



Combined effect and amelioration of aflatoxin and citrinin in broilers: A histopathological study

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Abstract : Aflatoxin was produced from *Aspergillus parasiticus* and citrinin was produced from *Penicillium citrinum* and both were quantified using thin layer chromatography. The toxins, aflatoxin at the rate of 1ppm and citrinin at the rate of 25 ppm, were mixed in broiler feed. Activated charcoal (0.4%) and/or lyophilized yeast culture (0.2%) were added as adsorbents. Four diets for broilers were prepared – Diet 1- basal diet (control), Diet 2- basal diet added with aflatoxin (1ppm) and citrinin (25ppm), Diet 3- basal diet added with aflatoxin (1ppm), citrinin (25ppm) and activated charcoal (0.4%) and Diet 4- basal diet added with aflatoxin (1ppm), citrinin (25ppm), activated charcoal (0.4%) and lyophilized yeast culture (0.2%). These four diets were fed for 6 weeks to four group of broiler chicks with four replications of eight birds in each group using Completely Randomized Design. The histopathological studies revealed central vein congestion, focal lymphoid aggregates, and bile duct hyperplasia in the liver, degenerative changes, intertubular congestion and hemorrhages in kidney, cystic spaces and lymphoid depletion in bursa of Fabricius, depleted germinal centres and interfollicular arteries in spleen of birds fed on diet 2. On diet 3, the lesions were moderate degree and on diet 4 the lesions were very mild.

Key words : Aflatoxin, Citrinin, Activated charcoal, Lyophilized yeast, Amelioration

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INTRODUCTION

Mycotoxins in poultry feed is causing heavy economic losses due to effecting the health of the broilers and may lead to death of the poultry birds (Coman *et al.*, 2007). Aflatoxin, citrinin, ochratoxin, zearalenone, sterigmatocystin were identified in different parts of India (Devegowda and Murthy, 2005). Natural occurrence of aflatoxin B1, ochratoxin A and citrinin (Pereyra *et al.*, 2009; Anand kumar and Balachandran, 2005 and Markov *et al.*, 2013) observed in feeds. These hepato- nephrotoxic mycotoxins aflatoxin and citrinin are secondary metabolites of fungi *Aspergillus parasiticus* and *Penicillium citrinum*, respectively (Raja and Lakshmanachar, 1991 and Mahmoud, 1993). The natural occurrence of aflatoxin and citrinin in the feed ranged from 40 and 800ppb (Ahmad and Vairamuthu,

2000). Co-occurrence of these two mycotoxins affect the productivity of broiler chicken by producing lesions in many organs, lowering the growth rate, feed conversion and resistance to infectious diseases by impairing both cellular and humoral immunity (Coulombe, 1993). Combined effect of aflatoxin and citrinin showed significant decrease in hematological and serum biochemical values (Ahmad *et al.*, 2006 and Priyadarshini and Narasareddy, 2010). Aflatoxin and citrinin showed pathological effect on liver, spleen, bursa of fabricius, spleen and intestine (Ahmad and Vairamuthu 2006; Kumar and Balachandran, 2014 and Anand kumar and Balachandran, 2014).

The present study is to test the combined effect of aflatoxin and citrinin in contaminated broiler feed and the efficiency of two adsorbents, activated charcoal (0.4%) and lyophilized yeast culture (0.2%) on the performance of broilers and histopathology of vital organs in broilers.

RESEARCH METHODOLOGY

Aflatoxin was produced by growing *Aspergillus parasiticus* NRRL 2999 culture on broken rice using the method of (Shotwell *et al.*, 1966). Citrinin was produced by growing *Penicillium citrinum* (MTCC-2547) on maize flakes, extracted and estimated by method of (Chandrasekharan *et al.*, 1999) using thin layer chromatography.

One hundred and twenty eight day old female broiler chicks of vencob strain were randomly distributed into 4 groups with 4 replications of 8 birds in each replication. The experimental design was Completely Randomized Design with four groups of chicks which were fed the following experimental groups: Group 1-Basal group (control group), Group 2-Basal group + 1 ppm aflatoxin +25 ppm citrinin, Group 3-Basal group + 1 ppm aflatoxin +25 ppm citrinin+0.4% activated charcoal, Group 4-Basal group + 1 ppm aflatoxin +25 ppm citrinin + 0.4% activated charcoal + 0.2% lyophilized yeast culture. Body weight and feed consumption were taken weekly and feed conversion ratio was calculated. At the end of the experiment (6 weeks), the visceral organs like liver, kidney, spleen and bursa of Fabricius of representative birds of each group were collected and recorded the gross morphological changes.

RESULTS AND DISCUSSION

Gross pathology:

Liver:

Liver of group 2 birds was pale and enlarged with rounded borders (Anand kumar and Balachandran, 2014). This was observed in individual aflatoxicosis affected birds (Anand kumar and Balachandran, 2005) The livers of groups 3 and 4 were moderately pale with slight enlargement.

Kidney:

Kidney of group 2 birds was moderately enlarged with occasional hemorrhages. All the lobes of the kidneys were uniformly enlarged (Anand kumar and Balachandran, 2014). Mild to moderate enlargement of kidneys with mild hyperemic changes were observed in groups, 3 and 4.

Spleen:

Spleen of group 2 birds was moderately congested with occasional mottling appearance (Anand kumar and Balachandran, 2014). Splens from groups 3 and 4 showed mild congestion. The birds from group I did not reveal any lesions of significance in these organs.

Bursa of fabricius:

Bursa of Fabricius from group 2 showed hyperemic changes with mild enlargement in few birds and atrophy in few birds (Anand kumar and Balachandran, 2014). On sectioning bursal folds showed edematous appearance. Very mild hyperemic changes in group 3 birds and no apparent lesions of pathological significance were seen in-group 4.

Histopathology :

Liver:

Sections of the livers from this group 2 revealed moderate central vein congestion, focal lymphoid aggregates and bile duct hyperplasia (Plate 1) few sections also showed sinusoidal dilatation and round cell in filtration. These

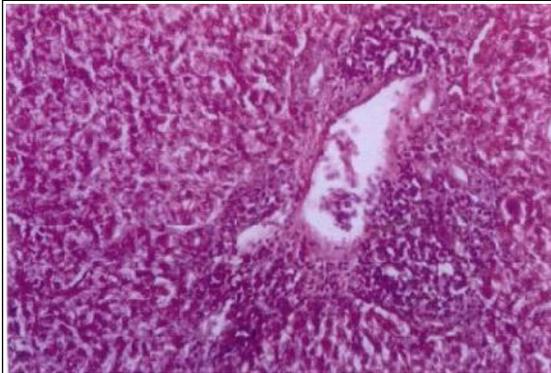


Plate 1 : Liver of group 2 showing moderate central vein congestion, focal lymphoid aggregates and bile duct hyperplasia

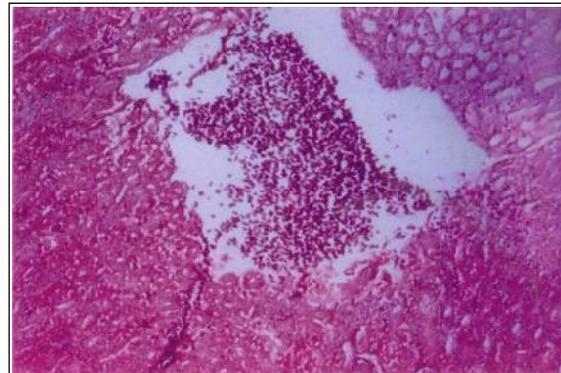


Plate 4 : Kidney of group 2 showing marked intertubular congestion and hemorrhages

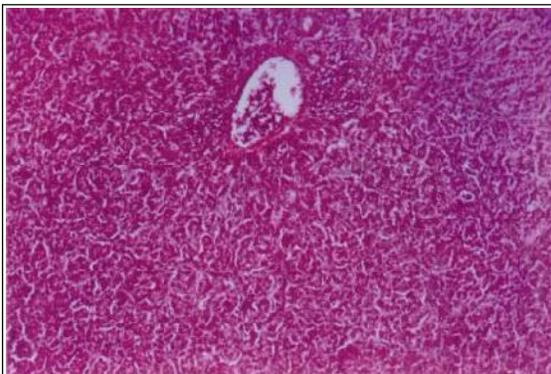


Plate 2 : Liver of group 3 showing moderate central vein congestion and mild dilatation of sinusoidal spaces

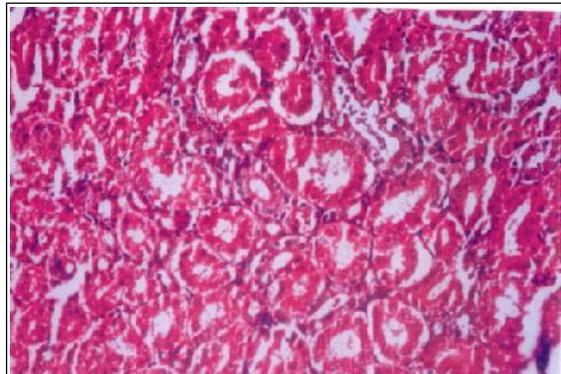


Plate 5 : Kidney of group 3 showing moderate degenerative changes and focal areas of infiltration by lymphocytes

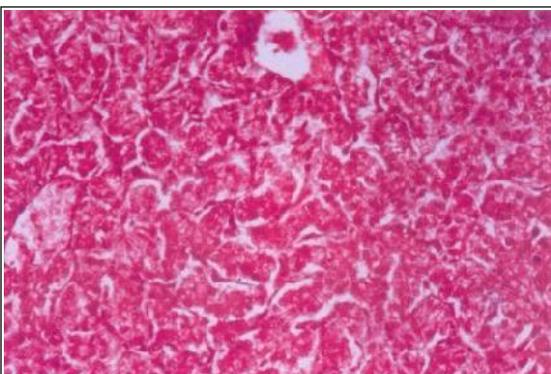


Plate 3 : Liver of group 4 showing ductular arrangement of hepatic cells indicating regeneration of hepatic cells

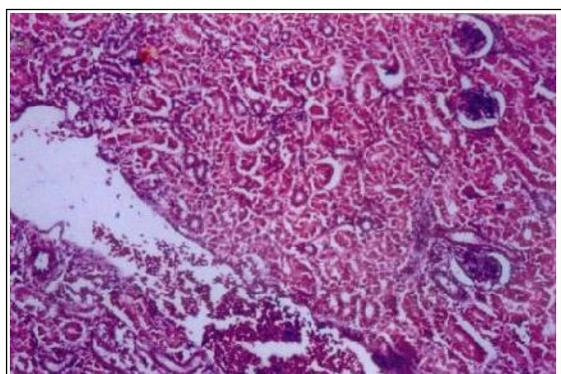


Plate 6 : Kidney of group 4 showing moderate intertubular congestion with swollen glomerular tufts

results were in agreement with Ahmad and Vairamuthu (2001) and Anand kumar and Balachandran (2014) which reported bile duct hyperplasia, periductular mononuclear cell infiltration and fibrosis. Liver section from group 3 showed moderate central vein congestion and mild dilatation of sinusoidal spaces (Plate 2). Sections from group 4 revealed mild focal areas of lymphoid aggregates with degenerative hepatic cells. Few sections revealed ductular arrangement of hepatic cells indicating regeneration of hepatic cells (Plate 3).

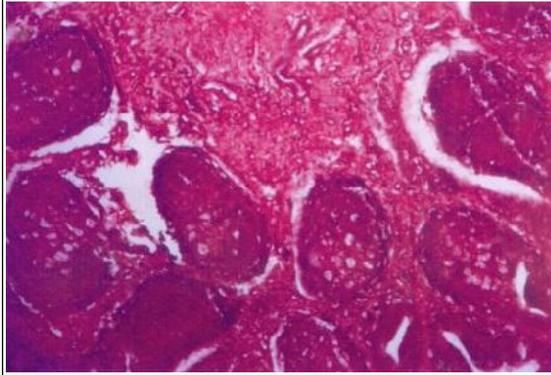


Plate 7 : Bursa of fabricius from group 2 showing areas of cystic spaces in the follicles

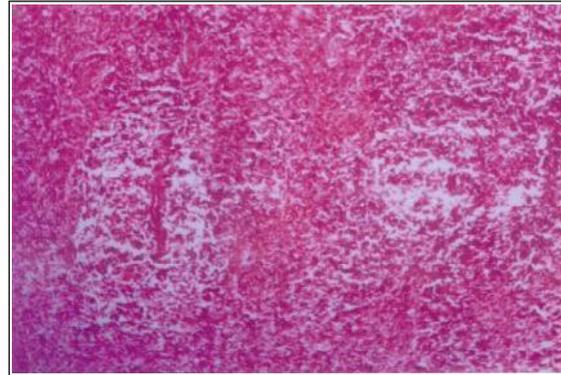


Plate 10 : Spleen of group 2 showing moderately depleted germinal centres

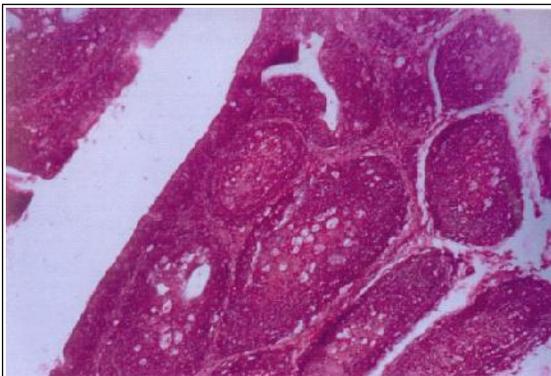


Plate 8 : Bursa of fabricius from group 3 showing moderate cystic spaces in the follicles

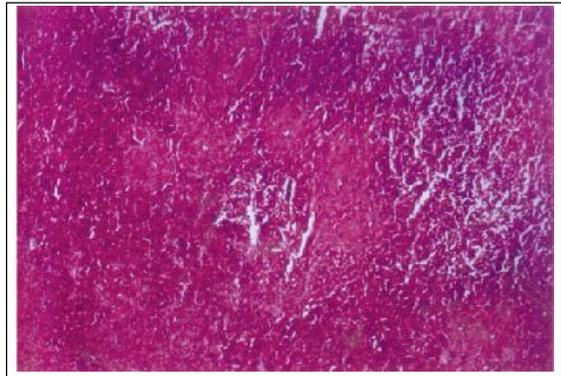


Plate 11 : Spleen of group 3 showing mild depleted germinal centres

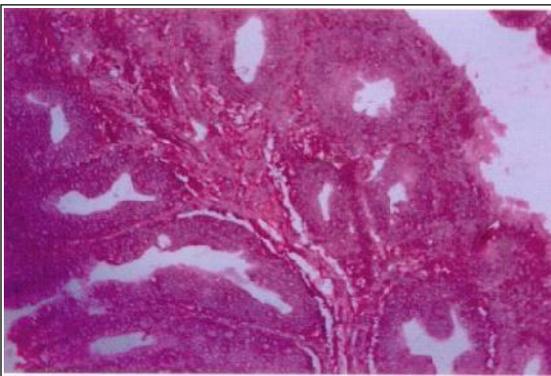


Plate 9 : Bursa of fabricius from group 4 showing cystic spaces with moderate interfollicular hemorrhages

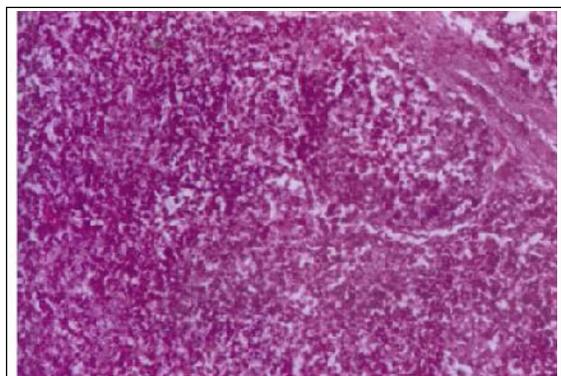


Plate 12 : Spleen of group 4 showing very mild depleted lymphoid follicles

Kidney:

Section from group 2 showed marked intertubular congestion (Plate 4) and hemorrhages. Few sections also revealed marked degenerative changes with some tubules showing casts. Anand kumar and Balachandran (2014) showed similar findings reporting that degeneration and necrotic changes in the tubular epithelial cells in kidney. Ahmad and Vairamuthu (2001) reported mild to moderate congestion or hemorrhage, diffuse degeneration, luminal dilatation with hyaline casts in kidneys. The sections of kidney from group 3 revealed moderate degenerative changes and focal areas of infiltration by lymphocytes (Plate 5). Kidney sections from group 4 showed moderate intertubular congestion (Plate 6) with swollen glomerular tufts. Few sections also revealed very mild intertubular fibrosis.

Bursa of fabricius:

Sections of Bursa of fabricius from group 2 revealed areas of cystic spaces in the follicles (Plate 7). Sections from few birds revealed marked inter follicular hemorrhages in addition to the cystic spaces. Similar findings observed by Anand kumar and Balachandran (2014) observed that lymphocytolysis, severe atrophy of follicles with corrugation of plical epithelium, interfollicular fibrosis and multiple cysts. Sections of bursa of fabricius of group 3 showed moderate cystic spaces in the follicles (Plate 8). Some areas also revealed mild interfollicular congestion. Sections of group 4 revealed areas of cystic spaces with moderate interfollicular hemorrhages (Plate 9). Cystic spaces were also seen in follicular epithelium in few sections.

Spleen:

Spleen section from group 2 birds revealed moderate congestion of trabecular arteries (Plate 10). Sections also revealed moderately depleted germinal centres. Ahmad and Vairamuthu (2001) reported that spleen showed mild to moderate lymphoid depletion and degeneration of lymphocytes. In contrary increase in germinal centres was observed by Anand kumar and Balachandran (2014). Peng *et al.* (2014) reported that increased necrotic cells and vacuoles in the splenic corpuscles and periarterial sheath. Sections of group 3 showed mild depleted germinal centres. Sections of group 4 also revealed very mild depleted lymphoid follicles (Plate 11). Some sections showed mild congestion of trabecular arteries (Plate 12).

Conclusion:

The research summarizes that activated charcoal partially adsorbed the toxins and could protect the birds from toxic effect partially, whereas activated charcoal and lyophilized yeast culture had complementary effect in ameliorating the effect of aflatoxin and citrinin in broiler chicks.

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