Pathological study of Stachybotrys chartarum that isolated from baths floor in Babylon province on some respiratory and digestive organs in white mice(Balb/c) .

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Abstract:

Stachybotrys chartarum is filamentous fungi that cause respiratory system infection in human and animals . This study was aimed to evaluate histological changes in some respiratory and digestive organs such as pharynx, trachea and lung induced by this fungus suspension . Detection of Stachybotrys chartarum fungus confirmed by cultured random samples that collected in clean containers from baths floor on potato dextrose agar in room temperature for 7-14 days. Then the suspension of positive samples of this fungus diluted by normal saline and counted the spores by using hemocytometer before used experimentally in lab animals (Balb mice) to study histological changes in some respiratory and digestive organs . Twelve mice used in this study divided into two groups . first group consist of six mice injected orally with 0.5 ml from one positive sample of fungus suspension contain 2×10^3 spore / mm^3(for month : one dose daily) according to count of hemocytometer to evaluate some pathological changes in pharynx ,trachea and lung .Second group injected orally with 0.5 ml from phosphate buffer saline only. The results revealed that no changes in the pharynx of mice infected with0.5 ml contain 2×10^3 spore / mm^3 from Stachybotrys chartarum fungus suspension. 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.

While the trachea of group mice treated with same concentration of fungus suffering sub mucosal chronic inflammatory cells and infiltration with increase thickness of mucosal basal layer .

Key word : Stachybotrys chartarum , white mice .

دراسة امراضية فطر ستاكيبوترس كارتارم المعزولة من ارضية الحمامات في محافظة بابل على بعض الأعضاء الهضمية والتنفسية في الفئران البيض

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الخلاصة :

ستاكيبوترس كاتارم فطريات خيطية تصيب الجهاز التنفسي في الإنسان والحيوان هدف الدراسة لتقييم التغيرات النسيجية لبعض أعضاء الجهاز التنفسي والهضمي المعاملة بمعلق الفطر أعلاه . تم التحري عن الفطر بزرع العينات المأخوذة عشوائيا من أرضية الحمامات على وسط دكستروز البطاطا في درجة حرارة الغرفة ولمدة سبعة إلى أربعة عشر يوم . تم استخدام معلق العينات الموجبة للفطر تجريبيا على الحيوانات المختبرية (الفئران) لدراسة التغيرات النسجية لبعض أعضاء الجهاز ين الهضمي والتنفسي . أستخدم اثنى عشر فأرا في هذه الدراسة قسمت الى مجموعتين . المجموعة الأولى تكونت من ستة فئران وعملت فمويا ب.5و. مل من معلق الفطر الموجب والذي يحتوي على $2\times10^3/\ mm^3$ بوغ (لمدة شهر بواقع جرعة واحدة يوميا) بناء على عد مقياس عداد الخلايا لتقييم التغيرات النسيجية في البلعوم , القصبات والرئة . المجموعة الثانية عملت ب5 و. مل من المحلول الدارئ للفوسفات . تشير النتائج أن بلعوم مجموعة الفئران المعاملة بالمعلق لم تعاني تغير نسيجي بينما الرئة عانت تغيرات واضحة بسبب معلق الفطر منها ذات الرئة البيني وزيادة في سمك المسافات الحويصلية إضافة إلى ترشيح والتهاب مزمن في الخلايا وخصوصا الخلايا وحيدة النواة. اما القصبات فقد عانت التهاب مزمن في الطبقة تحت المخاطية وترشيح مع زيادة في سمك الطبقة القاعدية المخاطية .

الكلمات امفتاحية : ستاكيبوترس كاتارم , فئران بيض

Introduction :

Stachybotrys chartarum is one of several species of filamentous fungi capable of producing mycotoxins under certain environmental conditions (1).

Stachybotrys chartarum called a black mold that produces its conidia in slime heads. found in soil and grain, In some observational studies, the growth of this toxigenic mold in the indoor environment has been implicated as a cause of building-related illness. Recent studies were reported a cluster of cases of pulmonary hemosiderosis and hemorrhage associated with exposure to *Stachybotrys* (2, 3) . Health problems related to this mold have been documented in humans and animals since the 1930. More recently, *S. chartarum* has been linked with so-called sick building syndrome.. (4, 5) .There are two chemotypes in *S. chartarum*, one that produce trichothecene

mycotoxins including satratoxins and one that produce atranones.(6,7).

Material and methods:

1-Samples collection and Lab. test :

Random Samples collected from the baths floor and preserve in clean container . tests Laboratory done by cultured the bath ground samples on potato dextrose media incubated at room temperature for 7-14 days . One positive samples of Stachybotrys chartarum fungus was further used for the experimental study on laboratory animals (mice) for evaluation the effects of Stachybotrys chartarum diseases in tissues sections of these mice.

2-Experimental study : A total of 12 males mice species Balb/c have aged one month and weight 20-30 g divided into two groups , the first group consist of six mice infected nasally with 0.5 ml contain 2×10^{3} spore / mm^3 from Stachybotrys chartarum fungus suspension for one positive sample by using one dose daily for one month . second group as control group was received 0.5 ml of sterile phosphate buffer saline (PBS) according to methods of (8). after 7-14 days clinical signs were recorded in infected animals . were observed Experimental mice were sacrificed after anesthetization by chloroform and open abdomen cavity by medical scissors, tissue . pharynx, trachea and lung tissue sections were collected for the experimentally infected mice and placed in formalin 10% for histopathological examination in later. Histological sections and staining were prepared according to methods described by (9). The pathological changes were reading by Dr. Nemah . H. AL-jabori /college of medicine / university of Babylon under the magnification power 10X and 40 X of light microscope.

Results:

1- **Lab. study result**s : culture of samples of bath floor shows Stachybotrys chartarum fungus on potato dextrose media and diagnose morphological on black and branched mold carried conidia (figure 1) . 0.5 ml contain 2×10^{3} spore / mm^3 from this fungus suspension by using hemocytometer to histological study in vivo (inside mice) to evaluation the histological changes in some respiratory and digestive organs **.(** pharynx ,trachea and lung) .

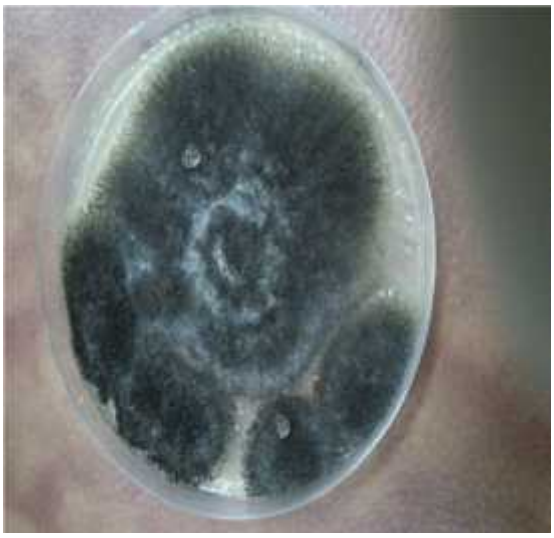

Figure (1) growth of Stachybotrys chartarum fungus on potato dextrose media after 7-14 days .

2-Histological changes : Results of the current study revealed histological changes in trachea of lung of mice infected with 0.5 ml contain 2×10^3 spore / mm^3 from Stachybotrys chartarum fungus suspension while in pharynx section no change were observed , these changes shown in figure2,4,6 while figures 3,5,7 represented control group of mice infected with 0.5ml phosphate buffer saline .

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In results figure (2) shows the pharynx of mice infected with0.5 ml contain 2×10^3 spore / mm^3 from Stachybotrys chartarum fungus suspension shows no changes in cells of pharynx infected with this concentration . figure (3) shows Control of pharynx of mice infected with 0.5ml phosphate buffer saline.

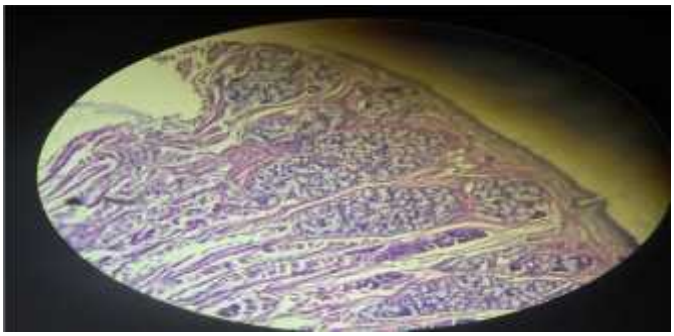

Figure (2) : Pharynx of mice infected with 2×10^3 spore / mm^3 from Stachybotrys chartarum fungus . This slide shows no changes in Pharynx cells . H&E. 10X.

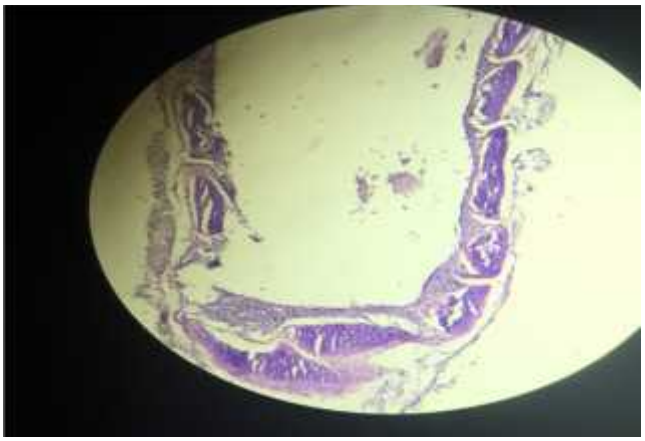

Figure (3) : Section in pharynx of control mice infected with 0.5ml phosphate buffer saline indicate to Normal cells of pharynx . H&E. 10X.

The results in figure (4) lungs of mice infected with same concentration of Stachybotrys 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) revealed to the lungs of control mice infected with 0.5ml phosphate buffer saline. No histological changes observed in control mice group..

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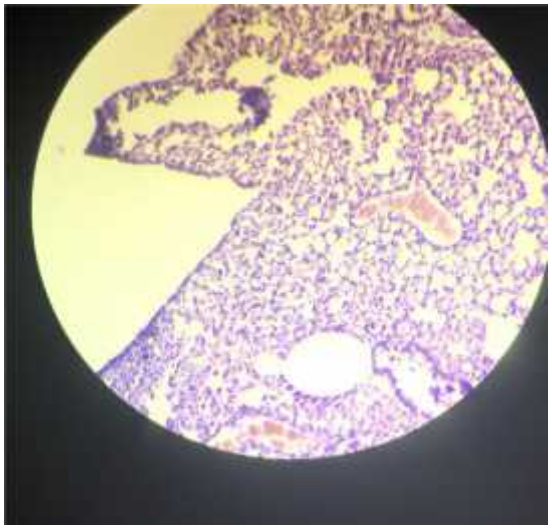

Figure (4): Section of lung of mice infected with 0.5 ml contain 2×10^3 spore / mm^3 from Stachybotrys chartarum fungus shows interstitial pneumonia with thickening of inter alveolar space due to infiltration and chronic inflammatory cells especially mononuclear cells . H& E . 20X.

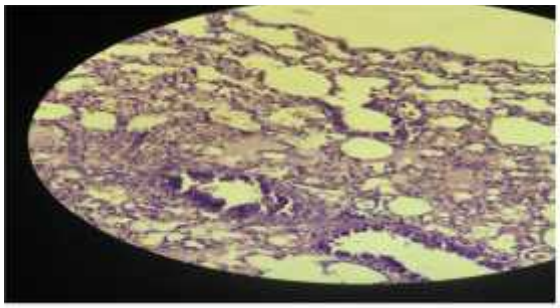

Figure (5) : Section in lung of control mice infected with 0.5 ml phosphate buffer saline . this slide shows normal inter alveolar space and normal cells of lung . H& E .20X.

The results in figure (6) trachea of mice infected with same concentration of Stachybotrys chartarum fungus suspension has sub mucosal chronic inflammatory cells and infiltration with increase thickness of mucosal basal layer.

While figure (7) shows control of trachea of mice infected with phosphate buffer saline.

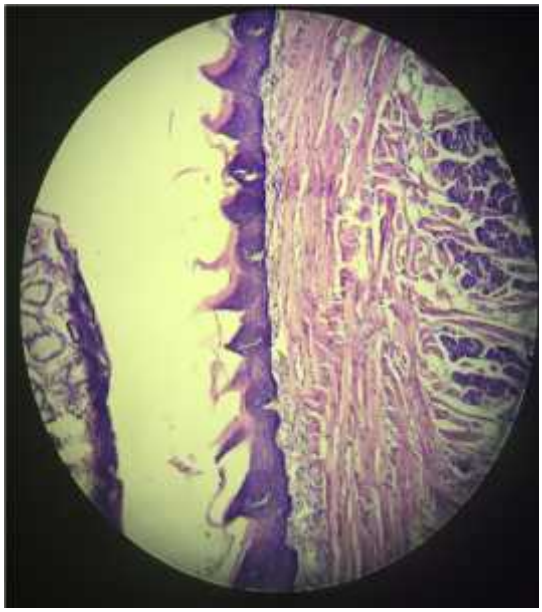

Figure (6) Section in trachea of mice infected with 2×10^3 spore / mm^3 from Stachybotrys chartarum fungus shows sub mucosal chronic inflammatory cells and infiltration with increase thickness of mucosal basal layer . H&E .10X

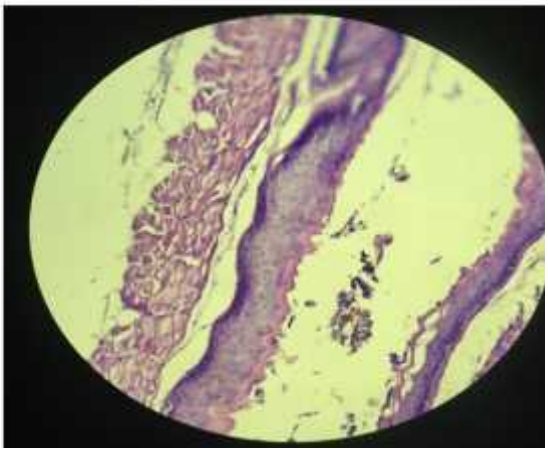

Figure (7) control of trachea of mice infected with phosphate buffer saline.

The slide shows the normal histology of the trachea cells . E&H stain. Magnification X10

Discussion:

Stachybotrys chartarum fungus cause disease in respiratory system infection in human and animals . Recent studies reported inducing sensory irritation, inflammatory, and/or pulmonary responses in mice and rats exposed via intranasal instillation, intra tracheal instillation, and inhalation with infection Stachybotrys chartarum (8)..

The results of present study about effects of S. chartarum suspension on pharynx of mice which experimentally infected with 2×10^3spore / mm^3 from this fungus suspension revealed to no histological changes . these result perhaps to the fungus incapable on living in this tissue .

This results similar to other studies mentioned that no inflammation or tissue damage was seen in the nasal cavity (10). 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 .

The results of trachea and lung mice infected with same concentration of this fungus shows in figure (4,6) trachea and lungs of mice infected with 2×10^3 spore / mm^3 from Stachybotrys 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 Stachybotrys chartarum cause severe alveolar, bronchiola and the higher concentration caused a significant

increase in monocytes, neutrophils, and lymphocytes in the lung (11, 12).

Causes of histopathological changes in trachea and lung of infected mice perhaps due to the toxin that produce by Stachybotrys chartarum effect on respiratory system only .

In Conclusion : the Stachybotrys chartarum fungus suspension caused clear histological changes in trachea and lung of mice (Balb /c)with 2×10^3 spore / mm^3

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