

Acute inflammatory responses and histological changes for *Stachybotrys chartarum* fungus in the lungs and nose for infant white mice

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Abstract

Stachybotrys chartarum in one of fungi that cause respiratory system infection to human and animals therefore this study it done to evaluation histological changes in some respiratory organs such as nose and lung induced by this fungus suspension. Detection of *Stachybotrys chartarum* fungus confirmed by cultured samples which collected from baths floor on potato dextrose agar in room temperature for seven days. Then the suspension of positive samples of this fungus diluted by phosphate buffer saline and counted the spores by using hemocytometer before used experimentally in lab animals (Balb/C mice) to study histological changes in respiratory system . Twelve mice used in this study divided into two groups. first group consist of six mice induced with 0.5 ml from one positive sample of fungus suspension contain 2×10^4 spore / mm^3 (for month : one dose daily) according to count of hemocytometer to evaluate some histopathological changes in nose and lung .Second group induced with 0.5 ml from phosphate buffer saline only. The results revealed that the nose of mice injected with 0.5 ml contain 2×10^4 spore / mm^3 from *Stachybotrys chartarum* fungus suspension cause focal superficial necrosis of the respiratory epithelial cells with acute inflammatory cells and infiltration of the mucosa .while the lung of group mice treated with same concentration of fungus has interstitial pneumonia with thickening of inter alveolar space due to infiltration and chronic inflammatory cells especially mononuclear cell.

الاستجابات الألتهابية الحاده والتغيرات النسيجية لستاكي بوتريس كارتارم في الرئتين والأنف بالنسبة للفئران البيضاء الرضع

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الخلاصة :

ستاكي بوتريس كارتارم احد الفطريات التي تصيب لجهاز التنفسي لذ لك نفذت هذه الدراسة لتقييم التغيرات النسيجية لبعض أعضاء الجهاز التنفسي المستحثة بمعلق الفطر أعلاه . للتحري عن الفطر قمنا بزرع العينات المأخوذة من أرضية الحمامات على وسط دكستروز البطاطا في درجة حرارة الغرفة ولمدة سبعة أيام . تم

استخدام معلق العينات الموجبة للفطر تجريبيا على الحيوانات المختبرية (الفئران) لدراسة تغيرات الجهاز التنفسي . اثني عشر فأرا أستخدم في هذه الدراسة قسمت الى مجموعتين . المجموعة الأولى تكونت من ستة فئران أستحثت بـ 5. مل من معلق الفطر الموجب والذي يحتوي على $2 \times 10^4 / \text{mm}^3$ بوع (لمدة شهر بواقع جرعة واحدة يوميا) بناء على عد مقياس عداد الخلايا لتقييم التغيرات النسيجية في الأنف والرئة . المجموعة الثانية استحثت بـ 5 و . مل من المحلول الدائري للفوسفات . تشير النتائج ان الأنف عانى تنخر سطحي بؤري بسبب تركيز معلق الفطر اعلاه . اما الرئة فتسبب معلق الفطر في احداث ذات الرئة البيئي وزيادة في سمك المسافات الحويصلية إضافة الى ترشيع والتهاب مزمن في الخلايا وخصوصا الخلايا وحيدة النواة.

Introduction:

Stachybotrys chartarum is a fungus that has become notorious as a mycotoxin producer that can cause animal and human mycotoxicosis (Nelson,2005).

S. chartarum called a black mold that produces its conidia in slime heads. found in soil and grain, but the mold is most often detected in cellulose-rich building materials from damp or water-damaged buildings (Samson ,2010) It requires high moisture content in order to grow and is associated with wet gypsum material excessive humidity, water leaks, water infiltration, or flooding as in bathrooms (Andersen ,2011).

(Bae *et al.*, 2009, Hastings *et al.*, 2005). Fungal division from Deuteromycota and its single class Deuteromycetes and is a member of the order Moniliales and the family Dematiaceae (Nelson, 2001). *S. chartarum* is capable of producing mycotoxins that include both macrocyclic tricothecenes and nonmacrocylic tricothecenes. There are very few reports that toxigenic molds found inside homes can cause health conditions such as pulmonary hemorrhage or memory loss. The (Institute of Medicine IOM,2004) found there was sufficient evidence to link indoor exposure to mold with upper respiratory tract symptoms, cough, and wheeze in otherwise healthy people, with asthma symptoms in people with asthma, and with hypersensitivity pneumonitis in

individuals susceptible to that immune-mediated condition. Other recent studies (World Health Organization issued additional guidance, 2009) have suggested a potential link of early mold exposure to development of asthma in some children, particularly among children who may be genetically susceptible to asthma development, and that selected interventions that improve housing conditions can reduce morbidity from asthma and respiratory allergies. Previous experiments used exposures of rats and mice to *S. chartarum* spores intranasally and intratracheally; lung tissue was examined for histological changes and bronchoalveolar lavage for evidence of injury and inflammation (Nikulina *et al.*, 1996, 1997; Rao *et al.*, 2000; Rosenblum Lichtenstein *et al.*, 2006). *S. chartarum* has been consistently reported to cause pulmonary hemorrhage ,extensive inflammation and chemokine levels in response to intratracheally instilled spores of *S. chartarum* a well as apoptosis, cytokine release, DNA damage, and changes in gene expression (Bae *et al.*, 2009, Chung *et al.*, 2003b, Dearborn *et al.*, 1999, Islam *et al.*, 2008)

S. chartarum is the only fungus that we know of to which BALB/c mice are more sensitive. (Jamie 2010) We have previously shown that BALB/c mice respond more to pulmonary exposure to *S. chartarum* spores than do other strain mice (Rosenblum Lichtenstein *et al.*, 2006).

Health problems related to this mold have been documented in humans and animals since the 1930s. More recently, *S. chartarum* has been linked with so-called sick building syndrome. (Heller, 2003; Page and Trout, 2001), Blood tests confirmed the presence of a toxin produced by *S. chartarum*, and severe mold contamination was found in the home. (Gao and Martin, 2002).

The aim of the current study: Evaluation the histological changes induced by *Stachybotrys chartarum* fungus suspension intranasally in mice (Balb/c).

Material and Methods:

1-Samples collection and Lab . tests :

Stachybotrys chartarum fungus samples were collected from the bath ground and testing microbiological Laboratory that done by cultured on potato dextrose media and incubated at room temperature for 3-7 days in order to obtaining on *S chartarum* fungus . Dilution done by two –fold serial diluents in 8 tubes and harvesting of this fungus with normal saline.

One positive samples of this fungus was further used for the experimental study on laboratory animals (mice) after infected with 0.5 ml from fungus suspension contain 6×10^3 spore / mm^3 counted by using hemocytometer chamber (white blood cells count chamber) for evaluation the effects of *Stachybotrys chartarum* on respiratory tissues sections taken from these mice.

2-Experimental study : A total of 12 males mice species Balb/c have aged one month and weight 25-30 g divided into two groups , the first group consist of six mice infected intranasal with 0.5 ml from

2- .

Stachybotrys chartarum fungus suspension for one positive sample contain 6×10^3 spore / mm^3 (for month as one time daily) . The other as control group was received 0.5 ml of sterile phosphate buffer saline (PBS) according to methods of (Scott, 2004). After 7-14 days clinical signs were recorded in infected animals . then the Experimental mice were sacrificed after anesthetization by chloroform and open abdomen cavity by medical scissors. Nose and lung tissue sections were collected for the experimentally infected mice and placed in formalin 10% for histological changes examination in later. Histological sections and staining were prepared according to methods described by (Luna 1968). The histological changes were read by Dr. Nemah . H. AL-jabori /college of medicine / university of Babylon under the magnification power 10X and 40 X of light microscope.

3- Statistical Analyses

Using statistical package for social science (SPSS) version 13.0, two-way analysis of variance was conducted to test the significance of effects of groups and periods post injection on the examined traits. The statistical differences among means of the different treatments were tested By Duncan's multiple range test.

Results:

1- **Lab. study results :** culture microbiological testing in laboratory observed *Stachybotrys chartarum* fungus on potato dextrose agar . 0.5 ml contain 6×10^4 spore / mm^3 from this fungus suspension used with histological study in vivo (inside mice) to evaluation the histological changes in some organs of respiratory system .(nose and lung) . figure 1and 2 shows these results the culture

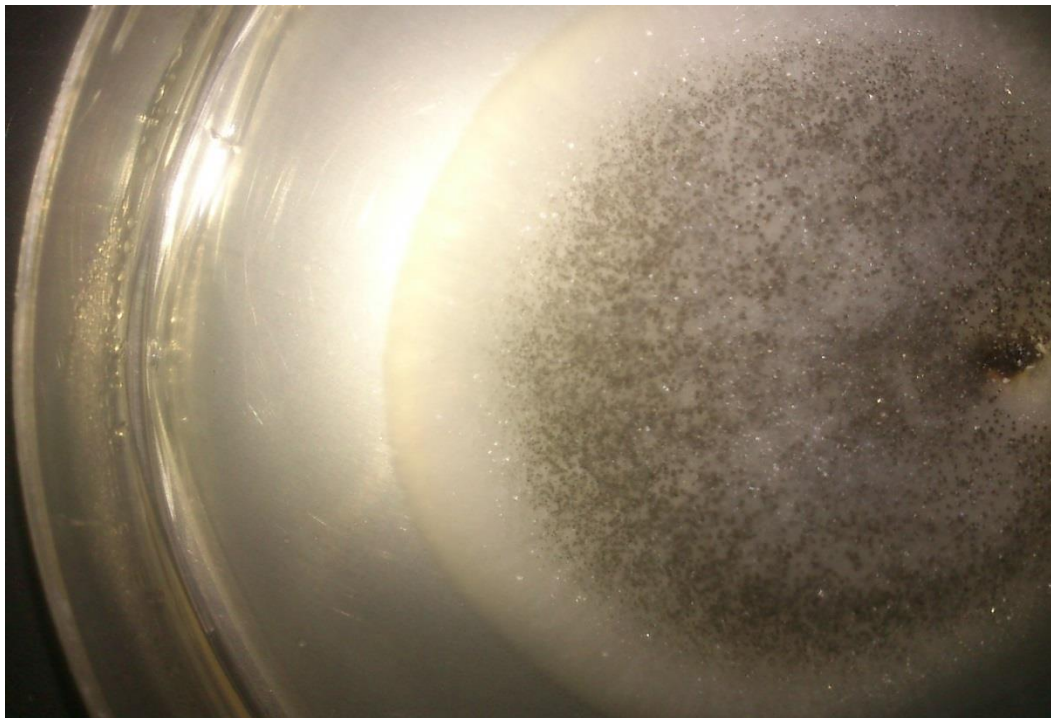


Figure (1) A culture of *S. chartarum* on PDA from top side.



Figure (2) A culture of *S. chartarum* on PDA from bottom side (see the roots).

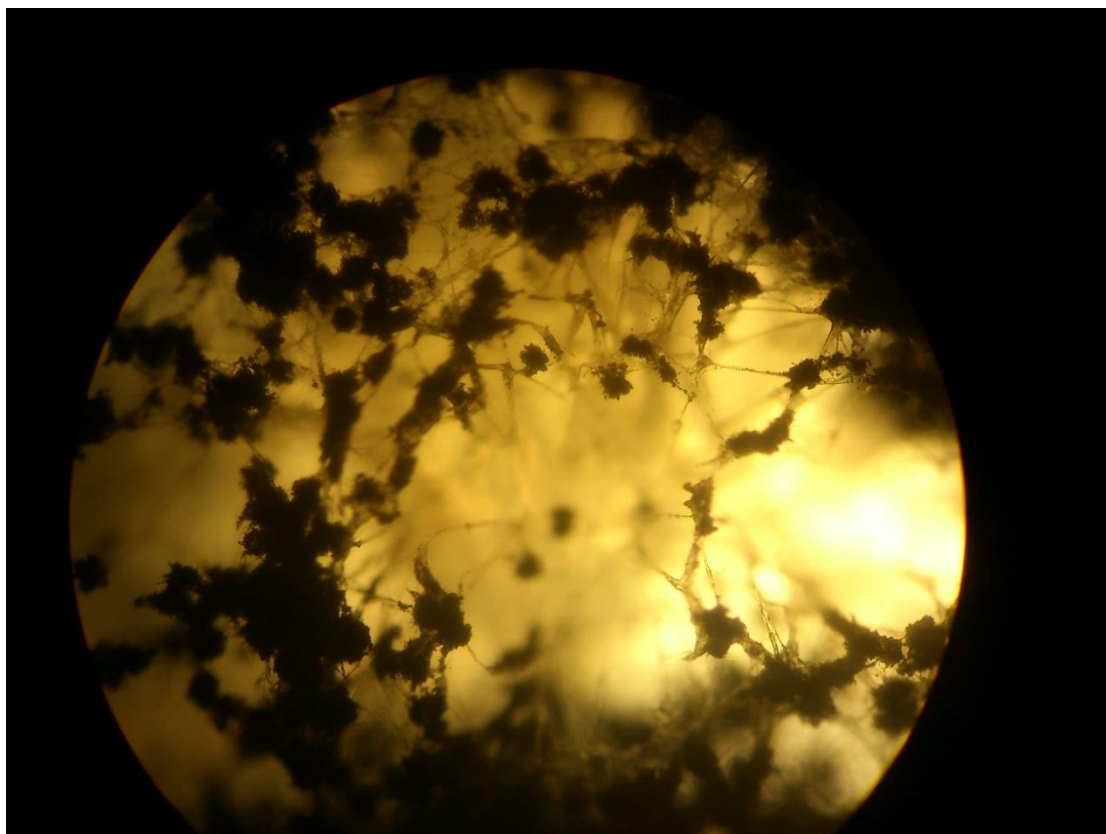


Figure (3) The mass of conidia of *S. chartarum* at the tip of the conidiophore networks. Under light microscopic magnification 4x.

3- **Histological changes** : Results of the current study revealed histological changes in nose and lung of mice infected with 0.5 ml contain 6×10^4 spore / mm^3 from *S. chartarum* fungus suspension , these changes shown in figure 4,6 while figures 5,7 represented control group of mice infected with 0.5ml phosphate buffer saline

In this results figure (4) the nose of mice infected with 0.5 ml contain 6×10^4 spore / mm^3 from *S. chartarum* fungus suspension shows focal superficial necrosis of the respiratory epithelial cells with acute inflammatory cells and infiltration of the mucosa .

The results in figure (6) lungs of mice infected with 0.5 ml 6×10^4 spore / mm^3 from *S. chartarum* fungus suspension indicated to interstitial pneumonia with thickening of inter alveolar space due to infiltration and chronic inflammatory cells especially mononuclear cell.

While the figure (5,7) revealed to the nose and lungs of control mice infected with 0.5ml phosphate buffer saline. No histological changes observed in nose and lung control mice group.

The graph (8) shows the histological resulting column for compensation between injection and control groups after one month ago (one dose/daily from *S. chartarum* fungus suspension).

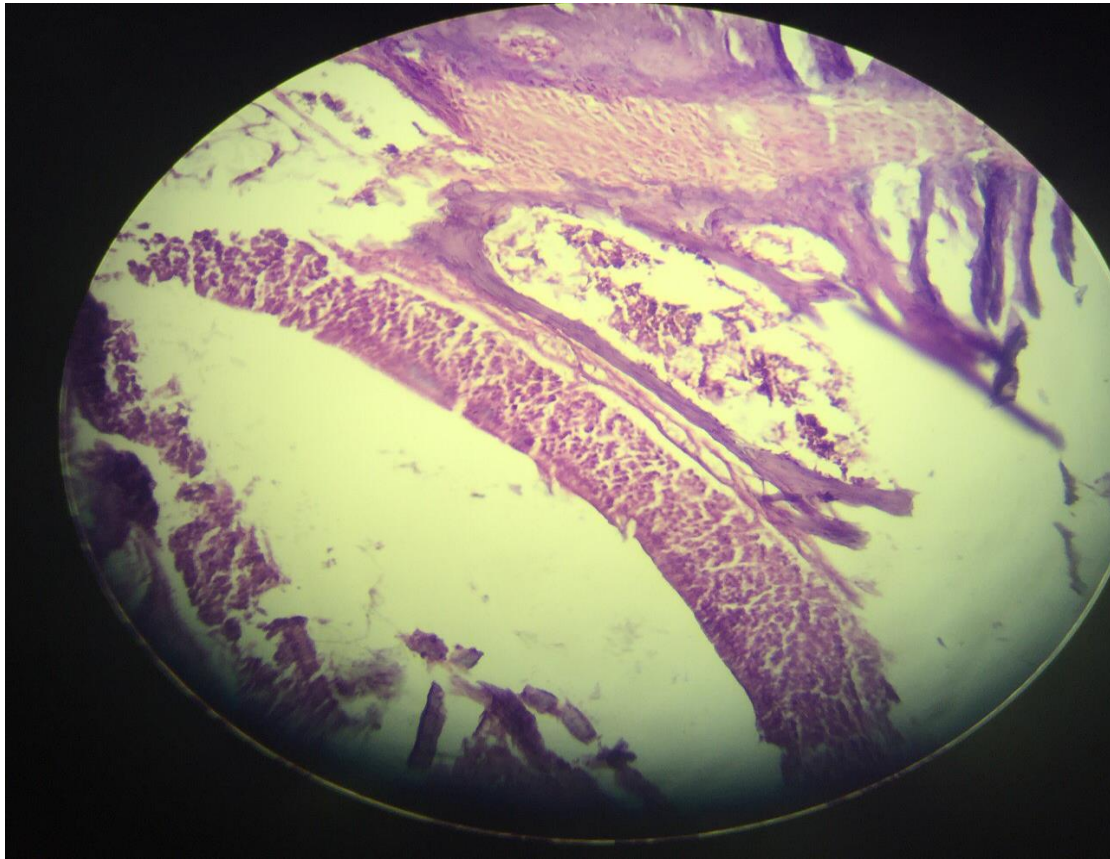


Figure (4): Nose of mice infected with 0.5 ml contain 6×10^4 spore / mm^3 from *Stachybotrys chartarum* fungus suspension . This slide shows focal superficial necrosis of the respiratory epithelial cells with acute inflammatory cells and infiltration of the mucosa . E&H stain. Magnifications 20X.

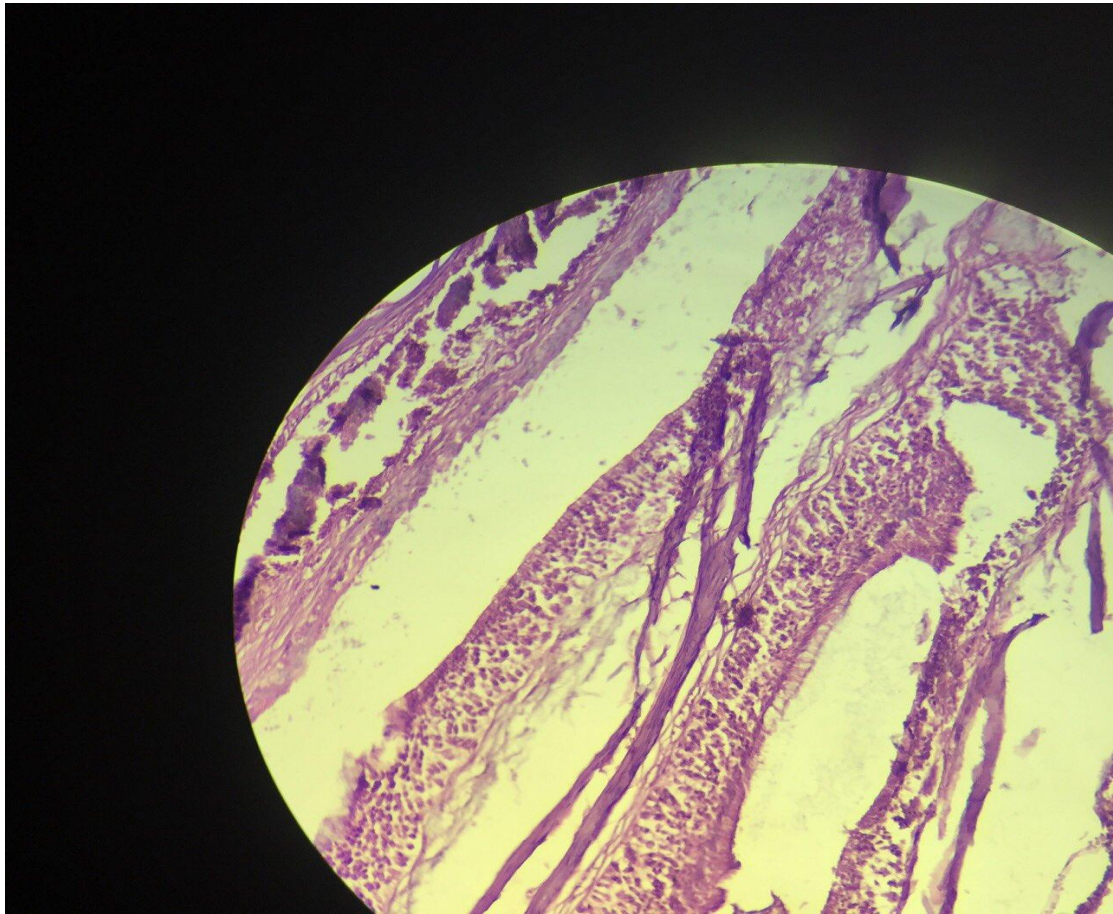


Figure (5) :Control of Nose of mice infected with 0.5 ml phosphate buffer saline . There is normal appearance in respiratory epithelial cells of nose without any damage or destruction of epithelium cells . E&H stain. Magnification 20x.

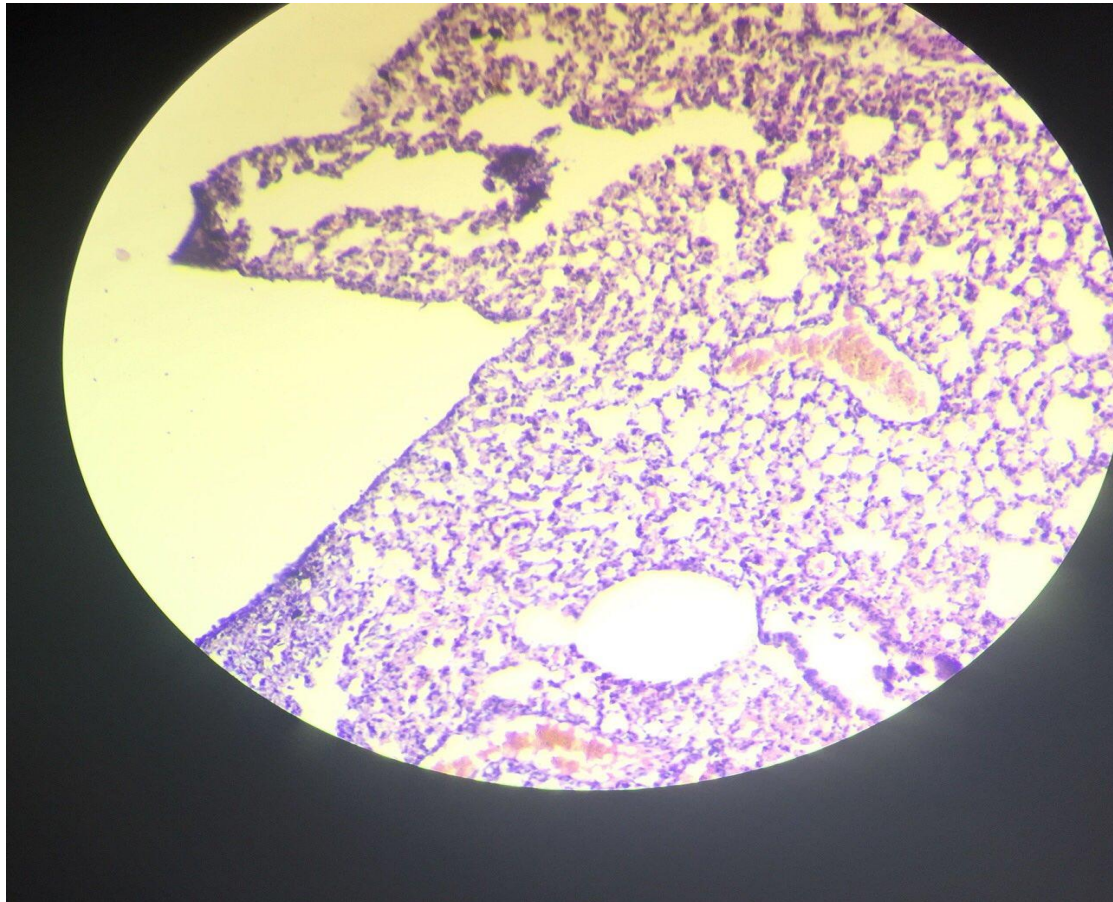


Figure (6): lung of mice infected with 0.5 ml contain 6×10^4 spore / mm^3 from *Stachybotrys chartarum* fungus . this slide shows interstitial pneumonia with thickening of inter alveolar space due to infiltration and chronic inflammatory cells especially mononuclear cells . E&H stain. Magnification 20x

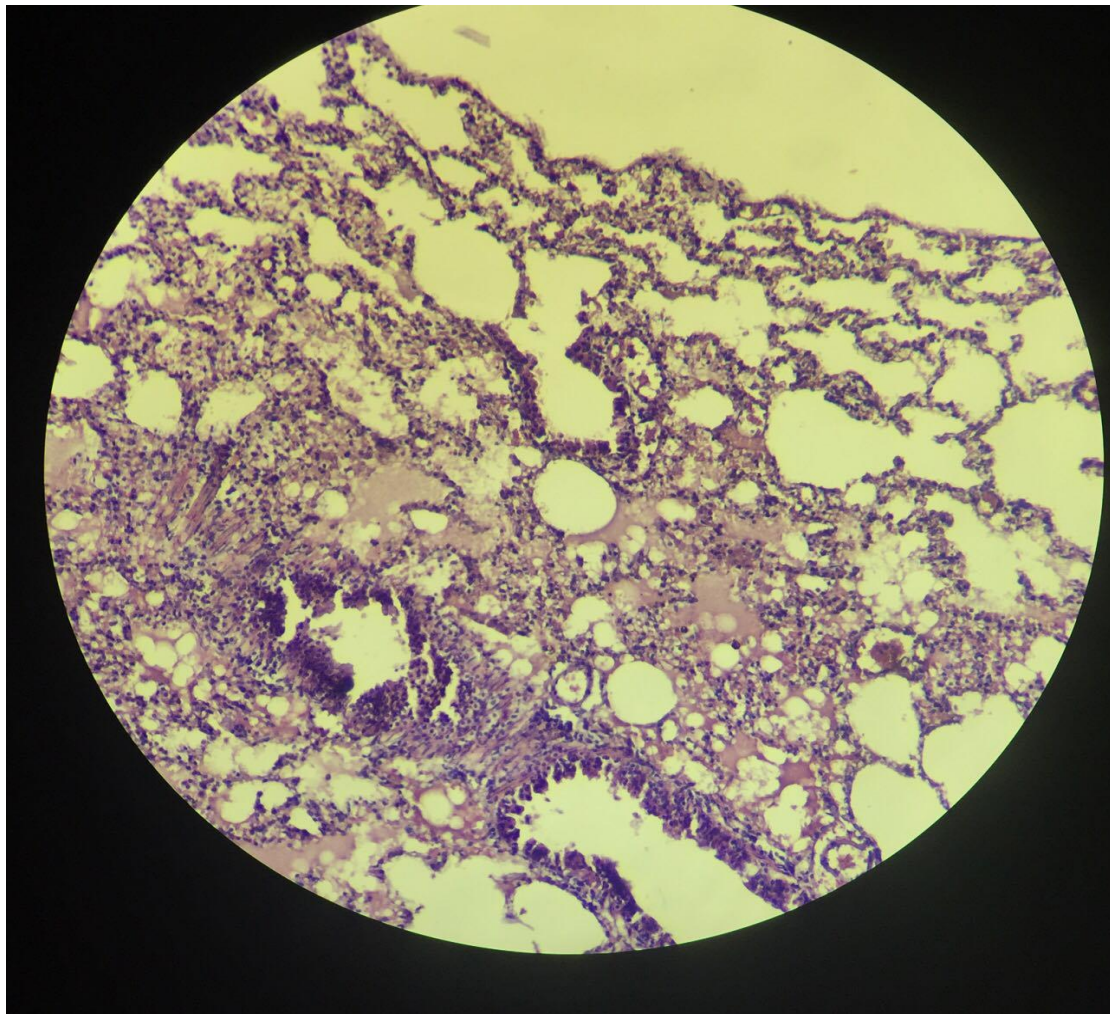
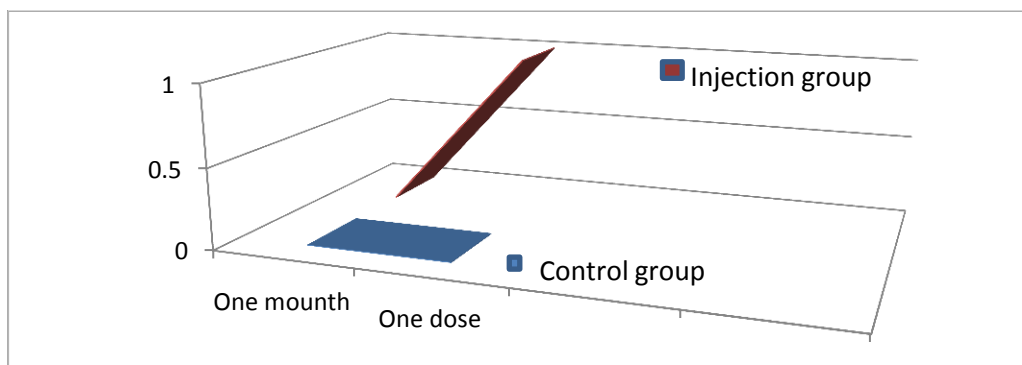


Figure (7) : Control of lung of mice infected with 0.5 ml phosphate buffer saline . this slide shows normal inter alveolar space and normal cells of lung . E&H stain. Magnification 20X.

The graph (8) shows the histological results column for compered between injection and control groups.



Discussion:

Stachybotrys chartarum was reported to induce sensory irritation, inflammatory, and/or pulmonary responses in mice and rats exposed via intranasal instillation, intratracheal instillation, and inhalation (Scott , 2004)..

The results of present study about effects of *S. chartarum* suspension on nose and lung of mice which experimentally infected revealed to histological changes within nose and lung mice infected with 6×10^4 spore / mm³ from this fungus suspension .These histological changes were observed in figure 3,5 . figure (3) showed histological changes in nose represented in focal superficial necrosis of the respiratory epithelial cells with acute inflammatory cells and infiltration of the mucosa . Spores or spore components in the lungs of BALB/c mice persisted longer and thus may have elicited a greater response. This persistence might also explain how an extract of *S. chartarum* can also induce allergic asthma in BALB/c mice after repeated pulmonary exposures (Viana *et al.*, 2002). The first evidence that differences in spore clearance and macrophage susceptibility to spore-induced death may contribute to strain differences in susceptibility to *S. chartarum* seen in vivo. (Dearborn *et al.*, 1999, 2002)

This results similar to other studies mentioned that no inflammation or tissue damage was seen in the nasal cavity. (Korpi *et al.*2002)in spite of the interstitial inflammation with luminal hemorrhagic exudates were observed in nose of animals infected with this dose from fungus, as well as toxicity

or mortality was seen (Nikulin *et al* , 1996) .

The results of lung mice infected with same concentration of this fungus shows in figure (5) lungs of mice infected with 6×10^4 spore / mm³ from *S chartarum* fungus suspension indicated to interstitial pneumonia with thickening of inter alveolar space due to infiltration and chronic inflammatory cells especially mononuclear cell.

This results similar to other studies mentioned that *S chartarum* cause severe alveolar, bronchiola and the higher concentration caused a significant increase in monocytes, neutrophils, and lymphocytes in the lung (Leino *et al* ,2003 ; Nikulin *et al*, 1997). Genetic variability in human populations may account for some of the wide variation among individuals responding to mold exposure in contaminated occupational and domestic settings. In addition, other concomitant conditions, such as cigarette smoke exposure or bacterial infection, might further exacerbate poor spore clearance in susceptible populations. A Centers for Disease Control (CDC) report concluded that *S. chartarum* was responsible for acute idiopathic pulmonary hemorrhage in a cluster of infants.

Causes of histopathological changes in nose and lung of infected mice perhaps due to the toxin that produce by *S chartarum* effect on some organs of respiratory system including the nose and lung .

Conclusion : the *Stachybotrys chartarum* fungus suspension caused clear histological changes in nose and lung of mice (Balb /c) infected with 6×10^4 spore / mm³ from fungus suspension .

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