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ORIGINAL ARTICLE

Effect of Esterified Glucomannan in Amelioration of Aflatoxin Induced Microscopic Changes in Broiler Chicks

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Abstract

A biological experiment was conducted to evaluate the efficacy of esterified glucomannan in counteracting aflatoxicosisin broiler chicks. The chicks were given aflatoxin (AF) free diet oraflatoxin (1ppm) containing diet or aflatoxin (1ppm) with esterified glucomannan (EG, 0.1%) containing diet continuously from day-old to six weeks of age. At the end of the study, the liver, kidneys, spleen and bursa of Fabricius were collected for histopathological studies. Microscopically, the liver of the AF fed broilers showed fatty change, degeneration and necrosis of hepatocytes and the cellular infiltration around the portal triads and blood vessels. Kidneys of AF intoxicated birds revealed degeneration and necrosis of tubular epithelial cells along with cellular infiltration, congestion and haemorrhages in the parenchyma, severe fatty change and mild infiltration of lymphoid cells in the interstitial spaces. In spleen, congestion, multifocal areas of haemorrhages and mild lymphocytic activity of periarteriolar lymphatic tissue with increased number of histiocytes were observed in AF fed birds. Mild to moderate degree of congestion, increased number of histiocytes with heterophil infiltration, connective tissue proliferation, sparse cellularity of the follicles with presence of large number of histiocytes containing cellular debris were observed in bursa of Fabricius of AF treated birds. Feeding of EG along with AF showed milder bile duct hyperplasia and hypertrophy in liver and lowered the severity of tubular necrosis in kidneys. However, supplementation of EG has no recognizable counteracting effect on histopathology of spleen. Moderate degree of lymphoid hyperplasia in bursa of Fabricius in broilers receiving both AF and EG compared to AF alone treated group is indicative of ameliorative action of EG.

Keywords: Broiler chicks, Aflatoxin, Esterified glucomannan, Histopathology.

Introduction

Fungi are ubiquitously present in the agricultural products and some of these produce toxic metabolites known as mycotoxins. Mycotoxins are a relatively large, diverse group of naturally occurring secondary metabolites secreted by toxigenic fungi of genera *Aspergillus, Penicillium and Fusarium*. They are produced in cereal grains as well as forages before, during and after harvest, in favourable environmental conditions such as hot and humid climate (Katole *et al.*, 2013). Aflatoxins (AF) are group of closely related mycotoxin that can be produced by three species of *Aspergillus; A. flavus, A. Parasiticus* and the rare *A. Romius* growing on a variety of feedstuffs, mainly maize, peanuts and cottonseed (Dersjant-Li *et al.*, 2003). Toxicological spectrum of various mycotoxins is very wide encompassing different kind of toxicities viz. acute and chronic toxicities, carcinogenicity, genotoxicity, immunotoxicity, mutagenicity, and teratogenicity in animals and poultry (Patil *et al.*,

2014). Aflatoxicosis in poultry also causes listlessness, anorexia with lowered growth rate, poor feed utilization, decreased egg production and increased mortality (Miazzo *et al*., 2000). Additionally, anaemia, reduction of immune function (Oguz *et al*., 2003), hepatotoxicosis, haemorrhage (Ortatatli and Oguz, 2001), teratogenesis (Sur and Celik, 2003), carcinogenesis and mutagenesis are associated with aflatoxicosis. These mycotoxins produces deleterious effects grossly and histopathologically in the liver, spleen, kidneys and other vital organs which leads to decreased growth and performance in poultry (Sandhu *et al*., 2005). The incidence of hepatocellular tumours, particularly in poultry, is considered to be one of the serious consequences of aflatoxicosis (Dalvi, 2005). Aflatoxicosis affects mainly liver and kidneys which leads to jaundice, generalized oedema, haemorrhages, tan or yellow colour discoloration of liver, periportal necrosis with bile duct proliferation and fibrosis; and depletion of lymphoid organs (Charlaton, 2006). Gross pathological investigation may reveal a swollen and yellowish fatty liver with haemorrhages on its surface and swelling of kidneys, while histopathological changes reveal disorganization of hepatic structure (dystrophy) and a severe necrosis of parenchyma cells (fatty necrosis infiltration) accompanied by a proliferation of bile vessels (Rosa *et al*., 2001).

Several physical, chemical and biological approaches have been proposed to detoxify mycotoxin contaminated feed and feedstuffs. Live yeast, *Sacchromyces cerevisiae* was found to alleviate the adverse effects of aflatoxins in poultry (Churchil *et al*., 2009). The beneficial effects of *Sacchromyces cerevisiae* have been attributed to mannan in the cell. The studies performed with esterified glucomannan (EG) (0.5 and1g/kg) of yeast cell wall with different concentrations of AF (0.05 to5 mg/kg) in broilers (Maldhure and Churchil, 2009) showed that EG supplementation can partially or completely reverse AF induced toxicity on performance, biochemistry, haematology and immune responses of birds. However, the ameliorating effect of EG on histopathology of vital organs has not been adequately studied during aflatoxicosis so far. The aim of this study was therefore to investigate the efficacy of EG against AF toxicity in broiler chicks by observing its effects on liver, kidneys, spleen and bursa of Fabricius.

Materials and Methods

*Aspergillus parasiticus var globosus**411 obtained from Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology (IMTEC), Chandigarh, India was used for the production of AF. The fungus was maintained by sub culturing it on potato dextrose agar at 10 days interval (Shotwell *et al*., 1966). Aflatoxin was grown on the rice culture and the quantitative measurement of AF in the mouldy rice was done by standard method (Romer, 1975). Feed ingredients (maize, wheat bran, soybean meal, unsalted dried fish and sesame oil cake) used for the preparation of basal diet were screened for the presence of AF in the same method (Romer, 1975). The feed ingredients free of AF were only used for the preparation of experimental diets. The basal diet (T1) was formulated as per Bureau of Indian Standards for poultry feeds (BIS, 1992). The mouldy rice containing known quantity of AF and an EG containing commercial product (Mycosorb® Alltechinc., Bangalore) were incorporated either alone or in combination, so as to prepare other experimental diets. The diet T2 contained 1ppm AF, whereas T3 contained 0.1% EG in addition to 1ppm AF.

A total of 18 day-old broiler chicks were randomly allotted to three treatment groups with six birds each. The treatment diets were fed to the experimental groups from day-old to six weeks of age. Standard management procedures were followed during the course of experiment. At the end of the study, tissue samples were taken from all the birds. Detailed necropsy was done and gross lesions observed were recorded. Representative tissue samples from liver, kidneys, spleen and bursa of Fabricius were collected and preserved in 10% neutral buffered formalin. The tissues were subjected to routine histopathological examination after staining with haematoxylin and eosin (Bancroft and Stevens, 1996).

Results and Discussion

Macroscopically, the lesions present in birds fed AF with or without EG were swollen, pale and friable liver indicating not recognizable ameliorating effect of EG on AF induced gross lesions. The gross lesions observed in AF treated birds were similar to the earlier observations (Ortatatli *et al*., 2005). Enlargement in liver due to aflatoxicosis has also been reported in other avian species including Japanese quails (Parlat *et al*., 1999), ducks (Ostrowski-Meissner, 1984) and water fowls (Robinson *et al*., 1982). Microscopically, liver of the AF fed broiler chicks showed diffuse fatty change characterized by a clear round vacuoles in the cytoplasm of hepatocytes as a prominent feature. Liver parenchyma also showed individual cell necrosis, congestion, dilated sinusoidal spaces and cellular infiltration around triads and blood vessels (Fig 1). Fatty changes in hepatocytes were noticeably higher in AF treated birds. The hepatocytes showing fatty vacuoles coalesced to form fatty cysts. The architecture of the liver was completely altered and the regenerating hepatocytes were arranged in acinar or ductular patterns. The acinar hepatocytes also showed fatty

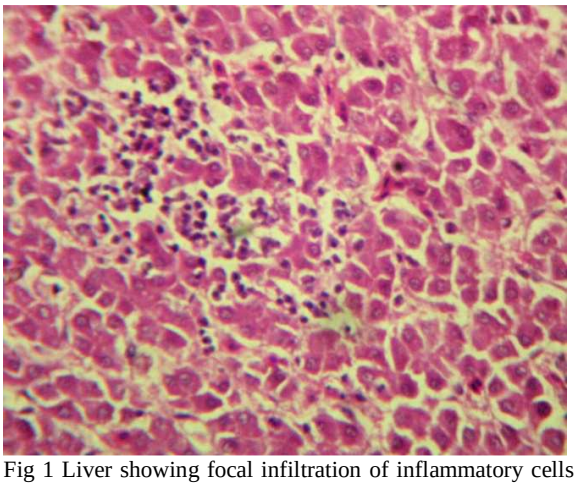

Fig 1 Liver showing focal infiltration of inflammatory cells in AF fed birds – H&E 100X

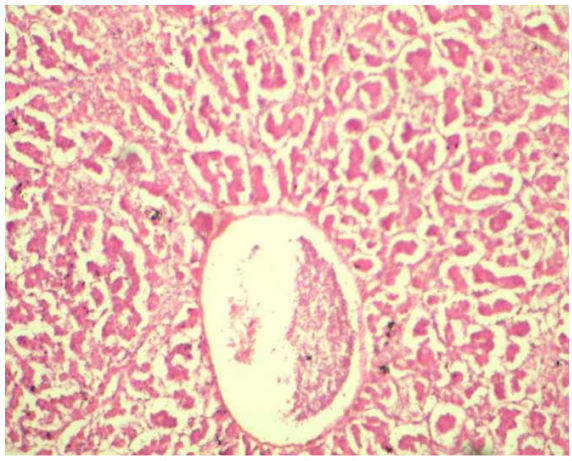

Fig. 2 Liver showing vacuolar degeneration, engorged central vein and necrosed hepatocytes in AF fed birds – H&E 100X

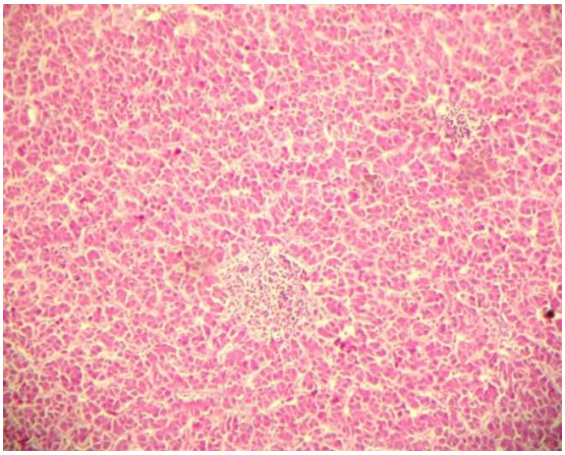

Fig 3: Liver showing congested central vein and increased sinusoidal space in AF +EG fed birds – H&E 20X

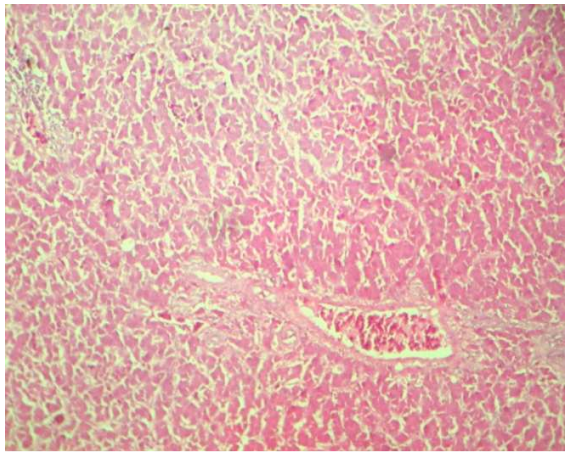

Fig 4: Liver showing engorged central vein and increased sinusoidal spaces in AF + EG birds- H&E 40X

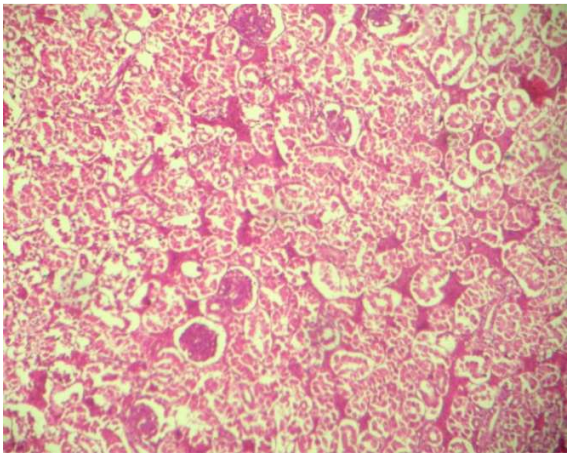

Fig 5: Kidneys of AF treated birds showing degenerated tubular epithelial cells and engorged blood vasculature – H&E 40X

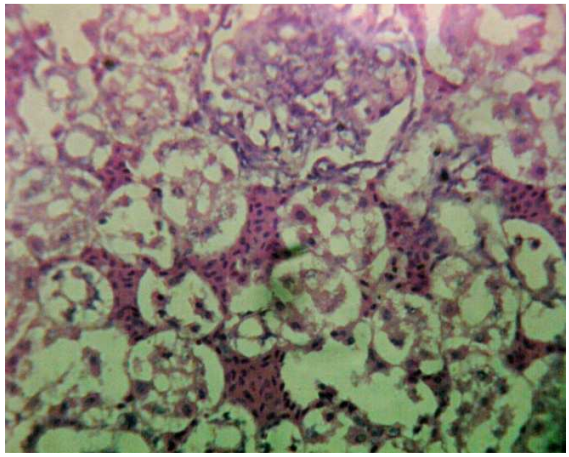

Fig 6: Kidneys of AF treated birds showing degenerated tubular epithelial cells and severe hemorrhagic infiltration in inter tubular space – H&E 100X

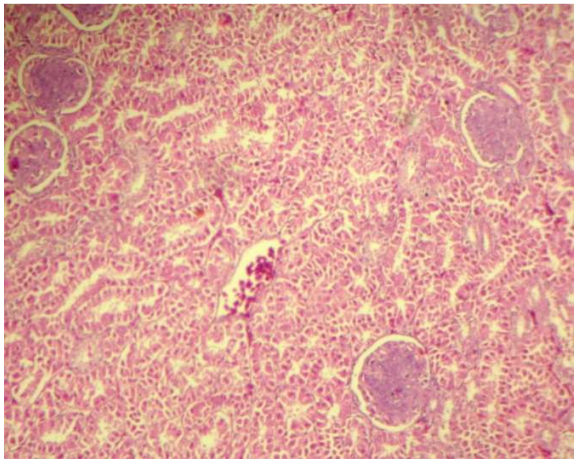

Fig 7: Kidneys of AF + EG treated birds showing less severe tubular damage – H&E 40X

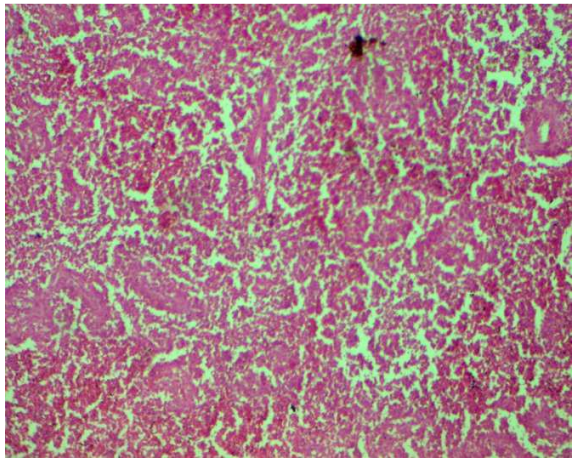

Fig 8: Spleen of AF treated birds showing depleted lymphoid follicles – H&E 40X

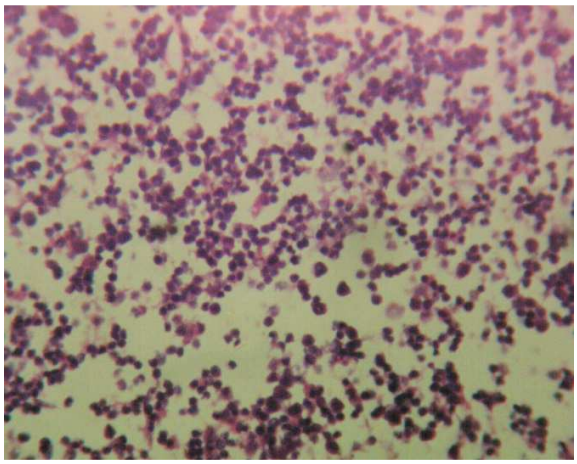

Fig 9: Bursa of Fabricius of AF treated birds showing depleted lymphoid follicle – H&E 100X

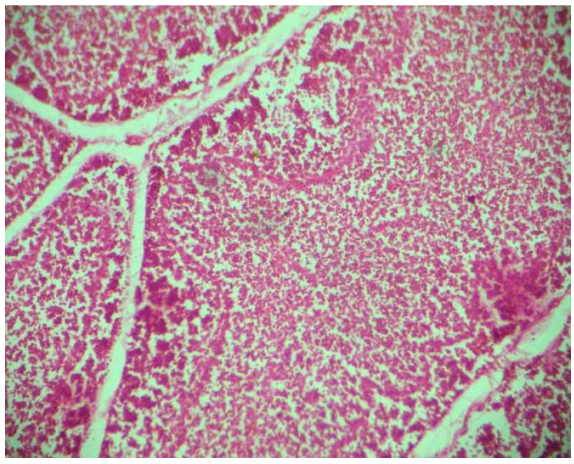

Fig 10: Bursa of Fabricius of AF + EG treated birds showing intact lymphoid follicles – H&E 40X

change in some areas similar to the earlier observations (Kumar and Balachandran, 2009). Many hepatocytes were swollen in periportal and mid-zonal areas. Severe haemorrhages were detected in some liver sections similar to the previous findings (Yildirim *et al*., 2011). Birds received EG along with AF showed moderate fatty changes with dilated sinusoidal spaces and necrosis of hepatocytes (Fig 4). The lesions were of milder degree in birds fed with EG along with AF. Supplementation of EG slightly avoided the lesions like vacuolar degeneration of hepatocytes and cellular infiltration. The lesions like bile duct hyperplasia and hypertrophy were ameliorated at a higher degree by the supplementation of EG.

Kidneys of AF intoxicated birds revealed degeneration and necrosis of tubular epithelial cells as a prominent feature along with cellular infiltration, congestion and haemorrhages in the parenchyma (Fig 5). In addition, occasional thickening of basement membrane was seen in the AF fed birds as reported earlier (Kumar and Balachandran, 2009). Hyaline casts within the lumens were also detected in some kidney sections similar to the earlier observations (Yildirim *et al*., 2011). Severe fatty change with considerable loss of architecture due to accumulation of large fat droplets in addition to mild infiltration of inflammatory cells in the interstitial spaces of kidneys were also evident in addition to vacuolar degeneration of renal tubular cells. Such changes have also been reported by earlier researchers in birds intoxicated with aflatoxins (Ortatatli and Oguz, 2001). Kidneys of birds supplemented with EG in feed containing AF showed histopathological lesions like thickening of the glomerular basement membranes with the presence of

hyaline droplets within the tubule. Tubular degeneration/damage was less severe (Fig 7). However, supplementation of EG decreased histopathological changes of tubular necrosis with 100% incidence in T2 but only 66.7% in T3 indicating the ameliorating effect of EG.

Gross examination of the spleen showed slight enlargement and congestion in AF fed birds. Histologically, congestion, multifocal areas of haemorrhages and mild lymphocytic activity of periarteriolar lymphatic tissue with increased number of histiocytes were observed in AF fed birds. The spleen showed lymphoid depletion, an increase in the number of germinal centres and reticulum cell hyperplasia in toxin treated birds. Lesions observed in the spleen of toxin treated birds were similar to earlier reports (Kumar and Balachandran, 2009). Supplementation of EG has not resulted in any significant ameliorative effect of histopathological changes in spleen.

Grossly, atrophy of bursa of Fabricius was consistently observed in the toxin fed birds and comparatively bigger size of the bursa of Fabricius was evident in EG supplemented group. The bursa of Fabricious of all the groups showed no other visible gross lesion. Microscopically, mild to moderate degree of congestion and increase in number of histiocytes with heterophil infiltration was observed in AF treated birds. Further prominent connective tissue proliferation, sparse cellularity of the follicles with the presence of large number of histiocytes containing cellular debris indicating pronounced lymphocytic activity in birds affected by aflatoxicosis (Fig 9). These findings were in conformation with the earlier findings (Ortatatli and Oguz, 2001; Kumar and Balachandran, 2009). Moderate degree of lymphoid hyperplasia with well-defined follicles filled with lymphoid cells was in EG supplemented groups indicating its beneficial effect (Fig 10).

Conclusion

In conclusion, dietary AF at 1 ppm caused extensive damage to the architecture of vital organs. It can affect liver severely which was evident during histopathological examination including disorganization of hepatic structure and a severe necrosis of parenchyma cells accompanied by proliferation of bile vessels. It also caused hepatic fibrosis and depletion of lymphoid organs. The supplementation of EG has no effect on AF induced macroscopic changes. However, it ameliorated the toxicity of AF in terms of microscopic lesions of liver, kidneys and bursa of Fabricius but with no noticeable effect on spleen. The results indicated that EG has the ability to adsorb AF with its subsequent elimination through faeces thereby conferring protection to the vital organs from AF induced microscopic changes.

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