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Research Article

Efficacy of *Curcuma longa* Extract in an *In-vivo* Model of *Rhizopus oryzae* Infection in Neutropenic Mice

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ABSTRACT

Mucormycosis, a life-threatening fungal infection caused by *Rhizopus oryzae*, disproportionately affects immunosuppressed individuals. Current treatments face significant limitations, necessitating alternative approaches. This study investigates the therapeutic potential of ethanolic extracts of *Curcuma longa*, known for its antifungal and immunomodulatory properties, in a neutropenic mouse model of mucormycosis. Mice were infected with *R. oryzae* and treated with *C. longa* extracts at 100, 200 and 400 mg/kg doses. Key parameters assessed included survival rates, body weight, hematological profiles (RBC, HGB, PLT), organ weights, fungal burden in kidney and brain tissues, and immunological responses. The 400 mg/kg dose significantly improved survival rates, hematological recovery, and normalized organ weights, with reductions in fungal burden comparable to standard treatment. Mice also exhibited dose-dependent recovery in systemic health. However, incomplete recovery and some mortalities were noted, highlighting the need for further optimization. These findings suggest *C. longa* extracts possess antifungal, immunomodulatory, and systemic protective effects, offering a promising natural alternative for mucormycosis management in immunosuppressed hosts. Future research should focus on refining dosing strategies and establishing clinical safety and efficacy profiles.

Introduction

Curcuma longa, commonly known as turmeric, is renowned for its bioactive compounds, particularly curcuminoids like curcumin, which exhibit diverse biological activities.[1] It demonstrates potent anti-inflammatory effects by preventing pro-inflammatory cytokines (e.g., TNF-α, IL-1β) and modulating the NF-κB pathway, while its antioxidant properties involve scavenging free radicals and boosting endogenous enzymes like SOD and catalase. Curcumin exhibits antimicrobial action contrary to bacteria, fungi, and viruses, disrupts biofilms, and possesses anti-cancer properties by inducing apoptosis, inhibiting angiogenesis, and suppressing metastasis.[2] Its hepatoprotective effects reduce lipid peroxidation and oxidative stress, showing promise in liver diseases like NAFLD. Neuroprotective benefits include crossing the blood-brain barrier to counter oxidative and inflammatory damage, potentially aiding Alzheimer's disease. It accelerates wound healing by promoting collagen deposition and reducing inflammation, supports cardiovascular health by enhancing endothelial function and reducing LDL oxidation, and exhibits anti-diabetic properties by improving insulin sensitivity and protecting β-cells.[3] Additionally, it prevents kidney stone formation, mitigates urolithiasis, and modulates immune responses, making it beneficial for both boosting immunity and managing autoimmune conditions.

C. longa extract (CL) has shown promising results in treating osteoarthritis (OA) symptoms. Multiple studies have demonstrated its efficacy in decreasing pain and improving function, as measured by the visual analog scale (VAS) and Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) scores.[1-3] CL's anti-inflammatory properties may contribute to its effectiveness in OA treatment.[4] Compared to placebo

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and non-steroidal anti-inflammatory drugs (NSAIDs), CL exhibits similar or superior efficacy with fewer adverse effects.[2] However, its impact on effusion-synovitis volume and cartilage composition remains inconclusive.[3] CL's safety profile is generally favorable, with adverse events comparable to placebo.[1, 3] While these findings are encouraging, larger multicenter trials are necessary in the direction of fully evaluating the clinical importance of CL in OA treatment.[3]

Rhizopus oryzae infection in neutropenic mice is a widely used model for studying mucormycosis, reflecting the aggressive and invasive nature of this fungal disease in immunocompromised hosts.[4] Neutropenia, induced using agents like cyclophosphamide, weakens the host's ability to combat the infection, allowing *R. oryzae* to invade tissues and blood vessels, leading to thrombosis and necrosis.[5] This model is valuable for evaluating antifungal therapies such as amphotericin B and posaconazole, understanding immune mechanisms, and studying fungal virulence factors like spore germination and siderophore production. It provides critical insights into the pathogenesis and treatment of mucormycosis in immunosuppressed conditions.[6]

Research on *R. oryzae* infection in mice has yielded important insights into treatment strategies and immune responses. Caspofungin, an echinocandin antifungal, showed limited efficacy in neutropenic mice, improving existence at lower doses but not at higher doses.[5,6] Caspofungin inhibited *R. oryzae* 1,3-β-d-glucan synthase and reduced fungal burden in the brain when administered prophylactically.[6] In diabetic mice with disseminated zygomycosis, liposomal amphotericin B significantly improved survival compared to amphotericin B deoxycholate.[7] Immunocompetent mice demonstrated effective fungal clearance associated with IL-17 and IL-2 production, highlighting the role of IL-17 signaling in immunity contrary to *R. oryzae*.[8] These studies provide valuable information on potential treatment options and immune responses in different mouse models of *R. oryzae* infection, contributing to our understanding of mucormycosis pathogenesis and management.

C. longa extract has demonstrated significant antifungal activity against various plant and human pathogens. *In-vivo* studies have shown its effectiveness against rice blast and tomato late blight, with demethoxycurcumin being the most potent curcuminoid.[9] Curcumin, isolated from *C. longa*, exhibited strong fungicidal properties against *Phytophthora infestans*, *Puccinia recondita*, and *Rhizoctonia solani* in greenhouse experiments.[10] Furthermore, *C. longa* extract inhibited spore germination and hyphal growth of *Mucor circinelloides*, a causative agent of mucormycosis, by up to 70 and 90%, respectively.[11] The plant's miscellaneous biological actions, comprising anti-inflammatory, antioxidant, and anti-carcinogenic properties, have been attributed primarily to curcumin.[12] These findings suggest that *C. longa* extract and its components may serve as promising candidates for developing novel antifungal treatments.

The study aims to evaluate the therapeutic potential of ethanolic extracts of *C. longa* against *R. oryzae* infection in neutropenic mice. The objectives include inducing neutropenia to establish a fungal infection model, preparing and characterizing *C. longa* extracts for consistency, and assessing their antifungal efficacy through survival rates, fungal burden, and histopathology. Additionally, the study will evaluate the immunomodulatory effects of the extract on cytokine levels and neutrophil function, compare its therapeutic efficacy with standard antifungal treatments, and investigate its safety profile by monitoring toxicity markers.

Materials And Methods

Materials

The *C. longa* rhizomes were collected in the Radhangari Villages Dist. Kolhapur in mid-rainy season (June 2022 and June 2023) with the help of local hellers and then identified by botanist. The test item used in this study was an ethanolic extract of *C. longa* (rhizome). The extract was characterized by its orange-yellow color and sticky texture. It was stored at room temperature to maintain its stability and integrity. During handling and usage, standard laboratory precautions were strictly followed to ensure safety and prevent contamination.

Methods

Animal used

All procedures complied with CPCSEA guidelines (The Gazette of India, Dec 15, 1998) and were approved by the IAEC under Project No. SC/IAEC/2324/018 (clearance: IAEC-17-015). Male Swiss albino mice, 6–8 weeks old and weighing between 20 to 30 g, were sourced from Sciore Research Private Limited. Male mice were used in this study to ensure consistency in physiological responses and minimize potential variability introduced by hormonal fluctuations in female mice. The animals were housed in polypropylene cages equipped with stainless-steel grill tops, food and water bottle holders, and corn cob bedding, with no more than four mice per cage. Environmental parameters were strictly controlled, including a temperature of 22 ± 3°C, relative humidity of 50 ± 5%, and a 12-hour light/dark cycle. The mice were provided with a standard pelleted maintenance diet and had unrestricted access to reverse osmosis (RO) water. [13,14]

In-vivo Experiment Study

The study aimed to assess the effectiveness of the test compounds in treating *R. oryzae* (The microbial type culture and gene bank MTCC with the collection Acc. No. 262) infection in neutropenic mice. The experimental

design followed previously reported methods. The ethanolic extract of *C. Longa* rhizome toxicity study has already been tested for acute, subacute and chronic toxicity studies and data is available in publications.[15] The safety dose of Rhizome extract is 1350 to 1450 mg/kg. Based on the reference, we selected three doses considering the mentioned lower dose of 100 mg/kg and safety dose.[16] Neutropenia was induced via intraperitoneal administration of cyclophosphamide at a dose of 150 mg/kg body weight (bw) four days prior to infection, followed by a second dose of 100 mg/kg bw one day before infection. Mice were arbitrarily separated into five groups: Disease Control (*R. oryzae* only, ~10^6 spores), standard (Amphotericin B at 1-mg/kg intravenously with *R. oryzae*), and Test groups treated with low (100 mg/kg), intermediate (200 mg/kg), or high (400 mg/kg) doses of ethanolic extract of *C. longa* administered orally. The *R. oryzae* inoculum (~10^6 spores) was injected via the tail vein. About 24 hours post-infection, treatments with the respective test items began, with doses selected based on prior studies. Mice were monitored daily for survival, and body weights were recorded every three days. The study continued until a minimum of three mice survived in the disease control group. Survival data were used to generate a survival plot. At the end of the experiment, blood samples (100 µL) were collected for hematological analysis, and mice were sacrificed for microbiological evaluations of the brain and kidney. [17,18]

Data Analysis

Data analysis was performed using statistical tools, with GraphPad Prism version 8.42 employed for the analysis. Results were presented as Mean ± SD.

Result And Discussion

Curcuma longa extract, particularly at 400 mg/kg, significantly improved survival rates, restored hematological parameters, and reduced fungal burden in a dose-dependent manner, though some mortality and incomplete recovery persisted. Previous studies have demonstrated the antifungal and immunomodulatory potential of *C. longa*, particularly its curcumin component, in various fungal infections. Our results align with studies reporting curcumin's efficacy in reducing fungal burden and improving host immunity. However, our findings also highlight certain limitations, as complete recovery was not achieved, necessitating further investigation into optimized dosing and potential synergistic treatments. This refinement will eliminate redundancy, strengthen the scientific rationale, and provide a clearer justification of our results in relation to existing research.

Plant Authentication

C. longa rhizomes were collected and authentication was done by a botanist. The details of authentication are presented in Figs 1 and 2.

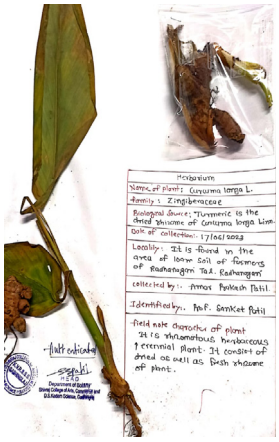

Fig. 1: Herbarium

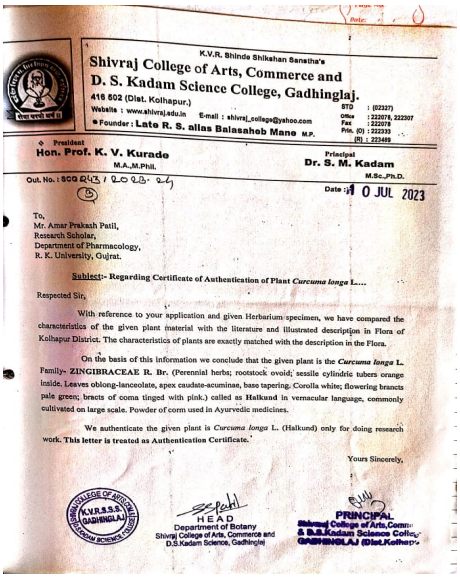

Fig. 2: Letter of authentication

Body Weight Management

The data reveal a variable impact of disease and treatments on the body weight of experimental animals over 12 days. The disease control group showed high mortality, with some animals surviving and demonstrating gradual weight gain, resulting in a mean weight increase of 5.87 g by day 12. In contrast, animals treated with standard drugs and *C. longa* extracts (100, 200 and 400 mg/kg) displayed a relatively consistent increase in body weight, with higher doses of *C. longa* (400 mg/kg) achieving the greatest improvement (mean increase of 5.4 g). Mortality rates remained high in the disease control and lower-dose treatment groups, suggesting the severity of the disease. While the 400 mg/kg dose of *C. longa* improved weight maintenance and gain, it did not eliminate mortality, underscoring the treatment's partial efficacy. Figs 3 and 4 illustrate these trends, with weight changes reflecting dose-dependent protective effects and highlighting the therapeutic potential of *C. longa* at higher doses.

Hematology Parameters

The hematological data indicate significant variations across groups, reflecting the impact of disease and the protective effects of *C. longa* treatment. The disease control group showed marked reductions in hematological para meters, with high mortality and reduced WBC, RBC, HGB, and PLT counts among surviving animals, indicating severe immune suppression and anemia. The standard-treated group demonstrated improved hematological profiles, with higher WBC, RBC, HGB, and platelet counts compared to the disease control group, suggesting partial recovery. *C. longa* treatment groups showed dose-dependent improvement in hematological parameters, with the 400 mg/kg dose displaying the most notable recovery, characterized by increased RBC (6.73×10^6/µL), HGB (13.13 g/dl), and PLT (530.67×10^3/µL). However, variations within the *C. longa* groups indicate that higher doses are more effective in mitigating hematological disruptions caused by the disease. Figs 5–8 highlight these trends, suggesting the potential of *C. longa* in restoring hematological parameters, although complete recovery was achieved.

Measurement of Organ Weight

The presented data illustrates the absolute and relative organ weights of rats in various treatment groups, highlighting significant differences among diseased, standard, and *C. longa* treatment groups. Disease control rats showed substantial mortality with notable reductions in organ weights in surviving animals, indicating severe systemic toxicity. Standard treatment and *C. longa* interventions demonstrated improvements, with higher mean organ weights and reduced variability compared to disease control, suggesting protective effects. Notably, *C. longa* at 400 mg/kg yielded the most pronounced recovery in relative weights of vital organs like the spleen, heart, lungs, and liver, signifying its dose-dependent therapeutic potential. However, some mortality persisted across *C. longa* groups, warranting further investigation into its safety profile. The consistent trends in brain and adrenal weights suggest minimal neurological or endocrine impact, underscoring its systemic specificity. These findings

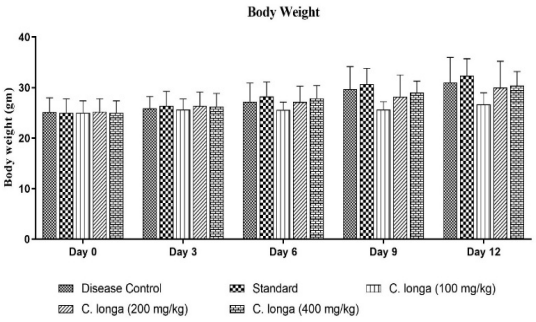

Fig. 3: Body weight

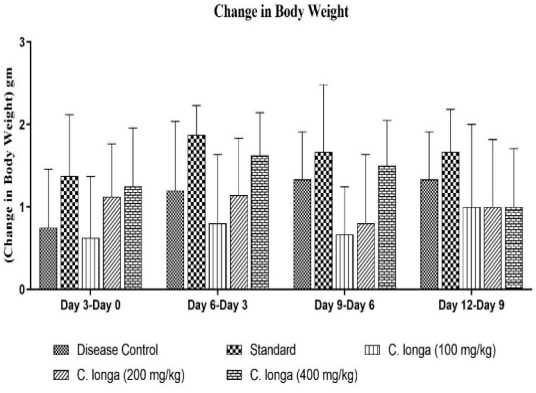

Fig. 4: Change in body weight

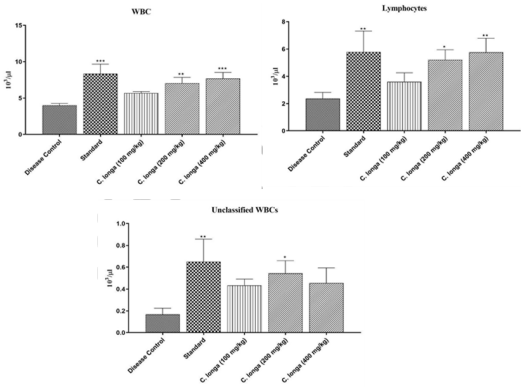

Fig. 5: Hematology parameters

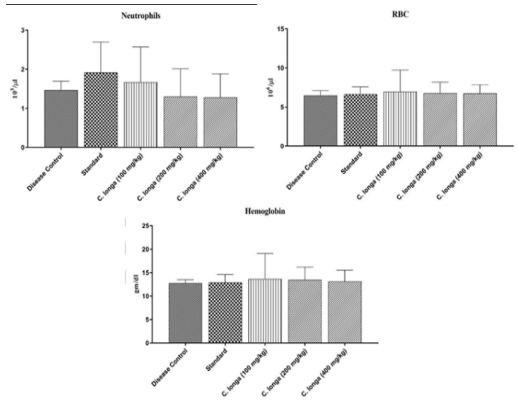

Fig. 6: Hematology parameters

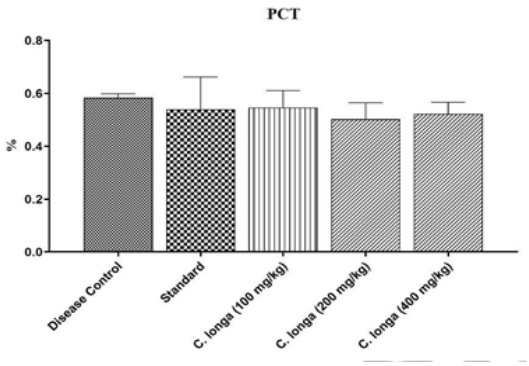

Fig. 7: Hematology parameters

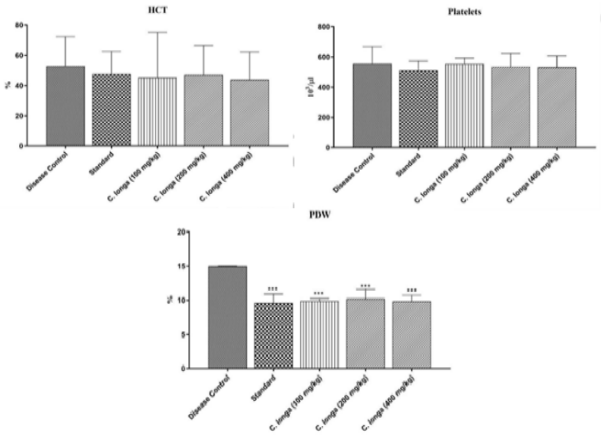

Fig. 8: Hematology parameters

affirm *C. longa's* potential in mitigating organ dysfunction associated with the disease while emphasizing the importance of optimizing dosing strategies for enhanced efficacy and safety as shown in Figs 9-11.

In-vivo Study

The survival data highlights the therapeutic potential of *C. longa* in improving survival rates in disease-induced rats. The disease control group exhibited the lowest mean survival (8.13 days), indicating the severity of the condition. Standard treatment significantly increased survival (11.00 days), demonstrating its efficacy. Among the *C. longa* groups, a dose-dependent improvement in survival was evident, with the 400 mg/kg dose achieving the highest mean survival (10.75 days) and the lowest variability (SD: 1.83). The 200 and 100 mg/kg doses also improved survival (9.75 and 8.75 days, respectively) compared to the disease control. These findings suggest that *C. longa* not only mitigates the disease's lethality but also offers comparable outcomes to the standard treatment, especially at higher doses. The data emphasizes the importance of dose optimization for maximizing therapeutic benefits while maintaining safety as shown in Fig. 12.

Fungal Load Measurement

The fungal load data indicates the efficacy of *C. longa* in reducing fungal colonization in the kidney and brain tissues of treated animals. In the disease control group, fungal burden was highest in both organs, with mean log CFU values of 6.44 and 6.20 for the kidney and brain, respectively. The standard treatment significantly reduced fungal load, achieving mean log CFU values of 4.89 in the kidney and 5.11 in the brain, demonstrating effective fungal suppression. Among the *C. longa* groups, the 400 mg/kg dose exhibited the most pronounced reduction in fungal load, with log CFU values of 5.56 in the kidney and 5.53 in the brain, compared to higher values in the 100 mg/kg and 200 mg/kg groups. The dose-dependent

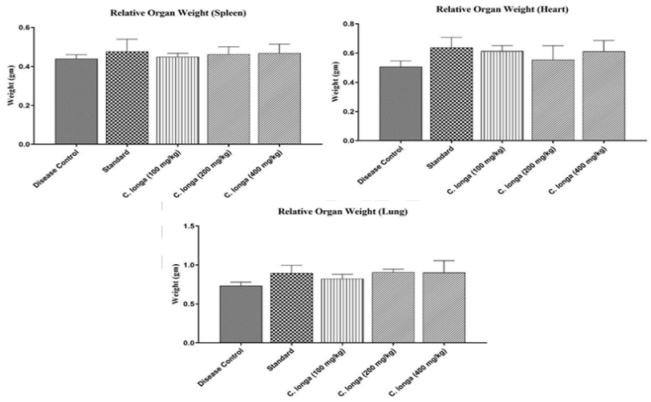

Fig. 9: Relative organ weights

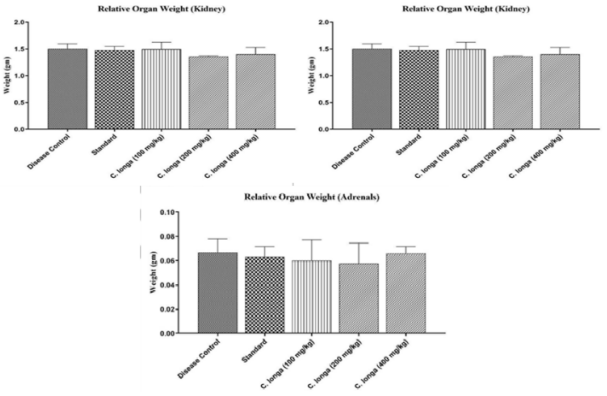

Fig. 10: Relative organ weights

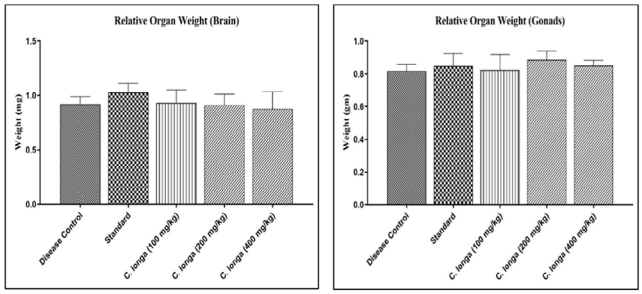

Fig. 11: Relative organ weights

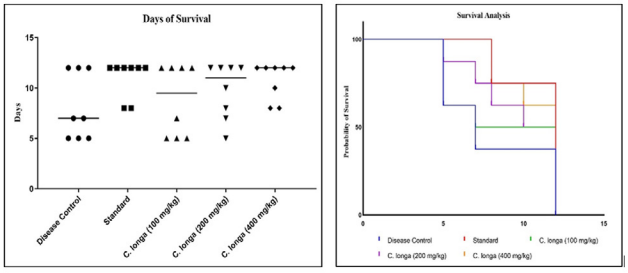

Fig. 12: Survival data and analysis

efficacy of *C. longa* highlights its antifungal potential, particularly at higher doses, suggesting its therapeutic promise for managing fungal infections in systemic and localized contexts as shown in Fig. 13.

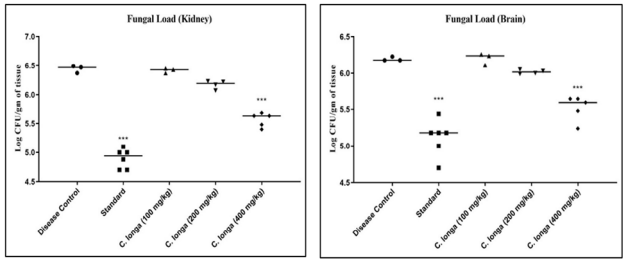

Fig. 13: Fungal load on kidney and brain

Data Interpretation and Analysis

Data were analyzed using one-way or two-way ANOVA, followed by Tukey's multiple comparisons test. Statistical significance was defined as *p <0.05, ** p <0.01, and *** p <0.001 compared to the disease control group in all instances.

Conclusion

The data collectively demonstrate the therapeutic potential of *C. longa* in mitigating the effects of disease in experimental animals, with a clear dose-dependent efficacy. The 400 mg/kg dose consistently outperformed lower doses in improving survival rates, maintaining body weight, restoring hematological parameters, and reducing fungal burden in vital organs such as the kidney and brain. While the standard treatment displayed greater efficacy overall, *C. longa* at higher doses provided comparable results in many parameters, highlighting its promise as a natural alternative. However, the persistence of mortality and incomplete recovery in some parameters, even at higher doses, underscores the need for further research to optimize dosing and evaluate safety profiles. Overall, *C. longa* exhibits significant potential in disease management, particularly in reducing fungal load and supporting systemic recovery, making it a promising candidate for further preclinical and clinical investigations.

The study highlights the significant therapeutic potential of *C. longa* in reducing fungal burden and supporting systemic recovery, demonstrating a clear dose-dependent efficacy as well as restore hematological parameters at higher doses underscores its promise as a natural alternative therapy. However, limitations include the persistence of mortality and incomplete recovery in some parameters, indicating the need for further dose optimization and comprehensive safety evaluations before clinical translation.

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