T0 (9.05) T1 (8.52) and T2 (8.73) (Table 1). PCV showed non-significant difference among the treatment groups. TEC (10^6/µl) and TLC (10^3/µl) were low in T1 (2.05, 18.1) and T2 (2.20, 19.8) as compared to T0 (2.37, 21.2) and T3 (2.55, 22.8) respectively.

Table 1: Effect of *E. officinalis* supplementation on hematological attributes of birds fed with AFB1

SN	Attributes	T0	T1	T2	T3
1	Haemoglobin(g/dL)	9.05 ± 0.10^{b}	8.52 ± 0.30^{c}	8.73 ± 0.05^{c}	9.5 ± 0.12^{a}
2	PCV (%)	30.4±0.37	29.4±0.29	30.1±0.17	30.9±0.75
3	TEC(10^6/µl)	2.37 ± 0.04^{ab}	2.05 ± 0.06^{c}	2.20 ± 0.06^{bc}	2.55 ± 0.13^{a}
4	TLC(10^3/µl)	21.2 ± 0.33^{b}	18.1 ± 0.35^{d}	19.8 ± 0.12^{c}	22.8 ± 0.58^{a}

[a,b,c] means bearing different superscript differ significantly

3.2 Serum Biochemical Parameters

SGOT (AST) and SGPT (ALT) of T1 group were significantly ($P<0.05$) higher than T0, T2 and T3 (Table 2). ALP (mg/dL) of group T1 (530) was significantly ($P<0.05$) higher than T0 (462) and T3 (404) had normal range. Serum total protein, albumin and globulin was significantly ($P<0.05$) lower in T1 (2.74, 1.45 and 1.29) as compared to other groups. Serum creatinine (mg/dL) was significantly higher in T1 (0.65) as compared to T3 (0.50) and was similar with T0 and T2. The serum cholesterol (mg/dL) and triglycerides (mg/dL) in T1 (78.4, 118) and T2 (28.0, 123) were significantly lower than T0 (109, 131) and T3 (120, 140).

Table 2: Effect of *E.officinalis* supplementation on biochemical attributes of birds fed with AFB1

Attributes	T0	T1	T2	T3
SGOT/AST (IU/dL)	186 ± 2.25^{c}	281 ± 2.56^{a}	272 ± 0.96^{b}	181 ± 2.71^{c}
SGPT/ALT (IU/dL)	$8.86\pm0.51b^{c}$	17.7 ± 1.09^{a}	10.8 ± 0.84^{b}	6.54 ± 0.71^{c}
ALP (IU/dl)	462 ± 4.54^{b}	530 ± 6.10^{a}	518 ± 2.94^{a}	404 ± 4.59^{c}
Total Protein (g/dL)	3.73 ± 4.43^{b}	2.74 ± 3.37^{d}	3.18 ± 2.34^{c}	4.71 ± 1.38^{a}
Albumin (g/dL)	1.58 ± 3.94^{b}	1.45 ± 2.67^{d}	1.50 ± 2.45^{c}	1.99 ± 0.97^{a}
Globulin (g/dL)	2.15 ± 6.91^{b}	1.29 ± 1.64^{d}	1.68 ± 2.86^{c}	2.71 ± 2.63^{a}
Creatinine (mg/dL)	0.57 ± 0.03^{ab}	0.65 ± 0.02^{a}	0.60 ± 0.02^{ab}	0.50 ± 0.05^{b}
Triglycerides (mg/dL)	109 ± 3.34^{b}	78.4 ± 3.85^{c}	82.0 ± 1.22^{c}	120 ± 3.73^{a}
Cholesterol (mg/dL)	131 ± 3.04^{b}	118 ± 0.97^{c}	123 ± 0.66^{c}	140 ± 0.70^{a}

[a, b, c, d]Means bearing different superscript differ significantly ($P<0.05$)

4. Discussion

Reduction of Hb, TEC and TLC inT1were in agreement with Sakhare *et al*. [23], Sawarkar *et al*. [24] and Rathod *et al*. [25]. Non- significant alteration of PCV in T1 group was found similar to the result of Sakhare *et al.* [23] and Sawarkar *et al*. [24]. Improvement of Hb, TEC and TLC were seen in *E. officinalis* fed group. Haematological parameters decreased in T1due to many factors that includes inhibition of protein synthesis [26], decrease of the total iron binding capacity [27] caused by hepatotoxic effect of AFB1.

Increased serum AST and ALT values in birds fed only AFB1 containing diet were similar to the results reported by Tessari *et al*. [28], Motawe *et al*. [29], Fani *et al*. [30] and Kumar *et al*. [31]. Increase in serum levels of AST can be attributed to liver damage caused by AFB1 [28, 32]. Similar result of increased ALT values in birds fed AFB1was observed by Fapohunda *et al*. [33] that may be due to leakage of enzyme into extra cellular fluid caused by altered endothelial permeability. Benjamin [19] and Ozer *et al*. [34] reported that the highest concentrations of ALT in damaged liver cells therefore, it can be termed as liver-specific alteration seen as hepatotoxic liver cell damage and fatty changes. An increase in serum ALP in AFB1 fed broilers was similar to result of Fapohunda *et al*. [33]. Benjamin [19] reported that elevation of ALP in hepato-biliary disease due to increase pressure within the biliary system and hepatotoxic liver necrosis and fatty changes. AST and ALT values were lower in T0, T3 and T2 as compared to T1. Thisindicated that feeding of *E. officinalis*toAFB1 fed and to apparently healthy broilers improved their liver health by ameliorating the harmful effects of AFB1on liver and kidney.

The serum total protein, albumin and globulin were significantly reduced in T1fed birdswas in similar pattern as reported by, Tessari *et al*. [28] Khatke *et al*. [35], Manegar *et al*. [36] and Wade *et al*. [37]. Reduction of total protein, albumin and globulin in T1 were due to impairment of amino acid transport, mRNA transcription and thereby inhibiting DNA and protein synthesis [38]. Also, it may be attributed to liver damage caused by AFB1 thereby decreasing the synthesis of plasma proteins and secretory capacity of liver [39]. AFB1 fed bird's revealed liver damage as noticed through gross and histopathological examination [11]. Supplementation of *E. officinalis* with AFB1 showed improvement in protein levels as compared to only AFB1 fed birds. There were increase of serum total proteins, albumin and globulins in only *E. officinalis*fed birds which may be attributed to its hepato-protective activity [40], similar results were reported by Gatne *et al*. [41] and Kumar *et al*. [42]. Moderate increase in the creatinine levels of AFB1 fed birds were in agreement with Motawe *et al*. [29] and Valchev *et al*. [43]. who reported that creatinine increaseddue to degenerative and necrotic changes in renal tubular epithelium. Cellular swellingwith degenerative changes was observed in the kidneys of AFB1 fed birds [11].

The reduction of serum cholesterol and triglycerides levels in AFB1 fed birds were in accordance with Raju and Devegowda [44] and Rathod *et al*. [25] reflected impaired liver metabolism, leading to reduced synthesis of cholesterol and triglyceride. The improvement in the serum total cholesterol and triglycerides in *E. officinalis* fed birds might be due to hepato-protective role and increased feed intake [41, 42, 45].

5. Conclusion

It was concluded that *E. officinalis* (Amla) feeding in broilers have beneficial effect on haemato-biochemical attributes in aflatoxin fed broilersthatcan be used in poultry as feed supplement forimproving the health status and reducing adverse effect of natural aflatoxincontamination in feed. The beneficial effect of *E. officinalis* could be attributed to the presence of antioxidant and hepato-protective properties paving way of its use towards better performance and health.

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Conflict of interest

Authors have no conflict of interest.

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