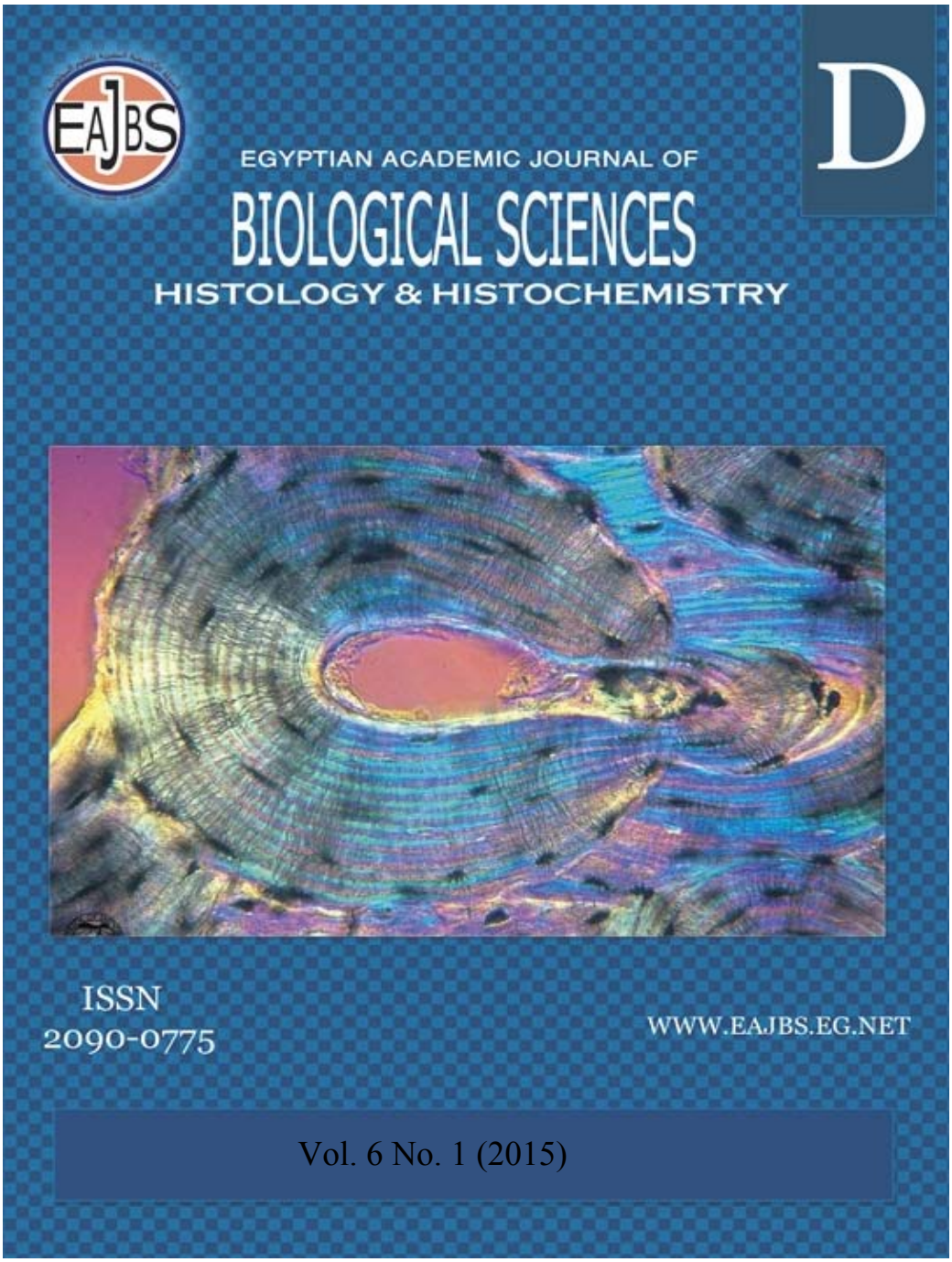

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Histopathological Changes in lung of Rabbit after exposure to Allergic fungi

Sanaa HuseeinMohamad[1], AhammdHamd[2], Batol ImranDheeb[3]

1- Kirkuk University ,College of Science, Biology Department
2- Kirkuk University, College of Education for Pure Science, Biology Department.
3- Iraqia University, College of Education, Biology Department

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ABSTRACT

Histological sections from lungs of rabbits inhaled with fungus species as *Aspergillus niger*, *Candidia albicans*, *Fusarium* spp., *Aspergillus fumigates*, *Aspergillus flavus* and *Alternaria spp,* showed histological changes represented by severe hematoma and inflammatory cells infiltration and stenosis in the alveoli.

INTRODUCTION

Primarily, infection results from inhalation of the airborne spores, which are small enough to reach the alveoli of the respiratory system. Most patients suffering from aspergillosis have an impaired immune system that is often evoked by leukemia, neutropenia or after prolonged treatment with steroids, such as solid organ transplantation patients (Ellis, 1999). The mortality rate of aspergillosis among these patients lies between 30 to 90% (Ellis, 1999; Latgé, 1999).The primary route of human infection is *via* the inhalation of these airborne spores,, followed by conidial deposition in the bronchioles or alveolar spaces. In healthy individuals, conidia that are not removed by mucociliary clearance encounter epithelial cells or alveolar macrophages, the primary resident phagocytes of the lung. Alveolar macrophages are primarily responsible for the phagocytosis and killing of *A. fumigates* conidia as well as the initiation of a proinflammatory response that recruits neutrophils (one type of polymorphonuclear cell (PMN) to the site of infection. Conidia that evade macrophage killing and germinate become the target of infiltrating neutrophils that are able to destroy hyphae (Denning, 1998).

MATERIALS AND METHODS

Histopathological Studies:

Animals

Only 28 intact and healthy adult male Albino rabbits are used aged 3-4 months; of 3 - 3.2 gm body weight. The rabbits obtained from the Voluntary College of Mosul University, and were kept in their ventilated wooden cages. The dimensions of the cages were (1.5x1.5x1.0 meter) and were cleaned on regular daily using detergent while the cage ground was sieved to allow elimination of urine and waste.

Maintenance of the experimental animals:

The 28 New Zealand male white rabbits were used to determine the pathogenic effects of fungi to lower respiratory tract, reared at an optimal room temperature ranged between 22-25°C at 60% - 70% humidity.

Animals were fed on locally prepared diet which formulated from natural ingredients suitable for growing maintenance.

Experimental Design:

All groups were inoculated by inhalation using swab from fungi into nasal twice daily.

The total 28 rabbits were divided into 7 groups, each of 4 rabbits as follows:

Group 1: Control animals without exposure.
Group 2: Animals were exposured with *A. niger*.
Group 3: Animals were exposured with *C. albicans*.
Group 4: Animals were exposured with *A. fumigatus*.
Group 5: Animals were exposured with *A. flavus.*
Group 6: Animals were exposured with *Fusarium* spp.
Group 7: Animals were exposured with *Alternaria* spp.

Histopathological examination:

All rabbits were sacrificed 2 weeks after the treatment. Lungs were dissected out and organs were fixed inside plastic containers filled with 100 mL of 10% formalin. Organ samples were dehydrated in progressively descending alcohol started with 100% alcohol; followed by embedding in paraffin and cut into section of 4-5 µm thickness and stained with haematoxylin and eosin (H&E) (Luna, 1968) as follows:

1- The sections were de-paraffinized by dipping them into hot xylene for 5-10 minutes (twice).
2- Descending graded alcohols (100%, 90% and 70%) were used for dehydration (5 minutes each).
3- The section was stained with haematoxylin for 2-3 minutes.
4- Washed with tap water for 5-10 minutes.
5- Differentiated a few second in 1% acidic alcohol (1% HCl in 70% alcohol) until the section looks red, usually 5-15 seconds.
6- Washed well in running tap water for 3-5 minutes to remove the acid.
7- Stained in 1% eosin for 10 minutes.
8- Graded alcohol (70%. 90%, 100%, and 100%) was used for dehydration, 5 minutes in each alcohol grade.
9- The section was cleared by xylene through three changes (15, 15 and 30 minutes).
10- Mounting using Disterne-Plastcizer Xylene (DPX) and cover slips.
11- For microscopically examination, the slides were examined at 200x magnifications using an optical microscope.

RESULTS AND DISCUSSION

Histological study

Control animals

The cross sections taken from the lungs of control animals showed a healthy structure of alveoli, normal septa with normal form bronchioles (Fig. 1).

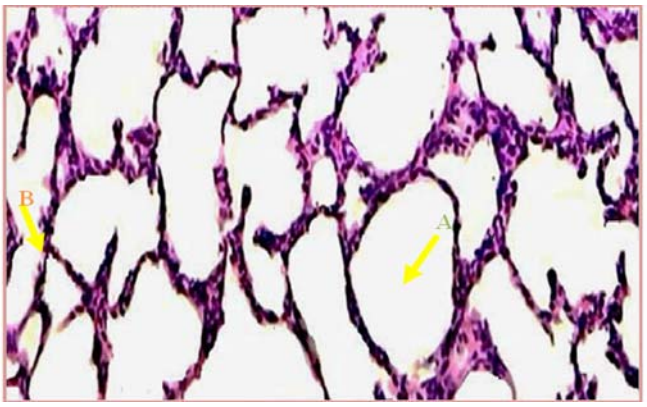

Fig. 1: Histological Section in the normal lung shows A-normal structure of alveoli; B- the alveoli wall and normal lung tissue (H&E stain, 100x) .

Morphological changes:

Lunges of rabbits infected with fungi *A. fumigatus*, A. *niger*, *A. flavus*, *C. albicans*, *Alternaria* and *Fusarium* showed severe hemorrhage (Figs. 1, 2, 3, 4, 5, 6).

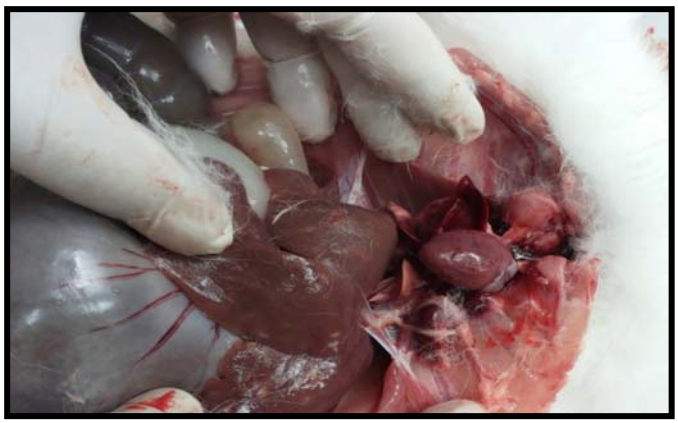

Fig. 2: Lungs of *A. fumigatus* demonstrates severe hemorrhage (arrow).

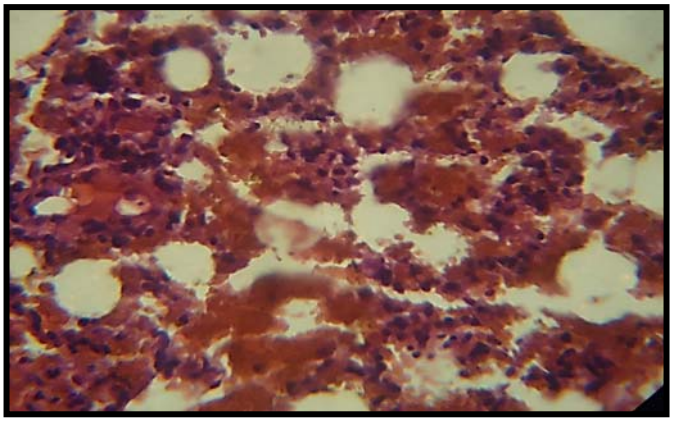

Fig. 3: Lung of *A. fumigatus* demonstrates fibroblast (fb), pneumocyte (blue arrow), Lymphocytes (L) and congested blood (co) (H&E 400X).

A. fumigatus

A section in the lung infected with *A. fumigatus* demonstrates damaged alveoli, blood congestion in the intercellular spaces and infiltration of WBC. Pneumocytes could be seen in the alveolar walls but the patches of the congested blood had filled the intercellular spaces obscuring the fibroblasts (Fig. 4)

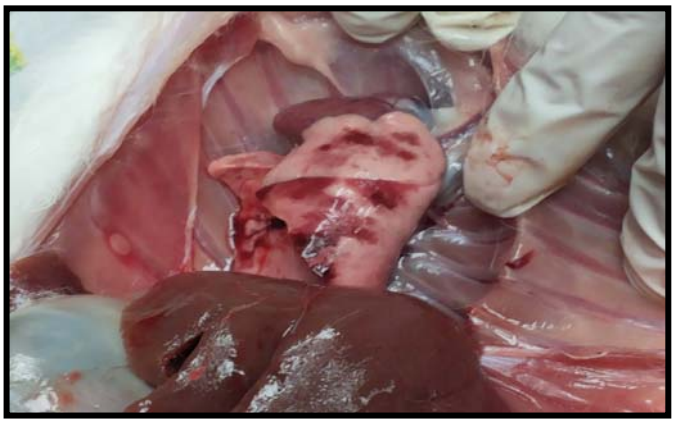

Fig. 4: Lung of*A.niger* demonstrates hemorrhage (arrows).

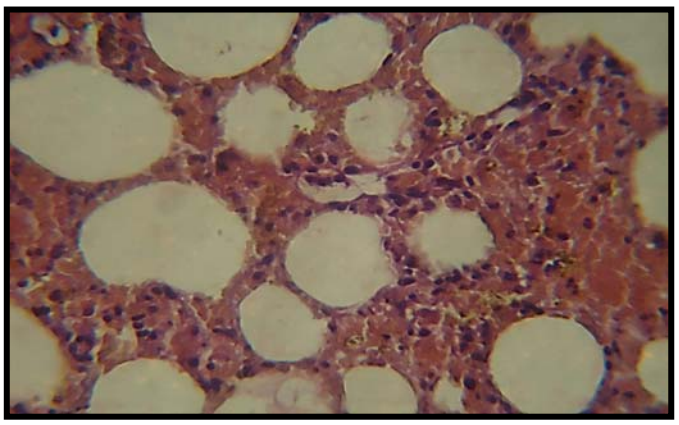

Fig. 5: Section in the lung of *A. niger* illustrates a thickened alveoli wall (tw), fibroblast (fb), pneumocyte (HC), lymphocytes (L) and congested blood (cb) (H&E 400X).

A. niger

A cross section in lung of a rabbit infected with *A. niger* demonstrates a thickened alveoli wall with congested blood patches with accumulation of immune cells filling the intercellular spaces of the pulmonary tissue. There seem to be severe inflammatory cells infiltration in (Fig. 6).

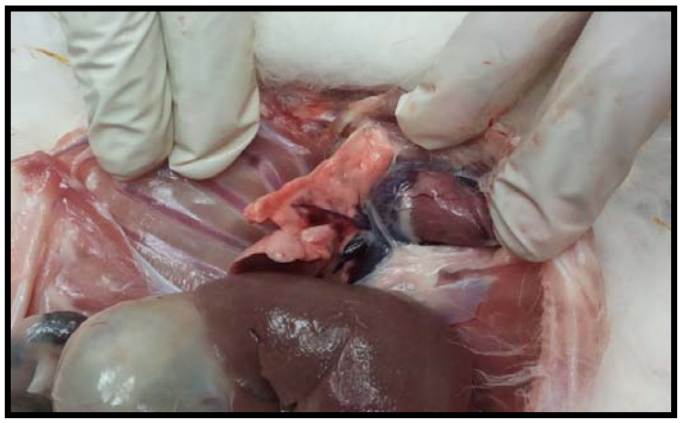

Fig. 6: Lung of *A. flavus* demonstrates severe hemorrhage (arrow).

A. flavus

Pulmonary tissues of rabbits infected with *A. flavus* had almost similar architecture of those infected with other fungi (Fig. 7). Tochigi*, et al*., (2009) reported the effects of an *Aspergillus* species *(Aspergillus flavus)* on the lung tissues as an acute hemorrhage in the septa due to destruction of blood vessels with a moderate to severe infiltration of mononuclear cells in the lungs. Similar results are found in the present results. The *Aspergillus fumigatus* had also caused an extensive infiltration of mononuclear cells to the pulmonary tissue mice (Murayama, *et al*., 2009). The effect of *Aspergillusniger* and *Aspergillus flavus* on the pulmonary tissues, had led to congestion and mild perivascular edema with thickening and hyalinization of the blood vessel walls in all animals while peri-bronchial and perivascular lymphocytic infiltration were detected in each individual animal (Jayabarathi and Mohamudha, 2010). Another study on birds demonstrated that *Aspergillus* species, particularly *Aspergillus niger* and *Aspergillus flavus* had led to infiltration of heterophils, lymphocytes and monocytes in the alveoli walls (Atkinson and Brojer, 1998). Results of these two studies are in agreement with this study.

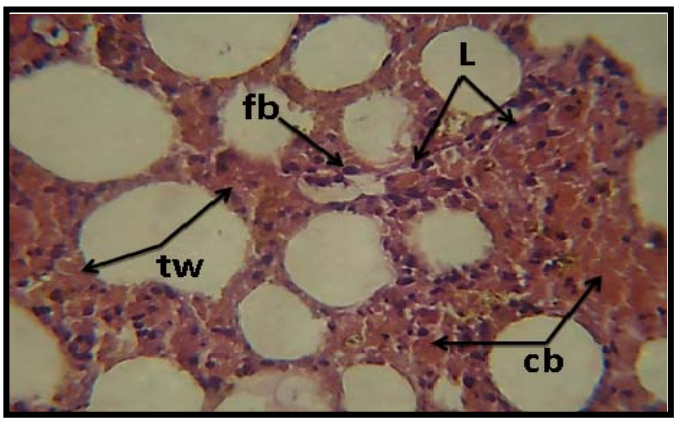

Fig. 7: Section in the lung of *A. flavus* illustrates a thickened alveoli wall (tw), fibroblast (fb), pneumocyte (HC), lymphocytes (L) and congested blood (cb) (H&E 400X)

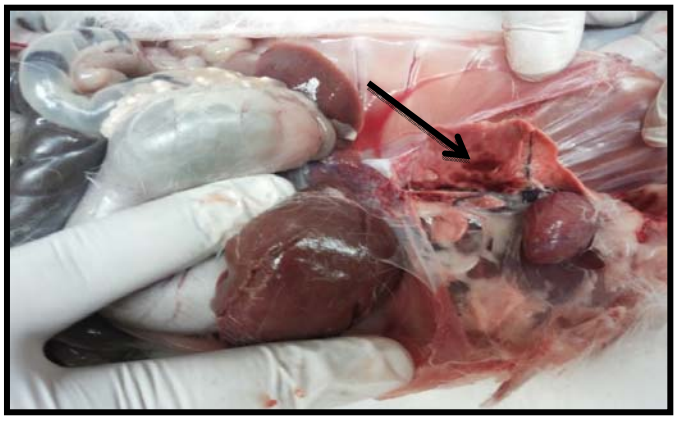

Fig. 8: Lung of *C. albicans* demonstrates severe hemorrhage (arrow).

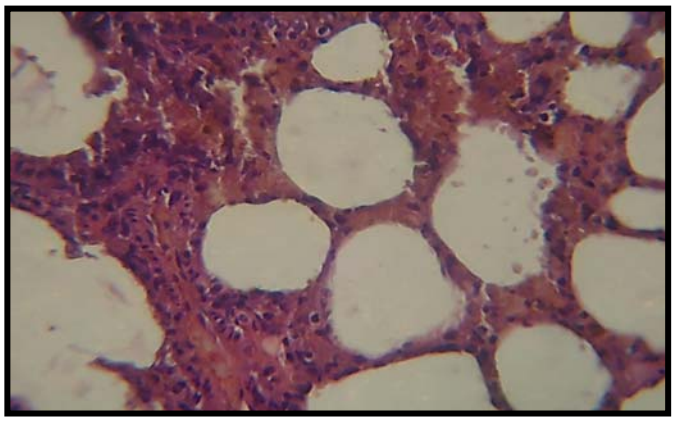

Fig. 9: Section in the lung of *C. albicans* the thickened alveoli wall (tw), fibroblast (fb), pneumocytes (L) and congested blood (cb) (H&E 400X).

C. albicans

Various lesions in pulmonary tissues i.e. thickening of alveoli walls with congestion of blood between the walls, mild inflammatory cells infiltration and accumulation of pneumocytes and lymphocytes appeared in most sections (Fig. 10).

An extensive interstitial pneumonic lesion characterize by thickening of alveolar walls due to congestion of alveolar capillaries and infiltration of mononuclear cells causing narrowing of alveolar lumen. Also there was a per-bronchial lymphoid tissue hyperplasia and in advance cases a pulmonary fibrosis infected with *Candida albicans*

were found in the pulmonary tissues in white mice (Inoue, *et al.,* 2009; Al-Jeboori and Al-Harreery, 2013). Both studies are in agreement with the present study.

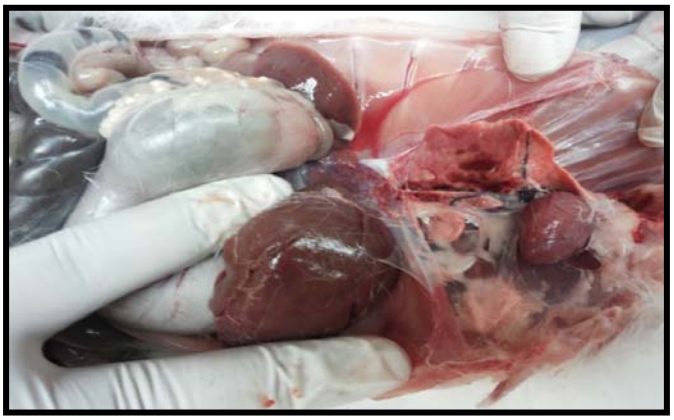

Fig. 10: Lung of *C. albicans* demonstrates severe hemorrhage (arrow).

C. albicans

Various lesions in pulmonary tissues i.e. thickening of alveoli walls with congestion of blood between the walls, mild inflammatory cells infiltration and accumulation of pneumocytes and lymphocytes appeared in most sections (Fig. 11).

An extensive interstitial pneumonic lesion characterize by thickening of alveolar walls due to congestion of alveolar capillaries and infiltration of mononuclear cells causing narrowing of alveolar lumen. Also there was a per-bronchial lymphoid tissue hyperplasia and in advance cases a pulmonary fibrosis infected with *Candida albicans* were found in the pulmonary tissues in white mice (Inoue, *et al.,* 2009; Al-Jeboori and Al-Harreery, 2013). Both studies are in agreement with the present study.

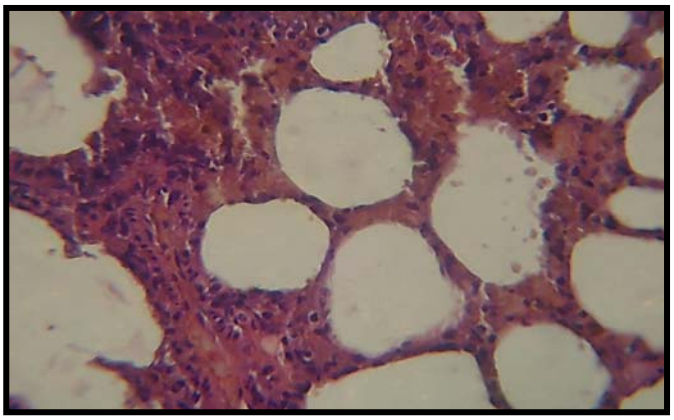

Fig. 11: Section in the lung of *C. albicans* the thickened alveoli wall (tw), fibroblast (fb), pneumocytes (L) and congested blood (cb) (H&E 400X).

Alternaria:

A cross section in lung of a rabbit infected with *A. alternaria* demonstrates a thickened alveoli wall with congested blood patches with immune cells filling the intercellular spaces of the pulmonary tissue. There seem to be severe inflammatory cells infiltration in (Figs. 12&13). The effect of *Alternaria alternate* on the lung tissues in white mice infected with *Alternaria alternate* had led to thickening of the septa and accumulation of infiltration mononuclear cells around the vessels and the bronchioles (Havaux, *et al*., 2005). This is again in an agreement with the results

of the present study. In horse *Alternaria* lead to infiltration mononuclear cells in the alveoli walls and around the vessels and the bronchioles (Beech, 1975). These results are similar to the present study.

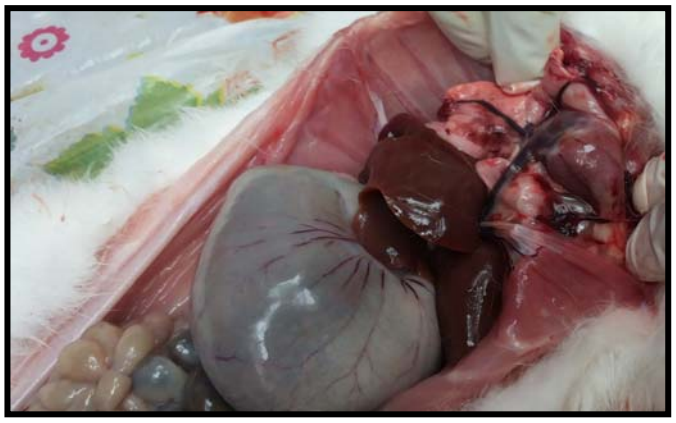

Fig. 12: The exposed lungs of *Alternaria* demonstrates severe hemorrhage (arrow).

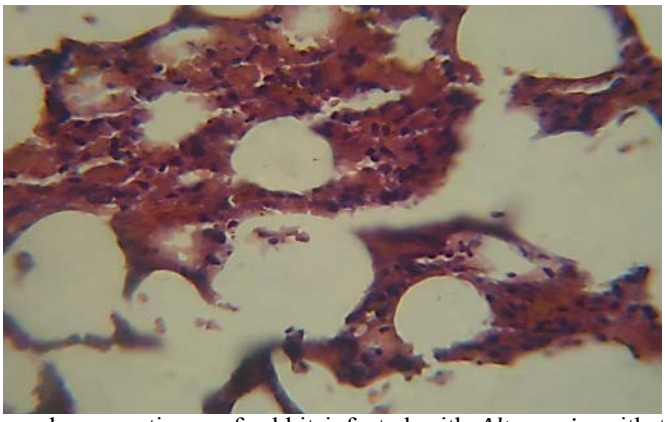

Fig. 13: A section in pulmonary tissue of rabbit infected with *Alternaria* with thickening of alveoli walls (tw), Lymphocytes (L) and congestion (co) (H&E 400X).

Fusarium

Considerable damage to the alveoli walls with congestion of blood between the walls were detectable in most sections of lungs infected with *Fusarium*. In addition, severe and clear inflammatory cells infiltration of lymphocytes and pneumocytes were visible between the cells of the septa with fibroblasts (Figs. 14&15).

The effect of *Fusarium kyushuense* on lung tissues in white mice infected *with F. kyushuense* caused infiltration of plasma cells and lymphocytes in the alveoli walls Koichi, *et al., (*2000) while it led to infiltration of mononuclear cells in the alveoli walls and widening of interlobular septa of weaned piglets Dilkina, *et al., (*2000). Both studies are in agreement with the results of the present study.

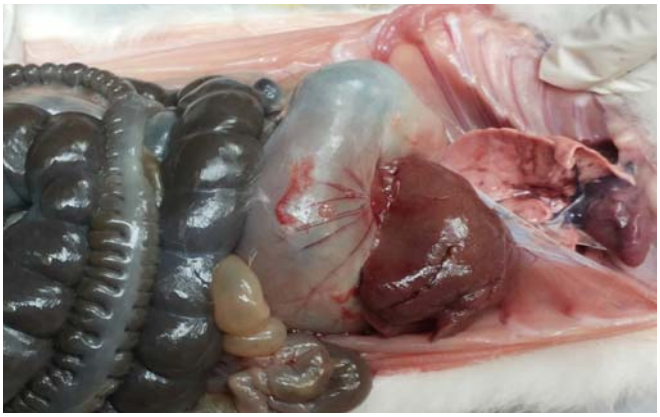

Fig.14: Lung of rabbit infected with *Fusarium* group showed severs hemorrhage (arrow).

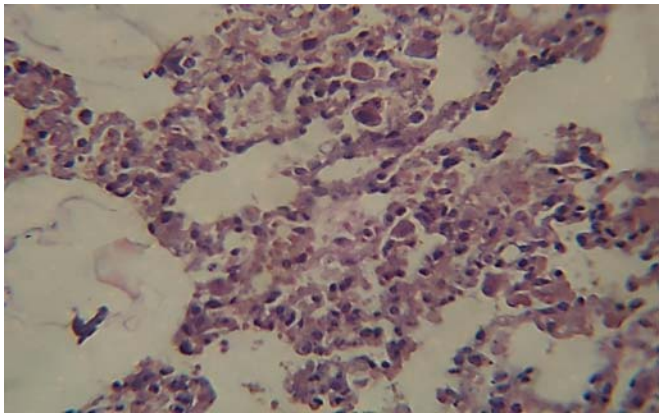

Fig. 15: A section in the lung of rabbit infected with *Fusarium* illustrates a thickened alveoli walls (tw), fibroblast (fb), pneumocytes (HC) and Lymphocytes (L) (H&E 400X).

CONCLUSION

It is concluded that:

Various histological changes appeared in the lung of rabbits. The *Aspergillus fumigatus* had caused an extensive infiltration of mononuclear cells to the pulmonary tissue rabbits. The effect of *Aspergillus niger* and *Aspergillus flavus* on the pulmonary tissues, had led to congestion and mild perivascular edema with thickening and hyalinization of the blood vessel walls in all animals while peri-bronchial and perivascular lymphocytic infiltration were detected in each individual animal.

The effect of *Candida albicans* on pulmonary tissues had led to thickening of alveoli walls with congestion of blood between the walls, mild inflammatory cells infiltration and accumulation of pneumocytes and lymphocytes appeared in most sections. *Alternaria* leads to infiltration mononuclear cells in the alveoli walls and around the vessels and the bronchioles. *Fusarium* caused damage to the alveoli walls with congestion of blood between the walls in most sections of lungs infected. In addition, severe and clear inflammatory cells infiltration of lymphocytes and pneumocytes were visible between the cells of the septa with fibroblasts.

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