Histopathological change induced by experimantaly infecion of fungi isolated from pet animals in mice

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Introduction

A Fungus is one of the most diverse microorganisms that inhabit different environmental sources such as soil, plant parts (leaves, root and fruits), water and food sources .The growth and distribution of fungi are affected by different environmental factors such as temperature, pH, moisture, degree of aeration, amount and type of nutrients (Alsohaili and Bani-Hasan, 2018). In latest years, numerous opportunistic fungal infections like Aspergillosis, Candidiasis, Zygomycosis, Cryptococcosis, Geotrichosis, Rhodotoruliosis and Fusariosis have been documented as an imperative cause of mortality and morbidity in developed in addition to developing nations ,these fungi are broadly common in environment and are recovered from air, plant, substrates , water, soil. Fungus can distress a lot of organs of body for example eye, ear, sinus, lung, brain, bone, skin, kidney and heart. Fungi are widespread in both indoor and outdoor environments as mycelial fragmentation, spores or dissociated extracellular and intracellular components. They are generally saprophytic and not dangerous but under extra ordinary situation like lung diseases weakened immune systems or lung diseases, they can cause infections (Jalil et al ; 2020).

The majority common fungi that infected mucosal tissues like *Candida albicans , Cladosporium, Aspergillus, Fusarium, Cryptococcus,* and *Malassezia* (Krüger et al; 2019). These Fungi can reach to the sinonasal cavities via spores inhaled from animals (Deutsch et al., 2019).

Aspergillosis is caused by filamentous fungi belonging to the genus Aspergillus(Ulloa-Avellán et al., 2023), that include Aspergillus fumigatus, A. flavus, A. terreus, A. niger and others ,these diseases cover a wide range of infections from localized conditions through allergic reactions to fatal disseminated infections in humans and animals(Gnat et al., 2021). And Aspergillus species have multiples virulence causes like phospholipase enzymes and gliotoxin, make this species to invading tissue of the host causing infections(Abad et al., 2010). In animals, aspergillosis is primarily a respiratory infection that may become generalized (Zmeili and Soubani, 2007). Avian aspergillosis is noted predominantly as diseases of the respiratory tract, but all organs can be involved, leading to a variety of acute or chronic manifestations (Arné et al. , 2011). Sinonasal, bronchopulmonary, and disseminated infections are the major forms of aspergillosis in dogs and cats (Elad and Segal, 2018).

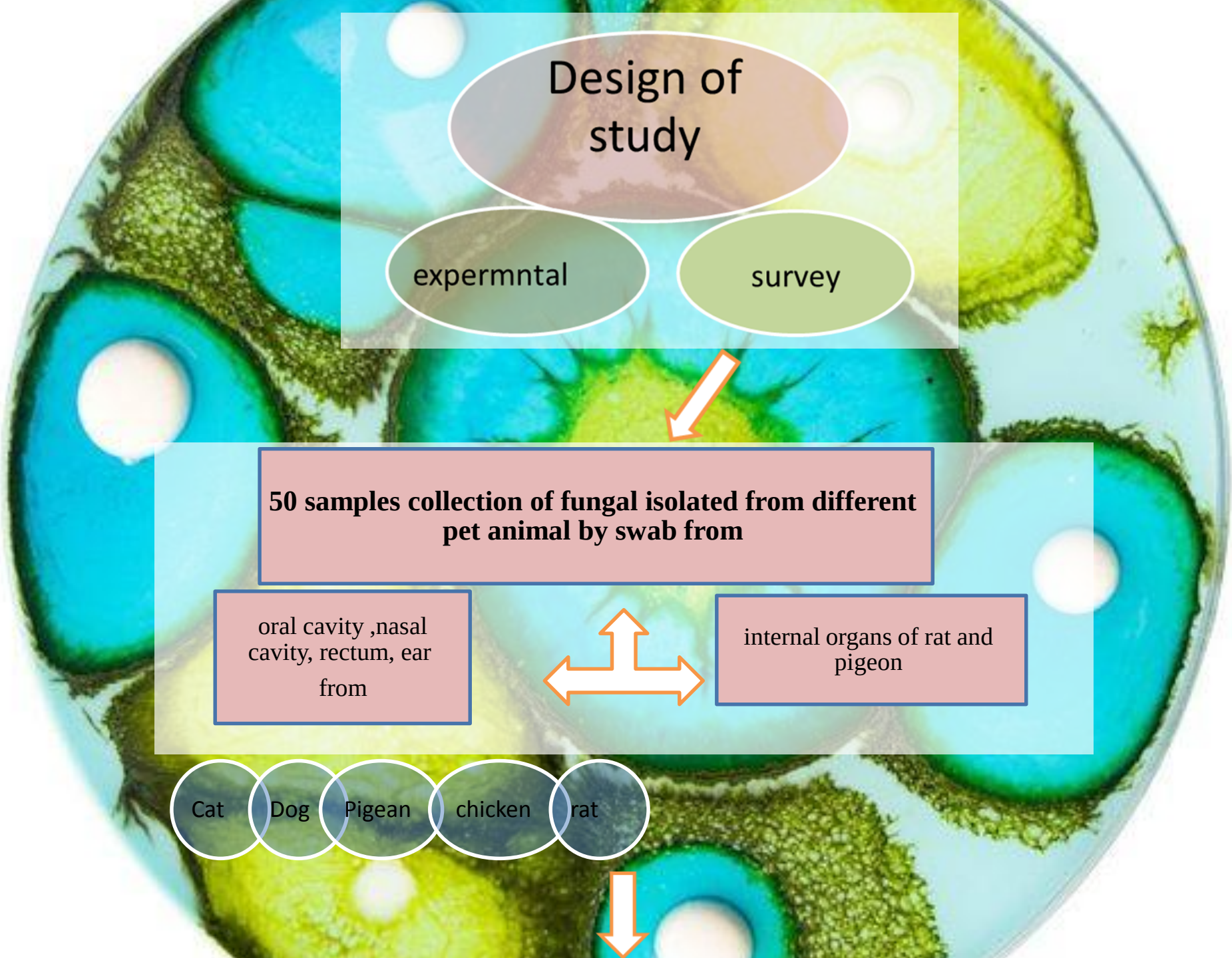
Aim of study

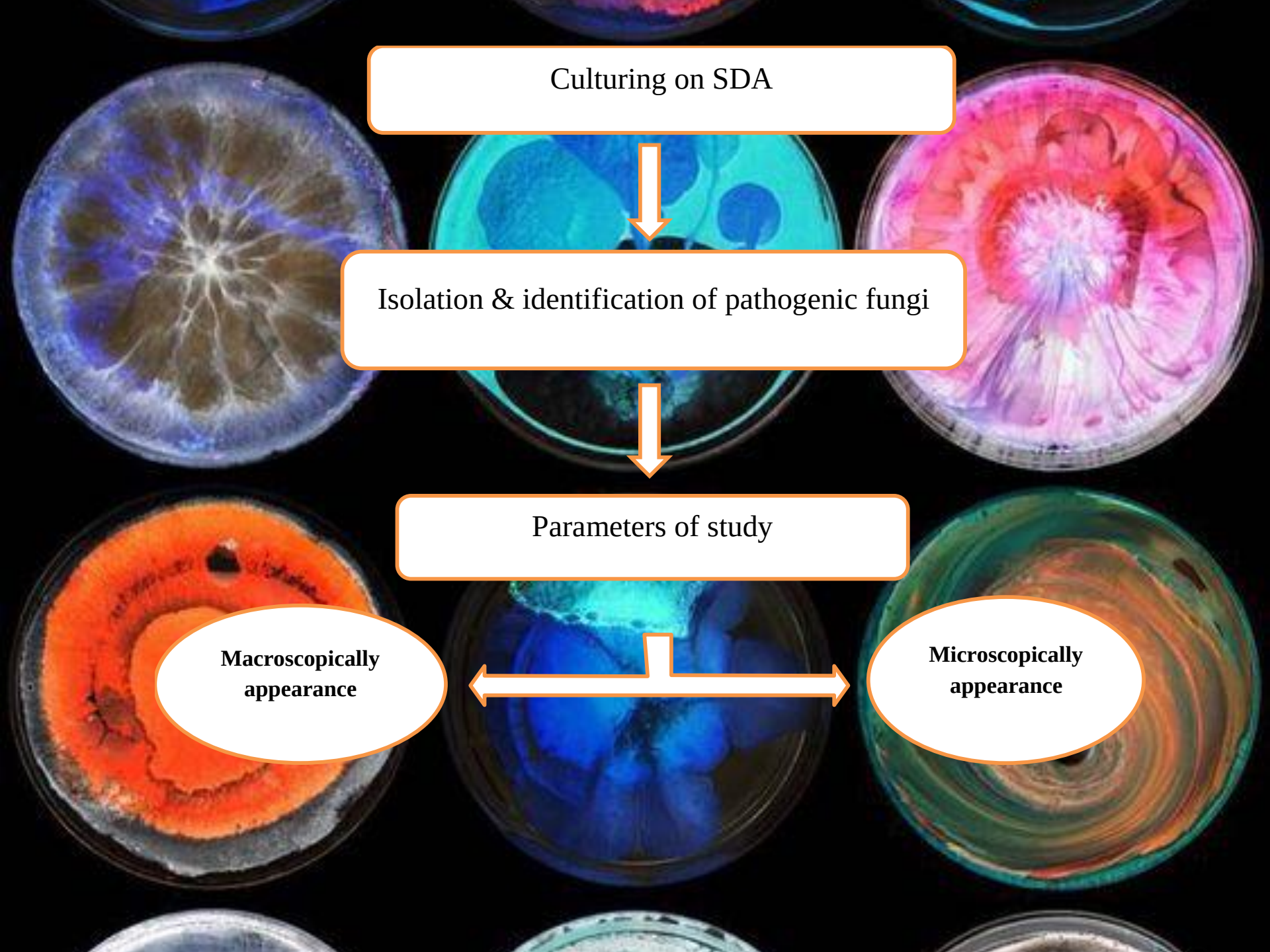
1- Isolation and identification of the most important pathological fungi from collection oral, nasal, rectal samples from cats, dogs, poultry and from members of wild rats from different places in the city of Baghdad.

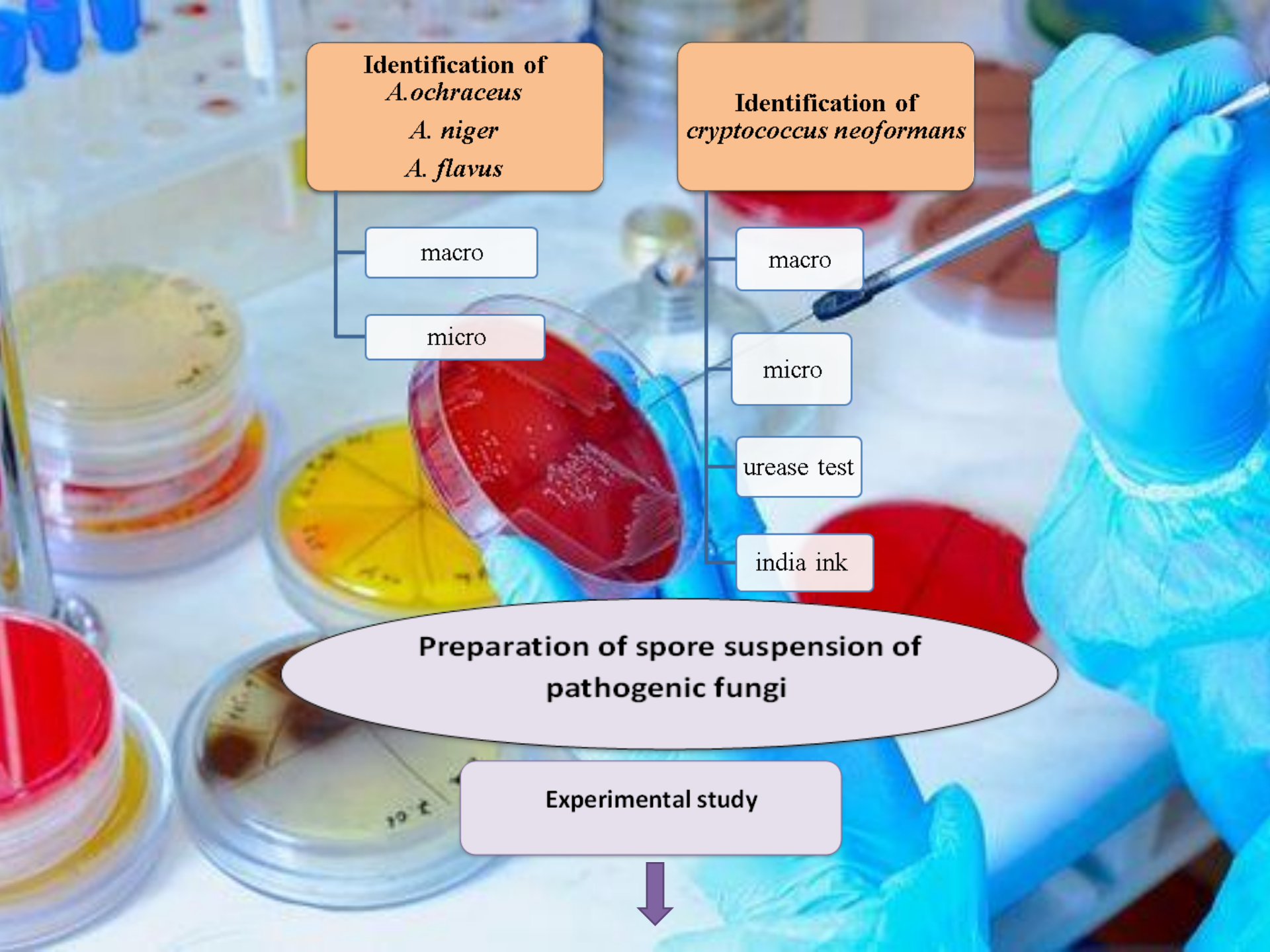
2- Investigation the pathogenisity of highest proportion of fungal isolates in mice.

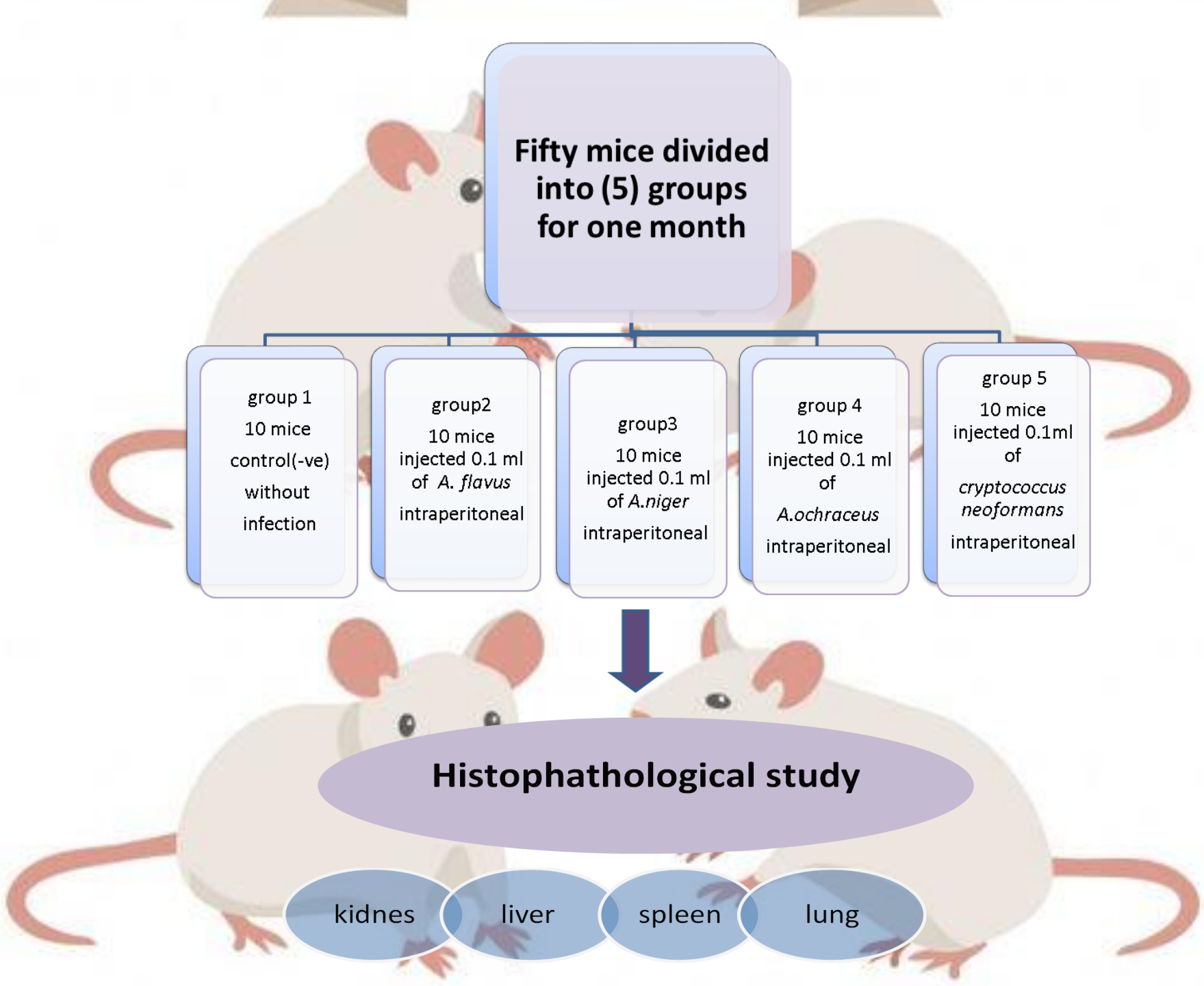
3- Histopathological examination of lung, liver, spleen and kidney.

Materials and Methods

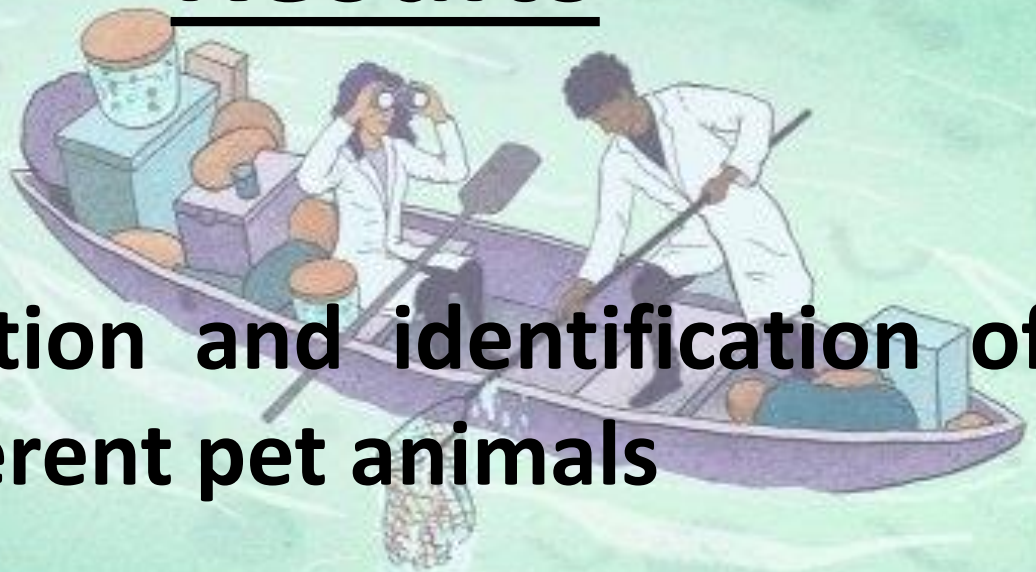

Design of study
expermntal
survey
50 samples collection of fungal isolated from different pet animal by swab from
oral cavity ,nasal cavity, rectum, ear from
internal organs of rat and pigeon
Cat
Dog
Pigean
chicken
rat


Culturing on SDA
Isolation & identification of pathogenic fungi
Parameters of study
Macroscopically appearance
Microscopically appearance


Identification of
A.ochraceus
A. niger
A. flavus
macro
micro
Identification of
cryptococcus neoformans
macro
micro
urease test
india ink
Preparation of spore suspension of pathogenic fungi
Experimental study


Fifty mice divided into (5) groups for one month
group 1
10 mice
control(-ve)
without
infection
group2
10 mice
injected 0.1 ml
of A. flavus
intraperitoneal
group3
10 mice
injected 0.1 ml
of A.niger
intraperitoneal
group 4
10 mice
injected 0.1 ml
of
A.ochraceus
intraperitoneal
group 5
10 mice
injected 0.1ml
of
cryptococcus
neoformans
intraperitoneal
Histophathological study
kidnes
liver
spleen
lung

Results



The isolation and identification of fungi from different pet animals

Table (4-1):- The percentage of the different fungi isolated from different pet animals:-

	Types of fungi isolates	Number	Percentage %
1	*Aspergillus flavus*	7	14
2	*Aspergillus fumigatus*	1	2
3	*Aspergillus niger*	7	14
4	*Aspergillus ochraceus*	3	6
5	*Aspergillus ustus*	1	2
6	*Aspergillus terreus*	3	6
7	*Cladosporum spp.*	8	16
8	*Chrysosporium spp.*	2	4
9	*Cryptococcus neoformans*	2	4
10	*Fusarium spp.*	1	2
11	*Penicillium spp.*	3	6
12	*Penicillium chrysogenum*	3	6
13	*Rhizopus spp.*	4	8
14	Unknoun fungi	5	10
Total		50	100

Table (4-2):-The percentage of fungi isolated from dogs which collected from different sample

	Types of fungi isolates from dogs	Number	Sample	Percentage (%)
1	*Aspergillus flavus*	٣	• Oral cavity (2) • Rectum	33.3
٢	*Aspergillus fumigatus*	1	• Oral cavity	11.1
٣	*Aspergillus niger*	1	• Rectum	11.1
٤	*Aspergillus ochraceus*	1	• Ear	11.1
5	*Aspergillus terreus*	1	• Nasal	11.11
6	*cladosporam spp.*	2	• Nasal	22.3
total		9		100

Table (4-3):- The percentage of fungi isolated from cat which collected from different samples:-

	Types of fungi isolates from cats	Number	sample	Percentage %
1	*Aspergillus terreus*	1	• Oral	11.1
2	*Aspergillus ustus*	1	• Rectum	11.1
3	*Aspergillus niger*	1	• ear	11.1
4	*Cladosporum spp.*	1	• rectum	11.1
5	*Fusarium spp.*	1	• rectum	11.1
6	*Penicillium spp.*	2	• rectum • oral	22.25
7	*Panicillium chrysogenum*	2	• oral • ear	22.25
total		9		100

Table (4-4):- The percentage of fungi isolated from rat which collected from different samples:-

	Types of fungi isolates from rats	No.	Sample	Percentage %
1	*Aspergillus flavus*	2	• Liver • Intestine	16.7
2	*Aspergillus niger*	3	• -genitalttract • -Brain • -nasal	25
3	*Chrysosporium spp*	2	• Rectum • Spleen	16.7
4	*Cladosporium spp*	2	• Recturm • Spleen	16.6
5	*Rhizopus spp*	3	• Spleen • Liver • Abdomen	25
total		12		100

Table (4-5):- The percentage of fungi isolated from pigeon which collected from different samples:-

	Types of fungi isolates	Number	Sample	Percentage %
1	*Aspergillus flavus*	1	• Trachea	10
2	*Aspergillus niger*	2	• Liver • Lung	20
3	*Aspergillus terrus*	1	• Spleen	10
4	*Cladosporum spp*	2	• Intestine • Heart	20
5	*Cryptococcus neoformans*	2	• Oral • Feces	20
6	*Peuicillium spp.*	1	• Kidney	10
7	*Penicillium chrysogenum*	1	• Intestine	10
total		10		100

Table (4-6):- The percentage of fungi isolated from chicken which collected from different samples:-

	Types of fungi isolates from chickens	Number	Sample	Percentage %
1	*Aspergillus flavus*	1	• Rectum	20
2	*Aspergillus ochraceus*	2	• Rectum • Spleen	40
3	*Cladosporum spp*	1	• Oral	20
4	*Rhizopus spp.*	1	• Oral	20
total		5		100

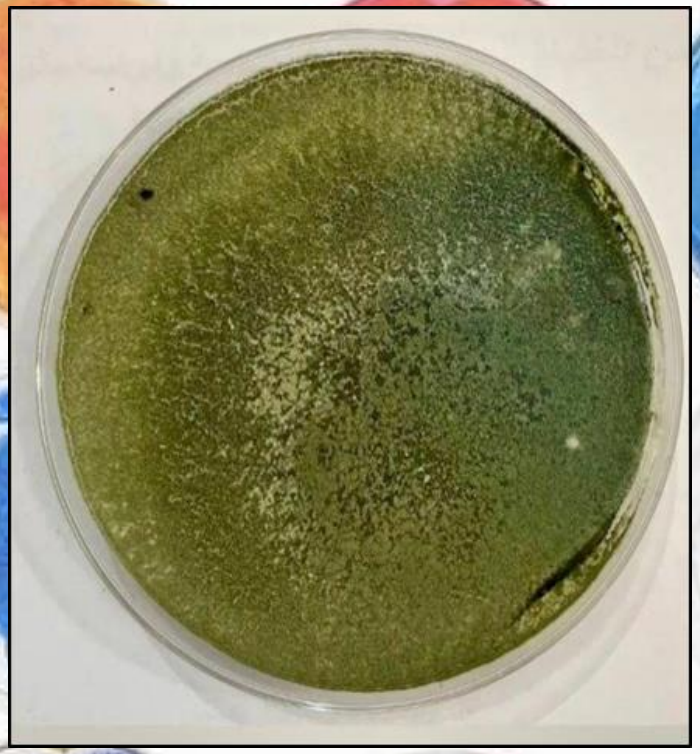


Figure (4-1): Macroscopic appearance of *Aspergillus flavus* on SDA at 25˚C for 5-7

Figure (4-2): Microscopic appearance of *Aspergillus flavus* by using lactoph ---enol cotton blue stain 40X

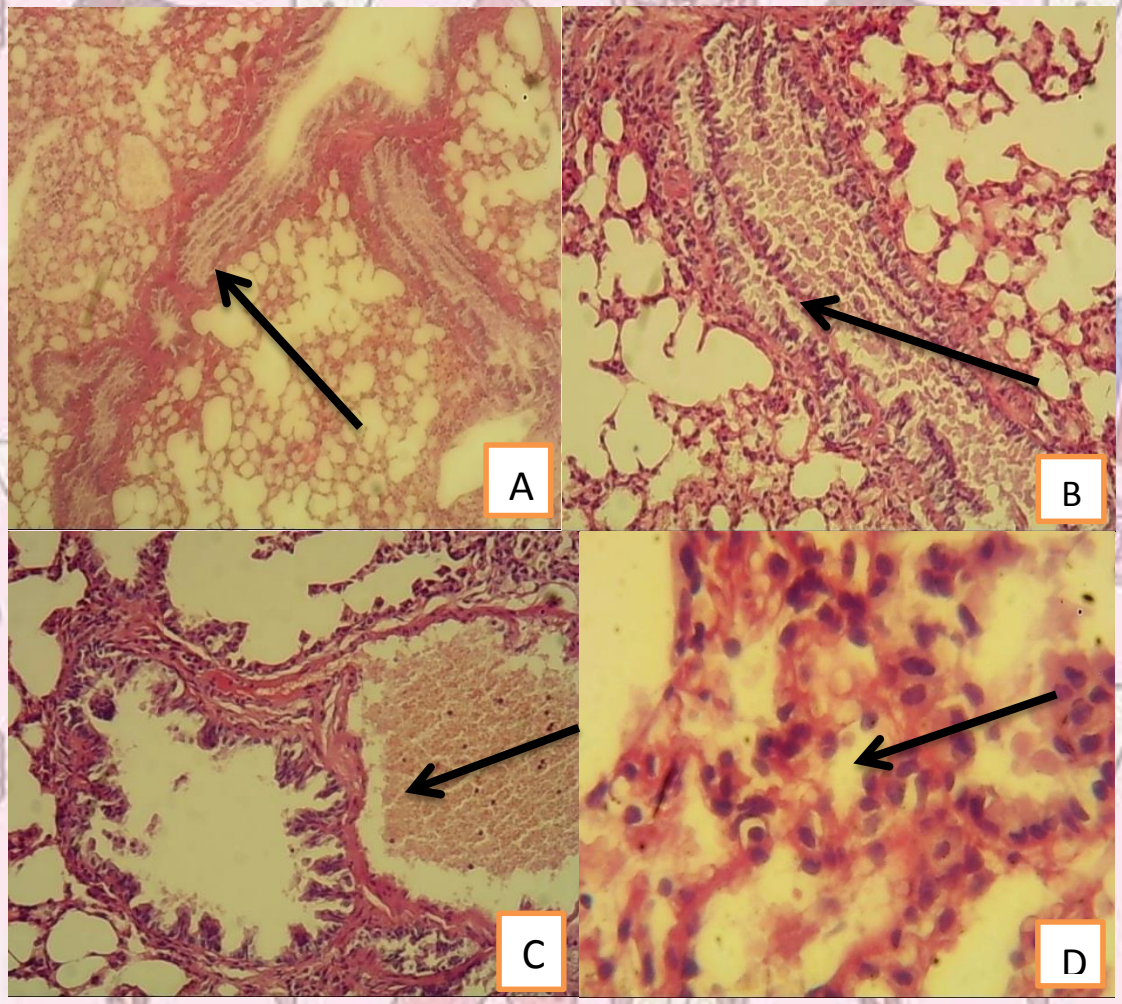


Figure (4-19): A;B;C;D lung of mice infected with *Aspergillus Flavus* (1 X 10^7 cells/mL).at dose of 0.1 ml/mouse .IP. .showed A&B: bronchial dilation near emphysematous alveoli (arrow).). H&E10X C: marked clotting near to degenerated bronchioles which have papillary projection (arrow):H&E40X D: inflammatory cells attached to fibrinous exudation within lumen of degenerated alveoli (arrow). H&E40X

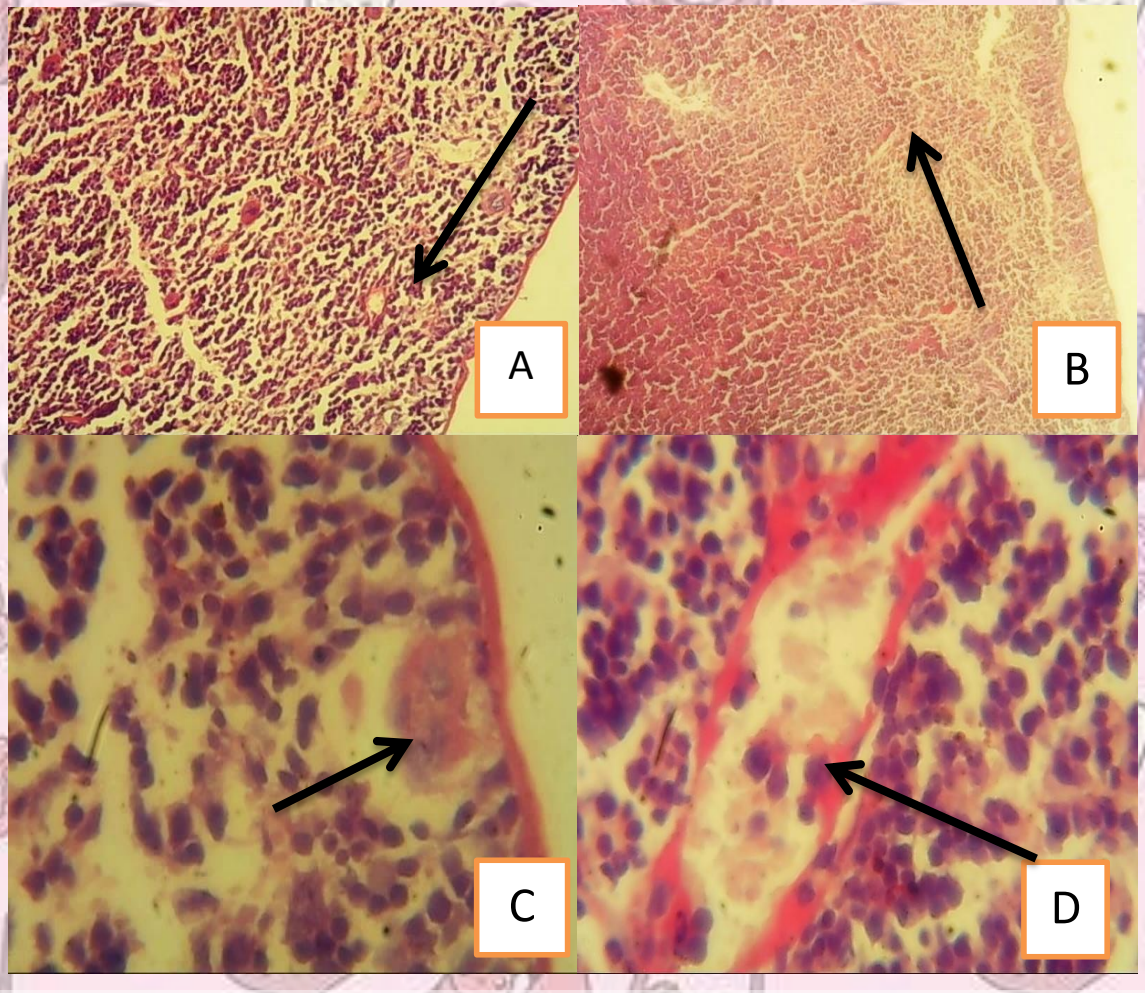


Figure (4-15): A;B;C;D : Spleen of mice infected with *Aspergillus Flavus* ((1 X 10^7 cells/mL).at dose of 0.1 ml/mouse .IP.. .showed splinitis A&B: white pulp depletion with proliferative megakaryocytes infiltration (arrow)10X H&E. C:: inflammatory cell infiltration around activated megakaryocytes seen(arrow) H&E 40X. D: Hemorrhagic and congestive changes near inflammatory reaction seen (arrow):H&E 40X.

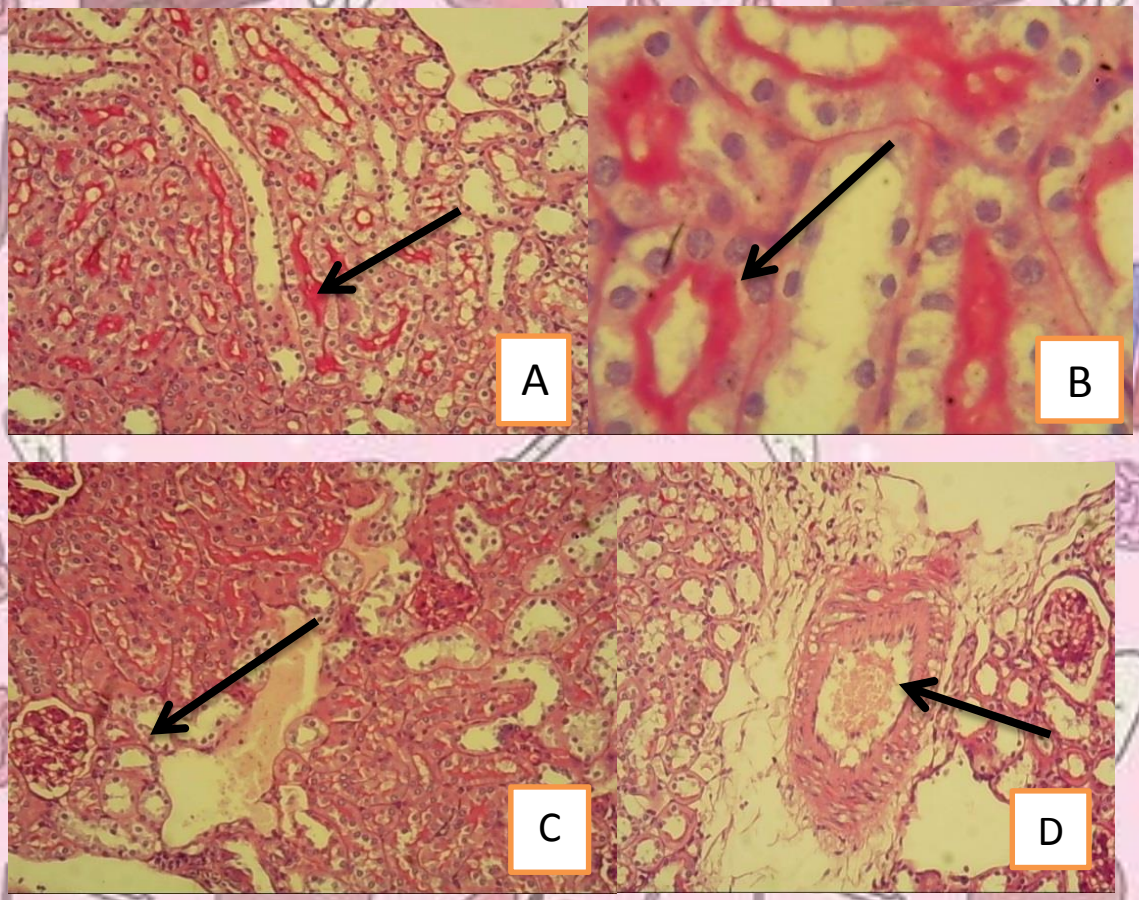


Figure (4-16): A;B;C;D :kidney of mice infected with *Aspergillus Flavus* (1 X 10^7 cells/mL).at dose of 0.1 ml/mouse. IP. Showed; A&B: :degeneration of renal tubules with sever hyalinization (arrow)). H&E.10X &40X : C: swelling of tubules with occluding lumen (arrow) H&E 10X ; D: pyelonephritis due to sever inflammatory reaction nearto congested and thickened blood vessels H&E 10X .

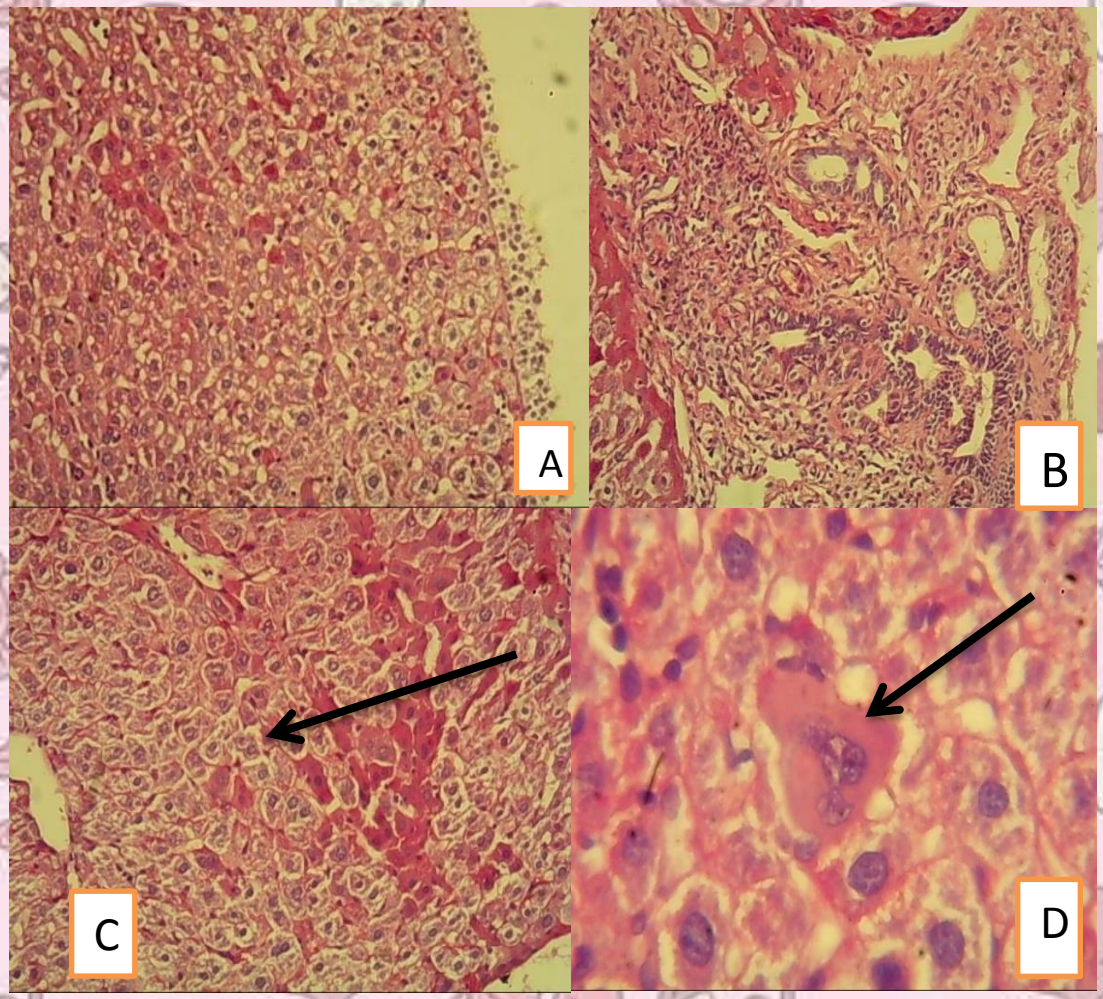


Figure (4-17): A;B;C;D :Liver of mice infected with *Aspergillus Flavus* (1 X 105 cells/mL).at dose of 0.1 ml/mouse. IP. Showed; A: :hepatocytes vacoulation and sever inflammatory reaction (arrow). H&E.10X B:bile duct hyperplasia t due to seve inflammatory infiltration mainly kuffer cells : C: lobular necrosis (arrow) H&E 10X, D: necrosis of hepatocytes with evidence of apoptosis(arrow) . H&E 40X ..

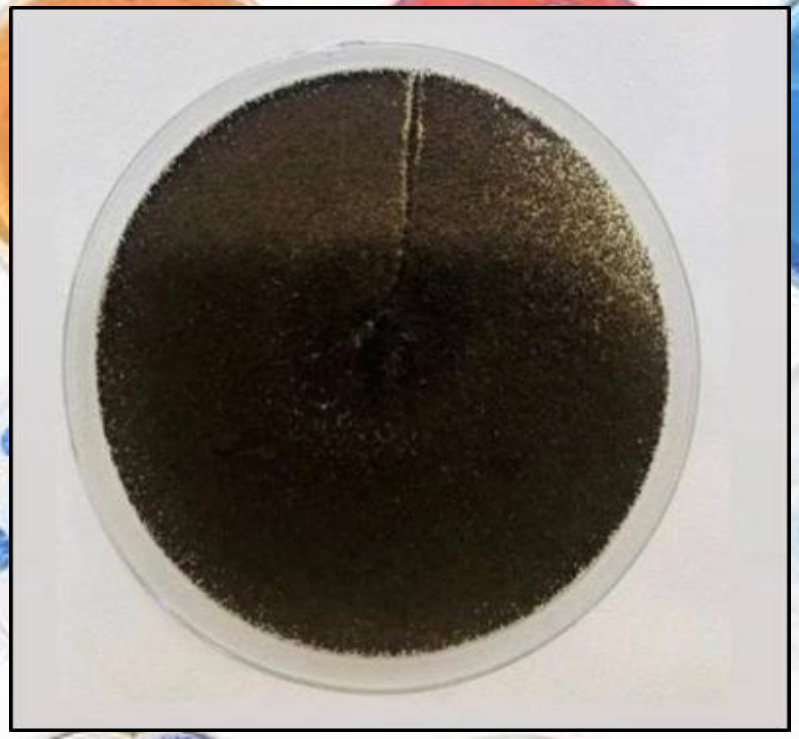


Figure (4-3): Macroscopic appearance of *Aspergillus niger* on SDA at 25˚C for 5-7 days.

Figure (4-4): Microscopic appearance of *Aspergillus niger* by using lactophenol cotton blue stain 40X

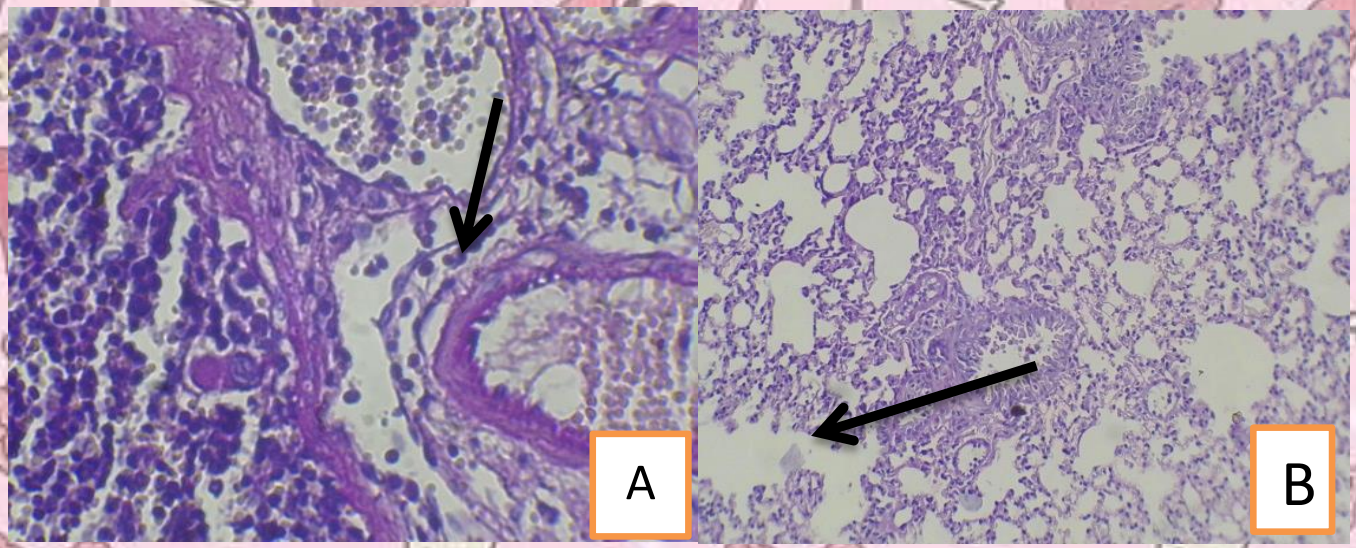


Figure (4-19): A&B : Lung of mice infected with *Aspergillus Niger* (1 X 10^7 cells/mL).at dose of 0.1 ml/mouse.IP.. .showed A: Hemorrhagic and congestive changes associated with intestinal Pneumonia enclosed to pleura which appear inflamed B : emphysematous alveoli with bronchitis (arrow) .PAS 40X & 10X.

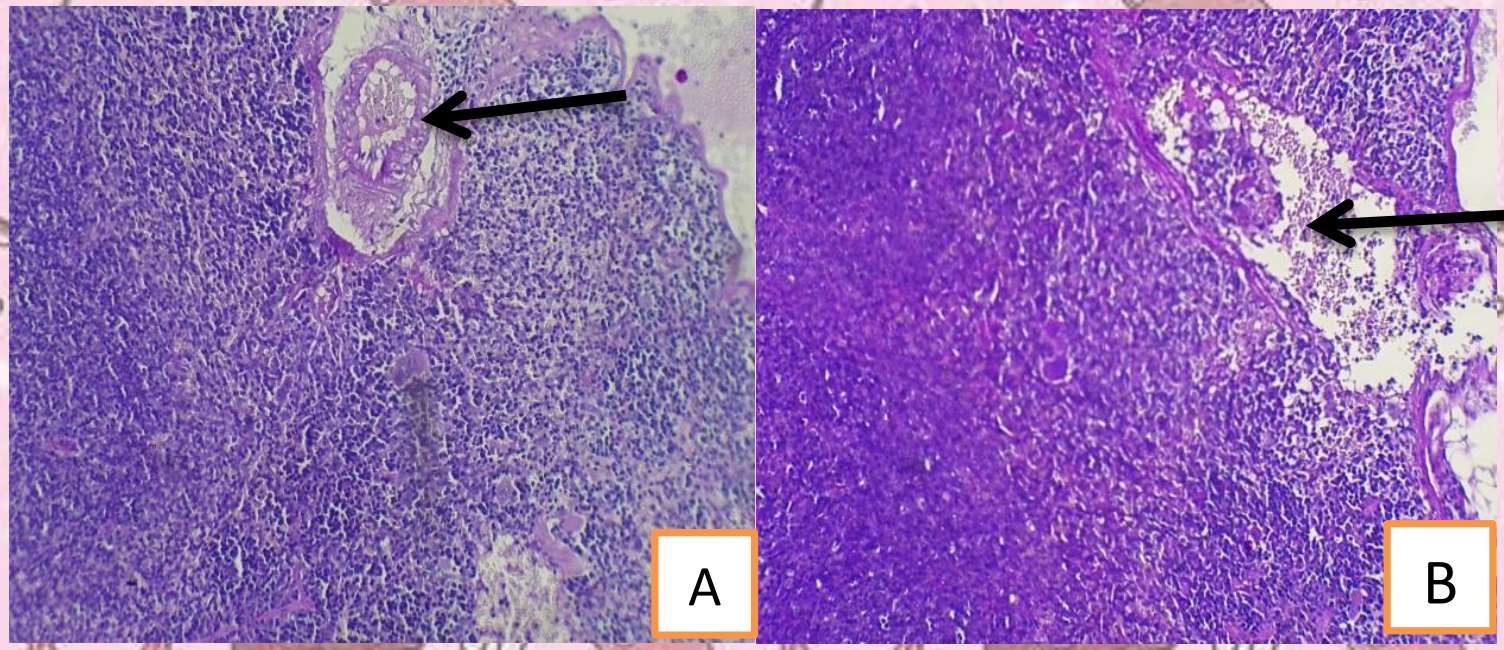


Figure (4-18): A&B : Spleen of mice infected with *Aspergillus Niger* (1 X10^7 cells/mL).at dose of 0.1 ml/mouse.IP.. .showed A: sever splenitis appear as depletion with inflamed artery and inflammatory cell infiltration and granuloma (arrow) and B : necrotic foci with clear congestive changes fungi colonies appear enclosed to necrotic foci (arrow) .PAS 10X.

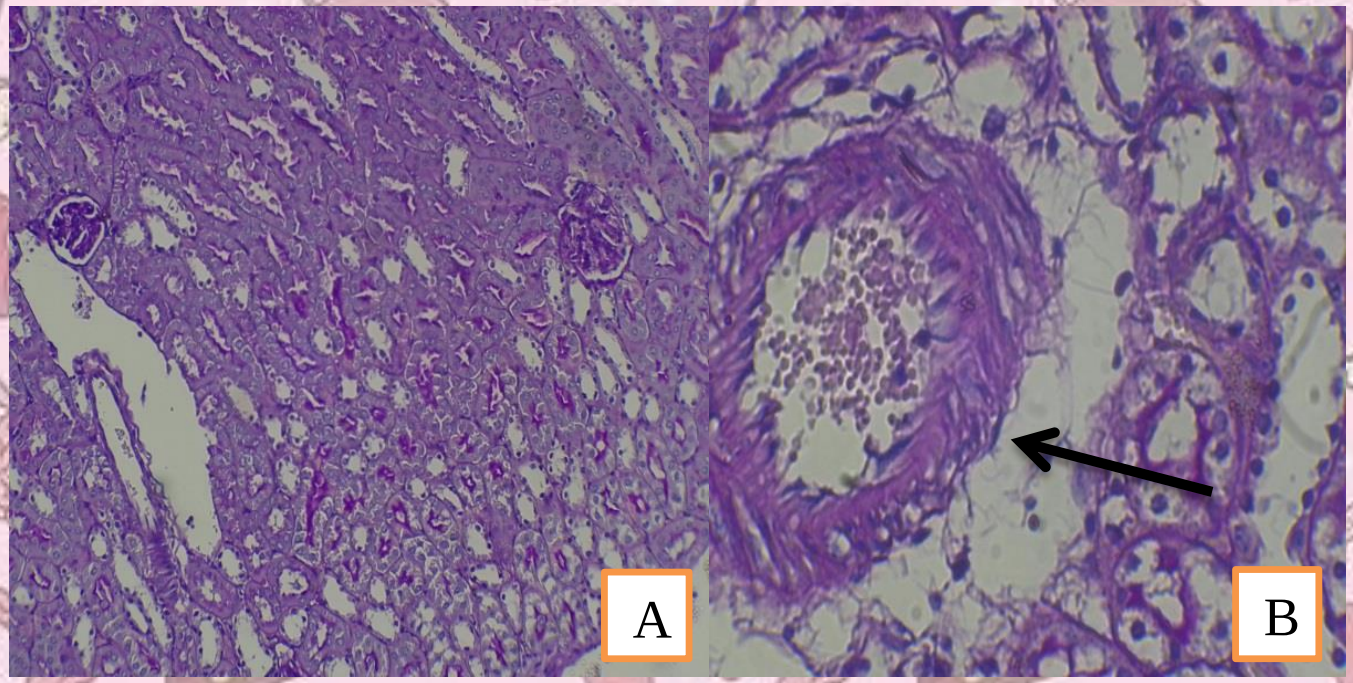


Figure (4-20): A&B : Kidney of mice infected with *Aspergillus Niger* (1 X 10^7 cells/mL).at dose of 0.1 ml/mouse.IP. .showed A: atrophy of glomeruli with hyalinization of renal tubules (arrow) B : congested and thicken inflamed blood vessel near to invasive fungi hyphae (arrow) .PAS 40X & 10X.

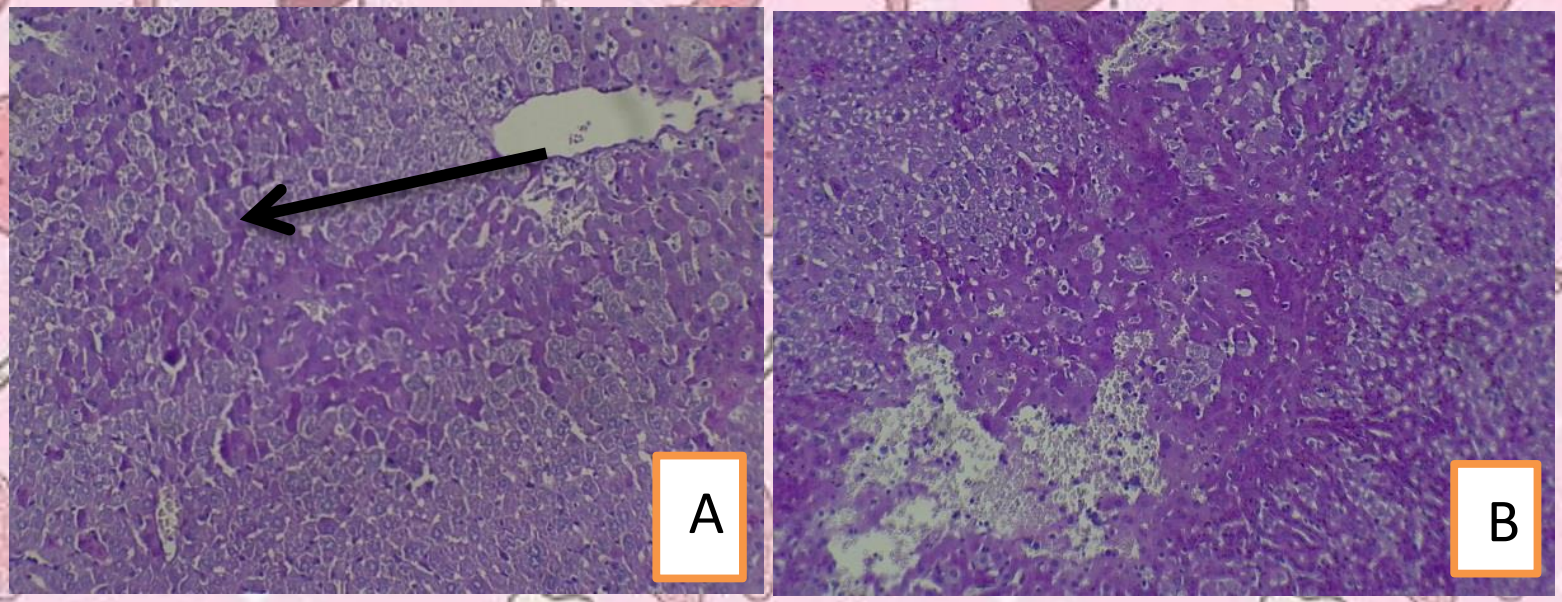
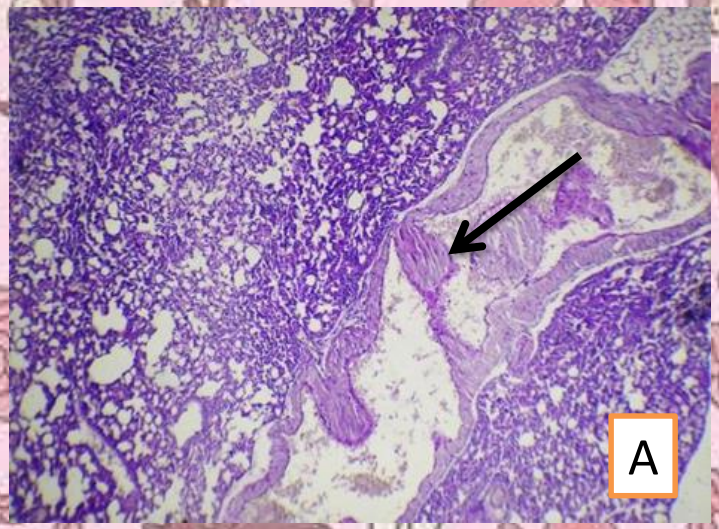


Figure (4-21): A&B : Liver of mice infected with *Aspergillus Niger* (1 X 10^5 cells/mL).at dose of 0.1 ml/mouse.IP.. showed A: multi zonal lobular necrosis with amyloid like substances deposition(arrow) B : necrosis beneath fungal hyphae(arrow)..PAS 40X & 10X.

Figure (4-5): Macroscopic appearance of *Aspergillus ochraceus* on SDA at 25 ° C for 5-7 days.

Figure (4-6): Microscopic appearance of *Aspergillus ochraceus* by using lactophenol cotton blue stain 40X



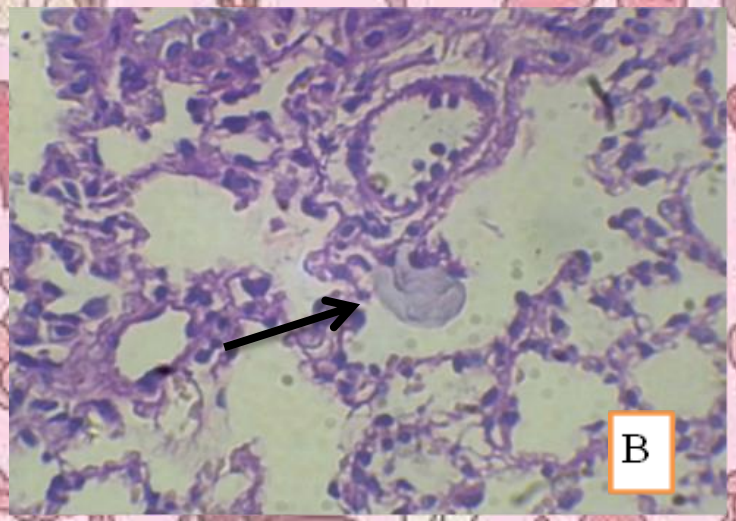


Figure (4-24): A&B :Lung of mice infected with *Aspergillus Ochraceus* (1 X 10^7 cells/mL).at dose of 0.1 ml/mouse. IP. shows A :interstitial pneumonia due to thickens exudation and absence of inter alveolar space enclosed to elongated bronchiole which have fibrotic lesion within lumen (arrow) B: necrotic alveoli and inflammatory cells attached to fibrinous exudation near to broken it's wall (arrow).PAS 10X &40X.

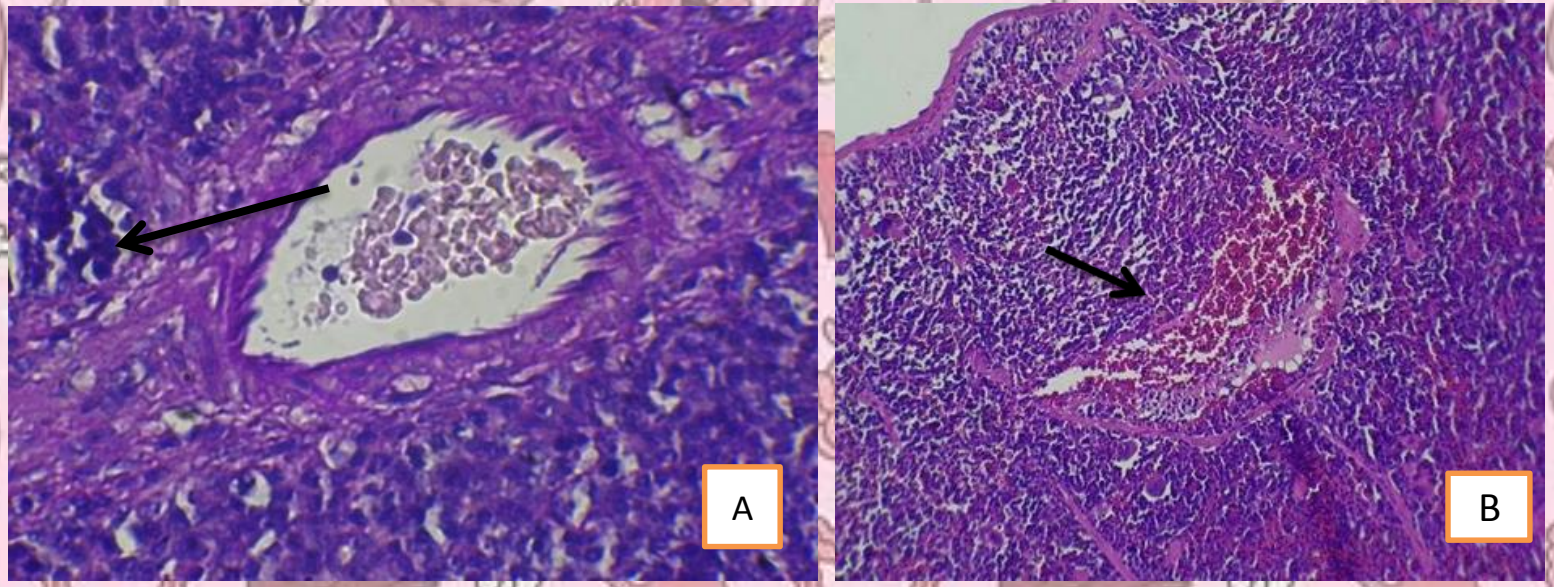


Figure (4-22): A&B :Spleen of mice infected with *Aspergillus Ochraceus* (1 X 10^7 cells/mL).at dose of 0.1 ml/mouse. IP. Showed; A : splenitis with granuloma enclosed to thickened and congested inflamed blood vessels (arrow) B: white pulp depletion with hemorrhagic changes congested blood vessels in red bulp (arrow).PAS 40X &10X.

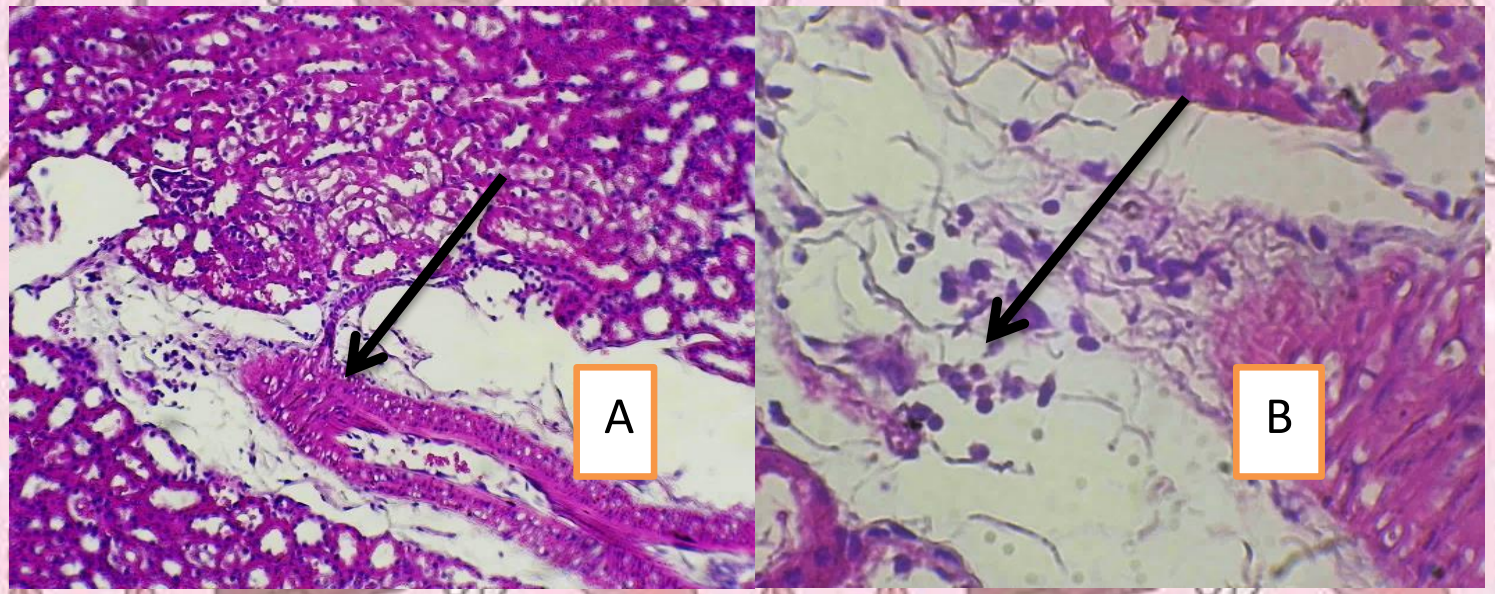


Figure (4-) A&B :kidney of mice infected with *Aspergillus Ochraceus* (1 X 10^7 cells/mL).at dose of 0.1 ml/mouse. IP. Showed; A: pyelonephritis due to necrotic descending renal tubules and dilated inflamed and congested blood vessels (arrow) B: fungi spores and hyphae also seen (arrow).PAS 10X &40X.

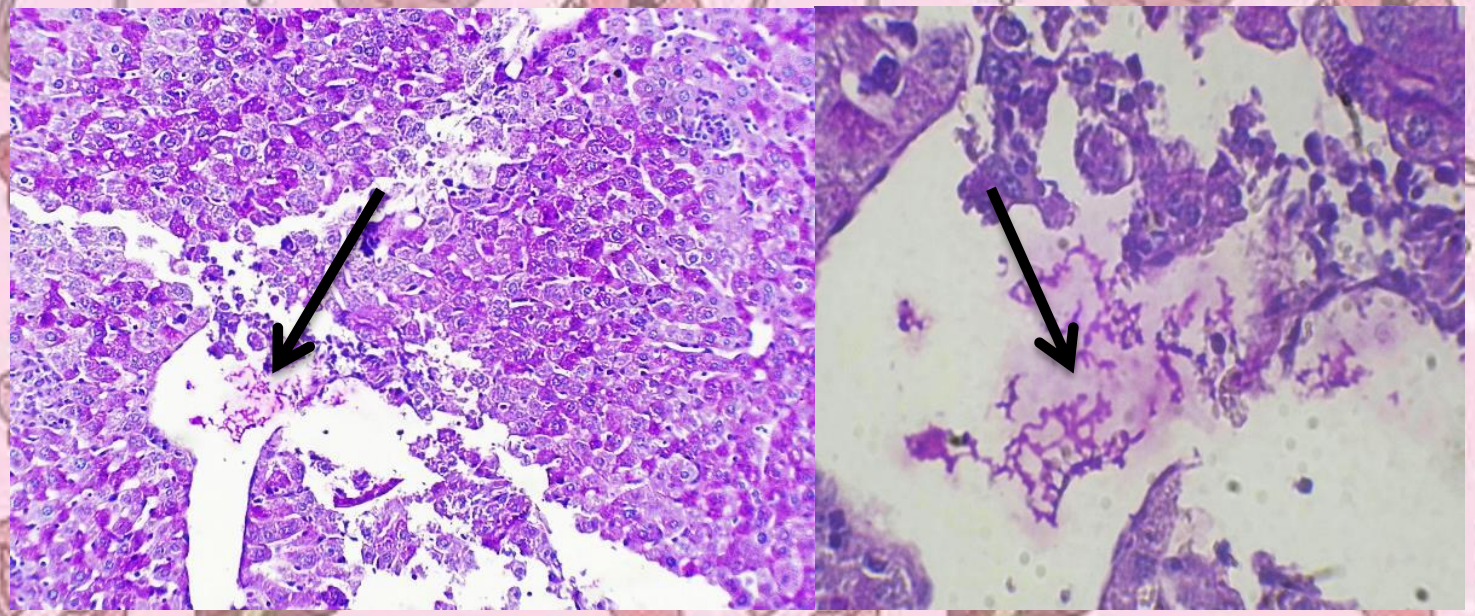


Figure (4-23): liver of mice infected with *Aspergillus Ochraceus* ($1X10^7$ cells/mL).at dose of 0.1 ml/mouse. IP. Two pictures shows area of necrosis at the center of degenerated and necrotic liver lobules shows fungi filamentous hyphae (arrow).PAS 10X &40X.

Figure (4-7): Macroscopic appearance of *Cryptococcus neoformans* on SDA at 37 ° C for 5 days.

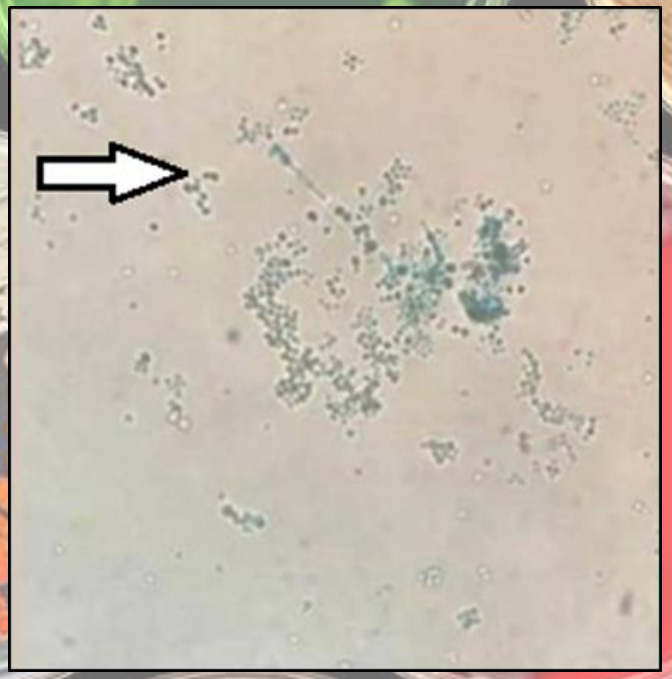


Figure (4-8): Microscopic appearance of *Cryptococcus neoformans* by using lactophenol cotton blue stain 40X show spherical budding yeast

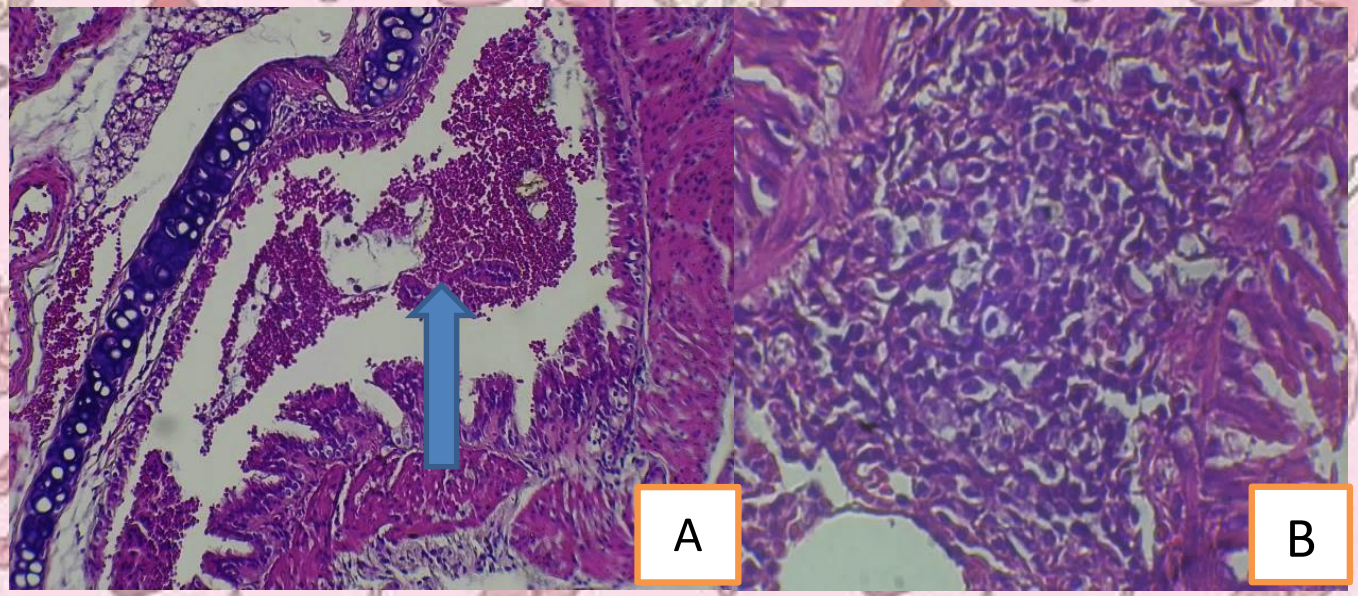


Figure (4-26): A&B Lung of mice infected with *Cryptococcus spp* (1 X 10^7 cells/mL).at dose of 0.1 ml/mouse. IP. showed A: sever bronchi-pneumonia characterized by thick exudation with desquamation of epithelial lying bronchioles (arrow) and B: granuloma (arrow) .PAS 10X &40X.

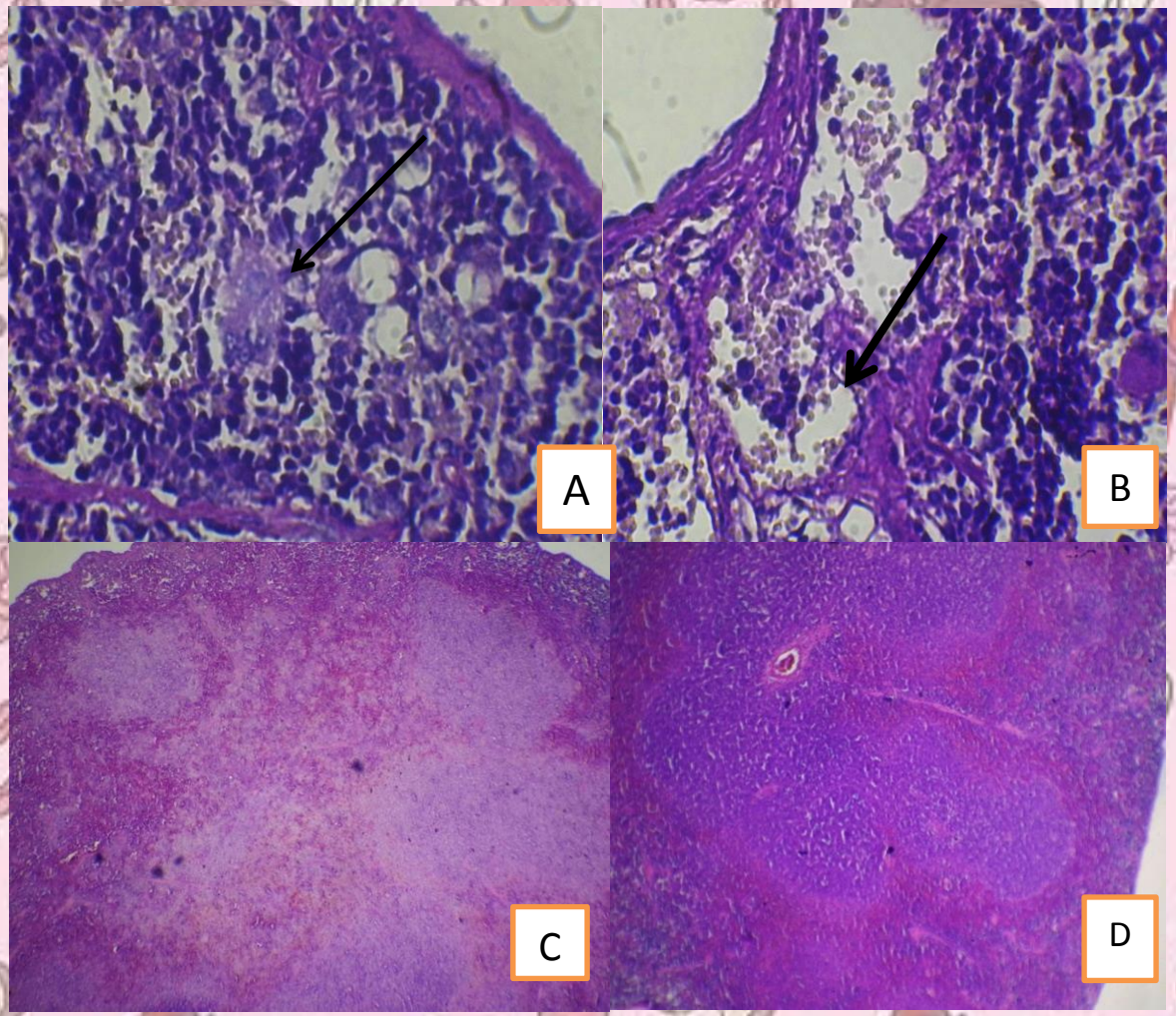


Figure (4-25): A;B;C;D : Spleen of mice infected with *Cryptococcus spp* (1 X 10^7 cells/mL).at dose of 0.1 ml/mouse .IP.. .showed A&B: sever splenitis appear as depletion with necrosis and inflammatory cell infiltration (arrow)40X PAS. C&D: marked deposition of amyloid like substances in spleen with depletion changes in white pulp (arrow):PAS 10X.

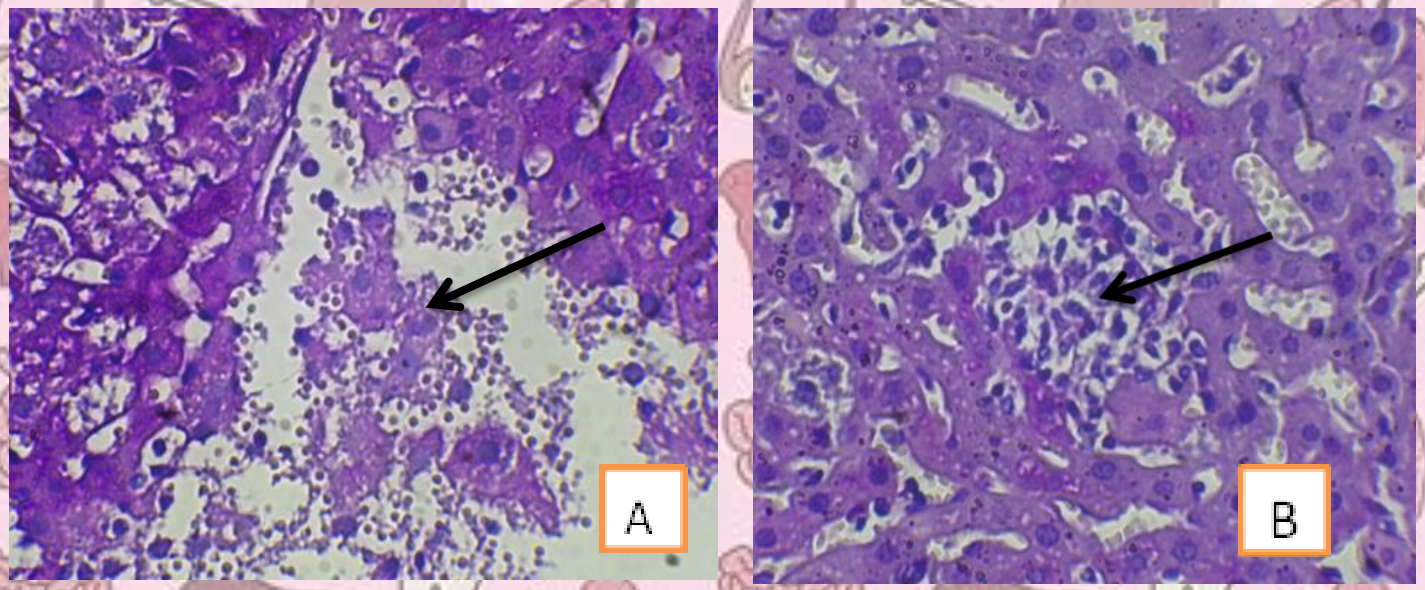


Figure (4-27): A&B : Liver of mice infected with Cryptococcus spp (1 X 107 cells/mL).at dose of 0.1 ml/mouse. IP. Showed ; A :necrotic area contained colonies of yeasr and dead hepatocytes (arrow)

B: granulomatous foci within sinusoidal space (arrow).PAS 10X &40X.

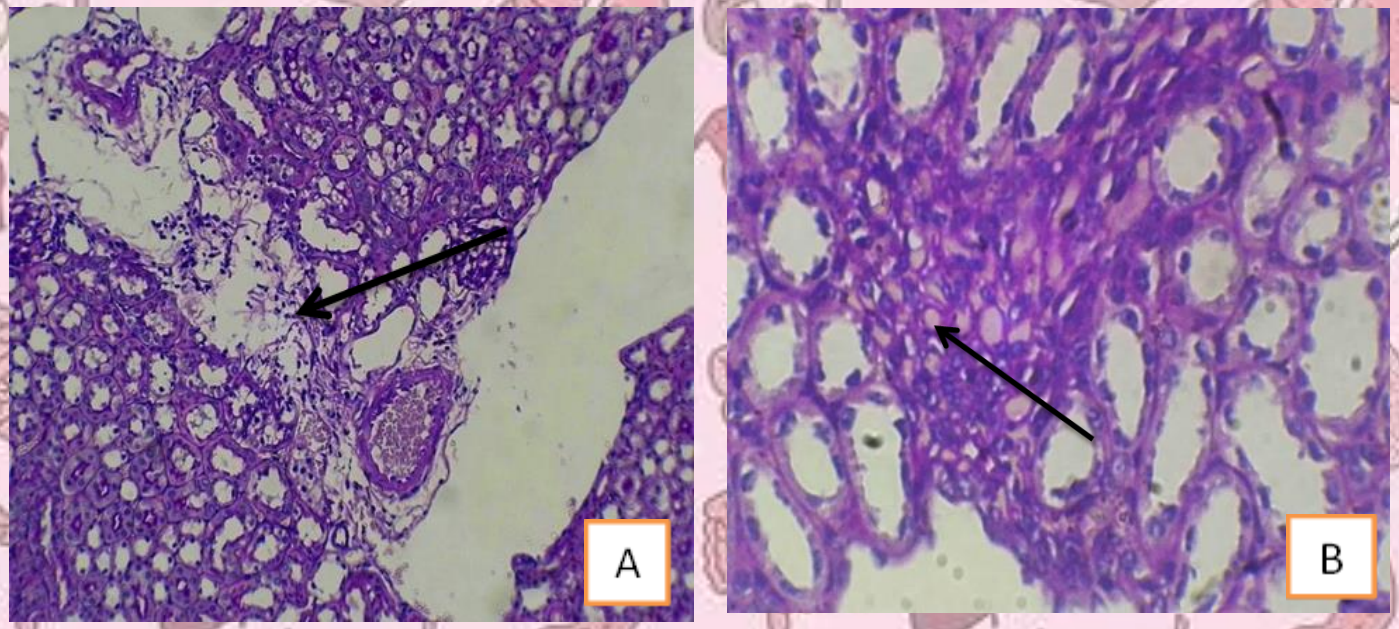
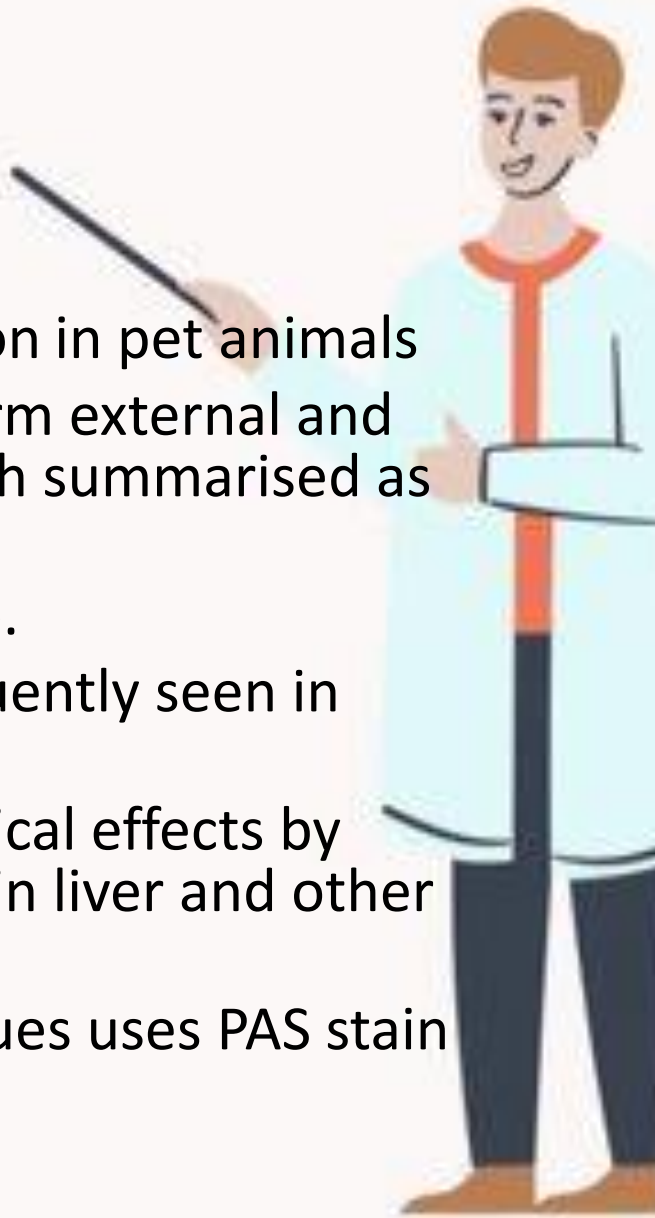


Figure (4-28): A&B :kidney of mice infected with *Cryptococcus spp* (1 X 10^7 cells/mL).at dose of 0.1 ml/mouse. IP. Showed ; A :pyelonephritis and congested blood vessels (arrow) B: granulomatous foci within tubules lumen (arrow).PAS 10X &40X.

Conclusion



1. Pathogenic fungi presence naturally infection in pet animals
2. Different types of fungi can be detected form external and internal samples the animal of this study which summarised as following-:

   a-Main fungal isolates were Aspergellus Spp.

   b-Yeast Crepotoccous neofrmans was frrequently seen in different samples.

3. Experimental study reported hitopathological effects by fungal infection that causednecrosis specially in liver and other tissues samples , within few days.
4. Fungal hyphes can be seen in affected tissues uses PAS stain

Recommendations

1. Study other types of fungi may be diagnosed in pet animal in another locations .
2. Use another laboratory animals for make experimental infection
3. Using another evaluation parameters against fungal infection like immunity
4. Make comparison between human and animals models of infection
5. Use multiple types of therapy like (plant extracted) for treatments


Thank you