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ORIGINAL ARTICLE

A Histological Study To Investigate The Effectiveness Of *Sacchromyces cerevisiae* Yeast In The Reduction Of Aflatoxin B1 Toxicity In White Rat Animals

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ABSTRACT

Objective: The study included the use of bio-method and environmentally friendly effective in the reduction of the toxicity of Aflatoxin B1.

Methods: A study of the effect of *Sacchromyces cerevisiae* yeast in the effectiveness of AflatoxinB1 included the preparation of three groups of white rat animals include Negative control Is represented four lab animals treated with Aflatoxin only (concentration 100µg /kg w.b.) , Positive control Is represented four animals treated with normal saline only (1 ml / kg w.b.)and four animals treated with mixture of yeast and toxin B1 with 1ml/ kg w.b. for 48 h , The animals were then anatomy by opening the abdominal cavity and taking organs (liver, intestines, kidneys, spleen) into study histological changes.

Results: The results of the histological study in the treatment of toxin with yeast was the safety of the tissues studied from the injury and were normal compared to the treatment of control while the treatment of toxin only, The study showed injuries and histological changes represented by necrosis , hemorrhage and vascular congestion of all studied organs.

Conclusion: *Sacchromyces cerevisiae* yeast provided complete protection for the organs under study (liver, intestine, spleen and kidney) of the harmful effect of AflatoxinB1.

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INTRODUCTION

Aflatoxins refers to group of bisfuranocoumarin metabolites produced by strains of *Aspergillus flavus* fungal group, Aflatoxins contaminate of agricultural products before or after harvest , also continue with crops to the storage , also found in soil , plants and animals with ther products like eggs and meat[1,2]. The exposure to Aflatoxin B1 exhibits two main pathways, both are correlated to gastrointestinal tract ,first path includes direct ingestion of aflatoxin B1 contaminated plant origin foods like maize, peanut ….etc. the second path is through ingestion of meat, eggs, milk containing aflatoxin B1 carried over through consuming contaminated feed by animals, after absorption in gastrointestinal tract the toxin will transfer to the liver[3]. Liver is the target organ for aflatoxin B1, so the metabolism of protein, carbohydrates and lipids in liver will be affected. after Aflatoxin B1 conversion to Aflatoxin–8,9- epoxide (The product of Aflatoxin oxidation) by cytochrome P450 it will react with guanine in DNA and RNA leading to depurination[4,5].

Aflatoxins are found to be the cause of liver cancer, stomach cancer, colon cancer, and the acute toxicity characterized by acute liver damage, hemorrhage, high fever, vomiting, jaundice, edema and death in human[1,3].

Aflatoxins cannot be easily removed from contaminated food by detoxification, thus the interest of developing biological control method that can decreases toxin content and increases food safety is of a major concern noe days[6]. After the studies proved the inefficiency of chemical and physical methods in the reduction of the toxicity of aflatoxin, the studies aimed at the biological methods and their high effectiveness in removing the

toxicity of Aflatoxins through the use of microorganisms such as bacteria, yeast and fungi. Biological factors can be classified depended on their source and mechanism of action like biodegradation, isolates of yeasts including *Candida krusei* and *Sacchromyces cerevisiae* are tested for aflatoxin binding, the yeast strains bound more than 15% (w/w) of aflatoxin B1 and the toxin binding is highly strain specific, there are many reports on use of physically separated yeast cell walls obtained from brewery as feed additive in poultry diet resulting in reducing of toxic effects of Aflatoxins, yeast cell walls were added to rat diet along with aflatoxin B1, a significant reduction in the toxicity was observed In an in vitro study with the cell wall material, oligosaccharides derived from the *Sacchromyces cerevisiae* cell resulted in as much as 95% (w/w)[7].

MATERIALS AND METHDS

Laboratory animals

Used males of the albino rats of the species (*Rattus rattus*) and age 8-12 weeks of weight ranged between 200-210 gm, obtained from the animal house in pharmacy College/ University of Karbala. It was put in cages and in groups. Prepare suitable conditions of suitable temperature and lighting.

Preparation of Culture Media

Preparation Potato Dextrose Agar (PDA) and Potato Dextrose Broth (PDB) according to instructed by the manufacturer (Hi-Media – India).

Biodegradation of Aflatoxin

a- Isolation of fungus *Aspergillus flavus*

It was obtained *Aspergillus flavus* isolated and diagnosed by Dr. Sami Abdul Ridha Al-Jumaili, department of clinical laboratories, college of applied medical sciences, Karbala university, Iraq, depending on the taxonomic keys that put both[8].

b- Testing the fungus *A. flavus* susceptibility to the production of aflatoxin B1 by Thin Layer Chromatography (TLC)

Is the development of isolation fungi *A. flavus* on central PDA and by puting the tablets of the fungi studied and a diameter of 5 mm old a week in the center of each dish then incubated at 25 ± 2 C° for one week after this cutting the middle and transported the pieces by a sterile needle to a blender container then 20 ml of choloform and blending the mixture for 10 minutes and then is filtrerd mix by filter paper then taking the filtrate and placed in a beaker is clean sterilized then move to electric oven at a temperature of 40 C° where quantitative focus to approximately 1ml only, the presence of Aflatoxin B1 was detected using TLC (20-20 cm) with platelets activated in the 120 C° oven for an hour before use the separation system was used chloroform: methanol (98: 2). A straight line was made on a TLC plate 1.5 cm from the base of the plate, and 15 microliters were taken by a capillary tube of standard AFB1 and put on the line 2 cm from the left of the plate and 2 cm from the spot of the standard toxin, then the leaves were left to dry then move to the cell basin containing the mobile phase and were monitored until the solution reached about 2 cm from the upper end of the plate, the plates were removed and dried and then examined under ultraviolet radiation and wavelength 360 nanometers and was detected only aflatoxin B1 matched the transfer factor (Rf) and the fluorescence color of the aflatoxin extract content with the standard material of AFB1[9].

c- Extraction and purification of aflatoxin B1

After the definitive diagnosis of isolated *A. flavus* producing AFB1 by method mentioned above, 70 petridish were prepared from the PDA and all dishes were vaccinated with *A. flavus* isolates. The sample was carried out in a Streak form on a TLC plate. The AFB1 was then separated from the TLC plate by scaling the silica gel containing B1 by a sterile blade and collected in sterile, sterile test tubes. Each tube was then added to 10 mL of chloroform and was well prepared and then centrifuged at 6000 cycles/minute for 15 minutes.

Collect the leachate and leave the precipitate, and then transfer the leachate to a small baker size 20 ml and put in the oven at 50 m temperature until the dry sample and so repeated the process several times to obtain the 4 gm from toxin and storage it in deep freeze at 20 C˚ for its treated of animal lab[10].

Test the toxicity of AFB1 pre-treated with *Sacchromyces cerevisiae* yeast in white rat animals

This study conducted as the following steps:

1. Yeast ground in 10 ml of PDB at 25 C˚ for 24 h after that added the aflatoxin B1 with 100 µg/ml and incubated at 25 C˚ for 72 hours.
2. Prepare 12 white male rat age 8-10 weeks and were divided into three groups each group included four animals, treatment of animals lab as the following:

A- Negative control: four animals labs treated with aflatoxin only (concentration 100 µg /kg w.b.).

B- Positive control: four animals treated with normal saline only (1 ml / kg w.b.).

C- Yeast + aflatoxin B1: four animals treated with mixture of yeast and toxin B1 (which prepare in step A) with 1ml/ kg w.b. for 48 h. This processes repeated five times in 10 days, followed by the clinical symptoms that can be seen in the treated animals during the period of dosing, and then left for two days. The animals were anesthetized by chloroform. The animals were then anatomy by opening the abdominal cavity and taking organs (liver, intestines, kidneys, spleen) and preserved in formalin at 10% concentration for the to study histological changes.

Histological study

Histological sections were prepared in Al Sadr General Hospital –city in Al-Najaf and followed the method[11]. The kidney, liver, spleen and intestine were fixed in 10% formalin and embedded in paraffin. Four micron thick section were prepared and stained with hematoxylin and eosin dyes. The specimens were examined under an light microscope. The tissue sections were microscopically identified by Dr. Haider Jabr Kahyush / Specialist in Histopathology (Iraqi Board of Pathology) Al Hussein Medical City.

RESULTS

Testing the isolation of fungi *A. flavus* on the production of Aflatoxin

Use of thin layer chromatography TLC .

The isolation was subjected is the use of TLC to detect the poison and the results of this test showed the isolation of *A. flavus* on the production of aflatoxin through. The Relay Factor of 0.56 and the color of the fungus extract of the fungus isolate with the standard toxin of B1 (Figure 1).

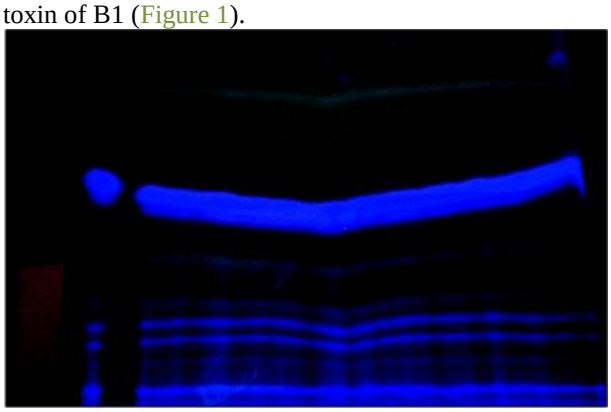
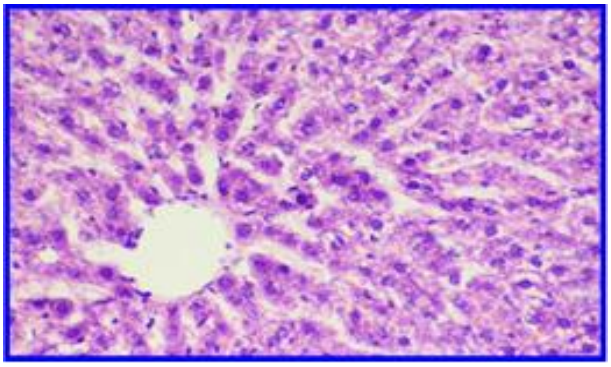


Fig. 1 **The use of TLC technology to detect *A. flavus* ability to produce Aflatoxin B1**

Test the toxicity of AFB1 pre-treated with *Sacchromyces cerevisiae* yeast in white rat animals

The results of microscopic diagnosis of tissue segments taken from kidneys, liver, small intestine and spleen of male rats treated with aflatoxin B1 showed clear changes in the tissues of these organs and severe clinical effects in both. The liver has undergone changes in the tissue, including congestion of the blood vessels and hemorrhagic state, in addition to necrosis in certain cells and vasoconstriction, either in the kidneys has been in the atrophy of the glomerulus with hypertrophy in the wall and congestion of blood vessels in addition to hemorrhage and the case of cell death of glomerular and renal tubes, these effects caused by metabolic products of fungi in the kidneys are due to the role of these compounds in the inflammation and oxidation of fat in the membranes of the cellular and thus increases the metabolism of constituents of plasma blood, leading to the contraction glomerular and cell death[13]. For the intestines, pathological changes were represented with decomposition and molecular necrosis of the glan Gastrointestinal and analysis intestinal villus. In the spleen there has been a state of necrosis in the white lobe and red scaly of the spleen. Comparing these cases with the control treatment and The animals treated with the yeats and Aflatoxin B1 did not show any satisfactory symptoms in the histological sections of the kidneys, liver, small intestine and spleen (Figure 2, 3, 4 and 5).



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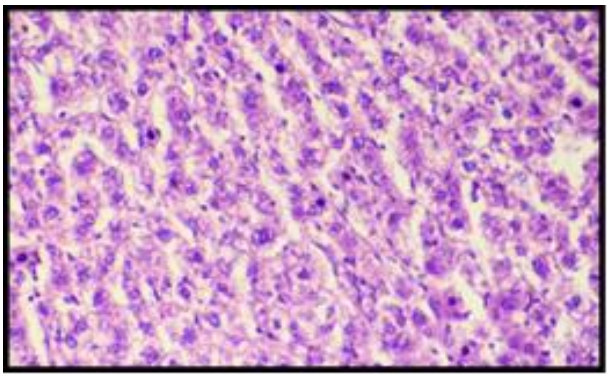

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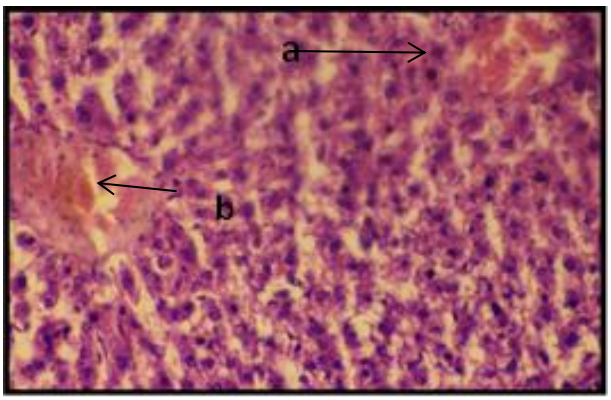

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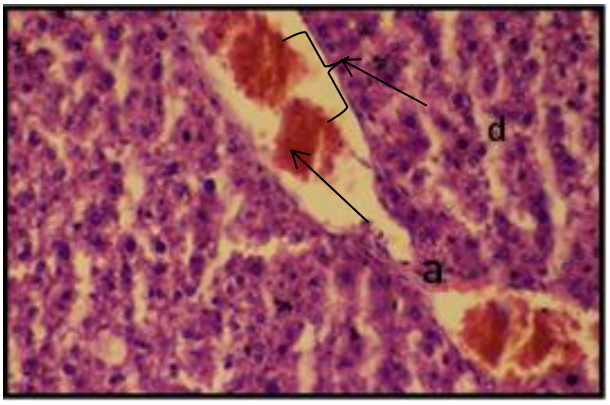
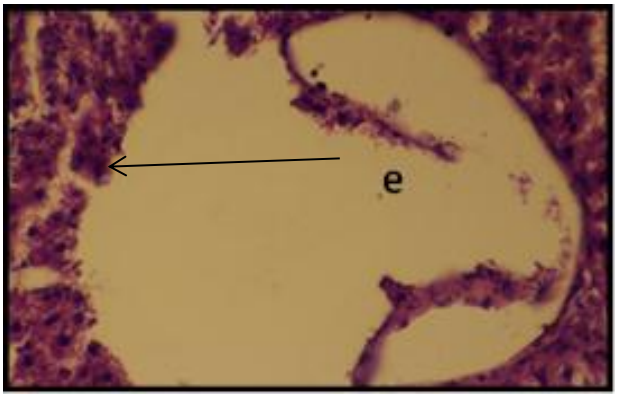


C_2



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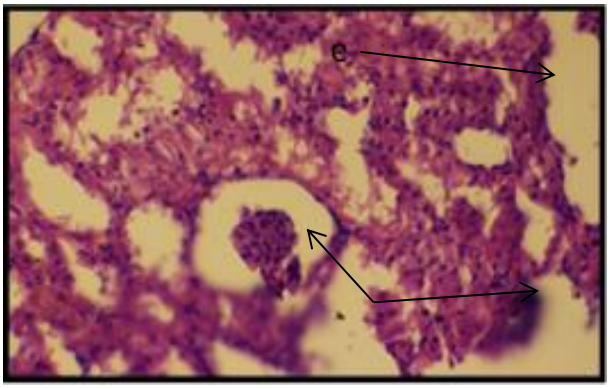
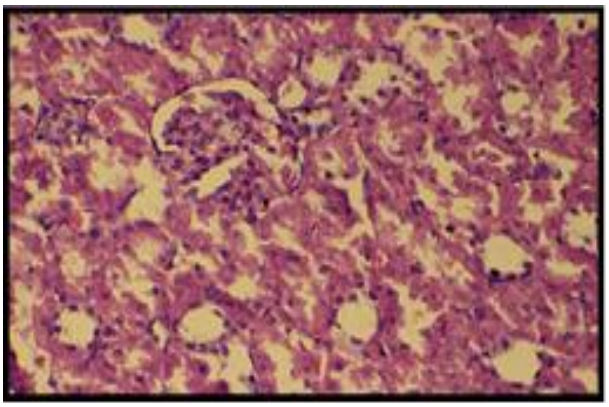


C_1

Fig. 2 **Section in the liver tissue of male White Rat A- Control treatment. B- Aflatoxin B1+ yeast treatment. C1, C2, C3 - only aflatoxinB1**
a. congestion b. hemorrhage e. necrosis d . dilation of the blood vessel



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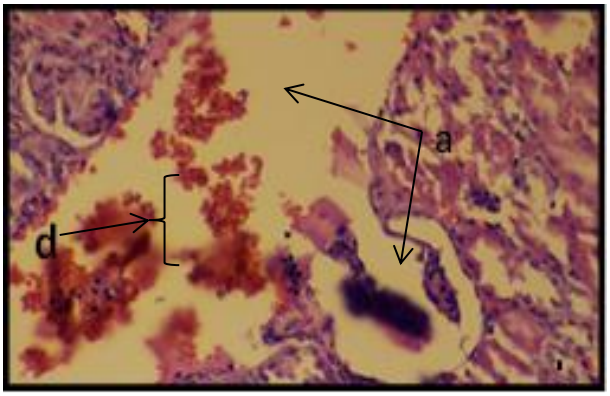
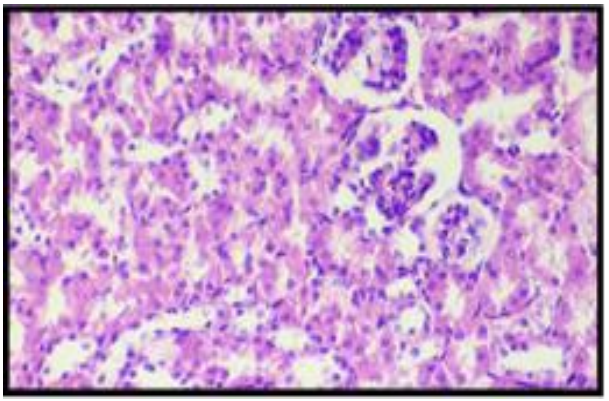


C_2

Fig. 3 **Section in the kidney tissue of male White Rat A- Control treatment. B- Aflatoxin B1+ yeast treatment . C1, C2 -only aflatoxinB1 .**
a- dilation blood vessels b-Atrophy of the glomerulus e-Necrosis d- congestion



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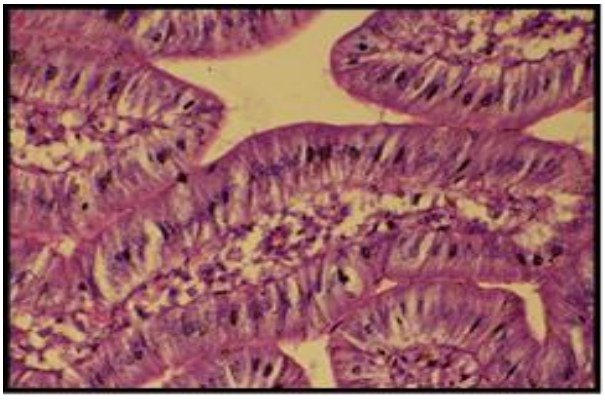
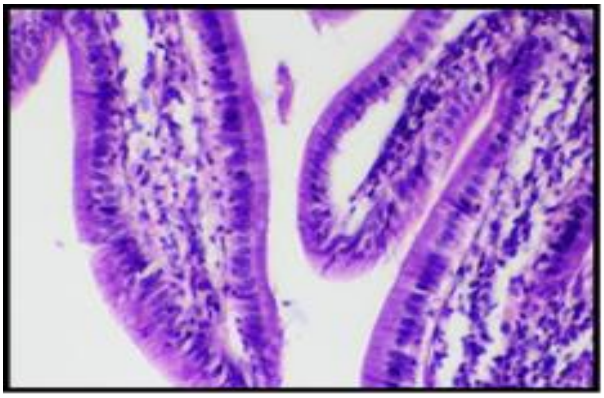


A



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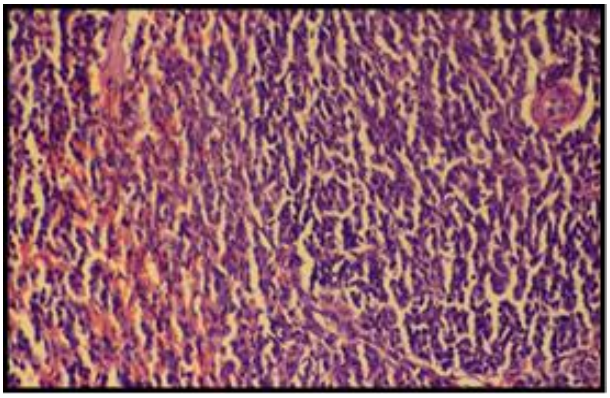
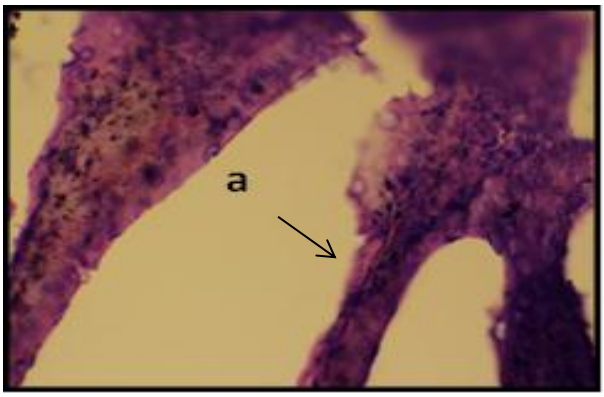
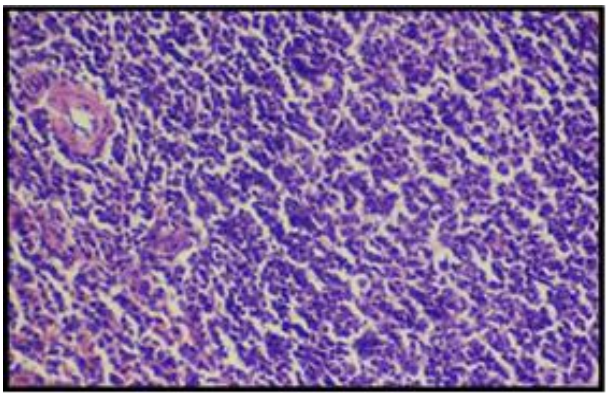


A



C_1



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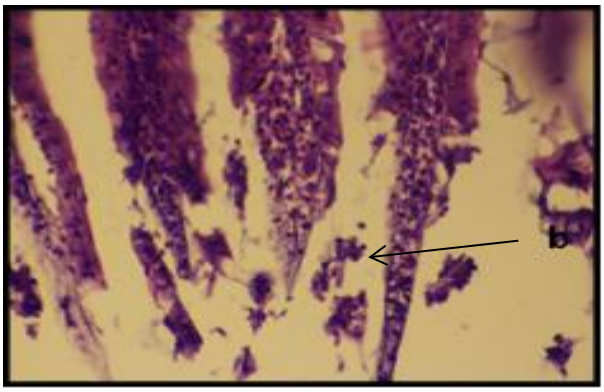
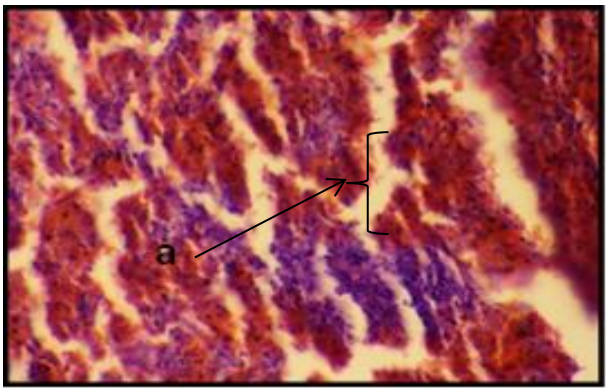


C_2

Fig. 4 **Section in the intestine tissue of male White Rat A- Control treatment. B- Aflatoxin B1+ yeast treatment . C1, C2 -only aflatoxinB1 .**
a –analysis intestinal villus b- Necrosis



C

Fig. 5 **Section in the spleen tissue of male White Rat A- Control treatment. B- Aflatoxin B1+ yeast treatment . C-only aflatoxinB1.**
a – Necrosis

DISCUSSION

In what is related to the test TLC, these results were somewhat consistent with the results of other studies [12], indicated that the highest percentage of fungal isolates produced by Aflatoxin using TLC was A. flavus, which reached 100%. As for histological study The results were similar to that of the[10]. The results of the microscopic examination of the treatment of aflatoxin B1 showed a satisfactory change in the tissues of the liver, kidney, intestines and spleen organs of male white

rats. In the kidney, glomerulosclerosis and congestion of the glomerular plexus with necrosis Hemorrhagic hemorrhage in the renal blood vessels. The histological changes of the small intestine showed the fall of the droplets and their dissolution with a concentration of inflammatory cells in the liver. An enlarged central vein appeared as well as congestion and bleeding in both the arteries and hepatic veins.

The use of *saccharomyces cerevisiae* yeast in the removal of aflatoxin toxicity from the trends in modern, as[14] indicated that giving a yeast concentration of 5.0% can to reduce the appearance of symptoms of amoxicillin B1 poisoning at a concentration of 5 parts per million (ppm). Yeast effect of *cerevisiae* may be due. In the removal of the toxicity of aflatoxin B1 to the yeast binding this poison to its cellular wall, which is made up of Oligosaccharide [15].

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