Since 1983
EPITHEORESE KLINIKES FARMAKOLOGIAS KAI FARMAKOKINETIKES | 42(Sup1): 83-91(2024)

Received: 23 February 2024 | **Accepted**: 15 April 2024 | **Published**: 27 December 2024

Open Access | **RESEARCH**

Detection of histopathological changes in the lung tissues of mice caused by *Cryptococcus neoformans*: therapeutic effect of endophytic fungus extract

Sahary M. Mahmoud[1,*], Milad A. Mezhar[1], Rashid K. Shaban[1]

[1]Department of Biology, College of Education for Pure Sciences, Tikrit University, Iraq

***Corresponding author:** Sahari Moufaq Mahmoud: Department of Biology, College of Education for Pure Sciences, University of Tikrit, Iraq; Tel.: +964-7710012402; e-mail: saharemoufaq23@gmail.com

ABSTRACT

Background: Cryptococcus neoformans is a significant pathogenic yeast that causes severe infections in immunocompromised individuals, such as those with respiratory diseases, cancer, kidney failure, and meningitis. Early diagnosis and treatment are crucial in managing the infections. The study was conducted at Al-Batoul Hospital and Baqubah Teaching Hospital to isolate and diagnose *Cryptococcus neoformans* and assess potential antifungal treatments. *Aim:* This study aimed to isolate and identify *Cryptococcus neoformans* from clinical samples, determine the distribution of *Cryptococcus* species, and evaluate the effectiveness of fungal filtrate extract as a therapeutic agent compared to Amphotericin B in treating *Cryptococcus* infections in a mouse model. *Methodology:* The study spanned from September 2022 to May 2023 and included samples from sputum, urine, and cerebrospinal fluid (CSF). Traditional culture methods, phenotypic characterization, and Vitic device technology were used for diagnosis. The antifungal efficacy of *Cladosporium cucumerinum* filtrate extract was evaluated and compared to Amphotericin B. Mice infected with C. neoformans were treated with either the fungal filtrate or Amphotericin B, and histopathological analysis of lung tissues was performed to assess treatment outcomes. *Results:* A total of 15 isolates of *Cryptococcus* were identified, including 6 from CSF, 5 from sputum, and 4 from urine samples. Three species were identified: *Cryptococcus neoformans*, *Cryptococcus albidus*, and *Cryptococcus laurentii*. The *Cladosporium cucumerinum* filtrate at 100% concentration exhibited higher antifungal activity than Amphotericin B. Mice infected with C. neoformans displayed loss of appetite and hyperactivity. Histological examination of lung tissues showed inflammatory cell infiltration, blood vessel congestion, and thickening of bronchi and alveolar walls in the infected mice. Mice treated with the fungal filtrate showed reduced pathological changes compared to those treated with Amphotericin B, with nearly normal alveolar structures. *Conclusion:* The study successfully identified *Cryptococcus neoformans* and other species from clinical samples. The *Cladosporium cucumerinum* filtrate exhibited promising antifungal activity, outperforming Amphotericin B in reducing histopathological damage in an experimental mouse model. This suggests the potential for using fungal filtrates as an effective treatment for *Cryptococcus i*nfections.

KEYWORDS

Cryptococcus neoformans, antifungal treatment, *Cladosporium cucumerinum* filtrate, histopathology, amphotericin B

How to cite this article: Mahmoud S. M., Mezhar M. A., Shaban R. K.: Detection of histopathological changes in the lung tissues of mice caused by *Cryptococcus neoformans*: therapeutic effect of endophytic fungus extract. *Epitheorese Klin. Farmakol. Farmakokinet.* 42(Sup1): 83-91 (2024).
DOI: 10.61873/NPHG8547

Publisher note: PHARMAKON-Press stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

1. INTRODUCTION

Cryptococcosis, caused by the yeast *Cryptococcus neoformans*, is a dangerous disease globally [1]. This disease is more common in people with weakened immune systems, especially those infected with AIDS [2]. *Cryptococcosis* is a highly widespread systemic fungal disease, and it is a fatal fungal disease. In addition to the fact that it infects humans, it infects several species of animals at different ages. In addition to infecting humans, it infects several species of animals around the world Ages. These yeasts have been isolated from cerebrospinal fluid and some animals [3].

The infection with these yeasts is unclear and without any apparent symptoms, or it can be severe infections such as meningitis and pneumonia, depending on the nature of the host's immune system. Infection occurs by inhaling live yeast cells or their spores spread in the environment, where these cells colonize the lungs but often spread to the rest of the body's organs, mainly affecting the central nervous system, causing meningitis [3].

Recently, interest in *Cryptococcus neoformans* yeast has increased. As a cause of cases of cerebral meningitis resulting from *cryptococcosis*, as statistics indicates, the incidence of this disease is high globally, especially in developing countries such as India and East Asia [4,5]. It is believed that the key to the infection with *cryptococcosis* is the interaction between these yeasts and the large phagocytic cells. These yeasts begin to multiply immediately after entering the phagocytic cells and are released from them, whether with or without lysis of the infected cells. In most cases, they appear without any symptoms as long as the immune system is healthy, but the symptoms begin to appear after immunosuppression [6].

Cryptococcosis affects various body systems, especially the respiratory system, represented by the nasal cavity and lungs [7]. Yeast can often reach the central nervous system after an infection of the lungs, or after an infection of the nose and sinuses, or through white blood cells through the bloodstream. When it reaches the brain, stromal nodes containing the yeast are formed as an immune response by the host. There may be one or more, and these nodes may enlarge. They expand with treatment, and this does not represent a treatment failure, as it shrinks over time with continued treatment in some cases [8].

Endophytes are extremely diverse microorganisms that inhabit plant tissues inside and between cells. There are approximately one million fungi [9]. Endophytic fungi have the ability to produce numerous bioactive substances that can produce compounds similar to specific pharmacological activities of plants [10]. It produces various chemoreceptor complexes, such as alkaloids, flavonoids, steroids, terpenoids, and phenolic compounds, and these compounds have interesting and multidirectional pharmacological activities including antimicrobial, antioxidant, antidiabetic, antimalarial, and antineoplastic properties. The discovery of these new and diverse active compounds is valuable for the study of natural medicinal products [11].

The fungus *Cladosporium* spp is a genus found everywhere and belongs to the *Cladosporiaceae* family[10]. This mushroom contains compounds that are of medical importance or are relevant as biological control agents for plant diseases[11,12]. It contains many effective secondary compounds, including alkaloids, phenols, sterols, flavonoids, and other effective compounds that act as antibiotics against types of bacteria and fungi such as *Aspergillus flavus, Candida albicans, Staphylococcus aureus,* and *Pseudomonas syringae* [13].

The aim of the study was to isolate and identify *Cryptococcus neoformans* from clinical samples and evaluate the antifungal efficacy of *Cladosporium cucumerinum* filtrate compared to Amphotericin B. The study also aimed to assess the histopathological effects of these treatments in a mouse model infected with *C. Neoformans.*

2. METHODOLOGY

2.1. Ethical approval and consent

Ethical approval for the study was obtained from the Ethics Committee at Baqubah Teaching and Batoul Hospital in Diyala Governorate. The collection of clinical samples from patients was conducted under strict ethical guidelines, following the hospital's protocols for research involving human subjects. Informed consent was obtained from all participating patients or their legal guardians, after a thorough explanation of the study's objectives and the procedures involved. The consent process ensured that participants were aware of their voluntary participation and the confidentiality of their personal and medical data.

2.2. Sample collection

A total of 130 clinical samples were collected from patients diagnosed with various conditions, such as meningitis, pulmonary tuberculosis, and kidney failure. The patients were attending the chest and respiratory diseases clinic, the cancer center, and the dialysis center in Baqubah Teaching and Batoul Hospitals. The collection period spanned

from October 2022 to January 2023, and the samples included:

- 50 cerebrospinal fluid (CSF) samples from patients with meningitis,
- 30 sputum samples from patients with pulmonary tuberculosis,
- 50 urine samples from patients with kidney failure.

2.3. Fungal culture and identification

The collected samples were cultured on Sabouraud Dextrose Agar (SDA), Trichosporon Agar (TOC), and Potato Dextrose Agar (PDA), with *Cryptococcus* differentiation media. The plates were incubated at 37°C and monitored daily for fungal growth. Microscopic examination of the grown colonies was performed using India ink staining. Diagnosis of *Cryptococcus* species was confirmed using the Vitek device, a rapid identification system. The *Cladosporium cucumerinum* fungal strain was sourced from Tikrit University, College of Education, Department of Life Sciences, and its identification was confirmed using Polymerase Chain Reaction (PCR).

2.4. Preparation of fungal filtrate *e*xtract

The *Cladosporium cucumerinum* filtrate extract was prepared by cultivating the fungal colonies in liquid Aspergillus medium. The medium was prepared in 250 ml beakers, which were sterilized before the addition of chloramphenicol to prevent bacterial contamination. Fungal inoculation was performed by adding 0.5 mm diameter discs of *Cladosporium* colonies into the medium. The beakers were incubated at 25°C for 28 days, and regular shaking (every 2-3 days) was done to promote uniform growth. The fungal culture was then filtered using sterile filter paper to obtain the fungal filtrate extract.

2.5. Experimental animal model

A total of 50 male mice, weighing between 25-30 grams, were used for the in vivo studies. Ethical approval for conducting experiments on mice was obtained from the Institutional Animal Care and Use Committee (IACUC). The mice were housed in standard laboratory conditions with a controlled environment (12-hour light/dark cycle, temperature of 22-25°C) and were provided with food and water ad libitum. The experiments were conducted from February to mid-March 2023.

2.6. Experimental design

The mice were divided into three groups for treatment studies:

Table 1. Groups of Mice, Treatments, and Number of Mice per Group.

Group	Treatment description	Number of mice
Control group	Infected with *Cryptococcus neoformans* but received no treatment	10
Amphotericin B group	Infected with *C. neoformans* and treated with Amphotericin B	10
Fungal filtrate group	Infected with *C. neoformans* and treated with *Cladosporium cucumerinum* fungal filtrate	10

The infection was induced by inoculating the mice with *C. neoformans*, and treatments were administered post-infection. The mice were monitored daily for clinical signs, including appetite and activity levels. After the experiment, the mice were euthanized, and lung tissues were isolated for histopathological examination.

2.7. Histopathological examination

The lung tissues were fixed in formalin, processed, and embedded in paraffin. Thin sections (4-5 µm) were stained using hematoxylin and eosin (H&E) for histological analysis. The sections were evaluated under a light microscope to assess the degree of inflammatory infiltration, blood vessel congestion, bronchiole thickening, and alveolar wall alterations in the lung tissues.

2.8. Statistical analysis

Data from the histopathological findings and the efficacy of the fungal filtrate and Amphotericin B treatments were statistically analyzed using SPSS software (version 25). A one-way ANOVA test was performed to compare the means between different experimental groups. Post hoc analysis was conducted using Tukey's test to determine the significance of the differences between the control, Amphotericin B-treated, and fungal filtrate-treated groups. Statistical significance was set at $p < 0.05$.

3. RESULTS

3.1. Phenotypic characteristics and growth patterns of *Cryptococcus neoformans*

In Figure 1, the two images depict phenotypic characteristics and growth patterns of *Cryptococcus neoformans* under different conditions: Phenotypic characteristics using India ink dye: The left panel shows the *Cryptococcus neoformans* yeast cells observed under a microscope with India ink staining. India ink is commonly used to visualize the polysaccharide capsule surrounding the yeast cells. The halo-like appearance around the cells is a result of the capsule, which does not absorb the dye, thus leaving a clear zone around each yeast cell. This is a hallmark feature of *Cryptococcus neoformans*, aiding in its identification [14,15].

Growth on *Cryptococcus* differentiation medium: The right panel shows the growth of *Cryptococcus neoformans* on a differentiation medium. The distinct colonies on the plate indicate the organism's ability to grow on selective media, which helps differentiate *Cryptococcus* species based on colony morphology, texture, and color. The medium supports the yeast's growth, and the different morphologies of the colonies suggest possible variations in phenotypic traits or environmental responses [16].

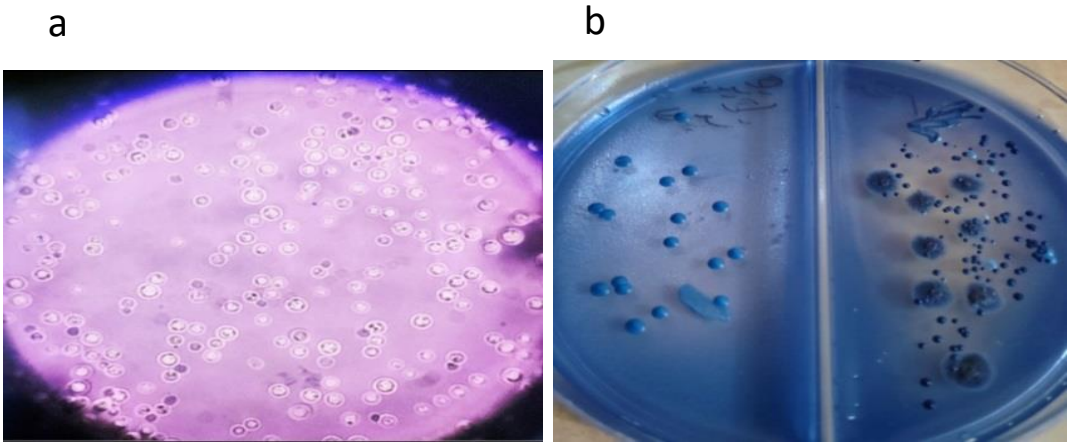

Figure 1. (a) Phenotypic characteristics of *Cryptococcus neoformans* yeast using India ink dye. (b) Growth of *Cryptococcus neoformans* yeast on Cryptococcus differentiation medium.

3.2. Histological study

In Figure 2, various histopathological sections of lung tissues from different treatment groups are presented, stained with Hematoxylin and Eosin (H & E). Panel (a) shows a control group with normal lung architecture, including visible alveoli and bronchioles. Panel (b), representing the group treated with yeast only, displays a thickening of the alveolar walls and bronchioles, suggesting inflammation or infection-induced remodeling. In panel (c), the lung section from the cortisone-treated group exhibits prominent inflammatory cell infiltration, indicating an immune response. In contrast, panel (d), from the group treated with antifungals, also shows alveolar wall thickening and blood congestion, hinting at a partial therapeutic response but with residual inflammation. Panel (e), corresponding to the group treated with fungal filtrate, shows largely intact alveoli and bronchioles, suggesting some degree of protective or restorative effect. Panel (f) illustrates a section from the group treated with cortisone, yeast, and antifungals, where alveolar thickening persists, indicating an incomplete resolution of the infection. Finally, in panel (g), the group treated with cortisone, yeast, and fungal filtrate shows almost normal lung morphology, with well-preserved alveolar sacs, reflecting the potential effectiveness of the fungal filtrate in promoting lung recovery. These images provide comparative insights into the effects of various treatments on lung tissue integrity.

The histopathological findings presented in Table 2 show clear differences across treatment groups. The control group has normal alveolar integrity, with low alveolar wall thickness (2.5 ± 0.2), minimal inflammatory cell infiltration (0.8 ± 0.1), and minimal blood congestion (0.5 ± 0.1), representing healthy lung tissue.

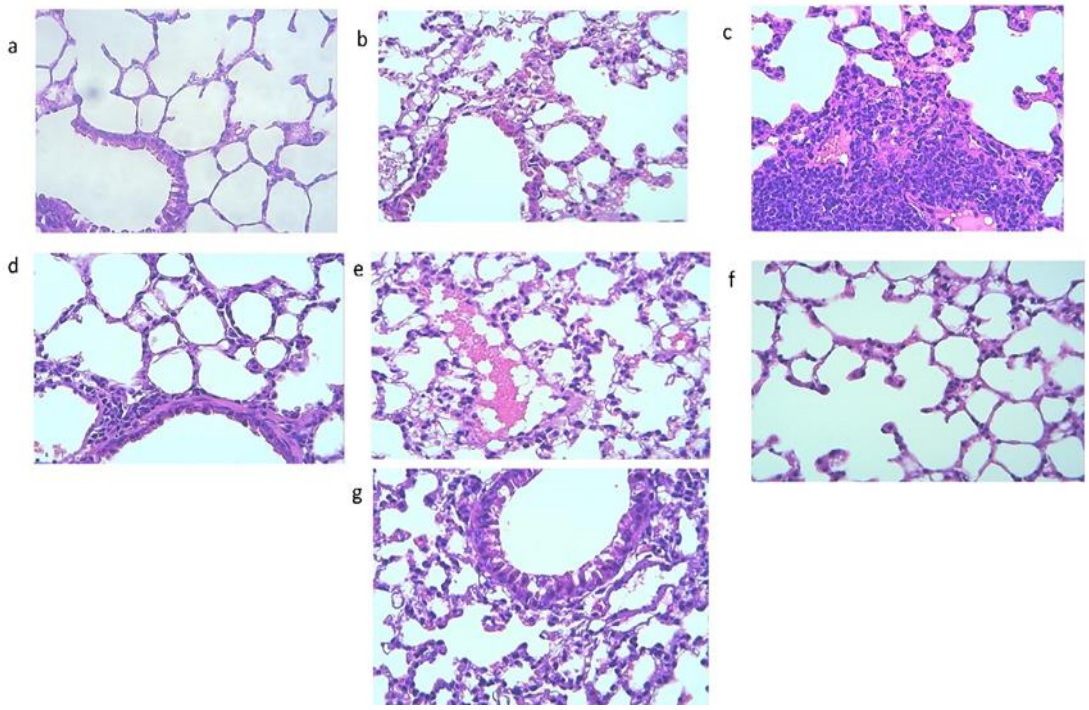

Figure 2. (a) A section of the lung of the control group showing the alveoli (Alv) and bronchioles (Br). H & E 400X. (b) A lung section of the group treated with yeast only shows thickening of the walls of the alveoli (TW) and bronchioles (Br). H & E 400X. (c**)** Lung section of the group treated with cortisone only shows inflammatory cell infiltration (IF). H & E. (d) Lung section of the group treated with antifungals only shows thickening of the alveolar walls (TW) and blood congestion (CON). H & E 400X. (e) A section of the treatment with fungal filtrate only, showing the alveoli and bronchioles. (f) A section of the lung of the group treated with cortisone, yeast, and antifungals shows the bronchioles (Br) and thickening of the alveolar walls (TW). H & E 400X, (g) A section of the lung of the group treated with cortisone, yeast, and fungal filtrate shows the pulmonary alveoli (Alv) and alveolar sacs (AVS) in an almost normal manner. H & E 400X.

In the yeast-only group, there is a marked increase in alveolar wall thickness (5.8 ± 0.5), significant inflammatory cell infiltration (4.5 ± 0.4), and considerable blood congestion (3.9 ± 0.3), indicating that infection with *Cryptococcus neoformans* leads to severe inflammatory and structural damage. The cortisone-only group also shows heightened pathological changes with high alveolar wall thickening (4.9 ± 0.4), increased inflammatory infiltration (5.2 ± 0.4), and severe blood congestion (4.8 ± 0.4), likely due to the immunosuppressive effects of cortisone exacerbating the infection.

The antifungal-only group demonstrates moderate improvement, with reduced alveolar wall thickness (3.7 ± 0.3), inflammatory cell infiltration (3.8 ± 0.3), and blood congestion (2.5 ± 0.3), although the lung integrity remains altered. In contrast, the fungal filtrate-only group shows near-normal histology with minimal alveolar thickening (2.7 ± 0.2), low inflammatory infiltration (1.2 ± 0.2), and reduced blood congestion (0.7 ± 0.1), indicating that the fungal filtrate may offer significant therapeutic benefits.

The group treated with cortisone, yeast, and antifungals shows persistent pathology, with high alveolar wall thickening (4.6 ± 0.3), inflammatory infiltration (4.3 ± 0.3), and blood congestion (4.1 ± 0.3), suggesting that the antifungals alone were insufficient to fully mitigate the effects of cortisone-induced immune suppression. Conversely, the group treated with cortisone, yeast, and fungal filtrate shows substantial improvement, with alveolar wall thickness (2.8 ± 0.2), inflammatory infiltration (1.5 ± 0.2), and blood congestion (0.9 ± 0.1) approaching normal levels, indicating that the fungal filtrate could be effective in restoring lung health, even in the presence of immunosuppression.

Table 2. Histopathological findings.

	Alveolar wall thickness (mean Â± SD)	**Inflammatory cell infiltration** (mean Â± SD)	**Blood congestion** (mean Â± SD)	**Alveolar integrity** (normal/altered)
Control group	2.5 Â± 0.2	0.8 Â± 0.1	0.5 Â± 0.1	Normal
Yeast only	5.8 Â± 0.5	4.5 Â± 0.4	3.9 Â± 0.3	Altered
Cortisone only	4.9 Â± 0.4	5.2 Â± 0.4	4.8 Â± 0.4	Altered
Antifungals only	3.7 Â± 0.3	3.8 Â± 0.3	2.5 Â± 0.3	Altered
Fungal filtrate only	2.7 Â± 0.2	1.2 Â± 0.2	0.7 Â± 0.1	Normal
Cortisone, yeast, and antifungals	4.6 Â± 0.3	4.3 Â± 0.3	4.1 Â± 0.3	Altered
Cortisone, yeast, and fungal filtrate	2.8 Â± 0.2	1.5 Â± 0.2	0.9 Â± 0.1	Normal

4. DISCUSSION

The results of the microscopic examination of tissue sections taken from the lungs of mice infected and immunosuppressed with the drug hydrocortisone showed severe histological-pathological changes represented by the presence of cell infiltration, thickening of the alveolar wall, haemorrhage, and tumour masses in the lung. This is due to the yeast's possession of the enzyme hemolysin, which decomposes red blood cells.

This is why a clot forms inside the blood vessel, which is a defensive process carried out by the body to avoid bleeding [16-18]. Yeast can live and multiply after being eaten inside the microglial cell, and it is difficult for the system to overcome the infection explained that it reaches the brain tissue and that the reason for its infiltration into cells is due to the accumulation of polysaccharides that make up the yeast capsule, which leads to an increase in osmosis, and as a result of the yeast infecting the lung tissue, and this stimulates the inflammatory response and causes a defect in stimulating the secretion of chemoattractive factors [17,18]. Initially related to neutrophil cells, which failed to eliminate the yeast due to the virulence factors it possesses, such as capsule and melanin, which work to inhibit the phagocytosis process [19, 20]

As for the cause of blood vessel congestion, it occurs due to the content of the bacterial suspension of fungal secretions, such as the yeast's possession of the enzyme hemolysin, which decomposes red blood cells, causing an increase in blood pressure inside the capillaries, while the cause of degenerative and necrotic changes in the cells lining the bronchi is attributed to the ability of the yeast to On the production of protease and phospholipase enzymes that work to degrade the membranes of these cells, leading to the release of lysosomes that have a necrotic effect on the tissues [21].

Chen *et al.* [22] in a study he conducted on a person suffering from pulmonary *cryptococcosis*, showed that the tissue changes were represented by thickening of the septa between the lung alveoli with the presence of yeast in them, and this is consistent with what he found [23].

It was indicated that Amphoterici B acts on the production of free radicals within the fungi, leading to the depletion of super-antioxidants, which in turn affects the cellular pathways of the fungi [24,25]. Also mentioned that this antibody has immunomodulatory effects, as it can stimulate inflammatory mediators such as IL-6, TNF, nitric oxide, and prostaglandins from immune cells in mice and humans [25]. These results are consistent with what was reported by [26]regarding the occurrence of thickening of the walls of the pulmonary alveoli and infiltration of the lung tissue [26].

It was stated in a study evaluating the effective role of endophytic fungi as antioxidants and antimicrobials, where they discovered that fungal extracts isolated from within the plant *Alternaria alternata* and *Cladosporium cladosporioides* contained secondary metabolite compounds, including flavonoids represented by flavones and flavonols as well. It contains phenols such as Phosphomolybdic Phosphotungstic-Phenol [27].

This study agreed with a study on the inhibitory effectiveness of *Cladosporium cucumerinum* fungus extract on yeasts and bacteria [28]. The 100% concentration gave the highest inhibitory effectiveness against the yeasts *Candida albicans* and

Candida glabrata. The value of the diameter of inhibition was 24 and 19 mm, respectively, and the value of the least significant difference was 6.83. 6.37, respectively. For the bacteria *Enterococcus faecali* and *Staphylococcus saprophyticus*, the value of the inhibition diameter was 24 and 21 mm, respectively, and the value of the least significant difference was 6.31 and 7.01, respectively. *Cladosporium mushrooms* are effective against bacteria and fungi because they contain effective secondary substances and compounds, including alkaloids and their derivatives, coumarins and their derivatives, flavonoids, naphthalene compounds and their derivatives, and sterols, as these compounds have many therapeutic biological activities[13].

In a study [29] it was shown that mushroom filtrate shows an inhibitory activity against bacterial species, as the 100% concentration gave the highest inhibitory effectiveness against *St.aureus* and *E.coli* bacteria, with an average diameter of inhibition of 26.9 and 25.3 mm, respectively. It also has an inhibitory effect on yeasts: *Candida guilliermondii*, *C.albicans, Trichosporon Asahii*, and *Cryptococcus laurentii*. This study is consistent with the current study. From the results of the current study, we note that they were consistent with a study [28], as they confirmed that the filtrate of the fungus *C. cucumerinum* shows inhibitory activity against pathogens, including the bacteria *S. pyogenes, E. faecalis*, and *St. sapophyticus* and *K. pheumoniae*. It also gave effectiveness against Candida sp. The reason for this effectiveness is that it contains biologically active compounds represented by alkaloids and phenols

In a study [28], conducted a study to estimate the total content of alkaloids and phenols in the filtrates of endophytic fungi, as the fungus *C. cucumerinum* had the highest content of alkaloids and also contained a large percentage of phenols, through a GC.MS analysis of a study [29] which showed that it contained It contains 19 alkaloid compounds, but in small proportions, and 8 phenolic compounds. The results of the current study were also similar to the findings of a number of studies, including the study of [30], which confirmed that the extract of the *Cladosporium* fungus showed inhibitory activity against many pathogens, as it was effective against the bacteria as effective as isocoumarin derivatives. It was [11] indicated that the *Cladosporium* genus has an antibacterial effect on the bacteria *Escherichia coli, Pseudomonas aeruginosa, Bacillus megaterium*, and *Staphylococcus aureus*, if the active secondary compounds destroy the bacterial cell wall and cause a defect in the cytoplasm, thus causing the formation of holes in the cell wall membranes [31].

This study is consistent with the results of a study [29]as it showed that the filtrate of the fungus *C. cucumerinum* has an inhibitory effect on the growth of cancer cells in the organism's body if these cells were treated at different concentrations, as the filtrate recorded the highest inhibition rate of 35.74% at the concentration of 50% and 29.36%. At a 100% concentration, this may be due to the *Cladosporium* fungus containing effective secondary substances and compounds, including alkaloids and their derivatives, coumarins and their derivatives, flavonoids, naphthalene compounds and their derivatives, and sterols, as these compounds have many therapeutic biological activities [13].

5. CONCLUSION

This study has shown that the extract of the fungal filtrate *Cladosporim* sp has an effective role in treating mice after they were infected with modern *cryptococcosis*, which caused fibrosis and thickening of the alveoli and bronchioles, and when treated with the antifungal amphotericin, it caused blood congestion in the lung, in contrast to the fungal filtrate, which gave the lung a normal appearance.

ACKNOWLEDGMENTS
The authors declare that there are no acknowledgments for this study.

CONFLICT OF INTEREST STATEMENT
The authors declare no conflicts of interest.

REFERENCES

1. Jarvis J. N., Casazza J. P., Stone H. H., Meintjes G., Lawn S. D., Levitz S. M., *et al*.: The phenotype of the *Cryptococcus*-specific CD4+ memory T-cell response is associated with disease severity and outcome in HIV-associated cryptococcal meningitis. *J Infect Dis*. 207(12): 1817-28 (2013).
DOI: 10.1093/infdis/jit099

2. Barnett J. A.: A history of research on yeasts 14: medical yeasts part 2, *Cryptococcus neoformans*. *Yeast*. 27(11): 875-904 (2010).
DOI: 10.1002/yea.1786

3. Gupta A. K., MacLeod M.A., Foley K. A., Gupta G., Friedlander S. F.: Fungal skin infections. *Pediatr Rev*. 38(1): 8-22 (2017).
DOI: 10.1542/pir.2015-0140

4. Labrecque O., Sylvestre D., Messier S.: Systemic *Cryptococcus albidus* infection in a Doberman Pinscher. *J Vet Diagn Invest*. 17(6): 598-600 (2005).

DOI: 10.1177/104063870501700615

5. Voelz K., Lammas D. A., May R. C.: Cytokine signaling regulates the outcome of intracellular macrophage parasitism by *Cryptococcus* neoformans. *Infect Immun*. 77(8): 3450-7 (2009).
DOI: 10.1128/iai.00297-09

6. Coelho C., Bocca A. L., Casadevall A.: The tools for virulence of *Cryptococcus neoformans*. *Adv Appl Microbiol*. 87:1-41 (2014).
DOI: 10.1016/B978-0-12-800261-2.00001-3

7. Jones T. C., Hunt R. D.: Patologia veterinária. *Piccin-Nuova Libraria*. 1816 (1987).

8. Liu R., Bao Z-X., Zhao P-J., Li G-H.: Advances in the study of metabolomics and metabolites in some species interactions. *Molecules*. 26(11): 3311 (2021).
DOI: 10.3390/molecules26113311

9. Rashmi M., Kushveer J. S., Sarma V. V.: A worldwide list of endophytic fungi with notes on ecology and diversity. *Mycosphere*. 10(1): 798-1079 (2019).
DOI: 10.5943/mycosphere/10/1/19

10. Abdollahzadeh J., Groenewald J Z., Coetzee M. P. A., Wingfield M. J., Crous P. W.: Evolution of lifestyles in *Capnodiales*. *Stud Mycol*. 95(1): 381-414 (2020).
DOI: 10.1016/j.simyco.2020.02.004

11. Khan M. I. H., Sohrab M. H., Rony S. R., Tareq F. S., Hasan C. M., Mazid M. A.: Cytotoxic and antibacterial naphthoquinones from an endophytic fungus, *Cladosporium* sp. *Toxicol Rep*. 3: 861-5 (2016).
DOI: 10.1016/j.toxrep.2016.10.005

12. Adorisio S., Fierabracci A., Muscari I., Liberati A. M., Cannarile L., Thuy T. T., *et al*.: Fusarubin and anhydrofusarubin isolated from a *Cladosporium* species inhibit cell growth in human cancer cell lines. *Toxins*. 11(9): 503 (2019).
DOI: 10.3390/toxins11090503

13. Salvatore M. M., Andolfi A., Nicoletti R.: The genus *Cladosporium*: A rich source of diverse and bioactive natural compounds. *Molecules*. 26(13): 3959 (2021).
DOI: 10.3390/molecules26133959

14. Lungran P., Vijaya D., Shashi S.: *Cryptococcosis*: its prevalence and clinical presentation among HIV positive and negative patients in Rims, Manipur. *IOSR J Dent Med Sci*. 13(7): 38 (2014).
DOI: 10.9790/0853-13743841

15. Hagen F., Illnait-Zaragozí M-T., Meis J. F., Chew W. H., Curfs-Breuker I., Mouton J. W., *et al*.: Extensive genetic diversity within the Dutch clinical *Cryptococcus neoformans* population. *J Clin Microbiol*. 50(6): 1918-26 (2012).
DOI: 10.1128/jcm.06750-11

16. Shah H. S., Patel D. A., Vegad M. M.: Evaluation of conventional and serological methods for rapid diagnosis of cryptococcal meningitis in HIV seropositive patients at tertiary care hospital. *National Journal of Community Medicine*. 2(3): 354-357 (2011).

17. Sharma V., Paliwal R.: Isolation and characterization of saponins from *Moringa oleifera* (moringaceae) pods. *Int J Pharm Pharm Sci*. 5(1): 179-83 (2013).

18. Bartges J. W.: Diagnosis of urinary tract infections. *Vet Clin Small Anim*. 34(4): 923-33 (2004).
DOI: 10.1016/j.cvsm.2004.03.001

19. Jung W. H., Hu G., Kuo W., Kronstad J. W.: Role of ferroxidases in iron uptake and virulence of *Cryptococcus neoformans*. *Eukaryot Cell*. 8(10): 1511-20 (2009).
DOI: 10.1128/ec.00166-09

20. Loane D. J., Byrnes K. R.: Role of microglia in neurotrauma. *Neurotherapeutics*. 7(4): 366-77 (2010).
DOI: 10.1016/j.nurt.2010.07.002

21. Shi M., Li S. S., Zheng C., Jones G. J., Kim K. S., Zhou H., *et al*.: Real-time imaging of trapping and urease-dependent transmigration of *Cryptococcus neoformans* in mouse brain. *J Clin Invest*. 120(5): 1683-93 (2010).
DOI: 10.1172/JCI41963

22. Janbon G., Doering T. L.: Biosynthesis and genetics of the *Cryptococcus* capsule. *Cryptococcus*: From Human Pathogen to Model Yeast. 27-41 (2010).
DOI: 10.1128/9781555816858.ch3

23. Vu K., Eigenheer R. A., Phinney B. S., Gelli A.: *Cryptococcus neoformans* promotes its transmigration into the central nervous system by inducing molecular and cellular changes in brain endothelial cells. *Infect Immun*. 81(9): 3139-47 (2013).
DOI: 10.1128/iai.00554-13

24. Chen Y-G., Lin T-Y., Lin G-M, Lin J-C.: Pulmonary *cryptococcus* infection after mono-chemotherapy with gemcitabine. *Respir Care*. 56(3): 339-41 (2011).
DOI: 10.4187/respcare.00652

25. Zhou H-X., Ning G-Z., Feng S-Q., Jia H-W., Liu Y., Feng H-Y., *et al*. *Cryptococcosis* of lumbar vertebra in a patient with rheumatoid arthritis and scleroderma: case report and literature review. *BMC Infect Dis*. 13: 1-7 (2013).
DOI: 10.1186/1471-2334-13-130

26. Sangalli-Leite F., Scorzoni L., Mesa-Arango A. C., Casas C., Herrero E., Gianinni M. J. S. M., *et al*.: Amphotericin B mediates killing in *Cryptococcus neoformans* through the induction of a strong oxidative burst. *Microbes Infect*. 13(5): 457-67 (2011).
DOI: 10.1016/j.micinf.2011.01.015

27. Mesa-Arango A. C., Scorzoni L, Zaragoza O.: It only takes one to do many jobs: Amphotericin B as antifungal and immunomodulatory drug. *Front Microbiol*. 3: 286

(2012).
DOI: 10.3389/fmicb.2012.00286

28. Khalifa S. A., Al-Elyani R. A., Domiaty D. M.: Histological and ultrastructural studies on the effect of Costus Plant and Amphotericin B on male lung rats infected by Aspergillus niger. *Life Sci J*. 9(4): 5321-38 (2012).

29. Gauchan D. P., Kandel P., Tuladhar A., Acharya A., Kadel U., Baral A., *et al*.: Evaluation of antimicrobial, antioxidant and cytotoxic properties of bioactive compounds produced from endophytic fungi of Himalayan yew (*Taxus wallichiana*) in Nepal. *F1000Res*. 9: 379 (2020).
DOI: 10.12688%2Ff1000research.23250.2

30. Erjaee Z., Shekarforoush S. S., Hosseinzadeh S.: Identification of endophytic bacteria in medicinal plants and their antifungal activities against food spoilage fungi. *J Food Sci Technol*. 56: 5262-70 (2019).

31. Msarhd M. A., Mezher M. A.: Detection of Chemical Compounds (Secondary Metabolites) of Alcoholic Extract of *Cladosporium Cucumerinum* using Gas Chromatogram-Mass Spectrometry GC-MS. *Scholast J Nat Med Educ*. 2(5): 339-43 (2023).