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Antioxidant effect of *Eclipta prostrata* (L.) leaf powder in broiler chicken during aflatoxicosis

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Abstract
The study was aimed to investigate the protective effect of *Eclipta prostrata* leaf powder against oxidative stress 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. On 42nd day all the birds were sacrificed, liver samples were collected and checked for its antioxidant status. Treatment with *E. prostrata* leaf powder revealed protection against oxidative stress caused by aflatoxicosis in dose dependent manner which is indicated by significant (P<0.05) increase in the level of reduced glutathione.

Keywords: Aflatoxicosis, broiler chicken, antioxidant status, *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 oxidative stress during aflatoxicosis in broiler chicken.

Materials and Methods
The study on "Protective effect of *Eclipta prostrata* (L.) leaves against oxidative stress caused by experimental aflatoxicosis in broiler chicken" was conducted at the Department of Veterinary Pharmacology and Toxicology, Mannuthy, Thrissur.

The experiment was approved by Institutional Animal Ethics Committee (IAEC), College of Veterinary and Animal Sciences, Mannuthy (order no: IAEC/CVASMTY/7/17-18).

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 leaves were collected; shade dried and pulverized using an electrical pulveriser. The powdered leaves were stored in air tight container at room temperature. The feed was pre-checked for the presence of aflatoxin. The mouldy maize containing 500 ppm of aflatoxin B1 was incorporated to prepare the experimental diet.

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.

On day 42, all the birds were sacrificed using carbon dioxide chamber and the freshly collected liver samples were washed with running tap water to remove blood clots, weighed and homogenized immediately for estimation of antioxidant status. The level of lipid peroxidation in liver was estimated by the method described by Fraga *et al.* (1988) [6]. Level of reduced glutathione in liver homogenate was determined by the method of Ellman (1959) [4]. Data obtained from the experiment were subjected to statistical analysis using SPSS software version 24.0.

Results

Lipid peroxidation level in liver tissue

The mean lipid peroxidation level in the homogenised liver tissues on the day 42 of entire groups is presented in table 1. The mean ± standard error values of lipid peroxidation level in liver for T_1 to T_6 were 80.40 ± 29.31, 161.74 ± 81.30, 91.96 ± 26.95, 117.20 ± 29.96, 117.77 ± 19.44 and 99.16 ± 32.63 nM MDA/g of tissue respectively. Lipid peroxidation level was significantly higher in T_2. But there was no significant difference between normal control and *E. prostrata* leaf powder treated groups.

Reduced glutathione level in liver tissue

The mean reduced glutathione level in the liver tissues of all the treated groups on day 42 are presented in table 2. The mean ± standard error values of reduced glutathione level in liver for T_1 to T_6 were 45.13 ± 2.53, 27.23 ± 4.47, 42.96 ± 3.45, 32.14 ± 5.41, 40.04 ± 4.46 and 42.23 ± 4.47 µg / mg of tissue respectively. Reduced glutathione level in the liver was significantly lower in T_2 compared with other groups. Among the powder treated groups, T_3 and T_6 showed a significant increase in reduced glutathione values which were similar to T_1 and T_5.

Table 1: Effect of *E. prostrata* leaves on lipid peroxidation (LPO) in birds fed with aflatoxin incorporated diet (Mean ± SE, n=10)

Groups	LPO (nM MDA/g of tissue)
T_1	80.40^{b}± 29.31
T_2	161.74^{a}± 81.30
T_3	91.96^{b}± 26.95
T_4	117.20^{b}± 29.96
T_5	117.77^{b}± 19.44
T_6	99.16^{b}± 32.63

Means bearing different superscripts in columns differ significantly (P< 0.05)

Table 2: Effect of *E. prostrata* leaves on reduced glutathione (GSH) in birds fed with aflatoxin incorporated feed (Mean ± SE, n=10)

Groups	GSH (µg / mg of tissue)
T_1	45.13^{a}± 2.53
T_2	27.23^{d}± 4.47
T_3	42.96^{ab}± 3.45
T_4	32.14^{c}± 5.41
T_5	40.04^{b}± 4.46
T_6	42.23^{ab}± 4.47

Means bearing different superscripts in columns differ significantly (P< 0.05)

Discussion

Lipid peroxidation (LPO) level in the liver

The overproduction of reactive oxygen radicals due to tissue damage results in oxidative stress and reduced antioxidant capacity causing an imbalance resulting in the damage of cellular biomolecules especially lipids. Lipid peroxidation is a free-radical mediated reaction leading to oxidative degradation of polyunsaturated fatty acids. The compounds obtained from LPO are very unstable and they tend to degrade quickly into variety of sub products. Malondialdehyde is one of the usual outcome of LPO, is considered as a popular indicator of oxidative damage to cells and tissues (Grotto *et al.*, 2009) [8]. The lipid peroxidation level (nM MDA/g of tissue) indicative of oxidative damage in liver was (P<0.05) elevated in the liver of T_2 compared to all other groups. This is in concordance with the findings of Eraslan *et al.* (2004) [5] who opined that aflatoxin caused liver damage which led to free radical formation over and above the capacity of antioxidant defence and thereby increased lipid peroxidation level in the aflatoxin treated groups. Similar results were observed by Gowda *et al.* (2008) [7] and Al-Zuhariy and Hassan (2017) [1]. The expression of intracellular antioxidant mechanisms is down-regulated by aflatoxin thereby increasing the lipid peroxidation level in liver (Da Silva *et al.*, 2018). The significantly (P<0.05) reduced lipid peroxidation levels in liver observed in powder treated group of birds (T_4, T_5, T_6) indicated that *E. prostrata* leaf powder had rectified the tissue damage caused by aflatoxin in a dose dependent manner. The group T_6 at 0.2 per cent showed greater inhibitory effect on lipid peroxidation. This result is supported by the work done by Arun and Balasubramanian (2011) [2] who reported that elevated lipid peroxidation level by ethanol induced oxidative damage was combated by administration of ethanolic extract of *E. prostrata* leaves in rats. Thirumalai *et al.* (2011) [12] also reported that administration of aqueous leaf extract of *E. alba* significantly reduced the lipid peroxidation level in CCl_4 induced oxidative damage in rats.

Reduced glutathione level in the liver

Reduced glutathione is the most common endogenous antioxidant present in animals. It is a thiol containing molecule capable of defending against free radicals, lipid peroxides and other reactive oxygen species and provides protection against oxidative stress. Reduced glutathione reacts with free radicals to form oxidised glutathione using enzyme glutathione peroxidases. The enzyme glutathione reductase helps to recycle reduced glutathione back from oxidised glutathione by utilising the reducing equivalents of NADPH (Pocernich and Butterfield, 2012) [10]. Reduced glutathione level reduced significantly in the aflatoxin (T_2) control group compared with all the other groups. This result is supported by the work done by Karaman *et al.* (2010) [9] who reported a reduction in the level of reduced glutathione and other antioxidant enzymes when the birds were fed with the feed contaminated with aflatoxin. The depletion of reduced glutathione might be due to its oxidation by binding with electrophiles produced from lipid peroxidation in liver (Sahu *et al*., 2014) [11]. Addition of *E. prostrata* leaf powder at the dose rate of 0.2 per cent in aflatoxin contaminated feed improved reduced glutathione level significantly. This result is in accordance with the previous study conducted by Arun and Balasubramanian (2011) [2] who reported that treatment with *E. prostrata* leaf extract significantly improved the depleted level of reduced glutathione in ethanol provoked hepatotoxicity in rats. Reported that administration of hydro alcoholic extract of aerial part of *E. alba* significantly improved the reduced glutathione level in cerebral ischemia induced oxidative damage in brain tissue of rats. Reported that flavonoids in cocoa upregulated the two antioxidant enzymes through the activation of kinases signalling pathways against oxidative stress in hepatic cell lines. Therefore the flavonoids in this leaf of *E. prostrata* might be responsible for antioxidant effect of this plant.

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