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Study on efficacy of diatomaceous earth to ameliorate aflatoxin induced patho-morphological changes in kidneys of broiler chicken

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Abstract
The present study was carried out at Department of Pathology, Veterinary College, Bangalore, India from July 2014 to December 2014. The ability of Diatomaceous earth (DAE) in reducing the detrimental effects of aflatoxin (AF) on kidneys of broiler birds was evaluated following supplementation of 2000 mg DAE along with 0.5 and 1 ppm of AF/Kg feed. A total of 240 healthy day old broiler chicks were divided into 6 groups comprising of control and treatment groups. AF feeding caused enlargement, friable texture, yellowish discoloration and congestion of kidneys. Histopathology revealed varying degree of congestion and haemorrhages, glomerulo-tubular degeneration and mononuclear as well as heterophilic cellular infiltration in renal parenchyma.
Co-treatment with DAE and aflatoxin resulted in significant decrease in severity and magnitude of renal lesions. The study concluded that 2000 mg of DAE in feed can be effectively used to reduce the histotoxic effects of aflatoxin in broiler chicken.

Keywords: Aflatoxin, 2% diatomaceous earth, amelioration, gross and histopathology, kidneys, broiler chicken

1. Introduction
Aflatoxins (AF) are secondary metabolites and a class of mycotoxins produced predominantly by *Aspergillus flavus* and *A. Parasiticus* [4]. These toxins are present worldwide in feeds and cause severe economic losses to the poultry and livestock industries [26]. Studies showed the adverse effects of aflatoxin in broiler chickens including decrease in body weight gain, efficiency of feed utilization, alteration in serum biochemical parameters, pathologic changes in visceral and lymphoid organs as well as altered immune responses [49, 20, 21, 22]. Aflatoxins disturb the renal function through increasing the relative weight of kidneys [35], inducing congestion in renal sinusoids [14], degenerative and necrotic changes in renal tubular epithelium [30, 14, 50] and reduce the glomerular filtration rate [11].

Producers, researchers and governments aim to develop effective prevention management and decontamination technologies to minimise the toxic effects of AF [37]. Approaches used have included the physical, chemical and biological treatment of contaminated feed and feed stuffs. The adsorbent materials (Aluminosilicates, Bentonite, Silicas, Zeolite, Mycosorb, etc) have been evaluated for their ability to remove or diminish the adverse effect of mycotoxins in animal feed. These compounds must have the ability to bind physically with chemical substances, precluding their absorption [48]. A variety of adsorbents such as bentonite [37], zeolite [25], hydrated sodium calcium aluminosilicate [2], *Saccharomyces cerevisiae* [5] and activated charcoal [16] have been used successfully in detoxifying AF in contaminated feeds.

Diatomite or diatomaceous earth (DAE) is a mineral that consists of 90% silicon dioxide. It is fine-grained, biogenic siliceous sediment, and is available in large quantities at relatively low cost [17]. DAE can be milled to a fine powder, the particle size will differ depending on the milling used and could affect performance. Diatomite is an inert dust formed by milling of fossilized remains of phytoplankton (diatoms), composed of silicon dioxide, commonly used for the control of insects infesting stored products [3]. Poultry farmers sometimes feed diatomaceous earth to the birds with the belief that the sharp edges of the fossilized diatoms will damage the parasites in turn it will reduce coccidiosis; however, there is no scientific data to support its use [24]. Earlier study indicated that diatomite is not effective in reducing the detrimental effects of aflatoxin in broiler [9]. However, Modirsanei *et al* [27] reported that Diatomaceous earth significantly increased body weight gain, feed intake and improved feed

conversion ratio as well as productive efficiency index, increased serum albumin and the activity of serum lactate dehydrogenase in the birds fed with AF mixed diet. The variations in results with DAE may be due to variations in sources of DAE and processing used in the earlier studies. Diatomites from different sources vary in their mineralogical composition, morphological characteristics and milled particle sizes. Diatoms vary significantly in size and shape as there are several thousand species of diatoms [39]. The diatomite used in this study contains beneficial levels of montmorrillonite comprising of hydrated sodium calcium aluminium magnesium silicate known in the prevention of aflatoxins [34]. Considering the beneficial properties of diatomaceous earth, the present study was undertaken to evaluate the efficacy of DAE in ameliorating aflatoxin induced experimental patho-morphological changes in kidneys of broiler chicken.

2. Materials and Methods

The present study was carried out at Department of Pathology, Veterinary College, Bangalore, India from July 2014 to December 2014. Two hundred and forty unsexed day-old healthy broiler chicks were procured from a reputed commercial hatchery and reared in battery cage system in experimental sheds with average temperature ranging from 27 to 31ºC and relative humidity of 59% to 62% with 16:8± 1h (Light : Dark) cycle of intensity of 10 to 20 lux. All chicks were vaccinated on days 7 and 11 of age with the LaSota strain of Newcastle disease virus and Infectious bursal disease (intermediate strain) respectively.

Optimum conditions of management were provided to the birds throughout the period of experiment. Toxin free and DAE free Starter and Finisher broiler feed was procured from Department of Poultry Science, Veterinary College, Bangalore, India as recommended by the National Research Council. Required quantities of cultured aflatoxin material were added to make the final concentration of aflatoxin in feed to be 0.5 ppm and 1ppm.

The approval of the Institute Animal Ethics Committee (IAEC) was obtained prior to the conduct of the experiment. The birds were randomly divided into 6 groups, each comprising of 40 chicks (Table 1).

Table 1: Experimental groups in the present study

Groups	Treatment
I	CONTROL (Toxin free & DAE free feed)
II	AGRIPOWER DAE 2000 mg/kg of feed
III	AFLATOXIN (1 ppm)
IV	AFLATOXIN (0.5 ppm)
V	AFLATOXIN (1 ppm) + DAE 2000 mg/kg of feed
VI	AFLATOXIN (0.5 ppm)+ DAE 2000 mg/kg of feed

All the birds were checked daily for the health and husbandry conditions. All the sanitary and hygienic precautions were strictly followed throughout the experiment. Prior permission of the Institute Animal Ethical Committee (IAEC) was obtained before the conduct of experiment. The birds were observed daily for clinical signs and mortality (if any). A complete record of the daily mortality (if any) was also maintained. Six birds selected randomly from each group were weighed individually and sacrificed on day 7, 14, 21, 28 and 35 of experiment.

All the six birds sacrificed on weekly intervals were subjected to detailed post-mortem examination and gross lesions if any were recorded. Representative tissue samples from kidneys were immediately collected and fixed in 10% buffered formalin for histological examination. Samples from kidneys were dehydrated in ascending grades of ethanol, cleared in xylene and embedded in paraffin. Sections of 3-5 µm were obtained and stained with haematoxylin/eosin (H&E) as per standard procedure [23]. Light microscopy was used to evaluate congestion, degeneration, necrosis, fatty changes and leucocytic infiltration [38].

The experimental data collected was analysed using the General Linear models (GLM) procedure using Statistical Package for the Social Sciences software 16 (SPSS) of 2010 version. Statements of statistical significance were based on $P<0.05$.

3. Results

3.1 Gross Pathology

In the present study, gross pathological changes were recorded in kidneys on day 7, 14, 21, 28 and 35 day of the experimental study. The birds of group I (Control) and group II (DAE treated) revealed normal morphological appearance of the organ throughout the experimental study. The aflatoxin fed birds of group III and IV were emaciated on all the days of observation. In Group III and IV birds, the kidneys appeared pale and swollen in the first and second weeks. Kidneys appeared mildly congested by the end of third week and thereafter moderately congested (Fig. 1). At the end of the experiment, kidneys were enlarged and pale with yellowish discoloration (Fig. 2). The changes observed in birds of group V to VI (aflatoxin with supplementation with DAE) were less pronounced and showed improvement (Fig. 3).

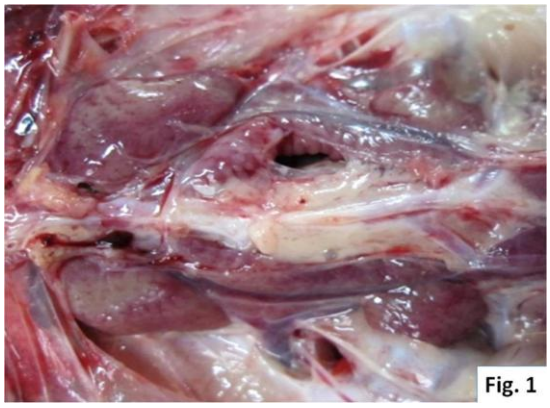

Fig 1: Kidneys from bird fed AF 0.5 ppm showing enlargement and congestion on day 28 of experiment.

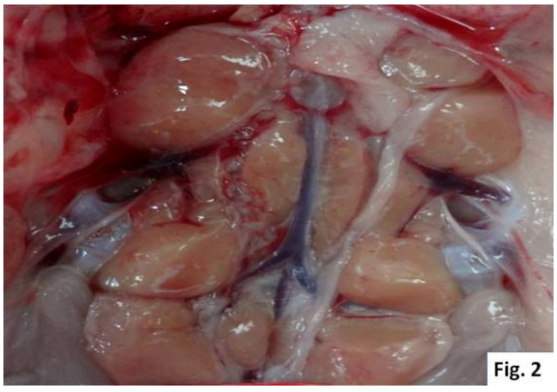

Fig 2: Kidneys from bird fed AF 1 ppm showing enlargement and pale discoloration on day 35 of experiment.

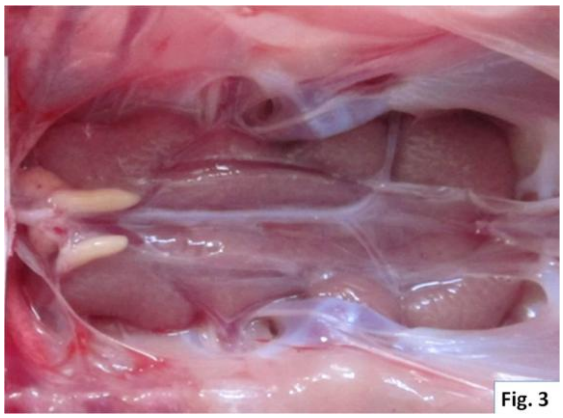

Fig 3: Kidneys from bird fed AF 1ppm + 2% DAE showing marked improvement in size and texture on day 35 of experiment.

3.2 Histopathology:

The birds of group I (Control), group II (DAE) revealed normal architecture of kidneys at different intervals of time.

Group III and IV (Aflatoxin): The renal parenchyma in aflatoxin fed birds at different time intervals revealed mild to moderate degree of vascular congestion and haemorrhages in occasional areas and mild to moderate degree of tubular epithelial degeneration predominantly in the proximal convoluted tubules. In addition, infiltration of mononuclear cells and heterophils was also observed from day 14 of the experiment (Fig 4, 5).

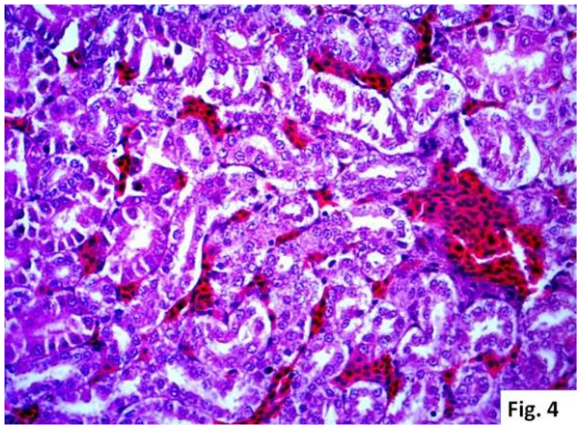

Fig 4: Kidneys from bird fed AF 0.5 ppm showing moderate degree of congestion, haemorrhages and tubular epithelial degeneration/desquamation on day 28 of experiment. H & E x 400.

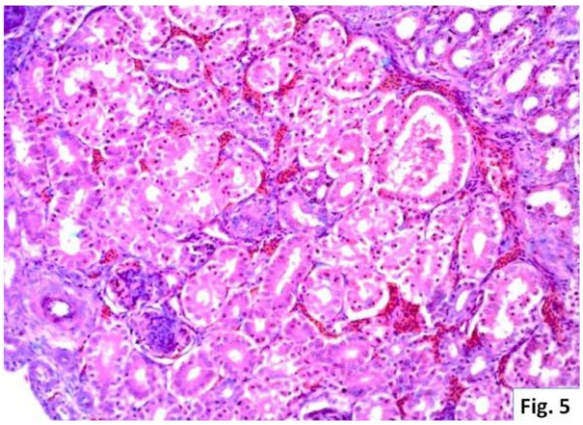

Fig 5: Kidney from bird fed AF 1 ppm showing moderate haemorrhages, glomerular degeneration and tubular epithelial degeneration/desquamation on day 35 of experiment. H & E x 200.

Group V and VI (Aflatoxin and DAE): The histopathological changes of different organs in birds fed with aflatoxin (0.5 and 1 ppm) and supplemented with DAE @ 2000 mg/kg feed at different time intervals revealed mild degree of congestion and haemorrhages, vacuolar degeneration of renal tubules epithelial cells with focal collections of mononuclear cells in the interstitium from day 14 of experiment. On day 35 of experiment, the kidneys revealed almost normal architecture with minimal vascular congestion and vacuolar changes in the tubular epithelium (Fig. 6).

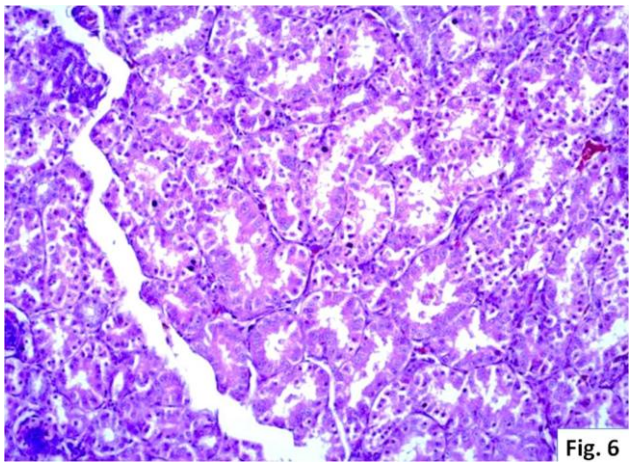

Fig 6: Kidney from bird fed AF 0.5 ppm supplemented with 2% DAE showing marked improvement in the glomeruli and tubules on day 35 of experiment. H&E X 200.

4. Discussion

Mycotoxins, particularly aflatoxin, are important to the poultry industry because of frequent occurrence in feed stuffs which can cause severe economic loss and health problems to poultry [6].

Several studies have been carried out to reduce the toxicity of AF in broilers and many commercial have been investigated to bind as well as absorption of AF in gastrointestinal tract [36]. The major advantage of these adsorbents includes expense, safety and ease of administration through feeds [7]. A variety of zeolites, phyllosilicates and synthetic aluminosilicates are capable of removing AFs from aqueous solutions however, major difference are observed in the strength of AFB_1 binding by the above sorbents [1].

Kidneys are the major excretory organs and about 20–25% of the total amount of circulating toxins, including mycotoxins, reaches renal parenchyma [13]. The demands of kidneys for nutrients and oxygen are high because of their functional load. The different parts of nephrons are exposed to the toxic effect of AF and its metabolites before being excreted, resulting in nephrotoxicity [41]. In birds exposed to the toxic effect of AFB_1, haemorrhagic and fatty kidney syndrome, thickening of glomerular membranes, degenerative changes in renal epithelial cells and congestion of renal parenchyma are reported [14].

The kidneys of aflatoxin fed birds revealed progressive changes characterised by mild to moderate degree of congestion and haemorrhages in occasional areas, mild to moderate degree of tubular epithelial degeneration, desquamation and necrosis, collections of mononuclear cells along with heterophils in interstitial spaces, varying degree of glomerular hypercellularity, degeneration and hyaline changes. These findings are very well supported by the observations of previous workers [44, 49, 45, 21].

The AFB induced generation of reactive oxygen species (ROS) occurs mainly in the mitochondria of hepatocytes and renal epithelial cells and results in damage of important biomolecules such as DNA, proteins and lipids [15]. Aflatoxins increase lipid peroxidation in liver and kidney tissues and induce cellular damage causing impaired morphology of the organs [46, 8]. Despite the fact that AFB_1 is eliminated mainly through the kidneys, the accumulation of a relatively high concentration of toxin impairs the excretory function and leads to congestion with subsequent patho-morphological alterations [11]. Low activity of glutathione-S- transferases involved in conjugation, detoxification and excretion of aflatoxins has also been reported in birds. The deficiency or the total lack of functional activity of these enzymes with affinity for binding to the major AF metabolite in birds is the mechanism of renal toxic effect of aflatoxins during their excretion [19]. Aflatoxins are known to inhibit the protein synthesis, forming adducts with DNA, RNA and proteins, inhibit RNA synthesis via binding DNA- dependent RNA polymerase, degranulate endoplasmic reticulum and thus, cause a variety of alterations in many organs namely liver, kidneys, heart and skeletal muscles [28, 41]. Aflatoxin induced nephrotoxicity is also assumed to be due to interference with transport function in collecting tubule cells together with diffused impairment of the proximal tubules function [31].

Supplementation with DAE could reduce the severity of lesions compared to toxin fed groups, which indicate the protective role of DAE. Further, birds of Group V and VI showed less severe lesions than Group III and IV indicating the protective role of DAE. However, the birds in Group V and VI revealed lesion with less intensity, which could be attributed to binding by DAE at higher dosage as suggested by Shi *et al.* [42] and Denli *et al.* [10]. The improvement in kidney lesions observed in the Groups V and VI were also in tandem with these findings thereby affirming the beneficial effect of DAE against toxic effects of aflatoxin.

The macroscopic and microscopic changes in kidneys of birds in groups V and VI fed with DAE in combination with aflatoxin included those that were observed in aflatoxin alone fed birds, but in reduced magnitude and severity. The gradual improvement in the gross and histopathological changes in DAE incorporated in AFB_1 fed birds are in agreement with earlier reports [42, 10].

Contrary to the present findings, no protective effect of DAE on the histopathological lesions in AFB_1 fed birds has also been reported earlier [40, 37, 9].

Differences among studies could be explained by different levels of adsorbents or the AF exposure dose tested. Based on the available scientific literature, the chemical complexity of mycotoxins means that a compound's effectiveness in sequestering one mycotoxin does not mean an equal ability to sequester other mycotoxins. Each of the mycotoxin has different functional groups; thus, the binding capacity of an adsorbent depends on its chemical and physical properties and its relation with the physical structure of the target mycotoxins. Thus, the physicochemical differences among the adsorbents used in the studies mentioned above could explain the higher or lower efficacy among them. However, the ability of the toxin binder to bind mycotoxins depends on other factors such as pH, molecular arrangement and its geographic region of origin [47]. Natour and Yousef [29] reported significantly higher in- vitro adsorption ability of DAE to AFB_1, which is directly proportional to the number of diatom valves. In- vitro study showed that DAE has high (94.71%) ability to adsorb AF from the feed at pH 6.5 [43].

In conclusion, the incorporation of DAE in the diet during the period of exposure to AF in the present study could plausibly reduce the toxic effects of aflatoxin. This result confirms that the protective effects of DAE, which might be due to its capability of specific chemisorption of AF in gastrointestinal tract leading to reduced bioavailability of this mycotoxin [33, 1]. DAE is a powerful natural adsorbent and which might effectively adsorb the toxins through its polar ends of the toxin [12]. DAE has a small mass (0.5-0.8 g/cm^3), high porosity and high content of silicon responsible for the high adsorption capacity [48]. These properties and ability of DAE might plausibly be responsible for the ameliorating action against this mycotoxin in broiler birds.

In respect of abundant availability, source and cost of DE as well as ability of this absorbent in reducing the moisture content in the poultry litter [17], it seems that using DAE at the level of 2000 mg/kg of feed could be effective and economic way of ameliorating the adverse effects of aflatoxin.

Perusal through the available literature reveal that clay, zeolite minerals and DAE are structurally and functionally diverse group of compounds; they vary considerably from source to source and may not have equal affinities and capacities for aflatoxin and other mycotoxins, thus they should be rigorously tested one by one and thoroughly characterised *in vivo*, paying particular attention to their effectiveness and safety for sensitive animal models and their potential for harmful interactions. Similarly, generalisations should be avoided for all potential mycotoxins detoxifying agents, as adsorbing compounds can differ in efficacy even within the same category. Considering the results of earlier work done on effect of different levels of DAE on aflatoxin, further studies seems to be necessary to determine whether lower levels of DAE in broilers diet will be effective in controlling or preventing the occurrence of aflatoxicosis in chicken.

5. Conclusion

In the present study, aflatoxin in the broiler diet at level 0.5 and 1 ppm of feed produced patho-morphological changes in kidneys indicating their adverse effect on general health of the experimental birds.

Incorporation of DAE at level of 2000 mg/kg of toxin free broiler feed showed no adverse effects on kidneys as compared to healthy control. The addition of Agripower DAE to aflatoxin containing feed revealed significant improvement in renal parenchyma. The protective effect of 2000 mg of Agripower DAE/kg feed could be useful to counter aflatoxicosis problem in field conditions that is probably due to its higher binding ability.

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