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Using *Agaricus bisporus* filtrate and calcium citrate against toxicity of *Aspergillus turcosus* in histological and biochemical changes in albino mice.

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Abstract - This study showed the effect of histopathology in the liver and kidneys of mice caused changes in the weights of the animals after ten days and after the end of the 21-day dosing process and changes in the levels of liver enzymes (GOT, GPT, ALP) where she reached her level (172,92 ,200) U/L respectively by comparing the levels of the control group, which had reached (160 ,54, 138)U/ L respectively. The activity of mycotoxins in the toxin-fed group also caused pathological changes in liver tissue, including -Dilation of blood vessels filled with red blood cells (RBC) . Lab results are shown when kidney function is checked. There are statistically significant differences in the levels of both urea and creatinine in the blood serum of experimental animals treated with pathogenic fungi. It reached a urea level of 51mg/dL and a creatinine ratio of1.19 mg/dL compared to the control group. The group in which urea level reached 31.66mg/dL and creatinine0.42 mg/dL resulted in kidney damage of laboratory animals. which appears in the form of expansion or congestion of the renal tubules and the presence of Bowman capsule with renal tubules that contain albumin (Protein). The results showed the success of the treatments of *A.bisporus* filtrate, calcium citrate, and the interaction of calcium citrate and *A.bisporus* filtrate in this study and their positive and opposite effect on the treatment and restoration of liver and kidney tissues.

Keywords -- Histopathalogy liver, kidney, *Agricus bisporus* , calcium citrate.

Introduction

Mycotoxins have low molecular mass (MW ~ 700 Da) and secondary metabolites produced by *Aspergillus, Penicillium*, and *Fusarium* are highly harmful to animals and humans (7). FAO has been estimated that 25% of the world's crops such as nuts, grains and rice are contaminated with mold and fungal growths, according to a review by the Food and Agriculture Organization of the United Nations and the World Health Organization(WHO)(15). the toxic effect of mycotoxins on animal and human health is referred to as mycotoxins. Exposure to mycotoxins occurs mostly via ingestion but also occurs through the skin and inhalation. The extent of the harmful effects of

mycotoxins on human or animal health depends primarily on the extent of exposure (dose and time period), the type of mycotoxin, physiological and nutritional status, as well as the potential synergistic effects of other chemicals to which animals or humans are exposed (6). Aflatoxin B1 is considered one of the highest toxins in carcinogenic fungi as Aflatoxin B1 can penetrate the cell membrane and bind to its DNA, causing irreversible mutations (12). The chemical nature of aflatoxin makes it a very fat-soluble compound that can be absorbed from sites of exposure such as the digestive and respiratory tracts into the bloodstream where it can move throughout the body (5). Exposure to these carcinogenic mycotoxins can be by eating contaminated food that ends up in the stomach and is absorbed, or by inhaling aflatoxin B1 particles from food contaminated with these mycotoxins Exposure to these toxins is likely to be prevalent in parts of the world where there are poor crop infestation control methods, transportation facilities, and storage facilities; However, it can also spread in societies with high levels of poverty(19)

Methods

A. turcosus toxin in vivo in live laboratory animals

The current study was conducted on in vitro albino mice obtained from the Infertility and Embryology Research Institute, Al-Nahrain University, with similar ages and weights ranging from 20-25 grams. Enter into a special laboratory in the Animal House of the College of Science at Al-Qadisiyah University, in cages, and in each cage (5) animals including control ,Appropriate conditions were created in terms of ventilation, lighting, and nutrition, and they were placed under observation to ensure their safety from diseases before the start of laboratory work, as no (external) disease was recorded. The cage floors were covered with clean sawdust. After exposing them to the sun for several days in order to get rid of the fungus, the cages were taken care of and cleaned by changing the sawdust twice a week for each cage. As for food and water, it was available throughout the period A dilution (0.5%) of the toxin concentration was chosen for *A. turcosus*, while the concentration was 10 mg/ml for calcium citrate filtrate and *A. bisporus*. Laboratory animals were vaccinated as of 3/8/2023 every two days for a period of (21) days (13,3).

Table(1) Distribution of laboratory rats and dosed materials

Group	Description of treatment and concentrations of substances
G.1	Control group was given only water and food
G.2	Oral instillation of *A.turcosus* filtrate in mice every 48 hours, up to 0.5 ml/ 25 g
G.3	Oral instillation of *A.bisporus* filtrate in mice every 48 hours, up to 0.5 ml/ 25 g
G.4	Mice were dosed with *A.turcosus* orally every 24 hours after dosing mice were injected with infiltrated doses of *A. bisporus* up to 0.5/ ml 25 g
G.5	Mice doses of *A. turcosus* orally infiltrate every 24 hours of dosing The dose of calcium citrate is about 0.5ml/25g

ISSN:0975 -3583,0976-2833 VOL14, ISSUE 06, 2023

G.6	Mice with *A.turcosus* orally infiltrated every 24 hours from calcium citrate infiltrates and *A.bisporus* were dosed up to 0.5 ml/ 25g.

Two days after the last dose of rats, where laboratory animals were sacrificed after anesthesia with chloroform, and they were given an intracardiac injection (I.P) of Xylazine and Ketamin at a dose of 5 and 50 mg of kg of weight. body for both drugs, respectively. Then its front and hind limbs are fixed by means of pins in an anatomy dish and its abdomen is opened with sharp scissors at the bottom of the abdominal skin area near the genital tract, and by making a longitudinal incision from the beginning of the posterior region to the anterior region after that the organs (liver and kidneys) of each mouse are extracted They are washed well in a physiological salt solution (Normal saline), after which they are fixed with 10% formalin for the purpose of studying histological changes in them.

chemistry analyses

Blood samples are collected using the method of drawing blood from the heart directly through the heart puncture of the animal and using a syringe with a wine (Needle Gauge23x1/4) where the drawn blood is placed in a tube that is special for the serum called the Jel Tube for the purpose of studying the functions of the liver function by measuring GPT GOT ALP enzymes as well as renal function; Measurement of urea and creatinine .After completing the blood withdrawal process, the samples were placed in a centrifuge for 25 minutes at a rate of (2500) rpm.

Histological study

Histological sections were prepared from the organ that was included in the study, depending on the method described which includes the following:

1. Fixation: Samples were fixed for 24 hours in a 10% formalin solution.

2.Sample Washing: the samples wash with water for two hours and changing the washing water every quarter of an hour to get rid of the excess fixation solution inside, which did not mix with the tissues after the completion of the fixation task.

3.Dehydration: After getting rid of formalin and washing it with water and getting rid of excess water in the tissues, the dehydration process is carried out in an ascending series of ethyl alcohol concentrations as follows: 90% 90% 100% 50% 100% 70% 80% for 1.5_2 hours for each concentration.

4.Removal: To get rid of the remaining ethyl alcohol from the tissue as a result of the dehydration process, liking the samples . In addition to making the tissues more transparent and removing unwanted fat, xylene is used in this process for (1.5-2) an hour each time.

5. Infiltration: The impregnation process was performed completely tissue with melted paraffin wax at a temperature of 58°C. In order to fill the voids in it, the process took place in two stages, an hour for the first stage, while a full night remained. for the second stage.

6. Burial: the samples are buried in special copper molds shaped like the letter (L). After that, the molds are placed on a flat surface to pour the wax molds, marking the molds and determining the direction of the sample. The wax molds are left in the freezer for 24 hours before the cutting process begins.

7 .Slicing: Dies were cut using a rotary microtome with a thickness of 6–7 µm .

8.Homogenization and Loading of Slides: The tissue sections are placed in a water bath at a temperature of 40 °C for the purpose of homogenizing the tissue sections and removing wrinkles and bumps resulting from the cuts. Then, the sections were loaded onto glass slides lightly coated with Mayers albumin to paste , and left on a hot plate for 24 hours at a temperature of 40 degrees until it dries from the water, thus the tissue is ready for manufacture process.

9- Staining: For the purpose of the staining process, the following dyes were used (Haris Hematoxline-Eosine stain)

10.Microscopy: After completing the staining process, the slide was fixed with Canada Balsam, thus the tissue was ready for microscopic examination and imaging.

11.Diagnosis of histological sections

Results and Discussion

Effect of aflatoxin B1 In-vivo

The results presented in Table (2) indicate that there are statistically significant differences between the weights in the second group. No increase was observed in the average weights during the dose period, as it reached 23.33 gm before the dose, and after ten days it was 24 gm. Before the carcass, it reached 25 gm. Compared with the control group, the average weight before the dose was 24.33 g, and after ten days the average weight was 24.66 g, and before the carcass 25 g, while significant differences were observed in the third group that took doses only with a dose of *A. bisporus* filter Before the dose the average weight was 24g. . As for after ten days, it was 25g, while before the carcass, the average weight was 25.33 g. This is consistent with what (9) suggest the presence of A. bispories. An enzyme system that takes part in the metabolic processes of food, so some food components are converted into Fat in the metabolic processes that occur in the organism's body, and in other groups, only a slight increase in weight was observed as a result of the toxic effect of *A. turcosus* fungus. This is consistent with what was reported by (2).

ISSN:0975 -3583,0976-2833 VOL14, ISSUE 06, 2023

Table(2) Effect of *A.turcosus* filtrate in weights of mice

Group	Treatment				
		Before dosing	After 10 days	Before sacrifice	Transaction rate ±standard deviation
G1	Control group	24.33±2.51	24.66±2.08	25±2.64	24.66±2.12
G2	*A.turcosus* filtrate	23.33±2.3	24±2	25±1	23.88±2.31
G3	*A.bisporus* filtrate	24±2.64	25±2	25.33±1.52	24.77±1.92
G4	*A.turcosus*+*A.bisporus* filtrate	26±1	25.66±1.52	25.66±1.52	25.77±1.20
G5	*A.turcosus* filtrate+calcium citrate	22.66±3.05	24±2.64	25±1	23.88±2.31
G6	A.turcosus+calcium citrate+A.bisporus filtrate	25.33±2.51	26±2.64	26±2.64	25.77±2.27
	Time average	24.27±2.34	24.88±1.99	25.33±1.66	24.83±2.01
	L.S.D	1.46	2.07	3.59	

To estimate the effectiveness of the liver enzymes (AST(GOT),ALT(GPT),ALP).

The effect of A. turcosus infiltrate on the level of liver enzymes in the blood of albino mice treated with it(Table3), where it reached a level of (200,92,172 U/ L) respectively, in the treatment of A. turcosus infiltrate only, compared with The control group reached (138,54,160 U/ L), and thus there was a significant increase in the group treated with toxins compared with the control group. The results also showed a significant decrease ($P < 0.05$) in the enzyme levels for the rest of the treatments compared to the second group as shown, and these results are consistent with the study by (1) the effect of mycotoxins, including aflatoxin on liver enzymes, which caused an increase in GPT and GOT enzymes, and the reason for the increase is due to the significant toxic effects of mycotoxins on liver cells and tissues that caused a peek in liver cells containing these enzymes , This leads to their liberation in the blood and an increase in their level in the blood serum. In terms of the effect of commercial mushroom filtration on laboratory animals, the level of enzymes was (98,41.6,283 U/ L), and the enzymes were not affected by filtration much and remained active and represented in the liver by comparisonWith the control group, this is consistent with the results of (14) knowing that any change, whether it is a sharp increase or decrease in the level of these enzymes, is an indication of the presence of a disease in the body of the animal. Also, As for the treatment of *A. turcosus* infiltrates with calcium citrate,

a slight increase in enzymes was observed compared to the treatment of *A. turcosus* infiltrate with *bisporus* infiltrates. The reason may be the control of *A.bisporus* infiltrate in survival hepatocyte activity during mycotoxin exposure.

Table (3) toxic effect of fungus A.turcosus filteratein the levels of liver enzymes in the blood of mice

Group	Treatment	AST U/L	ALT U/L	ALP U/L
G.1	The control	138±1.2	54±1	160±1
G.2	A.turcosus filtrate	200±1	92±1	172±1
G.3	A.bisporus filtrate	98±1	41.6±1.52	283±1
G.4	A.turcosus +A.bisporus filtrate	47±2	49.6±1.52	120.6±1.15
G.5	A.turcosus+calcium citrate	110±1.15	44.6±1.52	284.6±1.52
G.6	A.turcosus+ calcium citrate +A.bisporus filtrate	287±1.15	84±1	226±1.15
LSD 0.05		2.21	2.29	2.01

Determination of urea and creatinine levels in the blood of mice.

Aflatoxin caused a significant increase ($P < 0.05$) in the concentration of both urea and creatine in serum. White rats were treated with this poison, where the urea level was 51 mg/dL and creatine 1.19 mg/dL compared to the control group, where the urea level was 31.66 mg/dL and creatinine 0.42 mg/dL, where after creatine is a compound produced from the breakdown of creatine phosphate found in muscles and skeletal bone thus It appears in the blood and its appearance in the blood is an indication of the performance of the glomerulus because it is responsible for filtering it and changing its level in the blood in some pathological conditions, the most important of which is renal failure (4). As shown in Table (4), which shows the occurrence of significant differences in the levels of urea and creatine between the studied groups, and the reason is due to the effect of the toxin on the kidney tissues and causes atrophy in the kidneys. The phalanges and dilatation of the renal tubules. These results are similar to what was reported in a study (18), in which it was found that the toxin of Aflatoxin , caused an increase in the level of urea and creatinine concentrations in the blood of animals treated with it compared with a control group. 31.6 mg/dl and 0.35 mg/dl, respectively, within normal limits, and indicates the control of these fungi to maintain cell activity in the kidneys, but in the rest of the groups and treatment with oyster mushroom infiltrates or citrates, a significant decrease in urea and creatinine levels was observed compared to group of animals that have been dosed with toxic fungus filters.

ISSN:0975 -3583,0976-2833 VOL14, ISSUE 06, 2023

Table (4)Toxic effect of fungus *A.turcosus* Filterate in the levels of creatinine and urea in the blood of mice

Group	Treatment	Creatinine mg/dl	Blood uera mg/dl
G.1	The control	0.42±0.01	31.66±1.15
G.2	A.turcosus filtrate	1.19±0.005	51±1
G.3	A.bisporus filtrate	0.35±0.01	31.6±1.52
G.4	A.turcosus +A.bisporus filtrate	0.45±0.02	33.6±1.15
G.5	A.turcosus+calcium citrate	0.36±0.02	33.6±1.52
G.6	A.turcosus+ calcium citrate +A.bisporus+ filtrate	0.40±0.01	33.6±2
LSD0.05		0.022	2.58

Histopathological effect of aflatoxin B1 on mice.

1.The effect in liver

The results of the microscopic examination indicated the diagnosis of histological sections taken from the livers of experimental mice treated with a filter of the toxin *A. turcosus*, shown in Figure (1) The presence of clear pathological changes in the cross-section of the liver tissue, including: - Dilation of blood vessels filled with red blood cells (RBC). The reason for the appearance of these symptoms in the liver tissue is the intense effect of aflatoxin B1. When compared to the control group Figure(2) , It is placed when there is no expansion or congestion in the blood vessels, and the vessels are normal, as well as the absence of inflammatory cells, with the presence of large, proportional-shaped hepatocytes with a central nucleus . With regard to the third group of mice that were dosed with a filter of the commercial mushroom *A. bisporus* (Fig.3), If we notice normal liver tissue with proliferation of Kupffer cells with the appearance of the cells in the form of two nucleated nucleated hepatocytes , which is consistent with what (11) indicated about inoculating albino mice with *A. bisporus* infiltrates It does not affect liver tissue this study confirmed the presence of Kupffer cells of normal sizes and numbers, in addition to the proliferation and division of liver cells, so that some of them appeared to contain two nuclei In the group treated with *A. turcosus* infiltrate and commercial mushroom. *A. bisporus* (Fig.4). Large congestion in blood vessels (Dilation of blood vessels) with bleeding and proliferation of Kupffer cells with normal, hexagonal hepatocytes these effects explain the protective role of commercial mushroom filtrate in a way that reduces the toxic effect of *A.turcosus*. In the fifth group, where the rats were dosed from the infiltrates of the poisonous mushroom with calcium citrate (Fig.5) Slight congestion, no inflammatory cells, and most hepatic cells appearing with two nuclei,As for the last treatment, rats were injected with a poisonous mushroom filtrate, commercial mushroom *A. bisporus* filtrate, and calcium citrate shown in (Fig .6) Simple congestion in the blood vessels with proliferation of Kupffer cells with normal hepatocytes, some of which suffer from clear mitoses the appearance of some binucleated cells indicates the repair and

regeneration of damaged cells, as it was found that the percentage of damage was less severe compared to the group that was ingested with mycotoxins alone (16).

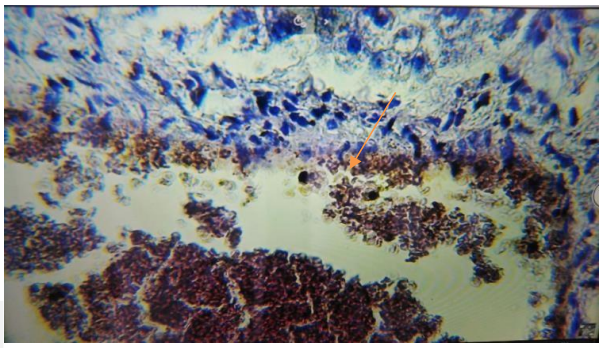


Figure (1) An enlarged cross-section of a mouse liver of a group treated with the poisonous fungus *A.turcosus*: large congestion in the size of the blood vessel, Dilation of blood vessels, and is filled with blood cells RBC red

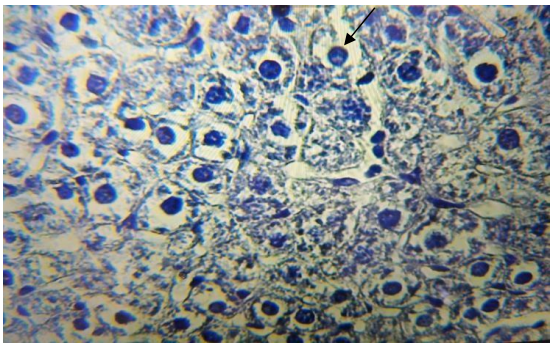


Figure (2) of an enlarged cross-section of a mouse liver of a control group: there is no dilatation or congestion in the blood vessels and the vessels are normal, as well as the absence of inflammatory cells with large hepatocytes of hexagonal shape with a central nucleus

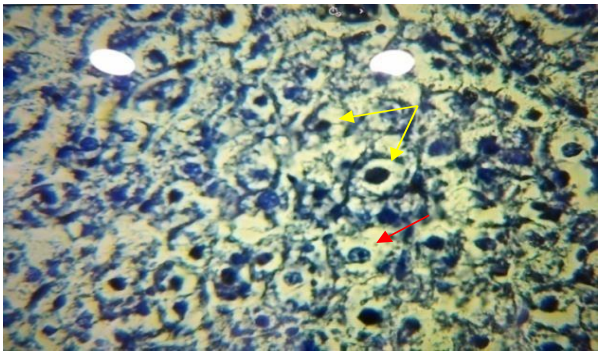


Figure (3) Magnified cross-section of mouse liver of the group treated with commercial mushroom *A. bisporus* infiltrates: Normal liver tissue with proliferation of Kupffer cells cells (yellow color) with the appearance of hepatic cells in a hexagonal shape (red color)

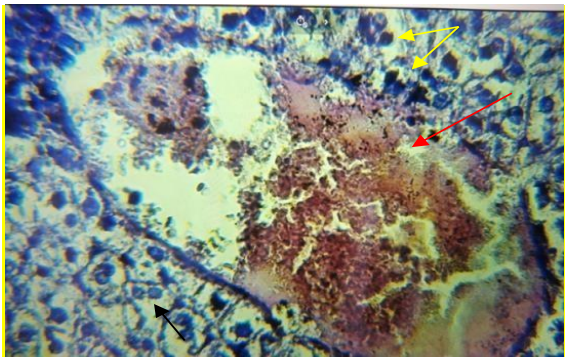


Figure (4) Magnified cross- section of mouse liver of a group treated with infiltrates fungus *A. turcosus* and commercial mushrooms *A. bisporus*: large congestion in blood vessels with (red color)bleeding and proliferation of Kupffer cells in (yellow color) with normal hepatocytes in hexagonal shape in (black color)

ISSN:0975 -3583,0976-2833 VOL14, ISSUE 06, 2023

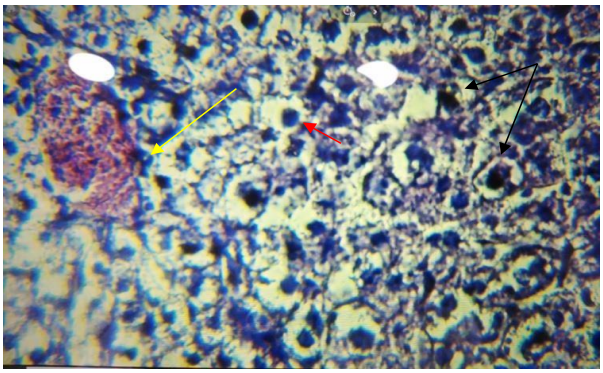


Figure (5) Magnified cross section of a mouse liver for a group treated with A. *turcosus* infiltrates and calcium citrate: There are blood vessels of normal size with slight congestion (yellow color) no presence of inflammatory cells and most liver cells appear with two nuclei, which indicates a process of repair and reproduction of Kupffer cells(Black Color)

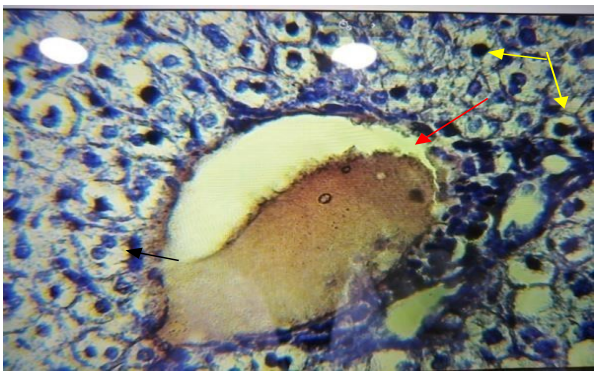


Figure (6) Magnified cross-section of a liver treated with a toxic fungus *A.turcosus* infiltrate and a commercial fungus *A.bisporus* infiltrate With calcium citrate: a slight congestion in the blood vessels a (red color) with a proliferation of Kupffer cells a(yellow color) and the presence of hepatocytes in a binuclear form a (black color).

2.The effect on Kidney

where the showed results diagnostic of histological sections taken from the kidneys on presence of histopathological changes in the group treated with the candidate of the poisonous fungus *A. turcosus* (Fig.7) which appears in the form of expansion or congestion of the renal tubules and the presence of Bowman capsule with renal tubules that contain albumin (Protein) as a result of the effect of the mycotoxin aflatoxin B1, compared to a control group shown in (Fig 8) Which are free from pathological changes in the tissues of the kidneys, where the renal tubules are devoid of albumin (protein) with normal renal tubules and lined with normal cuboidal cellsFigure (9) Which represents the group treated with *A. bisporus* commercial mushroom filtrate the renal tubules are normal, that is, there is no congestion in them and the renal tubules are devoid of albumin (protein), a normal shape of the kidney these results indicate that the resistant filtrate is not toxic when administered to mice and this is consistent with the findings of (10), and that the result of the microscopic examination of the mouse kidney in the group that was treated with the toxic filtrate *A.turcosus* and *A.bisporus* is dilatation of the renal tubules with slight congestion in some regions, as shown in the(fig10) As for the fifth group, which was treated with *A. turcosus* infiltrate and calcium citrate. , as shown in (Fig 11) Slight dilatation of renal tubules, no inflammatory cells, normal appearance of the kidney .In the latter group, treatment with the poisonous fungus *A. turcosus* and the infiltrated commercial mushroom *A.*

*bispo*rus was treated with calcium citrate together (Fig.12). Partial effect in terms of congestion of the renal tubules, and some renal tubules are present with Bruin and some The other is no protein (albumin). indicating the ability of the two treatments to reduce or limit the effect of filtration of toxic fungus in a laboratory animal(17).

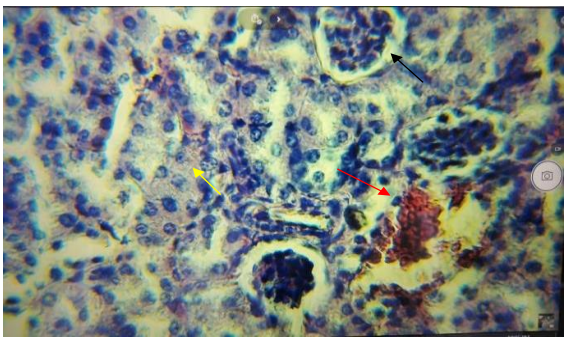


Figure (7) Magnified cross-section of a mouse kidney of a group treated with a toxic fungus *A.turcosus* infiltrate: Expansion or congestion of the renal tubules (red color) presence of Bowman capsule(black color) and renal tubules containing albumin (protein) (yellow color.)

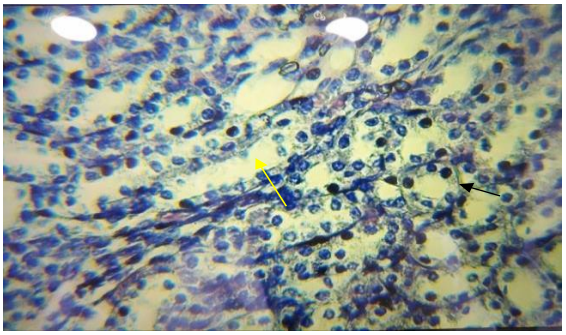


Figure (8) An enlarged cross-section of a mouse kidney for a group Control The renal tubules are free of albumin, i.e. protein, (yellow color), with normal renal tubules lined with normal cuboidal cells, (black color)

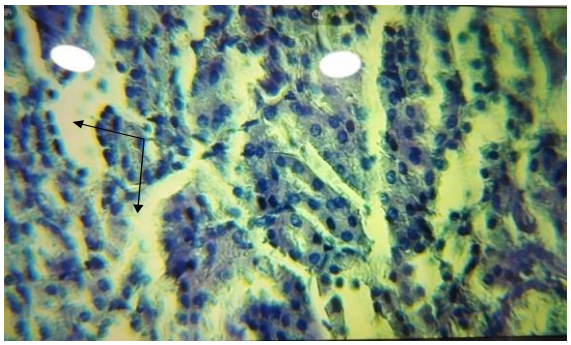


Figure (10) Magnified section of a mouse kidney for a group treated with a toxic fungus *A.turcosus* filter and a commercial fungus *A.bisporus* filter: expansion of the renal tubules with slight congestion in the Some areas .

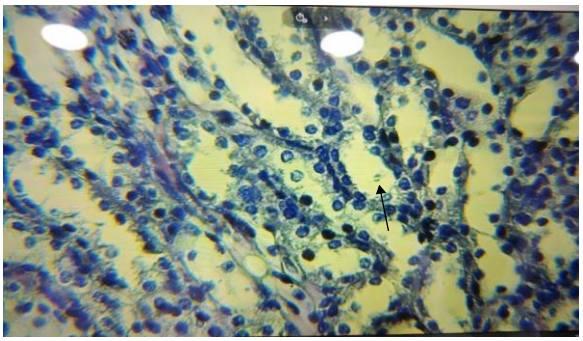
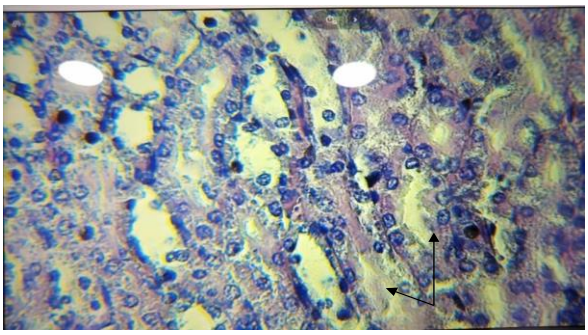


Figure (9) Magnified cross-section of a mouse kidney for a group treated with a commercial fungus *A.bisporus* filter: The renal tubules are normal that is there is no congestion in them, and the renal tubules are devoid of albumin (protein), a normal form of the kidney

ISSN:0975 -3583,0976-2833 VOL14, ISSUE 06, 2023



Figure(12) an enlarged cross-section of a mouse kidney for a group treated with a poisonous mushroom *A. turcosus* filter and a commercial mushroom *A.bisporus* filter with citrate calcium a partial effect in terms of congestion of the renal tubules, and some renal tubules have protein and others do not have albumin protein

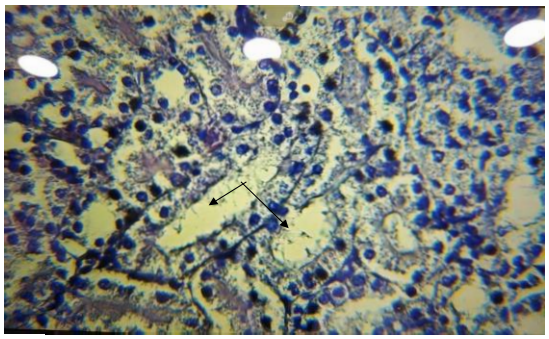


Figure (11) Magnified cross-section of mouse kidney of a group treated with toxic fungus *A. turcosus* and citrate Calcium: Slight dilatation of renal tubules and no cells Inflammatory is a normal form of the kidney.

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