Histopathologic changes in liver and kidney tissues induce by fungal toxins and treated by filtered fungi Agaricus bisporus and Sodium bicarbonate

Noor Jasim Mohammed
Al-Qadisiyah University/College of Science/Diwaniyah/Iraq, scie.bio.ph.20.16@qu.edu.iq

A. S. Saadon
Al-Qadisiyah University/College of Science/Diwaniyah/Iraq

Abstract

The study has the selection of isolates of Aspergillus niger fungus and examining their ability to produce the Ochratoxin A and defining the toxic effects on some of the anatomic properties of the females of white rat. Moreover assessing the ability of filtered fungi Agaricus bisporus in reducing its toxicity, and when testing the ability of isolates of the fungus that is examined to produce the Ochratoxin A by using of the technology High Performance Liquid Chromatography (HPLC).The results of the histological study for the animal of the white rat that is treated with the Ochratoxin A where the tissue sections that have been taken from the liver shown the existence of pathological effects like a sever congestion in the tissues of the liver and loss of the radiative arrangement of the liver cells and an expansion in the liver pockets and breeding of the kupffer cells , while the kidney has been affected by the Ochratoxin A and the effect is represented in atrophy of the glomeruli and an expansion of the renal tubule twisted with (Degredation) and alienation in the cells of lining the renal tubules twisted Ochratoxin A with the existence of slight changes of the tissue in the organs of the rats treated with it. In the tissues of the liver and kidney, the results of test have shown and the laboratory diagnosis for the tissue sections for the mentioned organs that there is no occurrence of change of pathological tissue in the treatment of filtered fungi Agaricus bisporus and sodium bicarbonate and it is the symmetry of the tissue sections of the comparison treatment that referred to few pathological changes.

The effect of Ochratoxin A on levels of liver enzymes (ALT, AST, ALP) with levels of (44.57, 39.52 , 36.54) U/L respectively compared to control group (12.42 , 15.25 , 12.25)

U/L respectively The result of laboratory studay of the kidney function showed significant differences in the concentration of urea and creatinine rat tested (62.53) mg/dl and creatinine (1.852)mg/dl compared to control group (33.55)mg/dl and (0.621)mg/dl respectively. The result showed the effect of treatments Agaricus bisporus filtarate and sodium carbonate on liver and kidney tissues.

Keywords: *Aspergillus niger, Agaricus bisporus, Ochratoxin A, Sodium bicarbonate.*

INTRODUCTION

The pollution of soil and dried is one of the problems that threatens many of the developing countries that lacks the circumstances of the provide good environment for people. The majority of people eat small quantities of mycotoxins in their diet and do not show clear symptoms of disease, but when eating food that contains high concentrations of toxins or exposure to them by inhaling toxic fungal spores, it can cause serious health problems, including kidney dysfunction and tissue

damage liver tissue and nervous system disorders (Bhat and Vasanthi, 2003).

The immune system is unable to distinguish and detect mycotoxins because of their low molecular weight, which leads to their accumulation in the tissues of certain organs such as the liver, kidney and spleen (Bennett, 1987; Bennett and Klich, 2003). Mycotoxins are also among the strongest known toxins, and small concentrations of them amount to less than (10ppm) cause dangerous diseases because they are not affected It is not damaged by the high temperatures used in cooking. In this study, HPLC technology was used to detect ochratoxin A toxins, and Agaricus bisporus were used as a biological control agent, due to the increase in scientific and clinical interests towards the use of this Agaricus bisporus, which is edible and is a valuable source of biologically active compounds with potential therapeutic effects. It is natural, less expensive, and has few side effects. Agaricus bisporus show their efficiency against many diseases, and their therapeutic effects are emphasized through complex and multiple cellular and molecular procedures (Wasser, 2011).

Sodium bicarbonate was used as a chemical control agent in order to increase the pH, and the use of sodium bicarbonate would be the best and possible option because it is safe and does not cause any health risks (Lee et al., 2021).

Materials and Methods:

Use in this study, filtered fungi Agaricus bisporus and sodium bicarbonate. This is to control the fungus A.niger pollutant of road soil and dried fruits, in addition to diagnosing the isolated fungus by PCR technique.

Examining the ability of the isolates of the fungus A.niger on producing Ochratoxin A Developing the Isolates of the Fungus A.niger. It has been creating Petri dishes contain the medium (PDA) where isolate the fungus tablets A.niger with an average diameter 5 ml. planted at a rate of three repeaters for each isolate and put these tablets in the petri dishes centers then incubated at a temperature of 28C ^{o} for a period of ten days.

Extracting Ochratoxin A, Extract the Ochratoxin A according to (Macdonald and others, 1999) identifying the Ochratoxin A.

The technology of High Performance Liquid Chromatography to detect the fungus isolates A.niger that are able to produce Ochratoxin A (OTA) and according to (Sobolev and Dorner, 2002).

Experimental design:

Potions were prepared and a dilution was chosen %0.5 from the filtrate of the poisonous fungus, taking into consideration the lethal dose for half of the number LD50 and according to what was mentioned (Mohammed et al., 2017).

When studying the determination of the toxicity of ochratoxin A on female albino mice, as for sodium bicarbonate and Agaricus bisporus filtrate, the concentration was chosen %10 to find out the effect of the two treatments and interaction of it on the laboratory animal and the lowest concentrations.

Laboratory animals began to be dosed every two days for a period of 21 days as indicated in the table(1):

Table (1): Description of coefficient and material treatment

Grouping	Description of coefficients and material concentration
G.1	Only water and diet were given to the control group

G.2	Mice were dosed with fungus filtrate *A.niger* orally every 48 hours at a rate of 0.5 ml/25 gm
G.3	Mice were dosed with fungus filtrate *Agaricus bisporus* orally every 48 hours at a rate of 0.5 ml/25 gm
G.4	The rats were dosed with a fungus filter *A.niger* and after 24 hours of dosing, they were dosed with a fungus filter *Agaricusbisporus* at a rate of 0.5 ml/25 gm
G.5	Mice were dosed with a fungus filtrate *A.niger* and after 24 hoursthey were dosed with sodium bicarbonate at a rate of 0.5 ml/25 gm
G.6	Mice were dosed with a fungus filtrate *A.niger* and 24 hours afterdosing, they were dosed with a fungus filtrate *Agaricus bisporus* and sodium bicarbonate orally at a rate of 0.5 ml/25 gm

Then, the laboratory animals were sacrificed two days after the last dose, after anesthesia with chloroform, and they were excised through an opening in the abdominal cavity.

Histological Test:

1- Formaldehyde (buffered form)

To prepare 10% formalin solution, add 100 ml of formaldehyde 37% to 900 ml of distilled water and dissolve 6.5 g of sodium phosphate monobasic and 4 g of sodium phosphate dibasic salt in solution and PH (7-7.5). Formalin 5% was prepared by diluting the 10% formalin in distilled water in 1:1 ratio.

2- Hematoxylin- Eosin Staining

2.1 Hematoxylin:

Dissolve 1 g of Hematoxylin powder Merck in 100 ml of absolute ethanol and consider it as solution one.14.5 g dissolve ferric chloride 29%in 50 ml distilled water and mix 4 ml of this solution with 95 ml of distilled water and 1 ml of hydrochloric acid 37% and is considered as solution two. Then mix the solution one and two and apply for six days in direct sunlight to be coated.

2.2 Preparation of Eosin Solution

Dissolve 1 g of Eosin powder (Merck) in 100 ml of distilled water and serve as a solution for eosin storage. 1 gram of fluxion dissolved in 100 ml distilled water and considered as a fluxion storage solution. To prepare a working solution, 23 ml of eosin-containing solution with 2.3 ml of fluxion and 176 ml of alcohol 96 and 0.9 ml Acetic acid glacial was mixed.

3- Tissue Sampling

To isolate the ovary, the experimental and control female rats were sacrificed and the ovary were fixed for use in tissue processing in 10% formalin buffer.

3.1 Tissue Fixation

Each piece of tissue was placed in a small capsule with a corresponding volume of 10% formalin buffer. After 24 hours, a better fixation of the fixate solution tissue was changed. After 72 hours, the Fixation formalin solution was replaced with 5% and stored in the refrigerator for tissue processing.

4- Dehydration

After fixing the sample in Formalin, the samples were placed in alcohol with increasing degrees. The volume of alcohol was 50 to 100 times higher than the volume of the tissue.

No.	Alcohol concentration	Times
1	**Alcohol 70%**	**100 minutes**
2	**Alcohol 80%**	**55 minutes**

3	**Alcohol 90%**	**55 minutes**
4	**Alcohol 96%**	**55 minutes**
5	**Alcohol 100%**	**110 minutes**
6	**Alcohol 100%**	**110 minutes**

5- Clearing

A texture that contains alcohol cannot be mixed with paraffin and it is necessary to remove alcohol and replace it with xylene that can be exchanged with paraffin.

No.	Xylene concentration	Times
1	**Xylene ı**	**35 minutes**
2	**Xylene ıı**	**35 minutes**
3	**Xylene ııı**	**35 minutes**

6- Infiltration

After clearing the samples were infiltrated by paraffin:

No.	Concentration	Times
1	**Xylene-paraffin**	**30 minutes**
2	**Paraffin ı**	**30 minutes**
3	**Paraffin ıı**	**120 minutes**
4	**Paraffin ııı**	**120 minutes**

7- Blocking Out

To do this, select the metal molds called Leuchkarts L pieces in a suitable size and use a paraffin bath where molten paraffin 58 ° C and melted. Immediately, the samples were placed vertically inward. Paraffin content should be 25 to 50 times higher than the volume of the tissue sample.

8- Cutting

To prepare a better cut, the sample levels in the paraffin block were reduced using a sharp blade. The blocks were transferred to the refrigerator for 30 minutes, and then placed on a rotating microtome after cooling. Finally, 5 micrometers were cut into sequential slices.

9- Fixing the Slices on the Slides

In order to remove wrinkles, the cut pieces containing the tissue samples were pulled and gently floated in a distilled water bath at 40 ° C. In order to better adhere the slices to the lamina, a few drops of the albumin glue were also poured into the water bath. After 2 to 3 minutes, the slices were placed on a clean slur and was placed to dry in the mist.

10- Staining

The lamina prepared at the end of the cross-sectional phase will show the tissue-forming cells. But due to the solidity of the background, cells, cytoplasm and cellular nucleus, their diagnosis will be difficult. Therefore, by employing color combinations and creating an optical contrast, it is possible to separate the cell boundary and determine the position and appearance of the cell nucleus in the cytoplasm. In this study, staining method was used for dyeing of tissues using H & D (Hematoxylin-Eosin method). In this method, the nucleus is violet and the cytoplasm becomes pink. The connective fibers are also pale to pale in their own form. The main points for staining and paraffin embedding are as follows:

A- Deparaffinization

No.	Concentration	Times
1	**Xylene ı**	**1 minutes**
2	**Xylene ıı**	**1 minutes**

Lamellas that are prepared in the cross-sectional stage have tissues filled with paraffin inside and around them. Therefore, in order to replace color solutions, paraffin should be

removed from the inside and outside of the tissues, so that the cut is completely transparent.

B- Watering

No.	Concentration	Times
1	Alcohol 100	2-3 minutes
2	Alcohol 96%	2-3 minutes
3	Alcohol 80%	2-3 minutes
4	Alcohol 70%	2-3 minutes

To replace the colored solutions, it is necessary to return the water collected in the tissue passage to the tissue. In this regard, the water-solubility regime begins with absolute alcohol.

C- Staining

No.	Stain	Times
1	Hematoxylin	10-15 minutes
2	running water	5 minutes
3	Alcoholic acid	3-2 seconds
4	running water	10 minutes
6	Eosin	10 minutes

D- Dehydration

No.	Concentration	Times
1	Alcohol 70%	2-3 minutes
2	Alcohol 80%	2-3 minutes
3	Alcohol 96%	2-3 minutes
4	Alcohol 100%	2-3 minutes
5	Alcohol 100%	2-3 minutes

E- Clearing

Due to the fact that xylene has a stable composition in relation to alcohol, it will preserve the quality of the lamellas for a long time.

No.	Concentration	Times
1	Xylene ı	2-3 minutes
2	Xyleneıı	2-3 minutes
3	Xylene ıı	2-3 minutes

11- Optical Microscope Studies

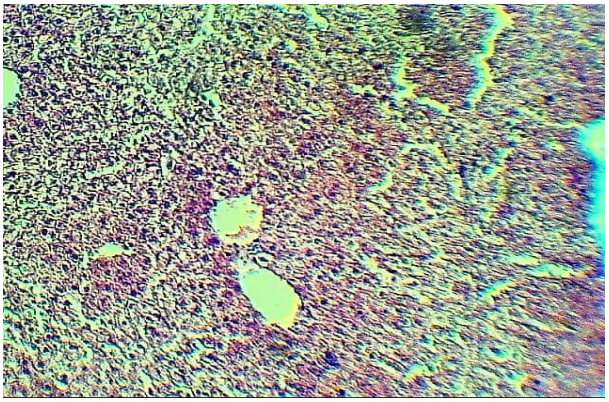
It should be noted that in each treatment group, 5 slides were prepared from each ovary, and 10 slams were taken on each slide at different levels of tissue and each section was examined under consideration were considered.

The Results and Discussion:

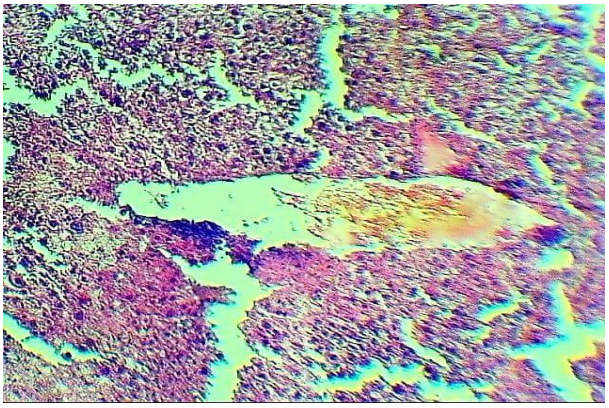
The Histological Study:

The results of the microscopical test for the tissue sections that was taken from the organs like liver , kidney for the white female rats that was treated with ochratoxin A fig(2), The presence of a large clot in the liver tissue with severe degeneration and necrosis of the liver cells with the loss of the radial arrangement of the cells around the central vein, and there is an enlargement of the top of the hepatocytes and congestion of the central vein, as for the tissues of the kidneys, it was noted that there is noticeable atrophy in the glomeruli with severe necrosis and erosion in the epithelial line of the renal coiled tubules, with expansion in the lumen of these tubes. There is also bleeding in the tissues of the kidneys. As in the pictures below, it shows the damage to the tissues, as well as the effectiveness of sodium bicarbonate and Agaricus bisporus filtrate in reducing the damage of the poison.

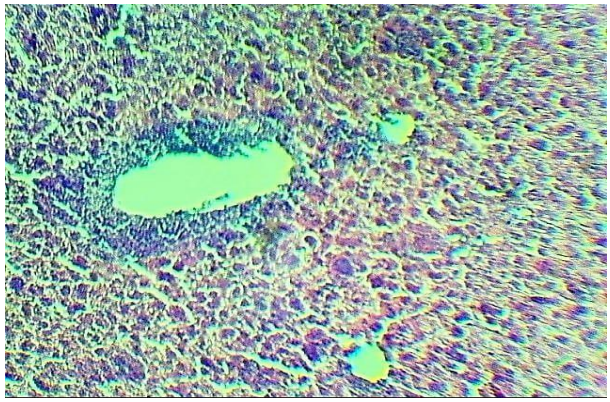
Fig(1) The control group showed normal hepatocytes, the cells were arranged in a hexagonal radial shape around the central vein, and the bile ducts appeared normal



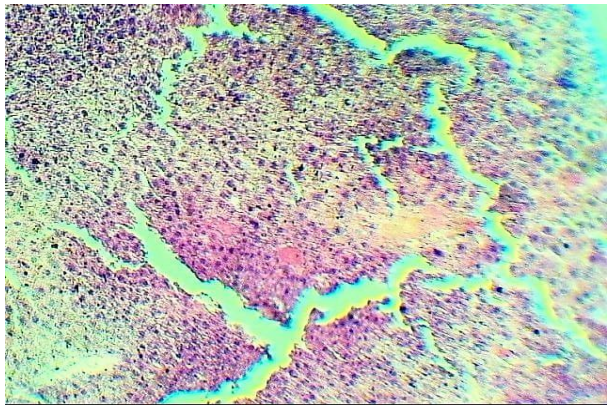
Fig(2) The group treated with ochratoxin A showed a large clot in the liver tissue with congestion of the central vein



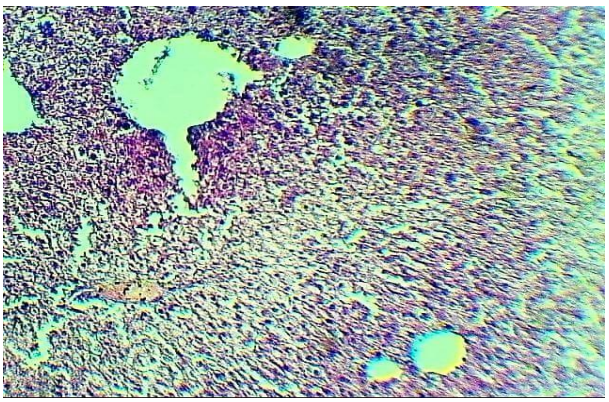
Fig(3) No change was observed in the histological examination of the group treated with Agaricus fungus filtrate, as the hepatic cells appear normal, the nucleus is clear in central location, and the bile duct is normal.



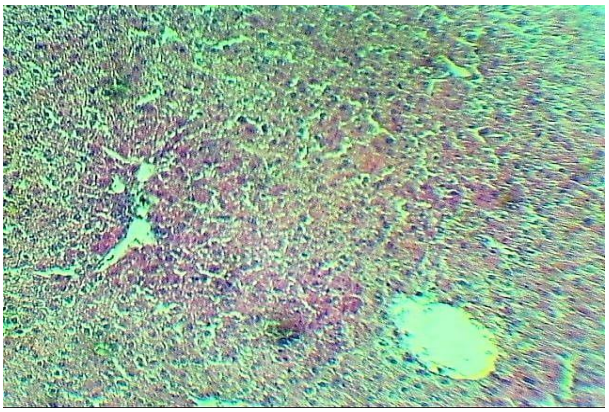
Fig(4) The group treated with sodium bicarbonate showed congestion in the liver cells with slight infiltration of the cells, while the bile ducts appeared normal.



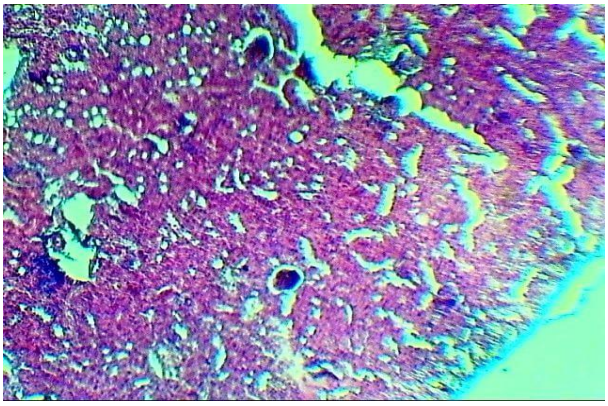
Fig(5) The group treated with Agaricus fungus filtrate and toxic fungus filtrate showed proliferation of kupffer cells with slight histopathy of bile duct cells, normal radial arrangement of hepatocytes around the central vein, and fat accumulation on some hepatocytes



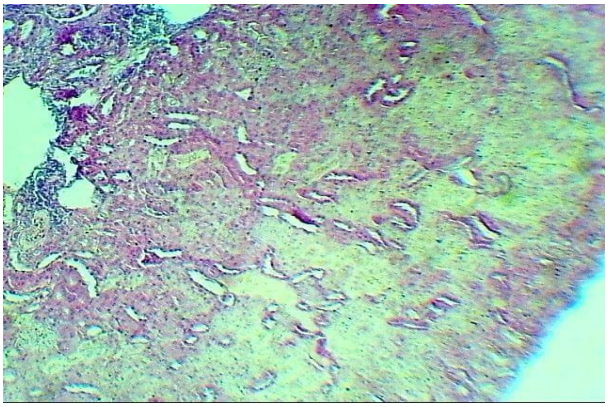
Fig(6) Shows the synergistic effect of Agaricus fungus filtrate and sodium bicarbonate in reducing the effect of the toxin, where the hepatocytes appear normal with clear centrally located nuclei, as well as the presence of normal radial arrangement of hepatocytes around the central vein with a slight infiltration of cells and proliferation of kupffer cells



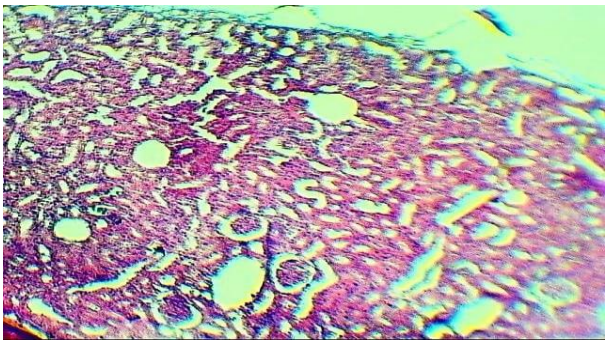
Fig(7) The renal tissue appears in the control group and the convoluted renal tubules are normal, as they are lined with normal epithelial cells and the glomerulus appears medium and large



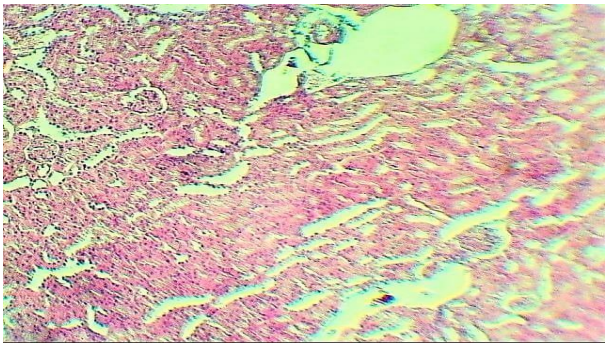
Fig(8) The picture shows the group treated with the filtrate of the poisonous mushroom, where there is a heavy infiltration of the macrophage cells inside the renal tissue with the expansion of the convoluted renal tubules and the shedding of the cells lining these tubules



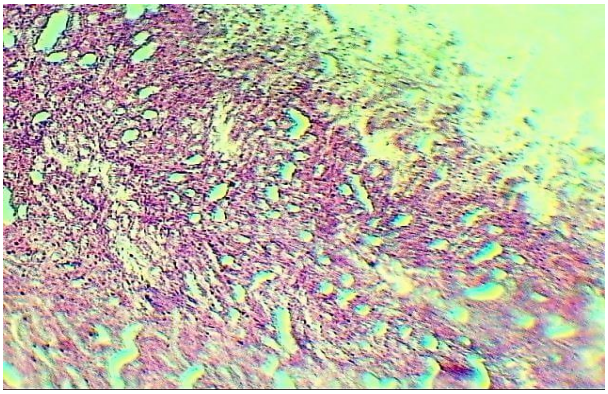
Fig(9) Convoluted tubules appear normally and are lined with cuboidal cells, as well as the glomeruli are large, round, and normal



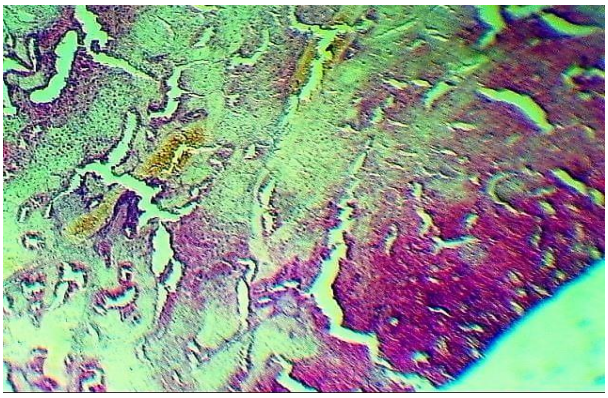
Fig(10) The picture shows the effect of sodium bicarbonate in reducing the effectiveness of ochratoxin, where a slight infiltration of macrophage cells, hyperplasia of the cells lining the tubules, and a clear expansion of the twisted renal tubules were observed.



Fig(11) The picture shows the group treated with poisonous mushroom filtrate and Agaricus fungus filtrate, where a slight infiltration of macrophage cells was observed within the renal tissue and thickening in the walls of the artery, and the glomerulus appeared rich in cells and expanded



Fig(12) The picture shows the group treated with poisonous fungus filtrate and Agaricus fungus filtrate with sodium bicarbonate. It is noted that the renal cells appear normal and the glomerulus is large and round surrounded by narrow and elongated renal tubules. Hyperplasia of some cells is noted.



The effect of ochratoxin A in level of urea and creatinine concentration

The effect of ochratoxin A in level of urea and creatinine concentration in the serum of female mice treated with it as the creatinine level reach to 1.852mg/dl, and the urea level 62.53mg/dl

compare with the control group whose urea level 33.55mg/dl and creatinine 0.621mg/dl which indicate significant differences in the level of creatinine and urea between the studied group and the reason of effect of poison on the kidney tissues which causes clear changes in the renal glomeruli, also lymphocyte in filteration and bleeding in the kidney appeard. In addition to this is presence of decomposition of the cells of the urinary tubules with fluid exist between the urinary tubules which in turn effects the functions of kidney and then leads to kidney failure and a decrease in the process of excretion of toxic substances (Guyton, 1989). In the percentages of urea and creatinine when compared to the control group, as for the treatment of the filtrate of Aspergillus niger fungus with sodium bicarbonate, a slight increase in the rates was observed, while in the treatment of Aspergillus niger, Agaricus bisporus and sodium bicarbonate, no increase was observed in the percentages of urea and creatinine. The average levels may be due to the control of Agaricus bisporus filtrate in maintaining renal cell activity during the period of exposure to mycotoxins.

Table(2)- effect of filtered A.niger in rate of creatinine and urea in blood mice

Groups	Description	Blood urea mg/dl Average ±standard error	P value	Creatinine mg/dl average±standard error	P value
G.1	Control	33.55±0.20		0.621±0.03	
G.2	Aspergillus niger filterate only	62.53±0.16		1.852±0.03	
G.3	Agaricus bisporus	36.85±0.03		0.756±0.002	
G.4	Aspergillus niger+ Agaricus bisporus	48.02±0.32	<0.001	1.231±0.01	<0.001
G.5	Aspergillus niger+ sodium bicarbonate	34.85±0.31		1.245±0.003	
	Aspergillus niger+				

G.6	sodium bicarbonate+ Agaricus bisporus	41.02±0.31		0.985±0.003	

The reason may also be due to the effect of the poison on the tissues of other organs in the body in which these enzymes are present (Volmer, 2004). The results agreed with (Pradhan et al.,2019) in the study of the effect of Agaricus bisporus filtrate on laboratory animals in the treatment of Agaricus bisporus, the enzymatic levels reached (ALT 35.24), (ALP 32.85), (AST 32.05). The enzymes were not affected by the iltrate and remained on their activity and representation in the liver compared to the control group. Note that a high or low level of enzymes indicates the presence of pathological device in the human or animal body, and no change in the enzymes ratios examined was observed in the study and this is in agreement with (Qassem, 1998). Aspergillus niger with Agaricus bisporus filtrate. The reason may be the control of Agaricus bisporus filtrate in maintaining the activity of liver cells during exposure to mycotoxins. The table that shown below includes of the average of liver enzyme and effect by biological and chemical treatment.

Table(3)- effect of filtered A.niger in rate of liver enzymes in blood mice

Groups	Description	ALT u/l	P value	AST u/l	P value	ALP u/l	P value
G.1	Control	12.42±0.14		15.25±0.09		12.25±0.09	
G.2	Aspergillus niger filterate only	44.57±0.02		39.52±0.01		36.54±0.02	
G.3	Agaricus bisporus	35.24±0.08		25.69±0.05		25.85±0.31	
G.4	Aspergillus niger+ Agaricus bisporus	39.42±0.31	<0.001	32.05±0.31	<0.001	32.85±0.63	<0.001
G.5	Aspergillus niger+ sodium bicarbonate	29.54±0.31		19.52±0.37		29.52±0.36	
	Aspergillus niger+						

G.6	sodium bicarbonate+ Agaricus bisporus	26.47±0.32		18.42±0.32		30.14±0.31	

Reference

Bennett, J.W & Klich.M, (2003). Chotoxins. C lin. Microbial. Rev, 16, 497-516.

Bennett, J.W. (1987). Mycotoxins, mycotoxicoses, mycotoxicology and Mycopathologia, Springer.

Bhat R.V. and Vasanthi S., (2003). Food safety in food security and food trade: mycotoxin food safety risk in developing countries. Washington D.C. International Food Policy Research Institute.

Guyton, E. (1989). Guidelines for developing educational programs for cooperating teachers. Action in teacher Education,11 (3), 54-58.

Lee, H.J, Li, S., Gu., K., & Ryu., D. (2021). Reduction of ochratoxin A during the preparation of porridge with sodium bicarbonate and fructose. Toxins, 13(3), 224.

Mac Donald, S.Wilson, p .; Barnes, K. ; Damant.;Massey ,R .;Mort by E;Shepherd,M.(1999). Ochratoxin A in dried fruit : method Development and Survey, Food .addit .cntam.16:253.

Mohammed S.W.; Khalid A.H.; Salim R.Al.; Hanan J.N. and Noorulhuda K, (2017). Determination of the toxicity of fumonisin B1 on male albino mice. Tropical biological research unit, College of Science-University of Baghdad.

Park, D.L.; NjapauH, and BoutrifE. (2009). Minimizing risks posed by mycotoxins utilizing the HACCP councept.

Pradhan, M., Rai, D., & Bhandari, R. (2019). Metastatic Cutaneous Melanoma: A Rare Entity with a Unique Presentation. Journal of Nobel Medical College,8(2),67-69.

Qassem W., Jarrah M. & Othman, (1998). Heart response to horizontal impulse.Journal of medical engineering & technology,22 (2), 82-90.

Sobolev.V.S, and Dorner.J.W, (2002). Cleanup procedure for determination of aflatoxin in major agriculture commoditiesby liquid chromatography.J. Associated of official analytical chemist international, 85:642-645.

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.

Wasser, S.P. (2011). Current findings, future trends, and unsolved problems in studies of medicinal mushrooms. Applied microbiology and biotechnology, 89(5), 1323-1332.