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Article

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

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ABSTRACT

This study provides a brief review of approaches for the detection of histopathological effects of *Cryptococcus neoformans* on the liver and kidney in mice that were 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 the liver and kidney were prepared and stained with Haematoxylin and Eosin by the P.A.S. method. The results show that the liver was infiltrated with inflammatory cells, primarily mononuclear cells, in the portal. In addition to the activation of Kupffer cells and vacillation of hepatocytes, most blood vessels were congested. The section of the kidney shows sluffing of epithelia lining tubules and complete destruction of glomeruli, in addition to infiltration of mononuclear cells. These results suggested that the fungus invasiveness of mice has substantial effects on vital organs and may lead to death.

Keywords: *Cryptococcus neoformans*, Hepatic cryptococcal infection, Cryptococcuria.

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 accounts for around 95% of reported cryptococcal infections; the remaining 5% are caused by other serotypes or *Cryptococcus gattii* [2]. It is an illness contracted by inhaling 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 concerning 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's* 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 [7]. Cryptococcosis is brought on by inhaling spores or dried yeast cells, and it results in approximately 180,000 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 in immunocompetent persons, this often results in an asymptomatic lung infection 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 to the skin, eyes, or central nervous system. Nasal cryptococcosis is frequently seen in clinical signs including sneezing, snoring or snorting, dyspnea, nasal deformities, or a mucopurulent, serous, or sero-sanguineous nasal discharge [10,11]. *Cryptococcus* infections have been reported in many 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]. Cryptococcosis is a relatively uncommon presentation in human patients in urinary tract infections or a diffused illness manifestation (U.T.I.). Patients frequently have an underlying, immune-compromising illness when cryptococcosis is diagnosed in individuals [14]. Renal involvement with cryptococcosis in animals is rare, and it has only sporadically been demonstrated in cats with systemic cryptococcosis by detecting fungi during necropsy or urine sediment analysis [15]. Therefore, this research aimed to study the histopathological effects of *C. neoformanson* on the liver and kidney.

MATERIALS AND METHODS

Animals utilized in the experiment

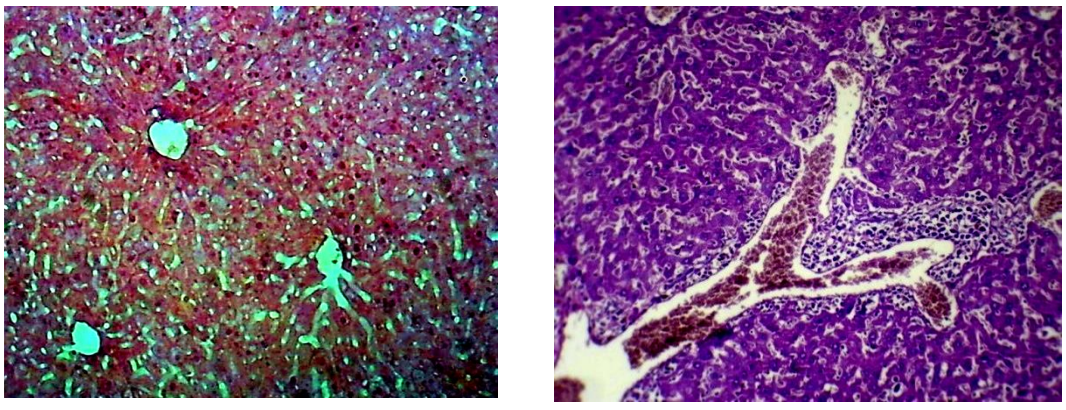
Twenty albino BALB/C female mice weighing an average of 22–25g were used. Animals were housed in cages in pairs and were fed water. A total of 10 mice from the first group (G1) were utilized as the control group and not given any treatment, whereas 10 mice were injected intraperitoneally with a single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

Histopathological sections

Samples of liver and kidney were collected after scarification of mice at 14 days of a 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 stockinette [16]. The specimens were washed with distilled water several times in order to remove a large proportion of fixative and dehydration by passing the specimens through ascending gradual of ethanol (50,70,80,95 and 100) % in each run of the treatment with methyl benzoate 24 hrs. Then, they were rehydrated by gradual ethanol (100, 95, 80, 70, and 50) % in each run. The samples were cleared by xylol, embedded in paraffin wax at 70ºC, and sectioned by microtome at 5-6 microns of thickness. The slides were mounted and covered with a coverslip using albumin at 56ºC, stained with Eosin and Hematoxylin, and examined under a light microscope at 400X [17, 18].

RESULTS

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



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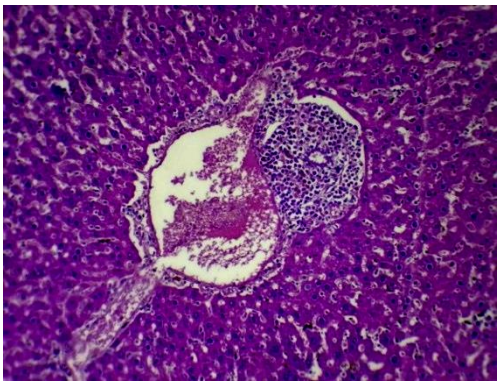

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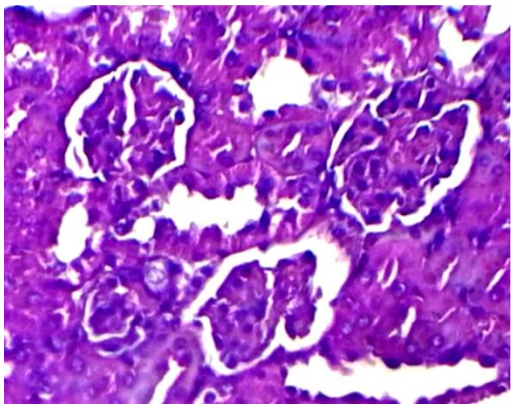
Figure 1: Histopathological section of liver of (a) Control group showing many lobules each lobule contains central vein surrounded by hepatic cord and separated by sinusoid; (b & c) Infected mice with *C. neoformans* showing infiltration of inflammatory cells, primarily mononuclear cells in the portal area in addition to activation of Kupffer cells and vacillation of hepatocytes, most blood vessels were congested, (H & E stain, 400X).

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



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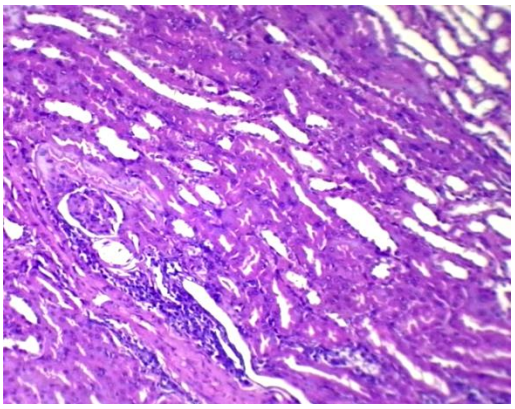

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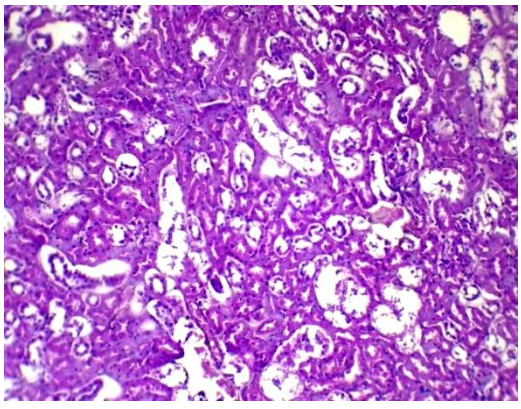

(**c**)

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 destruction of glomeruli with infiltration of mononuclear cells, (H & E stain, 400X).

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 P.M.N.s, 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, inflammatory cells were infiltrated, especially mononuclear cells, and activation of Kupffer cells in hepatic lobules; fungi can enter the liver or even the whole body through the damaged mucosal membrane, causing aggravated liver damage [20]. As observed in figure (2), the renal section of mice treated with *C. neoformans* revealed destruction of glomerular tuft, sluffing, and convoluted tubules proximal and distal epithelial linings, which are deteriorating with infiltration mononuclear cells. These defects are also seen in another study [21-23]. These findings might be due to the dissemination of cryptococcosis. Fungal infection is associated with animals losing weight, their blood cell and leukocyte counts dropping, their plasma glucose levels dropping, and their stomach, liver, and kidneys developing pathological abnormalities [24].

CONCLUSIONS

Cryptococcus neoformans causes severe damage to the liver and kidneys, suggesting it 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 preventing or reducing their severe effects.

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

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