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Using sodium citrate and Agaricus bisporus filterate to reduce Aspergillus flavus harmful effects on laborotary mice's histological sections and biochemistry

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Summary

The isolated of ***Aspergillus flavus*** from the chickpea sample had the greatest quantity of aflatoxin B1 toxin, measuring 164 ppb, according to an analysis utilizing HPLC technology. This strain was identified phenotypically using keys and then molecularly diagnosed using in order to compare the contaminated fungal isolate A. flavus that was isolated together with the isolates that have already been officially recognized by the National Center for Biotechnology Information (NCBI)

the isolate was first molecularly diagnosed using the Phylogenetic tree analysis and base sequence analysis are combined with the polymerase chain reaction (PCR) method.

induced by Aspergillus flavus, thirty female mice were experimentally injured in six groups, and each group received 0.5 lμ oral dosage administered once through a stomach tube.

Findings from the investigation pathological .macroscopicand the toxic fungus's histopathological ability to contaminate. 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 ,appearanceThese 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 pathogenic 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 (ALT, GPT, and GOT) with values of 234.0, 152.0, and 288.0 IU/L, respectively, in comparison to the control group's values of 122.0, 44.0, and 191.0 IU/L, As opposed to the control group, which had concentrations of 37 mg/dl and 0.5 mg/dl of urea and creatinine, 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 sodium citrate and A. bisporus filtrate treatments,

as well as the interaction between the two substances. 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 .

INTRODUCTION

Before mycotoxins were identified, fungus 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 etal, 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 69–100 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 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

is attributed Its 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 aflaoxin 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).

2. Materials and Methods

Investigation of A. flavus extract's effects in an organism's body in vivo:

Using Mouse Mus musculus species, commonly known as BALB/c albino 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 day 63-56 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 was 0.5%. Ten percent was the chosen concentration for both the A. bisporus extract and citrat sodium. 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:

Table (1) Laboratory white mouse distribution with ,coefficients 1 ,2,3,4 5, 6,

Only filtrate *A. flavus* + filtrate *A. bisporus* + filtrate *A. flavus* + Sodium Citrate are treated in transactions involving filterate *A.flavus* and *A. bisporus* treatment and extraction with sodium citrateAn explanation of the material transactions in ,ArcelesGive the mice purified water as their only dosage, and give them 0.5 ml or 30 g of *A. flavus* fungus orally every 48 .hoursA. flavus fungus is dosed orally to the mice, then after a day, *A. bisporus* 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 sodium ,citrateFollowing a 24-hour period, the mice were given an oral dose of *A. flavus* fungus and 0.5 ml / 30 g of sodium citrate every ,day Dosage: 0.5 ml or 30 g of *A. bisporus* 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

Totals	Transactions	Description of parameters and material concentration
1	the control	Treat animals with distilled water only
2	*A. flavus* fungus filtrate	Dosing mice with *A.flavus* filtrate orally every 48 hours: 0.5 ml/30 g per mouse/day.
3	*A.bisporus* fungal filtrate	Dosing mice with *A.bisporus* filtrate orally 48 hours 0.5 ml/30 gm per mouse/day
4	*A.flavus* + *A.bisporus* fungus	Mice were given a dose of *A. flavus* fungal filtrate, followed 24 hours later by a dose of *A. bisporus* filtrate (0.5 ml/30 g each mouse/day).
5	*A.falvus* + sodium citrate	The mice were given a dose of *A. flavus* fungal filtrate, and then they received a dose of sodium citrate + 0.5 ml/30 gm per mouse/day after a 24-hour period.
6	*A.flavus* + *A.bisporus* filtrate Sodium citrate	*A. flavus* fungal filtrate was given to the mice. They received an oral dosage of 0.5 ml/30 g of sodium citrate each mouse day after being dosed with *A. bisporus* filtrate for 24 hours.

Examinations in life chemistry

AST, ALT, ALP, blood urea, and creatinine levels in Serrum 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 the purpose of collecting blood samples directly from the heart. gel tube containing serum was filled with blood in order to investigate how the liver functions inthe 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).

Results and Discussion:

The weight rates of laboratory mice during the dosing period decreased as a result 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 31.20. The average weights decreased by

29.60 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' .weightsHowever, prior to the initiation of the dosage, there was no discernible change. Additionally, prior to the sacrifice, the average weight was 30.40 g. In contrast, the control group's average weight was 25.0 g before dosing, 28.20 g after ten days, and 30.80 g prior to the sacrifice.

There were significant differences in the third group, which took place only due to the scarcity of the commercial mushroom *A.bisporus*. Before dosing, the weight growth rate reached 30.40 g, but after ten days it reached 28.80 g, while before sacrifice, the weight average reached 26.40 gm

This is what contributed, along with what Ibrahim and others (2006) did, to the formation of the fungus *A.bisporus*. , 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

Totals	***Transactions***	***Average weights (g) ± standard error***			**Transaction rate ± standard deviation**
		Before sacrifice	After ten days	Before dosing	
1	the control	25.0 ± 1.58	28.20 ± 1.92	30.80 ± 1.78	28.00 ± 2.95
2	*A. flavus* filtrate	31.20 ±2.56	29.60 ± 2.04	30.40 ± 2.40	30.40± 2.89
3	*A. bisporus* filtrate	30.40 ± 2.60	28.80 ± 2.94	26.40 ± 2.51	28.53 ± 2.66
4	*A. bisporus* + *A.flavus*	24.20 ± 0.83	26.80 ± 1.33	29.20 ± 1.17	26.73 ± 2.25
5	*A. flavus*+ sodium citrate	22.60 ± 1.14	24.40 ± 1.51	26.80 ± 1.30	24.60 ± 2.16
6	*A. bisporus* + *A.flavus* + sodium citrate	26.40 ± 3.36	28.60 ± 3.20	30.80 ± 3.56	28.60 ± 3.64
Average times ± standard deviation		26.63 ± 3.46	27.73 ± 1.87	29.06 ± 2.02	

LSD value	for periods = 1.08	Coefficients = 1.56	for overlap = 2.91	

Examining how *A. flavus* toxins 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 152.0±4.5, GOT 288±3.5, ALP 234.0±7.89 IU/L, respectively) in an extract-based *A. flavus* treatment reached the The liver enzyme test findings (44.0±2.0, 191.0±5.0, 122.0.±4.1 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 table (3) giving *A. bisporus* to lab animals at at the enzymatic levels (ALP 198 ±1.15, GOT 39±1, and GPT 32±1.52) 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 The obtained enzymatic values (GOT 39 ± 1), GPT 32 ± 1.52), and ALP 1.15 ± 198; in the investigation of *A. bisporus* filtrate's impact on lab animals treated with *A. bisporus*, 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.

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 54.0 ±6.1 mg/dl and 1.73 ± 0.3 mg/dl, respectively, and 37.0 ± 3.0 mg/dl and 0.5± 0.1 mg/dl, respectively, for the control group. Between the groups under consideration Table (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 (3):The Filtrate of the poisonous Mushroom A.flavus on the amount of liver enzymes present in mice's blood

Totals	*Transactions*	GOT (IU/L)	GPT (IU/L)	ALP (IU/L)
1	the control	191.0 ± 5.0	44.0 ± 2.0	122.0 ± 4.1
2	*A. flavus* filtrate	288.0 ±3.5	152.0 ± 4.5	234.0 ± 7.89
3	*A. bisporus* filtrate	166.0 ± 4.3	40.0 ± 2.0	100.0 ± 3.51
4	*A. bisporus* + *A.flavus*	176.0± 3.5	62.0 ± 3.0	117.0 ± 7.23
5	*A. flavus*+ sodium citrate	329.0 ± 9.23	261.0 ± 7.0	74.0 ± 3.56
6	*A. bisporus* + *A.flavus* + sodium	318.0 ± 6.0	121.0 ± 5.1	103.0 ± 6.43

	citrate			
Average times ± standard deviation		244.66 ± 131.62	133.33 ± 69.61	125.0 ± 52.63
LSD value		7.63	8.31	4.89
Mean values		< 46 IU/L	< 34 IU/L	< 240 IU/L

Table(4):The Filtrate of the poisonous Mushroom A.flavus blood mice's amount of urea and creatinine

Totals	***Transactions***	**Creatinine mg/dl**	**Blood urea mg/dl**
1	The Control	0.5 ± 0.1	37.0 ± 3.0
2	*A.flavus*	1.73 ±0.3	54.0 ± 6.1
3	*A. bisporus*	0.7 ± 0.12	35.0 ± 2.0
4	*A. bisporus* + *A. flavus*	0.6± 0.09	52.0 ± 3.8
5	*A. flavus*+ sodium citrate	0.7 ± 0.15	37.0 ± 3.9
6	*A. bisporus* + *A. flavus* + sodium citrate	0.65 ± 0.08	48.0 ± 5.7
Average times ± standard deviation		0.813 ± 0.43	44.94 ± 8.66
LSD value		0.381	3.01
Mean values		0.5-0.8 mg/dl	19.0-34.8 mg/dl

Examining the levels of creatinine and urea in albino mouse blood

Regarding the administration of *A. bisporus* 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 citrat sodium, a marginal increase in levels was noted. Regarding the administration of citrat sodium, filtrate *A. flavus*, and filtrate *A. bisporus*, There was no discernible rise in levels; this could be attributed to the filtrate *A. bisporus's* ability to keep renal cells functional after mycotoxin exposure.

histological sections

Liver:

Tissue sections from white mice's livers treated with *A. flavus* filters were examined under a microscope to diagnose the condition. The results (Figure 1) There is central vein with radially arranged cords of hepatocytes. The hepatocytes showed with acidophilic cytoplasm with prominent nuclei. There is disruption of hepatic plates and loss of hepatic cord organization. Severe vaculation and ballooning hepatocytes and hepatocellular degeneration and there are inflammatory cells aggregations. 10X H&E.

KIDNEY:

Figure (1): Kidney section of rat in group (G1)as control group. Note normal arrangement of cortical and medullary elements (high cellularity glomeruli and normal proximal convoluted tubules. 10X H&E.

GROUP 1 (CONTROL GROUP)

LIVER

Figure (1): Liver section of rat in group (G1). There is central vein with radially arranged cords of hepatocytes. The hepatocytes showed with acidophilic

GROUP 2 LIVER

Figure (2): Liver section of rat in group (G2) treated with A. flavus filtrate. Higher magnification, there is vacuolation of the hepatocytes, the cytoplasm showed as coarse, pink with high vacuoles. Also there is severe necrosis of hepatocytes. 40X H&E

GROUP 3

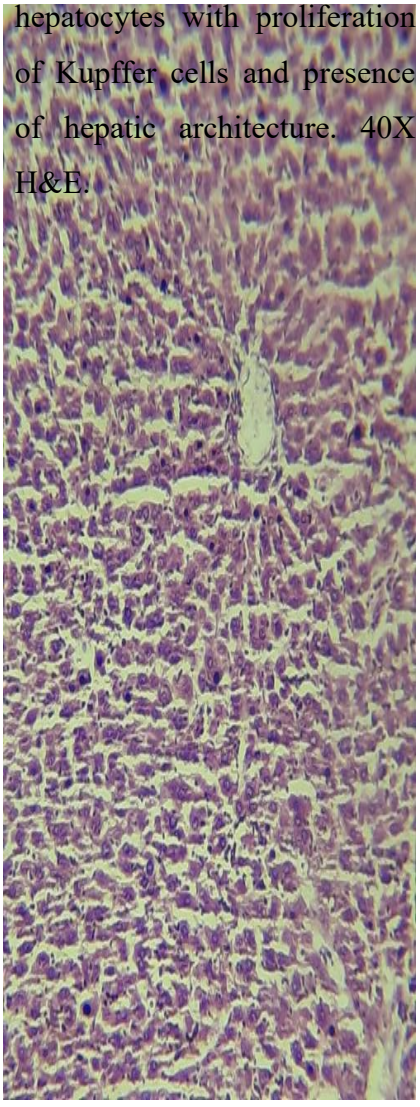
LIVER

Figure (3): Liver section of rat in group (G3) treated with A. bisporus filtrate . There is central vein with radially arranged cords of hepatocytes with dilated sinusoids lined by flat endothelial and Kupffer cells located between them. There are hepatocytes with

GROUP 4

LIVER

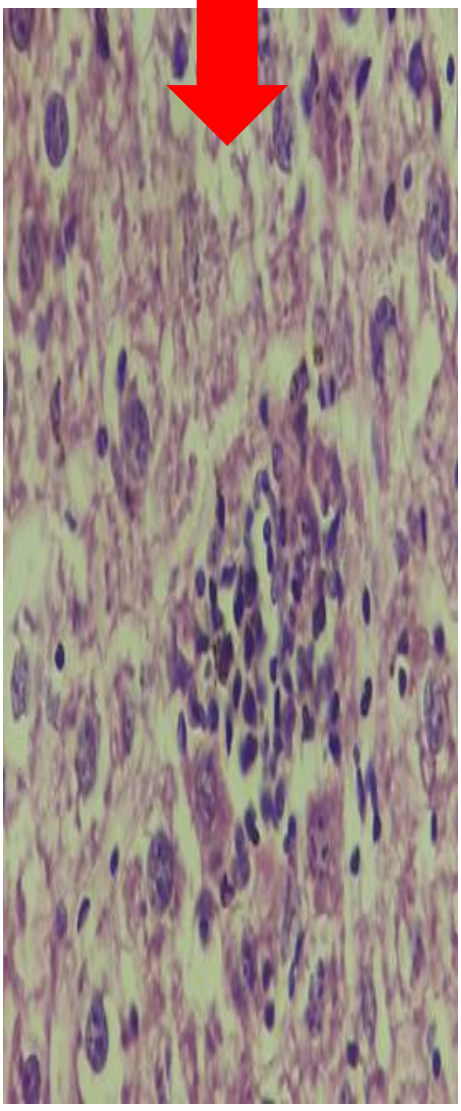
Figure (4): Liver section of rat in group (G4) treated with A. flavus filtrate and A.bisporus filtrate. Note there are normal hepatocytes with proliferation of Kupffer cells and presence of hepatic architecture. 40X H&E.



GROUP 5

LIVER

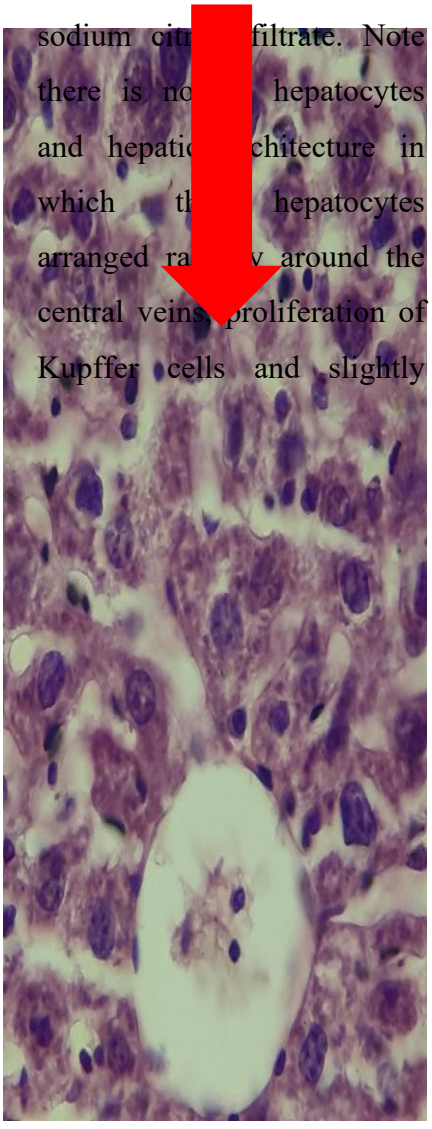
Figure (5): Liver section of rat in group (G5) treated with A. flavus filtrate and sodium citrate filtrate. Note there are

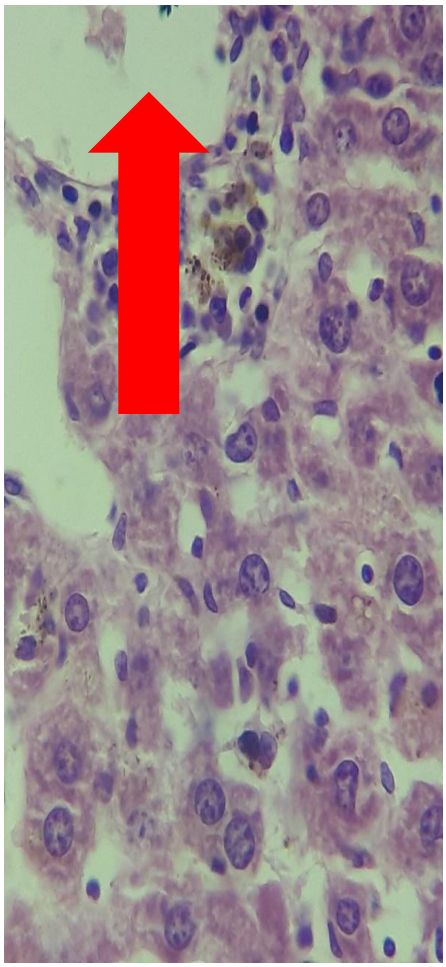


GROUP 6

LIVER

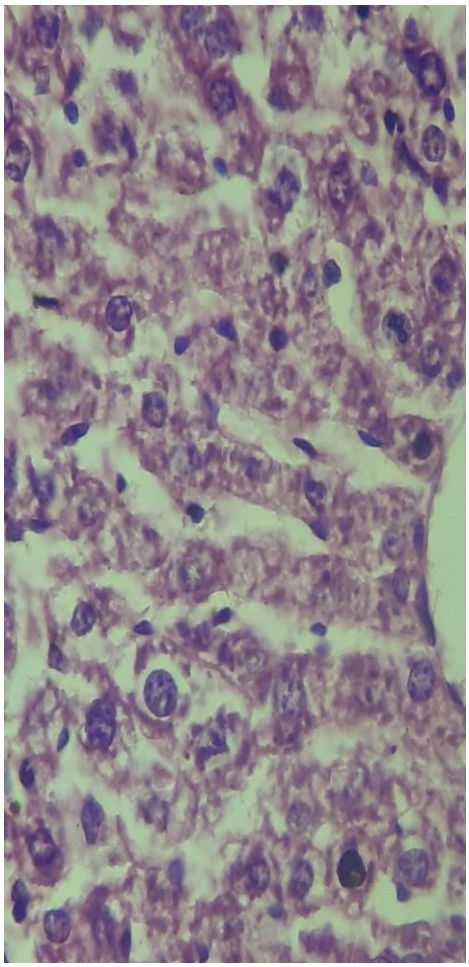
Figure (6): Liver section of rat in group (G6) treated with A. flavus filtrate and A.bisporus filtrates and sodium citrate filtrate. Note there is normal hepatocytes and hepatic architecture in which the hepatocytes arranged radially around the central veins, proliferation of Kupffer cells and slightly





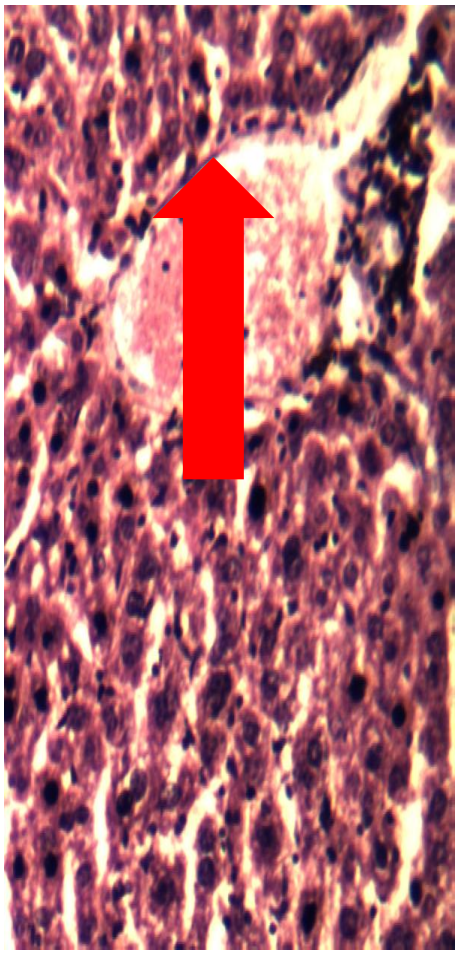
GROUP 1 (CONTROL GROUP)
KIDNEY

Figure (1): Kidney section of rat



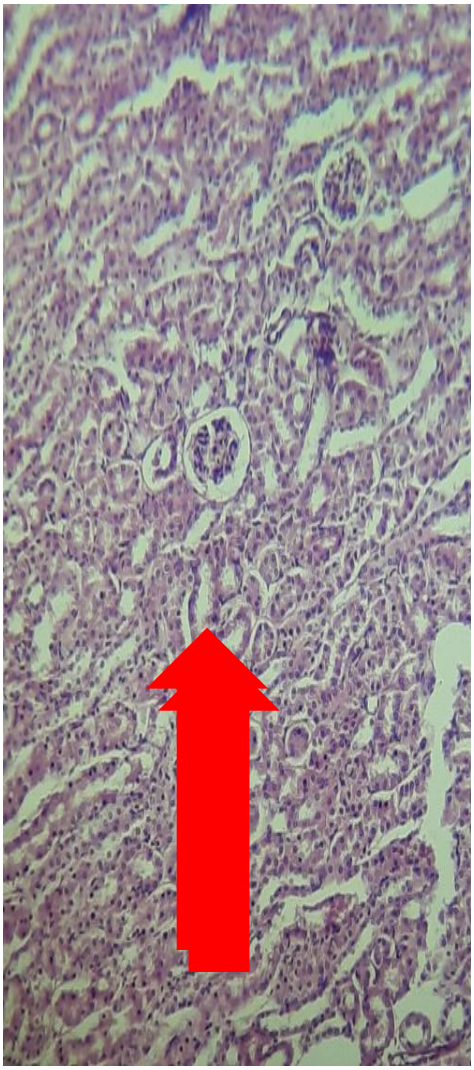
GROUP 2
KIDNEY

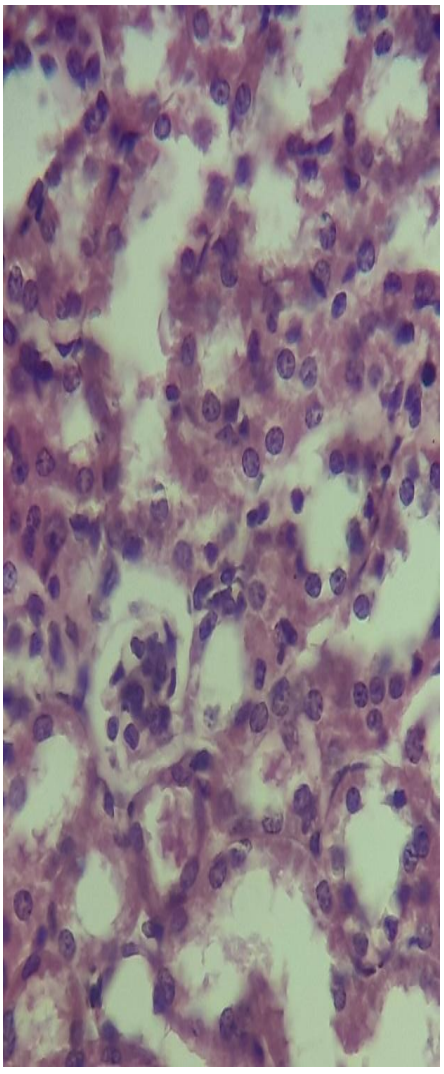
Figure (2): Kidney section of rat in group (G2) treated with A. flavus filtrate .

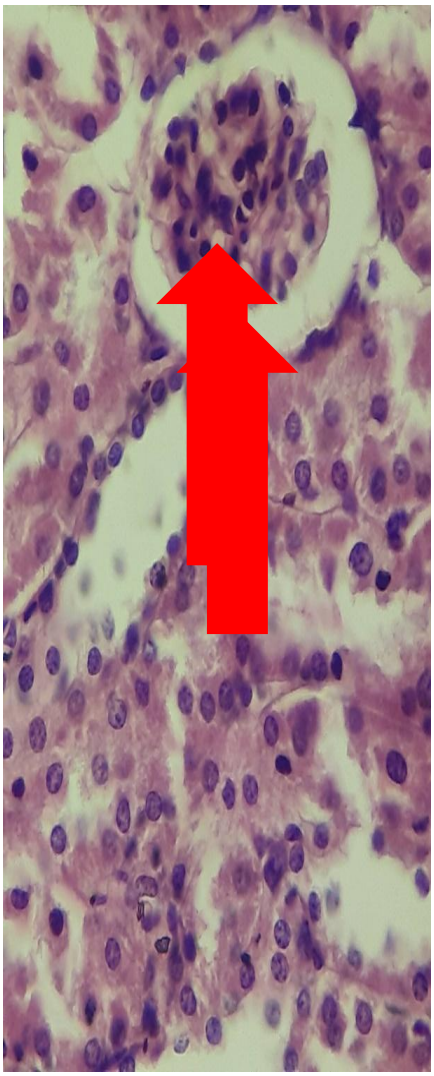


GROUP 3
KIDNEY

Figure (3): Kidney section of rat in group (G3) treated with A. bisporus filtrate. Normal







GROUP 4

KIDNEY

Figure (4): Kidney section of rat in group (G4) treated with A. flavus filtrate and A.bisporus filtrate. Note normal and rounded glomerulus with hyperplasia and regeneration of the renal tubules epithelium. 40X H&E.

GROUP 5

KIDNEY

Figure (5): Kidney section of rat in group (G5) treated with A. flavus filtrate and sodium citrate filtrate. Note atrophied glomerulus and mild necrosis and few sloughing of the epithelial cells of renal convoluted tubules. 40X H&E.

GROUP 6

KIDNEY

Figure (6): Kidney section of rat in group (G6). treated with A. flavus filtrate and A.bisporus filtrates and sodium citrate filtrate. There is regenerative tubular basophilia with normal, organized, and high cellularity glomeruli. 40X H&E.

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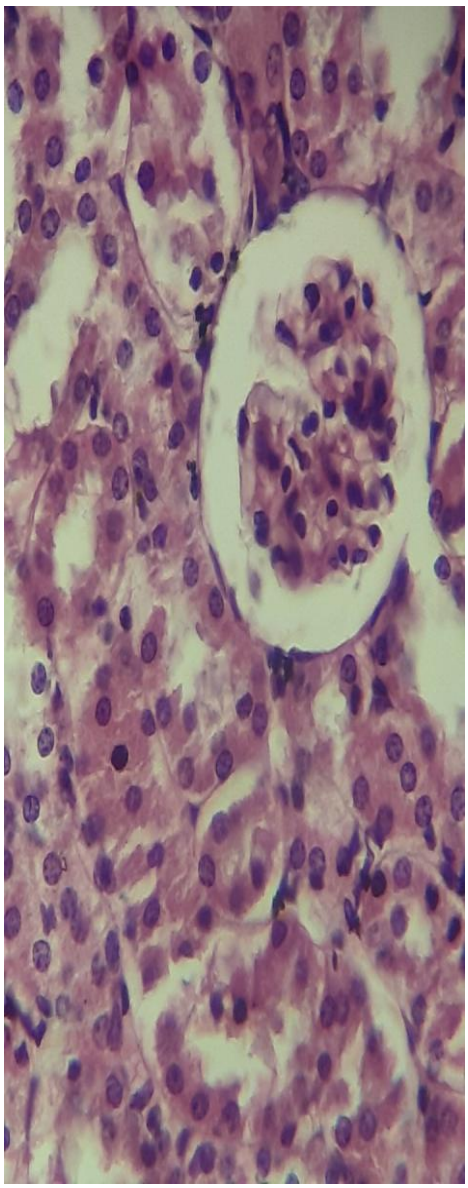

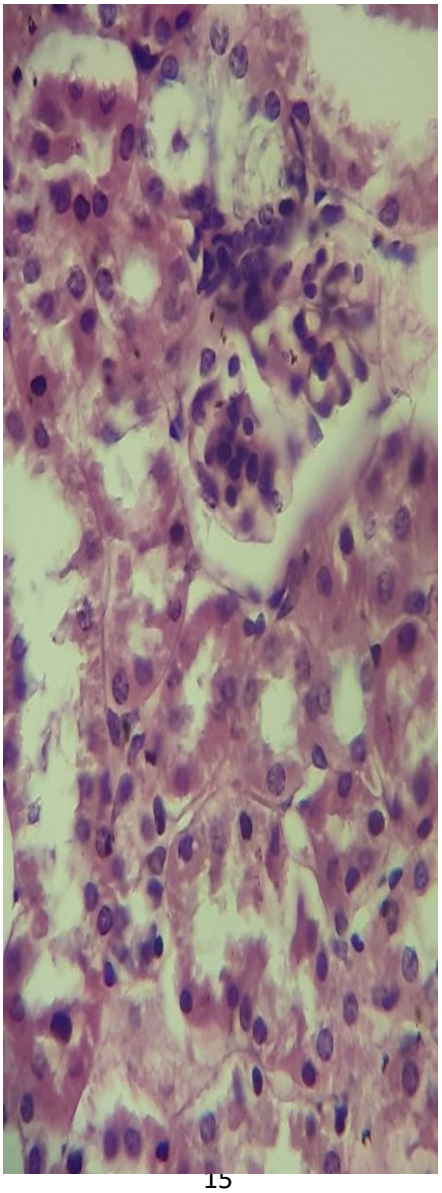

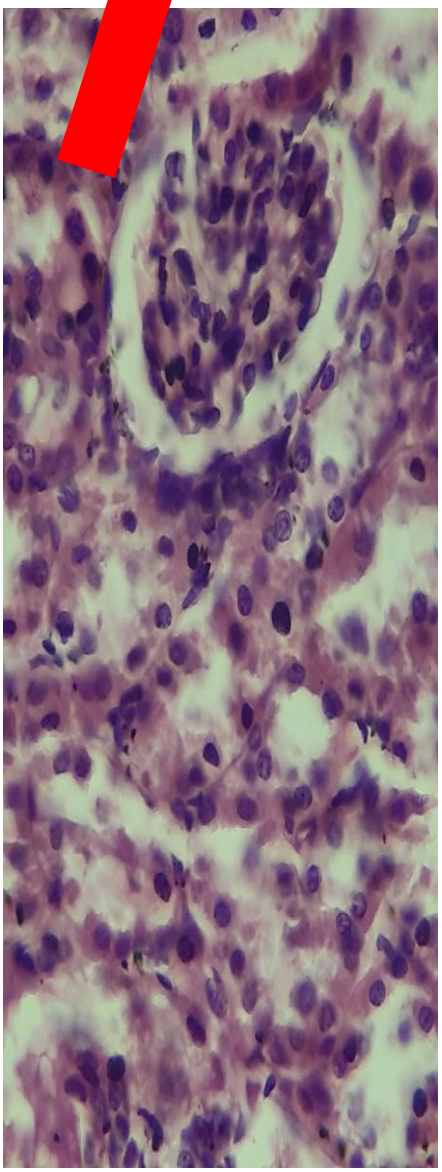

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