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The possibility of controlling on Aspergillus *terreus* produciny Aflatoxine isolation from some Chips local and imporet *pleurotus ostereatus* plauate and Sodium bicarbonate

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Abstract---After study HPLC technique showed that the highest concentration of Aflatoxin B1 toxin was due to the filterate of Aspergillus *terreus* isolate of the chips sample, which amounted to 193.9 ppb, and this isolate was phenotypically diagnosed using taxonomic keys as well as molecularly diagnosed by polymerase chain reaction (PCR) technique in addition to base sequence analysis phylogenetic tree analysis for the purpose of comparing the isolate of the contaminated fungus A.*terreus* with the isolates previously registered in the National Center for Biotechnology Information (NCBI*). P.ostreatus* filtrate and sodium bicarbonate proved their inhibitory ability for both radial growth and dry weight of the contaminated fungus, This study show effect toxin inside histo pathology of liver and kidney in mice.

Keywords---Histopathalogy liver, kidney, pleurotus ostereatus, sodium bicarbonate.

Introduction

The Mycotoxin are hydrocarbon chemical metabolic compounds with low molecular weights around (100_797) Daltons that have special genes for the production of toxins when appropriate conditions are provided of temperature around (20_30 C) and humidity of (13-18) (12) and the difference in composition is attributed Its chemical properties vary according to the damages and effects it causes on the host, including neurological disorders, some are carcinogenic and

mutagenic, as well as immunosuppressive and tissue damage, and they have strong effects on fetuses and placenta with carcinogenic activity in women (14). 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 (7).

Digestion that occurs in the human digestive system, where it resists high temperatures, as it does not have a great effectiveness in breaking down, as some toxins do not break down except at temperatures at 250 degrees Celsius (1) The International Agency for Research on Cancer has classified a number of these toxins as human carcinogens and have mutagenic activity at low concentrations (15) in a study indicated by (8). Increasing rates of genetic mutations in DNA as the toxin affects cells If a person increases from colon cancer at concentrations 1m, 25m, it can also be toxic, mutagenic, interfering with hormonal and immune functions. Toxins are generally toxic to cells where they interfere with the physiological processes of nervous system cells: digestive Pulmonary, genital, as well as opportunistic cutaneous infections (11) and include the internal organs represented by the heart, liver, spleen, kidneys, lungs and others.

The main target organ is the liver more than the rest of the organs, so hepatic necrosis occurs in those lobules. Most field studies on the effect of toxins on human health were conducted on the laboratory animal represented by the white mouse to confirm the effect of these toxins .Toxins are cumulative as a result of continuous exposure to toxins and occur when the poison is swallowed or after ingesting the poison with food as a result of absorption through the intestines and its arrival in the blood, then the liver and kidneys within approximately 60 minutes of ingestion as a result of interaction with the protein of the liver cell, so there is a defect in the work of liver enzymes in addition to Damage to these cells (the liver) as a result of the severity of the poison, the liver may succeed in getting rid of these toxins by converting them into less toxic compounds, as well as the exit of their products through excretion, urine or milk, while another procedure accumulates in the fatty organs represented by glands under the skin and in the muscles, so the danger lies

Materials and Methods

The A. terreus toxin inside the body of the organism In vivo laboratory animals

To study the effect of the fungus A. terreus toxin filterate on animal tissues, which is one of the important biological methods. For this experiment, white albino mice were used with a number of 30 mice, their ages ranged from 8-9 weeks, with an average weight of 30-40 g, after which it was distributed to six groups, including the control group, which was given water and food only for the duration of the study. Appropriate conditions of light and temperature were provided, and the floor was covered with sawdust after exposure to the sun for the purpose of getting rid of the fungi contaminating it, and 0.5% dilution was chosen. Of the poison concentration of A. terreus, as for sodium bicarbonate and

the filter of resistant mushrooms, the concentration was chosen 10%, taking into account the lethal dose of half the number LD50.The dose was started 6/2/2022 orally for 21 days.

The weights of the laboratory animals were measured before starting the experiment, during the experiment, after completion, and for the purpose of knowing or. Follow-up the effect of mycotoxins on the weights of laboratory animals during the experimental period

Table 1
Laboratory animals treatments

Group	Description of Treatment and concentration of substances
G. 1	The control group was given water and food only
G.2	Oral instillation of A. terreus infiltrates into mice every 48 hours, up to 0.5 ml / 30 g
G. 3	Oral instillation of P. ostreaus infiltrates into mice for 48 hours, up to 0.5 ml / 30 g
G.4	Mice dosed with infiltrates of A. terreus orally every 24 hours after dosing Mice were dosed with infiltrates of P. ostreaus up to 0.5 ml/30 g
G. 5	Mice dosed with A. terreus infiltrates orally every 24 hours of the dosing dose with sodium bicarbonate about 0.5 ml / 30 g
G.6	Mice dosed with A. terreus orally infiltrate every 24 hours of dosing with sodium bicarbonate and P. ostreaus infiltrate up to 0.5 ml / 30 g

After the end of the dosing period and two days after the last dose, the laboratory animals were sacrificed, using chloroform, and explained through an opening in the abdominal cavity, after blood was drawn directly from the heart by stabbing the heart using a medical syringe. Centrifugation at a speed of 3000 revolutions per minute and for a period of 10 minutes, the purpose of which is to obtain the serum, to study the functions of the liver, including AST, ALT, and ALP enzymes, as well as the renal function, including urea and creatinine (Blood urea creatinine).

Life chemistry exams

Calculation of the levels of liver enzymes AST, ALT, ALP and Blood urea, Creatinine in Serrum using the R Krey device of Japanese origin Very short, the serum is placed in the place designated for it inside the device, and after the test is completed, the results appear in the form of paper tapes for about 15 minutes.

Preparation of histological slides

After the blood was drawn, the Livers and Kidney organs were collected after washing them with normal saline, and the organs were fixed by placing them in 10% diluted formalin, for the purpose of preparing tissue sections.

- Fixation: Implant the organs for 24 hours in dilute formalin solution
- Washing the samples: the tissue sections were washed from formalin with water for two hours for the purpose of dispose of the solution

- Dehydration: The dehydration process is carried out in an ascending series of concentrations of Ethyl alcohol 100, 90, 80, 70% for a period of two hours in each concentration.
- Clearing: the samples are thinned by placing them with xylene, and the purpose is to make the tissue more transparent.
- And remove unwanted fat for 1.5-2 hours each time
- Infiltration: The filtration process was carried out for the purpose of saturating the tissue with a degree of melted paraffin wax.
- At 58 °C for a quarter of an hour, the samples are left inside the wax for two hours or more
- Embedding: The samples are embed in L-shaped copper molds.
- The molds are placed on a flat surface for pouring the wax molds, marking and defining the direction of the sample, and the molds are left
- To freeze up to 24 hours before cutting work
- Sectioning The cutting process for the molds is done by a Rotary Microtome device with a thickness of 6-7 micrometers.
- Homogenizing the slides and loading them: the tissue sections are placed in a water bath at a temperature of 40 °C for the purpose of
- Homogenize the tissue sections and remove the bends resulting from the cutting, then load the sections onto the glass slides
- Coated with a light smear of Mayer s albumin for the purpose of mounting, and leave
- On a hot plate for 24 hours at a temperature of 40 °C to dry it from the water after which the fabric is ready for the process
- Staining For the purpose of the dyeing process, the following dyes were used (Haris Hematoxline- Eosine stain)
- Microscopic examination: After the dyeing process was completed, the slides were fixed with Canada Balsam, then the tissue became
- Ready for examination and imaging
- Diagnosis of tissue sections.

Results and Discussion

Measuring the weights of white mice during the treatment that exposure to the fungal filtrate *A.terreus* led to a decrease in the weight rates of laboratory mice during the dosing period. The results shown in Table No. (2) showed that there were differences between the weights. In the second group, the average weight before dosing was 34.33. A decrease in the average weights in the period of dosing, ie after ten days, amounted to 32.66 g. The significant differences were observed in the weights of the animals after ten days and before sacrificing the laboratory animal, while there was no significant difference before the start of the dosing. And before the sacrifice, the average weight was 31.0 g, and compared to the control group, which averaged 29.66 g before dosing and after ten days, the average weight was 32.33 g and before the sacrifice was 33. 33 g.

These results agree with what was mentioned (6) and it is noted that there are Significant differences in the third group that were dosed only with *p.ostreaus* mushroom infiltrates. Before dosing, the average weight was 33 g, while after ten days it was 33.33 g, while before the sacrifice, the average weight was 33.66 g,

that p.ostreasr mushrooms have an enzymatic system that is involved in the metabolic processes, which leads to the conversion of some food components into fats from the metabolic processes that take place inside the body of the organism, and a very small decrease was observed in the other groups as a result of treatment with filtrate of poisonous mushrooms, and this is consistent with another study that showed that rats dosed With AFLB1 mycotoxin, it has an effect on the weights of laboratory animals and their renal and hepatotoxicity (Hepato- and nephrotoxicity

Table 2
Measuring the white length weights during the treatment

Group	Treatment	Average weight of mice (gm)			
		before dosing	After 10 days	before the sacrifice	Transaction rate +- standard deviation
G.1	control group	29.66+1.15	32.33+0.57	33.33+1.15	31.77+1.85
G.2	A. *terreus*Filtrate	34.33+1.15	32.66+0.57	31+0	32.66+1.58
G.3	*p.ostreaus*Filtrate		33.33+0.57	33.66+1.15	33.33+0.70
G.4	A.*terreus* +*p.ostreus*filtrate	32.33+0.57	32.83+1.04	33+1	32.72+0.83
G.5	A.*terreus*FiIltrate+ sodium bicarbonate	31.66+0.57	32+2	34.66+0.57	32.77+1.78
G.6	A.*terreus*++ sodium bicarbonate *p.ostreaus*filtrate	36+2	36.66+0.57	36+2	36.22+1.09
	Time average	0.94	0.67	1.64	
	L.S. D	0.67	1.64	0.94	

Estimation of the activity of liver enzymes ALP, ALT (GPT), AST (GOT)

The results of the table (3) showed the effect of aflatoxin toxin on the level of liver enzymes in the blood of white mice treated with this toxin, as it reached its level in the treatment of mushroom infiltrate, respectively, compared with the control group if the enzyme level reached (50.33, 31, 120) compared with the control group (91, 35,175, which led to a clear and significant decrease in the group treated with this toxin compared to the control group. The reasons for the decrease are attributed to the damage and change in tissues, which leads to necrosis and damage to liver cells and blood congestion to the liver tissue, as well as infiltration of white blood cells due to the metabolic products of the fungus that secretes aflatoxin. Affect the lipids of the plasma membrane, and thus there is a defect in the membrane permeability, so the membrane loses its optional property.

The reason for the decrease in GPT and GOT enzymes is due to the great toxic effects of mycotoxins present in his tissues and liver cells, which caused decomposition in hepatocytes containing these enzymes, which led to a lack of The release of liver enzymes in the blood serum As for the effect of p.ostreaus mushroom filtrate on the level of enzymes in the blood of laboratory animals, it

was (77.3) 3,31.,156) and these enzymes were not significantly affected by the p.ostreaus mushroom filtrate and remained on their representation and activity in the liver compared to the control group and this is consistent with what was reached (13), and the level of enzymes in the other treatment groups of the poisonous fungus A. terreus with Sodium bicarbonate or p.ostreaus mushroom filtrate recorded a significant (P<0.05) decrease compared to the group treated with toxic mushroom filtrate. As any change in the level of these enzymes, whether it is a sharp decrease or increase, indicates the presence of a pathological condition in the animal's body

Table 3
The Filtrate of the poisonous Mushroom A.terreus on the level of liver enzymes in the blood of mice

Group	Treatment	GOT	GPT	ALP
G.1	the control	175.6+3.78	35.66+1.15	91+2.88
G.2	A. *terreus* filtrate	120+2	31+1	50.33+8.32
G.3	*p.ostreaus* filtrate	156.3+4.61	31.33+4.93	77.33+0.57
G.4	A.*terreus* +*p.ostreaus*filtrate	252+5.29	153.33+4.93	80+4.93
G.5	A.*terreus*+sodium bicarbonate	117+5.29	16+2.64	67.66+1
G.6	A.*terreus*+ sodium bicarbonate +*p.ostreaus*filtrate	136.3+4.16	46.66+0.57	63.33+1.73
LSD0.05		7.72	5.54	7.48

Determination of the level of urea and creatinine in the blood of albino mice

The aflatoxin toxin caused a significant (P<0.05) increase in the level of urea and creatinine concentration in the blood of white mice treated with this toxin, as the urea level reached 49 mg/dl and the creatinine ratio was 1.75 mg/dl compatred with the control group, whose urea level was 40 mg/dl and creatinine 0.3 mg. / dl, and the reason is due to the effect of aflatoxins on the work of cytochrome cp450, which is secreted from the cells of the liver and kidneys, and therefore it is a complex that causes deformations in the tissue cells and increases the level of urea and creatine. Release from the muscles and decrease in excretion from the kidneys (17) Creatine is a compound that results from the breakdown of creatine phosphate present in the muscles and skeletal system, and therefore appears in the blood. Some of the pathological conditions, most notably renal failure (3), which is shown in the table (4) which shows the occurrence of significant differences in the levels of urea and creatinine between the studied groups, due to the reasons B, in this to the effect of the poison on the tissues of the kidneys, which results in atrophy of the glomerulus and expansion of the renal tubules, which in turn indicates a defect in the functions of the kidneys, leading to a decrease in the process of excreting toxic substances outside the body, thus leading to kidney failure and a rise in the level of urea (5). These results are close to what was stated in the study (9), where it was found that the mycotoxin of aflatoxins causes an increase in the concentration of urea and creatinine in the blood of treated animals compared to the control group. dl 27.4 and 0.50 mg/dl, respectively, within the limits of normal ratios, and this indicates the control of this fungus in maintaining the activity of cells in the kidney, while in the rest of the groups treated with p.ostreaus mushroom filtrate or bicarbonate, a significant

decrease was observed in the levels of urea and creatinine compared to the group of animals treated with filtrate. poisonous mushrooms.

Table 4
The Filtrate of the poisonous Mushroom A.terreus on the level ofcCreatinineand urea in the blood mice

Group	Treatment	Blood uera	Creatinine
G.1	the control	40.22+0.38	0.33+0.15
G.2	A. *terreus*filtrate	49.4+0.25	1.75+0.20
G.3	*p.ostreaus*filtrate	27.4+1.25	0.50+o
G.4	A.t*erreu*s + *p.ostreaus*filtrate	36.5+0.81	1.40+0.36
G.5	A.*terreus*+ sodium bicarbonatefiltreate	44.41+1.42	1.73+0.15
G.6	A.*terreus*++ sodium bicarbonate+ *p.ostreaus*filtrate	38.72+0.95	1.50+0
LSD0.05		1.71	0.338

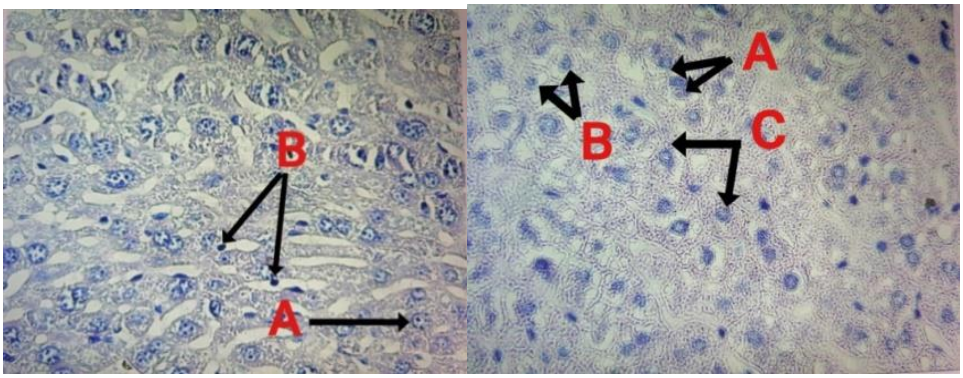
Liver

A.terreus filtrate is a strong carcinogen and causes liver tumors in laboratory mice. The microscopy examination demonstrated the diagnosis of tissue sections taken from the livers of laboratory mice treated with infiltrates of the poisonous fungus A. terreus in the form of (2) the presence of clear histopathological changes, including: Infiltration of hepatocytes, especially macrophage cells (macrophages). The vaculation of hepatocytes, which occurs due to a disturbance in protein metabolism, and then the accumulation of water inside the hepatocytes, as well as some of the cells suffer from clear necrosis, where they appear devoid of nuclei, and others appear to contain binucleated hepatocytes. and this study showed the effect of *A.terreusfiltrate* on liver and kidney tissues of orally dosed male mice. The appearance of these symptoms in the liver tissue is caused by the severe effect of aflatoxins.

The liver is the largest gland and comes in the second place in terms of complexity after the brain, the largest internal organ and the most vital organ, as it secretes metabolic products and removes toxins from various compounds, and the loss of the liver's basic functions leads to death after a short period of time (10). In comparison with the second group, which represents the control group (1) where the hepatocytes appear without any changes and are of natural shapes and arranged radially, and the hepatic cords are clear from the central vein lined with squamous cells, and the hepatocytes appear large with a hexagonal shape and a central nucleus. With regard to the third group of mice that were instilled with infiltrates of mushroom *P. ostreaus* (Fig.3), we notice the hepatic cords formed from the arrangement of hepatocytes radially from the central vein. Which is consistent with what was stated by (13) that feeding white mice with infiltrate of mushroom *P. ostreaus* does not affect the liver tissue. This study confirmed the presence of cover cells of normal sizes and numbers, in addition to the proliferation and division of liver cells so that some of them appear containing two nuclei.

In the group treated with infiltrate of poisonous fungus *A. terreus* and infiltrate of *P. ostreatus* (the presence of hepatic cords from the central vein and moderate

rupture of those cells, some of them show binucleated hepatocutes and others are heapgonal shape with a central nucleus) Clear and cells arranged radially around the central vein with very slight necrosis of some of these cells, and proliferation of Kupffer cells, these effects explain the protective role of *p.ostreausmushroom* infiltrate in a way that reduces the toxic effect of A. With sodium bicarbonate (form) it was observed that some hepatocytes appear binuclear, and this indicates repair in the hepatic tissue. Hepatocytes appear radially arranged around the central vein, and clear ruptures in hepatocytes with slight necrosis of some of them, and a slight infiltration of inflammatory cells macrophages appears in the form of a tumor lesion early granulomatous lesion, and proliferation of Kupffer cells, as for the last treatment in which mice were dosed The filtrate of toxic mushrooms and *p.ostreaus* mushrooms with sodium bicarbonate are shown in(Fig6), in which the hepatocytes appear with a normal shape and a clear, central nucleus and radially arranged forming hepatic chordates around the central vein. Proliferation of Kupffer cells and a slight expansion of the hepatic sinusoids. The appearance of some binuclear cells indicates repair and regeneration of damaged cells, as it turns out that the percentage of damage is less severe compared to the group that was dosed with mycotoxin alon



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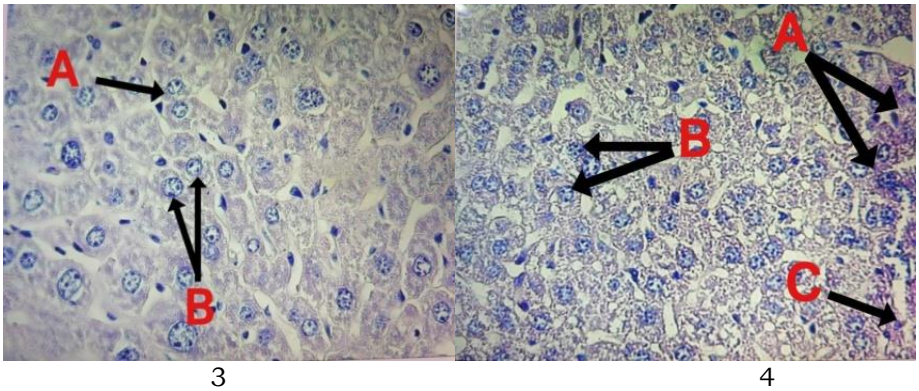

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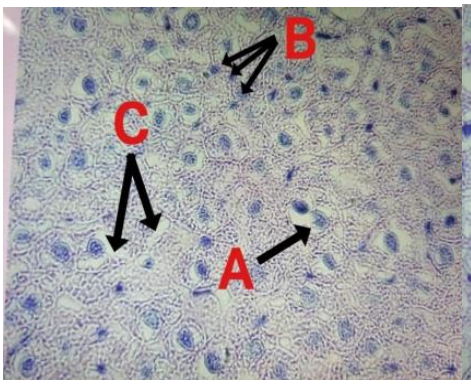

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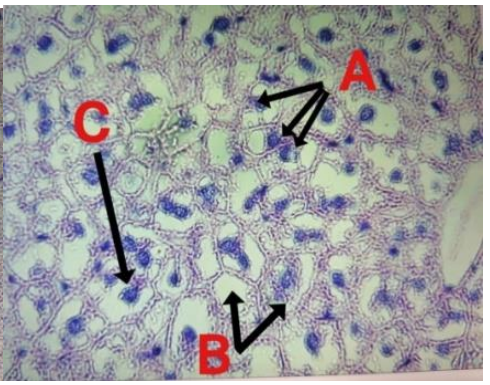

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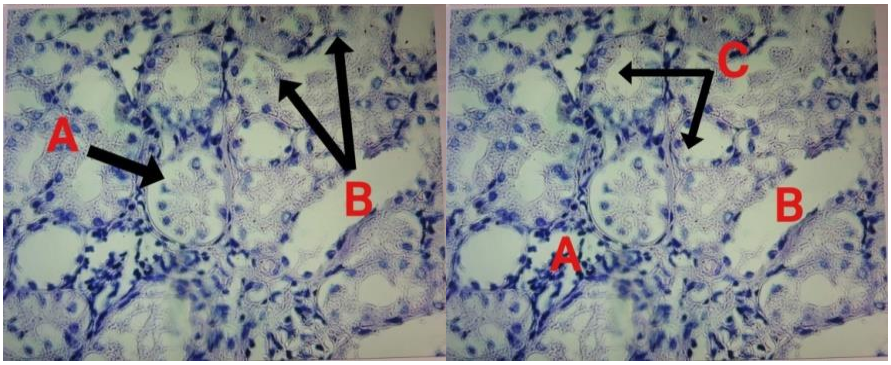
Kidney

The results of the diagnosis of tissue sections taken from the kidneys of laboratory mice treated with a filter of the poisonous fungus *A. terreus* (Figure (2) revealed the presence of histopathological changes which appear in the form of a dense infiltration of inflammatory cells within the renal tissue with congestion in the blood vessels and the renal glomerulus is slightly atrophied with an expansion of the convoluted renal tubules Renal convoluted tubules, and shedding of cells lining these tubules, and these changes occur as a result of the effect of *A.terreus* filtrate on renal cells and tissues, in comparison with the control group shown in Figure (1), which is free from pathological changes in the kidney tissue, where the glomeruli appear medium and large and the renal convoluted tubules are normal Glomerulars and lined with normal epithelium (cubic cells).

As for the group treated with infiltrate of mushroom *P.ostreatus* shown in the figure (4) the glomerulus appears large and round, and the convoluted renal tubules appear elongated with obvious hyperplasia in some of them as they are lined with normal cubic cells. For those mice, The result of microscopic examination of the kidneys of mice in the group treated with the infiltrate of toxic *A. terreus* and the infiltrate of mushroom *P.ostreatus* indicates slight hemorrhage in the renal tissue. The glomerulus appears rich in high cellularity, dilated and large cells. In addition, hyperplasia is evident in the cells lining the tubules with necrosis of a small number of them and the occurrence of bloody bleeding in some areas as shown in the figure (3), while in the fifth group treated with the infiltrate of the poisonous fungus *A. terreus* and sodium bicarbonate shown in the figure (5), which shows necrosis Of the cells, a clear expansion of the renal convoluted tubules is noted with hyperplasia of the cells lining the tubules, and some of these cells suffer from degeneration and shedding within the endothelium. The results of the infiltrates of the poisonous fungus *A. terreus* and the infiltrate of mushroom *P.ostreatus* and sodium bicarbonate (Fig.6) indicate the presence of a large and round glomerulus. The glomerulus appears surrounded by narrow twisted renal tubules lined with normal cuboidal cells. A clear hyperplasia of these cells is noted from the above. The results of the intervention treatment indicate the ability of Leachate of *p.ostreaus* mushrooms when used with sodium bicarbonate on reducing the effect of toxic leachate of mushrooms on tissues of laboratory animals.



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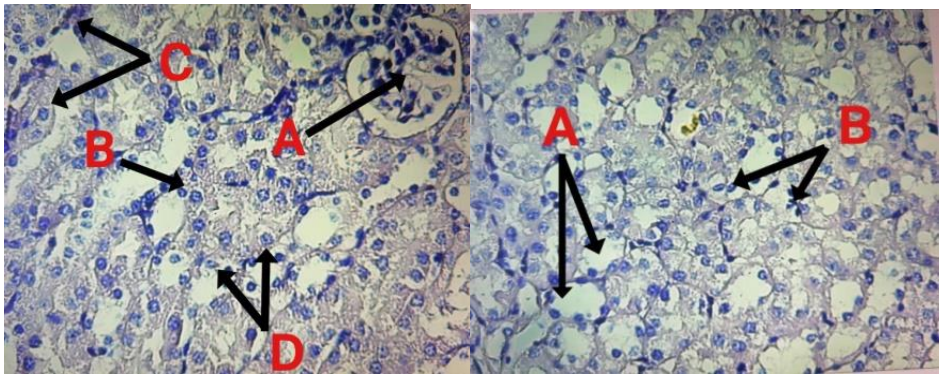

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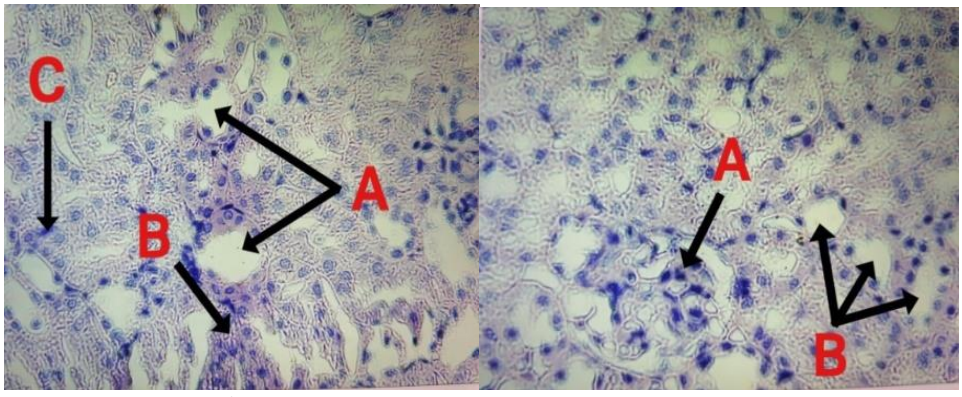

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References

Alghasham,A.A.(2013).Cucurbitacin –apromising target for cancer therapy International journal of health science .7(1).pp:77-89.

AL-Jumay,Sami Abdul –Ridha Ali.(2014),Mycotoxin.printing and publishing-Iraq,p.32.

Bamanikar, S.A;Bamanikar, A. A. andArora, A. (2016). Study of serum urea and creatinine in diabetic and non-diabetic patients in in a tertiary teaching hospital. Journal of Medical Research, 2(1), 12-15.

Carlson D. B. ;Williams D.E.; Spitsbergen J. M.; Ross P. F.; Bacon C.W.; Meredith F.I.and Riley R.T.(2001). Fumonisin B1 promotes aflatoxin B1 and N-methyl-N'-nitrosoguanidineinitiated liver tumors in rainbow trout, Toxicol. Appl. Pharmacol., 172, 29–36

Guyton, A. (1986).Text book of medical physiology. 7thEd. W.B. SaunndersCompany

JavedT.;BennettG.A.;RichardJ.L.;Dombrink-Kurtzman M.A.;CoteL. M. and Buck W.A. (1992).Mortality in broiler chicks on feed amended with a Fusarium proliferatum culture or with purified fumonisin B1 and F.moniliformin, Abst. 106th AOAC Int. Ann. Mtg. Aug 31–Sept. 3, 1992, Cincinnati, OH, p. 232.

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

Mohammed ,S.W.(2015). Detection of Fusarium Species Producing Fumonisin B1 on Maize Using Molecular Methods and Study Its Histopathological Effects on Albino Mice. PhD thesis. College of Science for Women –Baghdad University

of fungal allergy.Int Arch Allergy Immunol . , 145(1) :58 -86.

Ozougwa, J. C. andEyo, J.E. (2014). Hepatoprotective effects of Allium cepa (onion) extracts against paracetamol–induced liver damage

Pedersen,N,B.,P.A.MardhT.Hallberg,N.Josson,M.,Radic,B.,Lucie,A.andpavlovic,M(1999).Toxic effect of mycotoxic in human .Bulletin of the Word Health Organiza

Reproductive health effects of aflatoxins: a review of the literature.

Reproductive Toxicology, 9: 262-270

Schrader,T.J.,Cherry,W.,Soper, K.,Langlois,I.,(2006).Further examination of the effects of nitrosylation on Alternaria alternate mycotoxin mutagenicity in vitro. Mutation Research.606.61-71

Shuaib, F.M.; Ehiri, J.; Abdullahi, A.; Williams, J.H. and Jolly, P.E. (2010)

Simon –Nobbe,B.;Denk,U.;Poll,V.;Rid,R. and Breitenbach,M.(2008) The spectrum

the role of liver transplantation .Amri. J. Med. 3(2): 25 – 29

Timoz,SulafHamid.(2015).Molecular diagnosis of protstreaus mushroom and effectiveness of some industrial waste in its productivity andits ability totreasury.Doctoral thesis of college of EducationQadisiyah .University.

Wang, H. and Zhang. X. (2000). Recent progress in the study of common Fusarium mycotoxon apoptosis of cells. Wei Sheng Yan Jiu. 29(3):181-193

Yassein,S&Zghair,Z.R.(2012).Study ofToxicityand pathogenicity ofAflatoxinB1and G1inMice .Al-Anbar j,2(1),23-31