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

Priya K, Preethy John, Usha PTA, Kariyil BJ and Uma R

**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 T₁ and T₅ by giving 500 ppb of aflatoxin B₁ (AFB₁) contaminated maize from eighth day of age onwards. The group T₁ was kept as normal control and T₂ as toxic control. T₅ was fed with *E. prostrata* leaf powder at 0.2 per cent level. The leaf powder of *E. prostrata* was given to T₅ and T₆ 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 T₅ 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 B₁, B₂, G₁, G₂, M₁, M₂ are the different types of aflatoxin and are differentiated by their fluorescence under ultraviolet light. Among these, aflatoxin B₁ 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 B₁ - 8, 9 - epoxide. This metabolite intercalates with DNA forming DNA adducts which can be carcinogenic. Aflatoxin B₁ 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 Bomin 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 T1 and T3 by giving 500 ppb of aflatoxin B1 (AFB1) from eighth day of age onwards. The group T1 was kept as normal control and T2 as toxic control. T2 was fed with E. prostrata leaf powder at 0.2 per cent level. The leaf powder of E. prostrata was given to T1, T2 and T3 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.

The mean± standard error values of CK 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 182.78± 1.56, 184.21± 2.46, 190.87± 3.95, 183.02± 3.33, 184.30± 4.72 and 184.60± 8.40 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 198.57± 4.66, 232.18± 6.52, 306.15± 8.81, 235.23± 10.94, 259.16± 6.11, 226.74± 10.94, 287.40± 8.40 and 226.74± 10.94 IU/L respectively. There was no significant difference noted between T2 and T3. In the T6, there was significant (P<0.05) reduction in the AST values compared with T2, T4 and T5 though AST value couldn’t reach those of the normal group.

On day 42, the mean AST levels (IU/L) values for T1 to T6 were 232.18± 6.52, 235.23± 10.94, 306.15± 8.81, 226.74± 10.94, 259.16± 6.11, 226.74± 10.94, 287.40± 8.40 and 226.74± 10.94 IU/L respectively. There was no significant difference noted between T2 and T3. In the T6, there was significant (P<0.05) reduction in the AST values compared with T2, T4 and T5 although AST value couldn’t reach those of the normal group.

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 T1, 184.21± 2.46, 259.16± 8.81, 183.02± 3.33, 184.30± 4.72 and 259.16± 6.11 IU/L in T2, 190.87± 3.95, 250.94± 8.81, 228.60± 10.94, 287.40± 8.40 and 228.60± 10.94 IU/L in T3 respectively.

There was significant (P<0.001) decrease in the AST level in the T3 to that of toxic group T2 which was not to that level of normal control. There was no significant difference between T2, T4 and T5.

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) groups recorded on day seven were similar for T1 to T3 and the values were 993.54± 11.89, 1149.43± 33.86, 1219.36± 38.55, 1025.12± 86.18, 1119.16± 76.11 and 1272.26± 51.70 IU/L for T1 to T6 respectively.

On day 21, the CK values were 1139.44± 13.89, 1231.62± 36.08, 1194.43± 33.86, 1272.26± 51.70 IU/L in T1, 993.54± 22.02, 1219.36± 38.55, 1272.26± 51.70 IU/L in T2, 1025.12± 86.18, 1119.16± 76.11 and 1272.26± 51.70 IU/L in T3 respectively.

On day 42, the CK values for T1 to T6 were 1259.27± 10.75, 1462.24± 101.21, 1272.26± 51.70, 1273.08± 35.01, 1302.05± 34.69, 1286.24± 25.81 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 in T1, 1011.88± 67.74, 1231.62± 36.08, 1462.24± 101.21 IU/L in T2, 977.47± 11.89, 1149.43± 33.86, 1272.26± 51.70 IU/L in T3, respectively.

There was significant (P<0.001) reduction in the CK values compared with T2, T4 and T5 although CK value couldn’t reach those of the normal group.

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>7th day</th>
<th>21st day</th>
<th>42nd day</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>182.78± 1.56</td>
<td>198.57± 4.66</td>
<td>232.18± 6.52</td>
<td>7.298**</td>
</tr>
<tr>
<td>T2</td>
<td>184.21± 2.46</td>
<td>250.94± 8.81</td>
<td>306.15± 18.29</td>
<td>30.329**</td>
</tr>
<tr>
<td>T3</td>
<td>190.87± 3.95</td>
<td>204.54± 6.11</td>
<td>228.60± 10.94</td>
<td>10.021**</td>
</tr>
<tr>
<td>T4</td>
<td>183.02± 3.33</td>
<td>259.16± 10.06</td>
<td>287.40± 8.40</td>
<td>84.860**</td>
</tr>
<tr>
<td>T5</td>
<td>184.30± 4.72</td>
<td>226.74± 5.33</td>
<td>264.90± 8.77</td>
<td>31.765**</td>
</tr>
<tr>
<td>T6</td>
<td>184.30± 4.72</td>
<td>226.74± 5.33</td>
<td>264.90± 8.77</td>
<td>31.765**</td>
</tr>
<tr>
<td>F value</td>
<td>0.321**</td>
<td>9.189**</td>
<td>7.298**</td>
<td>31.765**</td>
</tr>
</tbody>
</table>

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
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Within the groups comparison revealed that, there was not differed significantly in T
 groups. In T
 decreased in aflatoxin treated group to that of all the other groups. In T
 ± 0.16 and
 ± 0.10. The TP level was significantly decreased in the toxic T
 and T
 compared to that of other treated groups. In T
 there was significant increase in the CL level but not to the extent of normal birds and T
.

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

Table 3: Effect of E. prostrata leaves on serum creatine kinase (IU/L) in birds fed with aflatoxin incorporated feed (Mean ± SE, n=10)

<table>
<thead>
<tr>
<th>Groups</th>
<th>7th day</th>
<th>21st day</th>
<th>42nd day</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>174.22±7.20</td>
<td>204.69±9.00</td>
<td>219.42±8.62</td>
<td>11.631**</td>
</tr>
<tr>
<td>T2</td>
<td>179.67±7.74</td>
<td>161.36±7.17</td>
<td>118.14±5.01</td>
<td>18.786**</td>
</tr>
<tr>
<td>T3</td>
<td>173.50±9.74</td>
<td>185.76±6.28</td>
<td>206.38±8.67</td>
<td>4.393**</td>
</tr>
<tr>
<td>T4</td>
<td>174.78±11.74</td>
<td>164.42±5.52</td>
<td>126.63±2.38</td>
<td>11.118**</td>
</tr>
<tr>
<td>T5</td>
<td>178.52±1.48</td>
<td>167.88±5.40</td>
<td>146.56±4.40</td>
<td>21.915**</td>
</tr>
<tr>
<td>T6</td>
<td>190.01±8.60</td>
<td>184.32±7.90</td>
<td>151.43±6.12</td>
<td>12.124**</td>
</tr>
</tbody>
</table>

Mean bearing capital letter as superscript differ significantly within a row (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 T
 to T
 were 2.6687±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
 ± 0.15989, 2.89530 ± 0.099648 g/dL respectively. The TP level was significantly decreased in aflatoxin treated T
 than that of all the other groups. Among the treated group, T
 and T
 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
, the TP level was significantly decreased but not to the level of aflatoxin treated groups. The total protein levels were not differed significantly in T
, T
, T
 and T
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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
 and T
. In T
, there was significant decrease in total protein level at 0.05 levels. In T
 no significant difference could be noted while in T
 and T
, 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)

<table>
<thead>
<tr>
<th>Groups</th>
<th>7th day</th>
<th>21st day</th>
<th>42nd day</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>2.66±0.10</td>
<td>2.90±0.18</td>
<td>3.42±0.20</td>
<td>4.76**</td>
</tr>
<tr>
<td>T2</td>
<td>2.63±0.13</td>
<td>2.39±0.06</td>
<td>2.19±0.10</td>
<td>3.73*</td>
</tr>
<tr>
<td>T3</td>
<td>2.64±0.15</td>
<td>3.14±0.12</td>
<td>3.45±0.12</td>
<td>10.51**</td>
</tr>
<tr>
<td>T4</td>
<td>2.61±0.08</td>
<td>2.94±0.10</td>
<td>2.86±0.11</td>
<td>2.45**</td>
</tr>
<tr>
<td>T5</td>
<td>2.69±0.02</td>
<td>2.72±0.07</td>
<td>3.21±0.16</td>
<td>7.04*</td>
</tr>
<tr>
<td>T6</td>
<td>2.61±0.06</td>
<td>2.89±0.09</td>
<td>3.33±0.10</td>
<td>23.31**</td>
</tr>
</tbody>
</table>

Mean bearing capital letter as superscript differ significantly within a row (P<0.05); * significant at 0.05 level; ** significant at 0.01 level.

Table 2: Effect of E. prostrata leaves on serum total protein (g/dL) in birds fed with aflatoxin incorporated feed (Mean ± SE, n=10)

<table>
<thead>
<tr>
<th>Groups</th>
<th>7th day</th>
<th>21st day</th>
<th>42nd day</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>988.18±7.21</td>
<td>1139.44±13.89</td>
<td>1259.27±10.75</td>
<td>148.10**</td>
</tr>
<tr>
<td>T2</td>
<td>1011.88±67.74</td>
<td>1231.62±36.08</td>
<td>1462.24±101.21</td>
<td>7.783**</td>
</tr>
<tr>
<td>T3</td>
<td>977.47±11.89</td>
<td>1149.43±33.86</td>
<td>1272.26±51.70</td>
<td>16.957**</td>
</tr>
<tr>
<td>T4</td>
<td>993.54±22.02</td>
<td>1219.36±38.55</td>
<td>1273.08±35.00</td>
<td>25.182**</td>
</tr>
<tr>
<td>T5</td>
<td>948.78±6.00</td>
<td>1025.12±68.18</td>
<td>1302.05±34.69</td>
<td>13.775**</td>
</tr>
<tr>
<td>T6</td>
<td>968.92±6.69</td>
<td>1119.16±76.11</td>
<td>1286.24±25.81</td>
<td>11.31**</td>
</tr>
</tbody>
</table>

Mean bearing different small letter as superscript differ significantly within a columns (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
 to T
 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
 to T
 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
 to that of normal birds T
. Among the treated groups, T
 showed a significant reduction in the CL levels when compared with that of T
 and T
. The CL level of T
 is comparable to that of T
 and T
.

On day 42, the CL values of T
 to T
 were 219.4220± 8.62860, 118.1406± 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
 and T
 compared to that of other treated groups. In T
 and T
 there were significant increase in the CL level but not to the extent of normal birds and T
.

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

Means bearing different small letter as superscript differ significantly within a columns (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 aflatoxin. 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) [10] who reported that the administration of E. prostrata reduced the AST level in paracetamol, galactosamine and CCl4 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 herbal ethanolic extract consisting of E. alba and Piper longum improved the decreased serum cholesterol level caused by CCl4 induced hepatotoxicity.

In the current study, the total cholesterol level was found significantly increasing from day 21 to 42 in T1 and T3. 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 aflatoxin induced rats. 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 CCl4 induced hepatic damage in rats.

Within the groups, the total protein level was significantly increased from 21st to 42nd day in T1 and T3. 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 T3, the total protein level had constantly decreased which could be the consequence of progression of liver damage induced by aflatoxin. In T3 and T5, the total protein level was increased which might be due to restoration of liver functions by E. prostrata leaf supplementation.

Acknowledgement

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the research work. The content of this article based on the thesis submitted to Kerala Veterinary and Animal Sciences University for the award of master’s degree.

References


10. Piotrowska et al. noted increase in serum total protein level in broiler chicken when its age advanced, 2011.


