TOXICITY STUDY OF FUSARIAUM XYLARIIOIDES INFECTED GROUNDNUT HAY IN CALVES

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ABSTRACT

Toxicity study of groundnut hay infected with Fusarium xylarioides was conducted in calves. The fungi was isolated from the contaminated groundnut hay which caused mycotoxicosis in 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 fungal infected groundnut hay at the dose level of 50, 75 and 100 g/kg daily for 7 days along with normal fodder. Clinical signs observed were diarrhea, weakness, severe arching of back, swollen forehead and conjunctival hemorrhage. Cutaneous hemorrhagic patches on back, scrotum and abdomen region were seen. The calves lost balance on hind and 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 respectively, which was further confirmed by histopathological lesions. There were lesions in heart, brain and GI tract of the treated calves. The present study indicated the toxic feature of Fusarium xylarioides infected ground nut hay in calves.

Key words : Toxicity, Groundnut hay, Calf, Fusarium xylarioides.

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 as mycotoxicosis (Nelson et al.,1993).

During the disease investigation process in Pattanayakanahalli of Tumkur District, Karnataka, India, in cross bred cows and buffaloes which were fed with fungal contaminated groundnut hay affected with toxicity. Clinical signs noticed in the affected animals were, loss of body condition, colic, tenesmus, ruminal atony, anorexia and bleeding disorders. There was bleeding from nostrils, rectum and fly bite site. Analysis of serum samples from these animals revealed a significant increase (P<0.05) in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) 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 groundnut hay was evaluated in calves. The present study was conducted

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with an objective of isolation and identification of the fungi present in the fungal contaminated groundnut hay, experimental induction of toxicity in calves and studying the blood serum chemistry, gross pathological and histopathological changes.

**MATERIALS AND METHODS**

**Groundnut hay**

Fungal contaminated groundnut hay was obtained from Pattanayakanahalli of Tumkur District, Karnataka, India where there was a natural episode of toxicity of fungal infected groundnut hay in cattle. 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 was added. Finally, distilled water was added to make up the volume to one liter.

The medium thus prepared was poured into sterile conical flasks and sterilized in an autoclave at 120°C, 15 lb for 15 min. After cooling, 25 ppm of streptomycin was added into the flasks before pouring into Petri plates to prevent the bacterial contamination.

**Plates**

The sterilized molten PDA was poured into the sterile Petri plates under the laminar flow and 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 broth thus prepared was sterilized in an autoclave at 120°C, 15 lb for 15 minutes.

**Isolation of the fungi**

Bits of fungal contaminated groundnut hay of 4-5 mm size were placed on the PDA plates and incubated at 26°C for 5-7 days. The colony characters were studied and pure culture of individual colonies was raised by the hyphal tip isolation method (Joffe and Palti, 1975). 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. Fungus identified as *Fusarium xylarioides* (*F.xylarioides*).

**Preparation of infection free groundnut hay**

Fungal contaminated groundnut hay was collected in polypropylene plastic covers and was sterilized and autoclaved at 120°C, 15 lb for 45 min. It was infected with pure culture of *F. xylarioides* under standard laboratory conditions. Hay became completely infected with fungus within 15-20 days which was visualized by the growth of the fungus. Fungus infected hay thus obtained was stored in polypropylene plastic covers in room temperature and was used for further study.

**Experimental animals**

**Calves**

Apparently healthy young Holstein Friesian Cross bred male 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 calves were acclimatized to the experimental conditions for a week. They were maintained under hygienic conditions, providing
good hay and water ad libitum. The calves were grouped (n=4) and housed in animal shed during the experiment.

**Design of the experiment**

Calves were grouped into four groups (n=4) in which the Group A served as control and was fed with normal groundnut hay @ 100 g/kg, whereas the Group B, C and D were fed with infected groundnut hay at the dose level 50, 75 and 100 g/kg for 7 days.

**Clinical observations**

General clinical observations were made thrice in a day. The health condition of the animals was recorded. All calves were observed for morbidity and mortality. Necropsy of dead calves was conducted and the post mortem lesions were observed.

**Clinical biochemistry**

The blood was collected on day 0, 3, 5 and 7 and the serum was used to estimate the concentrations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine (CRT) and serum urea nitrogen concentration to assess the toxic effect of the fungal infected ground nut hay on liver and kidney.

**Pathological study**

During the necropsy, representative tissue samples of liver, kidney, spleen, heart, lung, brain, intestines and stomach from the calves were collected in 10% neutral buffered formalin (NBF) for histopathological study (Luna, 1968).

**RESULTS AND DISCUSSION**

In the present study, one of the predominant fungal species isolated from the fungal contaminated ground nut hay was *Fusarium xylarioides*. Perusal of the literature revealed no reports of the same fungal species identified on groundnut hay. The fungal culture filtrate was negative for the presence of aflatoxins (B1, B2, G1 and G2), ochratoxin, T2, citrinin, sterigmatocystin and zeralenone by TLC method.

### Table 1: The effect of *F. xylarioides* infected groundnut hay on serum ALT and AST concentration (U/L).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Days</th>
<th>ALT</th>
<th>AST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal groundnut hay, Control, 100 g</td>
<td>0</td>
<td>34.75±0.51</td>
<td>100.22±0.84</td>
</tr>
<tr>
<td><em>F. xylarioides</em> infected groundnut hay, 50 g</td>
<td>3</td>
<td>38.75±0.65</td>
<td>106.78±1.71</td>
</tr>
<tr>
<td><em>F. xylarioides</em> infected groundnut hay, 75 g</td>
<td>5</td>
<td>39.92±1.11</td>
<td>108.00±0.91</td>
</tr>
<tr>
<td><em>F. xylarioides</em> infected groundnut hay, 100 g</td>
<td>7</td>
<td>40.80±0.76</td>
<td>112.29±1.45</td>
</tr>
</tbody>
</table>

Values are mean ± SE, n = 4, **P < 0.01, ***P < 0.001.
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, subcutis of back, scrotum and abdomen.

The gross changes in the heart seen were ecchymotic hemorrhagic spots. 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 are in accordance with the findings of Sharma et al. (1983), who reported that Fusarium toxins cause congestion and hemorrhage of liver in dairy animals.

There was a significant increase (P<0.001) in serum ALT and AST concentrations on day 3, 5 and 7 in groups fed with 75 g/kg (Group B) and 100 g/kg (Group C) of the hay (Table 1) compared to the control group. The elevated serum AST and ALT concentration was suggestive of the possible role of unidentified 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, vacuolar degeneration, mild biliary hyperplasia, fibrotic change at periporal areas and karyomegaly in some of the hepatocytes. Such hepatic damage in calves due to mycotoxicosis was also reported by many workers with elevation of serum AST concentration who reported fumonisin B1 induced liver damage in mice, rats and horses (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 5 to 7 in Group B (75 g/kg) and Group C (100 g/kg) and serum urea nitrogen concentration in these groups increased significantly (P<0.001) from day 5 to day 7 (Table 2). The elevated serum creatinine and urea nitrogen concentration in comparison to control

Table 2: The effect of F. xylarioides infected groundnut hay on serum creatinine and urea nitrogen concentration (mg/dl).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Creatinine</th>
<th>Urea nitrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal hay</td>
<td>75g/kg hay</td>
</tr>
<tr>
<td>Control</td>
<td>0.48±0.01</td>
<td>0.54±0.02</td>
</tr>
<tr>
<td>3</td>
<td>0.44±0.01</td>
<td>0.59±0.01</td>
</tr>
<tr>
<td>5</td>
<td>0.44±0.01</td>
<td>0.59±0.01</td>
</tr>
<tr>
<td>7</td>
<td>0.44±0.01</td>
<td>0.59±0.01</td>
</tr>
</tbody>
</table>

Values are mean ± SE, n = 4, **P < 0.01, *** P < 0.001.
group concentration is suggestive of the possible role of the toxins in causing kidney damage. This was further supported by histopathological lesions like, congestion along with vacuolar degeneration, necrosis of the tubules and fibrosis in the interstitium (Fig. 2). Similar findings were also reported by Fodor et al. (2006) where in, it was observed that fumonisin caused kidney damage.

In the present study, the lesions in the brain comprised of petecchial hemorrhages, congestion of blood vessels, perivascular cuffing with mononuclear cells, multiple focal areas of necrosis with infiltration of few inflammatory cells and occasional glial cell aggregation (Fig. 1). The changes in the CNS described as necrosis, liquefaction and hemorrhages in the present study were similar to those of earlier reports of Uhlinger (1997), who reported lecoencephalomalacia in the horse brain.

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 indicated 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 rumen was observed in all groups of the calves. Similar findings were reported by Junsuk et al. (1999), in rats which were fed with fungal contaminated the diets.

Further studies are essential to confirm the changes seen under natural disease process in large animals by considering many factors including dose, quantity of the infected groundnut hay and the form in which the test material is administered.

CONCLUSION

In Fusarium xylarioides infected hay administered group, calves were diarrheic and 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 the culture filtrate of F.xylarioides in calves at the dose of 50, 75 and 100 g/kg in the acute toxicity study. The toxin/s present in the culture filtrate of F.xylarioides has to be identified, isolated and quantified.

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