Bionatura
Latin American Journal of Biotechnology and Life Sciences

Article

Histopathological effects of *Cryptococcus neoformans* on liver and kidney in mice

Sara Saad Hussamaldeen Al-Bakir [1], and Dalia Abdalkareem Abdalshaheed [2,*]

[1] Department of Microbiology, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq; saadsara434@gmail.com . https://orcid.org/0000-0003-0633-6616
[2] Department of Microbiology, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq; dalia@covm.uobaghdad.edu.iq . https://orcid.org/0000-0003-1369-8826
* Correspondence, dalia@covm.uobaghdad.edu.iq

Abstract, This study provides a brief review of approaches fordetection of histopathological effects of *Cryptococcus neoformans* on liver and kidney in mice which was injected I/P with 10^5 yeast cells of *C. neoformans* suspended in 1 ml phosphate-buffered saline, at a single dose. After 14 days the mice were sacrificed and histopathological sections from liver and kidney were prepared and stained with Haematoxylin and Eosin as well as by the PAS method. The results shows that the liver was infiltrated of inflammatory cells mostly mononuclear cells in portal area in addition to activation of Kupffer cells and vacillation of hepatocytes, most blood vessels were congested. While, the section of kidney shows sluffing of epithelia lining tubules, and complete destruction of glomeruli, in addition to infiltration of mononuclear cells.

Keywords, *Cryptococcus neoformans*; Hepatic cryptococcal infection; Cryptococcuria

1. Introduction

A fungal illness known as cryptococcosis is brought on by the pathogens *Cryptococcus neoformans* and *C. gattii*, which resemble yeast [1]. *Cryptococcus neoformans* serotype A is responsible for around 95% of reported cryptococcal infections; the remaining 5% are caused by other serotypes or by *Cryptococcus gattii* [2]. It is an illness that is contracted by the inhalation of spores or yeast cells that have been dried from environmental sources such as plant matter, soil, and bird excrement [3]. *Cryptococcus* spp. is widespread and the most significant species with regard to medicine [4]. When seen under a microscope, the fungus appears as an oval or globular yeast with a diameter of 3mm to 8 mm. It is often encased in a mucopolysaccharidal capsule. The phenoloxidase enzyme causes the capsule to create a significant amount of melanin. The abundance of substrates for phenoloxidase activity in brain tissue may help to partially explain *Cryptococcus* preference for the central nervous system [5]. As an encapsulated yeast known as Torulahistolytica or European blastomycosis, *Cryptococcus* spp. may avoid the immune system's defense mechanisms and spread primarily from the lungs and central nervous system to the blood, skin, eyes, skeletal system, and urinary tract [6,7]. Cryptococcosis is brought on by inhaling spores or dried yeast cells, and it results approximately 180,000 of fatalities globally each year, including nearly 15% of all AIDS-related deaths [8]. Although a population of *C. neoformans* may remain dormant for long periods of time in immunocompetent persons, this often results in an asymptomatic lung infection that is managed by the host immune response [9]. The disease progression results in a highly lethal form of meningoencephalitis [8]. Cryptococcosis in animals is a systemic fungal infection of worldwide significance that usually initially infects the nasal cavity, paranasal tissues, or lungs. It can then disseminate, most commonly to the skin, eyes, or central nervous system. Nasal cryptococcosis is frequently seen clinical signs including sneezing, snoring or snorting, dyspnea, nasal deformities and/ or a mucopurulent, serous or sero-sanguineous nasal discharge [10,11]. *Cryptococcus* infections have been reported in a broad range of animals, including cats, dogs, horses, birds, and koala bears [12]. The most common symptom of hepatic cryptococcal infection is cholestatic jaundice, which can quickly proceed to liver failure and death in cases of widespread illness [13]. In urinary tract infection or a diffused illness manifestation, cryptococcuria is a rather uncommon presentation in human patients (UTI). Patients frequently have an underlying, immune-compromising illness when cryptococcuria is diagnosed in individuals [14].

2. Materials and Methods

2.1. Animals utilized in experiment

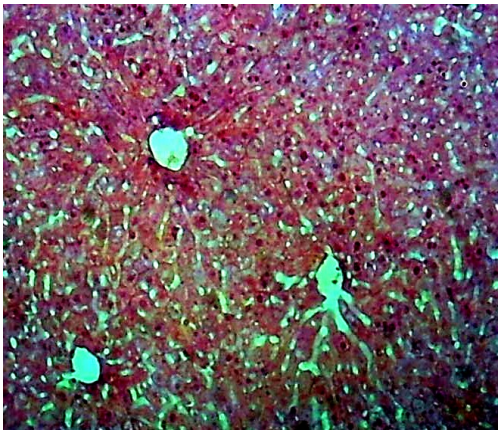
Twenty albino BALB/C female mice, weighing an average of 22–25g, were used. Animals were housed in cages in pairs and were fed water. A total 10 mice from the first group (G1) were utilized as the control group and not given any treatment; wgereas, 10 mice were injected intraperioteally a single dose of *Cryptococcus neoformans* suspension that contain 10^5 yeast cells into each 1 ml of PBS.

2.2. Histopathological sections

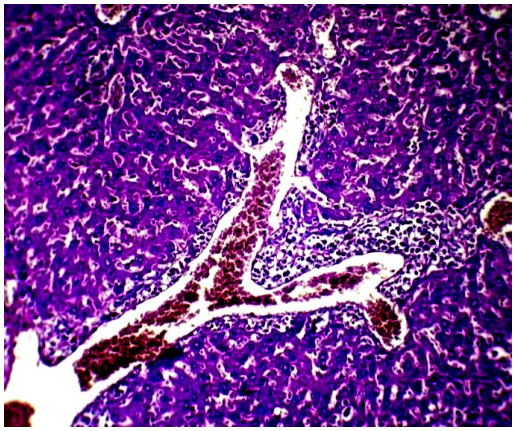
Samples of liver and kidney were collected after scarification of mice at 14 days of single administration of *C. neoformans*, and kept in 10% formaldehyde solution for fixation in order to preserve the figures, size, and tissue for specimens, then processed routinely using the histokinette [16]. The specimens were washed with distilled water several times in order to remove large proportion of fixative, and dehydration by passing the specismens through ascending gradual of ethanol (50,70,80,95 and 100) % in each run the treat with methylbenzoate 24 hrs and then was rehydration by gradual of ethanol (100, 95, 80, 70 and 50) % in each run. The samples were cleared by xylol, embedded in paraffin wax at 70ºC, sectioned by microtome at 5-6 micron of thickness. The sildes were mounted and covered with a coverslip using albumin at 56ºC, stained with Eosin and Hemtoxylin,and examined under light microscope at 400X [17, 18].

3. Results

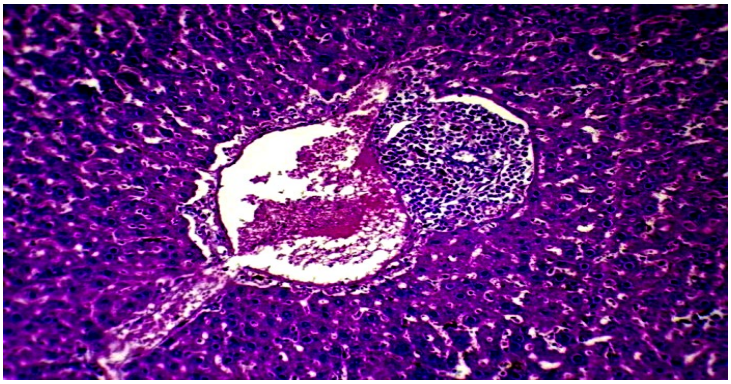
Microscopic examination of liver sections for the control group was revealed normal architecture of hepatic lobules and sinusoids lined by thin capillaries, and surrounded by portal area composed from portal vein, portal artery, and bile ductules in interstitium (Figure 1a). While, liver of mice which infected with *C. neoformans* showing liver with infiltrated of inflammatory cells mostly mononuclear cells in portal area in addition to activation of Kupffer cells and vacillation of hepatocytes, most blood vessels were congested (Figure 1b & c).



(a)



(b)



(c)

Figure 1. Histopathological section of liver of, (**a**) Control group showing many lobules each lobules contain central vein surrounded by hepatic cord and separated by sinusoid; (**b & c**), Infected mice with *C. neoformans* showing infiltration of inflammatory cells mostly

mononuclear cells in portal area in addition to activation of Kupffer cells and vacillation of hepatocytes, most blood vessels were congested, (H & E stain, 400X).

For kidney, microscopic examination for histological section of control mice were showed a normal renal tubules. As well as, the cortex and normal glomerular tufts were covered by a thin dense connective tissue capsule with adipose tissue (Figure 2a). While, the kidney section of infected mice with *C. neoformans* showing kidney with sluffing of epithelia lining tubules, and complete destruction of glomeruli, in addition to infiltration of mononuclear cells (Figure 2b, c).

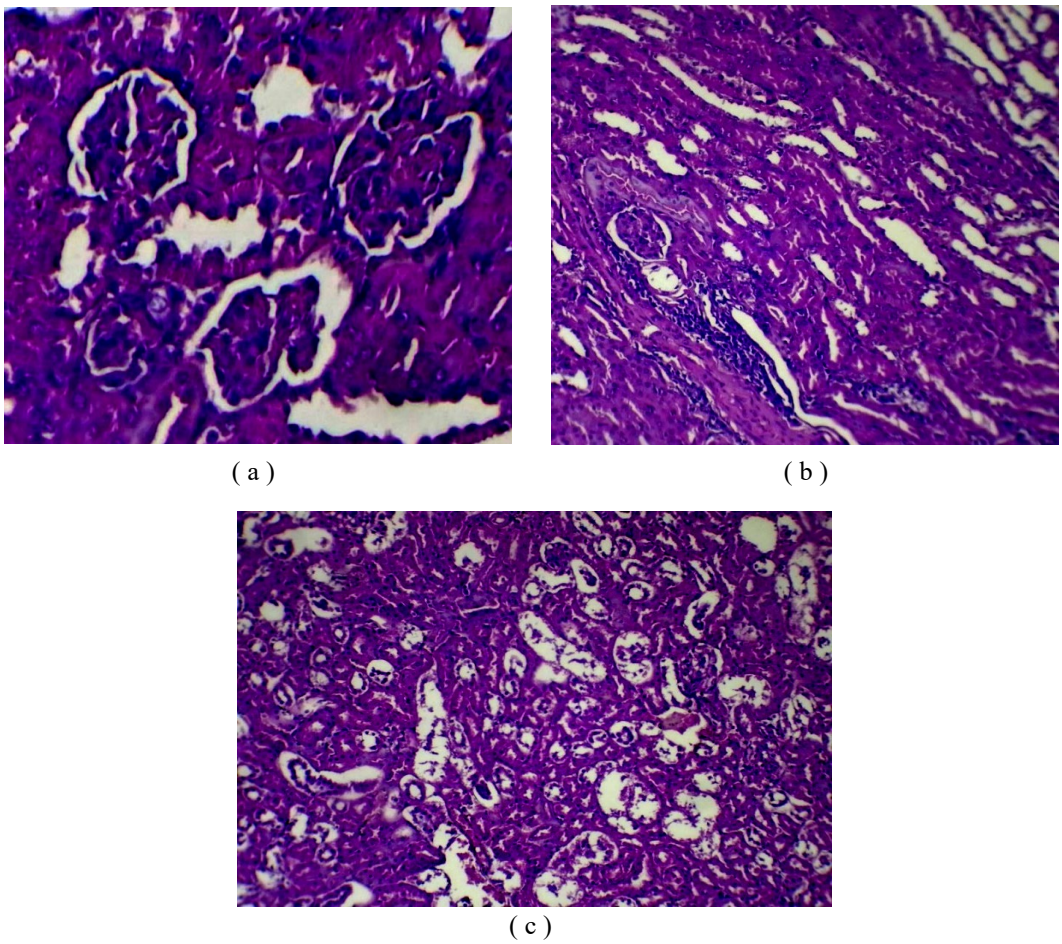


Figure 2. Histopathological section of kidney of, (a) Control group showing normal glomeruli and tubules; (b) Infected mice with *C. neoformans* showing kidney with sluffing of epithelia lining tubules, and complete destruction of glomeruli with infiltration of mononuclear cells, (H & E stain, 400X).

4. Discussion

The results showed the hepatic tissue section of mice infected with *C. neoformans* with vacuolation of hepatocytes, dilation of sinusoids and central veins as well as portal veins that containing the fibrinous network trapped few PMNs, fibrilles of fibrin precipitated on the endothelial layer of blood vessel cause thickening of vascular wall and congestion of blood vessels which agreement with Al Kaaby (2009) [19]. Additionally, there were an infiltration of inflammatory cells especially mononuclear cells and activation of Kupffer cells in hepatic lobules, fungi can enter the liver or even whole body through the damaged mucosal membrane cause aggravate liver damage [20]. As observed in figure (2), the renal section of mice were treated with *C. neoformans* revealed a complete destruction of glomerular tuft, sluffing and convoluted tubules proximal and distal epithelial linings are deteriorating with infiltration mononuclear cells these defect also seen in other study [21,22,23]. These findings might be due to the disseminated of cryptococcosis. Fungal infection is associated with animals lose weight, their blood cell and leukocyte counts drop, their plasma glucose levels drop, and their stomach, liver, and kidneys develop pathological abnormalities [24]. Finally, renal involvement with cryptococcosis in animals is rare, and it has only sporadically been demonstrated in cats with systemic cryptococcosis by the detection of fungi during necropsy or urine sediment analysis [15]. Therefore, the aims of this research were to study the histopathological effects of *C. neoformanson* on liver and kidney.

5. Conclusions

This study concludes that the fungus invasiveness of mice have strongly effects on vital organs and may lead to death, as well as the histopathological effects of Cryptococcus neoformans on liver and kidney in mice Cryptococcus neoformans causes severe damage liver and kidney suggesting impacts public health. Furthermore studies are of great importance to estimate the effect of this bacterium on other tissues and organs and to invent active methods for prevention or reducing their serious effects.

Author Contributions, Conceptualization, S.S.H.A. and D.A.A.; methodology, S.S.H.A.; software, S.S.H.A.; validation, S.S.H.A.; and D.A.A.; formal analysis, S.S.H.A.; investigation, S.S.H.A.; resources, S.S.H.A.; writing-original draft preparation, S.S.H.A.; writing-review and editing, S.S.H.A.; and D.A.A.; visualization, D.A.A.; supervision, D.A.A.; project administration, S.S.H.A.. All authors have read and agreed to the published version of the manuscript.

Funding, This research received no external funding.

Institutional Review Board Statement, The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics of the Scientific Committee of the Department of Microbiology, in the College of Veterinary Medicine, University of Baghdad (Baghdad, Iraq).

Acknowledgments,In this section, you can acknowledge any support given which is not covered by the author contribution or funding sections. This may include administrative and technical support, or donations in kind (e.g.; materials used for experiments).

Conflicts of Interest, The authors declare no conflict of interest.

References

1. Kwon-Chung, K.J.; Varma, A. Do major species concepts support one, two or more species within Cryptococcus neoformans? *FEMS Yeast Res* **2006**, 6 (4), 574–587.
2. Maziarz, E.K.; Perfect, J.R. Cryptococcosis. *Infect Dis Clin* **2016**, 30 (1), 179-206.
3. Kwon-Chung, K.J.; Fraser, J.A.; Doering, T.L.; Wang, Z.A.; Janbon, G.; Idnurm, A.; Bahn, Y.S. *Cryptococcus neoformans* and *Cryptococcus gattii*, the etiologic agents of cryptococcosis. *Cold Spring Harb Perspect Med* **2014**, 4 (7), 197-216.
4. Liu, X.Z.; Wang, Q.M.; Göker, M.; Groenewald, M.; Kachalkin, A.V.; Lumbsch, H.T.; Millanes, A.M.; Wedin, M.; Yurkov, A.M.; Boekhout, T.; Bai, F.Y. Towards an integrated phylogenetic classification of the Tremellomycetes. *Stud Mycol* **2015**, 81, 85–147.
5. Moretti, M.L.; Resende, M.R.; Lazéra, M.S.; Colombo, A.L.; Shikanai-Yasuda, M.A. Guidelines in cryptococcosis. *Rev Soc Bras Med Trop* **2008**; 41, 524–544.
6. Setianingrum, F.; Rautemaa-Richardson, R.; Denning, D.W. Pulmonary cryptococcosis, a review of pathobiology and clinical aspects. *Med Mycol* **2019**, 57(2),133–150.
7. Kwon-Chung, K.J.; Boekhout, T.; Fell, J.W.; and Diaz, M. Proposal to conserve the name *Cryptococcus gattii* against *C. hondurianus* and *C. bacillisporus* (Basidiomycota, Hymenomycetes, Tremellomycetidae). *Taxon* **2002**, 51(4), 804-806.
8. Rajasingham, R.; Smith, R.M.; Park, B.J.; Jarvis, J.N.; Govender, N.P.; Chiller, T.M.; Denning, D.W.; Loyse, A.; Boulware, D.R. Global burden of disease of HIV-associated cryptococcal meningitis, an updated analysis. *Lancet Infect Dis* **2017**, 17,873–881.
9. Najim, Y. S., Mohammed, Th. T. & Al-Khalani, F. M. H. The effect of the use of different levels of Azolla to male broilers diets in the production and physiological performance and economic feasibility. *Biochemical and Cellular Archives*. **2020**, 20(1): 573-580. Doi: 10.35124/bca.2020.20.1.573..
10. Meng, H.C.; Lu, C.H.; Wang, H.C.; Chen, H.L.; Tsai, N.W.; Li, S.H.; Hsu, N.W.; Lin, W.M.; Kung, C.T.; and Lin, W.C. Long-Term Neuropsychological Sequelae in HIVSeronegativeCryptococcal Meningoencephalitis Patients with and without Ventriculoperitoneal Shunts, A Cine MRI Study. *Behav Neurol* **2015**, 1-10.
11. Pimenta, P.; Sofia, A.; João, B.; Maria, J.; Pereira, L.; Paula Maduro, A.; LuísCardoso, C.C. Blepharitis due to *Cryptococcus neoformans* in a cat from northern Portugal. *J Feline Med Surg* **2015**, 1, 20-25.
12. Malik, R.; Krockenberger, M.B.; Brien, C.R.O.; Carter, D.E.E.A.; Meyer, W.; Canfield, P.J. Veterinary insights into cryptococcosis caused by *Cryptococcus neoformans* and *Cryptococcus gattii*. In, Heitman J, Kozel T, Kwon-ChungKJ, Perfect JR, Casadevall A, eds. Cryptococcus, From Human Pathogen to Model Yeast. Washington, DC, ASM Press, **2011**, 489–502.
13. Suwaid, A. H. .; Rashid, M. A. .; Taha, M. M. . Genetic Analysis For Combining Ability And Estimation Of Some Genetic Parameters Of Yield And Its Components In Maize Using Half Diallel Cross. *JLSAR* **2020**, 1, 60-64.
14. Kiertiburanakul, S.; Sungkanuparph, S.; Buabut, B. Cryptococcuria as a manifestation of disseminated cryptococcosis and isolated urinary tract infection. *Jpn J Infect Dis* **2004**, 57, 203-205.
15. Malik, R.; Krockenberger, M.; O'Brien, C. Cryptococcosis. In, Greene CE, ed. Infectious Disease of the Dog and Cat. 3rd ed. Missouri, Saunders, *Elsevier* **2006**,584-598.
16. Noaman A I, Khalaf R M, Emad GH, Al-Abbasy, Mohammed Th. T. Effect of flaxseed oil dosing on fertility, growth characteristics and some physical, biochemical, and hormonal blood parameters during the early pregnancy of Awassi ewes. *Revis Bionatura*. **2022**;7(4) 5. http://dx.doi.org/10.21931/RB/2022.07.04.5.
17. Gharban, H.A.; Al-Shaeli, S.J.; Al-Fattli, H.H.; Altaee, M.N. Molecular and histopathological confirmation of clinically diagnosed lumpy skin disease in cattle, Baghdad Province of Iraq. *Vet World* **2019**, 12 (11), 1827-1832.
18. Al-Shaeli, S.J.; Ethaeb, A.M.; Gharban, H.A. Molecular and histopathological identification of ovine neosporosis (Neospora caninum) in aborted ewes in Iraq. *Vet World* **2020**, 13(3), 597-603.
19. Al Kaaby, H.T. Isolation of Cryptococcus neofrmans from pigeon dropping and study of some pathogenesis aspects. *J Kerbala Univer* **2009**, 7 (2), 316-321
20. Romani, L. Immunity to fungal infections. *Nat Rev Immunol* **2011**, 11(4), 275-288.
21. Bollée, G.; Touzot, M.; Mechai, F.; Royal, V.; Lefrère, F.; Bougnoux, M.E. Plasma exchange for disseminated cryptococcosis. *Am J Kidney Dis* **2009**, 53,673-676.
22. Ramdial, P.K.; Sing Y.; Deonarain, J.; Bhimma, R.; Chotey, N.; Sewram, V. Pediatric renal cryptococcosis, novel manifestations in the acquired immunodeficiency syndrome era. *Int J Surg Pathol* **2011**, 19, 386–392.

23. Pongmekin, P.; Chongtrakool, P.; Santanirand, P.; Kiertiburanakul, S. Clinical characteristics and mortality risk factors of cryptococcal infection among HIV-negative patients. *J Med Assoc Thai* **2014**, 97,36-43.
24. Abbas, O.S.; Abdualshahed, D.A. Effect of Grape Seed Extract on the T-2 Toxicity in mice. *Iraqi J Cancer Med Gene* **2013**, 6(2), 120-125.