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Immunopathological Evaluation of Protective Potential of *Cajanus indicus* on Aflatoxin Induced Toxicity in Broilers

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A B S T R A C T

The aim of the present study was to elucidate the protective potential of herb, *Cajanus indicus* on aflatoxin (AF) induced immunopathological alterations in commercial broilers. A total of 168 Vencobb day old broiler chicks were randomly divided into four equal groups of 42 each. Group I broilers were given plain feed and served as control whereas, group II and III were given *Cajanus indicus* leaf powder (CLP) @ 2 g/kg and AF @ 1 ppm with normal feed respectively. Group IV broilers were fed on diet containing AF @ 1 ppm and CLP @ 2 g/kg for 42 days. The results revealed a significant ($P \leq 0.01$) increase in skin thickness in the control groups I and II at 24 and 48 hrs post challenge (1% Dinitroflurobenzene in vehicle) as compared to intoxicated groups III and IV. AF toxicity significantly reduced the development of humoral immune response in broilers and supplementation of *C. indicus* could partially protect the reduction in HA titers of AF intoxicated broilers. Aflatoxicosis significantly suppressed the both cell mediated and humoral immune responses. But CLP supplementation had a potent immunomodulatory effect. So, it helps not only in controlling aflatoxicosis but also can play a pivotal role in correcting chicken immune dysfunction.

Introduction

Poultry industry is designated as most dynamic and fastest growing segment amongst agricultural and livestock sectors in India. In comparison to agriculture and other livestock farming, poultry now-a-day serves as an income stabilizer and provides a regular and lucrative income to farmers. High quality nutrition is a primary requirement for healthy poultry birds in excellent condition with optimal production levels. Recently the feed production technology has paid a good deal of attention for all extrinsic and intrinsic quality factors.

However, feed safety is a concern for achieving the optimum productivity and consumer confidence. Aflatoxin (AF) is a collective term for a group of extremely toxic and carcinogenic secondary metabolites produced by some strains of *Aspergillus flavus*

and *Aspergillus parasiticus* during their growth on feeds and foods is a major concern for the poultry production. Aflatoxins are potent carcinogens and cause heavy economic losses in poultry due to growth depression, poor feed conversion efficiency, carcass yield and carcass quality and reduced disease resistance (Bedre *et al.,* 2010). Aflatoxin, the most common hepatotoxin, can cause impairment of humoral and cellular immune responses and increase susceptibility to some environmental and infectious agents (Ibrahim *et al.,* 2000; Oguz *et al.,* 2003). Eminent researchers and producers aim to develop effective prevention, management and decontamination technologies to minimize the toxic effects of AF. Previously, the adsorbent based studies have been conducted to remove AF from contaminated feed to minimize the toxicity of AF in poultry[2]. Recently there has been a growing interest in establishing the therapeutic potentials of plants and plant derived molecule for the drug development.

A nontoxic edible herb, *Cajanus indicus* (Kundu *et al.,* 2008) locally available in India is one of those plants which have many folk medicinal uses. A 43 kDA protein isolated from *Cajanus indicus* has recently been demonstrated to have efficient hepatoprotective effect (Kundu *et al.,* 2008; Sarkar *et al.,* 2005; Manna *et al.,* 2007). In view of the above medicinal properties, the present study is designed to investigate the ameliorative activity of *Cajanus indicus* against induced aflatoxicosis in broilers.

Materials and Methods

Production of aflatoxin

The AF was produced from *Aspergillus fl*avus 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 AFB_1, 28 ppm AFB_2 and 20 ppm AFB_2. 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

Leaves of *Cajanus indicus* were collected and botanically identified. The mature leaves of *Cajanus indicus* was shade dried and reduced to fine powder using electric grinder and the powder was stored in airtight containers.

Chickens and diet

The experimental investigation was planned for immunopathological evaluation of protective potential of *Cajanus indicus* on Aflatoxin induced toxicity in broilers after obtaining approval from Institutional Animal Ethics Committee. Clinically healthy one hundred and sixty eight one-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 upto14 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.

Experimental Design

Individually weighed chicks were randomly divided into 4 groups of 42 chicks; each group consisting of 3 replicates of 14 chicks. The experimental design consisted of four dietary treatments: 1. Control: Basal diet; 2. Basal diet + 2g CLP kg^{-1} diet; 3. Basal diet + 1 ppm AF; 4. Basal diet + 1 ppm AF +2g CLP kg^{-1} diet. When the chicks reached 42 days of age, the feeding trial was terminated.

Cell mediated immunity (CMI)

Cell mediated immune response was measured by Di Nitro Fluro Benzene (DNFB) test as described by Phanuphak *et al.,* (1974) and later slightly modified by Tamang *et al.,* (1988). Featherless area was marked on both sides of abdomen and cleaned thoroughly with acetone and air dried. Right lateral side of abdomen was used for DNFB application whereas left side served as control. 2000µg of DNFB in 0.1 ml of acetone and olive oil (4:1) was applied on the right marked area on the abdomen using a plastic ring to avoid spillage. The sensitized birds were challenged with 50µg of DNFB in 0.1 ml of acetone and olive oil (4:1) on the same area on 14^{th} day after initial sensitization. The response to DNFB was assessed by measuring the skin thickness using engineer's micrometer on 0, 24 and 48 hours of post challenge with three readings each and the overall mean skin thickness was calculated.

Humoral immunity

Humoral immune response was assessed by micro haemagglutination test according to the method of Thaxton *et al.,* (1974). HA plates were thoroughly dried and cleaned. Sheep blood was collected in equal volume of Alsever's solution and allowed to stabilize for one week. Sheep RBC's obtained after centrifugation was washed thrice in normal saline solution (NSS) and finally a 7% suspension of SRBC was prepared. For immunization 1ml of this suspension was injected intravenously in six birds from each group and the birds were bled on 10 days following injection. Blood was allowed to clot at 37ºC for few hours and refrigerated. Serum was collected and heated in a water bath to inactivate the complement fraction. The antibody production due to immunization was assessed by micro haemagglutination test. The reciprocal of the highest dilution of serum that caused complete haemagglutination was considered as HA titre and expressed as $\log_2$ values.

Results and Discussion

The mean skin thicknesses of broilers in different groups have been given in Table 1. The cell mediated immune response *in vivo* in broilers was estimated by chemical contact sensitization with DNFB**.** The skin reaction of broilers challenged with DNFB includes swelling, erythema, oedema, vesiculation, scab formation and sloughing. Overall skin reaction was more pronounced at 24 hours than 48 hours post challenges. The present study revealed a significant ($P \leq 0.01$) increase in skin thickness in the control groups I and II at 24 and 48 hrs post challenge as compared to intoxicated groups III and IV which indicate immunosuppression in terms of cellular immunity during aflatoxicosis. However, induced aflatoxicosis significantly suppressed the cell mediated immune response of broilers in both periods of post challenge in comparison to control. CLP supplemented birds of group IV showed better CMI status than the respective control groups, although the difference was insignificant statistically.The results on humoral immune status of broilers were summarized in Table 2. The AF treated broilers (group III) exhibited a highly significant ($p \leq 0.01$) decrease in HA titer as compared to groups.

Table.1 DNFB response (mean increase in skin thickness in mm) of broiler chicks exposed to aflatoxin and *C. indicus* (Left side served as vehicle control and right side treated with the DNFB) (n=6)

Groups	Abdominal side	Before sensitization	After sensitization	
			24 hr	48 hr
Gr I	Left	0.55±0.01	0.57±0.01	0.55±0.01
	Right	0.55±0.02[a]	3.58±0.03 [a]	3.51±0.03[a]
Gr II	Left	0.56±0.01	0.6±0.02	0.59±0.02
	Right	0.58±0.03[a]	3.52±0.13[a]	3.40±0.12[a]
Gr III	Left	0.56±0.02	0.57±0.13	0.56±0.01
	Right	0.57±0.02[a]	2.32±0.06[b]	2.20±0.07[c]
Gr IV	Left	0.66±0.02	0.67±0.02	0.62±0.03
	Right	0.61±0.02[a]	2.52±0.06[b]	2.44±0.07[b]
Level of significance		NS	**	**

Table.2 Effects of *C. indicus* supplementation on haemagglutination titres of Aflatoxin treated broilers (n=6)

	Group I	Group II	Group III	Group IV	Level of significance
HA Titre (log_2 values)	5.05±0.12[a]	5.27± 0.30[a]	3.4±0.21[c]	4.22±0.18[b]	**

However, broilers of group IV had significantly higher HA titer than group III. It indicates that AF toxicity significantly reduced the development of humoral immune response in broilers and supplementation of *C. indicus* could partially protect the reduction in HA titers of AF intoxicated broilers.

Values indicate Mean ± S.E., Superscript may read column wise for mean comparison. Similar superscript showing means do not differ significantly. (*P ≤0.05, **P≤0.01)

Presently induced aflatoxicosis significantly reduced CMI response to DNFB of broilers in both periods of post challenge in comparison to control. Previously Shivchandra *et al.,* (2003) observed reduced CMI response to DNCB in AF treated broilers. A similar decreased CMI response was recorded by Bakshi *et al.,* (1998) and Sawale *et al.,* (2009) in AF induced broilers and ochratoxin induced layers, respectively. A significant decrease in CMI response of the present study might be attributed due to the adverse effect on chemotactic and phagocytic action of lymphocytes (Ghosh *et al.,* 1991). However, birds of group IV revealed better CMI status as compared to group III which might be due to the immunomodulatory effect of *C. indicus* (Datta *et al.,* 1999).

The present findings of reduced humoral immune response due to aflatoxicosis and partial protection in reduction of HA titers due to supplementation of *C. indicus* were in accordance with those of Kurkure *et al.,* (Kurkure *et al.,* 2000) who observed reduced immune response of broilers during

aflatoxicosis. Reduced immune response recorded in the present study could be accounted for decreased protein and globulin synthesis, impaired processing of antigen due to depressed phagocytosis during aflatoxicosis in broilers and direct lymphotoxic activity of mycotoxin (Kalorey *et al.*, 2005). In the present study, lymphocytolysis of lymphoid organs might be responsible for reduced HA titers. So, it can be concluded that aflatoxin @ 1ppm caused significant immunosuppression and broilers may experience mortality due to secondary infection resulting from AF induced immunosuppression. However, supplementation of *C. indicus* revealed significant improvement of immune status of the broilers and provided a moderate amelioration in AF toxicity.

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