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INFLUENCE OF AFLOTOXIN B1 ON PROTEIN AND DNA CONTENT OF LYMPHOID ORGANS OF ONE WEEK OLD BROILERS DURING INDUCED AFLOTOXICOSIS

P. J. R. NATHANAEL*, S. LALITHA KUMARI AND V. VIVEKA VARDHANI
Department of Zoology and Aquaculture,
Acharya Nagarjuna University, Nagarjuna Nagar Guntur - 522 510, A. P., INDIA
E-mail: nathanael_pj@yahoo.co.in

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***Corresponding author**

ABSTRACT
Metabolic exudates liberated by *Aspergillus flavus* and *Aspergillus parasiticus* are referred as mycotoxins. Among them Aflotoxin B1 (AFB1) is prominent and is categorized as the group 1 carcinogen along with its mutagenic and terrotogenic behavior. AFB1 is hepatotoxic, nuerotoxic, and nephrotoxic causing a high degree of immunosupression in poultry birds having a broad spectrum of lethal impact as carcinogenic, hepatotoxic, mutagenic, and nephrotoxic agent. Our present investigation is focused on determining the levels of protein and DNA in bursa fabricus and cecal tonsils of one week old broilers suffering due to induced chronic aflotoxicosis thereby to know the intensity of alterations and anomaly of cellular mechanisms and to understand the immune response of the individual in the light of its physiological state. The organs that are playing a pivotal role are highly affected by AFB1 which causes adverse and disturbed levels of protein and DNA. Alterations in the metabolism of proteins in bursa fabricus and cecal tonsils of broilers treated with AFB1 are the significant toxic effects of AFB1.

INTRODUCTION

In India the availability of animal proteins in human diet is extremely low; hence, there is an indispensable need to produce meat that yields at low costs. The most imperative of human diet is the poultry meat with high quality protein and essential amino acids. The poultry venture has been grossly defeated by the out-break of fungal diseases, and aflotoxicosis pose a perpetual threat to turkeys, ducklings, goslings and chickens (Bakshi *et al.,* 1997). Aflotoxins are natural poultry dietary contaminant, which are a major risk factor of hepatocellular carcinoma (HCC) (Partanen *et al.,* 2010) and enhances a threat to public health scenario (Phillips *et al.,* 1998). Aflotoxicosis is primarily characterized by mortality, listlessness, anorexia, opisthotonous condition, decreased growth rates, negative feed conversions, decreased and delayed egg production, poor pigmentation and increased susceptibility to other diseases (Quareshi, 1988; Toro *et al.,* 2000). Aflotoxins after entering into the alimentary canal of birds exert systemic effects and cause specific damage to certain vital organs like liver (principal target organ and centre for detoxification), thymus and bursa of fabricus (primary lymphoid organs), kidney (principal organ of excretion) likewise. AFB1 causes induced DNA adduction and mutagenesis(Besaratinia *et al.,* 2009) Thymus and bursa of fabricus play a vital role for the production of cellular and humoral antibodies (Copper *et al.,* 1965; Glick, 1970) and AFB1 brings the alteration in cellular integrity in these tissues.

With regard to the biochemical changes caused by aflotoxins the most important initial event appears to be the interaction of AFB1 with DNA and protein metabolism resulting in the inhibition of cell mitosis. The suppression of DNA synthesis may result in the production of cells in a non dividing stage and this may lead to the abnormal production of cells (Panda and Johri, 1983; Ranchal *et al.,* 2009). High levels of AFB1 exposure clearly manifested altered physiological responses associated with altered gene expression in liver and thymus (Yarru *et al.,* 2009). Therefore, a new vista has been opened to study the level of protein and DNA in thymus and bursa fabricus of one week old broilers infected with two varied doses of AFB1.

MATERIALS AND METHODS

One week old broilers belonging to *Plymouthrock* strain were procured and kept in open litter system, and acclimatized to laboratory environment. All the animals were feed with the standard balanced diet and water was given *ad libitum*. 0.5mL of AFB1 suspension at a dose of 0.0l ng/bird (20 birds, group A), and 0.1ng/bird (20 birds, group B), was intubated orally by using a 16 gauze oral feeding needle. These doses were administered after a preliminary study. Control birds (20 birds, Group C) which are kept for comparison received 0.5. mL of distilled water only. Infected and control birds are separately kept throughout the study. All the experimental groups were sacrificed at day 1, 4, 8 and 11 of infection. Five animals from

the control group were also sacrificed on the same designated days. Tissues of bursa fabricus and cecal tonsils were processed for protein and DNA evaluation. Estimation of proteins and DNA was done following respectively Lowry *et al*., (1951) and Diphenylamine methods (Burton, 1971).

RESULTS AND DISCUSSION

The one week old broilers (groups A and B) treated with AFB1 manifested all the etiological signs confirming an induced chronic aflotoxicosis. They appeared dull, depressed and reluctant to feed and water. Prolonged asthenia, lethargy and reduced appetite resulting in decreased body weight were noticed. Persistent spleenomegaly, fatty liver syndrome with multiple hemorrhages and molted texture of hepatic organ was observed in all the experimental fowls during day 8 to 11 of infection. Autopsies performed clearly gives an insight of liver necrosis, odematous bursa, and other in vital organs, hydro pericardium syndrome and severe inflammation. Enlargement of cecal tonsils and atrophy of bursa was evident in chicks which received 0.01ng of AFB1. All these changes are similar to that of Smith and Hamilton (1970), Sivachandra *et al*., (2004); Zadrozny *et al.,* (2010), and other who also reported odematous bursa (in low doses), hydropericardium syndrome and atrophy of vital organs respectively during chronic aflotoxicosis.

Protien content (mg/mL) in bursa fabricus (Fig. 1)

In case of group a broilers which are being administered with 0.01ng of AFB1, the level of proteins was found to be increased during the whole length of the experimental period, and they were at peak on day 1. The level of proteins decreased gradually from day 1 to 11 when compared to the other days of infection. The broilers which were treated with 0.1ng of AFB1 (group B) also showed an enhancement in the content of proteins from day 1 to day 11, in comparison with that of the untreated

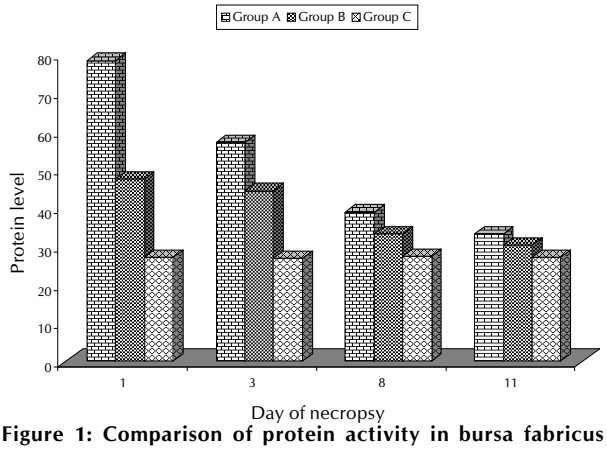

Figure 1: Comparison of protein activity in bursa fabricus of experimental groups A (0.01ng/bird), B (0.1ng/bird) (treated with AFB1) and control (untreated) © group of one week old broilers

ones. However the initial marked rise of proteins decreased gradually from day 1 to 11. The increased protein content vividly reveals the effect of aflotoxins disturbing the protein synthesis mechanisms in animal cells (Goldblatt, 1969).

DNA content (mg/mL) in bursa fabricus (Fig. 2)

It is found that in the one week old broilers of group A which

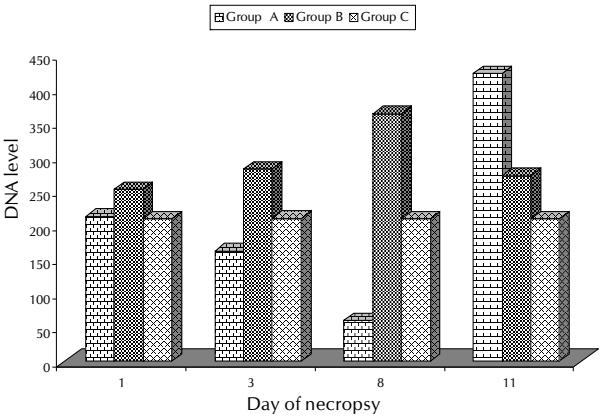

Figure 2: Comparison of DNA activity in bursa fabricus of experimental groups A (0.01ng/bird), B (0.1ng/bird) (treated with AFB1) and control (untreated) group© of one week old broilers

received a small dose of aflotoxin *i*.e. 0.01ng/bird showed a slight increase of DNA level on day 1, but fallen below the normal value on day 3 and 8, but there was a sudden rise of DNA on day 11. In case of group B, which were treated with 0.1ng of AFB1, showed a rise in the levels throughout the experimentation. The increase of DNA was gradual from day 1-8, but though it decreased on day 11, but this value is still higher than control value. It is clear from the results that the important initial events appear to be the increase of the DNA levels in fowls treated with a lowest dose which is due to the survival and increase of cells in a non dividing stage. The increased value clearly manifests the mutagenic property of AFB1, which gave rise to abnormal increase in cells with DNA content.

Protein content (mg/mL) in cecal tonsils (Fig. 3)

When the extracts of cecal tonsils of experimental and control broilers were analyzed for protein levels, they showed much variation. In case of group A (treated with 0.01ng/bird), a considerable enhancement was manifested throughout experimental period in comparison to untreated ones. Though there was a gradual decrease from day 1 to 11, the decreased level of proteins remained higher when compared to controls.

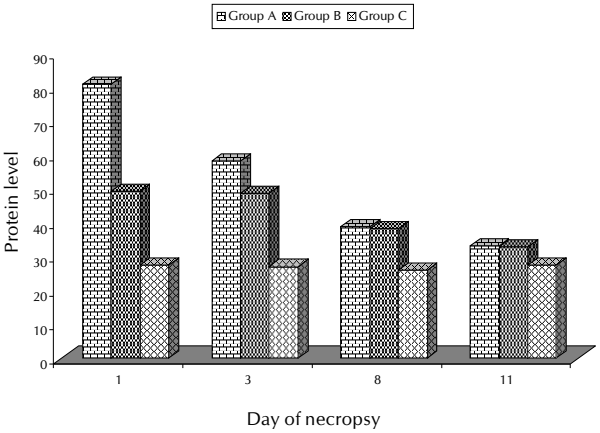

Figure 3: Comparison of protein activity in cecal tonsils of experimental groups A (0.01ng/bird), B (0.1ng/bird) (treated with AFB1) and control (untreated) group© of one week old broilers

In comparison with controls there was an increase of proteins from day I to 11 in broilers of group B treated with 0.1ng of AFB1, there was a gradual decrease of proteins from day 1 to 11. The aberration in the protein levels in the Cecal tonsils of experimental animals clearly explain the involvement of biochemical reactions to alter the protein metabolism.

DNA content (mg/mL) in cecal tonsils (Fig. 4)

In case of broilers treated with 0.01ng of AFB1 the level of DNA was increased slightly on day 1 when compared to control value, and decreased to below the normal level on day 3 and 8 and was again raised on day 11. In case of group B (0.1ng), the level of DNA was found to be below normal from day 1 to 8 and increased considerably on day 11. It is interesting to note that the below normal level of DNA on day 1(60.6mg) increased gradually (though these values are below normal value) and reached a higher level by day 11.

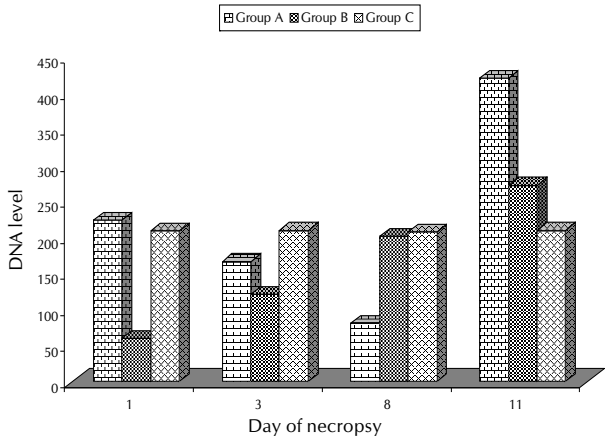

Figure 4: Comparison of DNA activity in cecal tonsils of experimental groups A (0.01ng/bird), B (0.1ng/bird) (treated with AFB1) and control (untreated) group© of one week old broilers

It is clear from these investigations that Aflotoxin B1 is capable of interacting with DNA of cells of both bursa fabricus and cecal tonsils. It is also observed that the cellular effects of aflotoxin can be directly attributed to the interaction with DNA. The primary lymphoid organs of broilers like thymus and Bursa, the secondary lymphoid organ like cecal tonsils and spleen are essential for the development of humoral response. The organs that are playing a pivotal role are highly affected by AFB1 which causes adverse and disturbed levels of protein and DNA. Alterations in the metabolism of proteins in bursa fabricus and cecal tonsils of broilers treated with AFB1 are the significant toxic effects of AFB1. In these studies, the AFB1 will provide a useful model and the investigations may provide additional information into the biochemical mechanisms underlying the carcinogenic or mutagenic process of AFB1.

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