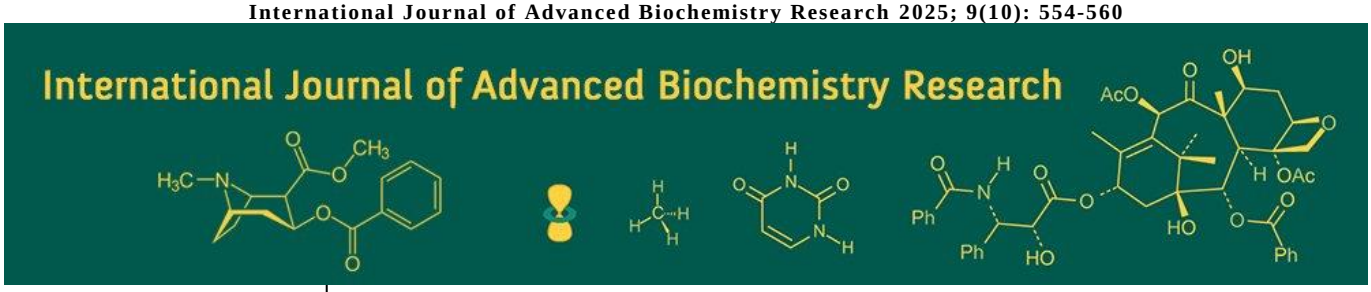

ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2025): 5.29
IJABR 2025; 9(10): 554-560
www.biochemjournal.com
Received: 21-07-2025
Accepted: 25-08-2025

Manjunath Uppar
PG Scholar, Department of Veterinary Pharmacology and Toxicology, Veterinary College Shivamogga, Karnataka, India

Vinay P Tikare
Associate Professor and Head, Department of Veterinary Pharmacology and Toxicology, Veterinary College Gadag, Karnataka, India

Shridhar NB
Professor and Head, Department of Veterinary Pharmacology and Toxicology, Veterinary College Shivamogga, Karnataka, India

Sunilchandra U
Professor, Department of Veterinary Pharmacology and Toxicology, Veterinary College Shivamogga, Karnataka, India

Corresponding Author:
Vinay P Tikare
Associate Professor and Head, Department of Veterinary Pharmacology and Toxicology, Veterinary College Gadag, Karnataka, India

Sub acute toxicity study in rats by the *Aspergillus niger* culture filtrates isolated from maize stalks of Shivamogga district, Karnataka

Manjunath Uppar, Vinay P Tikare, Shridhar NB and Sunilchandra U

DOI: https://www.doi.org/10.33545/26174693.2025.v9.i10h.6105

Abstract
The present study was conducted to evaluate the effects of the toxicity caused by consuming the fungal contaminated maize stalk by the cattle. Identification of the fungi was done by molecular identification of the fungi species using Sanger Sequence Chromatogram method and the fungus identified as *Aspergillus niger*. To imitate the field conditions, a repeated-dose 28-days oral sub acute toxicity study was performed with three groups (n = 6) of Wistar Albino rats. The rats were weighed individually on Day 0, Day 14 and Day 28. The group I (control) received oral gavage of potato dextrose broth @ dose of 2 ml/100 g, where as groups II and III received fungal culture filtrate of *Aspergillus niger* at the doses of 1 and 2 ml/100 g respectively. Rats showed clinical signs of gradual reduction in the feed intake, water intake, diarrhoea and loss of body weight. Treatment groups (group II and III) showed decrease in hematological parameters (Packed cell volume, Hemoglobin, Total leucocyte count and Total erythrocyte count) and increased serological parameters (Aspartate Transaminase, Alanine Transaminase, Creatinine, Blood urea nitrogen). At the end of the study period, the survived experimental rats were humanely sacrificed and subjected to detailed postmortem examination and observed for any gross changes in the vital organs. The findings of the present study concluded that the fungal culture filtrates of fungus *Aspergillus niger* isolated from contaminated maize stalk caused toxicity in rats, which was attributed for the presence of a toxic principle components in the gavaged fungal culture filtrates.

Keywords: Sub acute, *Aspergillus niger*, toxicity, rats

Introduction
Dairy cattle are susceptible to mold infection, particularly during stressful times when their immune system is weakened. Molds produce toxic compounds known as mycotoxins, which affect animals when they ingest feed contaminated with mycotoxin. The development of mold and the formation of mycotoxin are linked to plant stress brought by harsh weather, pest damage and poor storage techniques. The Food and Agriculture Organization (FAO) has estimated that worldwide, about 25% of crops are affected annually with mycotoxins (Yu and Pedroso, 2023) [1]. According to Cheli *et al.* (2013) [2], roughages like hay, straw, maize, wheat, and grass silage are important sources of mycotoxins in ruminants because they make up over 60% of the dry matter. Mycotoxins are low molecular weight secondary metabolites produced by certain strains of filamentous fungi such as *Penicillium*, *Fusarium* etc. Mycotoxins can be produced either before or after harvest, *i.e.*, during storage, transportation, processing, or feeding.

The primary factors influencing the production of mycotoxin in feedstuffs after harvest are temperature, water content, and insect activity. Environmental conditions such as high temperature and humidity increase the risk of fungal growth and mycotoxin production. Mycotoxin metabolism in the rumen does not correspond to total detoxification (Rodrigues, 2014) [3]. The basic and efficient measure in the reduction of fungal contamination at storage facilities of susceptible crops is to control environment by manipulation of ecological factors.This will minimize the entry of fungal-derived mycotoxins in feed and food chain and ultimately reduce their adverse effects on animal and human health (Agriopoulou *et al.*, 2020) [4].

Fungi not only have the potential to cause animal mycosis but, also reduce feed palatability. Feed may be contaminated by fungal species and mycotoxins at the same time, and the toxicological effects can be different according to the type of mycotoxin interaction: less than additive, additive, synergistic, enhanced, or antagonistic (Streit *et al.*, 2012) [5]. During the disease investigation, unusual symptoms were noticed in the cattle of dairy farms and other cattle at the Sominakoppa, Maisavalli and surrounding villages of Shivamogga district, Karnataka state. The detailed clinical investigation and history revealed that the dry maize stalks were fed to these animals had blackish spots indicative of fungal infection. Hence, the present study was undertaken to evaluate the possible toxic potential of fungal contaminated maize stalks in rats.

Materials and Methods

Fungal contaminated maize stalk fodder samples were collected from two dairy farms and from farmers field. Prior to performing of mycological procedures, laboratory was fumigated with Potassium permanganate and formalin. The laminar airflow was UV sterilized. 25 ppm of streptomycin was added to the Potato Dextrose Agar (PDA) containing flask to avoid bacterial contamination and mixed well. The PDA (10 ml) was poured into the sterilized Petri-plate in front of a Bunsen burner in the UV sterilized vertical laminar flow inoculation chamber. The Petri-plates were then allowed to cool for 30 min or until the media solidifies. Maize stalk parts were placed on prepared Petri plates in front of a Bunsen burner. The inoculation plates were incubated for 3-5 days at room temperature. In around 5 days, the fungal growth on the sample became visible. The fungal colonies were moved to another plate with PDA media and cultured for 5 days at 37 °C, for obtaining pure isolates of fungi. The pure culture was inoculated into conical flasks with Potato dextrose broth. For optimal fungus growth, the flasks were kept at 37 °C for 28 days. The mycelial-free content was decanted carefully into a new sterile container and used according to the preset protocol.

Identification of the fungi

Identification of the fungal species was done by molecular identification of the fungi species in the culture using Sanger sequence method (Petria Life Science, Bengaluru). The fungus identified was *Aspergillus niger.*

Sub-acute toxicity study in rats

In the present study, apparently healthy young Wistar albino rats in the age group of 6 to 7 weeks with the body weight ranging from 160±20 g were used. The animals were obtained from Kedhar Biolabs, D.No.17-2-2-/2, Telugu Geri, Boyapally, Mahbubnagar, (Reg No: 2150/PO/Bt/S/2022/CCSEA). All animals were maintained as per the guidelines of the Committee for Control and Supervision of Experiments on animals, in the small animal house, Veterinary College, Shivamogga at controlled temperature of 22±3 °C and relative humidity of 55±5 % and 12 h light/dark cycle. The animals were acclimatized for one week prior to the actual experiment. The study was conducted after obtaining approval by the Institutional Animal Ethics Committee, Veterinary College, Shivamogga with the approval Number: No VCS/IAEC/SA-101/2024-25 Dated 30-08-2024.

Experimental design

Table 1: Groupings of experimental rats for sub-acute oral toxicity study with *Aspergillus niger* fungal contaminated maize stalk

Sl. No	Groups	Animals per group	Treatment	Dosing
1	Group I (Control)	n = 6	Control	Administered (*per os*) with potato dextrose broth (Dose: 2 ml/100 g)
2	Group II	n = 6	Culture filtrate of *Aspergillus niger*	Administered (*per os*) with culture filtrate of *Aspergillus niger* (Dose: 1 ml/100 g)
3	Group III	n = 6	Culture filtrate of *Aspergillus niger*	Administered (*per os*) with culture filtrate of *Aspergillus niger* (Dose: 2 ml/100 g)

The Subacute oral toxicity was conducted in Wistar Albino rats for a period of 28 days as per OECD guidelines (OECD No: 407).

Procedure

The *Apergillus niger* culture filtrate was gavaged based on the hypothesis that, in the field conditions, the animals might have consumed the maize stalks contaminated with *Aspergillus flavus* in equal or different proportions. In the Group II, *Apergillus flavus* was administered at the dose rate of 1ml/100 g as a fungal infected material once daily and for the Group III *Apergillus flavus* was administered at the dose rate of 2ml/100 g as a fungal infected material once daily. The rats were weighed individually on Day 0, Day 14 and Day 28. The rats in the test group *ie.*, Group I were gavaged with the PD broth at the dose rate of 2ml/100 g once daily for a period of 28 days (OECD, 2001; Vinay, 2007) [6, 7].

Clinical observations

General clinical observations in the rats were recorded daily. The health condition of the animals was recorded. Daily all the animals were observed for morbidity and mortality. At the end of the study, the survived animals were sacrificed humanely and necropsy was done and organs were collected for further histopathological studies.

Clinical hematology

Present study was carried out to investigate the toxic effect of the fungal culture filtrate on different organs of the body of the animals, especially on the liver, heart and kidney functions. The blood samples were drawn from rats by retro-orbital plexus puncture method using Micro-Hematocrit capillary tubes. Using automatic haematological analyzer, the haematological parameters *viz.*, Haemoglobin (Hb), Total leukocyte count (TLC), Total erythrocyte count (TEC) and Packed cell volume (PCV) were estimated on day 0, 14th and 28th day.

Clinical biochemistry

The purpose of this study was to look at the hazardous effects of fungal culture filtrate on vital organs of the body *viz.*, liver, heart, kidney functions etc. Blood was obtained

from rats by retro-orbital plexus puncture approach using Micro Haematocrit capillary tubes. Serum biochemical parameters were analyzed on day 0, 14th and 28th day using biochemical analyzer Alphachem Pro AVECON Semi Auto Biochemistry Analyzer and ALPHA LINE Clinical chemistry reagents by Alpha technologies, using these the serum biochemical parameters *viz.,* Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Creatinine (Cr) and Blood urea nitrogen (BUN) were estimated.

Pathological study

At the end of the study period, all the survived experimental rats were humanely sacrificed and subjected to detailed postmortem examination and observed for any gross changes in the organs. For histological investigation, representative tissue samples of the vital organs *viz.,* liver, kidney, spleen, heart, lung and intestine (duodenum) were collected in 10% neutral buffered formalin (NBF).

Tissue processing

The collected tissues were treated using the paraffin embedding procedure for histological investigation. Using a microtome and disposable blades, the sections (4-5µ) were cut and then sliced sections were put into the tissue floatation bath then on to the slides to the next step. Hematoxylin and Eosin dyes were used to stain the sections (Alghamdi *et al.,* 2021) [8].

Results and Discussion

Identification of the fungi

In the present study, isolated pure fungi stained with lactophenol cotton blue was subjected to direct microscopic examination in the laboratory. The microscopic identification result was shown in Fig. 1.The molecular identification result was shown in Fig. 2. The fungus isolated was *Aspergillus niger*.

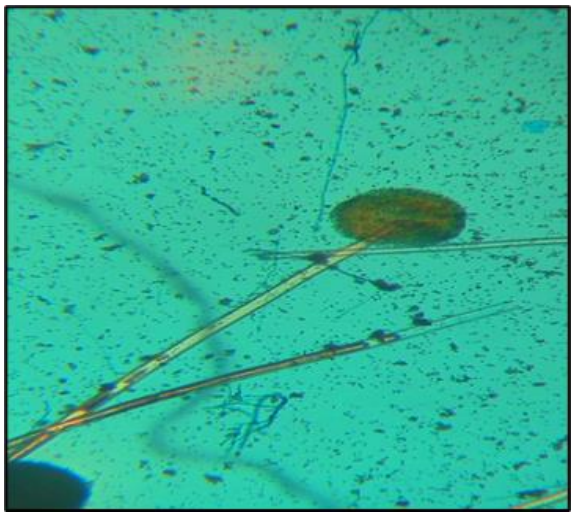

Fig 1: Microscopic appearance of *Aspergillus niger* showing stipe and vesicle.

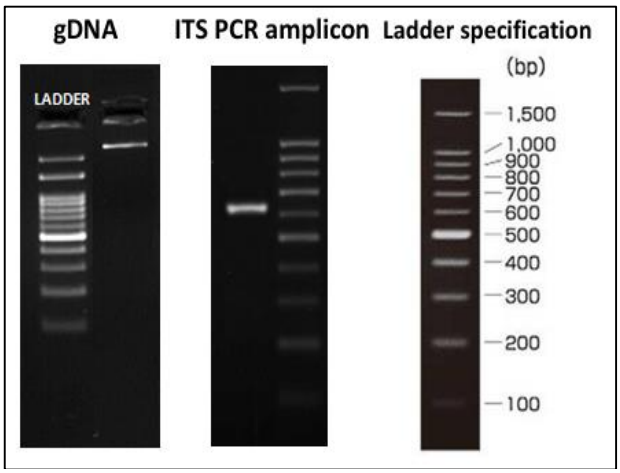

Fig 2: gDNA and ITS Amplicon QC data of *Aspergillus niger*

Broth harvesting and toxicity study

After 28 days of post inoculation, the mycelium free culture filtrates were decanted into sterile containers. They were stored at 4 °C and brought to room temperature before administration to Wistar Albino rats. In the present study, the fungal culture filtrates were administered to induce the toxicity in rats, since it was appropriate method to administer the desired dose of the broth culture filtrate containing major secondary metabolites or mycotoxins (Bayman *et al.,* 2002) [9]. The dose selected in rats was based on the maximum allowable dose to be administered to these animals as per the standard protocols (OECD, 2001) [6].

Clinical signs

All the test group rats showed various types of clinical signs. They showed gradual reduction in the feed intake, water intake, diarrhoea and loss of body weight. Animals were weak and depressed. Diarrhoea was more prominent in the Group III.

Similar findings were reported by Vinay *et al.* (2011) [10]; Chandrashekar (2012) [11]. In a study of aflatoxicosis in cattle conducted by Elgioushy *et al.* (2020), cattle that suffered from aflatoxicosis was reported exhibit clinical signs of depression and inappetence predominantly.

Toxicity studies in rats was conducted using fungal culture filtrates of various fungal species isolated from paddy straw (Shivaprasad, 2008) [12], that had caused toxicity in the cattle also shown similar clinical signs.

Body weight of rats

All the animals in control group and treated groups were weighed individually on the Day 0, 14 and 28 of experimental period. The body weight (g) of rats is given in Table 2.

The body weight of rats of all the treated groups on day 0, was not statistically (p>0.05) significant compared to control group values. On day 14th body weight (g) of rats was significantly (p<0.05) decreased and on 28th body weight (g) of rats was significantly (p<0.01) decreased in groups II and III compared to control group values.

The decrease in body weight (g) of rats might be attributed to reduced feed intake, due to the toxic content in culture filtrates. The decreased body weight in rats treated with mixed *A. flavus* and *A. niger* fungal culture filtrates correlated with the findings of Shivanand (2013) [13], where in the decrease in body weight in the sub-acute toxicity study in rats fed with fungal culture filtrates of *Aspergillus niger* isolated from paddy straw was reported.

Table 2: Effect of different fungal culture filtrates at different doses on body weight (g) in rats during repeated dose 28-day oral toxicity study.

Groups	Body weight (g)		
	Day 0	Day 14	Day 28
Group I (Control)	175.33±1.66	191.16±1.73	233.50±1.98
Group II	170.66±4.17	179.33±0.57*	209.33±3.86**
Group III	174.66±3.59	180.66±0.41*	201.66±3.65**

Values are mean ± SEM, n = 6, *p<0.05,**p<0.01,*** p<0.001.

Biochemical parameters

Serum alanine aminotransferase (ALT)

The serum ALT concentration values are given in Table 3.

The serum ALT concentrations on day 0 was not statistically significant (p>0.05) compared to control group. On day 14th and 28th serum ALT concentrations were significantly (p<0.05, p<0.01) increased in groups II and III. Udeani *et al.* (2013) [14], concluded in his study of effect of *A.flavus* on the liver of experimental rats administered with anti-retroviral drugs, immune suppression caused by antiretroviral drugs allowed infiltration of *A.flavus* to liver of the rats causing hepatotoxicity leading to significant rise in ALT, AST and ALP. Increased serum ALT levels in the fungal-culture filtrates gavaged groups indicated that the toxins present in the culture filtrates had caused significant damage to liver tissue, as confirmed by histopathological findings.

Table 3: Effect of different fungal culture filtrates at different doses on ALT (U/L) in rats during repeated dose 28-day oral toxicity study.

Groups	ALT (U/L)		
	Day 0	Day 14	Day 28
Group I (Control)	37.26±0.47	40.87±0.64	43.94±0.75
Group II	39.80±0.53	46.80±1.35*	53.83±0.93**
Group III	41.24±0.73	48.63±0.66**	55.48±0.70**

Values are mean ± SEM, n = 6, *p<0.05,**p<0.01,*** p<0.001.

Serum Aspartate aminotransferase (AST)

Serum AST values are given in result section Table 4.

On day 14th and 28th serum AST concentrations were significantly (p<0.01) increased in groups II and III compared to control group.

The rise in AST levels could be traced back to liver injury and muscular degeneration by muscle wasting, evidenced by decrease in body weight, where the toxin might have changed permeability and caused enzyme leakage. Venkanna (2008) [15], reported the significant increase (p<0.05) in AST in rats fed with the fungal culture filtrate of *A. clavatus* isolated from maize hulls.

Table 4: Effect of different fungal culture filtrates on AST in rats during repeated dose 28-day oral toxicity study.

Groups	AST (U/L)		
	Day 0	Day 14	Day 28
Group I (Control)	102.51±1.41	102.68±1.28	102.03±0.37
Group II	102.85±0.77	116.23±0.47**	121.91±1.88**
Group III	101.66±1.74	121.03±1.71**	130.18±1.32**

Values are mean ± SEM, n = 6,** p<0.01, *** p<0.001.

Blood urea nitrogen

The blood urea nitrogen concentration values were given in Table 5.

The serum BUN concentrations on day 0 was not statistically (p>0.05) significant compared to control group. On day 14th and 28th BUN concentrations significantly (p<0.01) increased in group II and group III, compared to control group values.

The elevated blood urea nitrogen concentration in comparison to control suggested the possible role of the toxins in causing kidney damage. This was further supported by histopathological observations like, congestion along with vacuolar degeneration, necrosis ballooning of the some of the tubules and fibrosis in the interstitium. Eraslan *et al.* (2017) [16], reported the significant increase in BUN values in most of the Wistar rats treated with metabolites *of A. niger* and *A. terreus.*

Table 5: Effect of different fungal culture filtrates at different doses on BUN (mg/dl) in rats during repeated dose 28-day oral toxicity study.

Groups	BUN (mg/dl)		
	Day 0	Day 14	Day 28
Group I (Control)	31.82±0.49	29.84±1.55	31.40±0.54
Group II	31.33±1.44	32.21±0.51*	35.77±2.35**
Group III	31.98±1.96	37.94±0.73**	39.27±1.31**

Values are mean ± SEM, n = 6, * p<0.05, ** p<0.01, *** p<0.001

Serum Creatinine

The serum creatinine concentration values are given in Table 6.

The serum creatinine concentrations on day 0 was not statistically significant (p>0.05) compared to control group. On day 14th and 28th, serum creatinine concentration was significantly (p<0.05, p<0.01) increased in group II and group III compared to control group.

The findings of the present study were also supported by the observation of Rekha (2014) [17], who stated that there would be increased serum creatinine concentration in the rats treated with the fungal culture filtrate of *Trichoderma harzianum*, *Penicillium citrinum* and *Aspergillus versicolor.*

Table 6: Effect of different fungal culture filtrates at different doses on creatinine in rats during repeated dose 28-day oral toxicity study.

Groups	Creatinine (mg/dl)		
	Day 0	Day 14	Day 28
Group I (Control)	0.24±0.01	0.35±0.01	0.35±0.02
Group II	0.26±0.01	0.43±0.40*	0.54±0.19**
Group III	0.26±0.01	0.51±0.25**	0.56±0.22**

Values are mean ± SEM, n = 6, * p<0.05, ** p<0.01*** p<0.001,

Hematological parameters

Total leucocyte count (TLC)

Total Leucocyte Count (TLC) values were given in Table 7.

There was no statistically significant ($p>0.05$) change in the total leucocyte count (TLC) on day 0 compared to control group values. On day 28, TLC value in rats was significantly ($p<0.05$, $p<0.01$) decreased in groups II and III compared to control group values.

Decrease in total leukocyte count (TLC) might be due to immunosuppression known to be caused by aflatoxins and also microscopic histopathologic lesions of lymphoid depletion in spleen also support the decrease in total leukocyte count.

In the study of effects of aflatoxin on some hematological parameters and protective effectiveness of esterified glucomannan in Merino rams by Donmez *et al.* (2012) [18], resulted in decreased erythrocyte, leukocyte count, hemoglobin and hematocrit levels. In a study of effect of aflatoxin on hematological and biochemical alteration in broilers by Rathod *et al.* (2017) [19], exhibited that broilers fed with aflatoxin diet revealed values of hemoglobin, total erythrocyte count and total leukocyte count significantly decreased when compared with control diet.

Table 7: Effect of different fungal culture filtrates on TLC (10^3cells/mm^3) in rats during repeated dose 28day oral toxicity study.

Groups	TLC (10^3cells/mm^3)		
	Day 0	Day 14	Day 28
Group I (Control)	8.86±0.21	8.49±0.11	8.22±0.01
Group II	8.90±0.17	7.96±0.79*	7.21±1.13**
Group III	8.73±0.08	7.76±0.26**	7.43±0.71**

Values are mean ± SEM, n = 6, * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Total erythrocyte count (TEC)

TEC values are given in Table 8.

There was no statistically significant ($p>0.05$) change in the total erythrocyte count (TEC) on day 0 compared to control group values. On day 28, TEC value in rats was significantly ($p<0.01$) decreased in groups II and III compared to control group values.

Sharma *et al.* (2011) [20], found in the study of influence of *Curcuma longa* and curcumin on blood profile in mice subjected to aflatoxin B1 reduced the level of total erythrocyte count (TEC).

In the study of broilers induced by exposure to aflatoxin B1 and ochratoxin A, biochemical and hematological changes on day 40, showed significant ($p<0.05$) reduction the total erythrocyte count (TEC) and RBC (Umar *et al.*, 2012) [21].

Table 8: The effect of different fungal culture filtrates at different doses on TEC (10^6cells/mm^3) in rats during repeated dose 28-day oral toxicity study.

Groups	TEC (10^6cells/mm^3)		
	Day 0	Day 14	Day 28
Group I (Control)	8.24±0.21	8.91±0.01	8.62±0.01
Group II	8.62±0.17	8.62±0.79	7.36±1.13**
Group III	8.65±0.08	8.55±0.66	7.29±0.71**

Values are mean ± SEM, n = 6, *$p<0.05$, ** $p<0.01$, *** $p<0.001$.

Haemoglobin

Haemoglobin concentration values were given in the Table 9.

There was no statistically significant ($p>0.05$) change in the haemoglobin concentration on day 0 compared to control group values. On day 28, haemoglobin concentration was significantly ($p<0.01$) decreased in groups II and III compared to control group values.

Similar findings were recorded by Sharma *et al.* (2011) [20] and Rekha (2014) [17] in their studies and indicated decreased content of hemoglobin.

Table 9: The effect of different fungal culture filtrates at different doses on Haemoglobin (g/dl) in rats during repeated dose 28-day oral toxicity study.

Groups	Hb(g/dl)		
	Day 0	Day14	Day 28
Group I (Control)	16.65±0.21	17.30±0.01	17.20±0.01
Group II	16.33±0.17	16.24±0.79	15.26±1.13**
Group III	16.53±0.08	16.23±0.66	15.24±0.71**

Values are mean ± SEM, n = 6, *$p<0.05$, ** $p<0.01$, *** $p<0.001$.

Packed cell volume (PCV)

Packed cell volume (PCV) values of rats were given in the Table 10.

There was no statistically significant ($p>0.05$) change in the Packed cell volume (PCV) on day 0 in rats compared to control group values. On day 28, packed cell volume (PCV) in rats was significantly ($p<0.01$) decreased in groups II and III compared to control group values.

The study conducted by Venkanna (2008) [15] and Chandrashekar (2012) [11] was in similar to present findings and was confirmed by the reduction in RBCs count, Hb, PCV, MCH and MCHC with increased MCV.

In the investigation of oxidative stress in the extrahepatic tissues of rats co-exposed to aflatoxin B1, Rotimi *et al.* (2018) [22], found that rats treated with aflatoxin had dramatically reduced body weight gain and PCV.

Table 10: Effect of different fungal culture filtrates at different doses on PCV (%) in rats during repeated dose 28-day oral toxicity study.

Groups	PCV (%)		
	Day 0	Day14	Day 28
Group I (Control)	45.65±1.21	45.30±0.51	48.20±0.52
Group II	46.33±0.17	43.24±0.79	40.24±1.13**
Group III	45.53±0.80	42.32±0.66	40.26±0.71**

Values are mean ± SEM, n = 6, *$p<0.05$, ** $p<0.01$, *** $p<0.001$.

Pathology

Gross pathology

In all the treated groups of rats there was congestion of lungs, liver, kidney and heart. There were no prominent gross pathological lesions in the spleen and intestine of all the experimental groups.

Histopathology

Control group

All the organs of control group rats revealed normal architecture in the histopathological findings.

Group III observations

Heart: Degeneration and necrosis of cardiomyocytes was observed (Fig.3).

Lung: Moderate congestion, emphysema and interstitial pneumonia was predominant (Fig.4).

Liver: Central venous congestion and sinusoidal congestion (Fig. 5).

Kidney: Subscapular hemorrhages, glomerular hemorrhages and degenerative changes (Fig.6).

Spleen: Subcapsular hemorrhages (Fig.7).

Intestine: Increased goblet cell activity, destruction of epithelial cells and lot of debris (Fig.8).

The groups II and III had the treatment with culture filtrate of *Aspergillus niger* at 1ml/100 g and 2 ml/100 g body weight respectively. Histopathological lesions were more pronounced in group II compared to group I.

In the study of histopathological and biochemical analyses of the preventive effects of honey in rats treated with experimental aflatoxicosis, Yaman *et al.* (2016), observed dysplastic, necrotic, and hydropic alterations in hepatocytes. Essa *et al.* (2017) [23], studied the modulating effect of MgO-SiO2 nanoparticles on immunological and histopathological alterations induced by aflatoxicosis in rats. Microscopic lesions in the liver showed patchy areas of hepatocytes, ballooning degeneration with cytoplasmic reticulation and pyknotic nuclei.

Yaman *et al.* (2016) [24], noticed degeneration and coagulative necrosis of proximal tubule epithelial cells and hyperemia in arterioles in histopathological investigations of protective role of honey in rats with experimental aflatoxicosis in kidney.

Chandrashekar (2012) [11] and Rekha (2014) [17], observed similar histopathological changes in the intestine of rats *viz.*, increased goblet cell activity, destruction of epithelial cells and lot of debris in their toxicity studies .

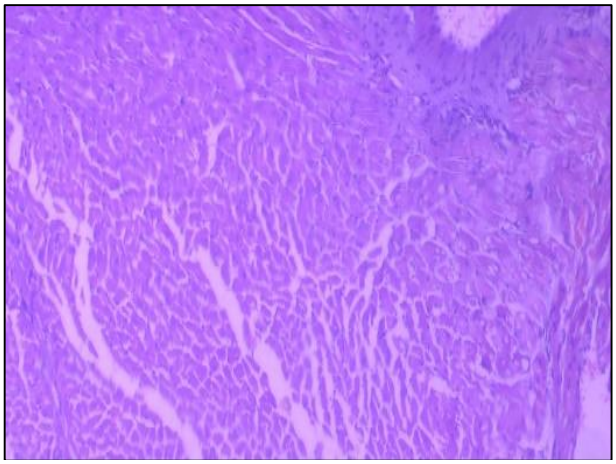

Fig 3: Heart: Degeneration and necrosis of cardiomyocytes

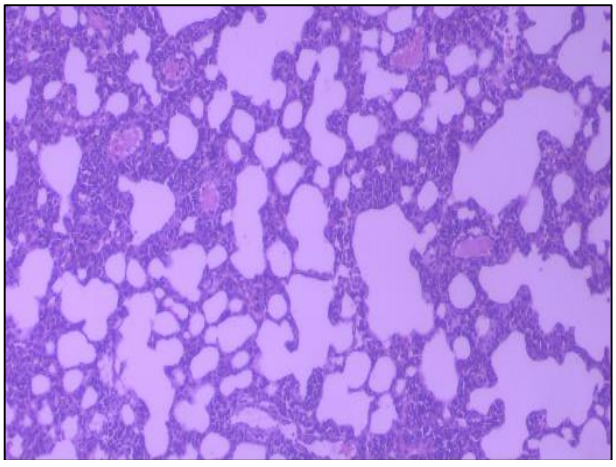

Fig 4: Lungs: Moderate congestion, emphysema

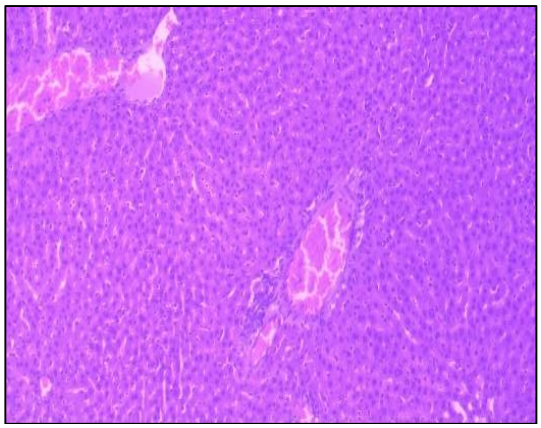

Fig 5: Liver: Central venous congestion and sinusoidal congestion

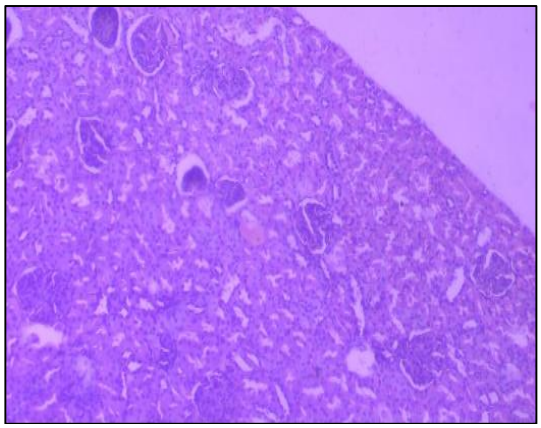

Fig 6: Kidney: Subscapular hemorrhages, glomerular hemorrhages

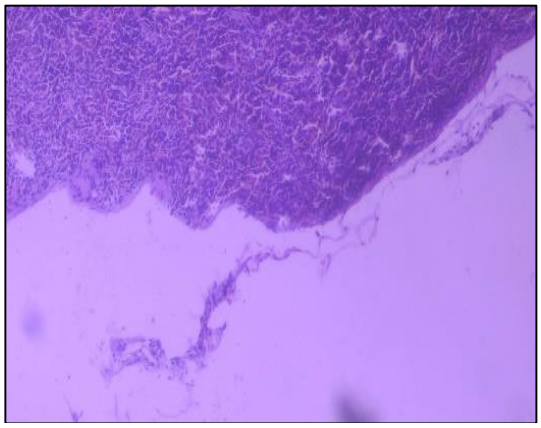

Fig 7: Spleen: Subcapsular hemorrhages

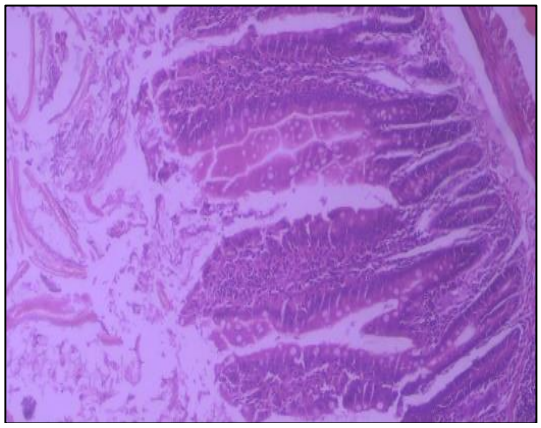

Fig 8: Intestine: Increased goblet cell activity, destruction of epithelial cells

Conclusion

The findings of the present study inferred that the fungal culture filtrate of fungus *Aspergillus niger* isolated from contaminated maize stalk resulted in toxicity in rats, which was attributed for the presence of a toxic principle component in the fungal culture filtrate**.** Further studies need to be carried out to identify exact nature of the culture filtrate and its metabolites.

Acknowledgments

The authors are grateful to the Dean, Veterinary College, Shivamogga, for providing the necessary facilities to conduct the study.

Disclosure of potential conflicts of interest

The authors have no conflict of interest to declare.

References

1. Yu J, Pedroso IR. Mycotoxins in cereal-based products and their impacts on the health of humans, livestock animals and pets. Toxins. 2023;15(8):480-495.
2. Cheli F, Campagnoli A, Dellorto V. Fungal populations and mycotoxins in silages: from occurrence to analysis. Anim Feed Sci Technol. 2013;183(1):1-16.
3. Rodrigues I. A review on the effects of mycotoxins in dairy ruminants. Anim Prod Sci. 2014;54(9):1155-1165.
4. Agriopoulou S, Stamatelopoulou E, Varzakas T. Advances in occurrence, importance, and mycotoxin control strategies: prevention and detoxification in foods. Foods. 2020;9(2):137-149.
5. Streit E, Schatzmayr G, Tassis P, Tzika E, Marin D, Taranu I, *et al.* Current situation of mycotoxin contamination and co-occurrence in animal feed—focus on Europe. Toxins. 2012;4(10):788-809.
6. OECD. Organization for Economic Cooperation and Development guidelines 403, 408, 425 and 426 for testing of chemicals. Paris, France; 2001.
7. Vinay PT. Toxicity studies of fungal isolates from groundnut hay in rats and mice [M.V.Sc. thesis]. Bidar: Karnataka Veterinary, Animal and Fisheries Sciences University; 2007.
8. Alghamdi RS, Alharbi TS, Alsubaie WR. Quality standards of histopathology laboratory and work facilities in a developed country. Arch Pharm Pract. 2021;12(1):90-97.
9. Bayman P, Baker JL. Ochratoxins: a global perspective. Mycopathologia. 2002;162(3):215-223.
10. Vinay PT, Shridhar NB, Narayanaswamy HD, Shrikrishna I, Jayakumar K. Subchronic toxicity study of *Fusarium xylarioides* culture filtrate from groundnut hay in rats. J Lab Anim Sci. 2011;1(1):49-53.
11. Chandrashekar G. Toxicity study of fungal isolates from maize stalks in rats [M.V.Sc. thesis]. Bidar: Karnataka Veterinary, Animal and Fisheries Sciences University; 2012.
12. Shivaprasad GR. Toxicity studies of fungal isolates in rats and mice from paddy straw suspected to cause necrosis of extremities in buffaloes [M.V.Sc. thesis]. Bidar: Karnataka Veterinary, Animal and Fisheries Sciences University; 2008.
13. Shivanand M. Toxicity study of fungal isolates from paddy straw in rats causing nervous disorders in goats [M.V.Sc. thesis]. Bidar: Karnataka Veterinary, Animal and Fisheries Sciences University; 2013.
14. Udeani TK, Okwuosa CN, Adiele EU. Effect of Aspergillus flavus on the liver of experimental rats administered with anti-retroviral drugs. Am J Phytomed Clin Therapeut. 2013;6:443-456.
15. Venkanna B. Toxicity studies of fungal isolates from maize hulls in rats and mice [M.V.Sc. thesis]. Bidar: Karnataka Veterinary, Animal and Fisheries Sciences University; 2008.
16. Eraslan G, Sarica ZS, Bayram LC, Tekeli MY, Kanbur M, Karabacak M, *et al.* The effects of diosmin on aflatoxin-induced liver and kidney damage. Environ Sci Pollut Res. 2017;24:27931-27941.
17. Rekha Y. Toxicity study of fungal isolates from maize straws in rats [M.V.Sc. thesis]. Bidar: Karnataka Veterinary, Animal and Fisheries Sciences University; 2014.
18. Donmez N, Donmez HH, Keskin E, Kisadere I. Effects of aflatoxin on some haematological parameters and protective effectiveness of esterified glucomannan in Merino rams. Scientific World J. 2012;2012:1-5.
19. Rathod P, Gangadhar K, Gangane G, Bhojane N. Effect of aflatoxin on haematological and biochemical alterations in broilers. Int J Sci Environ Tech. 2017;6:824-831.
20. Sharma V, Sharma C, Sharma S. Influence of Curcuma longa and curcumin on blood profile in mice subjected to aflatoxin B1. Int J Pharm Sci Res. 2011;2(7):1740-1745.
21. Umar S, Arshad A, Ahmad B, Arshad M. Clinico-biochemical and haematological changes in broilers induced by concurrent exposure to aflatoxin B1 and ochratoxin. J Public Health Biol Sci. 2012;1(3):79-85.
22. Rotimi OA, Rotimi SO, Oluwafemi F, Ademuyiwa O, Balogun EA. Oxidative stress in extrahepatic tissues of rats co-exposed to aflatoxin B1 and low-protein diet. Toxicol Res. 2018;34(3):211-220.
23. Essa SS, El-Saied EM, El-Tawil OS, Mahmoud MB, Abd El-Rahman SS. Modulating effect of MgO-SiO2 nanoparticles on immunological and histopathological alterations induced by aflatoxicosis in rats. Toxicon. 2017;140:94-104.
24. Yaman T, Yener Z, Celik I. Histopathological and biochemical investigations of protective role of honey in rats with experimental aflatoxicosis. BMC Complement Altern Med. 2016;16(1):1-11.