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Sub chronic toxicity study of *Fusarium xylarioides* culture filtrate from ground nut hay in rats

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Abstract

Sub chronic toxicity study of culture filtrate of *Fusarium xylarioides* isolated from the fungal contaminated ground nut hay was carried out 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. The rats were gavaged with culture filtrate at the dose level of 0.25, 0.5, 1 and 2 ml daily for 90 days. Clinical signs observed were diarrhea, weakness, severe arching of back, swollen forehead and conjunctival hemorrhage. Cutaneous hemorrhagic patches on back, scrotum, abdomen, ears and legs region seen. There was a significant increase ($P \leq 0.05$) 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 xylarioides* isolated from ground nut hay.

Key words: sub chronic toxicity, rat, *Fusarium xylarioides*, groundnut hay,

Introduction

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 especially during winter season.

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 (Pier, 1992). Mycotoxicosis is well documented in poultry. However, there is scanty information on cattle mycotoxicosis.

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

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, Karnataka. Groundnut hay was cultured on potato dextrose agar. Among the grown fungal isolates, *Fusarium xylarioides* (*F. xylarioides*) was mass cultured on potato dextrose broth (Fig. 1). The fungus was identified and confirmed at National Facility, Agharkar Research Institute, Pune. 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 (B_1, B_2, G_1 and G_2),

ochratoxin, T_2, citrinin, sterigmatocystin and zeralenone by TLC method.

Experimental design:

Apparently healthy young Wistar albino rats aged five weeks weighing 100 ± 10 g were used. Animals were divided into 5 groups with 12 animals in each group and housed in standard polypropylene cages during the experiment. Group I served as control which was gavaged with 2 ml of potato dextrose broth where as group II, III, IV and V were gavaged with *F. xylarioides* culture filtrate at the dose rate of 0.25, 0.5, 1 and 2 ml daily respectively for 90 days. The rats were weighed individually at the beginning of the study and once in a week for three weeks and at fortnight interval till day 90. All the rats were observed daily 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 alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine (CRT) and blood urea nitrogen (BUN) using Semi-Automatic Biochemical Analyzer (ARTOS, Bangalore) and commercially available diagnostic kits (Swemed Diagnostics, Bangalore).

Gross and histopathological studies:

Necropsy of the rats were 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) GraphPad Software, San Diego, California, USA.

Results

In the present study, one of the predominant fungal species isolated from the fungal contaminated ground nut hay was *F. xylarioides*. 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 nine mycotoxins analyzed.

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 and 2). 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 3 and 4) in groups treated with 1 and 2 ml of culture filtrate.

Clinical signs in rats observed were diarrhea, 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 (Fig 2 and 3).

Fig 1.Growth of Fusarium xylarioides in potato dextrose broth

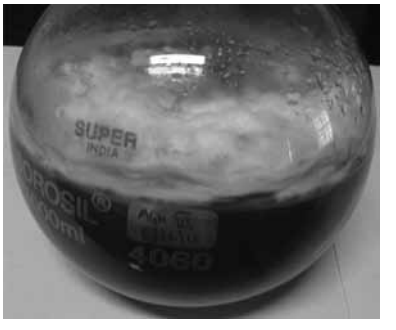


Table 1: The effect of *Fusarium xylarioides* culture filtrate on serum ALT concentration (U/L)

Treatment	Days							
	0	7	14	21	35	50	75	90
Group I (PD broth, Control)	24.7 ± 1.89	27.25±1.52	30.44±1.20	32.91±1.48	35.58±0.40	38.22±1.10	40.62±1.37	37.57±1.86
Group II (0.25 ml)	24.23±1.56	28.17±1.14	31.69±0.84	35.38±1.19	39.32±1.12	45.22±1.71	57.62±2.36***	52.61±2.42**
Group III (0.5 ml)	23.51±1.85	27.86±1.44	31.36±1.43	35.27±1.59	40.59±1.44	46.76±3.45	62.30±5.96***	55.97±3.16***
Group IV (1 ml)	24.92±0.89	28.31±1.04	32.25±1.75	37.63±2.30	46.61±4.91	56.98±3.83***	68.12±7.60***	62.62±6.84***
Group V (2 ml)	23.50±1.98	28.65±1.49	34.67±2.69	42.30±3.00	53.00±4.38***	63.27±6.65***	83.85±7.45***	73.32±6.78***

Values are mean ± SE, n = 12, *** P ≤ 0.001, * * P ≤ 0.01

Table 2: The effect of *Fusarium xylarioides* culture filtrate on serum AST concentration (U/L)

Treatment	Days							
	0	7	14	21	35	50	75	90
Group I (PD broth, Control)	90.16±2.95	93.67±3.29	98.21±3.07	103.41±2.52	107.02±2.32	111.37±2.69	120.68±4.30	114.22±3.77
Group II (0.25 ml)	87.67±3.26	91.56±2.39	97.08±2.77	101.75±2.60	114.0±5.13	135.66±7.92**	164. ±5.59***	157.09±5.11***
Group III (0.5 ml)	88.56±3.77	93.92±2.29	99.32±2.52	104.8±2.79	122.94±4.92	149.97±8.39***	179.58±8.74***	171.46±8.43***
Group IV (1 ml)	90.92±3.47	97.23±2.02	109.45±2.07	128.09±4.71**	143.13±5.49***	171.86±8.34***	200.58±9.55***	189.08±6.82***
Group V (2 ml)	90.03±2.68	97.60±1.80	115.38±2.95	137.09±5.34***	161.75±6.47***	188.94±7.35***	224.97±11.99***	214.37±12.35***

Values are mean ± SE, n = 12, *** P ≤ 0.001, * * P ≤ 0.01

Table 3: The effect of *Fusarium xylarioides* culture filtrate on serum creatinine concentration (mg/dl)

Treatment	Days							
	0	7	14	21	35	50	75	90
Group I (PD broth, Control)	0.45±0.03	0.54±0.034	0.60±0.026	0.66±0.02	0.71±0.020	0.80±0.031	0.73±0.02	0.67±0.014
Group II (0.25 ml)	0.44±0.038	0.52±0.035	0.64±0.049	0.86±0.040	0.87±0.023	0.95±0.057	0.90±0.052	0.86±0.046
Group III (0.5 ml)	0.45±0.030	0.54±0.037	0.64±0.038	0.91±0.049	0.96±0.057	1.08±.058**	0.98±0.060	0.92±0.0602
Group IV (1 ml)	0.45±0.032	0.57±0.017	0.71±0.04	0.97±0.029**	1.05±0.049**	1.15±0.043**	1.07±0.04**	0.98±0.0431**
Group V (2 ml)	0.47±0.032	0.61±0.020	0.74±0.049	1.16±0.04***	1.22±0.054***	1.30±0.069***	1.17±0.05***	1.05±0.028**

Values are mean ± SE, n = 12, *** P ≤ 0.001, * * P ≤ 0.01

Table 4 : The effect of *Fusarium xylarioides* culture filtrate on serum urea nitrogen concentration (mg/dl)

Treatment	Days							
	0	7	14	21	35	50	75	90
Group I (PD broth, Control)	35. 0±0.83	36.0 ±0.89	38.93±0.82	41.08±0.72	42.82±1.37	44.0 ±1.48	45.7±1.26	42.05±0.70
Group II (0.25 ml)	35.33±1.12	37.30±0.66	38.23±.63	40.16±0.55	44.75±0.83	47.56±1.11	49.5±1.26	46.32±1.42
Group III (0.5 ml)	34.99±1.13	36.57±0.82	38.64±0.87	41.68±0.94	46.30±1.77	50.64±1.43**	54.85±1.71***	52.70±2.08***
Group IV (1 ml)	35.28±1.05	37.91±0.85	41.30±1.22	44.56±1.54	50.66±1.55**	55.93±1.76***	60.12±2.11***	56.65±2.40***
Group V (2 ml)	35.31±1.25	38.31±1.17	43.06±1.39	48.95±1.41**	55. ±2.97***	62.54±3.28***	69.87±3.70***	65.97±3.01***

Values are mean ± SE, n =12, *** P ≤ 0.001,* * P ≤ 0.01.

The gross changes in the liver comprised of hemorrhage, congestion and the typical histopathological lesions confirmed the liver damage due to *F.xylarioides* in all treated groups of rats. This was further supported by the presence of severe congestion, centrilobular necrosis, vacuolar degeneration, mild biliary hyperplasia, fibrotic change at periportal areas and karyomegaly in some of the hepatocytes (Fig 4 and 5). Further, kidney showed histopathological lesions like, congestion along with vacuolar degeneration, necrosis of the tubules and fibrosis in the interstitium (Fig 6). 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 7). In the present study, histopathological lesions observed in the brain included necrosis, liquefaction and hemorrhages. Further, severe congestion of intestinal mucosa and hemorrhage was observed. Congestion of blood vessels of stomach was also observed in all groups of the rats.

Discussion

The gross changes in the liver comprised of hemorrhage, congestion and the typical histopathological lesions confirmed that the liver damage is due to *F.xylarioides* treatment. The result of the present study is in accordance with the findings of Sharma *et al*. (1983).

The elevated serum AST and ALT concentration compared to control group is suggestive of possible role of mycotoxins present in gavaged culture filtrate. This was further supported by the gross and histopathological lesions in the treated groups, by the presence of lesions like severe congestion, centrilobular necrosis, vacuolar degeneration, mild biliary hyperplasia, fibrotic change at periportal areas and karyomegaly in some of the hepatocytes. Such hepatic damage in rats and mice due to mycotoxicosis was also reported by many workers with elevation of serum AST concentration (Kellerman *et al*. 1990; Voss *et al.* 1995; Fodor *et al*. 2006).

Fig 2: Hemorrhagic patches on ears in rats treated with *Fusarium xylarioides* culture filtrate

Fig 3: Hemorrhagic patches on legs and scrotum in rats treated with *Fusarium xylarioides* culture filtrate

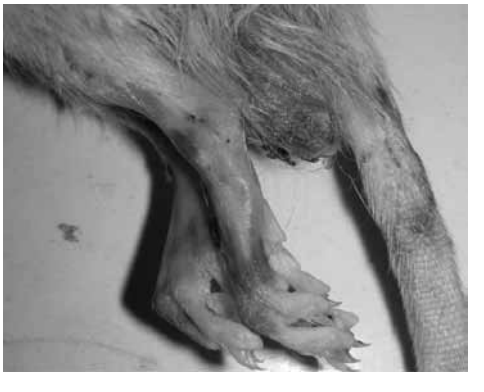
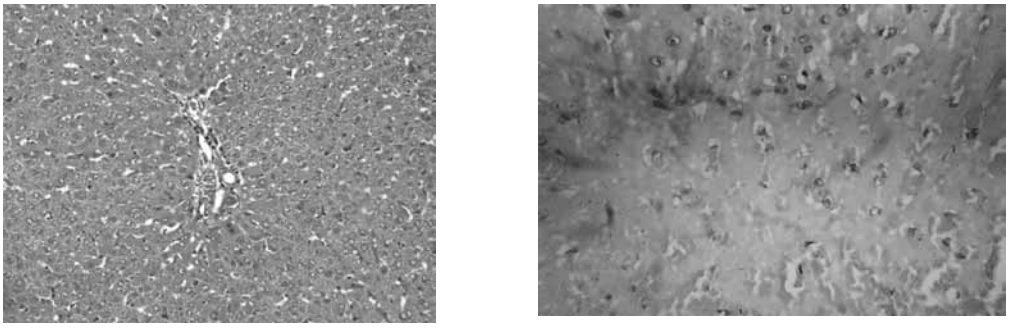
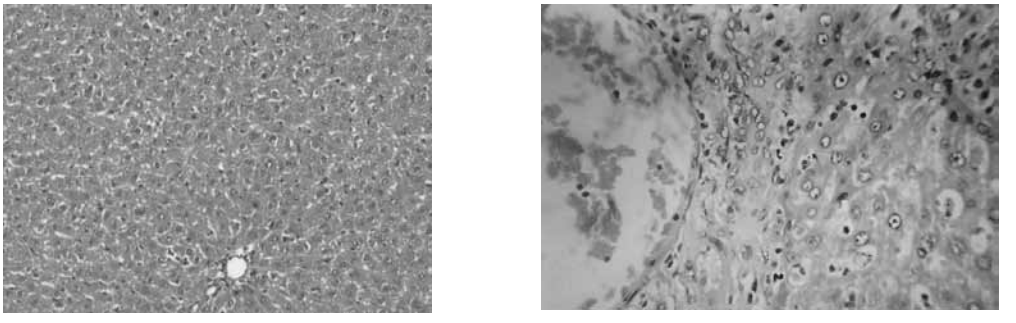


Fig 4: Section of liver from rat treated with Fusarium xylarioides culture filtrate showing extensive with loss of normal architecture. Note hepatomegaly in some of hepatocytes adjacent to normal cells (b) (H&E x 500)



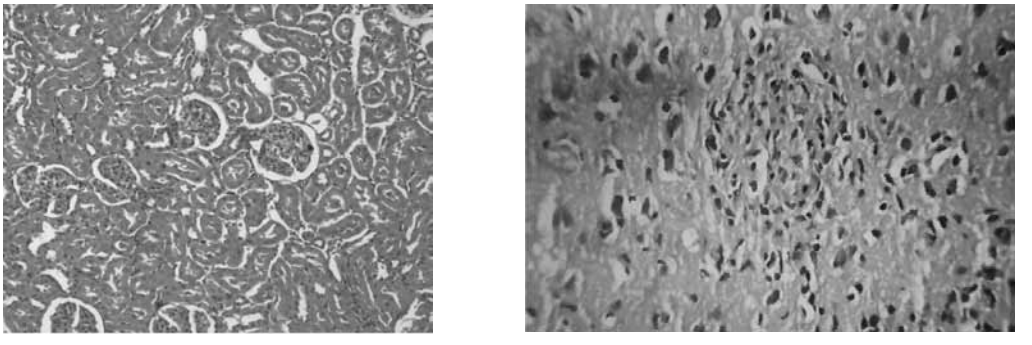
(a) Control (b) Experimental

Fig 5: Section of liver from rat treated with Fusarium xylarioides culture filtrate showing prominent biliary hyperplasia in periportal region (b) (H&E x 500)



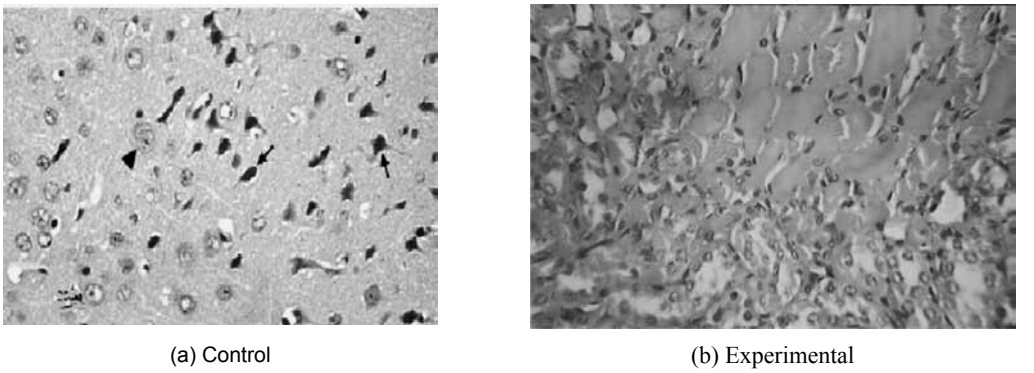
(a) Control (b) Experimental

Fig 6: Section of kidney from rat treated with Fusarium xylarioides culture filtrate showing severe congestion of inter tubular vessels and focal areas of tubular necrosis with loss of architecture (b) (H&E x 500)



(a) Control (b) Experimental

Fig 7: Section of brain from rat treated with Fusarium xylarioides culture filtrate showing severe congestion of blood vessels and perivascular mononuclear cuffing (b) (H&E x 500).



(a) Control (b) Experimental

The elevated serum creatinine and urea nitrogen concentration in comparison to control concentration is suggestive of the possible role of 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. Similar findings were also reported by Fodor *et al.* (2006). The changes in the brain described as necrosis, liquefaction and hemorrhages in the present study were similar to those of earlier reports of Uhlinger (1997).

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 rats might be susceptible to such low concentration. Congestion of blood vessels of stomach seen in all rats. 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, concentration of the fungal culture extract and the form in which the test material is administered.

Conclusion

In *Fusarium xylarioides* culture filtrate administered group, rats exhibited diarrhoea, severe arching of back and paraplegia. The clinical symptoms like swollen forehead, conjunctival hemorrhages, cutaneous hemorrhagic patches on back, scrotum, abdomen, ears and legs were observed along with pronounced nervous disorders. The present study revealed the hepatotoxic and nephrotoxic properties of the culture filtrate of *F. xylarioides* in rats at the dose tested (0.25, 0.5, 1 and 2 ml).

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