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Effect of *Eclipta prostrata* (L.) leaf powder on serum biochemical parameters of broiler chicken during aflatoxicosis

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Abstract
The study was aimed to investigate the protective effect of *Eclipta prostrata* leaf powder on experimentally induced aflatoxicosis in broiler chicken. Sixty Cobb400 day old broiler chicks were randomly divided into six groups comprising 10 birds in each group. Aflatoxicosis was experimentally induced in all groups except T_1 and T_3 by giving 500 ppb of aflatoxin B_1 (AFB_1) contaminated maize from eighth day of age onwards. The group T_1 was kept as normal control and T_2 as toxic control. T_3 was fed with *E. prostata* leaf powder at 0.2 per cent level. The leaf powder of *E. prostrata* was given to T_4, T_5 and T_6 at dose rates of 0.05, 0.1 and 0.2 per cent respectively. Serum was separated on days 7, 21 and 42 and used for the estimation of aspartate transaminase (AST), creatine kinase (CK), cholesterol (CL) and total proteins (TP). In T_2 group, there was significant increase in the AST and decrease in the CL and TP. Treatment with *E. prostrata* leaves powder revealed protection in dose dependent manner which is indicated by significant (P<0.05) reduction in the level of serum AST and increase in the level of cholesterol and total protein.

Keywords: Aflatoxicosis, broiler chicken, biochemical parameters, *Eclipta prostrata*

Introduction
Aflatoxicosis is a major devastating problem affecting poultry to a greater extend which imposes a huge economic burden upon the livestock farmers. Aflatoxins are secondary metabolites produced by the fungi *Aspergillus flavus* and *A. parasiticus.* Aflatoxins B_1, B_2, G_1, G_2, M_1, M_2 are the different types of aflatoxin and are differentiated by their fluorescence under ultraviolet light. Among these, aflatoxin B_1 is considered to be the most toxic and common contaminant in feed. It gets metabolised in liver by cytochrome P450 3A4 enzyme and gets converted to the toxic product aflatoxin B_1 - 8, 9 - epoxide. This metabolite intercalates with DNA forming DNA adducts which can be carcinogenic. Aflatoxin B_1 has been proven to be hepatotoxic, carcinogenic, mutagenic and to cause immunosuppression as well as oxidative stress in animals.

Aflatoxicosis occurs mainly through feed. About 25 per cent of grains and legumes, that form the vital sources of poultry feed, are estimated to be contaminated with mycotoxins. As per Biomin mycotoxin survey 2017, in South Asia, 81 per cent of feed samples were contaminated with aflatoxin and 64 per cent of samples had aflatoxin above threshold level.

In broiler chicken, the aflatoxin affects liver, kidney, gut morphology, spleen and thymus which lead to reduction in production performances as well as alteration in biochemical parameters. It also causes bruising of carcasses leading to discarding of poultry meat. The aflatoxicosis causes mortality either directly or by lowering the immunity against several other infectious diseases such as Newcastle disease and Infectious Bursal disease.

Since the globe is spanning towards organic livestock production, the use of the therapeutic potentials of plants and plant derived products to curb the toxic effects of aflatoxins is a widely accepted concept.

Eclipta prostrata (L.) previously called as *E. alba* (known as Kayyonni in Malayalam) belonging to Asteraceae family is reported to have many medicinal properties Perusal of literature revealed few reports on the protective effect of *E. prostrata* against aflatoxicosis in broilers. Hence the study was designed with the following objective of protective effect of *Eclipta prostrata* (L.) leaf powder on altered biochemical parameters during aflatoxicosis in broiler chicken

Materials and Methods

Aflatoxin was produced in maize using the culture *Aspergillus flavus* NRRL 6513 as per the method of the maize culture powder yielded 143.48 ppm of aflatoxin. This mouldy maize was incorporated in experimental feed to arrive 500 ppb of aflatoxin.

The fresh plants of *Eclipta prostrata* were procured locally from Thrissur district of Kerala and were authenticated by Botanical Survey of India (BSI), Coimbatore. The voucher specimen was deposited at the Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Mannuthy. The leaves were collected; shade dried and pulverized using an electrical pulveriser. The powdered leaves powder was stored in air tight container at room temperature.

Sixty Cobb400 day old broiler chicks weighing 50 ± 5 g were randomly divided into six groups comprising 10 birds in each group. The birds were maintained under deep litter system and provided with *ad libitum* water and feed throughout the experimental period. All the birds were vaccinated as per the standard schedule. Aflatoxicosis was experimentally induced in all groups except T_1 and T_3 by giving 500 ppb of aflatoxin B_1 (AFB_1) from eighth day of age onwards. The group T_1 was kept as normal control and T_2 as toxic control. T_3 was fed with *E. prostata* leaf powder at 0.2 per cent level. The leaf powder of *E. prostrata* was given to T_4, T_5 and T_6 at dose rates of 0.05, 0.1 and 0.2 per cent respectively.

The blood was collected from the wing vein on days 7, 21 and 42. Serum was separated and used for the estimation of biochemical parameters such as aspartate transaminase (AST), creatine kinase (CK), cholesterol and total proteins using ready to use diagnostic kits procured from Agappe Diagnostics Ltd, Ernakulam, Kerala.

Results

Aspartate transaminase (AST)

The mean along with standard error values of AST of all the treated groups are presented in the table 1. The mean AST levels (IU/L) recorded on day seven for groups I to VI were 182.780 ± 1.5678, 184.210 ± 2.4643, 190.870 ± 3.9579, 183.020 ± 3.3368, 184.860 ± 10.3977 and 184.320 ± 4.7271 IU/L respectively. There was no significant difference in the AST level values between the groups on day seven.

On day 21, AST (IU/L) values increased significantly (P<0.05) in all the groups with the mean value of 198.570± 4.6652, 250.940± 8.8185, 204.540± 6.1106, 259.160± 10.0621, 235.230± 10.9476 and 226.740± 5.3340 IU/L respectively. There was no significant difference noted between T_2 and T_4. In the T_6, there was significant (P<0.05) reduction in the AST values compared with T_2, T_4 and T_5 though AST value couldn't reach those of the normal group.

On day 42, the mean AST levels (IU/L) values for T_1 to T_6 were 232.180± 6.5298, 306.150± 18.2904, 228.600± 10.9456, 287.400± 8.4071, 279.670± 12.0226 and 264.960± 8.7702 IU/L respectively. There was significant (P<0.001) decrease in the AST level in the T_4 to that of toxic group T_2 which was not to that level of normal control. There was no significant difference between T_2, T_4 and T_5.

There was significant increase in the AST values in all the groups when compared between the days 7, 21 and 42 with the mean value of 182.78 ± 1.56, 98.57± 4.66, 232.18± 6.52 IU/L in T_1, 184.21± 2.46, 250.94± 8.81, 306.15± 18.29 IU/L in T_2, 190.87± 3.95, 204.54 ± 6.11, 228.60 ± 10.94 IU/L in T_3, 183.02± 3.33, 259.16± 10.06, 287.40 ± 8.40 IU/L in T_4, 184.86 ± 10.39, 235.23 ± 10.94, 279.67± 12.02 IU/L in T_5, 184.30 ± 4.72, 226.74± 5.33, 264.96 ± 8.77 IU/L in T_6 respectively.

Table 1: Effect of *E. prostrata* leaves on serum aspartate transaminase (IU/L) in birds fed with aflatoxin incorporated feed (Mean ± SE, n=10)

Groups	7th day	21st day	42nd day	F-value
T_1	$182.78^{C} \pm 1.56$	$198.57^{dB} \pm 4.66$	$232.18^{cA} \pm 6.52$	28.750**
T_2	$184.21^{C} \pm 2.46$	$250.94^{aB} \pm 8.81$	$306.15^{aA} \pm 18.29$	37.183**
T_3	$190.87^{C} \pm 3.95$	$204.54^{cdB} \pm 6.11$	$228.60^{cA} \pm 10.94$	10.021**
T_4	$183.02^{C} \pm 3.33$	$259.16^{aB} \pm 10.06$	$287.40^{abA} \pm 8.40$	84.860**
T_5	$184.86^{C} \pm 10.39$	$235.23^{abB} \pm 10.94$	$279.67^{abA} \pm 12.02$	32.067**
T_6	$184.30^{C} \pm 4.72$	$226.74^{bcB} \pm 5.33$	$264.96^{bA} \pm 8.77$	31.765**
F value	0.321^{ns}	9.189**	7.298**	

Means bearing small letter as superscript differ significantly within a columns (P<0.05)
Means bearing capital letter as superscript differ significantly within a row (P<0.05)
ns- non-significant (p>0.05); * significant at 0.05 level; ** significant at 0.01 level

Creatine kinase (CK)

The mean± standard error values of CK of all the groups are presented in the table 2. The mean CK levels (IU/L) recorded on day seven were similar for T_1 to T_6 and the values were 988.180± 7.2152, 1011.880± 67.7460, 977.470± 11.8941, 993.540± 22.0294, 948.780± 6.0090, 968.920± 6.6992 IU/L respectively.

On day 21, the CK values were 1139.440± 13.8982, 1231.620± 36.0876, 1149.430± 33.8631, 1219.360± 38.5540, 1025.120± 86.1844, and 1119.160± 76.1165 IU/L for T_1 to T_6 respectively.

On day 42, the CK values for T_1 to T_6 were 1259.270± 10.7505, 1462.240± 101.2173, 1272.260± 51.7020, 1273.080± 35.0010, 1302.050± 34.6997, and 1286.240± 25.8106 IU/L respectively. There was no significant difference noted in the CK between the treated groups on day seven, 21 and 42.

There was significant increase in the CK values within the groups on the days 7, 21 and 42 with the mean value of 988.18± 7.21, 1139.44± 13.89, 1259.27± 10.75 IU/L inT_1, 1011.88± 67.74, 1231.62± 36.08, 1462.24± 101.21 IU/L in T_2, 977.47± 11.89, 1149.43± 33.86, 1272.26± 51.70 IU/L in T_3, 993.54± 22.02, 1219.36± 38.55, 1273.08± 35.00 IU/L in T_4, 948.78± 6.00, 1025.12± 86.18, 1302.05± 34.69 IU/L in T_5, 968.92± 6.69, 1119.16± 76.11, 1286.24± 25.81 IU/L in T_6 respectively.

Table 2: Effect of *E. prostrata* leaves on serum creatine kinase (IU/L) in birds fed with aflatoxin incorporated feed (Mean ± SE, n=10)

Groups	7th day	21st day	42nd day	F-value
T_1	988.18^{C}± 7.21	1139.44^{B}± 13.89	1259.27^{A}± 10.75	148.10**
T_2	1011.88^{B}± 67.74	1231.62^{A}± 36.08	1462.24^{A}± 101.21	7.783**
T_3	977.47^{C}± 11.89	1149.43^{B}± 33.86	1272.26^{A}± 51.70	16.957**
T_4	993.54^{C}± 22.02	1219.36^{B}± 38.55	1273.08^{A}± 35.00	25.182**
T_5	948.78^{B}± 6.00	1025.12^{B}± 86.18	1302.05^{A}± 34.69	13.775**
T_6	968.92^{B}± 6.69	1119.16^{AB}± 76.11	1286.24^{A}± 25.81	11.31**
F value	0.529^{ns}	1.943^{ns}	2.170^{ns}	

Means bearing different small letter as superscript differ significantly within a columns (P<0.05)
Means bearing different capital letter as superscript differ significantly within a row (P<0.05)
ns non-significant (p>0.05); * significant at 0.05 level; ** significant at 0.01 level

Cholesterol (CL)

The results on cholesterol level of all the treated group are presented in table 3. The mean values of CL levels on day seven for T_1 to T_6 were 174.220± 7.2079, 179.670± 7.7451, 173.500± 9.7405, 174.780± 11.7448, 178.520± 1.4871, 190.010± 8.6058 mg/dL respectively. There was no significant difference noted in all the groups on day seven

On day 21, the CL values of T_1 to T_6 were 204.690± 9.0076, 161.360± 7.1766, 185.760± 6.2831, 164.420± 5.5291, 167.880± 5.4004 and 184.330± 7.9086 mg/dL respectively. There was significant reduction in the CL level in the toxic T_2 to that of normal birds T_1. Among the treated groups, T_6 showed a significant reduction in the CL levels when compared with that of T_4 and T_5. The CL level of T_6 is comparable to that of T_1 and T_3.

On day 42, the CL values of T_1 to T_6 were 219.4220± 8.62860, 118.1460± 5.01390, 206.3800± 8.67671, 126.6300± 2.38211, 146.5600± 4.40765 and 151.4300± 6.12402 mg/dL respectively. There was significant decrease in the CL level in T_2 and T_4 compared to that of other treated groups. In T_5 and T_6, there were significant increase in the CL level but not to the extent of normal birds and T_3.

Within the groups on comparison between different days, there was significant (P<0.05) increase in the level of cholesterol in T_1 and T_3 on day 42 when compared to the days 7 and 14 whereas it decreased significantly (P<0.05) in the T_2, T_4, T_5 and T_6.

Table 3: Effect of *E. prostrata* leaves on serum cholesterol (mg/dL) in birds fed with aflatoxin incorporated feed (Mean ± SE, n=10)

Groups	7th day	21st day	42nd day	F-value
T_1	174.22^{B}± 7.20	204.69^{aA} ± 9.00	219.42^{aA} ± 8.62	11.631**
T_2	179.67^{A}± 7.74	161.36^{cA} ± 7.17	118.14^{cB}± 5.01	18.786**
T_3	173.50^{B}± 9.74	185.76^{abAB} ± 6.28	206.38^{aA} ± 8.67	4.393*
T_4	174.78^{A}± 11.74	164.42^{bcA} ± 5.52	126.63^{cB} ± 2.38	11.118**
T_5	178.52^{A}± 1.48	167.88^{bcA} ± 5.40	146.56^{bB} ± 4.40	21.915**
T_6	190.01^{A}± 8.60	184.33^{abA} ± 7.90	151.43^{bB} ± 6.12	12.124**
F Value	0.544^{ns}	5.610**	44.412**	

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ns non-significant (p>0.05); * significant at 0.05 level; ** significant at 0.01 level

Total proteins (TP)

The results on TP level values of the entire treated group are presented in table 4. The mean values of TP levels on day seven for the groups T_1 to T_6 were 2.66870± 0.104802, 2.63470± 0.137795, 2.64250± 0.156633, 2.61410± 0.086630, 2.69350± 0.020641 and 2.61560± 0.069147 g/dL respectively. There was no significant difference noted among the groups in the level of TP on day seven

On day 21, the mean values of TP of treated groups were 2.90170 ± 0.187459, 2.39280 ± 0.063676, 3.14500 ± 0.122299, 2.94300 ± 0.105382, 2.72110 ± 0.071589, 2.89530 ± 0.099648 g/dL respectively. The TP level was significantly decreased in aflatoxin treated T_2 than that of all the other groups. Among the treated group, T_4 and T_6 were almost equal to that of normal control birds.

On day 42, the mean values of TP of treated groups were 3.42 ± 0.20, 2.19± 0.10, 3.45 ± 0.12, 2.86 ± 0.11, 3.21 ± 0.16 and 3.33 ± 0.10 g/dL respectively. The TP level was significantly decreased in aflatoxin treated group to that of all the other groups. In T_4, the TP level was significantly decreased but not to the level of aflatoxin treated group. The total protein levels were not differed significantly in T_1, T_3, T_5 and T_6.

Within the groups comparison revealed that, there was significant increase in the total protein level on days 21 and 42 when compared with day seven in T_1 and T_3. In T_2, there was significant decrease in total protein level at 0.05 levels. In T_4 no significant difference could be noted while in T_5 and T_6, there was significant increase in total protein level on day 42 at 0.05 and 0.01 levels respectively.

Table 4: Effect of *E. prostrata* leaves on serum total protein (g/dL) in birds fed with aflatoxin incorporated feed (Mean ± SE, n=10)

Groups	7th day	21st day	42nd day	F-value
T_1	2.66^{B}± 0.10	2.90^{aAB}± 0.18	3.42^{aA} ± 0.20	4.46*
T_2	2.63^{A}± 0.13	2.39^{cAB} ± 0.06	2.19^{cB} ± 0.10	3.73*
T_3	2.64^{B}± 0.15	$3.14^{a\ A}$± 0.12	3.45^{aA} ± 0.12	10.51**
T_4	2.61± 0.08	2.94^{ab} ± 0.10	2.86^{b} ± 0.11	2.45^{ns}
T_5	2.69^{B}± 0.02	2.72^{bB} ± 0.07	3.21^{abA} ± 0.16	7.04*
T_6	2.61^{C}± 0.06	$2.89^{ab\ B}$± 0.09	3.33^{aA} ± 0.10	23.31**
F value	0.087^{ns}	4.84**	11.83**	

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ns- non-significant (p>0.05); * significant at 0.05 level; ** significant at 0.01 level

Discussion

Aspartate transaminase (AST)

Aspartate aminotransferase previously known as SGOT (serum glutamic-oxaloacetic transaminase) is present in all tissues except bone with higher concentration in liver and skeletal muscles. Aspartate transaminase usually appears in the cytoplasm of hepatocytes. When there is an infection, necrosis, trauma or damage to the hepatocytes, it leaks into the extracellular components thereby increasing the serum AST activity (Nkosi *et al*., 2005) [8].

Relative tissue distribution of AST showed a prominent variation among the avian species which makes the interpretation of raised plasma AST activities a challenging one. But plasma AST has been reported to be single highly useful enzyme for detecting liver diseases in birds (Lumeij and Wasterhof, 1987) [6].

In the present study, the AST value on seventh day was same in all groups but the values were found to be increasing by 21st and 42nd day in aflatoxicosis induced groups and is significantly higher in group II which indicated the hepatic damage. This result highly correlates with the findings of Subhani *et al*. (2018) [15]. They reported that increase in AST level when broiler chicken fed with afaltoxin. Silva *et al*. (2007) [13] reported an increase in AST value as the age of the broiler advanced. According to them, increase in AST level might be due to increase in liver metabolism and muscle development as the age advances.

Treatment with leaf powder of *E. prostrata* at the dose rate of 0.2 per cent showed significant reduction in the AST value compared to all groups but not to the level of normal control. This result highly correlates with the findings of Lin *et al*. (1996) [5] who reported that the administration of *E. prostrata* reduced the AST level in paracetamol, galactosamine and CCl_4 induced hepatotoxicity. Similar observations were also noted in the work done by Prabu *et al*. (2011) [11].

Creatine kinase (CK)

Creatine Kinase mainly used as biomarker for diagnosis of certain diseases mainly in muscles where it is found abundantly. In the present study, the CK was assessed to ensure whether the increase in AST is solely due to liver damage because AST could be elevated during liver damage as well as muscle damage. Estimation of CK could rule out the possibility of muscle damage.

In the present study, no significant difference could be noted in CK activity of entire treated groups on 7, 21 and 42 days. So it could be confirmed that increase in AST activity is solely due to liver damage. These findings are correlated with the reports of Bailey *et al*. (2006) [2] who found no significant differences in CK level among the aflatoxin (4 ppm) fed birds.

Creatine kinase value had significantly increased with age in the present study. This result was supported by the findings of Silva *et al.* (2007) [13] reported increase in CK value as age advanced. Pietruszynska *et al*. (2010) [9] opined that increase in CK level was a characteristic feature of fast growing broiler chicken.

Cholesterol

Liver is the principle organ where the cholesterol is synthesised, stored and metabolised. So liver damage can adversely affect the cholesterol level which can be used as an indicator of liver diseases.

According to Wade *et al.* (2018) [17], serum cholesterol level was significantly reduced when the birds were fed with 300 ppb of aflatoxin. These findings are in line with present study where cholesterol level was greatly reduced in aflatoxin control group on day 21 and 42.

Decrease in cholesterol level could be attributed to impaired cholesterol biosynthesis due to hepatotoxicity caused by aflatoxin, besides the shifting of circulatory cholesterol back into liver (Bailey *et al*., 1998) [1]. However, addition of *E. prostrata* leaves in the diet of aflatoxin fed groups significantly improved the serum cholesterol level in dose dependent manner. This result is in agreement with the work of Samudram *et al*. (2008) [12] in which they reported that biherbal ethanolic extract consisting of *E. alba* and *Piper longum* improved the decreased serum cholesterol level caused by CCl_4 induced hepatotoxicity.

In the current study, the total cholesterol level was found significantly increasing from day 21 to 42 in T_1 and T_3. This result was similar with the outcomes of Gilani *et al*. (2018) [3] stated an surge in cholesterol level as the age advanced. But a decrease in cholesterol level was noted in all the other groups which might be due to the progression of liver damage caused by aflatoxin in the feed.

Total proteins

Liver is the main organ responsible for the synthesis of many circulating proteins such as albumin and globulin. Hence damage to hepatocytes is manifested by reduced total serum protein levels which can be used as an indicator to assess liver damage (Monson *et al*., 2015) [7].

In the present study, on days 21 and 42, serum total protein levels were significantly reduced in toxin control group. These findings were correlates with the earlier reports by Wade *et al*. (2018) [17]. They reported the reduction in total protein level when broiler chicken was fed with aflatoxin.

The serum total protein repression might be due to metabolites of aflatoxin that adducts with DNA that could affect transcription and translation (Sridhar *et al*., 2015) [14]. Aflatoxin adducts with lysine also which results in protein degradation or excretion (Monson *et al*., 2015) [7].

Supplementation of *E. prostrata* leaves in the diet of aflatoxin fed groups significantly improved the total protein level on day 21 and 42 in a dose dependent manner. This result is similar with the observations of Kumar *et al*. (2013) [4] who stated that alcoholic extract of *E. alba* leaves significantly improved the serum total protein level in paracetamol induced hepatic damage in rats. Similar findings were reported by Vasuki *et al*. (2012) [16] in CCl_4 induced hepatic damage in rats.

Within the groups, the total protein level was significantly increased from 21st to 42nd day in T_1 and T_3. This result is in concordance with the findings of Piotrowska *et al.* (2011) [10] who noted an increase in total protein level from 21 to 42 days in normal birds. They reported that this increase might be a direct consequence of high demand for amino acids needed for intensive somatic growth. But in T_2, the total protein level had constantly decreased which could be the consequence of progression of liver damage induced by aflatoxin. In T_5 and T_6, the total protein level was increased which might be due to restoration of liver functions by *E. prostrata* leaf supplementation.

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