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Effects of *Rhizopus oryzae* on Kidney of Albino Male Rats: Experimental Infection

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Abstract

Background: Rhizopus oryzae stands out as a prominent pathogen within the Mucorales class, notorious for inciting mucormycosis, a severe and often perilous fungal infection that predominantly targets immunocompromised patients, especially those grappling with diabetes and hematologic cancers **. Materials and Methods:** After confirming identification of *R,oryzae*, twenty of albino male rats were tested in which ten animals were infected with spores of this mold while others was used as control group. All animals were left for 30 days, sacrificed, and their Kidney_ were selected to be submitted to histopathological process. **Results:** *R. oryzae* revealed pathological changes in Kidney_ of albino male rats. The glomeruli demonstrated marked dilatation of Bowman's space , accompanied by noticeable dilatation of the renal tubules ,atrophy of glomerul,necrosis of lining epithelial of renal tubules and necrosis of mesangial cells in glomeruli , Degenerative alterations were observed in the epithelial lining of the renal tubules , and some appeared as scattered foci of degeneration distributed across different areas of the renal cortex **Conclusion:** This study concluded that the *R. oryzae* has ability to occur effects on Kidney of albino male rats. This conclusion demonstrates dangerous effects caused by this mold particularly immuncomprised patients. It may lose functions of Kidney

I. Introduction

Rhizopus oryzae stands out as a prominent pathogen within the Mucorales class, notorious for inciting mucormycosis, a severe and often perilous fungal infection that predominantly targets immunocompromised patients, . The significance of investigating this organism stems from its rapid proliferation and aggressive characteristics, which contribute to dismal prognoses for afflicted individuals, even in the presence of established antifungal therapies. Research indicates [**1**] that Rhizopus species rank as the leading culprits behind mucormycosis, underscoring the necessity for concentrated exploration of their biology and pathogenic traits.Moreover, recent investigations have brought to light an alarming rise in Rhizopus oryzae as a concerning pathogen amidst post-COVID-19 complications indicating a substantial uptick in its prevalence. This situation accentuates the pressing need to delve deeper into its virulence factors. According to [2] Mucorales infect the host either through inhalation, ingestion and/or through direct inoculation of fungal spores through an abraded skin due to trauma.[3] These fungi are ubiquitous in the environment and are seen microscopically as broad septate or aseptate right angled branching fungi According to Patel et al diabetes, diabetic ketoacidosis, organ transplant, malignancy, corticosteroids, immunosuppression, trauma, and burns are known risk factors for mucormycosis [4] The primary mechanism of kidney damage in renal mucormycosis is ischemic injury resulting from angioinvasion.Both the cortex and the medulla 5 are affected when fungal hyphae invade the small and large renal arteries, causing extensive thrombosis and eventual renal parenchymal infarction. Acute kidney damage (AKI), which is seen in 92% of individuals with bilateral renal involvement, may result from this process[5] A crucial technique for understanding R. oryzae infection and pathophysiology is the animal model [6] (Jacobsen, 2019). In veterinary medicine, mucormycosis seems to be important for conditions such feline brain infections [7] (Marinelly et al., 2025). Furthermore, buffaloes have this illness, which has been reported by by[**8**] (Barbosa et al., 2024). Also, gastric mucormycosis was diagnosed in a cat [**9**](Mavilio and Bottero, 2025). Based on the mentioned information, mucormycosis has interesting observation in both humans and animal aspects. For these reasons, this research was designed to study pathogenesis of this mold using albino male rats.

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II. Materials and Methods

Identification of *Rhizopus oryzae*

The *R. oryzae* was provided by[**10**] (Al-abedi and Alhasan, 2023) who isolated it from poultry food .This mold was also re-identified depending on the methods of morphology and molecular techniques including sequencing and genetic analysis. Fungal DNA was extracted according to instruction of DNA Geneaid kit.PCR process was done that the forward primer (*ITS*1): TCCGTAGGTGAACCTGCGG and reverse primer (*ITS*4): TCCTCCGCTTATTGATATGC were designed. The PCR components were reacted using 13, 1, 1, 3-4, and 6-7 µl of green master mix 2X, forward primer, reverse primer, extracted DNA, and nuclease-free water, respectively in which total mixture was 25 µl. The PCR process was carried out according to protocol which included initial denaturation at 95 ºC during 5 min. through 1 cycle in 1 step while denaturation, annealing, and extension at 95 ºC, 56 ºC, and 72 ºC for 0.30 sec., 0.30 sec., and 0.45 sec. in 2, 3, and 4 steps, respectively through 35 cycles. Final extension was done at 72 ºC for 10 min. through 1 cycle in 4 step. After completing process, genes were submitted into agarose electrophoresis, then visualized using ultraviolet device. All nucleotide sequences were aligned using BioEdit software, which includes a graphic view tool to illustrate the genetic variations (such as mutations) among nucleotides sequences compared with the standard strain sequences of *R,oryzae*. Mega x software was also used to construct a phylogenetic tree (The neighbour-Joining (NJ) algorithm, which has been classified. The nucleotide sequences were analyzed and processed using NCBI-BLAST Alignment Identification.

Preparation of Fungal Spores

Rhizposus oryzae was growing on SDA at 25 ºC for 5 days and a part of the fungal colony was cultured in a tube containing brain heart infusion broth (BHIB) and incubated at 25 ºC for 5 days. After completing incubation, an amount of the fungal growth was added into a tube containing sterile distilled water, mixed well, and placing a drop of it on a clean slide which covered with a lid. Finally, fungal spores were enumerated under microscope (40 X magnification) using a haemocytometer chamber depending on the following equation (Y × 25 × 10 = Spore / ml) described by [**18**](Al-Bayati, 2005) that a number of the spores was adjusted to be 10^6 spore /ml . It is important to note. Y: a total number of the spores in one square of the hematocytometer chamber, 25: a number of the all squares, and 10: Constant.

Experimental Animal Infection

1. Twenty of albino male rats were used in this study. The animals were divided into control and infected groups. The animals were managed well in laboratory and housed in separated cages. They were treated according to Ethical Approval numbered 22 SU-EC-2024 and dated 10 / November /2024 The experimental infection was conducted during 1/ February/ 2024 to 6/ April/ 2024. All animals were injected with 0.5 ml of cyclosporine intraperitoneally for 3 days to get immune-comprised animals. Then, each one of the infected group was inoculated with 0.5 ml of suspension containing 10^6 spore / ml for three days in which inoculation was done each day. Concerning control group, each rat was inoculated with 0.5 ml of sterile phosphate buffer saline (PBS) for the same period intraperitoneally. All animals were left for 30 days as incubation period, sacrificed, livers were selected, kept in formalin (10 % in concentration), and subjected to histopathological process. Simultaneously, blood sample was collected from hearts animals directly through anesthesia of the animals. The collecting blood was obtained before few minutes of their sacrificing. Then, blood was separated to select serum which was used to detect Renal function which were urea and creatinine.

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III. Results

Identification of *Rhizopus oryzae*

Macroscopically, the *R. oryzae* produced a colored gray and feather-like colony as well as aerial growth on SDA at 37 ºC for 4- 5 days. Microscopically, this mold formed non-septate hyphae which possesses rhizoids and sporangium on the spornagiophore (**Figure 1**). PCR identified *ITS* region (**Figure 2**) and genetic sequencing revealed this mold belongs to *R. arrhizus* strain CMRC 066.

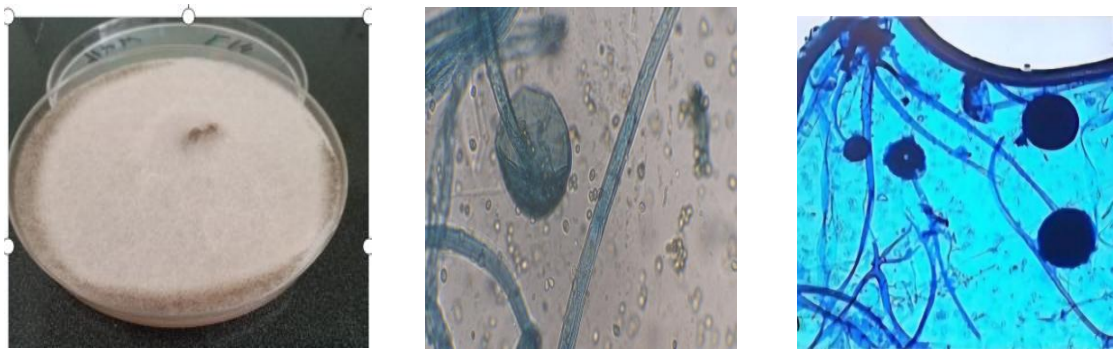

Figure1: Morphology of *R. oryzae*, from left to right: Growth on SDA agar and microscopic appearance.

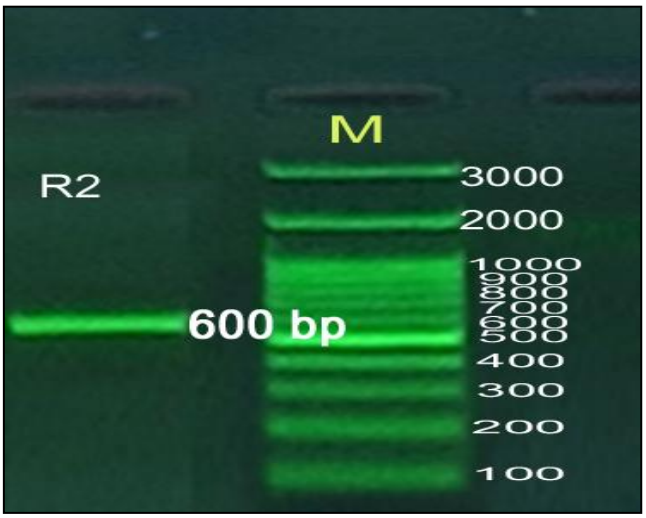

Figure 2. Detecting *ITS* region of *R. oryzae* by PCR.

Physio-pathological Effect of *Rhizopus oryzae* on Kidney

Physiological Effects on Kidney

Kidney function tests

As shown in the table 2 and figures 5,6 the analysis of the kidney function tests in the control and fungi groups. The fungi group showed significantly ($P<0.05$) higher levels of both Urea and Creatinine compared to the control group. The urea levels were 48.1 ± 1.89 for the fungi group and significantly ($P<0.05$) higher than the 32 ± 0.91 of the control group. In the same time, Creatinine levels were 0.73 ± 0.08 for the fungi group, a significant ($P<0.05$) increase over the control group's 0.25 ± 0.03.

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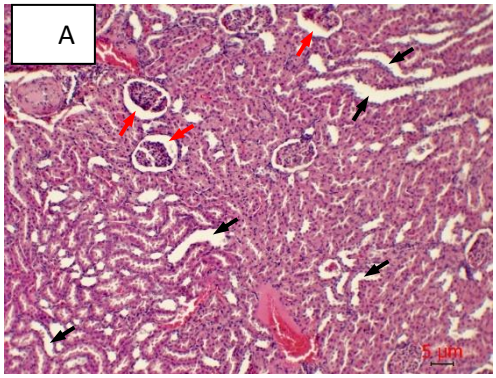
Table (2) Kidney function tests

Groups	Urea	Creatinine
Control	32±0.91c	0.25±0.03b
fungi	48.1±1.89a	0.73±0.08a
Calculated T value	7.64	5.43
Calculated P value	<0.0001	<0.0001

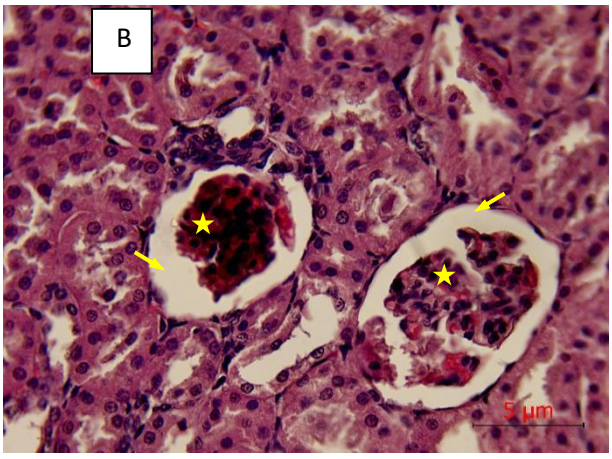
Means with different letters in the column are significantly different

A) Hitopathological Effects on

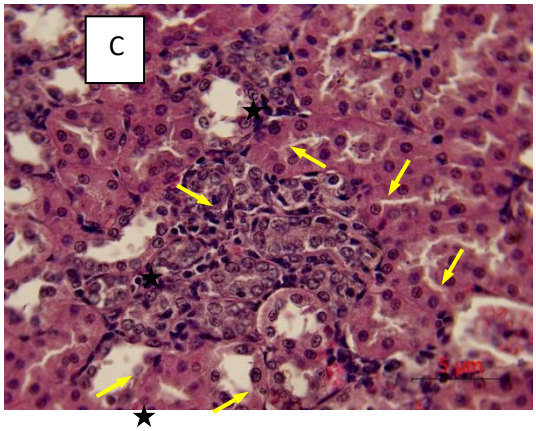
The microscopic examination show Most glomeruli demonstrated marked dilatation of Bowman's space , accompanied by noticeable dilatation of the renal tubules ,atrophy of glomerul,necrosis of lining epithelial of renal tubules and necrosis of mesangial cells in glomeruli , Degenerative alterations were observed in the epithelial lining of the renal tubules , and some appeared as scattered foci of degeneration distributed across different areas of the renal cortex **(Figures A,B,C,D,F 4.3.1.1)** comparing with normal control.(**Figure G).**



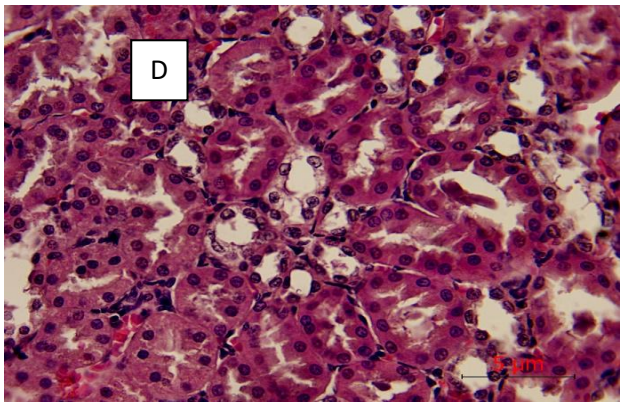
Kidney (fungi after 3-6): Most glomeruli demonstrated marked dilatation of Bowman's space (red arrows), accompanied by noticeable dilatation of the renal tubules (black arrows), H&E, 10x.



Kidney (fungi after 3-6): atrophy of glomeruli (asterisks) dilatation of Bowman's space (arrows), H&E, 40x.



Kidney (fungi after 3-6): Degenerative alterations were observed in the epithelial lining of the renal tubules (arrows), and some appeared as scattered foci of degeneration distributed across different areas of the renal cortex (asterisks), H&E, 40x.



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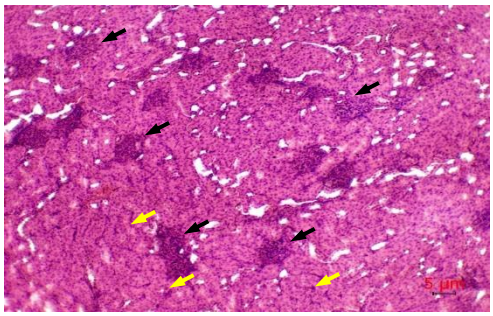

Kidney (control): normal of renal tubules (yellow arrows) and glomeruli (black arrows),

Kidney (fungi after 3-6): necrosis of lining epithelial

of renal tubules (arrows), H&E, 40x.

IV. Discussion

Concerning fungal identification, research findings indicated that *R. oryzae* develops colonies that are whitish-gray in color after five days on SDA. Microscopic examination reveals that this fungus produces sporangiospores, sporangiophores, non-septate hyphae, and rhizoids[**10**] (Al-abedi and Alhasan, 2023). The results of the present study align closely with those of [**10**] Al-abedi and Alhasan (2023) since *R. oryzae* was tested in their previous work, albeit with a different focus. Internal transcript spacer region including *ITS 1* and *ITS 4* have commonly used for identification of fungi including mentioned mold [**11**](Dolatabadi *et al.*, 20 22). Findings of these regions matched with results of the current work. Overview related identified *R. oryze* of this research indicated agreement with fungal identification which has been used.

The microscopic examination s show Most glomeruli demonstrated marked dilatation of Bowman's space , accompanied by noticeable dilatation of the renal tubules ,atrophy of glomerul,necrosis of lining epithelial of renal tubules and necrosis of mesangial cells in glomeruli Also, Degenerative alterations were observed in the epithelial lining of the renal tubules , and some appeared as scattered foci of degeneration distributed across different areas of the renal cortex **(Figures A,B,C,D,F 4.3.1.1) .**Mbility, or its capacity to infiltrate blood vessels. These findings are consistent with (Didehdar et al., 2022). 2 (Pahwa and others, 2013). The main cause of tissue pathology is this invasion. Rhizopus species infiltrate both big and small arteries, causing thrombucormycosis is characterized by the fungus's significant angioinvasive ai (blood clots) to develop and obstructing the vessels as a result, according to several studies. These results concurred with those of study number three (Gupta & Gupta, et al., 2012). (Ribeset al., 2000) . According to studies1 2 (Didehdar et al., 2022) and 13 (Prakash and Chakrabarti et al., 2019), vascular thrombosis causes a significant decrease in blood flow (ischemia) to the downstream tissues, which leads to infarction and widespread ischemic necrosis (tissue death from a lack of oxygen).Vascular impairment is the primary cause of the extensive tissue destruction rather than fungal growth inside the tissue alone. According to Gupta et al. (2012), "massive cortical and medullary infarction" from almost complete obstruction of the renal arteries and/or their branches is usually the cause of renal failure in mucormycosis. These findings align with 14 (Gupta , *et al.*, 2012). In the present study, which were in concordance with owning a larger family of genes encoding proteolytic enzymes, such as the gene families for secreted aspartic proteinase (SAP) and subtilases, is known to be present in R. oryzae, and several investigations have shown that the bacteria produces these enzymes. Compared to other fungi, R. oryzae has more genes that specify these lytic enzymes, which have been shown to contribute to the pathogenicity of other organisms (28 SAP genes and 23 subtilases genes). These genes are expressed in mucormycosis patients, and their products probably aid the organism's invasion of host cells and penetration through extracellular matrix proteins 15(Ibrahim et al., 2011).

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V. Conclusion

This study concluded that the *R. oryzae* has ability to occur effects on of albino male rats. This conclusion demonstrates dangerous effects caused by this mold particularly immuncomprised patients. It may lose functions of liver.

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Conflict of interest

Authors declare that no conflict of interest in this study.

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