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# Sub chronic toxicity study of *Fusarium verticilloides* culture filtrate from groundnut hay in rats

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ABSTRACT

Sub chronic toxicity study of culture filtrate of *Fusarium verticilloides* isolated from the fungal contaminated groundnut hay was conducted in rats. The fungi was isolated from the contaminated groundnut hay which caused mycotoxicosis in cross-bred cattle, exhibited the clinical signs of colic, tenesmus, ruminal atony,anorexia, bleeding from nostrils, rectum and fly bite site. Rats (20) were gavaged with culture filtrate at the dose level of 0.5, 1 and 2 ml daily for 90 days. Clinical signs observed were diarrhoea, weakness, severe arching of back, swollen forehead and conjunctival hemorrhage. Cutaneous hemorrhagic patches on back, scrotum, abdomen, ears and legs region were seen. 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 brain and GI tract of the treated rats. The present study indicated the toxic feature of the culture filtrate of *Fusarium verticilloides* isolated from ground nut hay.

**Key words**: Culture filtrate, Groundnut hay, Fusarium verticilloides, Rat, Sub chronic toxicity

Cross-bred cattle fed with contaminated groundnut hay for duration of 2–3 months exhibited the clinical signs of loss of body condition, colic, tenesmus, ruminal atony and anorexia, which was noticed in Pattanayakanahalli village of Tumkur District in Karnataka, India. There was bleeding from nostrils, rectum and fly bite site of the affected animals. Liver function tests of these animals revealed liver damage. The detailed clinical investigation and history revealed that the groundnut hay was fed to these animals and revealed blackish specks or spots indicative of fungal growth. The clinical signs were observed during winter.

Fungal infected hay 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 (Pier 1992). The total number of mycotoxins is not known, but the number of potential toxic metabolites of fungi has been estimated to be in the thousands, although to date only about 300 different mycotoxins have been identified (CAST 2003). Mycotoxicosis is well documented in poultry. However there is scanty information on cattle mycotoxicosis.

In the present study, the sub chronic toxicity of *Fusarium verticilloides* isolated from fungal affected groundnut hay was evaluated in rats.

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## MATERIALS AND METHODS

*Collection of material:* Fungal contaminated groundnut hay which was fed to the ailing animals was obtained from Pattanayakanahalli village of Sira taluk, Tumkur district of Karnataka. Groundnut hay was cultured on potato dextrose agar. The pure culture of isolated fungus was sent to Fungal Identification Service, Mycology and Plant Pathology Group, Agharkar Research Institute, Pune and fungal species was identified as *Fusarium verticilloides*. *F. verticilloides* was mass cultured on potato dextrose broth. After confirmation of complete growth of the fungus, the supernatant was discarded and the filtrate was used for gavaging the rats. The fungal culture filtrate was analyzed for the presence of aflatoxins (B1, B2, G1 and G2), ochratoxin, T2, citrinin, sterigmatocystin and zeralenone by TLC method.

*Experimental design:* As per OECD guidelines 408, apparently healthy young Wistar albino rats, aged 5 weeks having body weight of 100±10 g were used. Four groups of rats (n, 20) were made and housed in standard polypropylene cages during the experiment. Group 1 served as control which was gavaged with 2 ml of potato dextrose broth where as

group 2, 3 and 4 were gavaged with *F. verticilloides* culture filtrate at the dose level of 0.5, 1 and 2 ml daily respectively for 90 days. The rats were weighed individually at the beginning of the study and at fortnight interval till day 90. Daily all the rats were observed for the clinical signs of toxicity, morbidity and mortality.

*Clinical biochemistry:* The blood samples were obtained by retro-orbital plexus puncture method on day 0, 7, 14, 21, 35, 50, 75 and 90 and the serum was used to estimate serum concentrations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine (CRT) and blood urea nitrogen (BUN) using biochemical analyzer and commercially available diagnostic kits.

*Gross and histopathological studies:* Necropsy of the rats was conducted which succumbed during the experiment and the survived rats were sacrificed at the end of the study. Organs were weighed and representative tissue samples of liver, kidney, brain, intestines and stomach were collected in 10% normal buffer formalin solution (NBF) and were subjected to histopathology (Luna 1968).

*Statistical analysis:* The data was analyzed by one-way ANOVA with Tukey's post test using GraphPad Prism Software (Trial version 4.03 for Windows), California, USA.

## RESULTS AND DISCUSSION

In the present study, one of the predominant fungal species isolated from the fungal contaminated groundnut hay was *F. verticilloides*. Perusal of the literature revealed no reports of the same fungal species identified from groundnut hay. The fungal culture filtrate was negative for aflatoxins (B1, B2, G1 and G2), ochratoxin, T2, citrinin, sterigmatocystin and zeralenone.

Clinical signs in rats observed were diarrhoea, weakness, reduced feed /water intake, loss of body weight, recumbency, severe arching of back, swollen forehead and torticollis. The rats lost balance of hind limbs and rarely the forelimbs. Hemorrhages were seen on conjunctiva, sub cutis on back, scrotum, abdomen, ears and legs region (Figs 1 and 2). These results were similar to the earlier findings of Yiannikouris and Jouany (2002), who reported that mycotoxins caused weight loss, vomiting, severe skin problems, bleeding and death of animals.

The gross changes in the liver comprised of hemorrhages, congestion and the histopathological lesions like centrilobular necrosis, vacuolar degeneration, mild biliary hyperplasia, fibrotic change at periportal areas and karyomegaly in some

Table 1. The effect of *F.verticilloides* culture filtrate on serum ALT and AST concentration (U/L)

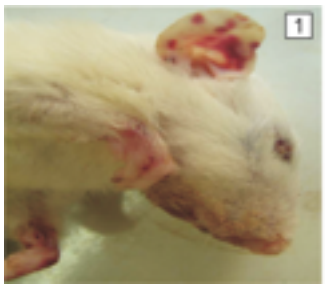
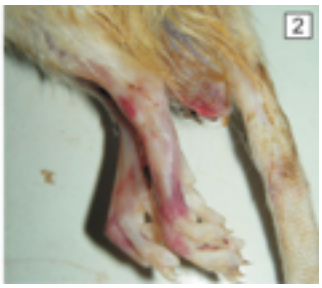
| Groups / Days | Group 1 (PD broth, Control) | | Group 2 (*F.verticilloides*, 0.5ml) | | Group 3 (*F.verticilloides*, 1ml) | | Group 4 (*F.verticilloides*, 2ml) | |
|---|---|---|---|---|---|---|---|---|
| | ALT | AST | ALT | AST | ALT | AST | ALT | AST |
| 0 | 34.05±1.15 | 100.43±1.41 | 34.84±1.23 | 101.28±1.59 | 36.61±1.59 | 102.84±1.22 | 35.63±1.68 | 102.21±1.53 |
| 7 | 36.22±1.04 | 104.16±0.61 | 37.90±1.53 | 111.77±2.35 | 41.60±1.86 | 116.76±3.21 | 41.61±1.86 | 116.95±4.62 |
| 14 | 38.63±1.06 | 105.88±0.70 | 42.57±0.92 | 138.79±3.44 | 47.90±1.33** | 146.68±4.63** | 50.49±1.26** | 156.62±5.33** |
| 21 | 39.41±0.97 | 107.98±0.69 | 44.95±1.08 | 181.17±3.52 | 50.30±1.27*** | 185.30±4.94*** | 53.15±1.38*** | 197.74±6.97*** |
| 35 | 43.06±0.64 | 110.40±0.72 | 47.87±0.74 | 215.88±5.12 | 53.21±1.09** | 230.18±5.25** | 56.56±1.18*** | 237.01±6.07*** |
| 50 | 45.49±1.18 | 115.05±0.78 | 58.75±1.64*** | 249.69±7.67*** | 63.04±2.13*** | 250.55±6.08*** | 68.44±1.53*** | 272.65±4.68*** |
| 75 | 47.74±1.15 | 108.08±0.77 | 64.22±2.16*** | 230.29±8.39*** | 67.58±1.92*** | 236.89±4.47*** | 72.65±1.14*** | 257.98±4.91*** |
| 90 | 46.55±1.85 | 101.46±0.66 | 59.70±1.57 | 218.14±7.51*** | 62.55±2.07** | 223.70±4.46** | 67.84±1.19*** | 241.12±6.37*** |

Values are mean ± SE; n, 20; *** P < 0.001; **P < 0.01.

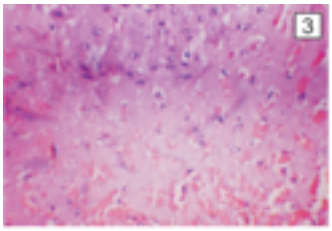
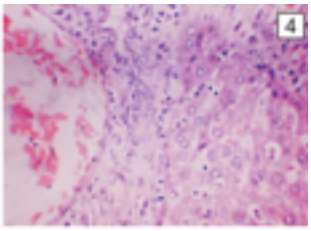
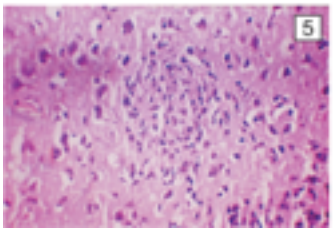
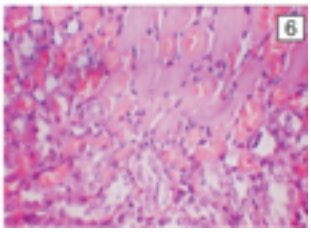
Table 2. The effect of *F.verticilloides* culture filtrate on serum creatinine and urea nitrogen concentration (mg/dl)

| Groups / Days | Group 1 (PD broth, Control) | | Group 2 (*F.verticilloides*, 0.5ml) | | Group 3 (*F.verticilloides*, 1ml) | | Group 4 (*F.verticilloides*, 2ml) | |
|---|---|---|---|---|---|---|---|---|
| | Creatinine | Urea nitrogen | Creatinine | Urea nitrogen | Creatinine | Urea nitrogen | Creatinine | Urea nitrogen |
| 0 | 0.49±0.033 | 38.38±1.30 | 0.40±0.018 | 36.90±1.08 | 0.46±0.027 | 39.37±1.19 | 0.49±0.024 | 37.74±1.26 |
| 7 | 0.54±0.030 | 39.66±1.33 | 0.45±0.016 | 37.41±0.97 | 0.49±0.023 | 40.39±1.18 | 0.55±0.017 | 40.52±0.86 |
| 14 | 0.57±0.036 | 40.99±1.14 | 0.50±0.016 | 37.43±0.87 | 0.58±0.014 | 41.32±1.11 | 0.61±0.022 | 42.50±0.95 |
| 21 | 0.61±0.025 | 42.43±1.39 | 0.56±0.01 | 38.39±0.93 | 0.640±0.016 | 42.44±1.37 | 0.71±0.026 | 46.22±1.37 |
| 35 | 0.58±0.044 | 44.06±1.75 | 0.62±0.01 | 43.52±1.43 | 0.720±0.015* | 47.77±1.25 | 0.79±0.026*** | 50.57±1.23 |
| 50 | 0.58±0.035 | 46.00±1.92 | 0.69±0.022** | 49.77±1.57 | 0.79±0.020*** | 54.63±1.87** | 0.93±0.010*** | 58.39±2.70*** |
| 75 | 0.56±0.03 | 41.75±1.72 | 0.72±0.04** | 46.60±1.37 | 0.93±0.04*** | 50.79±1.72** | 0.99±0.02*** | 54.43±1.77*** |
| 90 | 0.55±0.027 | 38.07±1.34 | 0.74±0.030** | 41.60±0.93 | 0.89±0.021** | 44.17±1.46 | 0.91±0.023*** | 48.37±1.45*** |

Values are mean ± SE; n, 20; *** P < 0.001; * *P < 0.01.

Figs. 1–2. **1.** Hemorrhagic patches on ears; **2.** Hemorrhagic patches on legs and scrotum

Figs 3–6. **3.** Section of liver from rat treated with *F. verticilloides* culture filtrate showing extensive haemorrhages with loss of normal architecture. Note: hepatomegaly in some of hepatocytes adjacent to normal cells H&E × 500 **4.** Section of liver from rat treated with *F. verticilloides* culture filtrate showing prominent biliary hyperplasia in periportal region. H&E × 500 × 500 **5.** Section of kidney from rat treated with *F. verticilloides* culture filtrate showing severe congestion of inter tubular vessels and focal areas of tubular necrosis with loss of architecture. H&E × 500 × 500 **6.** Section of brain from rat treated with *F. verticilloides* culture filtrate showing severe congestion of blood vessels and perivascular mononuclear cuffing. H&E × 500 × 500.

of the hepatocytes, confirmed the liver damage due to *F. verticilloides* treated groups. The results of the present study are in accordance with the earlier findings of Sharma *et al.* (1983). This is further supported by the findings of Smith and Thakur (1996) who reported that livers from treated calves had mild hydropic degeneration and cloudy swelling in a periacinar pattern throughout the liver. These changes were consistent with the mild to moderate elevation of liver enzymes.

There was a significant increase (P<0.001) in serum ALT and AST concentrations in the samples of day 21, 35, 50, 75 and 90 (Table 1). The elevated serum AST and ALT concentration compared to control group is suggestive of the possible role of presence mycotoxins present in gavaged culture filtrate in liver damage (Itakura *et al.* 1980, Vinay *et al.* 2011). 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 periportal areas and karyomegaly in some of the hepatocytes (Figs. 3, 4). Such hepatic damage in rats and mice 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 rats increased significantly (P<0.001) from day 35 to 90 and serum urea nitrogen concentration increased significantly (P<0.001) from day 50 to day 90 (Table 2) in groups treated with culture filtrate. The elevated serum creatinine and urea nitrogen in comparison to control concentration, is suggestive of the possible role of mycotoxins 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. 5). 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 glial cell aggregation (Fig. 6). The changes in the brain described as necrosis, liquefaction and hemorrhages in the present study were similar to those of earlier reports (Ross *et al.* 1993, Uhlinger 1997, Vinay *et al.* 2011).

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, Yiannikouris and Jouany 2002). However T2 toxin was negative in the screening of the culture filtrate which indicated that either the concentration of T2 might be too low to be detected by TLC method or rats might be susceptible to such low concentration or some other mycotoxins might be responsible for the toxicity.

Congestion of blood vessels of stomach was observed in all groups of the rats. Similar findings were reported when fed with fungal contaminated diets. (Junsuk *et al.* 1999, Yiannikouris and Jouany 2002).

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

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## REFERENCES

CAST (Council for Agricultural Science and Technology). 2003. *Mycotoxins: Risks in Plant, Animal and Human Systems*. Task force report No. 139, anuary, Ames, Iowa.

Fodor J, Meyer K, Riedlberger M, Bauer J and Posa R. 2006. The distribution and elimination of fumonisins after oral administration of *Fusarium verticillioides* fungal culture. *Magyar-Allatorvosok-Lapja* **128**: 334–42.

Itakura H, Yamashita H, Kawasaki Y and Mizunuma T. 1980. Toxic fungi isolated from Uganda foodstuffs, chronic toxicity of fungal culture filtrates. *Tropical Medicine* **22**(2): 95–100.

Junsuk P, Kyungrim L, Jincheol K, Sunhee L, Jeongah S and Yinwon L. 1999. A hemorrhagic factor (apicidin) produced by toxic Fusarium isolates from soybean seeds. *Applied Environmental Microbiology* **4**: 126–30.

Kellerman T S, Marasas W F O, Thiel P G, Gelderblom W C A, Cawood M J and Coetzer A W. 1990. Leukoencephalomalacia in two horses induced by oral dosing of fumonisin B1. Onderstepoort *Journal of Veterinary Research* **57**: 269–75.

Luna L G. 1968. Manual of histopathological staining methods of the Armed Forces Institute of Pathology. 3rd edn, Pp 25–78. McGraw Hill Book Co, New York.

Pier A C. 1992. Major biological consequences of aflatoxicosis in animal production. *Journal of Animal Science* **70**: 3964–70.

Petrie L, Robb J and Stewart A F. 1977. The identification of T 2 toxin and its association with a hemorrhagic syndrome in cattle. *Veterinary Record* **101**: 326–26.

Ross P F, Ledet A E, Owens, D Z, Ric L G, Nelson H A, Osweiler G D and Wilson T M. 1993. Experimental equine leukoencephalomalacia, toxic hepatosis and encephalopathy cause by corn naturally contaminated with fumonisine. *Journal of Veterinary Diagnostic Investigation* **5**: 69–74.

Sharma R B, Kumar A and Roy A N. 1983. Nature of hepatic damage induced by culture filtrates of seed-borne fungi in the laboratory rat. *Toxicology Letters* **19**: 1–2.

Smith J S and Thakur R A. 1996. Occurrence and fate of fumonisins in beef. *Fumonisin in Food*. Pp 39–55. (Eds) Jackson L S, Devries J W and Bullerman L B. Plenum Press, New York.

Uhlinger C. 1997. Leukoencephalomalacia in the veterinary clinics of North America. *Equine Practice* **13**: 13–20.

Vinay T, Shridhar N B, Narayanaswamy H D, Shrikrishna 1 and Jayakumar K. 2011. Sub chronic toxicity study of Fusarium xylarioides culture filtrate from ground nut hay in rats. *Journal of Laboratory Animal Science* **1**(1): 49–53.

Voss K A, Chamberlain W J, Bacon C W, Herbert R A, Walters D B and Norred W P. 1995. Sub chronic feeding study of the mycotoxin fumonisin B1 in B6C3F1 mice and Fischer 344 rats. *Fundamental Applied Toxicology* **24**: 102–10.

Yiannikouris A and Jouany J P. 2002. Mycotoxins in feeds and their fate in animals, a review. *Animal Research* **51**: 81–99.