TOXICITY STUDY OF FUSARIUM XYLARIOIDES INFECTED GROUNDNUT HAY IN CALVES

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Toxicity study of Fusarium xylarioides infected groundnut hay was conducted in calves. The fungi was isolated from the contaminated groundnut hay which caused mycotoxicosis in cross bred cattle which exhibited the clinical signs of colic, tenesmus, ruminal atony, anorexia, bleeding from nostrils, rectum and fly bite site. The calves were fed with Fusarium xylarioides infected groundnut hay @ 50, 75 and 100 g/kg daily for 7 days along with normal fodder. Aflatoxin B1, B2, M1, M2, citrinine, ochratoxin, deoxy nivalenol and T-2 toxins were not detected in TLC method. Clinical signs observed were diarrhea, weakness, severe arching of back, swollen forehead and conjunctival hemorrhage. Cutaneous hemorrhagic patches on back, scrotum and abdomen region seen. The calves lost balance on hind limbs and sometimes on forelimbs. There was a significant increase (P< 0.05) in serum concentrations of creatinine, urea nitrogen, ALT and AST indicated the renal and hepatic damage which was confirmed by histopathology. There were lesions in CNS and GI tract of the treated calves. The present study indicated the toxic feature Fusarium xylarioides infected ground nut hay.

Introduction: Molds are filamentous, fuzzy or dusty looking fungi that occur in many feedstuffs including roughages and concentrates. Fungal growth is typically triggered by warm and wet conditions. Fungi can infect dairy cattle, especially during stressful periods when they are immune suppressed, causing a disease referred to as mycosis. Molds also produce secondary metabolites or poisons called mycotoxins that affect animals when they consume mycotoxin contaminated feeds. This disorder is called mycotoxicosis. During the disease investigation process in Pattanayakanahalli of Tumkur District, Karnataka, India, cattle which fed with contaminated groundnut hay, showed disease especially in cross bred cows and buffaloes. Clinical signs noticed in the affected animals were loss of body condition, colic, tenesmus, ruminal atony, anorexia and bleeding disorder. There was bleeding from nostrils, rectum and fly bite site. Analysis of serum samples from these animals revealed an increase in aspartate aminotransferase (AST) values suggestive of liver damage. The detailed clinical investigation and history revealed that the groundnut hay fed to these animals’ revealed blackish specks or spots indicative of fungal growth. The clinical signs were predominantly observed during winter season. In the present study, the toxic potential of fungal affected ground nut hay was evaluated in cross bred calves. The objectives of the study were infecting the autoclaved groundnut hay with Fusarium xylarioides, experimental induction of toxicity in calves with this hay, studying the blood serum chemistry, to correlate the findings with gross and histopathological changes.

Materials and methods: Groundnut hay Fungal contaminated groundnut hay was obtained from the affected region of Pattanayakanahalli, of Tumkur District, Karnataka, India where the animals were affected after consuming naturally infected fungal contaminated hay. The representative samples of the hay were taken randomly for the isolation of the fungi. Preparation of solid and liquid media Potato Dextrose Agar (PDA) Pealed chopped potato 200 g Dextrose 20 g Agar agar 20 g in 1000 ml. The chopped potato was boiled in distilled water and to the extract dextrose and agar were added. Finally, distilled water was added to make up the volume to one liter. The medium thus prepared was poured into sterile conical flask and sterilized in an autoclave at 120 degree Celsius. 15 lb for 15 minutes. After cooling, 25 ml of streptomycin was added into the flasks before pouring into petri plates under the laminar flow and allowed to solidify. Plates The sterilized molten PDA was poured into the sterile petri plates under the laminar flow allowed to solidify. Slants The PDA prepared was dispensed into the 20 ml test tubes at the rate of 5 ml per test tube and autoclaved. These were kept in the slanting position and allowed to solidify. Potato Dextrose Broth (PDB) Pealed chopped potato 200 g Dextrose 20 g in 1000 ml The chopped potato was boiled in distilled water and to the extract dextrose was added. Finally, distilled water was added to make up the volume to one liter. The broth thus prepared was sterilized in an autoclave at 1200C, 15 lb for 15 minutes. Isolation of the fungi Fungal contaminated groundnut hay bits of 4-5 mm size were placed on the PDA plates and incubated at 28 degree Celsius for 5-7 days. The colony characters were studied and pure culture of individual colonies was raised by the hyphal tip isolation method. The pure cultures thus obtained were inoculated into PDA slants and kept in a refrigerator and maintained as stock culture. Identification of the fungi Identification of the isolated fungi was done by Fungus Identification Service, Mycology and Plant Pathology Group, Agharkar Research Institute, Pune, India. Preparation of Fusarium xylarioides infected groundnut hay The major fungi isolated was Fusarium xylarioides which was used for further study. Normal groundnut hay was collected in polypropylene plastic covers and was sterilized in autoclave at 1200C, 15 lb for 45 minutes. Next it was fed with pure cultures of Fusarium xylarioides under standard laboratory conditions. Hay became completely infected with fungus within 15-20 days which was confirmed by visual fungal growth. Fungus infected hay thus obtained was stored in air tight polypropylene plastic covers in room temperature and was used for further study. Screening of the mycotoxins The secondary metabolites of Fusarium xylarioides were screened for the presence of aflatoxin B1, B2, M1,M2, citrinine, ochratoxin, deoxy nivalenol and T-2 toxins by TLC method. Experimental Animals Calves Twelve apparently healthy young cross bred jersey calves were used in the present study. They were of the age group of two years and the body weight was 100 ± 20 kg. The animals were acclimatized to hygienic laboratory conditions, providing good hay and water ad libum. Design of the experiment Acute oral toxicity study was conducted to determine the toxicity of the infected groundnut hay in calves. Procedure The calves were grouped into 4 groups (n=3) namely group I, II, III and IV. Group II, III and IV were fed with fungal infected hay @ dose rate of 50,75 and 100g/kg respectively for 7 days. Group I served as control and fed with normal groundnut hay @ 100 g/kg body wt. Clinical observations General clinical observations were made thrice in a day. The health condition of the animals was recorded. Blood was collected every day from jugular vein. All animals were observed for morbidity and if any death was noticed, necropsy was conducted and organs were collected for histopathological studies. Clinical biochemistry Study was done to investigate the toxic effect of the fungal culture filtrate on different systems of the body, more specifically on the liver, heart and kidney functions. Serum biochemical parameters like alanine aminotransferase (ALT), aspartate
aminotransferase (AST), creatinine (CRT) and serum urea nitrogen concentrations were estimated. Pathological study At the end of the study period, representative tissue samples of liver, kidney, spleen, heart, lung, brain, intestines and stomach were collected in 10 % NBF for histopathological study (Luna, 1968).

Results and discussion: In the present study, the toxicity pattern of one of the predominant fungal species isolated from the fungal contaminated ground nut hay Fusarium Xylarioides was evaluated. Perusal of the literature revealed no reports of the same fungal species identified on groundnut hay. The fungal culture filtrate was negative for all the eight mycotoxins analyzed. Clinical signs in calves Calves were weak, depressed and recumbent. There was reduction in feed intake. The calves were diarrheic, had severe arching of back. The calves lost balance on hind limbs and sometimes on forelimbs. There was swollen forehead and torticollis. Hemorrhages were seen on conjunctiva, sub cuts on back, scrotum and abdomen. The gross changes in the liver comprised of hemorrhage, congestion and the typical histopathological lesions confirmed the liver damage due to F.xylarioides treated groups in calves. The results of the present study is in accordance with the findings of Sharma et al. (1983). There was a significant increase (P< 0.001) in serum ALT and AST concentrations in the samples of day 3, 5 and 7 in group III and IV. The elevated serum AST and ALT concentration compared to control group was suggestive of the possible role of mycotoxins present in infected groundnut hay in liver damage. This was further supported by the gross and histopathological lesions in the treated groups, by the presence of lesions of severe congestion, centrilobular necrosis, vascular degeneration, mild biliary hyperplasia, fibrotic change at periportal areas and cholestasis in some of the hepatocytes. Such hepatic damage in animals due to mycotoxicosis was also reported by many workers with elevation of serum AST concentration (Voss et al., 1995; Kellerman et al., 1990; Fodor et al., 2006). The serum creatinine concentration in calves increased significantly (P< 0.001) from day 3 to 7 in group III and IV and serum urea nitrogen concentration in these groups increased significantly (P< 0.001) from day 5 to day 7. The elevated serum creatinine and urea nitrogen concentration compared to control group was suggestive of the possible role of the toxins in causing kidney damage. This was further supported by histopathological lesions like, congestion along with vascular degeneration, necrosis of the tubules and fibrosis in the interstitium. Similar findings were also reported by Fodor et al. (2006). In the present study, the lesions in the brain comprised of congestion of blood vessels, perivascular cuffing with mononuclear cells, multiple focal areas of necrosis with infiltration of few inflammatory cells and occasional gial cell aggregation. The changes in the CNS described as necrois, liquefaction and hemorrhages in the present study were similar to those of earlier reports of Uhlinger (1997) who reported that leukoencephalomalacia in horses was caused by fumonisins. Mild congestion of intestinal mucosa and hemorrhage was observed. T-2 toxin is a very potent mycotoxin in cattle which was associated with gastroenteritis and intestinal hemorrhages (Petrie et al., 1977). However T2 toxin was negative in the screening of the culture filtrate which indicates either the concentration of T2 might be too low to be detected by TLC method or calves might be susceptible to such low concentration. Congestion of blood vessels of stomach was observed in all group of the calves. Similar findings were reported by Junsuk et al. (1999) in rats which were fed with fungal contaminated the diets. Further study is essential to confirm the changes seen under natural disease process in cattle by considering many factors including dose, concentration of the infected groundnut hay and the form in which the test material is administered.

Conclusion: Fusarium xylarioides was isolated from moulidy ground nut hay, which had caused toxicity in cattle has been used in this study. The present toxicity study revealed that Fusarium xylarioides infected hay administered to calves @ 75 and 100 g/kg where the calves were diarrheic, had severe arching of back and paraplegic. There was swollen forehead and conjunctival hemorrhages. Cutaneous hemorrhagic patches on back, scrotum and abdomen, were seen along with pronounced nervous disorders. The present study revealed the hepatotoxic, nephrotoxic and cardiotoxic nature of Fusarium xylarioides in calves in toxicity study. The toxin's present in the culture filtrate of F.Xylarioides has to be identified and isolated.

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