ISSN 1819-1878

Asian Journal of Animal Sciences

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OPEN ACCESS Asian Journal of Animal Sciences

ISSN 1819-1878
DOI: 10.3923/ajas.2022.10.15

Research Article
Mycotoxigenicity of Territrem B: Histopathological Investigations of Chick Ingesting *Aspergillus terreus* Infested Feed

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Abstract

Background and Objective: Territrem B, tremorgenic mycotoxin elaborated by *A. terreus* is neurotoxic and cause a variety of neurological disorders. Therefore, it was aimed to analyze histopathological changes in some of the vital organs of chicks consumed territrem B through the feed. **Materials and Methods:** A set of 5 days old checks were given measured quantity. *Aspergillus terreus* infested feed for 6 weeks. At the end of 2, 4 and 6 weeks a batch of chicks was sacrificed and pathological symptoms were examined by microtome sectioning of different vital organs. **Results:** There was a marked loss of body weight in chicks during 6 weeks of their growth receiving *A. terreus* infested feed. Intoxication was also observed by reduced emotive behaviour. On grass postmortem examination of liver and kidney revealed significant pathological changes. Consumption of *A. terreus* infested feed caused degeneration of nephrons and necrosis of renal tubular epithelium resulting in nephrotoxicity. It was also hepatotoxic as it caused necrosis of hepatocytes and dilation of bile ducts resulting in biliary hyperplasia. **Conclusion:** From the present investigations it is clear that territrem B is neurotoxic and causes malfunctioning of the liver and heart. The present study revealed that importance of providing healthy feed to poults and prevents health hazards of primary and secondary exposure of territrem B to livestock and man.

Key words: Chicks, *Aspergillus terreus*, infested feed, abnormalities and territrem B

Citation: Kiran, S., M. Surekha, S.M. Reddy and G. Benarjee, 2022. Mycotoxigenicity of territrem B: Histopathological investigations of chick ingesting *Aspergillus terreus* infested feed. Asian J. Anim. Sci., 16: 10-15.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Mycotoxins are poisonous chemical compounds and secondary metabolites produced by fungi, exhibit a variety of biological effects[1,2]. Aflatoxins, ochratoxins, zearalenone, T_2 toxins, tremorgens, trichothecenes and DON are found in food and feedstuffs all over the world[3-5]. In spite of worldwide awareness about different mycotoxins and health hazards they cause to human begins, only limited information is available on human and animal toxicity of territrem B[6,7]. Further, contamination of mycotoxins in foods and feeds and their management is being discussed extensively[8-10]. The tremorgenic mycotoxins which cause neurological disorders are more prevalent in livestock and comparatively less in human beings[11-13] as they are present extensively in feeds and fodders[14,15]. A recent review of literature on the health hazards of livestock and pet animals caused by tremorgenic mycotoxins stressed the need to take up the intrinsic and systemic study of their toxicosis. Infestation of food grains by tremorgenic mycotoxin producing moulds is reported to be prevalent in different parts of the world[16-18]. Mycotoxins have been reported to cause various embryonic disorders in chicks resulting in organ proliferation and ultimately leading to the death of the chick, thus resulting in serious economic losses in the hatchery[19,20]. Thus the above facts tempted the author to study the effect of territrem B in chicks.

Territrem B is reported to induce a variety of disease symptoms such as swollen, haemorrhages and degenerative changes in the nuclei of hepatocytes and disorders neurologic[21,22]. However, limited information is available on the pathological changes under the influence of territrem B. In the effect of the present investigation of territrem B infested feed given to chick for 6 weeks and histopathological changes in different vital organs were examined microscopically.

MATERIALS AND METHODS

Study area: *Aspergillus terreus* was isolated from paddy seeds collected from Godavari belt region of Telangana for 6 months during the period (January-June, 2019) and screened for the production of territrem B. Territrem B is a potent mycotoxin therefore, it was selected for detailed studies of its toxigenicity on chicks during July-January, 2020.

Feed collection: *Aspergillus terreus* producing territrem B was grown aseptically in trays containing moistened rice bran for 15 days at room temperature. The infested feed was air-dried and given as a feed for 5 days old chicks and examined for pathological symptoms regularly for 15 days.

Research procedure: The 5 days old chicks were procured from a local hatchery and maintained in the laboratory by regular feeding. A total of 4 batches of five chicks for each batch were maintained. Simultaneously control was maintained by giving a healthy feed. The other three batches of chicks were given the same amount of measured infested feed for 15 days. Out of these three batches, 1st batch was sacrificed at the end of 2 weeks, while the second batch was sacrificed after 4 weeks. The third batch was scarified on completion of 6 weeks and processed for histopathological studies. The visceral organs such as liver, kidney and brain were collected and fixed in Bouin's, Susa, Carnoy and Zenker's fluid, after washing the tissues in normal saline[23] for 48-72 hrs. The tissues were then processed for microtome sectioning and sections were cut at 3-6 microns thickness and stained with Heidenhain's Azan stain[23,24]. Finally, the sections were observed under a microscope for pathological conditions.

RESULTS

Intoxication of territrem B was observed by reduced emotive behaviour. There was a marked loss of body weight of chicks during 6 weeks of intake of *A. terreus* infested feed. Chicks fed with *A. terreus* infested feed showed the main symptoms such as weakness and shivering and feed refusal in Fig. 1a-b. On gross postmortem examination of liver, kidney, heart and brain revealed the following significant pathological conditions.

Kidney: The histological structure of the kidney of a healthy chick showed a clear Bowman's capsule, glomerulus, renal tubules in Fig. 2a, the lumen of the renal tubules, distinct nuclei of the epithelial cells and a distinct haemopoietic tissue. On the other hand, chick fed with *A. terreus* infested feed showed many histopathological abnormalities as evidenced by alteration of the histoarchitecture of the kidney which included congestion, the onset of degenerative changes in Bowman's capsule and haemopoietic tissue. The other histopathological changes observed in the kidney were hypertrophy of epithelial cells of renal tubules, pyknotic nuclei in the tubular epithelium, contraction of renal glomeruli and widening of Bowman's capsule. The prolonged feeding for 4-6 weeks with *A. terreus* infested feed caused dilatation of renal tubules, necrotic changes characterized by karyorrhexis in Fig. 2b and karyolysis of nuclei. Histopathological changes led to vacuolation, hypertrophy and necrosis of the kidney and finally affected other physiological processes resulting in the diseased condition.

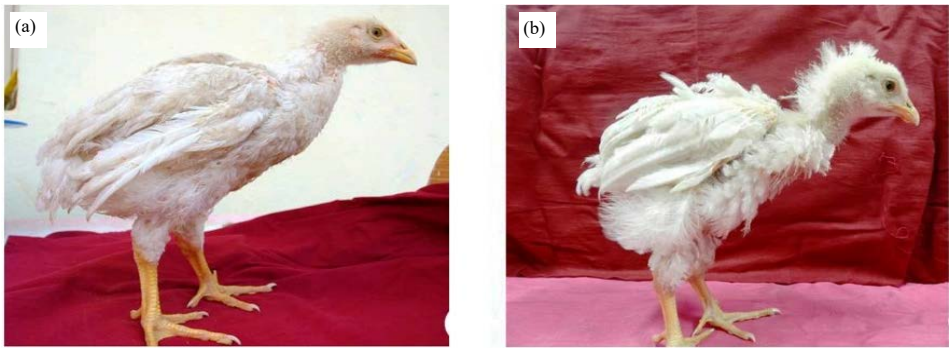

Fig. 1(a-b): Control and treated chick (a) Chick (control) Fed with normal diet and (b) Chick (treated) fed with infested a diet containing *A. terreus*

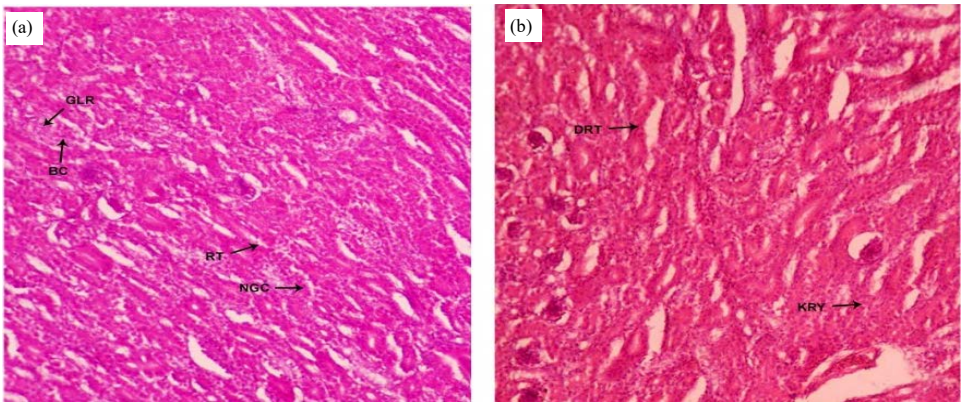

Fig. 2(a-b): Microphotograph of kidney of (a) Control chick and (b) Chick fed with *A. terreus* infested diet
GLR: Intact glomerulus, BC: Bowman's capsule, RT: Renal tubules, NC: Nuclei. This microphotograph evidenced by DRT: Dilatation of renal tubules, KRY: Karyorrhexis, (i.e., abnormal multiplication of nuclei)

Liver: The histological structure of the liver of a healthy chick consists of cords of hepatocytes which are arranged radially around the hepatic vein in Fig. 3a, while in chicks fed with *A. terreus* infested feed showed the polygonal, hepatocytes with homologues granular cytoplasm with the centrally located nucleus.

Chicks fed for two weeks with *A. terreus* infested feed have shown mild degenerative changes in both hepatocytes and hepato chords. The walls of the hepatic veins were ruptured, resulting in cloudy swelling of hepatocytes, nuclear hypertrophy and nuclear degeneration. Nuclear hypertrophy, vacuolization of hepatocytes and karyolysis further increased with the progress of the incubation period. The liver of chicks receiving *A. terreus* infested feed for 2, 4 and 6 weeks exhibited increased histopathological abnormalities which disintegrated parenchymatous tissue of the liver, the disintegration of hepato chords, widespread vacuolization and necrosis. The atrophy of the liver is a clear indication of its hepatotoxicity in Fig. 3b.

Brain: The brain which is the nerve centre of the body and co-ordinates the other functions of the body exhibited a variety of histopathological abnormalities. The brain of a healthy chick revealed the presence of hippocampus, neural cells with distinct nuclei in Fig. 4a, while chicks receiving *A. terreus* infested feed revealed swollen tissue, hyperplasia, dilation of epithelium and neurons leading to necrosis in Fig. 4b. However, these changes were perceptible significantly after prolonged exposure to *A. terreus* infested feed.

Heart: The histoarchitecture of control heart muscle showed that it consists of compactly packed muscle fibres with inter muscular spaces. The muscle fibres consists of spindle shaped nuclei in Fig. 5a. The chicks fed with *A. terreus* infested feed exhibited many histoarchitectural changes in the heart muscles. The histopathology of the heart muscles revealed the appearance of intermuscular spaces, cellular degeneration and degeneration of muscle fibres. These histopathological

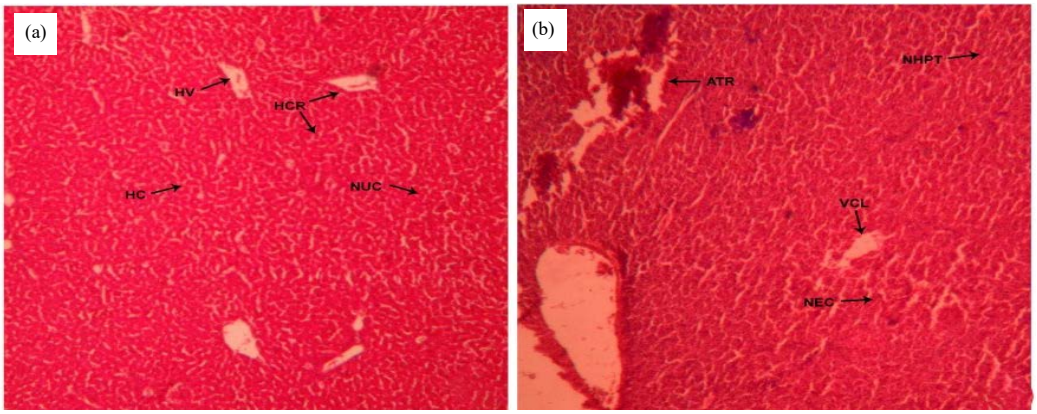

Fig. 3(a-b): Microphotograph of liver of (a) Control of chick and (b) Chick fed with *A. terreus* infested diet

HV: Intact hepatic vein, HC: Hepatocytes, HCR: Hepato chords, NUC: Nuclei, VCL: Vacuolization, ATR: Atrophy, NHPT: Nuclear hypertrophy, VCL: Vacuolization, NEC: Necrosis

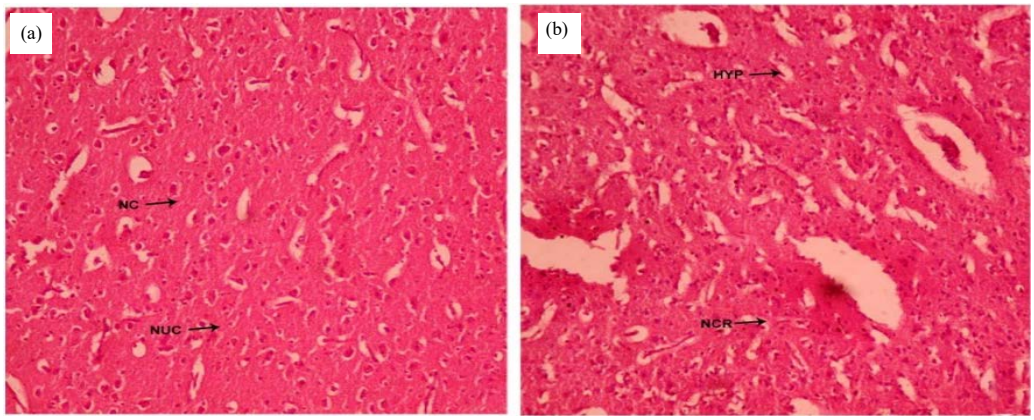

Fig. 4(a-b): Microphotograph of the brain of (a) Control chick fed with normal diet and (b) Chick fed with *A. terreus* infested diet

NC: Nuclei, NUC: Neural cells in the brain tissue, HYP: Hyperplasia, NCR: Necrosis of brain tissue

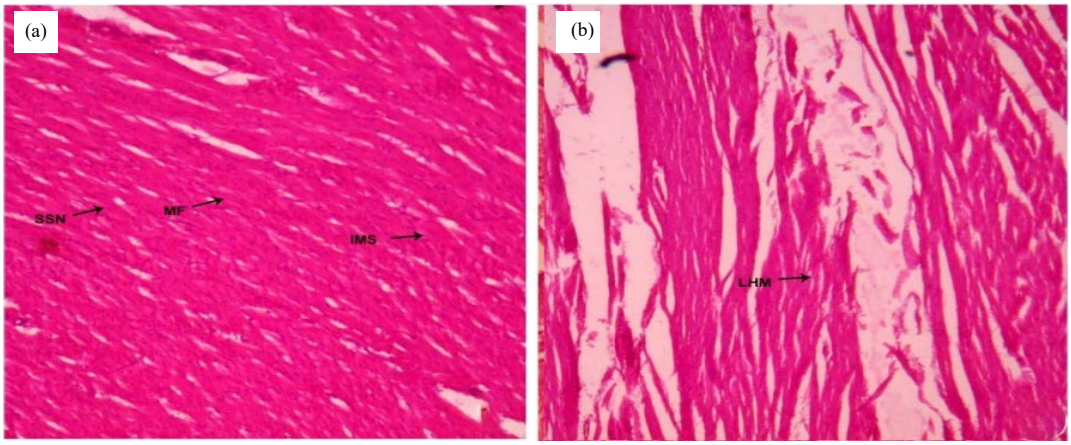

Fig. 5(a-b): Microphotograph of the heart of (a) Control chick fed with normal diet and (b) Chick fed with *A. terreus* infested diet

SSN: Spindle shaped nuclei, MF: Muscle fibres, IMS: Inter muscular spaces, dilation of cardiac muscle, LHM: Loosening of the heart muscle and formation intercellular space

changes led to the loosening of the heart muscle in Fig. 5b architecture which will have an impact on the functioning of heart muscles. Pyknotic nuclei and necrotic changes are the culmination of pathological change evidenced in the heart muscles of chick fed with *A. terreus* by the territrem B have a tremendous impact on the contraction and expansion of the heart resulting in the malfunctioning of the heart.

DISCUSSION

From the present investigations, it is clear that territrem B is toxic to chicks which increased both with the increase of territrem B intake and duration of consumption. A distinct malfunctioning of the liver and kidney was evident by pathological changes. The histopathological changes in the liver of chicks such as the disintegration of parenchymatous tissue of the liver, disintegration of hepatochords, widespread vacuolization and necrosis will certainly influence the different physiological processes and may lead to other health hazards and culminating in the death of chick. The histopathological changes in the heart muscles are evident by the appearance of intermuscular spaces, cellular degeneration and degeneration of muscle fibres. These histopathological changes led to pericardium with a dispose of tissue loosening of the heart muscle architecture which will have an impact on the functions of heart muscles of chicks Pycnotic nuclei and necrotic changes are the cumulative pathological change evidenced in the heart muscles of chick fed with *A. terreus* infested feed. The histopathological changes induced by the territrem B have a tremendous impact on the contract and relaxation of heart muscles that lead to malfunctioning of the heart. Necrosis of the kidney finally affected other physiological processes of chick resulting in the diseased condition. Sharma *et al.*[25] also observed hepatocellular and nephrotoxic damage in albino rats fed with culture filtrated of *A. terreus*. Territrem B also caused a variety of histopathological changes such as swelling, haemorrhages and degenerative changes in hepatocytes including neurologic symptoms. Valdes *et al.*[18] and Chen *et al.*[21] have also observed in different animals under the influence of territrem B. Arvanov *et al.*[26] reported the toxic nature of territrem B which induces tremors, salivation, convulsions and liver and kidney culminating in the death of the animal. Ling *et al.*[27] reported that territrem B increased the frequency and amplitude of spontaneous miniature endplate potentials of the mouse diaphragm. Arvanov *et al.*[26] believe that the territrem B might be a potent inhibitor of acetylcholinesterase in the molluscan neuron. It was demonstrated induction of tremors and convulsions by introducing territrem B intraperitoneally. Territrem B is reported to black potassium current in the nerve terminal which may contribute to its pharmacological action on synaptic transmission[28]. Territrem B is highly toxic and affects multi organs inducing tremors, salivation, convulsions, liver and kidney congestion[27].

Present investigations revealed a variety of maladies in different vital organs of chicks suggesting multi-organ failure. Biochemical reactions leading to failure of these organs need an in-depth study which facilitates the management of neurological disease of chick. Similarly, protecting the poultry feed from *A. terreus* growth and territrem B contamination will have to be probed. Further, epidemiological aspects of this mycotoxicosis have to be investigated to make the poultry industry economical.

CONCLUSION

Present investigations recorded the presence of potent tremorgenic mycotoxin, territrem B, in paddy samples which is one of the main ingredients of poultry feed of this region. The abnormal histopathological changes in different vital organs of chick may attribute to the presence of territrem b resulting in the proliferation of these symptoms which ultimately lead to the death of poults and serious economic loss to the farmer. The feed which is nutritionally rich in essential nutrients provides a congenial atmosphere for the proliferation variety of moulds including A. terreus resulting in the elaboration of territrem B which forms the root cause of tremorgenic disorders in poults. Therefore, providing healthy feed to poults is of primary importance. Providing *A. terreus* free feed to poults and prevent the health hazards due to primary and secondary exposure to territrem B.

SIGNIFICANCE STATEMENT

This study discovered the presence of potent tremorgenic mycotoxin territrem B in paddy samples which is one of the main- ingredients of poultry feed of this region. The adverse histopathological changes in the organs of chicks due to the effect of territrem B results from organ proliferation and ultimately leads to the death of the chicks, thus resulting in serious economic losses. This study will help the researchers to uncover the critical areas about the management and control of mycotoxins in feed. Present studies highlight the importance of providing *A. terreus* free feed to poults and prevent health hazards of primary and secondary exposure of territrem B.

ACKNOWLEDGMENT

Thanks are due to the Head, Department of Botany, Kakatiya University for providing Laboratory facilities and University Grant Commission, New Delhi for financial assistance.

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