The study of toxic effects of toxic isolate Alternaria alternata in vivo of white mice and the ability of Biological and Chemical treatments in the reduction of toxicity

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Abstract. The study included the detection of the effect of the Mycotoxins of the fungal isolates of Alternaria alternata, which was diagnosed both genetically and chemically by PCR. The analysis showed that the ability of A. alternata to produce toxins, including AltratoxinII, was found in one of the isolates 7.5499 μg / ml. This concentration is used in the experiment to determine its effect on the tissues and organs of white mice. In the study, the fungus Pleurotus ostreatus was used to reduce the toxicity of the fungus A. alternata. The fungus P.ostreatus gave high tolerance to A. alternata and evaluated the effect of sequential chemical treatments CaCO₃ on reducing the auditory effect on laboratory mice. The toxic isolation of pathogenic fungi is extract into the body of the organism .The effect on the levels of liver enzymes (GPT, GOT, ALP) has reached its level (IU / L) (84, 88, 264) respectively compared to control group which reached (22, 35, 147) IU / L respectively as well . And resulted in the effectiveness of the decomposition of liver cells containing enzymes and led to the liberation of the serum, as well as the cause of changes in the tissue pathological clear in the cross section of the liver tissue, including the occurrence of hepatocytes vaculosis and the presence of bubbles in the cells walls and the expansion of liver sinuses causing Hydropic degeneration. The results of the laboratory examination of the kidney function showed significant differences in the increase in the concentration of urea and creatinine in the serum of laboratory mice treated with urea level (59 mg / dl) and creatinine (1.73 mg / dl) compared to control group (29 mg / dl) And (0.7 mg / dl) respectively, and found a clear effect within the kidney tissues leading to atrophy in the glomeruli, infiltration of the lymphocytes and hemorrhage, in addition to the degradation of urinary tubule cells affecting kidney function leading to renal failure.

Keywords: Alteratoxin II , Pleurotus ostreayus , CaCO₃ , Alternaria alternata.

1. Introduction

Fungal growth in food was not a major health problem until Mycotoxins were discovered. The presence of fungus was a defect in the technology and production defects. Today, after the discovery of mycotoxins, the presence of fungi has become a major threat to consumer health. Food is a major health problem facing people, especially in developing countries, which suffer from poor food storage conditions and are a major concern, which has called on countries to provide healthy food for food security (Makum et al, 2010).
Most people consume a small amount of mycotoxins in their daily diet and show no obvious symptoms. Eating a high concentration of mycotoxins or taking them over a long period of time can lead to serious health problems. In liver and kidney functions, immunosuppression, congenital malformations, fetal deformities, cardiovascular disease and pulmonary systems (Bhat and Vasanthi, 2003).

The mycotoxins are the strongest known toxins and cause serious diseases with minimal concentrations of less than (10ppm). The reason for the strength of mycotoxinisis that they are heat resistant to the extent that they can't be destroyed by conventional heat treatments used in manufacturing and cooking (Jamili, 2014). Are rapidly spreading from the growing fungal colonies to the food, so removing the fungi-infected parts, as many people do, does not completely eliminate the innate toxins produced in these foods. Fungi are metabolized and produce mycotoxins when appropriate conditions of temperature and humidity (Mehmood et al., 20018) and A alternata of toxin-producing fungi indicate the increased importance and severity of this fungus (Oliveira et al., 2009).

Chronic infection occurs as a result of long-term consumption of small or medium amounts of fungal toxins, resulting in distinctive symptoms, thus making it difficult to diagnose and lead to imbalance in natural immunity and acquired against infectious diseases. This mushroom is responsible for human and animal diseases (Nishimura and Kohmoto, 1993) efforts have focused on dangerous toxic species, including A. alternata, due to its spread and toxicity.

That some species of the genus Alternaria produces several types of known toxins, including toxins Alternariol monomethyl ether, Alteratoxin II, Tenuazonic acid, Alteratoxin I, Alternariol and the most important fungi produced by A. alternata, A. tenuissim, A. Cucumerina (Stefanie, 2012; Sara et al., 2018).

Mycotoxins are a type of complex chemical compound produced by fungi in physiological processes called secondary metabolites, which are produced by various fungi characterized by low-molecular-weight compounds between 69-100 dalton that don't stimulate the immune system (Greisen and Schmidt, 2007). In addition, the mycotoxins are not synthesized in the sense that their molecular structure is free of the components that drive the organism to form antibodies. If the toxin molecule binds to the effective masses that it carries with a protein molecule to acquire an immunogenicity and this condition becomes chemically different, it causes different Biologic effects (Nakheel, 2011).

The Mycotoxins affect the vital functions of the organism's bodies in several forms. The most common cause of food is acute poisoning. In the end, it leads to destruction if eaten in quantities exceeding the internationally agreed limits allowed in human food or raw materials. (Creppy, 2002).

Biologic methods are based on changes in animal tissue after being dosage with the toxin such as mice. Biologic toxicity tests include feeding laboratory animals with toxins from A. alternata isolates whose effects are to be tested on white mice. The symptoms of the poisoning and its rapid appearance depend on the person's response to the compound, the duration of the exposure, as well as the duration of absorption and removal.

When the absorption is slow and continuous for a long time, it is deposited in the tissues of the various body organs (Yekeler et al., 2001). The study aims to use mice white albino mice to detect the effect of the mycotoxins on A. alternata on tissues and organs of the organism, both in terms of tissue or physiology, and to detect the ability of fungus Pleurotus ostreayus and CaCO3 to protect the laboratory animal from the toxic effect, which in turn indicates the possibility use P. ostreayus and CaCO3 with food consumed by humans.

The fungus P. ostreayus was used in the control of biologic because of its medicinal benefits because it contains chemical compounds. The fungus is highly competitive against a large number of fungi contaminated with food, including A. alternaria, as well as the use of CaCO3 in chemical control.

2. Materials and Methods

Study of the effect of extract A. alternata in the body of the organism in vivo:
Laboratory animals: White albino mice (BALB/c alibno, species: Mouse Mus musculus), were used to conduct a study of the effect of mycotoxins on laboratory animals, which are the biologic methods that depend on the changes that occur in the tissues of the laboratory animals after its dosage of extract. Thirty laboratory mice were used for males and females (8-9 weeks), the animals were divided into six groups (5 laboratory animals for each group), including the control group, which was given only water and diet. The dilution was chosen by 0.5% of the concentration of the toxins of the A. alternata. For CaCO₃ and the extract P. ostreayus, the concentration was selected 10%. The laboratory animals were started from 16/2/2018 every two days for 21 days (Al-Khafaji, 2017 and Rajani, et al., 2012) as shown in the table below:

| Totals | Transactions | Description of Arceles Material Transactions |
|--------|--------------|---------------------------------------------|
| First  | control      | Dosage the mice of distilled water only     |
| Second | extract A. alternaria | Dosage the mice extract A. alternata orally fungus every 48 hours 0.5 ml / 30 g / day |
| Third  | Treatment of only | Dosage the mice extract P. ostreus orally fungus every 48 hours 0.5ml / 30 g / day |
| Fourth | extract A. alternata + extract P. ostreayus | Dosage the mice extract A. alternata orally fungus and after 24 hours dosage the mice extract with P. ostreus fungus 0.5 ml / 30 g / day |
| Fifth  | extract A. alternata + CaCO₃ | Dosage the mice extract A. alternata fungus orally and after 24 hours dosage the mice CaCO₃ + 0.5 ml / 30 g / day |
| Sixth  | Treatment of interaction and extract P. ostreayus + Caco3 | Dosage the mice extract A. alternata fungus orally and after 24 hours, Dosage the mice extract P. ostreus fungus and CaCO₃ orally 0.5 ml / 30 g / day |

**Animal Sacrifice:** Two days after the last procedure, the laboratory animals were sacrificed after anesthesia with chloroform and explained through an opening in the abdominal cavity.

**Figure (1):** Demonstrates how to sacrifice laboratory mouse

**Preparation of the members of the study of tissue:** The organs that were elected to study and prepare after anesthesia, sacrifice and anatomy of the animals (liver and kidneys), and was placed in a substance proven (formalin 10%).

Blood collection samples from laboratory animals blood samples from the heart were collected directly by the heart puncture of the animal using a needle (Needle gauge 23x). The blood placed in a tube of the serum called the gel tube for the purpose of studying the function of the liver function in
the analysis of GPT ,GOT and ALP studying the functions of the kidneys Renal function measured by urea and creatinine.

**Estimation of the effectiveness of Liver enzymes GPT, GOT, ALP**

The level of liver enzyme, GOT, GPT and ALP was measured in serum using RANDOX. laboratory white mice using RANDOX.

**Estimation of the level effectiveness of urea in the serum of white mice:**

The level of urea in the blood serum was estimated using the cotton produced by SPINREACT.

**Determination of serum creatinine level in white mice:**

The level of serum creatinine was estimated using the plant produced by Bio Systems.

**Histopathological study:**

After the anesthesia was completed and the process of killing and dissecting the laboratory animals was carried out, blood samples were withdrawn from the right atrium of the heart. In order to obtain as many blood samples as possible for chemical tests, the following organs were isolated ( Liver, Kidney ) , which were subsequently preserved and fixed in Formalin 10%, for the purpose of preparation for textile preparations.

### 3. Results and Discussion

*Study of the effect of A. alternata toxins in the body of the organism*

*Chemical blood tests :

**Estimation of the effectiveness of Liver enzymes GPT, GOT, ALP**

Effect of *A. alternata* toxin in the levels of liver enzymes in the blood of white mice treated by the infected level (GPT 84±2.88 , GOT 88±1.52, ALP 264±3.6 )IU/L respectively in a extract treatment of *A. alternata* only in comparison with the control group of the infection reached the results of liver enzymes examination (22±1.52 , 35±1.52 , 147.33±2.84 IU/L), Table (2), which indicates significant differences between the levels of enzymes in the studied groups, that the cause of differences due to the toxic effects of large Mycotoxins in the cells and tissues of the liver, which caused the degradation of liver cells containing these enzymes led to emancipation in the blood and high and may be due to the effect of toxin on the tissues of other organs in the body where these enzymes are present (Meerdink, 2004).

The results were agreed with (Timoz, 2015) in the study of the effect of *P. ostreayus* on laboratory animals in the treatment of *P. ostreayus* at enzymatic levels ( GPT 32±1.52 ; GOT 39±1 ; ALP 198±1.15) that the enzymes were not affected by extract and remained active and represented in the liver compared with the group control, note that the high and low level of enzymes signify the existence of a satisfactory condition in the human body or animal, did not notice in the study a change in the ratios of enzymes examined and agree with Qasim (1998), in the treatment of extract fungus *A. alternata* with CaCO3 notes a slight rise in more enzymes than it is in the treatment of fungus *A. alternata* with extract *P. ostreayus*. The control of extract *P. ostreayus* may be due to the survival of liver cells during exposure to Mycotoxins.

| Totals | GPT | GOT | ALP |
|--------|-----|-----|-----|
| Transactions | IU/L | IU/L | IU/L |

Table (2) *A. alternata* toxin effect in the level of liver enzymes in the blood of white mice


Estimation of the level effectiveness of urea and creatinine in the blood of white mice:

The effect of *A. alternata* toxins increased the concentration of urea and creatinine in serum white laboratory mice treated with extract *A. alternaria* urea level 59 ± 1.52 mg/dl and creatinine ratio 1.73 ± 0.17 mg/dl compared with control group which reached urea level 29 ± 1 mg/dl and creatinine 0.7 ± 0.1 mg/dl table (3), which indicates significant differences in the level of urea and creatinine among the studied groups and the reason for the effect of toxins on the kidney tissues, which caused the apparent defect in the glomerular renal, extensive permeability in Macrophage cells and the occurrence of hemorrhage in the kidney, exfoliation of urinary tube cells with exit in sludge between urinary tubules and which in turn affects the functions of the kidney and then leading to renal failure and reduced toxicity (Guyton, 1986). In the treatment of extract *P. ostreayus* and its effect on laboratory animals, the levels of urea and creatinine were 33 ± 1.52 mg/dl and 0.9 ± 0.05 mg/dl, respectively within normal proportions.

As for the treatment of *A. alternata* with extract *P. ostreayus* there was no increase in urea and creatinine ratios compared to the control group. As for the treatment extract *A. alternata* with CaCO₃, A slight increase in levels was observed. As for the treatment of extract *A. alternata*, extract *P. ostreayus*, CaCO₃, No increase in levels was observe it may be the reason for this is the control of extract *P. ostreayus* in maintaining the effectiveness of renal cells during exposure to Mycotoxins.

| Totals | Transactions | Blood urea mg/dl average ± standard error | creatinin mg/dl average ± standard error |
|--------|--------------|------------------------------------------|------------------------------------------|
| First  | control      | 29±1                                     | 0.7±0.1                                 |
| second | Treatment of extract *A. alternaria* | 59±1.52                                | 1.73±0.17                               |
| Third  | extract *P. ostreayus* only          | 33±1.52                                | 0.9±0.05                                |
| Fourth | extract *A. alternata* + extract *P. ostreayus* | 36±2                                   | 1.3±0.05                                |
| Fifth  | *A. alternata* + CaCO₃               | 40±1.15                                | 1.6±0.15                                |
| Sixth  | Treatment of interaction Caco3 and extract *P. ostreayus* | 38±0.51                               | 1.4±0.05                                |
| LSD₀.₀₅ |               | 3.463                                   | 0.279                                   |
| The normal range Mean Values |       | 19-34 mg/dl                             | 0.5 – 0.8 mg/dl                          |

4. Results of the study:

**Effect of *A. alternata* in some white mice**

1. Liver

The results of the microscopic examination for the diagnosis of histological sections from the white mouse liver treated with the fungus *A. alternata* extract (3) the presence of clear histological changes in the cross section of the liver tissue, including: the occurrence of hepatocytes Vacuolation
is observed, there are bubbles in the cell walls (Hydrotic degeneration). The nucleus appears small and some of the other appear free of the nucleus, enlargement in Sinusoids. The cause of the appearance of these symptoms in the liver tissue is the severe effect of the fungus *A. alternata* extract, that the cause of hydric degeneration is a disorder in the metabolism of protein and thus lead to the pooling of water inside the cells compared with the cross section that represents the control group Figure (2) For hepatocytes around the central vein, which also appear naturally, the presence of normal Sinusoids and the proliferation of Kupffer cells, hepatocytes appear normal hexagonal and central nucleus. As for the group of mice treated with the, *P. ostreatus*, Fig.(4) did not show any tissue changes in this section. The hepatocytes appeared in their size and normal shape, which is consistent with (Timoz, 2015) that the use of *P. ostreatus* and gave it to the mouse white during a specified period does not affect the tissue of the liver, and has no toxic effect as well as the existence of Kupffer cells in numbers and normal sizes and the presence of proliferation in some of the hepatocytes and divisions in which contains two nuclei.

As for the treatment group with extract *A. alternata* and extract *P. ostreatus* (5), it appears in the section a splits and collects fat drops within hepatocytes so that the cells appear swollen and the nucleus is circumferential and resembles the shape of the ring Signate-like shape, light infiltration of Macrophage cells within the liver tissue.

The toxins cause a defect in the process of lipid metabolism, especially in the liver. It is one of the first signs that appear in the organism as a result of exposure to toxins, and the nucleus is circumstantial and resembles the. This can be explained by the protective effect and extract *P. ostreatus* control of the fungus on alleviating the toxic effects of *A. alternaria*. In the treatment of extract *A. alternata* with CaCO3 in Fig.(6) hepatocytes were shown to have a binucleated hepatocyte. While Fig.(7) most liver cells appear to be normal, Some hepatocytes suffer from hydrostatic degeneration, with intracellular bubbles, proliferation of Kupffer cells, and Sinusoid appear normal.

**Figure (2):** A cross section of a mouse liver the control group (40 X H & E):
A - Note the normal radiographic arrangement of hepatocytes around the central vein which also appears naturally, B - the presence of Sinusoid normal C - proliferation of Kupffer cells, D - hepatocytes appear normal hexagonal faces and central nucleus.

**Figure (3):** A cross-section of a mouse liver represents the treatment group with *A. alternata* (40 X H & E A): A - hepatocytes Vaculation is observed, B - There are bubbles in the cell walls (Hydrotic degeneration), C - Appear small and others appear free of the nucleus, D - enlargement in Sinusoids.
Figure (4): Cross-section of mouse liver represents a treatment group with only fungal filtrate (40XH & E): A - hepatocytes are not affected, B - proliferation of Kupffer cells, C - There is a proliferation in some hepatocytes because there is a split in which there are Binucleated hepatocyte.

Figure (5): A cross-section of a mouse liver representing a treatment group with A. alternata and 40x H & E: A - Splits and collects fat drops within hepatocytes so that the cells appear swollen and the nucleus is circumferential and resembles the shape of the ring Signate - like shape, B - Light infiltration of inflammatory cells within the liver tissue.

Figure (6): A hepatic representation of a group with fungi A. alternata and CaCO3 treatment hepatocytes indicate a Binucleated expansion.

Figure (7): Varicose cross section represents a treatment with A. alternata, P. ostreatus and calcium carbonate treatment CaCO3 (40XH & E): A - Most liver cells appear to be normal, B- Some hepatocytes suffer from hydrostatic degeneration, with intracellular bubbles, C- proliferation of Kupffer cells, D - Sinusoid appear normal.

I- Kidney

The results of microscopic diagnosis of the histological sections of the kidney indicate histopathological changes in the laboratory mouse, which represents the treatment group of A. alternata Only. Fig.(9) of the two sections (A,B) of the same tissue showed extensive permeability in Macrophage cells, the presence Severe hemorrhage in the renal tissue, necrosis of the cells Renal convoluted tube, severe congestion within the renal tissue, and a clear enlargement of the renal tubules. In the control group, no changes were observed in the renal tissue in Figure (8) the natural
histological section of glomerulars, Glomerular capsule, Capsule space, and Bowman’s capsule are safe from changes, clarity of the convoluted tube Proximal and distal.

As for the cross-section of the mouse’s kidney (Fig. 10), which represents the treatment group, the biological resistance of *P. ostreatus*. No significant change occurred in the kidney tissue. The cells are normal and not degraded. Normal Renal Convoluted tubules are normal cells of the epithelium lining the Convoluted tubes as well. On the non-toxic *P. ostreatus* fungus for those mice and this is consistent with (Timoz, 2015) that came to that. The result of the examination of the tissue of the kidney the mouse, which represents the treatment group with *A. alternata* and *P. ostreatus* fungi, causes a Simple hemorrhage in the renal tubule tissue and necrosis of the cells of Renal convoluted tubules with an Extensive permeability in Macrophage (Figure 11). The histological examination of the mouse kidney, which represents the treatment group with *A. alternata*, *CaCO3* shown to be Small glomeruli. Low atrophy in some renal glomerulonephritis is observed in some renal tubules with little necrosis in the cells lining the Renal Convoluted tubules (Fig. 12). In the treatment group of (intraction) fungus, *P. ostreatus* and *CaCO3* together (Fig. 13) hemorrhage occurs in the renal tissue, the glomeruli appear large, round and natural, but regarding the Renal Convoluted tubules are shown to be lined with natural cubic cells and mild infiltration in Macrophage cells.

**Figure (8)**: A cross-section of a mouse kidney control group showing tissue
A- Glomerulars, B- Glomerular Capsule space, Bowman’s capsule, D- Proximal and Distal convoluted tubule.

**Figure (9)**: A cross-section of mouse kidney treated with fungus only (40X H & E).

**Figure (10)**: A cross-section of a mouse kidney representing the treatment group with *P. ostreatus* mushroom only (40X H & E): A - Normal Renal Convoluted tubules, B - normal epithelial cells lining the Convoluted renal tubes.

**Figure (11)**: A cross-sectional macro-mouse representing treatment with *A. alternata* and *P. ostreatus* (40X H & E): A - Simple hemorrhage of the renal tubules, B - necrosis of the cells Renal convoluted tube, C - Extensive permeability in Macrophage cells.
B: Cross-section of mouse kidney treated with
*A. alternata* fungus only (10 X H & E). A – Extensive permeability in Macrophage cells
, B - the presence of severe hemorrhage in the renal tissue, C - necrosis of the cells Renal convoluted tube,
D - severe congestion within the renal tissue ,
E- clear expansion of renal tubules .

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