Study about environmental natural fungi infection in pet animals with experimental study

Buraq shakir salman[1*], Hashim M.S[1]. , Fadwa abdulrazaq Jameel[1] , Eman Hashim Yousif[1] , Hanaa Salih Abd Ali Alrammah[1], Shahad Ehsaan Aziz[1], Musab Imad Ali[1], Mueamar Natiq Muhsin[1], Zahraa S. Mahdi[2], Sikandar Khan[3] and Abdolmajid Ghasemian Affiliation[4]

[1] College of Veterinary Medicine University of Baghdad, Baghdad, Iraq.
[2] College of Health and Medical Technology Alzahraa University for Women, Kerbala, Iraq.
[3] Department of Chemistry, University of Malakand, Chakdara, Khyber Pakhtunkhwa, Pakistan.
[4]Noncommunicable Diseases Research Center, Fasa University of Medical Sciences, Fasa, Iran.
***Corresponding author's emails: barraaq.shaker1700a@covm.uobagh**
https://doi.org / 10.59658/jkas.v11i2.2066

Received:
Mar. 02, 2024

Accepted:
May 05, 2024

Published:
June 10, 2024

Abstract

The current study was designed to investigate Environmental and natural fungi infection in pet animals and then experimentally to study fungi effects on tissues. Naturally infectious samples were fifty swabs in different pet animals (dogs, cats, rats, pigeons, and chickens) from the veterinary hospital of Eden Square in January 202. The findings were *Cladosporum spp* at (16%) ; *Aspergillus flavus*, *Aspergillus niger* at (14%); *Rhizopus spp* at (8%); *Aspergillus ochraceus*, *Aspergillus terreus*, and *Penicillium chrysogenum* at (6%); *Chrysosporium spp*, and *Cryptococcus neoformans* at (4%); *Aspergillus fumigatus* , *Aspergillus ustus* and *Fusarium spp* at (2%) in percentage with other unknown fungi. In doge, only 9 (9/50) samples of fungi, mainly Aspergillus flavus at (33.3%) with cladogram *spp*.at (22.3%). In cats, also 9 (9/50), mainly *Penicillium spp* at (22.25 %). In wild rats, fifty cotton swabs showed 12 (12/50) mainly *Aspergillus niger*, besides *Rhizopus spp.* At (25%). While in pigeons, only 10 (10/50), mainly *Aspergillus niger*, *Cladosporum* spp., and *Cryptococcus neoformans* at (20%). In chicken, only 5 (5/50), mainly *Aspergillus ochraceus* at (40%). Fifty albino mice aged 6-8 weeks and 25± 3gm body weight. They were separated in plastic cages and kept for adaptation at 2-3 weeks in the Animal House of the College of Veterinary Medicine, and they were fed standard pellets and water. These animals were divided into 5 groups containing 10 mice each: 1st one: control group treated with normal saline; 2nd Group infected with 0.1ml of *Aspergillus flatus* intraperitoneally; 3rd group infected with *Aspergillus ochraceus* 0.1ml intraperitoneally (IP); 4th group infected with 0.1ml *Aspergillu niger* IP; 5th group mice infected with *Cryptococcus neoformans* 0.1ml IP at single dose. After 3 weeks animals were scarified.

Histopathological examination, in general, reports high incidences of necrotic lesions with granuloma and severe destructive changes, mainly in areas containing fungi hyphae that appear very clearly enclosed to necrotic and inflammatory foci, lung tissues suffering from prominent interstitial pneumonia with thick exudation, mainly fibrinous type. Liver tissues show massive necrosis with Kupfer cells and other inflammatory cell proliferation and evidence of apoptosis. Kidney samples appear with heavy degenerative changes ranging from swelling tubules to necrosis associated with severe inflammation caused by pyelonephritis and phlebitis. also, we saw fungi in the affected area. Spleen shows severe depletion and megakaryocyte proliferation and necrotic lesion. Fungi naturally infects occurs in pet animals and can transmitted to other animals in different environmental; area and can causes severe pathological lesions.

Key words: Rodent, mycosis, domestics animals.

Introduction

Fungi contaminated environmental areas such as animals field, cages, rooms , storages food's and causes different diseases as dermatitis, granulomatous lesions [1]. Fungi classify to two main types; molds as Aspergellosis Species and Yeast as candidiasis Species [2].

Main fungal infection reported as mold types and mainly Aspergillus sp. Others types also seen to be harmful pathogens for animals and humans [3]. Mycosis occurs when the spores and hyphae's inhaled by host' body and then spread intracellularlly in to different organs so can be causes' really disease's because of hitting of immune system [4].

The most common's mycotic pathogens were *Aspergillus sp* [5]. Many types of Aspergellosis were detected in different individual's manly domestic animals which can produced toxins [6]. Aflatoxin and Gliotoxin are the moat harmful toxins secreted from Aspergillus species mainly *Aspergillus flavus* [6,7].

Pulmonary Aspergillosis is a common incidence in animals due to weak immune systems and loosening of defense mechanisms like respiratory clearance activity [8]. As well as poultry, Aspergillosis is a highly spreading disease in wild and domestic poultry fields [9]. Small animals such as dogs and cats also suffer from deferment's fungal infection, mainly respiratory aspergillosis [10]. The aim of this study was to survey fungi infection in pet animals for the identification of pathological fungi in pet animals. Experiment with lab mice for the most frequent isolates to detect the harmful effects of fungi on histopathological tissues.

Material and methods:

Study design:

1.Survey work: Fungal isolated from different pet animal:

A:Samples collection: Fifty (50) swab from: oral cavity; nasal cavity; rectum and ear of some Pet animals as: Pigeon; Dog; Cat; Chicken and Rats(wild type).

B: Culturing on SDA.

C: Isolation & identification of pathogenic fungi (mold and yeast) as following: Macroscopically examination and Microscopically examination for diagnosis of of *A.ochraceus*; *A. niger* and *A. flavus* (molds). Identification of *cryptococcus neoformans*(yeast) Macroscopically and Microscopically as well as used urease test and Indian ink

Sabouraud Dextrose Agar (SDA)

This medium was prepared according to manufactures directions by dissolving of 65gm of (SDA) powder in a liter of distilled water and adding 0.05 g/l of chloramphenicol to prevent bacterial growth, mixed thoroughly and heated with frequent agitation then sterilized by autoclaving at 121˚C under 15 pound/inch² for 15 min. after that cooled sterile media to about 45- 50℃ and poured into plates or kept in universals tubes for maintaining the isolates [11].

Lactophenol cotton blue stain

This stain was prepared according to manufactures directions by taken small part of fungus from the fungal growth on SDA and mixed with one drop of this stain on clean slide, then covered by cover slip and examined by light microscope under 40X lenses [12].

Microscopic examination: This examination was performed according to science's [13] by putting one drop of lactophenol cotton blue stain on slide and mixed with small part of fungus by loop then covered with cover slip and examined by light microscope under 40X lens to determine the fungal elements microscopically.

D: Preparation of spore suspension of mold

Spore suspensions of mold fungi were prepared according to[14] with modification by adding 5ml of sterilizing PBS adding 0.01%tween-80 for facility separation of fungi proliferation using the loop then the solution's was filtered through sterile gauzes dowen in to test tubes , then filtration at 3000 rpm for 10 min. cloting was washing 2-4 times with PBS add 2ml of PBS ,and then add to precipitating spores with mixed by the vortex within 1-2 minutes then the turbid solution was tested by spectro-photometer's at 550 nm to an optical density of 0.144, which is equivalent to 1x 10^6 cells/ml or to ≠ 0.5 McFarland standard.

2. **Experimental study** .The period of this experiment's about one month

Fifty's white's mice were 25± 3gm body weight. And adapted in Veterinarians Medicine of Baghdad University and they were fed standard pellets and water. These animals were divided into 5 groups as following:

1. Ten mice as control group (-ve) without infection.

2. Ten mice infected with 0.1ml of *Aspergillus flavus* intraperitoneal
3. Ten mice infected with 0.1 ml *Aspergillus ochraceus* intraperitoneal's
4. Ten mice infected with 0.1ml *Aspergillus niger* intraperitoneal's
5. Ten mice infected with 0.1 ml *Cryptococcus neoformans* intraperitoneal's.

Histophathological study: At the end of experiment's animals were scarified mercifully used inhalation anesthesia(chloroforms). Small pieces of ($1cm^3$) lung, spleen, kidney and liver were taken and fixed in 10% neutral formalin buffer solution, with replacing after 24hrs. Routines tissues processed with ethanol alcohol and cleared by clearing solution and embedded with paraffin wax was done. Many slices were prepared for histopathological examination's and staining with routine stains Hematoxylin-Eosin (H&E) [15].

Results and Discussion 1: survey work:

Mycotic Examination for different types of fungi from pet animals:

Fifty cotton swabs were collected from different pet animals like (dogs, cats, rats, pigeon and chickens) from different region in Baghdad like veterinary hospital of Eden Square, veterinary clinic and animal breeders during January 2023. As mention : Cladosporum spp. (16%); *Aspergillus flavus*; *Aspergillus niger* at (14%); *Rhizopus spp* at (8%); *Aspergillus ochraceus*, *Aspergillus terreus* and Penicillium chrysogenum at (6%); *Chrysosporium spp*, and *Cryptococcus neoformans* at (4%); *Aspergillus fumigatus*, *Aspergillus ustus*, and *Fusarium spp* at (2%) in percentage with other unknown fungi. In doge only 9 (9/50) samples of fungi mainly *Aspergillus flavus* at (33.3%) with *cladosporam spp* at (22.3%), in cat, also 9 (9/50) mainly *Penicillium spp* at (22.25%). In wild rats, fifty cotton swabs showed 12 (12/50) mainly *Aspergillus niger*, and *Rhizopus spp* at (25%). In pigeon, only 10 (10/50) mainly *Aspergillus niger*, *Cladosporum spp*, besides *Cryptococcus neoformans* at (20%). In chiken, only 5 (5/50) mainly *Aspergillus ochraceus* at (40%).

Laboratory mycotic examination: (Macroscopic and Microscopic pictures)

Aspergillus flavus was isolated from pet animals by sub culturing on Sabouraud Dextrose Agar at 25˚C for 5-7 days, the colonies appeared as yellow- green in color with fluffy in texture as shown in Figure (1) using lactophenol cotton blue stain was complete flower (complete circle phialides) which were arranged on along conidiophores as shown in Figure (2).

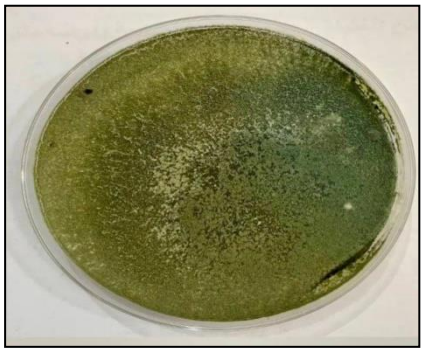

Figure (1): Gross appearance of *Aspergillus flavus* on SDA at 25˚C for 5-7 days.

Figure (2): Microscopically appearance of *Aspergillus flavus* by using lactophenol cotton blue stain 40X

Aspergillus niger was isolated from pet animals by sub culturing on SDA at 25˚C for 5-7 days, the colonies appeared as black in color with fluffy in texture as shown in Figure (3) using lactophenol cotton blue stain was black complete flower (complete circle phialides) which were arranged on along conidiophores as shown in Figure (4).

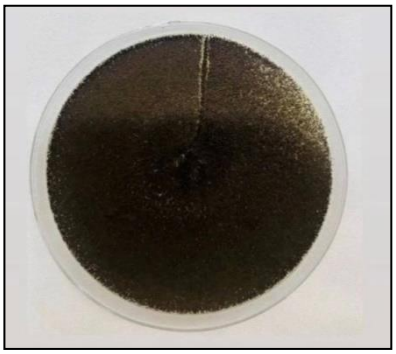

Figure (3): *Aspergillus niger*

Figure (4): Microscopically *Aspergillus niger*

Aspergillus ochraceus was isolated from pet animals by subculturing on SDA at 25˚ C for 5-7 days, the colonies appeared as pale brown to yellow in color with powdery in texture as shown in Figure (5) using lactophenol cotton blue stain was big complete flower (complete circle phialides) which were arranged on along conidiophores as shown in Figure (6).

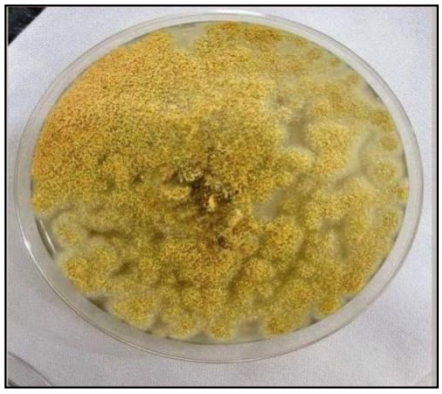

Figure (5): Gross picture of *Aspergillus ochraceus*

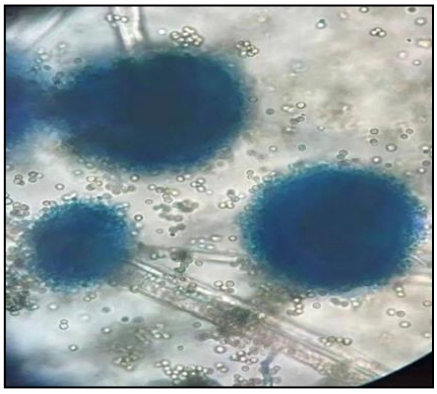

Figure (6): Microscopically of *Aspergillus Ochraceus*

Cryptococcus neoformans was isolated from pet animals by sub culturing on SDA at 37˚C for 5 days, the colonies were smooth, shiny, and white in color then become yellowish shade with very mucoid due to contain of capsule on SDA. as shown in Figure (7) using lactophenol cotton blue stain was spherical or ellipsoidal budding yeast as shown in Figure (8).

Figure (7): Gross picture of *Cryptococcus neoformans*

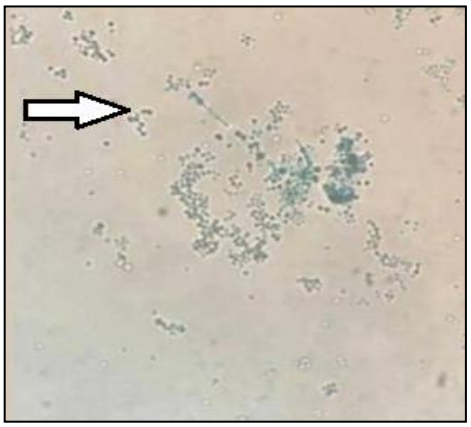

Figure (8): *Cryptococcus neoformans* microscopically

India ink :*Cryptococcus neoformans* was identification by using special stain that showed the capsule appears as a clear halo around the yeast cell as shown in Figure (9).

Figure (9): *Cryptococcus neoformans* microscopically

Urease test*: Cryptococcus neoformans* was identification by using urease test that appeared ability this yeast to hydrolyzed urea and produced pink color(+ ve) due to ammonia liberation as shown in Figure (10).

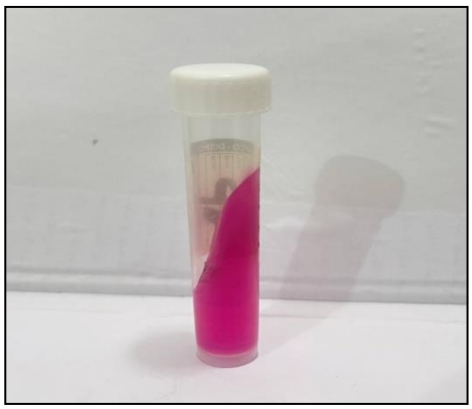

Figure (10): Hydrolysis of urea by *Cryptococcus neoformans* on urea agar or test show (+ ve) result

Macroscopic appearance of other fungi

The results of Macroscopic appearance of other fungi which isolated from pet animals in this study by sub culturing on SDA at 25 ˚C for 5-7 days,were include *A. terreus*, *Penicillium spp,* especially, *P. chrysogenum*, *Fusarium spp, Cladosporum spp*, *Rhizopus spp*, *Chrysosporium spp,* and other unknown fungi. The macroscopic apperance of *A. terreus* was appeared buff- cinamoon in color as shown in Figure (11A), The macroscopic apperance of *Penicillium spp* was appeared blue-green in color as shown in Figure (11 B), The macroscopic apperance of *Fusarium spp* was appeared white in color with cottony in texure as shown in Figure (11C), The macroscopic apperance of *Cladosporum spp* was olivaceous green in color with velvety in texure as shown in Figure (11D), The macroscopic apperance of *Rhizopus spp*. was appeared gray in color with black dot on the top of the hyphae and woolly in texure as shown in Figure (11E), The macroscopic apperance of *Chrysosporium spp.* was appeared white to gray in color with cottony to woolly surface as shown in Figure (11F).

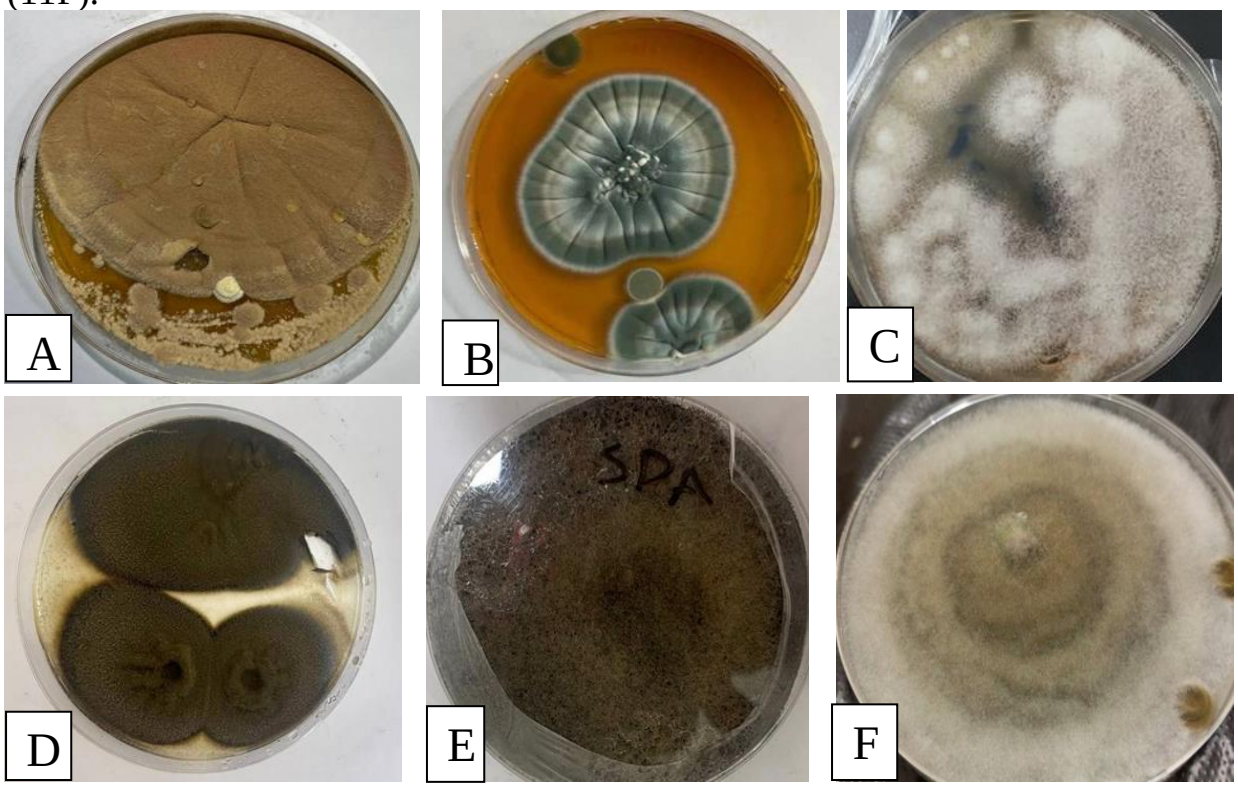

Figure (11): Macroscopic apperance of other fungi which isolated in this study A*: A.terreus*, B: *Penicillium spp*. C: *Fusarium spp*. D: *Cladosporum spp*. E: *Rhizopus spp*. and F: *Chrysosporum spp*

Microscopic appearance of other fungi

The results of Microscopic appearance of other fungi which isolated from pet animals in this study by using lactophenol cotton blue stain were include *A.terreus* was appeared half- floweras shown in Figure (12A), *Penicillium spp*.was appeared brush like arrangment as shown in Figure (12B), *Fusarium spp*.was appeared macroconidia as bannan shaped as shown in Figure (12C), *Cladosporum spp*was

appeared septate brown hyphae and the conidiophores bear swelling conidia which are ellipitical to cylindrical in shape which may be arrangement in chains as shown in Figure (12D), *Rhizopus spp.*was appeared umberulla like collapse (sporingum) with aseptated hyaphae as shown in Figure (12E) and *Chrysosporum spp.*was appeared one-celled conidia are produced directly on vegetative hyphae by non-specialised conidiogenous cells. Conidia are typically pyriform to clavate with truncate bases and are formed to produce (arthroconidia) as shown in Figure (12F).

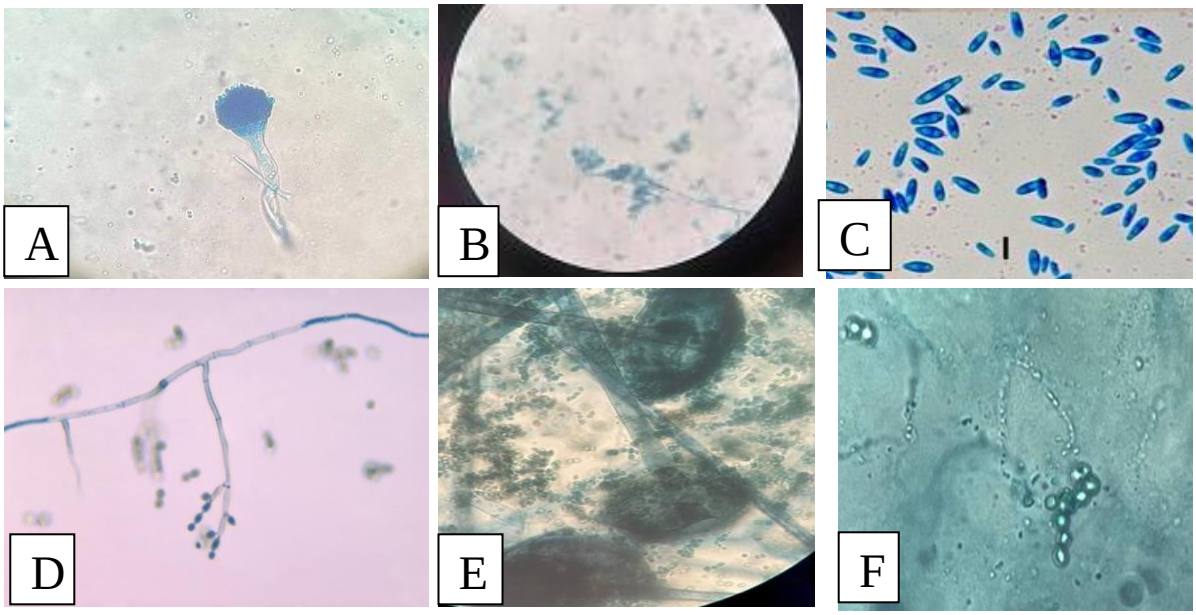

Figure (12): Microscopic appearance of other fungi which isolated in this study A*: A.terreus*, B: *Penicillium spp.*, C: *Fusarium spp*, D: *Cladosporum spp*, E: *Rhizopus spp*, and F: *Chrysosporum spp.*

The present study showed that the most prevalent fungal isolated were *Cladosporium spp*, and *Aspergillus spp.* in the percentage were (16%), and (14%) respectively due to these fungi were opportunistic nature with low food requirements for sporulation, opportunistic mold can be found almost anywhere, including the air, decomposing materials,and building walls. Fungi spread in environment and causes different diseases in different animals currents study report's different type of fungi in different pet animals, others authors make similar study in Poland [16], and in Iraq [17]. Fungi spreading according to intra specific variation, and natural distribution, growth or reproduction discussed by [18]. Isolation of fungi agree with authors opinion [16,19]. The isolation of mold from the lung, liver, spleen and kidney due to the fact that the spores of this mold are infinitesimal in size 3-2 mm. which enables him to invade different tissues and facilitate its spread among organs, that agree others researchers works [20,19,21].

Histopathological Examination: Lung section: shows evidence of fibrinous pneumonia with heavy clotting and edematous lesions associated with emphysema and thick inflammatory exudation still down on fibrinous exudation with hyperplasia of epithelial lying bronchioles' as shown in Figure (13). Spleen section: appears depleting

with inflammatory reaction appear as granulomatous foci with proliferative changes of megakaryocytes as shown in Figure(14) .Kidneys sections appears very inflamed with hailing renal tubules and have clear vacuoles associated with sever inflammatory infiltration and atrophied glomeruli as shown in Figure (15). Liver section: show severe infiltration of inflammatory cell with kuffer cells proliferations, other sections appears with necrotic changes in hepatocytes with evidence of apoptosis as shown in Figure (16).

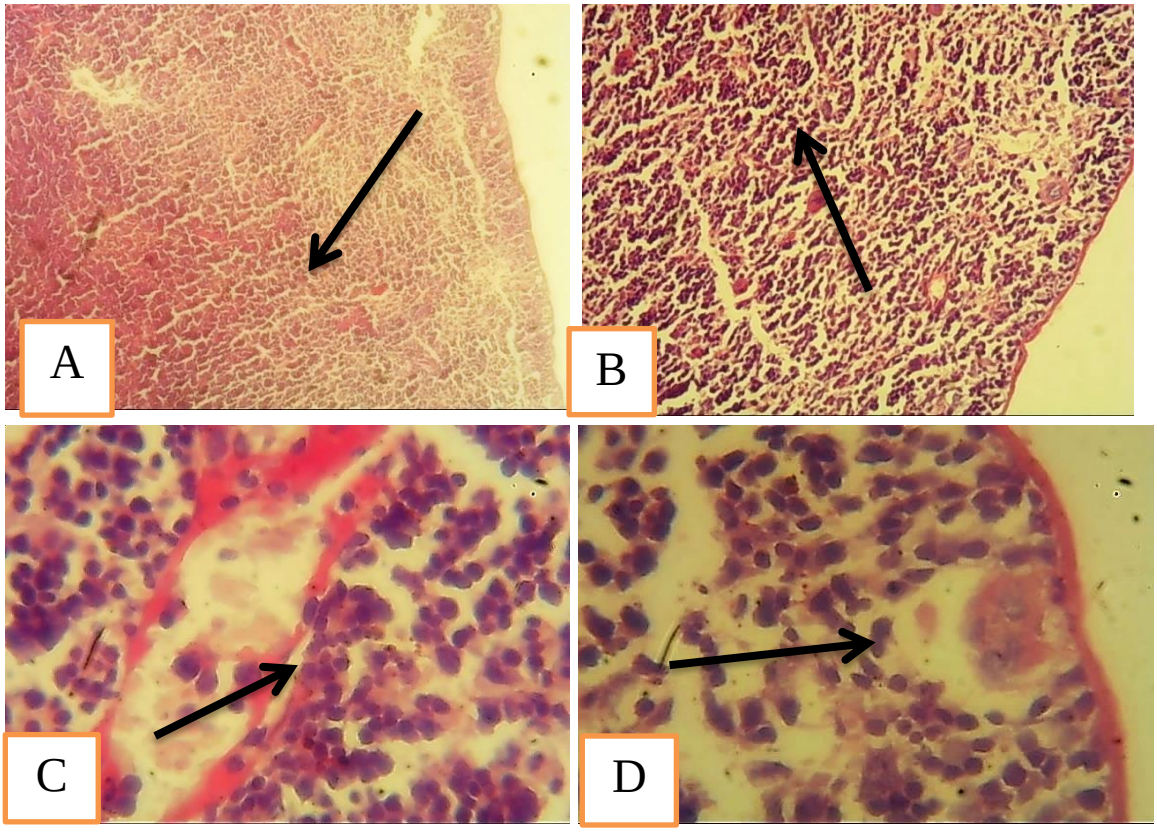

Figure (14): Spleen of mice infected with *Aspergillus Flavus* ((1X10^7 cells/mL).at dose of 0.1 ml/mouse. A&B: white's pulp's depletion's and megakaryocytes's (arrow) 10X H&E. C: inflamm- atory's cell infilt ration's (arrow) H&E 40X. D: Hemor-rhagic's and congestive changes (arrow): H&E 40X.

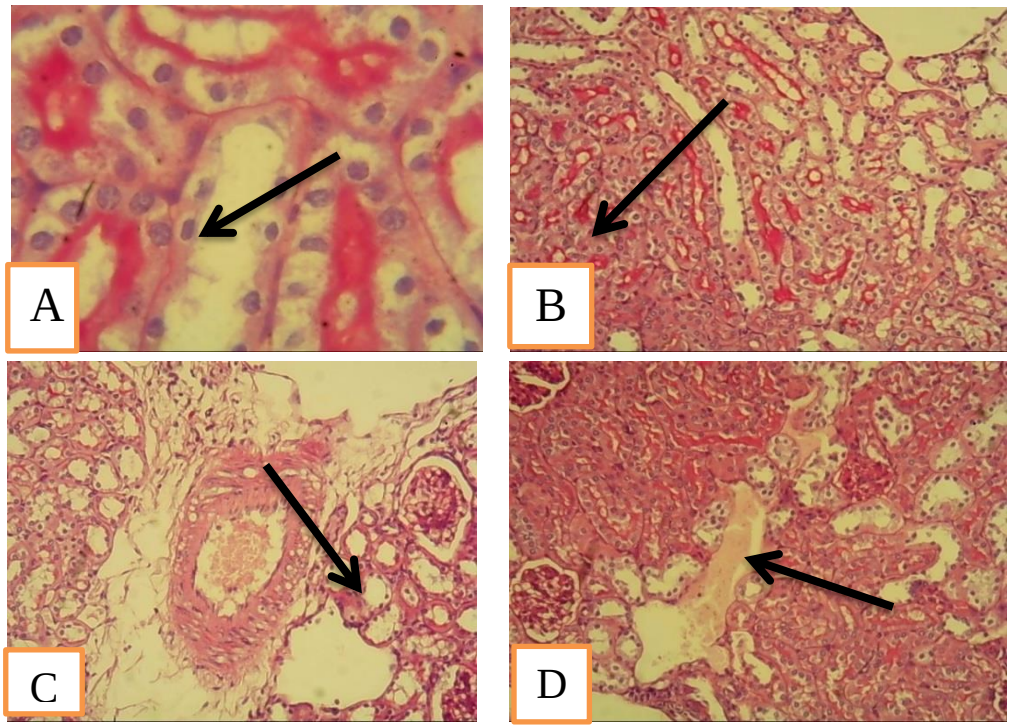

Figure (15): Kidney of mice infected with *Aspergillus Flavus* (1X10^7 cells/mL).at dose of 0.1 ml/mouse IP. A&B: renal tubules with severe hyalinization (arrow) H&E.10X &40X. C: swell-ling's of tubes nears thinking blood vessels (arrow) H&E 10X; D: pyelo-nephritis's H&E 10X

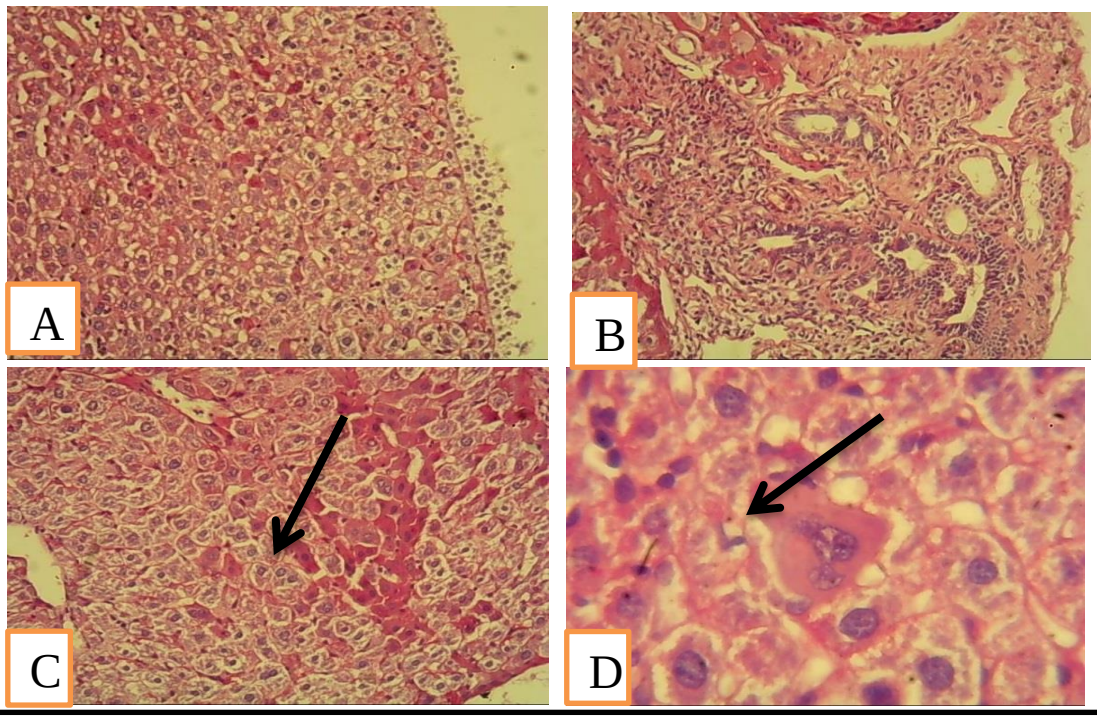

Figure (16): Liver's mice infected with *Aspergillus flavus* (1 X 10^5 cells/mL) at dose of 0.1 ml/mouse. IP. Showed; A: hepatocytes's vacoulation and severs inflammatory reaction's (arrow). H&E.10X B:bile duct hyperplasia's with inflammations associated kupffer cells. C: lobularing necrosis's (arrow) H&E 10X, D: apoptosis (arrow). H&E 40X

Aspergillus niger infection in lung section: caused Hemorrhagic and congestive changes associated with intestinal Pneumonia enclosed to pleura which appear inflamed emphysematous alveoli with bronchitis as shown in Figure (17) Spleen section: tissue specimen showed necrotic foci associated with granuloma in spleen and pneumonia, pleurisies with present of fungi mycelium in inflamed tissue as shown in Figure (18). Kidney section: showed nephritis with phlebitis associated with spread infection and fungi appearance in infected area figure (19). Liver section: Hepatitis related to coagulative necrosis is very clear with evidence of amyloidosis as well as Fungi (20).

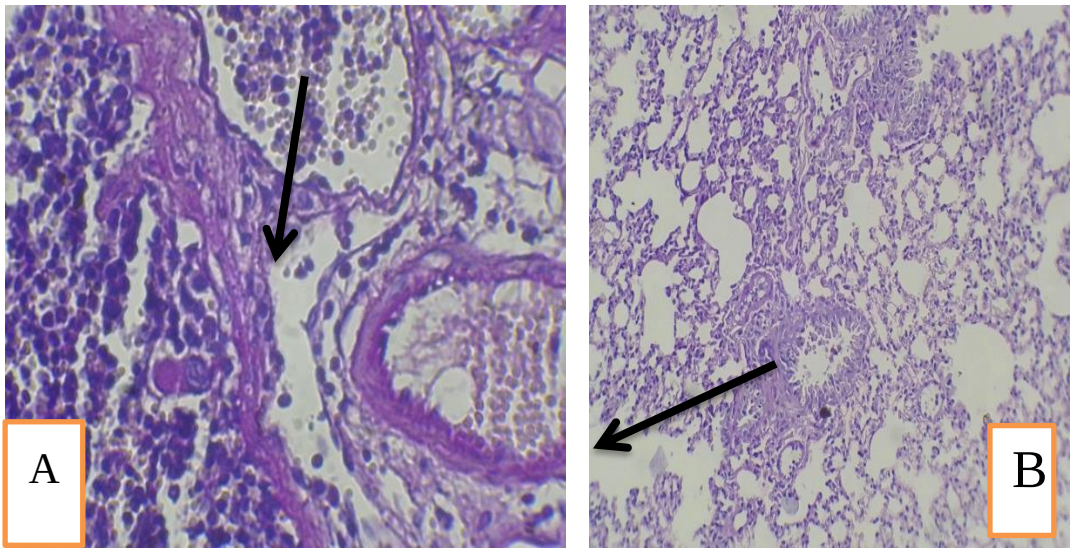

Figure (17): Lung of mice infected with *Aspergillus Niger* (1 X 10^7 cells/mL), at dose of 0.1 ml/mouse. IP. .showed A: Hemorrhagic changes associated with intestinal Pneumonia's pleura appear inflamed B: emphysimatous; bronchitis's (arrow). PAS 40X & 10X.

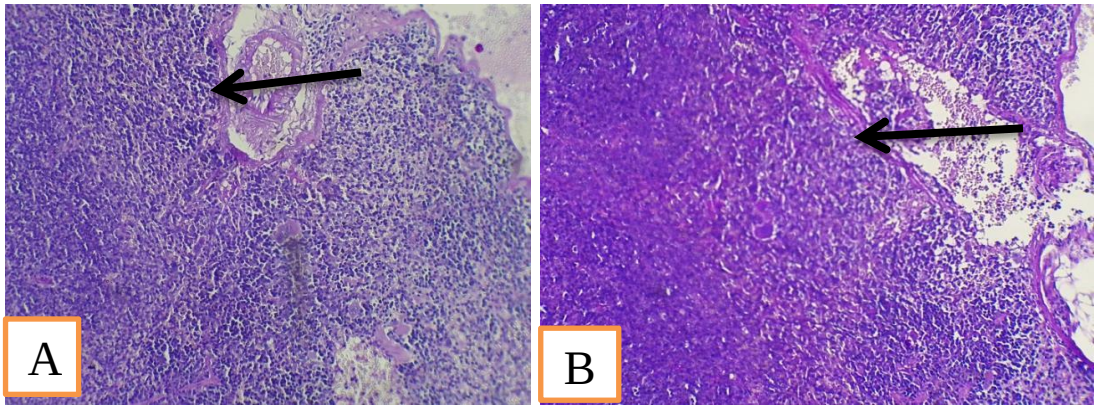

Figure (18): Spleen of mice infected with *Aspergillus Niger* (1 X10^7 cells/mL) at dose of 0.1 ml/mouse IP. showed A: spilinites's and granuloma's (arrow), and B: necrotic foci fungi colonies enclosing to narcotic's foci (arrow). PAS 10X.

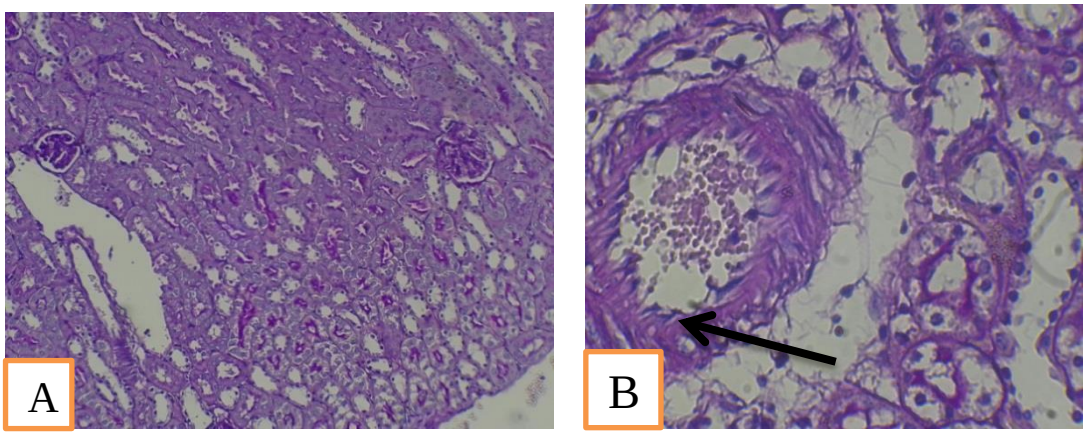

Figure (19): Kidneys of mice infected with *Aspergillus Niger* (1 X 10^7 cells/mL).at dose of 0.1 ml/mouse IP, showed A: atrophy's of glomeruli's with hyalinization's of renal's tubules's (arrow) B: congesting and inflaming blood vessels and fungi hyphae seen (arrow). PAS 40X & 10X.

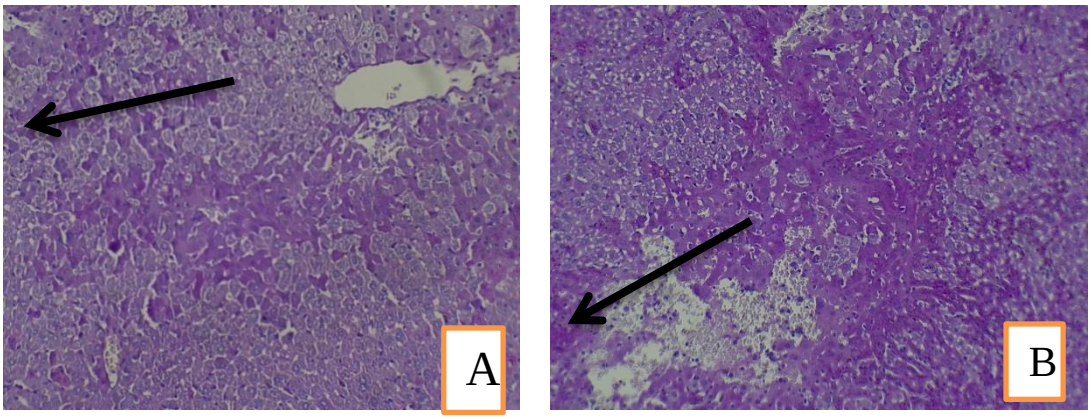

Figure (20): Liver's mice's with *Aspergillus Niger* (1X10^5 cells/mL) at dose of 0.1 ml/mouse IP A: necrosis with amyloid's like deposition's (arrow) B: necrosis beneath fungi's hyphaes (arrow). PAS 40X & 10X.

Aspergillus Ochraceus infection in Lung section: caused severe interstitial pneumonia with necrotic alveoli contained inflammatory cell attached to fibrinous exudations at affected area as shown in Figure (21). Spleen section suffering from heavy inflammatory reaction appear as granuloma and other section showed sever degenerative changes white and red pulp as shown in Figure(22). Kidney section: also appear inflamed and shows presence of fungal hyphae as shown in Figure (23). Liver show: necrosis of hepatocytes with sever degenerative changes fungi hyphae also seen as shown in Figure(24).

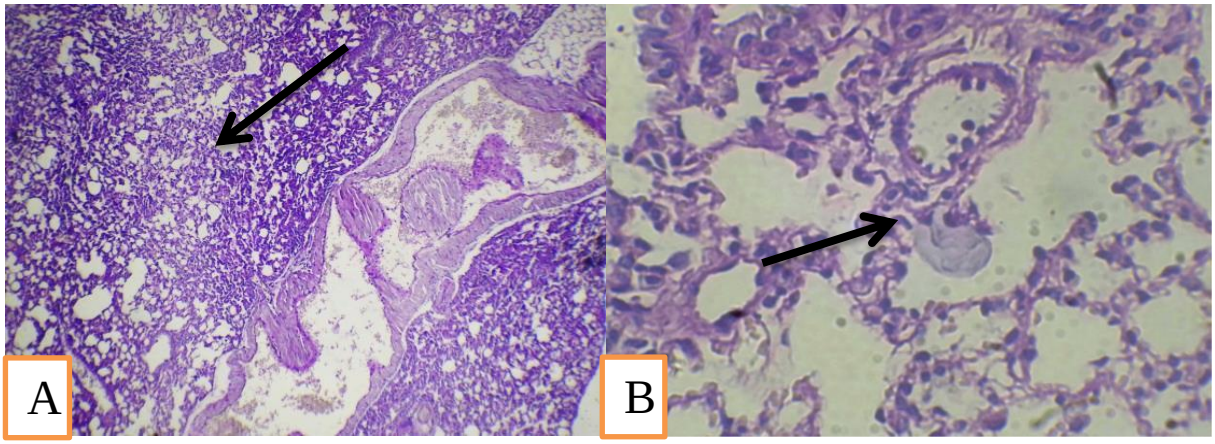

Figure (21): Lungs of mice infected with *Aspergillus Ochraceus* (1 X 10^7 cells/mL).at dose of 0.1 ml/mouse. IP, shows A: interstitials (arrow) B: narcotic's alveoli wall and inflammation's cells attached to broken it's wall (arrow). PAS 10X &40X.

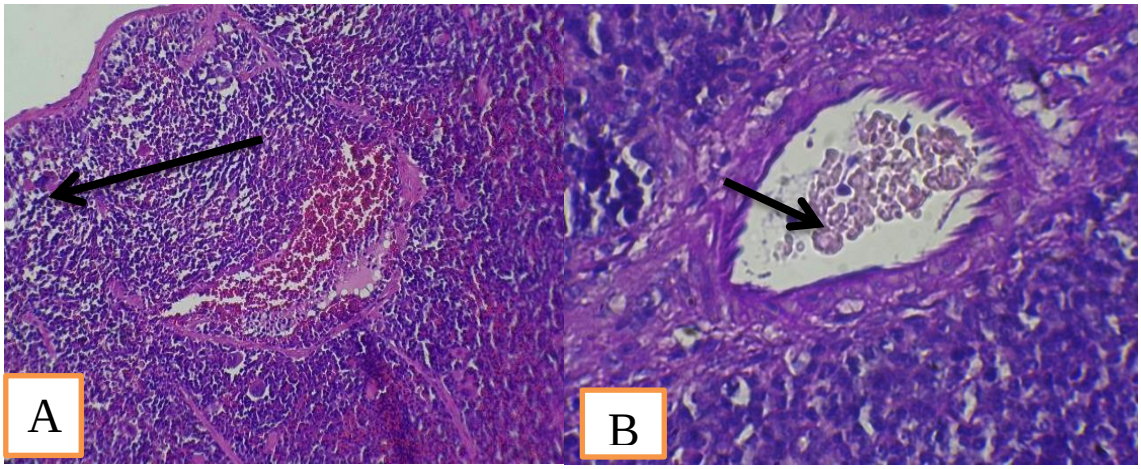

Figure (22): Spleen's of mice infected with *Aspergillus Ochraceus* (1X10^7 cells/mL) at dose of 0.1 ml/mouse. IP Showed; A: splenitis granuloma near to congested inflamed blood vessels (arrow) B: thickened congested blood vessels in red pulp (arrow). PAS 40X &10X.

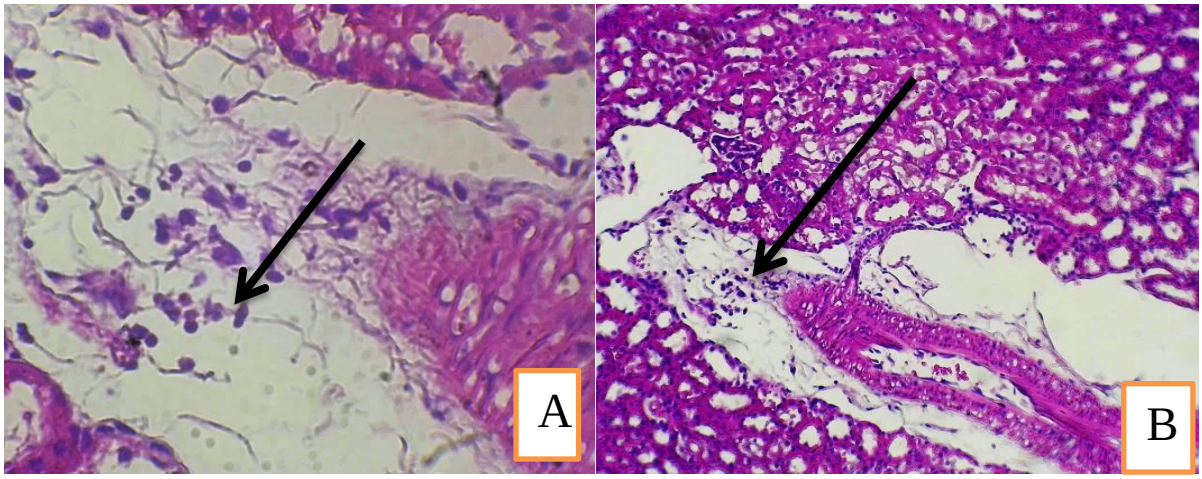

Figure (23): Kidney's mice infected with *Aspergillus Ochraceus* (1X10^7 cells/mL), at dose of 0.1 ml/mouse IP. Showed; A: pyelonecrotic-nephritis's, congested blood's vessel's (arrow) B: fungi colonies also seen (arrow).PAS 10X &40X.

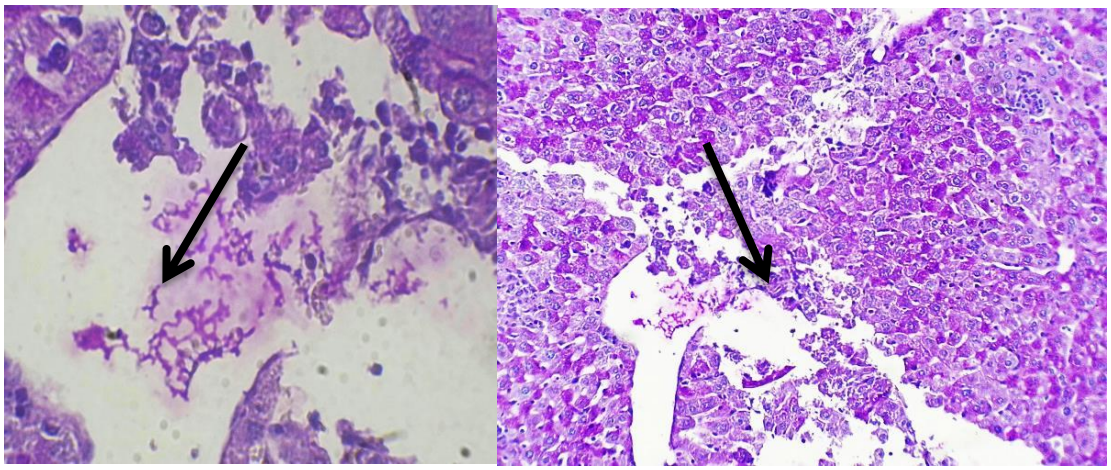

Figure (24): Liver's mice infected with *Aspergillus Ochraceus* (1X10^7 cells/mL) at dose of 0.1 ml/mouse. IP. Two pictures, show area of necrosis s fungi filamentous hyphae (arrow). PAS 10X &40X.

***Cryptococcus neoformans* infection caused** pyelonephritis and congested blood vessels, granulomatous foci within tubules lumen as shown in Figure (25). Liver section: Showed necrotic area contained colonies of yeas and dead hepatocytes granulomatous foci within sinusoidal space as shown in Figure (26).

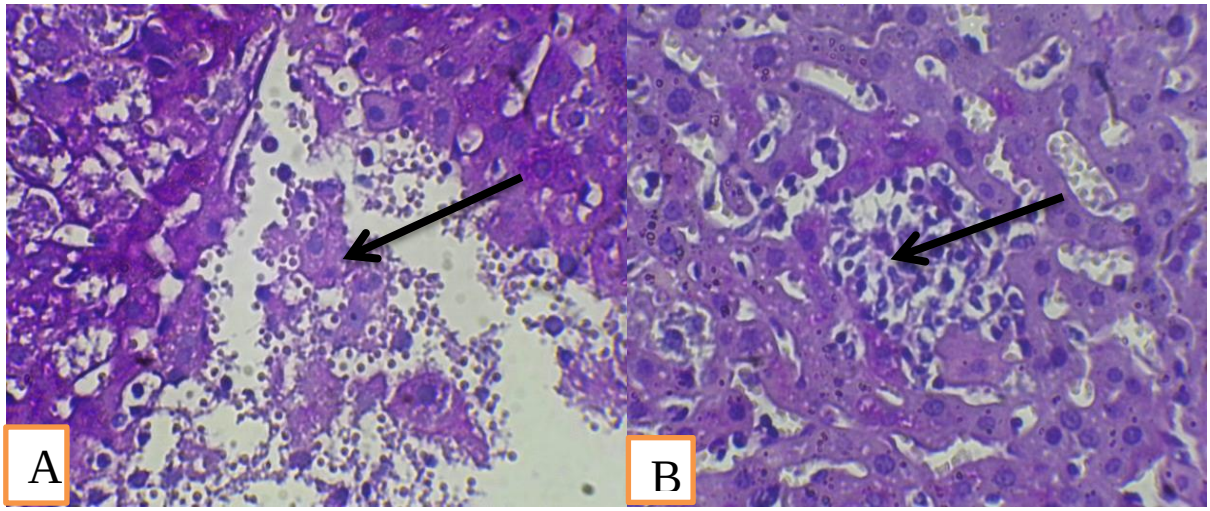

Figure (25): Liver of mice infected with *Cryptococcus spp* (1X10^7 cells/mL) at dose of 0.1 ml/mouse. IP. Showed ; A: necrosis and granuloma area fungal colonies within necrotic area (arrow) B: granuloma (arrow). PAS 10X &40X.

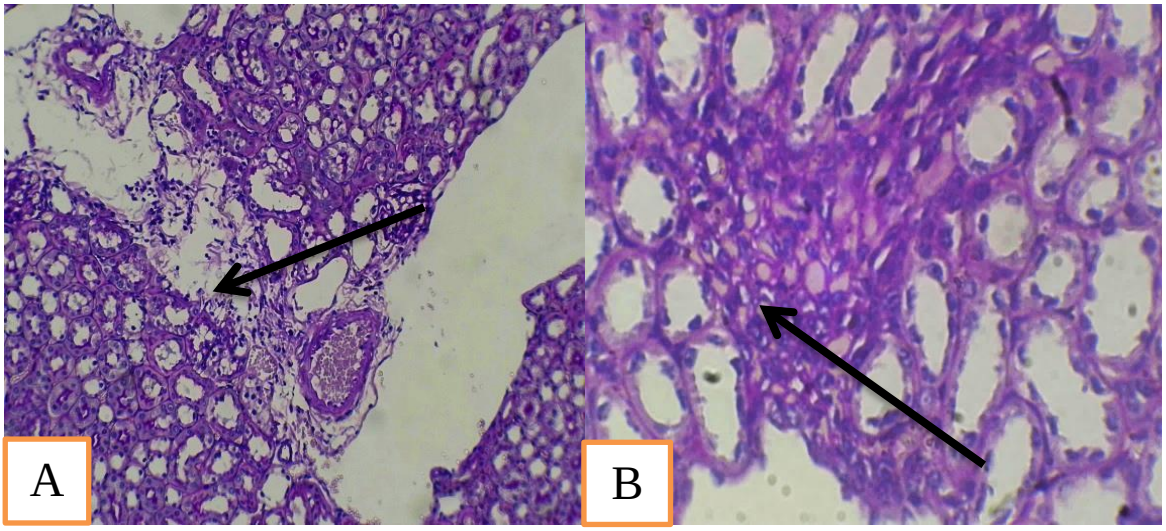

Figure (26): Kidneys of mice infected with *Crypococcus spp* (1X10^7 cells/mL) at dose of 0.1 ml/mouse. IP. Shows; A: interstitial nephritis (arrow) B: heavy granulomatous reaction attached to degenerated tubules (arrow). PAS 10X &40X.

Pathological changes in the kidney in cases of yeast infection is due to the presence of a large amount of yeast mannose & B-glucose receptors [22] that made the kidney one of the target organs for yeast. As for the appearance of signs of diffuse hemorrhage on the fungus-infected organs in wild rats, it is attributed to the penetration of spores. their secretion of toxins and lytic enzymes, and this is the reason for the rupture of the capsule for some organs, such as the liver and spleen, and ulceration of the intestinal wall with the appearance of petechial hemorrhage spots, in agreement with Mao Jedda [23] and the emergence of some cases of severe lymphatic drainage in the spleen is due to the occurrence of an inflammatory reaction resulting The stimulation of the immune response was represented by an overgrowth of lymphocytes and macrophages with an increase in the number of neutrophils and eosinophils, and

this led to an increase in the destruction of tissue cells and then the appearance of signs of cellular necrosis [24]. Respiratory lesions work on breathing and irregularity, shortness of breath to loss of appetite, weight loss, lethargy and withdrawn, watery eyes. Nasal and oral secretions, severe diarrhea, severe secretions, linear rashes [25] and with what he explained[26] and that the development and severity of pathological signs is due to the fact that the cause of virulence factors is infection with this bird from the body against infection with this disease [27]. The onset of a problem in the onset of tumor tumors is significant for those responsible for those diffuse granulomatous foci [28] and the appearance of signs of internal bleeding spread to the organs such as the liver, kidney, lung and intestines, is attributed to the penetration of this type of odor and the appearance of spots, and the diffuse leaflet, and this is what he mentioned previously [21]. Author's [29] make similar study in mice. Some worker's revealed that fungi experimental infection widely used in mammalian and mostly frequent in mice [30,31,32,33].

Fungi considers as pollutant's agents in environments and occurs as naturally infections in differing's types of pet's animals and caused severe pathological lesions.

References:

1.Alsohaili, S., & Bani-Hasan, B. M. (2018). Morphological and molecular identification of fungi isolated from different environmental sources in Northern Eastern Jordan Desert. Jordan Journal of Biological Sciences, 11(3), 329-337. ISSN: 1995-6673.

2.Jalil, W. I., Minnat, T. R., Walaa Mahmood Mohamed, & Anas A. Humadi. (2020). Isolation and identification of some fungi from dogs and assessment of antifungal activity of Nystatin and Metronidazole in vitro. Annals of Tropical Medicine and Public Health, 23(24), November 2020. DOI: 10.36295/ASRO.2020.2324433.

3.Krüger, W., Vielreicher, S., Kapitan, M., Jacobsen, I. D., & Niemiec, M. J. (2019). Fungal-bacterial interactions in health and disease. Pathogens, 8(2), 70.

4.Deutsch, P. G., Whittaker, J., & Prasad, S. (2019). Invasive and non-invasive fungal rhinosinusitis: A review and update of the evidence. Medicina, 55(7), 319. DOI: 10.3390/medicina55070319.

5.Ulloa-Avellán, O., Calderón-Hernández, A., Rubí-Chacón, R., & Vargas-Leitón, B. (2022). Aspergillus spp. isolated from lungs of poultry (Gallus gallus) at the Mycology Laboratory, School of Veterinary Medicine, Universidad Nacional, Heredia, Costa Rica between 2008 and 2021 and associated factors. Journal of Fungi (Basel), 9(1), 58. DOI: 10.3390/jof9010058.

6.Alves, S. H., Oliveira, M. G., Lobato, A. K., et al. (2010). Mycotoxicosis in animals: Clinical and pathological aspects. Journal of Venomous Animals and Toxins including Tropical Diseases, 16(3), 421-434.

7.Abad, A., Fernández-Molina, J. V., Bikandi, J., Ramírez, A., Margareto, J., Sendino, J., Hernando, F. L., Pontón, J., Garaizar, J., & Rementeria, A. (2010). What makes Aspergillus fumigatus a successful pathogen? Genes and molecules involved in invasive aspergillosis. Revista Iberoamericana de Micología, 27(4), 155-182.

8.Zmeili, O. S., & Soubani, A. O. (2007). Pulmonary aspergillosis: A clinical update. QJM, 100, 317–334.
9.Arné, P., Thierry, S., Wang, D., Deville, M., Le Loc'h, G., Desoutter, A., Féménia, F., Nieguitsila, A., et al. (2011). Aspergillus fumigatus in poultry. International Journal of Microbiology, 746356.
10.Elad, D., & Segal, E. (2018). Diagnostic aspects of veterinary and human aspergillosis. Frontiers in Microbiology, 9, 1303.
11.Jorgensen, J. H., Pfaller, M. A., Carroll, K. C., Funke, G., Landry, M. L., Richter, S. S., & Warnock, D. W. (2015). Manual of Clinical Microbiology, 11th Edition, Vol. 1.
12.Samson, R. A., & Van Reenen Hokstra, E. S. (1988). Introduction to foodborne fungi (3rd ed.). Grafisch Netherlands.
13.Baron, E. J., & Finegold, S. M. (1990). Bailey and Scott's Diagnostic Microbiology, 8th Ed. Mosby Co., pp. 172-173.
14.Faraj, M. K. (1990). Regulation of mycotoxin formation in Zea mays. Ph.D. Thesis, Department of Bioscience and Biotechnology, University of Strathclyde, Glasgow, U.K.
15.Luna, L. G. (1968). Manual of Histological Staining Methods of the Armed Forces Institute of Pathology, 3rd edition. McGraw-Hill Book Company, New York.
16.Wincewice, E. (2002). Microbiological examination of wild rats living in various environments in the epizootic aspects. Electronic Journal of Polish Agricultural Universities, 5(1), Series Veterinary Medicine.
17.Al-Amery. (2006). Pathological study of pathogenic fungi isolated from wild rats in Baghdad. M.Sc. in Pathology, College of Veterinary Medicine, University of Baghdad, pp. 1-70.
18.John & Gatherin. (2023). Introduction to Fungi, 3rd edition. Cambridge University Press. ISBN: 9780521807395.
19.Gow, N. A., Brown, A. J., & Odds, F. C. (2004). Fungal morphogenesis and host invasion. Current Opinion in Microbiology, 5, 366-371. DOI: 10.1016/j.mib.2004.06.003.
20.Camargo, Z. P., Ono, M. A., & Vicente, V. A. (2016). Histopathological changes in the lungs of cats experimentally infected with Cryptococcus neoformans var. grubii. Journal of Comparative Pathology, 155(3), 200-204.
21.Casadevall, Steenberger, A. J., & Nosanchuk, D. J. (2003). "Ready-made" virulence and "dual-use" virulence factors in pathogenic environmental fungi paradigm. Current Opinion in Microbiology, 6, 332-337.
22.Peter, J., & Ivan, M. R. (1998). Encyclopedia of Immunology, Vol. 2. Academic Press, San Diego.
23.Deng, S., Yang, R., Chang, C., et al. (2019). Aspergillus infections in animals. Veterinary Microbiology, 235, 193-203. DOI: 10.1016/j.vetmic.2019.07.017.

24.Cox, G. M., Mukherjee, J., Cole, G. T., Casadevall, A., & Perfect, J. R. (2000). Urease as a virulence factor in experimental cryptococcosis. Infection and Immunity, 68(2), 443-448.
25.Dagenais, T. R. T., & Keller, N. P. (2009). Pathogenesis of Aspergillus fumigatus in invasive aspergillosis. Clinical Microbiology Reviews, 22(3), 447-465. DOI: 10.1128/CMR.00055-08.
26.Almeida, T. N., de Lima, N. T., de Sousa, M. G., Dos Santos, W. O., de Oliveira, J. V., & Guerra, R. O. (2017). Histopathological changes induced by experimental infection with Malassezia pachydermatis in dogs. Journal of Comparative Pathology, 157(4), 244-248.
27.Tomee, J. F., & Kuffman, H. F. (2020). Putative virulence factors of Aspergillus fumigatus. Clinical and Experimental Allergy, 30(4), 476-484.
28.Jantumen, E., Pilonen, A., Volin, L., Pakkal, T., Koukila, P., & Runtu, P. (2000). Diagnostic aspects of invasive Aspergillus infections in allogeneic bone marrow transplant recipients. Bone Marrow Transplants, 25(8), 867-871.
29.Roeder, A. C., Kirching, J., Pupec, R. A., Schaller, M., & Korting, H. C. (2004). Toll-like receptors and innate antifungal responses. Trends in Microbiology, 12, 44-49. DOI: 10.1016/j.mib.2004.06.003.
30.Mirkov, P., Zolotarevski, L., Glamočlija, J., & Kataranovski, D. (2008). Experimental disseminated aspergillosis in mice: Histopathological study. Journal de Mycologie Médicale, 18(2), 75-82.
31.Farnell, E., Rousseau, K., Thornton, D. J., Bowyer, P., & Herrick, S. E. (2012). Expression and secretion of Aspergillus fumigatus proteases are regulated in response to different protein substrates. Fungal Biology, 116, 1003–1012.
32.Gathwan, K. H., Husein, S. I., Hashim, M. S., & Obaid, S. M. (2023). Evaluation of garlic efficiency on precipitated lead chloride in hepatocytes of mice. JUAPS, 17(2), 12-18. P-ISSN 1991-8941, E-ISSN 2706-6703.
33.Gnat, S., Łagowski, D., Nowakiewicz, A., & Dyląg, M. (2021). A global view on fungal infections in humans and animals: Opportunistic infections and microsporidioses. Journal of Applied Microbiology, 131(5), 2095–2113. DOI: 10.1111/jam.15032. Published: November 1, 2021.