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Using Agaricus bisporus filterate and sodium citrate against Alternaria arborescens toxicity in Histopathological and Biochemical change in laborotary mices

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***Abstract*---**This study was designed to find out the therapeutic efficiency of Argaricus bisporus fungi in the treatment of toxicity caused by fungus Alternaria aborescens, in the study (30) of female mice injury experimentally six groups of a single dose of (0.5)lμ size oral dosing using a stomach tube . Results of the study pathological macroscopic and histopathological ability of toxic fungus filtarate to the events of infection when an autopsy after (21days) from injury was infiltration of cells of the inflammatory and as well as caused histological clear in the cross section of the liver tissue, including the occurrence of vaculation of hepatic cells and the presence of bubbles in the cytoplasmic cells and the expansion of the hepatic siousoid , causing hydropic degeneration .Resalts showed a clear effect within the kidney tissues leading to atrophy in glomeruli, lymphocytic infiltration and hemorrhage, in addition to the degradation of urinary tubule cells , which affects the functions of the kidney leading to renal fialure . proved the results obtained from the study of histological sections impact the effective and positive role for the test in the treatment represented by a restoration of the Liver and kidneys tissues to be mounted typical natural.The results of the toxic effect isolation of pathogenic fungi with in the body of the organism showed a toxic effect in females of white laboratory mice of BALB / c albino species : Mouse Mus musculus , where changes were made in the tissues of the laboratory animals after their dosage and significant differences mice during the duration of the experiment period , and the effect on levels of liver enzymes(ALT,GPT,GOT)with levels of(306,162.6,294.3)IU/L respectively compared to control group (181.6,58,284.6)IU/ L respectively, The results of the laboratory

research of the kidney function showed significant differences in the increase in the concentration of urea and creatinine mice tested (51.66mg / dl) and creatinine (1.16mg / dl) compared to control group , (36 mg / dl)) And (80 mg / dl) respectively, The resuls proved the success of the treatments of the A.bisporus filtrate and sodium citrate and interaction of sodium citrate and the filtrate of the fungus A.bisporus in this study ,and its anti-and positive effect on the treatment and restoration of liver and kidney tissues and their normal functioning .

***Keywords*---**Aternaria arborescens, Agaricus bisporus, histological effects.

Introduction

Alternaria species are fungi widely distributed in the soil as normal components of its plant pathogens. They are widespread in both humid and semi-arid regions and can infect growing plants in the field. Alternaria species cause plant diseases on many crops. They are the principal contaminating fungi in wheat, sorghum and barley (Deshpande, 2002). The most commonly reported species include *Alternaria alternata, A. tenuissima, A. arborescens, A. radicina, A. brassicae, A. brassicicola, and A. infectoria.* They colonise a range of plants including cereal crops, such as sorghum and result in toxin contamination. *Alternaria* species have been reported to occur in oilseeds such as sunflower and rape seed, tomato, apples, citrus fruits, olives and several other fruits and vegetables. Many *Alternaria* species are host-specific. Due to their growth even at low temperature, *Alternaria* species are also responsible for spoilage of these commodities during refrigerated transport and storage. *Alternaria* species produce more than 70 secondary metabolites which are toxic to plants. A small proportion of these phytotoxins have been chemically characterised and reported to act as mycotoxins to humans and animals (Bottalico and Logrieco, 1992; Barkai-Golan, 2008). *A. arborescens* which is the most common *Alternaria* species in cereal crops,, is the most important mycotoxin-producing species. Based on their effect on plants, *Alternaria* toxins are divided into nonhost-specific toxins and host-specific toxins. Non-host-specific toxins are described to induce harmful effects in animals culture extracts of *A. aborescens* as well as individual mycotoxins such as alternariol and alternariol monomethyl ether are mutagenic and genotoxic in various in vitro systems. On the other hand, the animal toxicity of host-specific toxins high their possible harmful effects, *Alternaria* toxins are of concern for public health. They have been reported to cause animal toxicosis as a result of exposure from feed. This opinion deals with alternariol, alternariol monomethyl ether, tenuazonic acid, iso-tenuazonic acid, altertoxins, tentoxin and AAL-toxins. This state required researchers for examining cheaper and easily available antimicrobial stuffs from various sources which are the good sources of novel antimicrobial chemotherapeutic agents (Den Hollander *et al.*, 2022). *Agaricus bisporus* white button mushroom is a worldwide fungus with a distinguishing maturing body that is widely cultivated in the world, Edible mushroom characteristically contain

many different bio active compounds such as glycolipids, polysaccharides, sesquiterpenes etc., with miscellaneous biological activities , antibacterial, antifungal and antiviral agents.Investigators presented antimicrobial activity of several mushrooms (Giaccone, 2022), extracted from mycelia and fruiting bodies of various mushrooms have been reported for antimicrobial action against wide range of fungi toxicity (Fokunang *et al.*,2022).

Therefore, the current study aimed to evaluate the effect of chemical and biological treatments experimentally (in-vivo) on the physiological and histopathological effects of infection induced by *A. arborescens* filters. In the lungs of male laboratory albino rats, and the adoption of some criteria, such as measuring body weight and weight gain rate, and studying the biochemical and histopathological changes in the lungs of laboratory animals. In addition to recording the clinical signs appearing on the experimental animals through continuous monitoring throughout the study period and the signs or macroscopic changes in the lungs of the affected animals before and after treatment.

Materials and methods

- The research was conducted in the laboratories of the College of Science /University of Al-Qadisiyah .
- *Agaricus bisporus* was obtined from College of Agriculture ,.University of Al-Kufa

Tested fungal and prepare fungal suspension

Has been the development of fungi *A. arborescens* isolated from Corn Grains on the Potato's Dextrose Agar and incubated at a temperature (37˚C) for (7 days). Under sterile conditions developing colonies flooded about (2 ml) of physiological salt solution (0.85%) and using Loop separate the top of the colony and added a drop of material Tween 80 (0.01%) to reduce the surface tension. Collected spores in a glass tube and mixed device Vortex mixer . Spores suspended with a phosphate buffer solution to the became commentator absorbency of light (0.1) at wavelength 530 nm, equivalent to (107spore / ml)(Espinel – Ingroff,et.al.,1995) .

Experimental protocol

In order to bring about toxicity, Injected six groups of male albino mice type ofBALB/c alibno , species : Mouse Mus musculus weighing 40-50 grams, through oral dosing using a stomach tube of five mice per group, the first group injected with (0.5 lμ) of normal saline solution while animals remained second and third groups were injected with a single dose only amount (0.5 lμ) of *A. arborescens* and *A. bisporus* fungal respectively its focus(to mice every 48 hours 0.5 ml / 30 g per mouse / day. While the fourth group included inoculation of mice with filtrate of *A. arborescens,* and after 24 hours of dosing, mice were instilled with filtrate of *A. bisporus* 0.5 ml/30 gm for mice/day. The fifth group dosed mice with filtrate of *A. arborescens* and after 24 hours, sodium citrate + 0.5ml/30gm was administered to the mice/day and the sixth

group instilled mice with filtrate of *A. arborescens* and after 24 hours of dosing, mice were instilled with *A. bisporus* filtrate + oral sodium citrate 0.5 ml/30 gm for mice/day. Animals leave until the signs of fatigue, weakness, loss of appetite as evidence of the incidence of fungal. After the end of duration of the experiment had killed all the rats by intraperitoneal injection (I.P) at a dose of Ketamin and Xylazine about (2.5) and (25) mg / g of body weight for both anesthetists, respectively, were conducted anatomical, removed the Liver and kidneys to fixed in a solution of formalin regulator neutral concentration (10%), and then attended the tissue sections and dye Hematoxylin & Eosin dye for the purpose of studying the pathological changes of gross and histological caused by the fungus and the therapeutic efficiency of the plant extract solution alcohol according to Bancroft and Stevens method(Bancroft,. &Stevens, 1982).

Blood samples collection and Biochemical analyses

Blood samples were collected using the method of drawing blood from the heart directly by stabbing the heart of the animal using a wine syringe (Needle Gauge 23 x ¼). Renal function by measuring urea and creatinine, as shown: Alanine aminotransferease (ALT). Aspartate aminotransferease (ASP). Alkaline Phosphatase (ALP).blood urea . Seram creatinine

Statistical analysis

The results of the entire study were analyzed using the statistical software GraphPad Prism Institute, Inc. According to the data of the results of the study, the statistical test Chi-square test and the method of least significant difference (LSD) for one-way variance and two-way variance were applied to determine the least significant differences between study groups, as well as the adoption of a confidence interval equal to 95% and a value at the probability level less than 0.05 ($P < 0.05$) (Khalafi-Kheydani *et al.*, 2022).

Results and Discussion

Gross pathological changes

In the group of mice treated with *A arborescens* filtrate only for magnified was observed in the Liver and kidneys compared to the control group**.**

Histopathological changes

The results of the microscopic examination for the diagnosis of tissue sections taken from the livers of white mice treated with filters of the fungus *A.arborescens* (Figure 1) showed the presence of clear pathological changes in the cross-section of the liver tissue, including: Vaculation of hepatocytes, bubbles in the cytoplasm of cells, ascites degeneration. (Hydropic degeneration) The nuclei appear small and some of them appear devoid of the nucleus, as well as an expansion of the hepatic sinusoids. The reason for the appearance of these symptoms in the liver tissue is the severe effect of the poison Altratoxin, that the cause of the occurrence of ascitic degeneration is a

disorder in protein metabolism and thus leads to the accumulation of water inside the cells. On the production of enzymes, as *Alternaria* species have the ability to accumulate heavy elements such as calcium, zinc, copper, iron and others and work hard to break down lignin, cellulose, pectin, car-hydrate and complex hydrates, making them adapt to different types of materials and produce different enzymes accordingly (Singh and Kumari 2022). In comparison with the cross-section that represents the control group in Figure (2), the normal radial arrangement of hepatocytes around the central vein is noted, which also appears normal and the presence of normal sinusoids and proliferation of Kupffer cells, hepatocytes appear normal hexagonal with a central nucleus. As for the group of mice treated with filtrates of the fungus *A.bisporus* (Fig.3), no histological changes appeared in this section, as the hepatocytes appeared in their normal size and shape, which is consistent with the findings (Nalabotu *etal.*,2011; Al-Saidi and Saadon, 2019) that Using the filtrate of the pure fungus and giving it to the white mouse during a specific period does not affect the liver tissue, and it has no toxic effect, as well as the presence of cover cells in normal numbers and sizes and the presence of proliferation in some of the liver cells and division in them as they contain two nuclei. For the group treated with *A.arborescens* filtrate and *A.bisporus* filtrate, Figure (4) shows filtration and accumulation of fatty droplets inside the hepatocytes so that the cells appear swollen. Toxins cause a defect in the biosynthesis of fats, especially in the liver, and it is one of the first early signs which appear in the organism as a result of exposure to toxins, and the nucleus is peripheral and resembles a signate-like shape, a slight infiltration of macrophage cells within the liver tissue. This could be explained by the protective effect and the control of the filtrate of *A.bisporus* on mitigating the toxic effects of the toxins of *A.arborescens*. In the treatment of filtrate of pathogenic fungi with sodium citrate in Figure (5), hepatocytes with binucleated heptocytes were shown, indicating that Regeneration process, slight expansion of hepatic sinusoids and proliferation in Cover cells. In the cross-section of a mouse liver, which represents the group of overlapping treatment with *A.arborescens, A.bisporus* and sodium citrate, Figure (6), most of the hepatocytes showed normal, and Other hepatocytes suffer nosocomial degeneration, with intracytoplasmic bullae, and proliferation in Cover cells, while the hepatic sinusoids appear normal.It seems clear from the pathological changes This may be attributed the cause to the content solution casuals fungus from the secretions of fungal and which caused an increase blood pressure for (Microcirculation) and thus to increase the permeability of capillaries and hemoglobin free from red blood cell (Intravascular hemolysis) in addition to stimulating the production of (Proinflammatory mediators) such as(TNF-α) the charge for the crash in most parts of the body(Hirsch *et.al.*,2003). In addition to Hydrolytic enzyme promotes portability fungus disease , where the work of these enzymes to break down the cell membranes and the invasion of tissues host, leading to dysfunction or physical disruption which is caused by the imbalance in the homeostasis of Procedure of the body the host (Ferrer *et al.*,2012).

The results of the microscopic diagnosis of the histological sections of the kidney indicate the presence of histopathological changes in the kidney of the laboratory mouse, which represents the group treated with infiltrate of

A.arborescens only Figure (7). It showed a dense infiltration of Macrophage cells with severe heamorrhage in the renal tissue, and the presence of Necrosis of the cells lining the renal tubules of the renal tubules Renal convoluted tube, severe congestion within the renal tissue, as well as a clear expansion of the renal tubules, but in the control group, no changes were observed in the tissues of the kidneys in Figure (8), as the histological section appeared normal from glomerulars, Glomerulor capsule, capsule space, convoluted tubules, Bowman's capsule intact from changes and clarity of the maximum and minimum convoluted tube Proximal and distal. As for the cross-section of the mouse kidney, Figure (9), which represents the group treated with filters of the bio-resistance fungus only, there was no noticeable change in the kidney tissue. The results of the histological examination of the kidneys of mice representing the group treated with *A.arborescens* and *A.bisporus* filtrates indicate a slight hemorrhage in the interstitial tissue. Renal tubules and necrosis of cells lining the tubules with macrophage infiltration Figure (10). As for the histological examination of mouse kidneys, which represents the group treated with infiltrate of the fungus *A.arborescens* and sodium citrate, small glomeruli appeared, and a little atrophy was observed in some renal tubules with little necrosis in the lining cells. For renal convoluted tubules Fig. (11). As for the (interference) group treated with the infiltrate of the contaminated fungus and the infiltrate of the fungus *A.bisporus* and sodium citrate together, Figure (12) shows bleeding in the renal tissue, the glomeruli appear large, round and normal, as for the renal tubules, they appear lined with normal cubic cells and a slight infiltration in inflammatory cells.

Biochemical test

The effect of toxic fungal filtrate on the levels of liver enzymes in the blood of white mice treated with it reached (306±12.1, 126.6±2.15, 294±6.02 IU/L), respectively, in the treatment of *A. arborescens* filtrate only in comparison with the control group. Liver enzymes 52 ± 2 IU/L), (181.6 ± 7.6, 248.6 ± 3.5 Table (1), which indicates significant differences between the levels of enzymes in the studied groups, that the reason for the increase is due to the great toxic effects of mycotoxins in liver cells and tissues, as It caused a breakdown in the liver cells that contain these enzymes, which led to their release in the blood and an increase in their level in the blood serum.

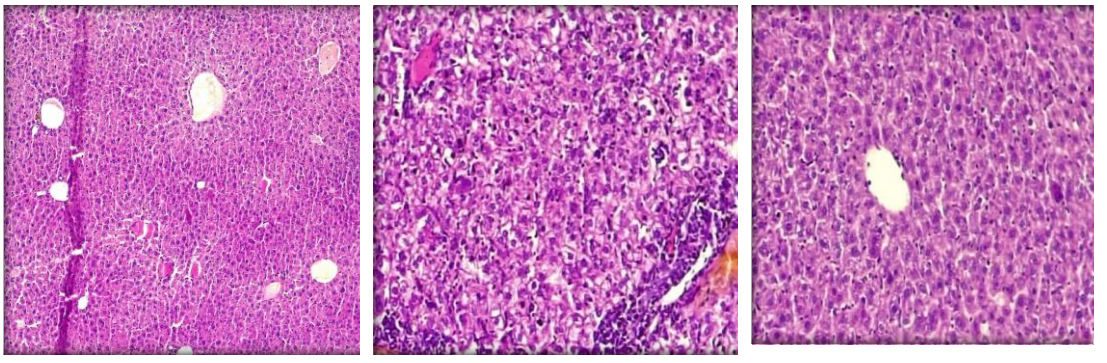

Figure (1): a cross-section of a mouse liver representing the control group (40 X H&E). It is noted that the normal radial arrangement of hepatocytes around the central vein appears normal as well. The presence of natural sinusoids. Hepatocytes appear normal, hexagonal, with a central nucleus.

Figure (2): A cross-section of a mouse liver representing the group treated with *A.arborescens* filtrate (40 X H&E). It is noted that hepatocytes vaccinate and the presence of bubbles in the cytoplasm of the cells (Hydropic degeneration). In addition to the nucleus appear small Others appear devoid of the nucleus, and a clear expansion of the sinusoid hepatic sinusoids.

Figure (3): A cross section of a mouse liver representing the group treated with *A.bisporus* filtrate only (40XH&E). Hepatocytes are undamaged kufr cells. The presence of proliferation in some of the liver cells due to the presence of divisions in them as they contain two nuclei.

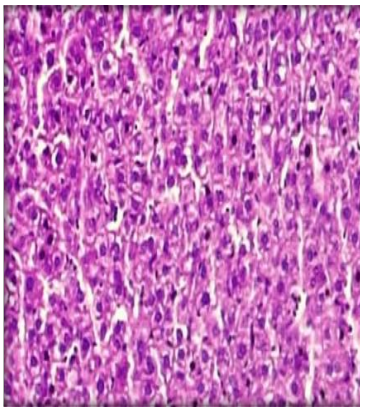

Figure (4): A cross-section of a mouse liver representing the group treated with *A.arborescens* filtrate and *A.bisporus* filtrate 40XH&E . Infiltration and accumulation of fatty droplets inside the hepatocytes so that the cells appear swollen and the nucleus is circumferential and resembles a signate-shaped ring, slight infiltration of inflammatory cells within the liver tissue

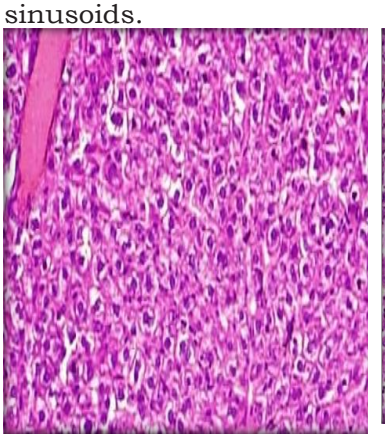

Figure (5): a cross-section of a liver representing the mice of the group treated with *A.arborescens* filtrate and sodium citrate (40XH&E) treatment. Most of the hepatocytes appear with two binucleated heptocytes, which indicates the process of regeneration, a slight expansion of the hepatic sinusoids, and the proliferation of Cover cells.

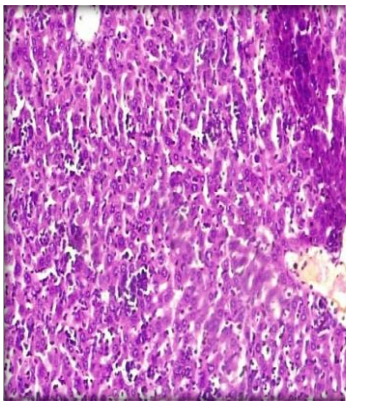

Figure (6): A cross-section of a rat liver representing the treatment of *A.arborescens* and *A.bisporus* filtrates and the treatment of sodium citrate (40XH&E). Most of the hepatocytes appear normal; some of the hepatocytes suffer from hydropic degeneration, with intracytoplasmic bubbles. The cells, proliferation of Kupffer cells, and hepatic sinusoids appear normal.

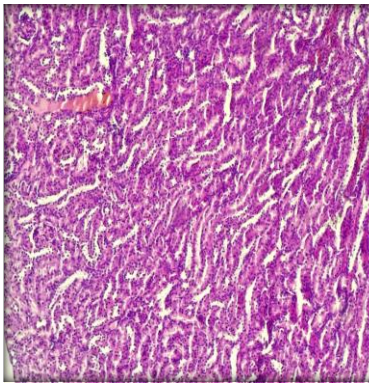

Figure (7): A cross-section of the kidney of a mouse representing the control group illustrating the normal histological structure of the kidney: (40 XH&E) glomeruli, glomerulars, glomerular capsule, capsule space, distal convoluted tubule, proximal convoluted tubule

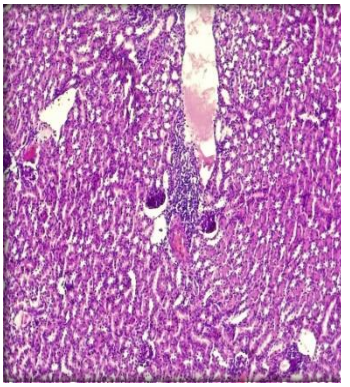

Figure (8) cross-section of the kidneys of mice treated with filtrate of *A.arborescens* Intensemacrophage infiltration, severe hemorrhage in the renal tissue, necrosis of the cells lining the renal tubules, severe congestion within the renal tissue, a clear dilatation of the renal tubules .

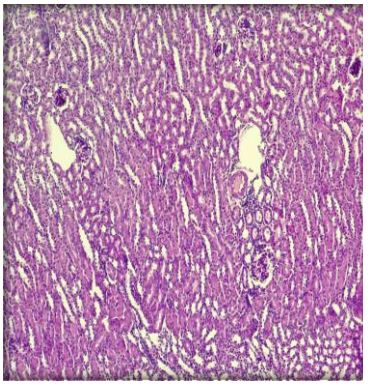

Figure (9): A cross section of a mouse kidney representing the group treated with *A.bisporus* filtrate only (XH&E) (40 Convenluted tubules Renal, normal epithelial cells lining the renal convoluted tubules

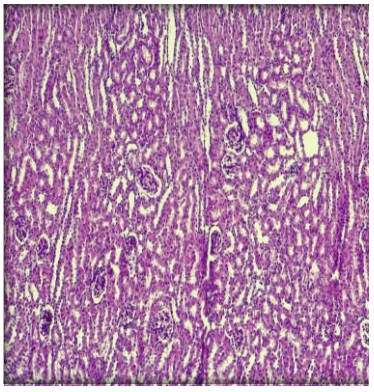

Figure (10) cross section of a mouse kidney representing treatment with filtrate of *A.arborescens* fungus and *A.bisporus* filtrate (40X H&E). Minor hemorrhage occurred in the interstitial tissue of

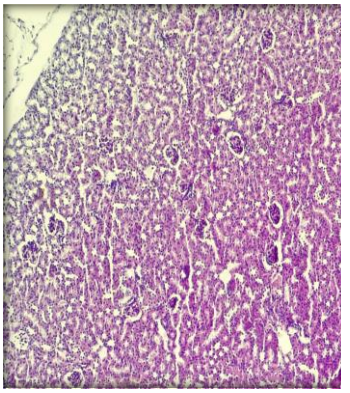

Figure (11): A cross section of a mouse kidney representing treatment with infiltrates of *A.arborescens* and sodium citrate (40XH&E). The presence of small

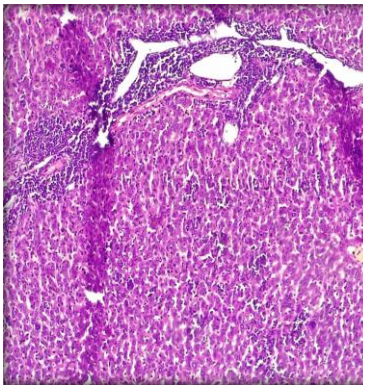

Figure (12): A cross section of a mouse kidney representing treatment with infiltrates of *A.arborescens* and sodium citrate and *A.bisporus* 40XH&E) hemorrhage occurs in the renal tissue and the

renal tubules, necrosis of cells lining the tubules, infiltration of macrophage cells.	glomeruli. A little atrophy is observed in some renal glomeruli. Little necrosis of the cells lining the renal convoluted tubules.	glomeruli appear large, round and normal, the convoluted tubules appear lined with normal cuboidal cells, infiltration Mild in macrophage cells.

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 *A. bisporus* filtrate on laboratory animals in the treatment of *A.bisporus*, the enzymatic levels reached (GOT 39 ± 1) (GPT 32 ± 1.52), (ALP 1.15 ± 198). The enzymes were not affected by the filtrate 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). *A. arborescens* with *A.bisporus* filtrate. The reason may be the control of *A.bisporus* filtrate in maintaining the activity of liver cells during exposure to mycotoxins.

The effect of Altratoxin toxin increased in the level of urea and creatinine concentration in the serum of laboratory white mice treated with it, as the urea level reached 51.66 ± 1.52 mg/dl and the creatinine ratio was 1.16 ± 0.15 mg/dl compared to the control group whose urea level was 36 ± 1 mg/dl and creatinine dl 0.80 ± 0.1mg/ table (2), which indicates significant differences in the level of urea and creatinine between the studied groups, and the reason is due to the effect of the poison on the kidney tissues, which caused clear atrophy in the renal glomeruli, so lymphocyte infiltration and bleeding in the kidney appeared, in addition to This is the presence of decomposition of the cells of the urinary tubules with fluid exit between the urinary tubules, which in turn affects the functions of the kidneys and then leads to kidney failure and a decrease in the process of excretion of toxic substances (Guyton, 1986). In the percentages of urea and creatinine when compared to the control group, as for the treatment of the filtrate of *A.arborescens* fungus with sodium citrate, a slight increase in the rates was observed, while in the treatment of *A.arborescens* + *A.bisporus* + sodium citrate , no increase was observed in the percentages of urea and creatinine. The average levels may be due to the control of *A.bisporus* filtrate in maintaining renal cell activity during the period of exposure to mycotoxins.

Table (1) Effect of toxic fungal filtrate on the average level of liver enzymes in the blood of white mice

Groups	Transactions	GOT(IU/L)	GPT(IU/L)	ALP(IU/L)
First	Control	284.6±3.51	58±2	181.6±7.6
Second	*A.bisporus* filtrate only	85±5	216.3±3.12	185±5
Third	*A.arborescens* + *A.bisporus* + sodium citrate	192.6±6.4	11.33±1.52	248±15.8

Fourth	*A.arborescens* + sodium citrate	122.3±6.8	209±8.54	311±5.2
Fifth	. *A.arborescens* filtrate only	294.3±6.02	162.6±2.51	306±12.1
Sixth	*A.arborescens* + *A.bisporus*	34±4	75.6±4.04	239.6±55.4
rate ± standard deviation		168.8±100.4	122.16±80.4	55.4
LSD $_{0.05}$		9.67	7.70	5.189
Normal Mean Values		<46 IU/L	<34 IU/L	<240 U/L

Table (2) Effect of toxic fungal filtrate on the average level of urea and creatinine in the blood of white mice

Groups	Transactions	creatinin mg/dl Average ± standard error	Blood uera mg/dl Average ± standard error
First	Control	0.80±0.1	36±1
Second	*A.bisporus* filtrate only	0.53±0.05	35±1
Third	*A.arborescens* + *A.bisporus* + sodium citrate	0.66±0.15	38±1
Fourth	*A.arborescens* + sodium citrate	0.63±0.05	34±1
Fifth	*A.arborescens* filtrate only	1.16±0.15	51.66±1.52
Sixth	*A.arborescens* + *A.bisporus*	0.40±0.01	42±2.64
rate ± standard deviation		0.70±0.26	39.44±6.34
LSD $_{0.05}$		0.196	2.65
Normal Mean Values		0.5 – 0.8 mg/dl	19-34 mg/dl

Conclusions

We conclude from this study that spossible use of the plant under study as an alternative tochemotherapy

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