MULTIMYCOTOXIN ANALYSIS USING LC-MS/MS AND SUB CHRONIC TOXICITY STUDY OF *Fusarium semitectum* FUNGUS ISOLATED FROM GROUNDNUT HAY

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Abstract: The present study was conducted to know the cause of the obscure disease found in the cattle of south Karnataka region since 2005. This study confirmed the disease in cattle, which occurred after feeding of the contaminated groundnut hay. Ailing cattle were showing the clinical signs of colic, tenesmus, bleeding from natural orifices, central nervous system abnormality symptoms, anorexia, chronic recumbancy and death after few days. Fungus was isolated from the groundnut hay, identified, characterized by molecular methods. The fungus contaminated wheat materials were subjected to multimycotoxin analysis using LC-MS/MS method. Apicidin and beauvericin mycotoxins found in high concentrations. To confirm further, toxicity studies were conducted in rats. Clinical signs of toxicity were observed. Body weight, blood clotting time, postmortem findings, histopathological changes, hematological parameters, and biochemical parameters abnormality were recorded and correlated. The present study indicated the toxic feature of the culture filtrates/fungi infected wheat material isolated from ground nut hay in rats. This study helped the farming community by identifying cause of the death of the cattle and reduced further mortality.

Key words: *Fusarium semitectum*, Fungal isolate, Groundnut hay

INTRODUCTION

The cattle in the south Karnataka region, especially of Tumkur district, Sira taluka and surrounding areas were suffering from peculiar type of disease. The cause of the disease was remained untraced. Cattle were showing the clinical signs toxicity, especially soon after monsoon. Clinical signs observed were colic, tenesmus, ruminal atony, anorexia, bleeding from nostrils, rectum and fly bite site, hemoglobinurea, anemia and blood oozing out all over the body even on pricking with a needle. The cattle were looking stressed and weak. After few days, animals were unable to get up, chronic recumbancy observed, ultimately ending in the death of the cattle. Preliminary studies were conducted to check possibility of infectious disease, especially anthrax, as blood was oozing out of the natural orifices. Laboratory findings of blood smears and lymph node biopsy sample did not reveal any specific pathogens. The blood smears were also negative for hemoproteozoan parasites. The histological study of vital organs showed marked changes. But the findings were not of conclusive and suffering of the animals continued.
After thorough screening, it was found that, farmers used to feed the animals exclusively on groundnut hay. When the suspected material was screened, mold growth was very much prominent. As during this particular season, there was much scarcity of the green fodder, farmers feed their animals with stored groundnut hay. We collected the samples from various places and the detailed study was undertaken. A preliminary investigation was conducted to evaluate the toxic features of the fungal infected ground nut hay in rats and mice. The study revealed monsoon harvested groundnut hay was susceptible for fungal contamination due to improper drying and humid environmental conditions. Fungal contaminants identified from infected hay were *Macrophomina*, *Puccinia*, *Cercospora*, *Fusarium*, *Aspergillus* and *Rhizopus* species [1].

Usually mycotoxicosis is of chronic type of disorder. As such there is very less emphasis given on ruminant mycotoxicosis especially affecting cattle, this will result in great production loss, by reducing the efficiency of the working and producing animals. But extensive research will be seen on poultry mycotoxicosis. In the country like India, livestock production is an important part of economy of the nation.

Each year million of tonnes of forage consumed by animals are invaded by variety of fungal species prior to harvest or during storage as hay, straw or silage [2]. Fungal growth is typically triggered by warm and wet conditions. The research conducted earlier indicated that in dairy cattle or swine, mycotoxin contamination of feeds reduces growth, milk production, lowered reproduction and immunity [3]. Sufficient high levels of these fungal metabolites in feeds can have toxic effects that range from acute (liver or kidney deterioration) to chronic toxicity (liver cancer), mutagenicity and teratogenicity, and resulting clinical signs ranging from skin irritation, birth defects, neurotoxicity, immunosuppression and death [4].

The present study was designed to elucidate toxic feature of fungal contaminated groundnut hay and effect of mycotoxins in rats and to correlate the findings to the toxicity observed in cattle.

**MATERIALS AND METHODS**

**Collection of fungal contaminated groundnut hay:** Fungal contaminated groundnut hay was collected from the affected region of Pattanayakanahalli, Sira taluk of Tumkur District, Karnataka, where the animals were showing signs of toxicity after consuming fungal contaminated groundnut hay.

**General laboratory procedures:** The present investigation of isolation of the fungi from the fungal contaminated groundnut hay was done in the Mycology laboratory of the Department of Pharmacology and Toxicology, Veterinary College, Bangalore. Potato dextrose agar (PDA) and potato dextrose broth were the medium of choice used to culture the fungi. All the glass wares, solid and liquid media used in the study were sterilized in an autoclave at 1.1 kg/cm² pressure for 20 minutes.

**Identification and quantification of the mycotoxins:** The fungal culture filtrates/ fungi infected wheat materials were analyzed for the presence of 17 mycotoxins viz., aflatoxins (B1, B2, G1 and G2), ochratoxin, T2 toxin, HT2 toxin, fumonisins (B1 and B2), diacetoxyscirpenol (DAS), deoxynivalenol (DON), 3-acetyl deoxynivalenol, 15-acetyl deoxynivalenol, zearalenone, citrinin, beauvericin and apicidin by using liquid chromatography mass spectrometry (LC-MS/MS) at the multimycotoxin analysis laboratory of the Department of Pharmacology and Toxicology, Veterinary College, Bangalore. The mycotoxin standards viz., aflatoxins (B1, B2, G1 and G2), ochratoxin, HT2 toxin, diacetoxyscirpenol (DAS), deoxynivalenol (DON), 3-acetyl deoxynivalenol, 15-acetyl deoxynivalenol, citrinin, beauvericin and apicidin used were of Sigma Aldrich chemicals. The mycotoxin standards T2 toxin, zearalenone and fumonisins (B1 and B2) used were of Ermentek, Israel.

**Experimental chemicals, reagents and mycotoxins standard preparation:** Acetonitrile, n-hexane, methanol, formic acid and acetic acid were used. All the chemicals used were of HPLC grade Merck chemicals. Water was ultra purified using Nanopure system (Barnsted, Thermoscientific Inc., Madison, WI, USA). All mycotoxins standards procured from Sigma and six different concentrations were prepared in acetonitrile.

**Apparatus:** LC-MS/MS instrument used was of 3200 Q TRAP LC-MS/MS system, Applied biosystems, MDS SCIEX (Foster city, CA, USA). HPLC was of L 200 series (Agilent Technologies, Waldbronn, Germany) consisted of degasser, binary pump, autosampler and column compartment. HPLC column was of Zorbax, Eclipse XDD-C 18, rapid resolution, HT 4.6x50mm, 1.8 micron, 600 bar column.
Design of the experiment: Sub chronic oral toxicity study was conducted as per OECD guidelines 408, to determine the toxicity of the fungal culture filtrate in rats. Four groups of rats were made as follows:

Group I - *Fusarium semitectum* (A) - 2 ml; Group II - *Fusarium semitectum* (B) - 1 ml broth; Group III - *Fusarium semitectum* (C) - 0.5 ml and Group IV - 2 ml PD Broth.

After 28 days of the growth, infected broth filtered in separate container, separated from the surface growth and stored at -15°C until further use for conducting toxicity in rats.

Toxicity study: As per OECD guidelines 408, apparently healthy young Wistar albino rats (procured from the laboratory animal house, Veterinary College, Hebbal, Bangalore) were used in the present study. They were of the age group of five weeks and the body weight was about 100 ± 10 g. The animals were acclimatized to the experimental laboratory conditions for a week. They were maintained under hygienic laboratory conditions, providing standard laboratory animal feed (Amruth Feeds, Bangalore) and water ad libitum. The rats were grouped (n=20) and housed in polypropylene rat cages during the experiment. The approval of the Institutional Animal Ethics Committee was obtained prior to the start of the experiment (No.62, LPM/IAEC/2010, dated 07-08-2010).

Clinical observations, hematology and biochemistry: General clinical observations were made thrice a day. The health condition of the animals was recorded. Daily all the animals were observed for morbidity and mortality. Necropsy was done and organs were collected for histopathological studies.

Blood samples were collected on 0, 45 and 90th day during the sub chronic toxicity study period. Blood clotting time was estimated, during blood collection itself. Using Automatic Blood Cell Counter (ERMA INC, Model PCE-210, Tokyo, Japan) and commercially available kits hematology parameters were estimated. Clinical biochemistry study was done to investigate the toxic effects. Using Microlab 300 (Vitalab Scientific, The Netherlands) and commercially available diagnostic kits from Merck (Ecoline®, Merck Specialties Limited, Plot No.11/1, Marvasado, Usagaon, Ponda Taluka, Goa, India), by following the manufacturer instructions furnished in the leaflet supplied along with the diagnostic kit, following biochemical parameters were estimated: Alanine aminotransferase (ALT) activity, aspartate aminotransferase (AST) activity, creatinine (CRT) concentration and urea nitrogen concentration.

Pathological study: At the end of the study period, surviving animals were sacrificed under ether anesthesia and gross changes in the organs were recorded. Representative tissue samples of brain, liver, kidney, spleen, heart, lung, intestines and stomach were collected in 10 % neutral buffered formalin (NBF) for histopathological study.

Toxicity study in rats using fungal infected wheat material: The isolated fungus was grown on autoclaved wheat. 25 ppm of streptomycin was added to the autoclaved wheat containing flask and mixed well to avoid bacterial contamination. The pure culture was inoculated into the conical flasks containing wheat, under UV sterilized condition. The flasks were kept at 37°C for a period of 28 days for optimal growth of the fungi. After that, the fungal infected wheat material was ground finely and stored at -15°C until further use for toxicity study.

Design of the experiment: Sub chronic oral toxicity study was conducted as per OECD guidelines 408, to determine the toxicity of the fungal culture infected wheat materials in rats. Four groups of rats were made as follows:

Group I - *Fusarium semitectum* (FS) (A) - 750 mg of contaminated wheat /rat; Group II - FS (B) - 263 mg of contaminated wheat /rat; Group III - FS (C) - 83 mg of contaminated wheat /rat and Group IV - Control - autoclaved wheat, 750 mg/rat. Clinical observations and other procedures followed will remain same to that of used in broth study.

Screening the fungal infected wheat material for the presence of coumarins: Fungal infected wheat material was screened for the presence of coumarins [5].

Statistical analysis: Mean values and standard error of means were calculated and expressed as mean ± SEM. The data were analyzed by two-way ANOVA...
with Dunnett’s post test using Graph Pad Prism Trial version 5.00 for Windows, Graph Pad Software, San Diego, California USA, www.graphpad.com.

RESULTS

Identification of the fungi: Both morphological and molecular identification of the fungi was done by Fungus Identification Service, Mycology and Plant Pathology Group, Agharkar Research Institute, Pune. The species identified was FS.

Macroscopic and microscopic morphology of fungi: Colonies of FS on PDA grew slowly, filled the Petri plate and matured in 4-6 days. From the front, colour of colony was dark yellow and as culture became old, yellow colour faded slowly and turned to orange colour. Canoe-shaped macro conidia with a long apical cell and a foot-shaped basal cell formed with 3 to 5 septa. Uni or bicellular, ovoid to ellipsoid micro conidia were abundant.

Identification of fungi and quantification of mycotoxins: The fungi was identified as FS. Molecular method of identification by using polymerase chain reaction (PCR) confirmed the same. The nucleotide sequence alignment, BLAST, correlated with NCBI accession numbers, confirmed the fungus identification up to the genus level.

The fungus infected wheat materials were subjected to mycotoxins screening and quantification by using LC-MS/MS, in the Multimycotoxin Analysis Laboratory, Department of Pharmacology and Toxicology, Veterinary College, Bangalore.

Method validation: The sensitivity of the developed method complies with the maximum levels as stated in the existing regulations. The apparent recovery varied from 88.00% to 92.00% for apicidin mycotoxin and 78.00% to 85.00% for beauvericin. For other analyzed mycotoxins, the apparent recovery period varied from 70.00% to 90.00%. A precision study was performed by determining the repeatability and the reproducibility at the four concentration levels.

Analysis of feed samples: The analysed wheat sample infected with FS had very high concentrations of apicidin and beauvericin mycotoxins i.e., apicidin 5375 µg/kg and beauvericin 2732.50 µg/kg.

Table 1: Report of the multimycotoxin analysis, using LC-MS/MS. The estimates expressed are in ng/g are equal to ppb or µg/kg, + Positive, - Negative

<table>
<thead>
<tr>
<th>Mycotoxins</th>
<th>Concentration</th>
<th>Sample Name</th>
<th>Wheat Matrix</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxin B1</td>
<td>ng/g</td>
<td>No Peak</td>
<td>No Peak</td>
</tr>
<tr>
<td>Aflatoxin B2</td>
<td>ng/g</td>
<td>No Peak</td>
<td>No Peak</td>
</tr>
<tr>
<td>Aflatoxin G1</td>
<td>ng/g</td>
<td>No Peak</td>
<td>No Peak</td>
</tr>
<tr>
<td>Aflatoxin G2</td>
<td>ng/g</td>
<td>No Peak</td>
<td>No Peak</td>
</tr>
<tr>
<td>Ochratoxin A</td>
<td>ng/g</td>
<td>No Peak</td>
<td>No Peak</td>
</tr>
<tr>
<td>T-2 Toxin</td>
<td>ng/g</td>
<td>No Peak</td>
<td>No Peak</td>
</tr>
<tr>
<td>HT-2 Toxin</td>
<td>ng/g</td>
<td>No Peak</td>
<td>No Peak</td>
</tr>
<tr>
<td>DAS</td>
<td>ng/g</td>
<td>No Peak</td>
<td>No Peak</td>
</tr>
<tr>
<td>DON</td>
<td>ng/g</td>
<td>No Peak</td>
<td>No Peak</td>
</tr>
<tr>
<td>3Ac-DON</td>
<td>ng/g</td>
<td>No Peak</td>
<td>No Peak</td>
</tr>
<tr>
<td>i5-AcDON</td>
<td>ng/g</td>
<td>No Peak</td>
<td>No Peak</td>
</tr>
<tr>
<td>Fumonisin B1</td>
<td>ng/g</td>
<td>No Peak</td>
<td>No Peak</td>
</tr>
<tr>
<td>Fumonisin B2</td>
<td>ng/g</td>
<td>No Peak</td>
<td>No Peak</td>
</tr>
<tr>
<td>Zearalenone</td>
<td>ng/g</td>
<td>No Peak</td>
<td>No Peak</td>
</tr>
<tr>
<td>Citrinin</td>
<td>ng/g</td>
<td>No Peak</td>
<td>No Peak</td>
</tr>
<tr>
<td>Apicidin</td>
<td>ng/g</td>
<td>-</td>
<td>5375.00</td>
</tr>
<tr>
<td>Beauvericin</td>
<td>ng/g</td>
<td>-</td>
<td>2732.50</td>
</tr>
</tbody>
</table>

Table 2: The effect of different fungal culture filtrates on various serum biochemical parameters in rats during sub chronic oral toxicity study. Values are mean ± SE, *** P < 0.001, ** P < 0.01, * P < 0.05, n = 20

<table>
<thead>
<tr>
<th>Biochemical parameter</th>
<th>Days</th>
<th>Type of culture filtrate</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>Serum creatinine (mg/dl)</th>
<th>Serum urea nitrogen (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>P D Broth (control)</td>
<td>Group IV</td>
<td>F. semitectum (A)</td>
<td>Group I</td>
<td>F. semitectum (B)</td>
</tr>
<tr>
<td>0</td>
<td></td>
<td>30.10±2.22</td>
<td>27.74±2.15</td>
<td>28.9±2.49</td>
<td>28.3±2.49</td>
<td>30.37±1.59</td>
</tr>
<tr>
<td>45</td>
<td></td>
<td>29.8±2.18</td>
<td>79.6±2.90</td>
<td>68.6±1.92</td>
<td>49.0±1.6</td>
<td>49.0±1.6</td>
</tr>
<tr>
<td>90</td>
<td></td>
<td>32.1±2.94</td>
<td>88.1±3.59</td>
<td>78.0±4.17</td>
<td>51.3±3.99</td>
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<tr>
<td></td>
<td></td>
<td>32.8±6.57</td>
<td>83.6±5.93</td>
<td>85.9±5.13</td>
<td>82.0±7.15</td>
<td>82.0±7.15</td>
</tr>
<tr>
<td>45</td>
<td></td>
<td>101.6±5.99</td>
<td>18.1±3.01</td>
<td>16.3±5.49</td>
<td>125.3±5.05</td>
<td>125.3±5.05</td>
</tr>
<tr>
<td>90</td>
<td></td>
<td>107.1±5.23</td>
<td>18.3±4.84</td>
<td>16.8±2.88</td>
<td>129.0±3.47</td>
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<tr>
<td>0</td>
<td></td>
<td>0.41±0.04</td>
<td>0.43±0.05</td>
<td>0.42±0.04</td>
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<td>0.38±0.03</td>
<td>0.3±0.22</td>
<td>0.78±0.01</td>
<td>0.69±0.03</td>
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<tr>
<td>90</td>
<td></td>
<td>0.36±0.03</td>
<td>0.9±0.44</td>
<td>0.85±0.09</td>
<td>0.79±0.05</td>
<td>0.79±0.05</td>
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<tr>
<td>0</td>
<td></td>
<td>38.0±2.16</td>
<td>71.4±3.23</td>
<td>59.3±1.91</td>
<td>48.4±2.00</td>
<td>48.4±2.00</td>
</tr>
<tr>
<td>45</td>
<td></td>
<td>39.2±1.73</td>
<td>81.6±5.27</td>
<td>74.1±4.86</td>
<td>61.1±5.30</td>
<td>61.1±5.30</td>
</tr>
</tbody>
</table>

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Fig 1: EPI spectra of Apicidin from *F. semitectum* in wheat matrix

Fig 2: EPI spectra of Beauvericin from *F. semitectum* in wheat matrix

Fig 3: Total ion chromatogram of Apicidin-Beauvericin from *F. semitectum* in wheat matrix
kg of infected material (Table 1).

The contaminated groundnut hay collected from the affected area was also subjected for mycotoxins screening. The two groundnut hay samples were also positive for only apicidin and beauvericin mycotoxins. The MS-MS spectra for apicidin mycotoxin from *F. semitectum* infected wheat matrix in ion trap mode showed fragmentations at 267.2, 294.2, 420.3, 464.3, 592.6 and 624.5 Daltons in chromatogram. The MS-MS spectra for beauvericin mycotoxin from *F. semitectum* infected wheat matrix in ion trap mode showed fragmentations at 134.1, 234.1, 244.0, 262.0, 280.1, 362.0, 541.4 and 623.5 Daltons in chromatogram (Figs. 1,2).

Total ion chromatogram of *FS* infected wheat matrix showed retention time (RT) of 17.66 min for apicidin mycotoxin and 18.96 min for beauvericin mycotoxin (Fig. 3). The MS-MS spectra of apicidin standard from chromatogram in wheat matrix in ion trap mode showed fragmentations at 267.2, 294.2, 420.3, 464.3, 592.6 and 624.5 Daltons in chromatogram. The MS-MS spectra for beauvericin mycotoxin from *F. semitectum* infected wheat matrix in ion trap mode showed fragmentations at 134.1, 234.1, 244.0, 262.0, 280.1, 362.0, 541.4 and 623.5 Daltons in chromatogram. These fragmentations were very much similar to those of fragmentations seen in the *FS* fungi (Fig. 3).

**Toxicity studies in rats with fungal culture filtrates/ broth material:** Except control group of rats, all experimental rats were weak, depressed and recumbent. They showed gradual reduction in feed intake and loss of body weight. Burrowing of the bedding material, mouth washing behavior, itching of body parts, huddling together and diarrhoea were common clinical signs observed in all treatment groups of rats. Rats exhibited severe arching of back. The rats lost of balance on hind limbs and sometimes on forelimbs. There was swollen forehead and conjunctival haemorrhage. Cutaneous haemorrhagic patches on back, scrotum, abdomen, ears and legs region were seen. But with less exaggerated nervous disorders. There were skin rashes and itching was much common in all the treatment rats.

There was a significant change in the body weight and blood clotting time in the treatment group of rats. Biochemical parameters alterations were given in Table 2. There were slight alterations observed in hematological parameters. Toxicity study with fungi infected wheat material also carried and results obtained were almost similar to that of broth study.

**Pathology:** Gross pathology of culture filtrate administered animals: In *Fusarium semitectum* culture filtrate gavaged rats, cutaneous hemorrhagic patches on back, scrotum, abdomen, ears and legs region were seen. Hemorrhagic spots on eyes, nose and paws were the common finding observed in all the rats. In some rats, serosal surface of stomach, cecum had patchy hemorrhages. There was severe congestion of heart, liver, lungs, stomach, spleen, testicles, cecum, kidneys and brain. Liver had petechial hemorrhages with necrotic points noticed.

**Screening the fungal infected wheat material for the presence of coumarins:** As the toxicity signs seen in the rats were of mainly hemorrhagic disorders, fungus infected material was screened for the presence of any sort of coumarins [5], but the contaminated materials were negative for the presence of coumarins.

**DISCUSSION**

In this particular field problem, all the causes of illness were ruled out and mycotoxicosis was suspected to be the most probable illness, based on the signs exhibited by the animals and isolation of the fungi from the contaminated groundnut hay during the preliminary investigation.

The present experiment was aimed to confirm that the toxins produced by the fungi were responsible for causing toxicity in cattle using rats as model, after isolation of the fungus from the fungal infected ground nut hay. There was a long history of toxicosis associated with the consumption of *Fusarium* infected cereals by both people and domestic animals [6]. Some reports are available on the toxicity of the fungal infected groundnut hay and the fungi responsible for it in cattle. The dose in the toxicity study selected in rats was based on the maximum allowable dose to be administered to these animals as per the standard protocols [7]. In the present study, both the fungal culture filtrate and fungi infected wheat materials were administered to induce the toxicity in rats, since it was an appropriate method to administer the desired dose of the broth / culture filtrate and fungi infected wheat material containing...
major secondary metabolites or mycotoxins [8-10]. The microscopic characteristics observed for *Fusarium semitectum* fungi in the present study was similar to earlier studies seen in red fleshed dragon fruit disease in Malaysia [11].

**Screening and quantification of mycotoxins:** The results of the present study were supported by the earlier findings, which carried out multimycotoxin analysis by liquid chromatography/tandem mass spectrometry after multitoxin immunoaffinity cleanup from maize, with slight modification method. The method was developed for the simultaneous determination of aflatoxins (B1, B2, G1, G2), ochratoxin A, fumonisins (B1, B2), deoxynivalenol, zearalenone, T-2 and HT-2 toxins in maize [12].

The method developed in the present study was in accordance with the multi-mycotoxin analysis of maize silage by LC-MS/MS earlier conducted with slight modification [13]. The present study concords with earlier findings that had isolated beauvericin, a cyclodepsipeptide from *Fusarium semitectum* and were toxic to Colorado potato beetles [14]. Production of apicidin mycotoxin from *FS* fungus was in accordance with the earlier reports [15].

Similar findings were also reported by earlier workers, where reduced feed intake and decreased body weight gain was observed in rats due to the presence of apicidin mycotoxin in the fungal infected wheat material [15]. In another study conducted confirmed the production of apicidin mycotoxin from *FS* fungus was in accordance with the earlier reports [15].

The present study supported the earlier report of where the clinical signs in rats included gastrointestinal effects such as, diarrhoea, haemorrhages in the stomach, intestine and bowel inflammation which was attributed to the toxic effects of apicidin [15]. The clinical signs of diarrhoea, weight loss were also reported in mice, when treated with apicidin derivative SD-2007 for 2 weeks [17]. Neurological disorders were also seen in experimental rats, which was due to the presence of beauvericin mycotoxin in very high concentration in *FS* culture filtrate/fungal infected wheat material was attributed to such condition. This was further supported by the gross and histopathological lesions in the brain of the affected animals like severe congestion and infiltration of lymphocytes, degeneration, swelling of ependymal cells and congestion. There was gliosis, focal area of glial cell proliferation, neuronal degeneration and vacuolar degeneration of neurons. This was correlated with the earlier findings [19].

The study conducted also concluded loss of body weight in mice during treatment with apicidin derivative SD-2007 [17]. Apicidin, a hemorrhagic mycotoxin, which was found in very high concentration in the *F. semitectum* infected wheat/culture filtrate might be responsible for this bleeding disorder [15].

The study concords with the earlier research conducted, where increased concentration of alanine aminotransferase (ALT) was observed in mice after treating with apicidin’s derivative SD-2007 for 2 weeks [17]. But these cultured materials had very high concentration of beauvericin, which might be responsible for causing hepatotoxicity, due to its inherent cytotoxic activity. The study concords with the earlier research conducted, where increased concentration of aspartate aminotransferase (AST) was observed in mice after treating with apicidin’s derivative SD - 2007 for 2 weeks (17). The increase in creatinine concentration in these particular groups indicated possible role of toxins apicidin and beauvericin in causing kidney damage. The earlier research reported increased creatinine concentration in rats and mice during administration of *Cladosporium oxysporum* culture filtrate and histopathological studies confirmed nephrotoxic feature of the culture filtrate [9]. Apicidin, beauvericin and citrinin in high concentrations, which might be responsible for the renal damage.

Based on the present study it could be concluded that, the culture filtrates/fungi infected wheat material of fungal isolate obtained from moldy groundnut hay was toxic to rats at the given dose and duration of treatment. The presence of very high concentration of apicidin and beauvericin mycotoxins were responsible for toxicity. Because of the similar clinical signs were exhibited by rats and were correlated with the biochemical, hematology parameters change and
during histopathology also. Therefore, toxicity observed in the cattle, during field conditions may be attributed to consumption of *F. semitectum* infected moldy groundnut hay.

**REFERENCES**


