

Original Research Article

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Ameliorative Effect of *Curcuma longa* in Aflatoxicosis Induced Hematological and Histopathological Changes in Broiler Birds

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ABSTRACT

The present study was undertaken to evaluate the ameliorative effects of *Curcuma longa* rhizome powder (1%) in induced aflatoxicosis in broiler chicken. In this study, commercially available broiler chickens of Vencobb strain were reared from day one to forty two days in the deep litter system of management and the birds were divided into three groups. Normal feed tested free of aflatoxin (AF), was given to the control (Group-1). Aflatoxin (1 ppm) was supplemented with the feed to Group 2 and *Curcuma longa* (1%) + AF was supplemented with the feed to Group 3. Hematological parameters like PCV, Hb, TEC and TLC were estimated. The results showed that the supplementation of *C. longa* (1%) + AF restored the hematological alterations induced by the AF. At the sixth week of age, two birds from each replicate were sacrificed to evaluate the gross and histopathological changes in the liver and kidney. Microscopically, congestion of liver parenchyma, cytoplasmic vacuolation/fatty change of hepatocytes and renal tubules, necrosis, mononuclear cell infiltration was observed in aflatoxicated birds (Group 2). Milder form of pathological lesions in treatment groups (group-3) birds reveal pallor discoloration of liver and hepatomegaly and mild lesion in kidneys. Present study revealed that supplementation of *Curcuma longa* rhizome powder could partially ameliorate aflatoxicity in broilers.

Keywords

Aflatoxicosis,
Curcuma longa,
Broiler,
Histopathology,
Clinical signs,
Hematological
changes.

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Introduction

Aflatoxicosis in poultry production has been a global problem and occurs due to the consumption of aflatoxin (AF) contaminated feed. Aflatoxin producing fungi like *Aspergillus parasiticus* and *Aspergillus flavus* flourish well in common feed ingredients under favourable environment. Among the animals the poultry has been recognized to be

the most susceptible. These mycotoxins produces deleterious effects grossly and histopathologically on liver, spleen, kidney and other vital organs which leads to decreased growth and performance in poultry. AF in broiler chicken has been widely investigated as carcinogenic, mutagenic, teratogenic (Ahmed *et al.*, 2009). Chronic

aflatoxicosis results in hepatocellular tumor, and these accounts for loss in production parameters revealed by loss of weight, decline in feed efficiency, drop in egg production and increased susceptibility to infections. However, practical and cost effective methods to detoxify / degrade mycotoxin containing feed stuffs on a larger scale basis are not available. Herbal drugs have been used traditionally in humans by physicians, herbalists and folk healers worldwide or for the prevention and treatment of liver diseases (Pattanayak *et al.*, 2016). Natural phytochemical compounds like coumarins, flavonoids and curcuminoids possess antioxidant property and inhibit the biotransformation of aflatoxin B1 to their active epoxide derivatives (Lee *et al.*, 2001). Turmeric (*Curcuma longa*) is a tropical Asian plant. The main yellow biologically active substance extracted from rhizomes of curcuma is curcumin, which is chemically diferuloyl methane. *Curcuma longa* has been reported to have anti-microbial, anti-inflammatory, anti-viral, anti-oxidant and anti-cancer effect on various laboratory animals (Waffa and Hatem, 2013). *Curcuma longa* is known for its medicinal use and little work has been done to assess its efficacy as a feed additive in poultry.

Therefore present study was undertaken to assess the ameliorative potential of *C. longa* in broiler birds when used as feed additive in induced aflatoxicosis birds

Material and Methods

Production of aflatoxin

The AF was produced from *Aspergillus flavus* NRRL - 18079 pure culture (Institute of Microbial Technology, Chandigarh, India) via fermentation of rice by the method of Shotwell *et al.*, (1966). Fermented rice was then steamed to kill the fungus, dried and

ground to fine crystalline powder. Hundred grams of powder from the culture substrate sample was sent to Animal Feed Analytical and Quality Control Laboratory, Veterinary College, Namakkal, TamilNadu, India for quantification of AF. The AF within the rice powder consisted of 165 ppm AFB₁, 28 ppm AFB₂ and 20 ppm AFB₂. The rice powder was added to the basal diet to provide the required amount of 1 ppm (1mg kg⁻¹).

Collection and preparation of the plant material

Curcuma longa rhizome was procured from the local market of Durg and identified from a botanist and was shade dried and reduced to fine powder using grinder and the powder was stored in airtight containers. The powder thus obtained was used in the present investigation.

Chickens and diet

The experimental investigation was planned for histopathological evaluation of protective potential of *Curcuma longa* on Aflatoxin induced toxicity in broilers after obtaining approval from Institutional Animal Ethics Committee. Clinically healthy ninety day-old, Cobb- 400 broiler chicks of both sexes, weighing 48- 50 g were obtained from a commercial hatchery and were reared on deep litter system of housing using rice husk with provision of artificial light at night. The chicks were fed a standard commercial feed starter upto 14 days, thereafter a grower diet upto 28 days and finisher upto 42 days.

Chickens were allowed access to the diets and fresh and clean drinking water *ad libitum*. The basal diets were tested for possible residual AF before feeding and there were no detectable levels present. All the experimental chicks were kept under close observation during entire period of study.

Experiment design

Chicks were weighed and randomly allotted into 3 groups of 30 chicks in each group having 3 replicates of 10 chicks in each group. Chicks of group-1 were kept as untreated control and were given only basal diet. Chicks of group – 2 were given fed diet with aflatoxin @ 1 ppm from day first of experiment and considered as experimental aflatoxicosis group. Group-3 birds were fed diet with aflatoxin @ 1 ppm along with *Curcuma longa* powder @ 10 gm/kg of feed as a treatment on mycotoxicosis from day first of experiment.

Clinical signs

Daily monitoring of all the birds in morning and evening for clinical sign was carried out. The severity of symptoms and mortality in chicks of different groups were recorded throughout the experimental period. Post mortem examination of all the birds of the different treatment groups died at different intervals was carried out systematically to rule out death due to toxicity.

Haematological study

Blood samples from jugular vein were collected in sterilized evacuated heparinised (Heparin @ 20 I. U. / ml of blood) tubes from the birds of all 3 groups at the end of the experiment i.e. day 42. The heparin admixed blood samples were used for haemoglobin (Hb), packed cell volume (PCV), total erythrocyte and leucocyte count (TEC, TLC) using conventional methods. Haemoglobin (Hb) content of blood was determined by Cyanmethaemoglobin method in spectrophotometer and the absorbance was read at 540 nm against Drabkins solution as blank and expressed in g/dl. Packed cell volume (PCV) was estimated by micro haematocrit method as described by Jain

(1986) and expressed in percent. Total erythrocyte count (TEC) and Total leucocyte count (TLC) were done as per the method described by Nambiar (1960) using diluting fluid recommended by Natt and Herrick (1954) and the values were expressed in million/ μ l and thousands/ μ l respectively. Two birds from each replicate were sacrificed and following parameters were studied by standard methods

Pathological study

All the chicks died during experimental period or sacrificed on day 42 were thoroughly examined by conducting post-mortem examination for the presence of gross pathological alteration and then representative tissue samples were collected in 10% formal saline solution for histopathological examination. Collected formal saline fixed tissues were processed by paraffin embedding. The paraffin embedded tissues were cut at 4 μ thickness and stained with Haematoxylin and Eosin (H & E) for histopathological study as per the method described by Singh and Sulochana (1997). Two birds from each replicate were sacrificed and following parameters were studied by standard methods

Results and Discussion

Clinical signs and mortality

The experimental birds in the toxin treated group were generally active up to 1 week of age after that loose droppings was observed. Loose droppings progressed in severity to watery droppings from 2nd to 4th week of age as the cumulative exposure to toxin was increased. In addition, progressive anorexia with increased water intake was noticed during 1st and 2nd week. In the 3rd and 4th week birds showed ruffled feathers, lethargies, emaciation with marked

depression, reduced appetite, stunted growth and reluctance to move (Plate 1, 2). In the *C. longa*+ AF treated groups, similar signs were also observed during 2nd and 3rd week but with the reduced intensity and at the end of 4th and 5th week the toxic signs like the emaciation, ruffled feathers and depression were improved considerably (Plate 3). Mortality of 16% and 10% were observed in the aflatoxin alone and the *C. longa* + AF treated groups respectively during 2nd week to 4th week of age.

The clinical signs observed above were also noted by Jayaprakash *et al.*, (1992). The clinical signs were due to the toxicity of aflatoxin. The observed mortality rate in the current study is in close agreement with Johri *et al.*, (1990) who recorded 8.3 per cent mortality in broilers fed with diet containing 0.5 ppm AF for 8 weeks. Similarly, 26.6 per cent in 1 ppm AF for 6 week (Jayaprakash *et al.*, 1992) was also reported. On contrary Giambrone *et al.*, (1985) do not observed mortality or morbidity in broilers chicks fed with diets containing aflatoxin B₁ at the levels of 0.1, 0.2, 0.4 or 0.8 ppm from 2 to 5 weeks of age. So the mortality and morbidity depends upon the age, genetic strain, and duration of toxic agent, concurrent diseases, environmental conditions nutrient and immune status of the bird.

The decreased intensity of clinical signs and mortality percentage observed in the birds treated with *C. longa* + AF is credited to hepatoprotective properties of *C. longa*, which resulted in overall improvement in the health condition of the bird.

The effects of supplementation of *curcuma longa* on haematological parameters in induced aflatoxicosis in broiler birds

The effects of supplementation of *C. longa* on haematological parameters (PCV, Hb, TEC and TLC) in induced aflatoxicosis in broiler

birds are presented in table 1 and figure 1. The PCV Values (Mean \pm S.E) observed at 6th week in the control, aflatoxin alone and *C. longa* + AF treated groups of birds were 30.17 \pm 0.31, 26.17 \pm 0.30, 28.67 \pm 0.33 per cent, respectively.

The Hb Values (Mean \pm S.E) observed at 6th week in the control, aflatoxin alone and *C. longa* + AF treated groups of birds were 9.88 \pm 0.11, 8.29 \pm 0.19, 8.98 \pm 0.12 (g/dl), respectively.

The TEC Values (Mean \pm S.E) observed at 6th week in the control, aflatoxin alone and *C. longa* + AF treated groups of birds were 3.06 \pm 0.03, 2.87 \pm 0.03, 2.91 \pm 0.03 ($\times 10^6$ /cumm), respectively.

The TLC Values (Mean \pm S.E) observed at 6th week in the control group, aflatoxin alone and *C. longa* + AF treated groups of birds were 19.86 \pm 0.23, 15.77 \pm 0.25, 16.74 \pm 0.37 ($\times 10^3$ /cumm), respectively.

The results showed that the supplementation of 1 ppm aflatoxin alone as well as supplementation of *C. longa* (1%) + AF (1 ppm) in feed caused a significant decrease ($p < 0.01$) in PCV, Hb, TEC and TLC at 6th week as compared to control. However, the haematological parameters like PCV, Hb, TLC (except TEC) in *C. longa* + AF treated group were significantly higher ($p < 0.01$) than the AF alone treated group.

It seems clear from the present results that the AF has a deleterious effect on the PCV, Hb and TEC counts. These findings are in concurrence with Tung *et al.*, (1975); Lanza *et al.*, (1980); Balachandran and Ramakrishnan (1987) and Vasan *et al.*, (1998).

The basis for PCV depression was primarily due to decrease in red blood cell numbers. Hemoglobin concentration was depressed

analogous to PCV, reflecting anaemia. Tung *et al.*, (1975) also observed anaemia and suggested that aflatoxin associated anaemia as hemolytic anaemia characterized by decreased erythrocyte counts, packed cell volume, hemoglobin levels. This type of

anaemia could result from inhibition of haematopoiesis, defective haematopoiesis, increased destruction of red blood cells, or a combination of all the three (Huff *et al.*, 1986).

Table.1 Effects of supplementation of *C. longa* on haematological parameters at 6 weeks in induced aflatoxicosis in broiler birds

Groups	PCV (%)	Hb (g/dl)	TEC ($\times 10^6$ /cumm)	TLC ($\times 10^3$ /cumm)
Control	30.17 \pm 0.31 ^a	9.88 \pm 0.11 ^a	3.06 \pm 0.03 ^a	19.86 \pm 0.23 ^a
Aflatoxin	26.17 \pm 0.30 ^c	8.29 \pm 0.19 ^c	2.87 \pm 0.03 ^b	15.77 \pm 0.25 ^c
Turmeric + aflatoxin	28.67 \pm 0.33 ^b	8.98 \pm 0.12 ^b	2.91 \pm 0.03 ^b	16.74 \pm 0.40 ^b

Values are mean \pm SE of 6 observations (n=6)

Mean with different superscript in column wise are differing significantly (P<0.01)

Fig.1 Effects of supplementation of *C. longa* on haematological parameters at 6th week in induced aflatoxicosis in broiler birds

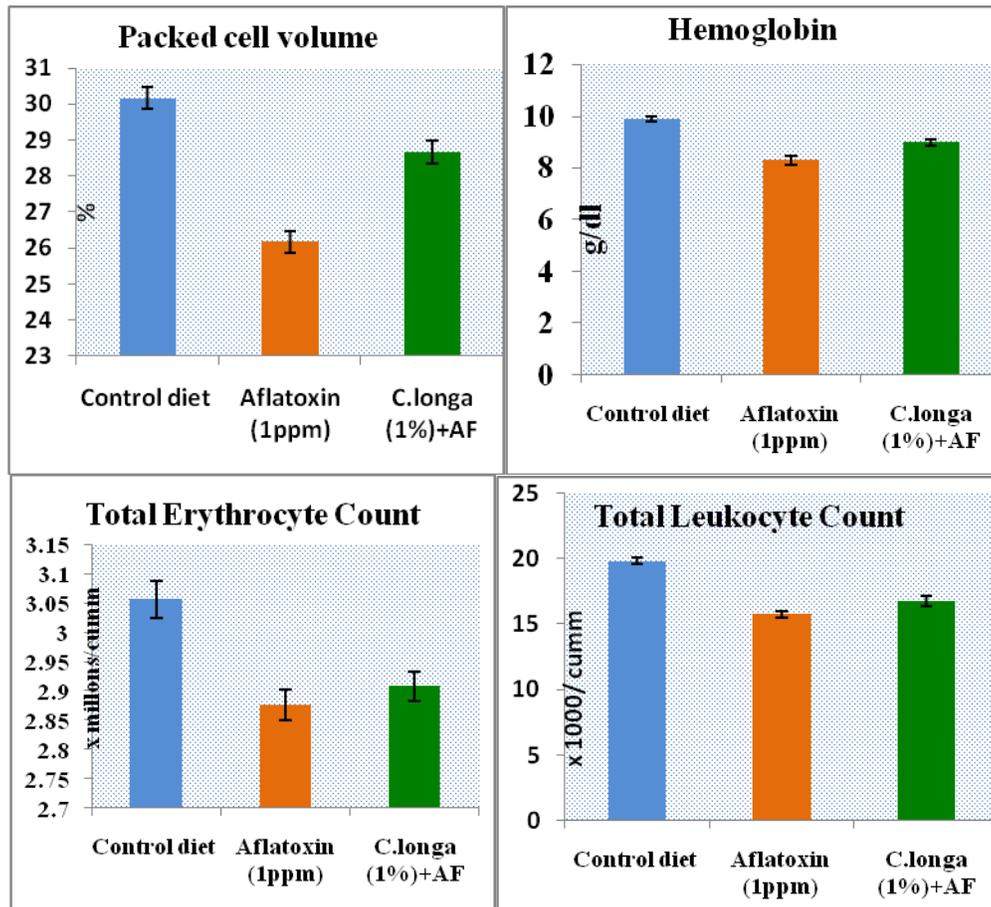


Plate.1 Photograph of broiler birds showing the normal signs in the control group



Plate.2 Photograph of broiler bird showing clinical signs like emaciation, stunted growth and ruffled feathers in the AF fed group



Plate.3 Photograph of the broiler bird showing clinical signs like marked depression, letharginess and stunted growth in AF+ *C. longa* fed group



Plate.4 Photograph of liver of broiler birds (Control) showing normal appearance



Plate.5 Photograph of liver of broiler birds (AF) showing mild enlargement and yellowish discoloration



Plate.6 Photograph of liver of broiler birds (AF+ *C. longa*) showing moderate degree of paleness



Plate.7 Photograph of broiler (Control) showing normal appearance of kidneys



Plate.8 Photograph of broiler birds (AF) showing enlargement and petechial hemorrhages in Kidneys

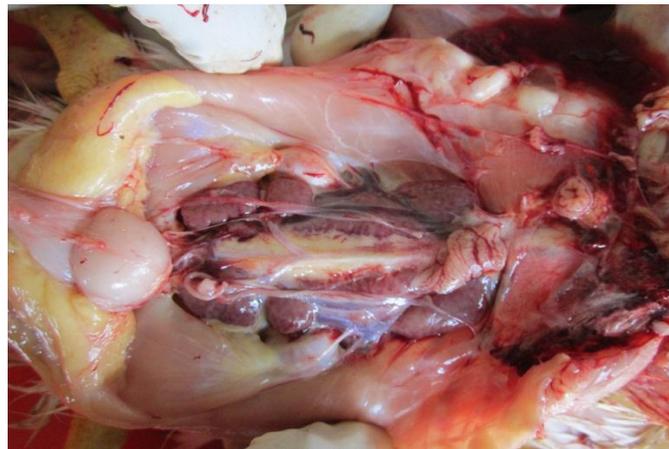


Plate.9 Photograph of broiler birds (AF + *C. longa*) showing mild degree of enlargement and hemorrhages in Kidneys



Plate.10 Section of liver of broiler birds (Control) showing normal appearance of hepatocytes. H&E X 400

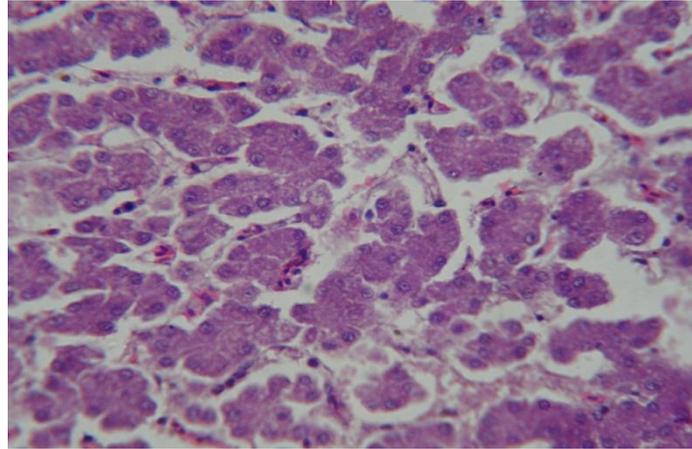


Plate.11 Section of liver of broiler birds (AF) showing severe fatty changes along sinusoidal dilatation. H&E X 400

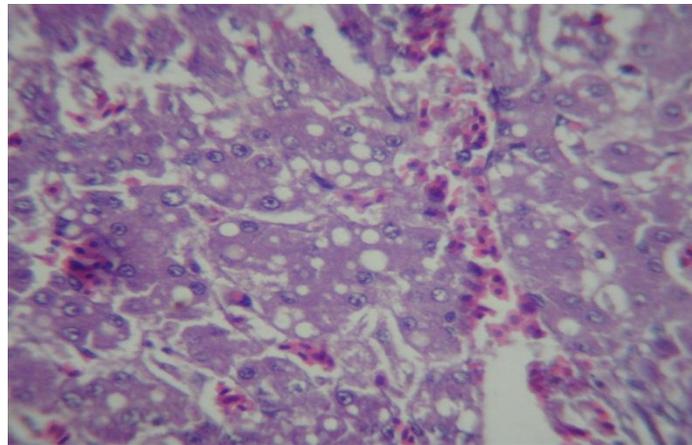


Plate.12 Section of liver of broiler birds (AF) showing lymphoid follicle formation. H&E X 400

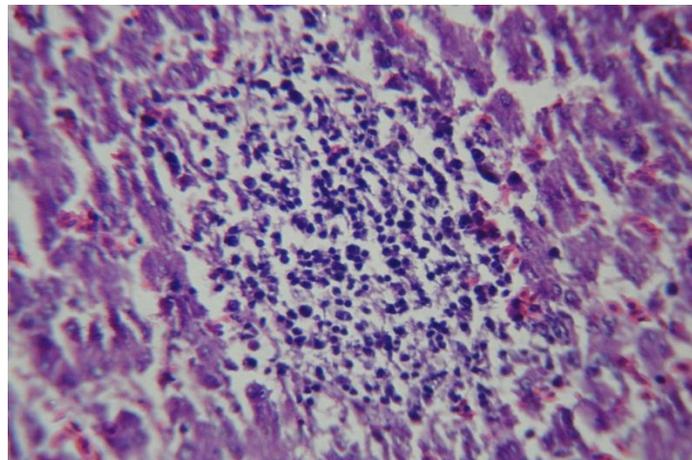


Plate.13 Section of liver of broiler birds (AF + *C. longa*) showing moderate fatty changes. H&E X 400

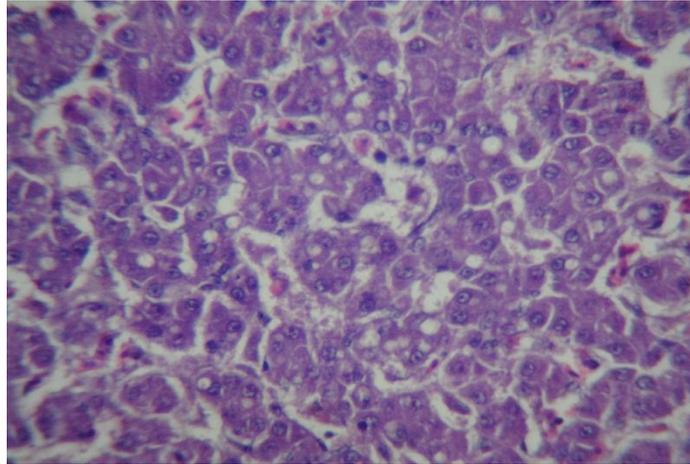


Plate.14 Section of the kidney of broiler birds (Control) showing the normal appearance. H&E X 400

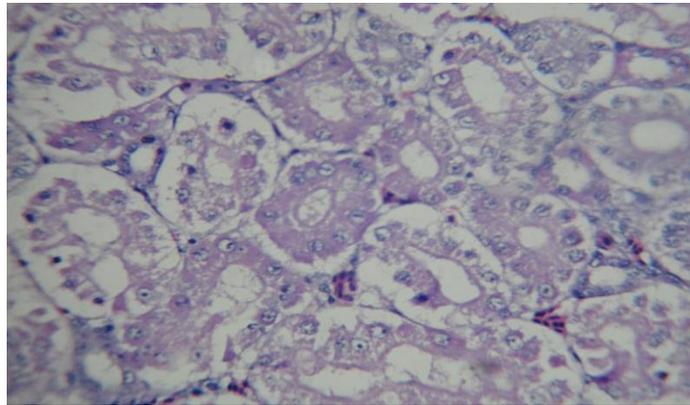


Plate.15 Section of the kidney of broiler (AF) showing the lymphoid follicle formation along with congestion. H&E X 400

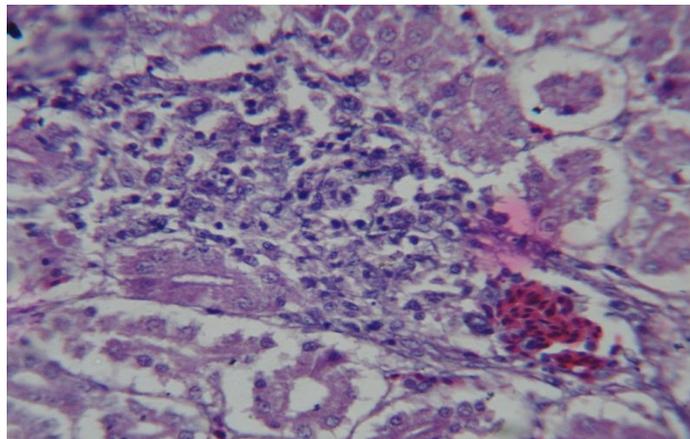


Plate.16 Section of the kidney of broiler birds (AF) showing severe haemorrhages. H&E X 400

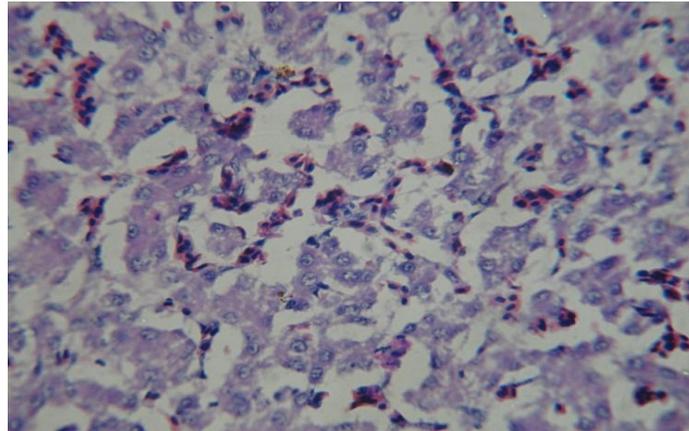


Plate.17 Section of the kidney of broiler birds (AF) showing severe degenerative and necrotic changes in the tubular epithelium. H&E X 400

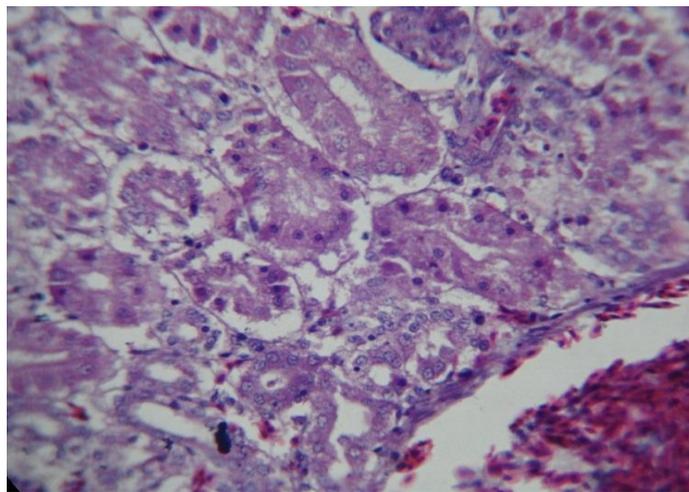
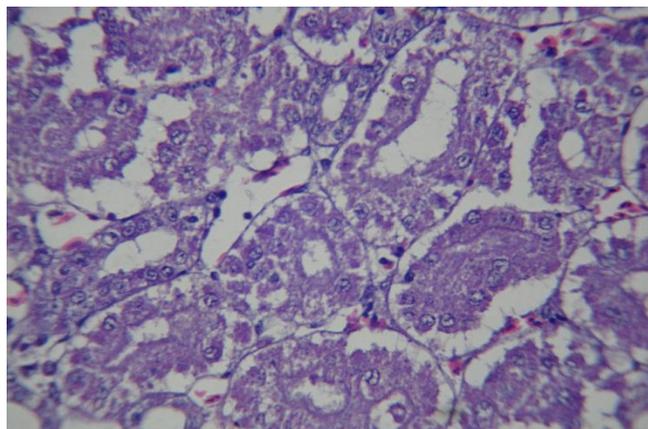


Plate.18 Section of the kidney of broiler birds (AF + *C. longa*) showing mild degenerative and necrotic changes in tubular epithelium. H&E X 400



Broiler birds treated with AF showed lower leucocyte levels; evidently this may be due to a considerable decrease in lymphocyte count. This may be indicative of the deterioration of the immune status. This finding comes in close agreement with Sharma *et al.*, (2011).

The co-administration of *C. longa* (1%) + AF (1 ppm) reduced significantly the magnitude of haematological alterations induced due to dietary AF (1 ppm) except the TEC counts. The improvement observed in haematological alterations were in agreement with the findings of Kurkure *et al.*, (2001); Mekala *et al.*, (2006); Sharma *et al.*, (2011).

Administration of *Curcuma longa*, were effective in reducing the adverse effect of AF on hemopoietic system supporting the hypothesis that plant products exhibits effective antioxidant agents. The active ingredient is Curcumin, essential oil (ptolymethylcarbinol), present in rhizome of the plant might contributed to the observed effects (Encyclopedia of Natural Medicine, 1998). Curcumin is an antioxidant and also a good scavenger of reactive oxygen species (ROS) and lowers its formation as well as the formation of inflammatory compounds such as prostaglandins and leukotrienes (Unnikrishnan and Rao, 1992) and protects erythrocyte membranes and phospholipid fatty acids from oxidation (Leela *et al.*, 1992).

Curcumin by virtue of its ability to inhibit AFB₁ adduct formation partially prevented the liver damage (Soni *et al.*, 1992). The protective effect on the haemopoietic system is also attributed to stimulating or protecting haematopoiesis in bone marrow and the subsequent increase of haematological constituents in the peripheral blood (Singh *et al.*, 2011). The increase in the TLC count could be attributed to the immunostimulating properties of the *Curcuma longa* (Grace *et al.*, 2010).

However, the supplementation of *C. longa* powder did not completely restore the haematological alterations induced by the AF, indicating its partial protection.

The effect of supplementation of *Curcuma longa* on pathological changes in induced in liver and kidneys aflatoxicosis of broiler birds

Effect on gross pathological changes

Grossly, liver of the aflatoxin induced broilers showed mild enlargement, yellowish discolouration and linear haemorrhages along with distension of gall bladder (Plate 4, 5). Kidneys had enlargement, congestion with mild petechial haemorrhages (Plate 7, 8). Spleen of the intoxicated birds exhibited marked enlargement and focal areas of haemorrhages.

The bursa of Fabricius showed mild to moderate atrophy. Severe haemorrhages and atrophy of thymus were also noticed in the present study. Mild congestion and watery contents were found in the intestinal mucosa. However, broiler of the group treated with AF + *C. longa* (1%) also had similar types of lesions with mild intensity (Plate 6, 9). The gross changes were in the close agreement with the findings of earlier researches Reddy *et al.*, (1982), Balachandran and Ramakrishnan (1987), Kurkure *et al.*, (2000) during aflatoxicosis in chicken.

Effect on the histopathological changes

Microscopically, the liver of aflatoxin intoxicated broilers revealed severe fatty changes, biliary hyperplasia, vacuolar degenerative and necrotic changes, sinusoidal congestion and infiltration of mononuclear cells (Plate 10-13). Whereas liver from the birds treated with *C. longa* + AF also had mild congestion and degenerative and

necrotic changes along with mild lymphoid aggregation. However, these changes were remarkably less in *C. longa* + AF group in comparison to AF alone fed group. These changes were in accordance with the findings of Ghosh *et al.*, (1989), Kurkure *et al.*, (2000) and Mekala *et al.*, (2007) in experimental aflatoxicosis. Thus, inclusion of turmeric powder resulted in lesser degree of hepatic changes indicating hepatoprotective and hepato regenerative effects (Mekala *et al.*, 2007).

Histopathologically, kidneys of the aflatoxin fed broilers exhibited atrophy of glomeruli, albuminous cast in the convoluted tubule, congestion and degenerative and necrotic changes and focal infiltration of mono nuclear cells.

However, birds of the group supplemented with *C. longa* + AF also showed very mild congestion, degenerative and necrotic changes along with infiltration of mononuclear cells (Plate 14-18). Similar changes were also reported earlier by Balachandran and Ramakrishnan (1987), Ghosh *et al.*, (1989), during mycotoxicosis in chicken.

Protective action of turmeric in induced aflatoxicosis is due to its active principle curcumin (Soni *et al.*, 1992) which might have contributed to partial protection of liver and kidneys as observed in the present study.

The present experiment showed that broiler chicks consuming an aflatoxin (1 ppm) supplemented diet experienced undesirable haematological alterations.

The addition of *C. longa* rhizome powder (1 %) to the aflatoxin-contaminated diet *C. longa* significantly improved the haematological parameters in induced aflatoxicosis and partially protected against the cytotoxic effects of AFB₁ in broiler birds.

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