AMELIORATIVE EFFECT OF *AMLA* IN BIOCHEMICAL AND HAEMATOLOGICAL PROFILE IN AFLATOXICOSIS IN BROILER CHICKEN

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Free radicals are constantly generated within the body during normal biochemical and immunological reactions. Reactive oxygen species like superoxide, hydrogen peroxide and hydroxyl radicals are the major free radicals involved. Enzymatic and non enzymatic methods within the body reduce the free radicals formed in the body. But this normal level of antioxidant effect may not be sufficient at times and result in free radical injury. It is possible to limit oxidative tissue damage and thus prevent or ameliorate disease progression by supplementing antioxidants exogenously. Liver is the major site of detoxification of toxins and so is particularly vulnerable to oxidative damage. Aflatoxin is a potent hepatotoxin, which is subjected to metabolic activation to its various toxic metabolites with the help of the hepatic microsomal enzymes like the P450 enzyme system. Fruits of *Emblica officinalis* popularly known as *amla* is used in many indigenous medical preparations used as general health tonic and also against a variety of diseases. It is reported to have antioxidant properties and exhibit *in vitro* inhibition of the oxygen radicals such as lipid peroxides, superoxides and hydroxyl radicals and the activity was comparable to that of known antioxidants such as ellagic acid, curcumin, alpha-tocopherol and ascorbic acid (Jose and Kuttan, 1995).

The present report is a study of the ameliorative effect of *amla* against the toxic effects of aflatoxin in chicken.

Materials and Methods

Fifty four, healthy day old Hubbard broiler chicks were obtained from the coastal Krishna hatcheries, Thrissur, Kerala. The chicks were randomly divided into three groups each having two replicates. All the six replicate groups were kept in separate pens under deep litter system throughout the experiment. Ideal brooding conditions were provided for the first four weeks. Each group was given scheduled experimental feed and water *ad libitum*.

Proximate principles of feed were analyzed as per AOAC (1990).

Feed samples were assayed for the presence of aflatoxin and ochratoxin by multimycotoxin analysis method (Tapia, 1985) using thin layer chromatography.

Broiler feeds, both starter(containing 100-150ppb of aflatoxinB_1)and finisher (containing 150-200 ppb of aflatoxinB_1) were used for the study.

Amla powder was prepared by powdering sun dried fruits of *Emblica officinalis* . All the three groups of birds were given feed as per the following schedule.

Group I – Birds of the control group were given commercial feed alone.

Group II – *Amla* powder was added at one percent level in the feed.

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Group III – *Amla* powder was added at two percent level in the feed.

The birds were reared for eight weeks. They were sacrificed at the end of the experiment.

Blood samples were collected at the end of the experiment for hematological and biochemical examination . Hemoglobin (Hb), Packed Cell Volume (PCV) and Erythrocyte Sedimentation Rate (ESR) were estimated as per Benjamin (1978). The Total Leucocyte Count (TLC) was determined as per the method described by Sastri (1976). The Differential Leucocyte Count (DLC) was done with the copper peroxidase method of Sato and Sekiya (1965).

Total serum protein was estimated by Biuret method (Gormall *et al.*, 1949). Serum albumin was estimated by Bromo Cresol Green dye binding method described by Doumas *et al.* (1971). Serum globulin and albumin-globulin ratio was calculated from these values. Serum Aspartate amino transferase (AST), Alkaline Phosphatase (ALP) and Gamma glutamyl transpeptidase (GGT) were estimated, using commercially available kits (M/s AGAPPE Diagnostics) and the final readings were taken spectrophotometrically at 405 nm.

The data obtained from various parameters were subjected to statistical analysis and analysis of variance (ANOVA) was conducted as per Rangaswamy (1995).

Results and Discussion

Feed analysis for proximate principles showed normal chemical composition. Analysis for mycotoxins indicated 100-150 ppb of aflatoxin B1 in starter rations and 150-200 ppb of toxin in finisher rations. Ochratoxin content was nil in all the samples. The hematological parameters are given in Table (1). The ESR values were significantly higher for the control group birds compared to the *amla* fed groups. The PCV and TLC were decreased in the control group birds compared to the *amla* fed groups. The DLC indicated significantly higher values for heterophil (H), lymphocyte (L) and monocyte (M) counts in the *amla* fed groups. No appreciable variation was seen in the eosinophil (E) and basophil (B) counts. The serum biochemical parameters recorded are given in Table (2). The serum total protein, albumin, globulin and albumin/globulin ratio showed a dose related increase in the *amla* fed birds compared to the control group birds. The liver specific serum enzymes like AST, ALP and GGT levels were high in the control group birds compared to the *amla* treated group.

The present study indicate that *Emblica officinalis* could limit the toxic changes produced by aflatoxin, which is evident from the improved haematological and serum biochemical response shown by the *amla* fed birds. Aflatoxin is a potent hepatotoxin. The active metabolites like the aflatoxin hemiacetals and 8-9 epoxides cause widespread non specific interference with the hepatocellular metabolic and physiological systems leading to hepato-cellular toxicity. The lower levels of serum protein and albumin noticed in the present study can be correlated to the hepatic damage. This might be due to poor absor-ption of amino acids from the intestine during aflatoxicosis or by the inhibitory effect of aflatoxin B_1 on protein synthesis in hepatic cells, inactivation of amino acids for protein synthesis in liver and blocking of RNA synthesis in the nucleolus (Mani *et al.*, 1993). The increase in the liver specific serum enzymes in the control group can be attributed to the pathological changes produced by the toxin in the hepatobiliary system, leading to increased cellular permeability and release of enzymes were lower in the *amla* treated groups. Similar findings have been reported where the extract of *Embilica officinalis* reversed the increase in serum enzyme level induced by carbon tetrachloride (Jose and Kuttan, 2000).

The increase in ESR, noted in control group birds, may be due to the decreased serum total protein and albumin. A loss of suspension stability of erythrocytes as a result of altered serum protein has been reported to cause increased erythrocyte clumping and rapid sedimentation rate (Jain, 1986). *Amla* treatment restored the enzyme systems and thus the protein synthesis in the liver, which is evident by the lowered ESR values seen in these birds. Low PCV and Hb values seen in the control group birds compared to *amla* fed birds indicate the inhibitory effect of aflatoxin on the haemopoetic system (Vasan *et al.*, 1998). *Amla* treatment has a protective effect on bone marrow cells (Kumar *et al.*, 1996).

Myelosuppressive effect of aflatoxin produced lowered leucocyte counts in the control birds. *Emblica officinalis* or its products can inhibit the myleosuppression

Table 1. Hemogram values (mean ±SE) of experimental birds at eight weeks of age

Group	Amla level (%)	ESR mm/h	PCV (%)	Hb (g%)	TLC (x10³/ cu.mm)	Differential leukocyte count (%)				
						H	L	M	E	B
I	0	2.42$^{a\pm}$ 0.07	26.56$^{a\pm}$ 0.10	9.06$^{a\pm}$ 0.19	18.13$^{a\pm}$ 0.08	30.61$^{a\pm}$ 0.12	59.39$^{a\pm}$ 0.25	1.72$^{a\pm}$ 0.18	0.94$^{a\pm}$ 0.13	0.11$^{a\pm}$ 0.08
II	1	1.82$^{b\pm}$ 0.04	28.80$^{b\pm}$ 0.08	10.28$^{b\pm}$ 0.16	19.50$^{b\pm}$ 0.16	32.61$^{b\pm}$ 0.16	61.00$^{b\pm}$ 0.20	1.56$^{b\pm}$ 0.15	1.06$^{b\pm}$ 0.13	0.22$^{b\pm}$ 0.10
III	2	1.66$^{c\pm}$ 0.04	31.12$^{c\pm}$ 0.12	11.82$^{c\pm}$ 0.15	22.75$^{c\pm}$ 0.13	37.61$^{c\pm}$ 0.20	65.89$^{c\pm}$ 0.34	2.22$^{c\pm}$ 0.15	1.28$^{c\pm}$ 0.12	0.28$^{c\pm}$ 0.11
	LSD	0.149	0.290	0.47	0.367	0.467	0.761	0.45	0.18	0.25

Means bearing the same superscript within a column does not differ significantly (P<0.05)

Table 2. Serum biochemical profile at eight weeks of age

Group	Amla level (%)	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Albumin: Globulin	Aspartate amino-Transferase (AST) (IU/L)	Alkaline Phos-phatase (ALP)(IU/L)	Gamma Glutamyl Transpe-ptidase (GGT)(IU/L)
I	0	2.97$^{a\pm}$ 0.06	1.30$^{a\pm}$ 0.05	1.63$^{a\pm}$ 0.06	0.81$^{a\pm}$ 0.05	23.85$^{a\pm}$ 0.28	38.39$^{a\pm}$ 0.17	11.75$^{a\pm}$ 0.09
II	1	3.42$^{b\pm}$ 0.07	1.75$^{b\pm}$ 0.04	1.73$^{b\pm}$ 0.07	1.05$^{b\pm}$ 0.03	22.15$^{b\pm}$ 0.25	34.55$^{b\pm}$ 0.12	9.92$^{b\pm}$ 0.09
III	2	4.51$^{c\pm}$ 0.07	2.32$^{c\pm}$ 0.01	2.19$^{c\pm}$ 0.01	1.06$^{c\pm}$ 0.07	20.84$^{c\pm}$ 0.29	31.00$^{c\pm}$ 0.11	7.61$^{c\pm}$ 0.11
	LSD	0.15	0.11	0.15	0.10	0.77	0.39	0.93

Means bearing the same superscript within a column does not differ significantly (P<0.05)

produced by chemicals (Pallabi De *et al.*, 1998) or radiation (Kumar *et al.*, 1996). This might be due to the activation of macrophages by *amla,* leading to enhanced production of colony stimulating factors, resulting in the proliferation of cells in the bone marrow (Chatterjee, 2001). The results of the present study indicate that *Emblica officinalis* can significantly limit the toxic changes produced by aflatoxin.

Summary

Aflatoxin is a potent hepatotoxin and causes widespread interference in the hepatic metabolic, synthetic and other functions. This is evidenced by the change in the haematological and biochemical parameters noted. *Emblica officinalis* significantly ameliorated the toxic changes produced by aflatoxin, in a dose dependant manner.

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