



A Study of The Histopathological Effect of White Albino Mice Lung Infected with *Aspergillus Fumigatus*, Treated with Amphotericin B, And *Trametes* Spp.

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Abstract

The present study was designed to investigate the histological lesions in the lungs of white mice infected with *Aspergillus fumigatus* by intraperitoneal injection, nasal implantation, the current research also examined an attempt to treat these tissue lesions by using both of the antifungal Amphotericin B and the alcoholic extract of *Trametes* for two weeks. The results showed that *A. fumigatus* led to tissue changes represented by the degeneration, desquamation the lining respiratory epithelium of the bronchiole, thickening, rupturing of the alveolar walls, congestion of the vessels and lymphocytic infiltration, the lungs of mice that infected with *A. fumigatus* and treated with the antifungal Amphotericin B and alcoholic extract of the *Trametes* spp. showed improvement, as degeneration and desquamation of the lining of the respiratory epithelium of the trachea, the presence of dust cells, lymphocytic infiltration, in addition to the appearance of semi-normal lung tissue in another histological also were observed. We conclude that Amphotericin B and alcoholic extract of *Trametes* spp. have activity in treating the histological lesions that resulted from *A. fumigatus* infection.

Keywords: *A. fumigatus*, Aspergillosis, Lungs, *Trametes* spp., Amphotericin B.

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I. INTRODUCTION

Fungi are a broad group of eukaryotic organisms made of chitin-containing heterotrophic cells, they constitute at least a million and a half and possibly more than 3 million fungal species, thus it ranking the second organism after the animal kingdom (Hawksworth, 2012). *Aspergillus fumigatus* is the most opportunistic pathogenic fungus; it is the widespread fungus in the nature, especially in soil, water, and rotting materials. It is able to grow at high temperatures (Fang and Latgé, 2018; Ahmed and Al-Shamary, 2019). It causes a range of diseases especially in immunosuppressed persons, such as recipients of organ transfusions, those with acquired immunodeficiency disease, and leukemia its consider the more pathogenic and causes aspergillosis, this term describes a wide range of diseases invasive to allergic diseases (Kosmidis and Denning, 2015).

The lung is the first place for the fungus to enter the host's body. Infection results from inhalation of the spores that are small enough to reach the respiratory alveoli, fungus spores are present in the air and everyone inhales estimated several hundred spores each day. Moreover, these spores quickly eliminated by the immune system of healthy individuals; recently, due to the increase in using of the immunosuppressants to treat human diseases. It is estimated

that *A. fumigatus* may be responsible for more than 600,000 deaths annually with a mortality rate of (25-90%) (Dhingra and Cramer, 2017).

The use of antifungal drugs as an amphotericin B antagonist has been the treatment of choice for pulmonary fungal infections for decades (Felton *et al.*, 2014; Al-Khikani and Al-Janabi, 2019). However, the increase in antibiotic resistance and toxicity is still the main obstacles against obtaining therapeutic effects, therefore, it was necessary to search for safe and useful materials against infectious diseases such as the large fungi (mushrooms), including the fungus *Trametes* spp. (Balan *et al.*, 2018; Kundakovic and Kolundzic, 2018), which is one of the large fungi with nutritional value rich in proteins active compounds, and has important therapeutic properties such as anti-microorganism, anti-inflammatory, antioxidant, and anticancer (Cruz, 2016).

A. Lungs

Lungs are located inside the thoracic cavity which lining by a thin membrane called the parietal pleura which is reflected in the hilum above the surface of the lung to form the visceral pleura, both the pulmonary artery and the lymphatic vessel are enter, and the pulmonary vein exits from the hilum region.

The right lung consists of three lobes, while the left consists of only two lobes (Pawlina, 2015).

B. Amphotericin B

It is a natural antibiotic belonging to the group of polene, it was extracted in 1955 from the strain (*Streptomyces nodusus*) Actinomycetes, it has a high molecular weight (924.08) g/mol, its molecular formula is $C_{47}H_{73}NO_{17}$ and it is also called Fungizone (Kaminski, 2014). It binds to the sterol-containing membranes of eukaryotic cells and penetrates into the lipid bilayer, forming pore-like structures that act as ionic transport channels, this leads to an increase in the permeability of the membrane, and the potassium ion leaching out of the cell and thus its death (Grela et al., 2018).

C. Trametes

The genus *Trametes* (60) includes a known and endemic fungal species in the world, it is found annually on the wood of trees and it forms fruiting bodies with a short, solid stem consisting of hardened cells (Zmitrovich et al., 2012). It is used in the treatment of various types of cancers, respiratory, urinary and digestive tract infections, chronic hepatitis, all these activities depend on the production of several active compounds such as polysaccharide krestin and phenolic compounds such as linoleic acid, which has antioxidant and bactericidal properties, Proteoglycans possessing immunostimulating activities (Sheikh et al., 2014). This study aimed to determine the effects of experimental infection with *A.fumigatus* fungus, and the therapeutic efficacy of alcoholic extract of *Trametes* spp. as a natural antifungal, to detect the effect of antifungal Amphotericin B on lung histological structure of white mice.

II. MATERIALS AND METHODS

Fungal isolation: The fungus used in the study was isolated from auditors of tuberculosis centers in the cities of Samarra and Tikrit, and those with respiratory diseases in the fungi lab - College of Science – University of Tikrit. The fungus was grown on Potato Dextrose Agar, Sabouraud Dextrose Agar, and diagnosed by traditional methods such as direct examination by KOH and colony morphological examination.

A. Preparation of *Aspergillus fumigatus* fungal suspension

Slanted cultures of the fungus under the study were taken at the age of 7 days and added to 5 ml of sterile distilled water with Tween 80 added at a concentration of (1)%, the tubes were stirred vigorously in order to separate the fungus spores well and filtered the resulting mixture using (Whatman. No.1) filter paper under aseptic conditions, and the filter representing the fungal suspension was transferred to a sterile vial, and then the number of spores was calculated by means of a (40X) blood cell count slide with a light microscope according to the following equation:

Number of spores per milliliter= average number of spores x 4 squares x 50

The fungal suspension at a concentration of (1×10^6) was used to induce infection in the experimental animals (Mohammed et al., 2017).

B. Animals and design of experiments

The study included (25) male white mice brought from Animal center- College of Veterinary Medicine- University of Tikrit . The Balb / c strain at the age of (6-8) weeks, and their weights ranged between (25-30) gm, they kept in Animal center- Pharmacology, Samarra Drugs Industries. Animals were randomly assigned to different groups (5 mice per group) they were:

- 1- The first group: The animals dosed (0.1) ml of normal solution (IP) and once daily for two weeks.
- 2- The second group: The animals were injected inside the peritoneum with (106) cells/ ml of the fungal suspension of *A. fumigatus* once only.
- 3- The third group: The animals were injected by the method of nasal implantation (instillation) with (106) cells/ ml of fungal suspension *A. fumigatus* once only.
- 4- The fourth group: This group include mice that were dosed with fungal suspension and treated with the antifungal Amphotericin B at a concentration of (1) mg/ kg orally after one week of infection.
- 5- The fifth group: This group of mice dosed with fungal suspension and treated with alcoholic extract of *Trametes* spp. at a concentration of (100) mg/ ml orally, for one week after infection.

All groups were immunosuppressed by subcutaneous injection of cortisone (250) mg/ kg two days before infection and a second dose two days after the start of treatment, except for the control group, to prevent secondary bacterial infection, Cefotaxime (50) mg/ kg was administered subcutaneously to all groups from the day of the first dose of the cortisone until the last day of treatment, and after the end of the experiment period, the animals were killed and dissected at the (15th) day, and the organ to be studied was taken.

C. Histological sections preparation

Histological Sections were prepared based on the method of Al-Hajj (2010), which included:

- 1- Samples Fixation: After the removal lung, it was transferred directly to the fixation medium, where the study samples were placed in plastic bottles containing formalin as a fixation medium at a concentration of (10%) (90 ml tap water + 10 ml of 40% formaldehyde) and kept for 24 hours.
- 2- Washing: After (24) hours of preserving the samples, they were washed with running tap water for (20-30) minutes to remove the fixative residue.
- 3- Dehydration: Samples were passed with a series of rising concentrations of ethyl alcohol (100%, 90%, 80%, 70%) to gradually remove water from the tissues, and the duration of each pass was (30) minutes, and the absolute alcohol step ((100%) was repeated twice to complete the final removal of water.

- 4- Clearing: To make the samples more transparent and completely withdraw alcohol from them, after removing the water from them, they were transferred to a pure xylene medium in two stages for a period of (15) minutes for each stage.
- 5- Infiltration: Samples were placed in paraffin wax, a melting point of (58) C°, where they were impregnated in three transfers at a rate of (60-30) minutes per transformation to ensure that the wax penetrates evenly into the tissue.
- 6- Embedding: The samples were embedded in the wax used for impregnation, as it was poured into special metal molds, and with forceps, the samples were carried and placed in it.
- 7- Trimming and Sectioning: After trimming the sample wax molds with a sharp blade, they were cut with a rotary microtome (4) micrometer thickness to obtain lung tissue sections. Then the clips were loaded onto glass slides using Mayer's albumen, with egg whites added to glycerol in equal volumes each. They were mixed in a small beaker and then the mixture was filtered several times using several layers of medical gauze and the filtrate was collected in an airtight bottle and placed in the refrigerator until use after adding (1) gm of thymol crystals to prevent rotting.
- 8- Staining: The wax was removed from the sections using hot xylol (45) C, then the glass slides loaded with the clips were passed in two changes of xylol for a period of (3) minutes for each change until the wax was completely removed, slides were passed with a series of descending concentrations of ethyl alcohol (50%, 70%, 90%, 100%) for a period of (5) minutes for each concentration, after which the slides were washed with distilled water for a period of (5) minutes as well, passed with the hematoxylin dye for (3) minutes, then they were washed with running water for (5) minutes, and then passed with distilled water for (3) minutes, passed with an alcoholic eosin dye solution for about (5) minutes, then were washed by immersing them once or twice in ethyl alcohol at a concentration of ((70%)), and then passed on with increasing concentrations of ethyl alcohol (100%, 90%, 70%, 50%) for a period (5) minutes each with the absolute concentration step repeated twice, and transferred to xylol for a period of (5) minutes for the purpose of purification.
- 9- Mounting: The dyed tissue sections were covered with Xylene Disterene Plasticizier (D.P.X) loading medium, then a glass cover was placed on them and left on a hot plate at a temperature of (37) C. for the purpose of drying, and then, they were examined and photographed under a microscope with a magnification power of (40X).

III. RESULTS

A. Control group

The results of control groups showed normal composition of the lung tissue containing the alveolar sacs, the alveoli, the respiratory trachea, and the blood vessels, (Figure, 1).



Figure 1. A micrograph of a mouse lung from the control group, in which the normal appearances of the alveolar sacs, pulmonary alveoli, as well as the respiratory bronchiole below the blood vessel were observed. (H&E, 40 X).

B. Group of mice injected (IP) with *A. fumigatus*

The results showed the occurrence of degeneration, desquamation of the lining of the respiratory epithelium of the trachea, expansion, thickening of the alveoli walls, and congestion of blood vessels and lymphocytic infiltration. (Figure, 2).

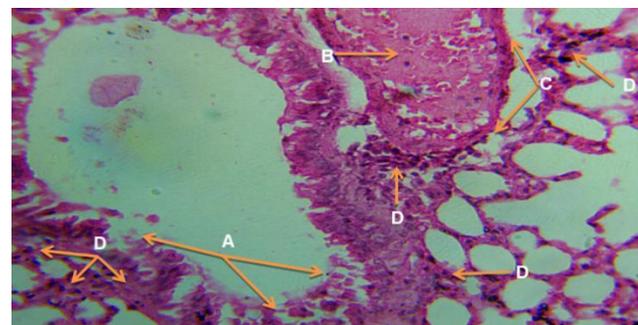


Figure 2. A micrograph of the lung of a mouse from the infected group with *A. fumigatus* by intraperitoneal injection, in which degeneration, desquamation of the lining of the respiratory epithelium of the bronchiole (A), blood vessel dilatation and congestion (B), Thickening in the wall of blood vessels (C), lymphocytic infiltration in tissue pan (D) (H&E, 40 X).

C. Group of mice infected with *A. fumigatus* inside the nasal implantation

The results showed the occurrence of degeneration, desquamation of the lining of the respiratory epithelium of the trachea, hemolysis of blood vessels and thickening of their walls, the presence of serous exudation, lymphocyte infiltration, in addition to the blurring of the features of the lung tissue (Figure 3).

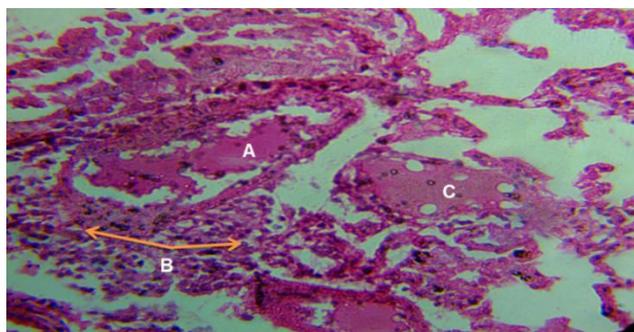


Figure 3. A micrograph of a mouse lung from the infected group with *A. fumigatus* by nasal implantation (instillation) method, in which hemolysis is observed in the blood vessels (A), acute lymphocytic infiltration (B), serum exudation inflammation (C), and general pulmonary blurring (H&E, 40 X).

D. Group of mice infected with *A. fumigatus* and treated with Amphotericin B one week after infection

The results showed the occurrence of degeneration, desquamation of the lining of the respiratory epithelium of the trachea, hemolysis of a blood vessel, as well as the lymphatic cell infiltration, and in another histological sections, the almost normal appearance of lung tissue. (Figures 4, 5).

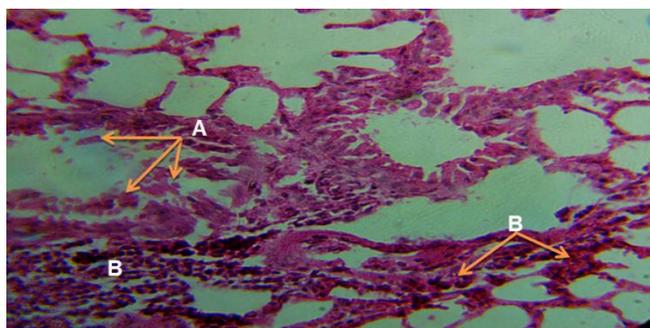


Figure 4. A micrograph of a mouse lung from the infected group with *A. fumigatus* and dosed with Amphotericin B a week after infection, in which degeneration, desquamation of the lining of the respiratory epithelium of the trachea (A), lymphocyte infiltration (B). (H&E, 40 X).

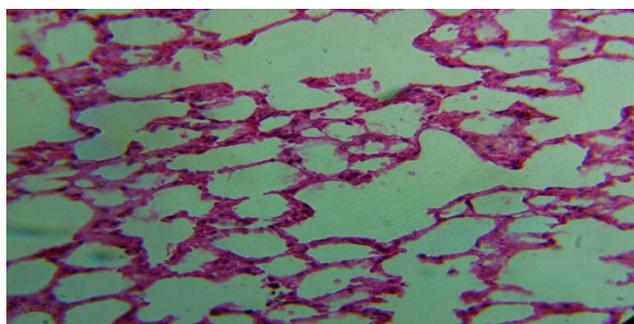


Figure 5. A micrograph of a mouse lung from the infected group with *A. fumigatus* and dosed with Amphotericin B a week after infection, in which almost normal appearance to all lung tissue. (H&E, 40 X).

E. Group of mice infected with *A. fumigatus* and treated with alcoholic extract of *Trametes* spp. one week after infection

The results showed the occurrence of degeneration, desquamation of the lining of the respiratory epithelium of

the trachea, in addition hemolysis in a blood vessel, lymphocytic infiltration, and the appearance of semi-normal, normal lung tissue in another histological sections. (Figure, 6,7).

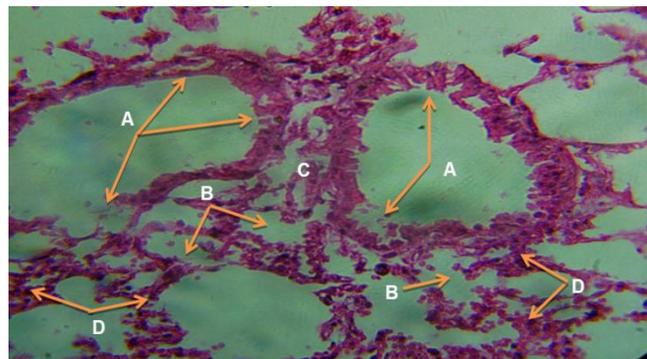


Figure 6. A micrograph of the lung of a mouse from the infected group with *A. fumigatus* and dosed with alcoholic extract of *Trametes* spp. a week after the infection, degeneration, necrosis of the lining of the respiratory epithelium of the trachea (A), Rupture of the walls of the alveoli (B), Fibrous necrosis inter bronchioles (C), lymphocytic infiltration (D). (H&E, 40 X).

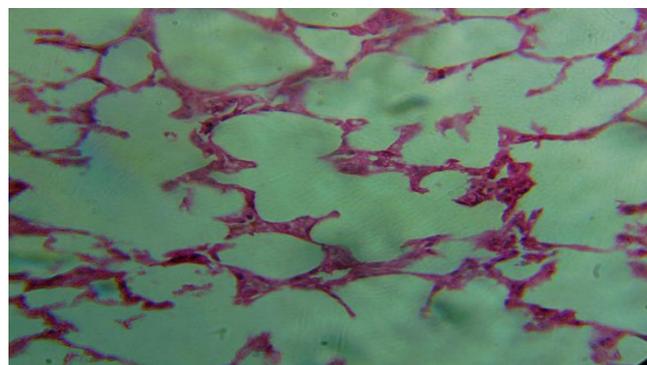


Figure 7. A micrograph of the lung of a mouse from the infected group with *A. fumigatus* and dosed with alcoholic extract of *Trametes* spp. a week after the infection, an almost normal appearance of all lung tissue. (H&E, 40 X).

IV. DISCUSSION

The lung histological examination of mice that infected by *A. fumigatus* showed the degeneration, desquamation of the lining of the respiratory epithelium of the trachea due to the virulence factors that secreted by fungus which make it to induce infection, such as the enzyme protease that stimulates the degradation of the basic synthetic proteins of the basal membranes, in addition to elastin and laminin in the host's lung (Chen *et al.*, 2000). Thickening of the alveoli walls, congestion of blood vessels which may be due to the local reduction in the venous blood flow as a result of blockage in the blood vessels supplying the part where the congestion occurred and lymphocytic infiltration (Al-Khateeb *et al.*, 1989), as well as helvonic acid, fumigillin, which facilitates its growth and survival in the lung, as the latter slowing the movement of cilia cells of the respiratory epithelium and thus facilitates the survival of spores. The ability of this fungus to produce phospholipid-degrading enzymes for cellular plasma membranes facilitates penetration of the vesicular expanses lined with the surfactant consisting of (80%) phospholipids

(Minji, 2013). These results were agreement with other studies such as Al-Qaisi and Khalid (2005), Ibrahim *et al* (2006), Al-Jubouri (2015), and Yassein (2020) were record the incidence of emphysema, thrombosis, cellular infiltration, thickening of the alveolar walls duo to congestion of capillaries.

The lung mice that infected with *A. fumigatus* and treated with Amphotericin B, and alcoholic extract of *Trametes* spp showed the degeneration, desquamation of the lining of the respiratory epithelium of the trachea, hemolysis of a blood vessel, as well as the lymphatic cell infiltration, which may be duo to the endotoxins and exotoxins that secreted from the fungus, or the side effects of this antifungal Amphotericin B, and in another histological sections, the almost normal appearance of lung tissue, Amphotericin B produces free radicals within the fungi, resulting in oxygen depletion and super antioxidants, which in turn affect the cellular pathways of the fungi; which able to induce inflammatory mediators such as IL-6, TNF- α , nitric oxide, and prostaglandins from mouse and human immune cells (Mesa-Arango *et al.*, 2012). Leenders *et al.*, (1996) record the ability of Amphotericin B to prevent the spread of *Aspergillus* from the affected left lung to the right one, liver, and spleen. Several studies have shown the use of *Trametes* to treat various cancers such as lung, stomach, and colon (Knezevic *et al.*, 2018; Roca-Lema *et al.*, 2019) because it contains Polysaccharide Krestin (PSK), Polysaccharide Peptide (PSP), when given to mice with cancer, it led to a decrease in the size and number of the resulting tumors. It contains many active compounds such as saponins, tannins which have the antimicrobial activity, stimulates the B and T lymphocytes proliferative, macrophages, and monocytes, and increase the antibodies and interferones secretion.

V. CONCLUSIONS

A. fumigatus fungus capable of causing histopathological lesions in white mice lungs, and antifungal Amphotericin B and alcoholic extract of *Trametes* spp. have proven effective in treating these histological lesions.

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