ISSN 2664-8075 (Print) & ISSN 2706-5774 (Online)
South Asian Research Journal of Applied Medical Sciences
Abbreviated Key Title: South Asian Res J App Med Sci

| Volume-7 | Issue-3 | May-Jun- 2025 | **DOI: https://doi.org/10.36346/sarjams.2025.v07i03.007**

Original Research Article

Using Calcium Citrate and *Ganoderma lucidium* Filterate to Reduce Toxicity of *Aspergillus flavus* Effects in Laborotary Mice's Histological Sections and Biochemistry Tests

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Article History
Received: 13.05.2025
Accepted: 18.06.2025
Published: 26.06.2025

Abstract: The isolate of *Aspergillus flavus* from the yellow corn and their products samples had the greatest quantity of aflatoxin B1 toxin, measuring 197.8 ppb, according to an analysis utilizing HPLC technology. This isolate was identified phenotypically and molecularly diagnosed and have already been officially recognized by the National Center for Biotechnology Information (NCBI, thirty female mice were experimentally injured in six groups, and each group received 0.5 lμ oral dosage administered once through a stomach tube. With six treatments toxic filtrate*, G.lucidium, calcium citrate, interference of them and control without treatment alone* Findings from the investigation pathological. The toxic fungus's in histopathological ability to effects. When an autopsy was performed 21 days after the injury, the inflammatory cells had infiltrated the liver tissue and caused liver tissue cross slice with a distinct histopathological, appearance these findings validated the positive and effective role that the part the test played in the therapy, as demonstrated by the return of the liver and kidney tissues to their normal, healthy states. The detrimental impact's outcomes a harmful effect was seen in female BALB/c albino laboratory mice upon isolation of toxic fungus within the organism. The mouse musculus, wherever notable alterations occurred in the lab animals' tissues subsequent to their medication administration and throughout the experiment's duration. The impact on liver enzyme levels (GPT, GOT, and ALP) with values of 145.0, 285.0and 238.0 IU/L, respectively, in comparison to the control group's values of 45.0, 190.0, and 124.0 IU/L, As opposed to the toxic fungus filtrate group, which had concentrations of 56.0. mg/dl and 1.75 mg/dl of urea and creatinine, respectively in comparision control group was 35.0 and0.6 mg/dl respectively. The results of the laboratory investigation on kidney function revealed substantial variations in the increase in these concentrations. The findings of this study demonstrated the effectiveness after using calcium citrate and *G. lucidium* filtrate treatments, as well as the interference between the two treatment. Additionally, the results demonstrated the anti- and beneficial effects of these interventions affecting the regular functioning and recovery of the tissues of the kidney and liver.

Keywords: Aflatoxin B1 (AFB1), Aspergillus flavus, BALB/c mice, Ganoderma lucidum, Calcium Citrate.

INTRODUCTION

Before mycotoxins were identified, fungi development in food was not a significant health concern. A flaw in the technology and in the production process was the existence of fungi. Fungal presence has emerged as a serious danger to consumer health following the discovery of mycotoxins. Food security has prompted calls for nations to supply wholesome food because it is a serious health issue for people, particularly in poorer nations where food storage conditions are inadequate and a big worry (Makum *et al*., 2010). The majority of people do not exhibit any overt symptoms when they ingest small amounts of mycotoxins through their regular diet. Significant health issues might arise from consuming large amounts of mycotoxins or doing so over a lengthy length of time. Bhat and Vasanthi (2003) talk about immunosuppression, fetal malformations, congenital anomalies, cardiovascular disease, and the liver and kidneys. With concentrations as low as 10 parts per million, Known to be the greatest poisons, mycotoxins lead to dangerous illnesses. The power of mycotoxins stems from their extreme heat resistance, which prevents them from being damaged by the common heat treatments

employed throughout the cooking and manufacturing processes (Jamili, 2014). The intrinsic poisons created in these foods are not entirely eliminated by removing the fungus contaminated areas, the same number of individuals, because the toxins are making their way quickly from the growing fungal colonies to the food. Under the right temperature and humidity conditions, fungi metabolize and produce mycotoxins (Mehmood *et al*., 2018).

Secondary metabolites, which include fungal physiological activities result in the production of low-molecular-weight compounds, or molecules with (250–800 daltons), which do not trigger the immune system. That are known as mycotoxins (Greisen and Schmidt, 2007). Different fungi produce these compounds. The mycotoxins are also not created in the sense that the elements that trigger the body to produce antibodies are absent from their molecular structure. According to Nakheel (2011), the toxin molecule can produce distinct biological consequences if it attaches to the protein molecule it contains to acquire immunogenicity and change chemically. Mycotoxins have a variety of effects on an organism's body's essential processes. Acute poisoning is the most frequent cause of food poisoning if consumed in amounts greater than the internationally established thresholds permitted in food for humans or as raw ingredients it ultimately results in destruction. (Creppy, 2002).The duration of exposure, the rate of absorption and elimination, and the individual's reaction to the substance all influence the symptoms of poisoning and how quickly they manifest. According to Yekeler *et al*., (2001), the substance is stored in the various body organs' tissues when absorption occurs gradually and continuously over an extended period. To determine the impact of the mycotoxins present in *A. flavus* on its tissues and organs, the, the study will employ white albino mice. Researchers demonstrated the antibacterial activity of a number of mushrooms (Giaccone, 2022). Antibacterial activity against a broad spectrum of fungal toxicity has been documented for fruiting bodies and mycelia of several mushrooms (Fokunang *et al*., 2022).the difference in composition chemical properties vary according to the damages and effects they have strong effects on fetuses and placenta with carcinogenic activity in women (Simon, 2008). Exposure to toxins, especially aflatoxin toxins for a long time, leads It leads to a defect in the work of the endocrine glands, including the endocrine, so the toxins of the fungi are considered one of the most dangerous poisons due to the low concentrations and the high resistance to temperatures, as they cause dangerous diseases for humans (Klein, 1989).

MATERIALS &METHODS

Investigation of *A. Flavus* Filterate Effects in an Organism's Body in Vivo

Using Mouse Mus musculus species, commonly known as BALB/c albino female white mice the impact of mycotoxins on lab animals was studied. The application of mycotoxin-based biologic techniques depends on the alterations brought about by an extract dosage in the tissues of laboratory animals. 30 sets of female laboratory mice were created. Six groups were created out of the animals (five lab animals each), one of which served as the control group and received nothing but food and water. *A. flavus* toxin concentration was used to determine the dilution's value, which 0.5 .ml. Ten percent was the chosen concentration for both the *G.lucidium* extract and citrate calcium. According to Al-Khafaji (2017) and Rajani *et al*., (2012), the laboratory animals were begun on 24/4/2024 and were kept every two days for 21 days. This is indicated within the following table(1) Laboratory white mice distribution with ,coefficient c,1,2,3,4,5,6 Only filtrate *A. flavus* + filtrate *G.lucidium* + filtrate *A. flavus* + calcium Citrate are treated in transactions involving *A.flavus* and *G.lucidium* treatment and extraction with calcium citrate An explanation of the material transactions in, Arceles Give the mice purified water as their only dosage, and give them 0.5 ml or 30 g of *A. flavus* fungus orally every 48 hours A. flavus fungus is dosed orally to the mice, then after a day, *G.lucidium* fungus is dosed orally to the mice (0.5 ml / 30 g / day),When the mice have been given the A. flavus fungus for 24 hours, they are given an oral dose of the extract and then 30 g/day of calcium citrate Following a 24-hour period, the mice were given an oral dose of *A. flavus* fungus and 0.5 ml / 30 g of calcium citrate every ,eid Dosage: 0.5 ml or 30 g of *G.lucidium* extract given orally to mice every 48 hours. Sacrifice of animals: Two days after the prior procedure, the lab animals were brought to their deaths by an abdominal cavity entrance, following a chloroform anesthetic. Therefore, the goal of the purpose of the current inquiry was to evaluate, experimentally (in-vivo), the impact of biological and chemical treatments concerning the physiological and histological consequences of infection caused by *A. flavus* filters. In the female laboratory albino rat lungs, as well as the implementation of certain standards, like body mass and rate of weight increase measurements, and the examination of the histological and biochemical alterations in the laboratory animals' lungs. Moreover, the indications of macroscopic alterations in the affected animals' lungs both prior to and following therapy are documented, as is the course of the experimental animals' clinical manifestations as observed by ongoing surveillance over the study period.

Table 1: Laboratory animals treatment

No.	Treatments	Description of parameters and material concentration
1	the control	Treat animals with distilled water only
2	*A. flavus* filtrate	Dosing mice with *A.flavus* filtrate orally every 48 hours: 0.5 ml/30 g per mouse/day.
3	*G.lucidium* filtrate	Dosing mice with *G. lucidium* filtrate orally 48 hours 0.5 ml/30 gm per mouse/day
4	*A.flavus* + *G.lucidium*	Mice were given a dose of *A. flavus* fungal filtrate, followed 24 hours later by a dose of *G.lucidium* filtrate (0.5 ml/30 g each mouse/day).

5	*A.flavus+calicium* citrate	The mice were given a dose of *A. flavus* fungal filtrate, and then they received a dose of calicium citrate + 0.5 ml/30 gm per mouse/day after a 24-hour period.
6	*A.flavus* + *G.lucidium* filtrate+ calcium citrate	*A. flavus* fungal filtrate was given to the mice. They received an oral dosage of 0.5 ml/30 g of calcium citrate each mouse day after being dosed with *G .lucidum.*filtrate for 24 hours.

Examinations in Life Chemistry

GPT, GOT, ALP, blood urea, and creatinine levels in Serum were calculated using the Japanese made R Krey instrument. Shortly after the test is finished, the findings display on paper tapes for roughly fifteen minutes. The serum is inserted into the specific location inside the ,apparatus putting the organs (liver and kidneys) that were chosen for study and preparation in a material that had been demonstrated to work (formalin 10%) following anesthesia and animal slaughter. Blood samples taken from experimental animals By means of a needle (Needle gauge 23x), the animal's heart was punctured for collecting blood samples directly from the heart. Gel tube containing serum was filled with blood in order to investigate how the liver functions in the examination of GPT, GOT, and ALP in order to research kidney function Urea and creatinine are used to measure renal function.

Making Histological Slide Preparations

Following the blood draw, the organs of the kidney and liver were removed and cleaned with normal saline. The organs were then preserved in 10% diluted formalin in order to prepare tissue sections.

1- Fixation: Implant the organs in a diluted formalin solution for a duration of 24 hours.
2- Sample washing: after two hours, the tissue sections were rinsed with water to remove the formalin solution.
3- Dehydration: A series of increasing ethyl alcohol concentrations (100, 90, 80, and 70%) are used in the dehydration process, with each concentration being held for two hours.
4- Clearing: To make the tissue more clear, the samples are diluted by putting them in xylene.
5- Infiltration: A certain amount of melted paraffin wax was intended to be saturate the tissue through the filtration process.
6- The samples are placed within the wax for at least two hours at 58 °C for thirty minutes.
7- Embedding: Copper molds shaped like a L are used to embed the samples.
8- Dividing A Rotary Microtome, which has a thickness of 6-7 micrometers, is used to cut the molds.
9- Loading and homogenizing the slides: the tissue sections are submerged in water bath at 40 °C to facilitate
10- The fabric can be dried from the water by placing it on a hot plate at 40 °C for 24 hours. Once dry, it is prepared for further processing.
11- Color Haris Hematoxline and Eosine stain were the dyes utilized in the dyeing procedure.
12- Microscopic analysis: Following the coloring procedure, the slides were fixed with Canada Balsam, and the tissue became.

Analytical Statistics

GraphPad Prism Institute, Inc.'s statistical software was utilized to analyze the entire study's findings. Utilizing the least significant difference (LSD) and the Chi-square test as statistical tests approach for both variance in both directions, the least significant differences between the research groups were determined. $P < 0.05$, which is a value at the probability level less than 0.05, and a 95% confidence interval were also used (Khalafi-Kheydani *et al*., 2022).

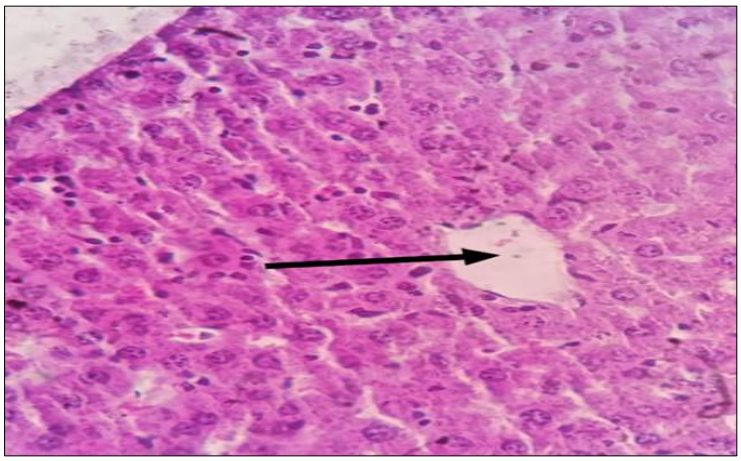

Fig. 1: Shows a cross-section of a mice liver, representing the control group (X400). The hepatic plates are clearly observed around the central vein (indicated by the black arrow). The hepatocytes are distinctly visible, hexagonal in shape, with a central nucleus

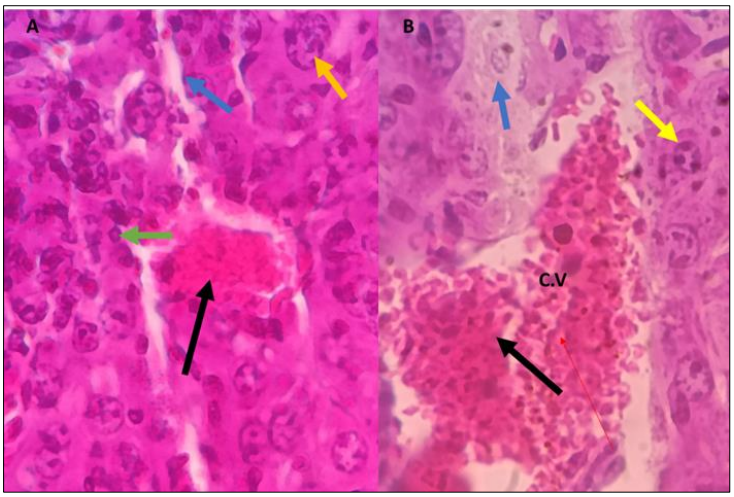

Fig. 2: A&B shows a cross-section of a rat liver representing the treatment group with a toxic A.flavus filtrate (H&E; X400). There is dilation of the central vein (CV) with hemorrhage (black arrow), cellular degeneration (blue arrow), and nuclear necrosis or hyperchromasia (yellow arrow)

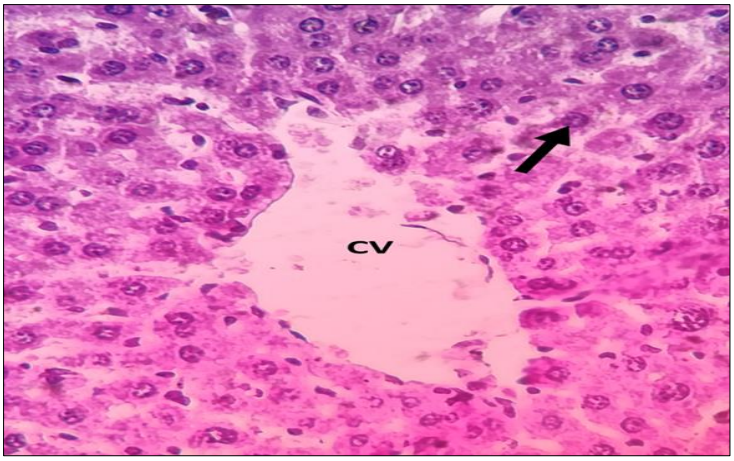

Fig. 3: Shows a cross-section of a rat liver representing the treatment group with only G.lucidium filtrate (H&E; X400). The central vein (CV) appears normal, and the hepatic cords are arranged in an orderly manner, with flattened endothelial cells and Kupffer cells interspersed (black arrow)

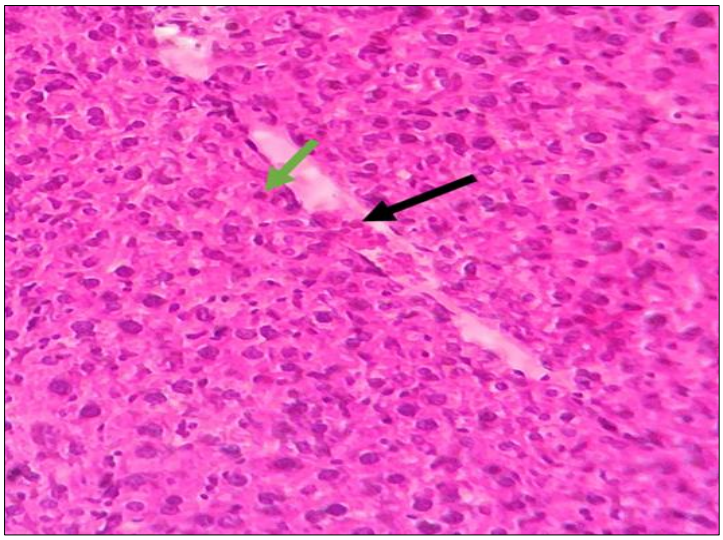

Fig. 4: Shows a cross-section of a mice liver representing the treatment group with both toxic A. flavus and G. lucidium filtrate (H&E; X400). A reduction in the degree of degeneration and inflammatory infiltration is observed (green arrows), along with some remaining vascular congestion (black arrows).

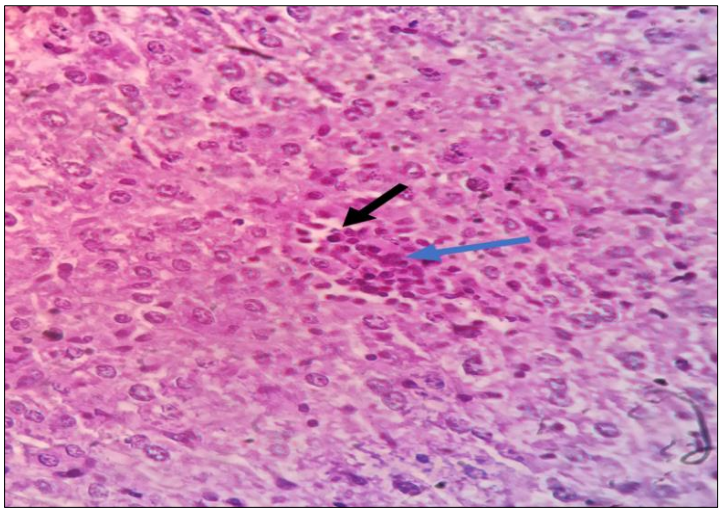

Fig. 5: Shows a cross-section of a mice liver representing the treatment group with both toxic A.flavus and calicium citrate (H&E; X400). A moderate improvement in hepatocytes is observed, with a significant reduction in signs of degeneration and necrosis (blue arrows) and proliferation of Kupffer cells (black arrow).

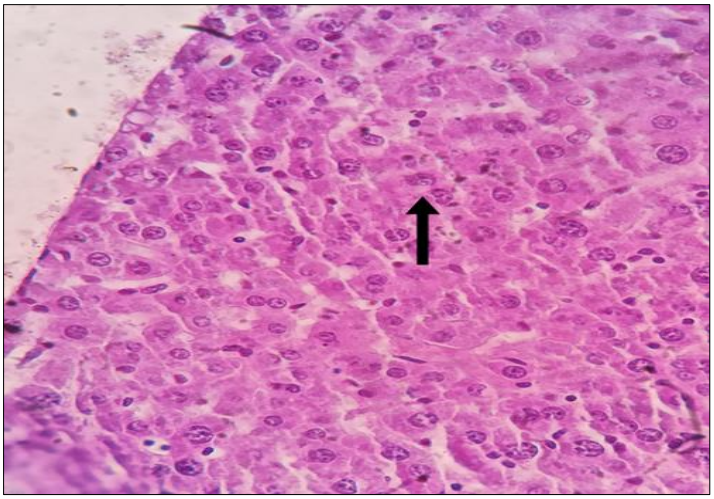

Fig. 6: Shows a cross-section of a mice liver representing the treatment group with both toxic A.flavus and interference of G.lucidium and calcium citrate (H&E; X400). Normal hexagonal cells are observed, along with proliferation of Kupffer cells (black arrow) and dilation of the sinusoids.

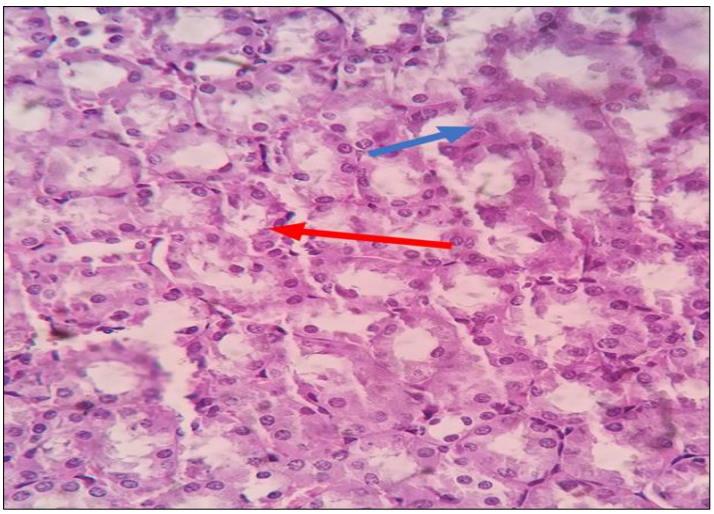

Fig. 7: Shows a cross-section of a mice kidney representing the control group (X400: H&E stain). The renal tissue appears normal, with glomeruli and renal tubules showing normal morphology (red arrow)

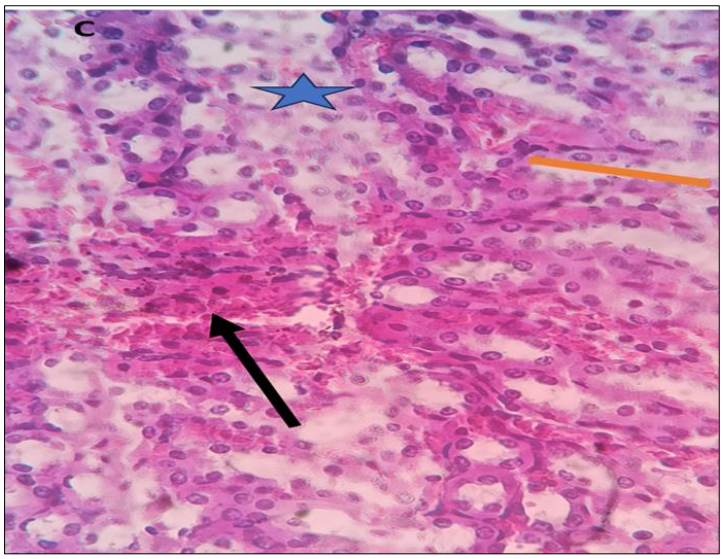

Fig. 8: Shows a cross-section of a mice kidney representing the treatment group with toxic A.flavus (X400: H&E stain). There is severe necrosis and shedding of epithelial cells observed on the lining of the convoluted renal tubules (orange arrow), with dilation (blue star) and obvious hemorrhage in the renal tubules (black arrow)

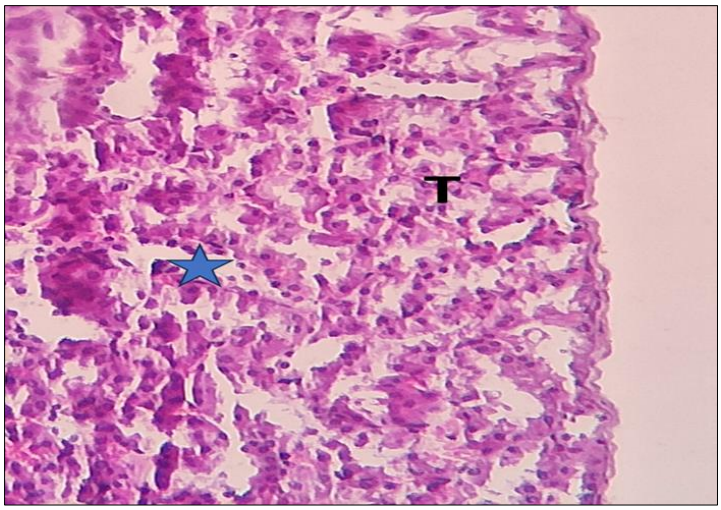

Fig. 9: Shows a cross-section of a rat kidney representing the treatment group with G. lucidium filtrate (X400: H&E stain). There is infiltration of large phagocytic cells (T) in the renal tissue and dilation of the convoluted tubules (blue star)

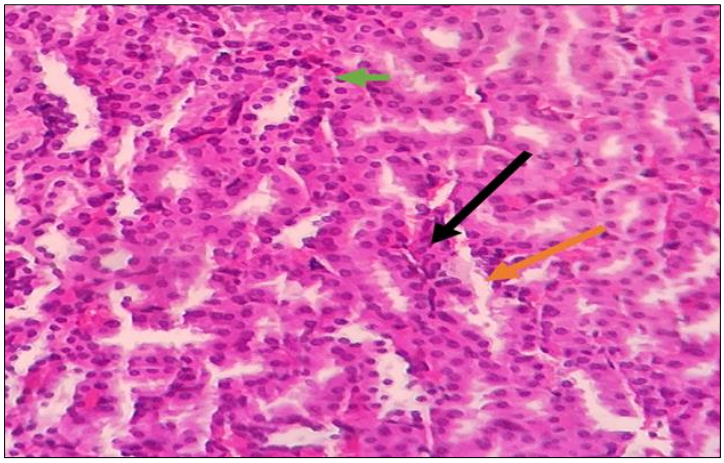

Fig. 10: Shows a cross-section of a rat kidney representing the treatment group with both toxic A.flavus filtrate and G.lucidium (X400: H&E stain). There is slight atrophy in some renal glomeruli (black arrow), mild necrosis in the lining cells of the convoluted renal tubules (orange arrow), and some minor hemorrhage in certain areas (green arrow)

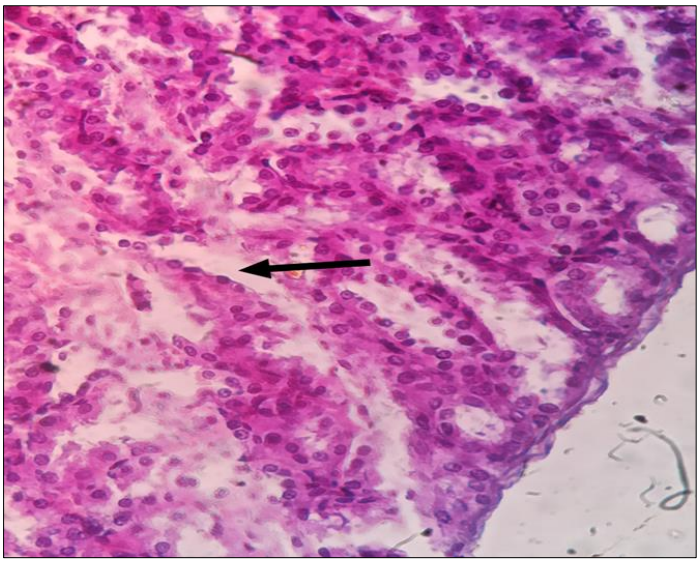

Fig. 11: Shows a cross-section of a rat kidney representing the treatment group with toxic A.flavus filtrate, and calcium citrate (X400: H&E stain). Regular, normally shaped glomeruli are observed, with slight dilation in the renal tubules (black arrow)

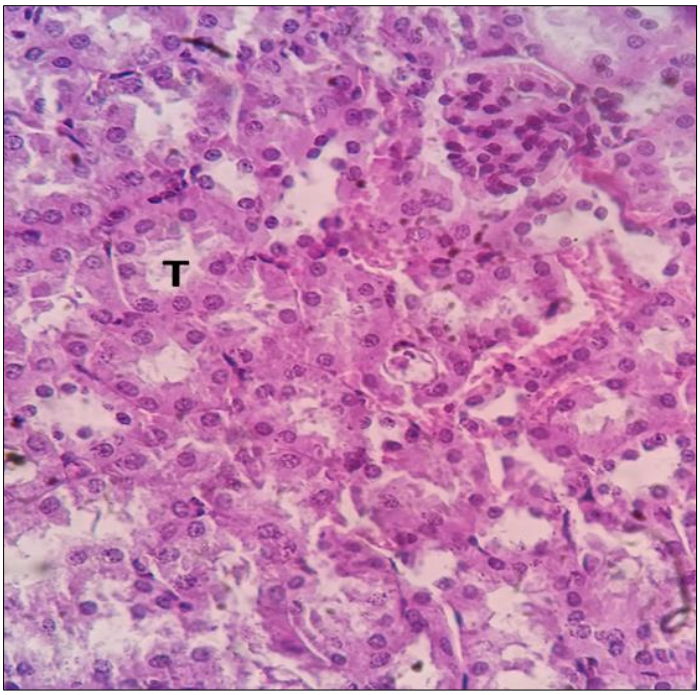

Fig. 12: Cross section of a mice kidney showing a characteristic collection of *A. flavus* + *G. lucidium* +calcium citrate (HE; X400) and normal cell growth, hexagonal and proliferation of cells, and proliferation of cells (black arrow) in the sinusoids.

RESULTS AND DISCUSSION

The weight rates of laboratory mice during the dosing period decreased because of exposure to the fungus filtrate *A. flavus*, according to measurements taken of the white mice during the therapy. There were variations in the weights, as indicated by the data in table No. (2). Prior to dosage, the average weight of the second group was 30.20. The average weights decreased by 28.50 g during the dosage period, or after 10 days. Before the laboratory animal was sacrificed and after ten days, there were notable variations in the animals'. Weights However, prior to the initiation of the dosage, there was no discernible change. Additionally, prior to the sacrifice, the average weight was 27.30 g. In contrast, the control group's average weight was 26.0 g before dosing, 29.20 g after ten days, and 31.90 g prior to the sacrifice. There were significant differences in the third group, which took place only due to the scarcity of the commercial fungus *G.lucidium*. Before dosing, the weight growth rate reached 28.40 g, but after ten days it reached 29.30 g, while before sacrifice, the weight average reached 30.90 gm. This is what contributed, along with what Ibrahim and others (2006) did, to the formation

of the fungus.*G. lucidium*, which is consistent with what was stated by (Yekeler *et al*., 2001). Additionally, this supports a different study that found that rats dosed Laboratory animal weights and their hepatotoxicity and renal toxicity are impacted by AFLB1 mycotoxin (Hepato- and nephrotoxicity).

Table 2: Measuring the white length weights during the treatment

	Treatments	***Average weights (g) ± standard error***			
No		**Before sacrifice**	**After ten days**	**Before dosing**	**Treatment rate ± standard deviation**
1	control	31.90±3.88	29.20±1.09	26.0±2.11	29.03±2.9
2	*A. flavus* filtrate	27.30±2.98	28.50±2.02	30.20±3.81	28.66±1.45
3	*G.lucidium* filtrate	30.90±4.1	29.30±2.3	28.40±2.91	29.53±1.27
4	*G.lucidium* + *A.flavus*	30.10±3.91	27.40±1.23	25.30±3.12	27.60±2.40
5	*A. flavus*+ calcium citrate	29.80±2.1	27.70±1.99	25.50±2.11	27.67±2.15
6	*G.lucidium* + *A.flavus* + calcium citrate	31.90±3.17	29.80±3.1	27.60±1.99	29.77±2.15
Average times ± standard deviation		30.31±1.81	28.65±1.22	27.17±1.99	
LSD value		for periods = 1.4	Coefficients = 1.1	for overlap = 1.3	

Examining how *A. flavus* toxin affect the organism's internal organs; doing chemical blood tests to gauge how well the GPT, GOT, and ALP liver enzymes. Are working. Impact of *A. flavus* toxin on liver enzyme levels in infected white mice (Table 3)GPT 145.0±5.65, GOT 285.0±10.32, ALP 238.0±10.71 IU/L, respectively) in *A. flavus* treatment reached the liver enzyme test findings (45.0±2.11, 190.0±3.45, 124.0.±8.88 IU/L), Table (2), which demonstrates considerable variations in the enzyme concentrations within the investigated groups for the infection that the reason for the variations may be attributed to the poisonous consequences of significant mycotoxins on the tissues and cells of the liver, which led to the breakdown of liver cells with these enzymes and their release into the blood and high. Alternatively, the reason Meerdink (2004) suggests that the cause might be associated with the effect of the toxin on the tissues of other organs in the body that house these enzymes. The results in tablet (3) giving *G.lucidum* to lab animals at the enzymatic levels of (GPT41±4.32,GOT161±7.62,ALP 100±4.56) consistent with Timoz's (2015) observation that the existence of both high and low levels of enzymes suggests that the human or animal body is in a suitable state. In contrast to the group control, the extract had no effect on the enzymes, which continued to be active and present in the liver. Another possible explanation is how the toxin affects the tissues of other body organs that contain these enzymes (Volmer, 2004). At the obtained enzymatic values GPT 41 ±4.32), (GOT 161 ± 7.62), and (ALP100±4.56;) in the investigation of *G.luciium* filtrate's impact on lab animals treated with *G.lucidium*, which was carried out in compliance with Pradhan *et al*., (2019). When comparing the filtrate group to the control group, the activity and representation of the liver's enzymes stayed the same.

Table 3: The poisonous filtrate of fungus *A.flavus* on the amount of liver enzymes present in mice's blood

Totals	***Treatment***	**GPT (IU/L)**	**GOT (IU/L)**	**ALP (IU/L)**
1	control	45.0±2.11	190±3.45	124 **.0**±8.88
2	*A. flavus* filtrate	145.0±5.56	285.0±10.32	238.0±10.71
3	*G.lucidium* filtrate	41.0±4.32	161.0±7.62	100±4.56
4	*G.lucidium* + *A.flavus*	60 ± 2.67	171.0±2.43	119.0±8.34
5	*A. flavus*+ calicium citrate	76.0±8.19	320.0±11.12	265.0 ±14.6
6	*G.lucidium*+ *A.flavus* + calcium citrate	125.0±10.31	315.0±14.6	105.0±6.54
Average times ± standard deviation		82.00±41.5	240.3±70.5	158.5±69.1
LSD value		18.0	23.0	17.0
Mean values		46 > IU/L	18 > IU/L	240 > IU/L

Examining the Levels of Creatinine and Urea in Albino Mouse Blood

Regarding the administration of *G.lucidium* extract to *A. flavus*, there was no discernible rise in the ratios of creatinine and urea relative to the control group. Regarding the *A. flavus* treatment extract with citrate calcium, a marginal increase in levels was noted. Regarding the administration of citrate calcium, filtrate *A. flavus*, and filtrate *G.lucidium*, There was no discernible rise in levels; this could be attributed to the filtrate *G.lucidium's* ability to keep renal cells functional after mycotoxin exposure. The administration of *A. flavus* toxin to laboratory white mice led to increased levels of urea and creatinine in their serum. Comparing the urea level and creatinine ratio of the experimental group, it was 56.0 ±3.41 mg/dl and 1.75 ± 0.23 mg/dl, respectively, and 35.0 ± 5.2 mg/dl and 0.6± 0.012 mg/dl, respectively, for the control group. Between the groups under consideration tablet (2) displays considerable variations in the amounts of creatinine and urea. because The poison's effects on the kidney tissues led to the development of renal bleeding and lymphocyte infiltration as well as visible shrinkage in the renal glomeruli. In addition to this refers to the breakdown of the urine tubule cells with

fluid exiting between them. This inhibits kidney function and ultimately results in renal failure and a reduction in the excretion of harmful compounds (Guyton, 1989). The administration of *A. flavus* toxin to laboratory white mice led to increased levels of urea and creatinine in their serum. Comparing the urea level and creatinine ratio of the experimental group, it was 56.0 ±3.41 mg/dl and 1.75 ± 0.23 mg/dl, respectively, and 35.0 ± 5.2 mg/dl and 0.6± 0.012 mg/dl, respectively, for the control group. Between the groups under consideration tablet (2) displays considerable variations in the amounts of creatinine and urea. because The poison's effects on the kidney tissues led to the development of renal bleeding and lymphocyte infiltration as well as visible shrinkage in the renal glomeruli. In addition to this refers to the breakdown of the urine tubule cells with fluid exiting between them. This inhibits kidney function and ultimately results in renal failure and a reduction in the excretion of harmful compounds (Guyton, 1989).

Table 4: The poisonous filtrate of fungus *A.flavus* in blood mice's amount of urea and creatinine

Totals	***Treatments***	**Blood urea mg/dl**	***Creatinine mg/dl***
1	control	35.0±5.2	0.6±0.012
2	*A.flavus*	56.0±3.41	1.75±0.23
3	G. lucidium	33.0±3.81	0.7±0.11
4	*G.lucidium + A. flavus*	54.0±8.91	0.65±0.09
5	*A. flavus*+ calcium citrate	35.0±5.88	0.6±0.071
6	*G.lucidium + A. flavus* + calcium citrate	41.0±7.2	0.7±0.10
Average times ±	standard deviation	42.33±12.7	0.83±0.41
LSD	value	19.0	0.38
Mean	values	0.6-0.9 mg/dl	19-34.8mg/dl

Histological Sections

Liver

The first treatment: Control. The histological examinations of the livers of the animals in the control group showed integrity of the general histological structure. Organized hepatic plates were observed surrounding a clear central vein, with regular blood sinusoids and multinucleated hepatocytes with clear central round nuclei. No pathological or degenerative changes were recorded. (Figure -1). The results of microscopic examination to diagnose the histological sections taken from the liver of the white mice treated with *A.flavus* filtrate (Figure -2) showed clear pathological histological changes in the cross-section of liver tissue, including: disorganization of liver plates and loss of hepatic cord arrangement, severe vacuolization and hepatocyte enlargement, and there were aggregates of inflammatory cells. Severe fatty degenerative infiltration was observed in the liver cells, appearing ring-like due to the peripheral location of the nucleus, with inflammatory cell clusters. At high magnification, vacuolization of liver cells occurs, and the cytoplasm appears coarse and pink with large vacuoles. There is severe necrosis of liver cells. This study showed the effect of A. flavus toxin on the liver tissues of female mice that were orally dosed, resulting in liver tissue symptoms caused by the severe effect of *A. flavus* compared to the first group the control group this result agree with Hussein and saadon(2022). The third group of mice that were administered with the filtrate of the commercial fungus *G. lucidum* (Figure -3), there was a central vein with hepatic cell cords arranged radially, with expanded sinusoids and proliferation of Kupffer cells. There was a central vein with hepatic cell cords arranged radially, with expanded sinusoids lined with flat endothelial cells and Kupffer cells. Hepatocytes with eosinophilic cytoplasm were present. The fourth group that was treated with *A. flavus + G. lucidum* filtrate (Figure -4), a very small number of inflammatory cell clusters was observed along with hepatic structure. Normal hepatocytes were observed with Kupffer cells and the presence of hepatic structure, which explains the protective role of the commercial mushroom filtrate by reducing the toxic effect of the toxic filtrate of *A. flavus* this result agree with Abdaljabar and saadon (2022). The fifth group that was administered the toxic filtrate of *A. flavus* with calcium citrate (Figure -5), mild infiltration of inflammatory cells was observed along with hepatic structure, and inflammatory cell clusters were observed with loss of hepatic structure around the congested central vein with mild fatty degeneration. As for the sixth treatment, in which mice were injected with doses of *A. flavus + G. lucidum* + calcium citrate as in (Figure -6), there was a cellular structure of the hepatic plate consisting of central veins and hepatocytes arranged radially around the central veins, and proliferation of Kupffer cells and slightly dilated sinusoids. The appearance of some binucleated cells indicates repair and regeneration of damaged cells, where it is evident that the extent of damage is less severe compared to the group that received fungal toxins alone.

Kidney

The control group showed histological sections of the kidney with intact structure of the cortex and medulla, with organized renal glomeruli and clear spaces between the renal tubules. The epithelial cells lining the proximal and distal convoluted tubules were normal, and there were no signs of congestion, degeneration, or necrosis. (Figure -7). The results of histological diagnosis of sections taken from the kidneys of experimental mice treated with the toxic filtrate of *A. flavus* (Figure -8) showed congestion of unorganized glomeruli with deformation of Bowman's capsule surrounding the glomerulus. Albumin was found inside the tubules, and there was severe necrosis of the epithelial cells lining the proximal convoluted tubules, which appeared dilated. There was also severe necrosis and shedding of epithelial cells lining the

convoluted renal tubules, and their nuclei showed either pyknosis or karyolysis, with glomerular atrophy. These changes occurred as a result of the effect of the toxic filtrate of *A. flavus* on kidney cells and tissues, compared to the control group. As for the third group that was treated with the commercial filtrate of *G. lucidum* (Figure -9), the renal tubules were normal with no congestion and the absence of albumin in the renal tubules. The shape of the kidney appeared normal, indicating that the commercial mushroom filtrate is not toxic when administered to mice. The result of the microscopic examination of the kidneys of the fourth group that was treated with *A. flavus* + *G. lucidum* filtrate (Figure -10) showed that most glomeruli appeared normal with high cellularity, while others showed mild atrophy and slight dilation of the convoluted renal tubules, with hypertrophy and regeneration of the epithelial lining of the renal tubules. As for the fifth group that was treated with the filtrate of *A. flavus* + calcium citrate (Figure -11), glomerular atrophy, mild necrosis, and slight shedding of epithelial cells in the convoluted renal tubules were observed. The results of the sixth group that was treated with the filtrate *of A. flavus* + *G. lucidum* + calcium citrate (Figure -12) showed a partial effect, with organized normal glomeruli, slight dilation of the convoluted renal tubules, and regenerated cortical tubular basal cells. There were regenerated tubule bases with cellular glomeruli.

REFERENCES

- Abd Aljabar, K. M, and Saadon, A. S. (2022). Using *Agaricus bisporus* filtrate and sodium citrate against Alternaria arborescens toxicity in Histopathological and Biochemical change in laboratory mice. International journal of health sciences,6(S4),5464-5475.
- AL-Khafaji, Nada Ahmed Fairouz. (2017) Molecular properties of fungus A.niger production of toxins and contaminated for feed grains and they are controlled by laboratory. Master Thesis. Faculty of Science - University of Qadisiya.
- Bhat, R. V., S. Vasanthi, (2003). Food safety in food security and food trade: mycotoxin food safety risk in developing countries washington D.C. International Food Policy Research Institute.
- Creppy, E.E. (2002). Update of survery, regulation and toxic effects of Mycotoxin in Europe. Toxicol. Lett. 127(1-3).
- Fokunang, E. T., Annih, M. G., Abongwa, L. E., Bih, M. E., Vanessa, T. M., Fomnboh, D. J., & Fokunang, C. (2022). Medicinal Mushroom of Potential Pharmaceutical Toxic Importance: Contribution in Phytotherapy.
- Giaccone, V. (2022). Identificazione e quantificazione delle Micotossine prodotte da Alternaria in alimenti e mangimi, mediante LC-MS/MS e LC/HRMS.
- Greisen and Schmidt-Hiydt. (2007). Gene expression as an early indication for mycotoxin biosynthesis. International Journal of Food Microbiology, Pages 277283.
- Guyton, E. (1989). Guidelines for developing educational programs for cooperating teachers. Action in teacher Education, 11(3), 54-58.
- Hussein,S.A. and Sadoon, A. S. (2022). The possibility of controlling on *Aspergillus trrreus* producing Aflatoxin isolation from some chips local and import by *Pleurotus ostareatus* filtrate and sodium biocarbinate. International journal of health sciences,6(S10),730-740.
- Khalafi-Kheydani, A., Mahmoodi, H., Sadat, Z., & Azizi-Fini, I. (2022). The effect o nettle root extract on urinary problems in older men with benign prostatic hyperplasia: A randomized clinical trial. Journal of Herbal Medicine, Jumaili, Sami Abdul Rida Ali. (2014). Mycotoxins. Dar Al Kutb for Printing and Al - Nashler –Iraq, p. 423.
- Klein, A. S.; Satio, J. and Reind, C. (1989). Aflatoxin poisoning : treatment and Marko ,M.,Hsieh,C.,Sch alek,R.,Frank, J.&Mannella,C.(2007) ocused –ion-beam thinning of frozenhydrated biology specimens for cryoelectron microscopy.NatureMethodsvol.4,p.215-217.
- Makun, H. A., S. T. Anjorin , B.Moronfoye, F. O. Adeje, O. A. Afolablil, G.Fagbayibo, B.O. Balogum and A.A.Surajudeen.(2010).Fungal and aflatoxin contamination of some human foo commodities d in Nigeria . African Journal of Food Science , 4:127-135.
- Meerdink ,G. L.(2004). Aflatoxin. In : Plumlee, K. H. (ed).Clinical Veterinary toxicology. Litter Rock, Arkansas, USA.PP:231-235.
- Mehmood,N. A. Riaz, F. Naz , H. Rosli, N. Jaabeen and S. Anwaar (2018). First Report of Strawberry Leaf Spot Caused by Alternaria alternata in Pakistan.Plant disease . no. 4 [12].
- Nakhilan, Abdel Aziz Majid. (2011). Mycotoxins Mycotoxins Dar Al-Dijla, Amman Jordan, I, p. 320.
- Pradhan, M., Rai, D., & Bhandari, R. (2019). Metastatic Cutaneous Melanoma: A Rare Entity with a Unique Presentation. Journal of Nobel Medical College, 8(2), 6769.
- Rajani , K.G. ; Zhenyuxu A. L. ; Bertrand S. L. and Steinmetz M . A. .(2012). Extensive Degradation of RNA Precursors by the Exosome in Wild-Type Cells Volume 48 . Pages 409-421 Issue.
- Volmer, P. A., Roberts, J., & Meerdink, G. L. (2004). Anuric renal failure associated with zinc toxicosis in a dog. Veterinary and human toxicology, 46(5), 276-278.
- Yekeler, H., Bitmitis, K., ÖzÇelik, N., Doymaz,M.Z. and Çalta, M. (2001). Analysis of Toxic Effects of Alternaria Toxins on Esophagus Of Mice by Light and Electron Microscopy .Toxicologic Pathology , no 4, pp 492–497.