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ORIGINAL ARTICLE

Antifungal effect of *Lacticaseibacillus rhamnosus* GG against oral candidiasis in diabetic rats

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ABSTRACT

Background and Objectives: Diabetes mellitus (DM) increases the risk of oral candidiasis by impairing immunity and enhancing *Candida* virulence. Although *Lacticaseibacillus rhamnosus* GG (LGG) has antifungal potential, its effect in diabetes remains unclear. This study evaluated the efficacy of LGG against *C. albicans* and *C. tropicalis* in diabetic rats.
Materials and Methods: A fungal suspension (1×10^7 CFU/mL) was applied to the tongue for three days in the controls and experimental groups of male alloxan-induced diabetic Sprague-Dawley rats. Then, LGG (0.3 mL of 1×10^8 CFU/mL) was administered for 28 days in the experimental groups. Weekly oral swabs were analyzed, and blood and tongue tissues were evaluated using biochemical, histopathological, and molecular methods (TLR2, TLR4, and Msp1/p75).
Results: Diabetic rats showed weight loss and increased oral *Candida* growth. LGG reduced *C. albicans* and *C. tropicalis* counts and stabilized body weight. Haematological and biochemical parameters improved with probiotic treatment. Histopathological examination showed reduced inflammation, and molecular analyses indicated modulation of Msp1/p75 and TLR2/TLR4. Overall, LGG provided antifungal and immunomodulatory benefits in diabetic rats with oral candidiasis.
Conclusion: This study assessed the efficacy of LGG against *C. albicans* and *C. tropicalis* in diabetic rats. Administration of LGG reduced *Candida* colonization and maintained normal physiological parameters. These results suggest that LGG has potential as a safe complementary antifungal therapy.

Keywords: *Candida albicans*; *Candida tropicalis*; *Lacticaseibacillus rhamnosus*; Probiotics; Toll-like receptors

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder associated with impaired glucose homeostasis resulting from defects in insulin production, or cellular responsiveness, ultimately leading to sustained elevations in blood glucose levels. DM represents a major global health issue, contributing substan-

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tially to morbidity, mortality, and healthcare costs. According to the World Health Organization (2024), over 422 million people currently live with diabetes, predominantly in low- and middle-income countries, with an estimated 1.6 million deaths annually. The International Diabetes Federation (2021) projects that this number will increase to approximately 743 million individuals aged 20-79 years by 2045, highlighting the urgent necessity for better preventive and therapeutic strategies. Among various opportunistic infections, *Candida albicans* and non-*albicans Candida* species such as *C. tropicalis*, *C. glabrata*, *C. krusei*, and *C. dubliniensis* (1) have been associated with oral candidiasis. The incidence of oral *Candida* carriage and infection is significantly greater in individuals with diabetes than in non-diabetic individuals - 68% versus 27%, respectively (2).

Multiple factors underlie the heightened susceptibility of diabetic individuals to *Candida* infections. Hyperglycemia alters salivary flow and salivary composition, weakens mucosal and neutrophil defenses, and promotes a shift from commensal to pathogenic *Candida* states (3). Enhanced epithelial adherence, increased enzymatic activity (phospholipase, esterase, and hemolysin), and robust biofilm formation further increase virulence and antifungal resistance (4).

Conventional antifungal agents such as azoles and polyenes remain effective but are increasingly challenged by drug resistance and adverse effects associated with disruption of the oral microbiota. Fluconazole resistance, now reported at 7-10%, underscores the need for safer, sustainable alternatives. Probiotics have emerged as promising adjuvant or alternative therapies. These live microorganisms confer health benefits when administered in adequate amounts. One such probiotic is *Lacticaseibacillus rhamnosus* GG (LGG), which demonstrates notable antifungal and immunomodulatory potential.

LGG inhibits *C. albicans* by suppressing growth, blocking hyphal formation, and reducing epithelial adhesion (5). It also provides benefits in diabetes by decreasing insulin resistance via enhanced glycogen storage and glucose regulation (6), lowering pro-inflammatory cytokines IL-1β and TNF-α (7), and improving the lipid profile by decreasing total and LDL cholesterol while increasing HDL (8). Despite these established metabolic benefits, studies investigating the effect of LGG on *Candida* species in diabetic models remain limited. The interaction between probiotics, host immunity, and fungal pathogens in a diabetic environment represents an underexplored yet clinically significant field of research.

The immunomodulatory effects of LGG are partly mediated by its major secreted protein (Msp1/p75), an O-linked glycosylated molecule involved in cell wall remodeling and immune regulation. Glycosylation enhances Msp1 stability and enables interactions with host immune cells, potentially modulating Toll-like receptor (TLR) signaling. TLR2 detects peptidoglycans and mannans from *C. albicans*, while TLR4 recognizes O-linked mannan (9). Activation of these receptors triggers MyD88-dependent and MyD88-independent pathways, inducing cytokines (IL-1β, IL-6, IL-8, and TNF-α). Although this promotes microbial clearance *via* Th1 responses, chronic hyperglycemia and inflammation can disrupt these pathways, leading to immune dysregulation in diabetes.

Prolonged exposure to probiotics such as LGG may beneficially modulate immune responses by stimulating microbial-associated molecular patterns (MAMPs) that regulate TLR2 and TLR4 signaling. This modulation enhances host defense while limiting excessive inflammation through negative regulators like Toll-interacting protein (Tollip), which attenuates overactive TLR pathways (10). Given the growing concern over antifungal resistance, along with the dual metabolic and immunomodulatory properties of LGG and the limited evidence regarding its antifungal efficacy in diabetic conditions, the current study aimed to evaluate the biotherapeutic potential of LGG against *C. albicans* and *C. tropicalis* in diabetic Sprague-Dawley (SD) rats with experimentally induced oral candidiasis.

MATERIALS AND METHODS

Healthy adult male SD rats (200 ± 20 g) were obtained from the Central Animal House, AIMST University, Malaysia. The rats were maintained under standard laboratory conditions at room temperature (20-25° C and 60-65% relative humidity) with a 12-h light and 12-h dark cycle. The rats were given water and fed a standard rodent pellet diet *ad libitum*. To reduce stress, the rats were acclimated to the laboratory environment for two weeks before the experiment. Prior approval was obtained from the AIMST University Human and Animal Ethics Committee (AUAEC/FOM/2021/02) before conducting the study.

Freshly prepared bacterial cultures [*L. rhamnosus* (Hansen) Collins. et al. ATCC 53103 - SPD Scientific (M) Sdn. Bhd. Company, Petaling Jaya, Malaysia] and fungal species [*C. albicans* ATCC 10231 and *C. tropicalis*, ATCC 1369 - Choice Care Sdn. Bhd. Company, Kuala Lumpur, Malaysia] cell suspensions were prepared from freeze-dried stocks and quantified using serial dilution and spread plate methods before administration. Colony morphology was verified at each step to ensure consistency with the original cultures. Rats inoculated with *C. albicans* only served as the control group. All other groups of rats were given alloxan monohydrate in normal saline (0.9% NaCl) at a single dose of 80 mg/kg (11) to induce diabetes. After 36 h of alloxan administration, blood samples were drawn from the tail vein and tested with an automated glucometer to confirm the induction of diabetes. Animals with glucose levels of >12.2 mmol/L were included in the study (12).

The rats were allocated into seven groups, each of 6 animals, as follows:

Group I: Control

Group II: DM control

Group III: DM + *C. albicans* (Immunosuppressed)

Group IV: DM + *C. tropicalis* (Immunosuppressed)

Group V: DM + LGG

Group VI: DM + *C. albicans* + LGG (Immunosuppressed)

Group VII: DM + *C. tropicalis* + LGG (Immunosuppressed)

Rats in Groups III, IV, VI, and VII were provided with dexamethasone (0.5 mg/L) in the drinking water for seven days before experimental infection to induce immunosuppression. Additionally, starting seven days before the infection, the rats were given a single dose of a 0.1% aqueous tetracycline solution. Then, the tetracycline hydrochloride concentration was lowered to 0.01% and administered as a single dose during the *candida* inoculation to suppress the natural bacterial flora and facilitate adequate *Candida* infection throughout the experiment period (13).

The animals of Groups I, II, III, V, and VI were infected with *C. albicans*, and Groups IV and VII were infected with a *C. tropicalis* suspension of 1×10^7 CFU/mL, respectively. Pathogenic *Candida* spp. were applied onto the dorsal surface (until the posterior one-third) of the tongue for 15 s using sterile cotton swabs. Food and water were withheld for 30 min after mucosal coating to facilitate the microbial colonization. Once a white, scrapable patch became evident, a mucosal swab was taken for microscopic analysis. The morphology was then confirmed by microscopic examination using Gram stain 3 days later. The rats in Groups V, VI and VII were administered LGG probiotics orally using a disposable syringe at a maximum tolerated dose of 0.3 mL containing 1×10^8 CFU/mL for 28 days (Fig. 1).

Microbial analysis was performed at regular weekly intervals during the study. On day 28, the blood samples from animals were collected from the animals via retro-orbital plexus puncture for haematological and biochemical evaluation, while tongue samples were collected for histopathological examination, immunohistochemistry and Western blot analysis. One animal from each of Groups VI and VII was lost during the experimental period on days 20 and 24, respectively.

Microbiological analysis. Mucosal swabs from the dorsal surface of the tongue was taken weekly for microbiological analysis. Tongue swabs were collected using a sterile swab, with the procedure performed three times per rat to standardize sample collection. Fungal counts (CFU/mL) for *C. albicans* and *C. tropicalis* were assessed on days 1, 7, 14, 21, and 28 using Sabouraud dextrose agar (SDA) and a manual colony counter. Viability and morphological changes of *Candida* species were periodically evaluated by Gram staining and compared with reference images from primary cultures.

Haematological analysis. Haematological analysis was performed on day 28 to assess red blood cell count (RBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), total white blood cell count (WBC), differential leukocyte count, and platelet count. Blood samples were collected into tubes containing sodium ethylenediaminetetraacetic acid (EDTA) as an anticoagulant and analyzed using an automated haematology analyzer (Sysmex XN-1000 Haematology Analyzer).

Biochemical analysis. Serum biochemical analysis was performed on day 28 using blood samples collected in plain tubes. The samples were allowed to clot and centrifuged to separate the serum, which was analyzed using a Roche Cobas c502 analyzer for param-

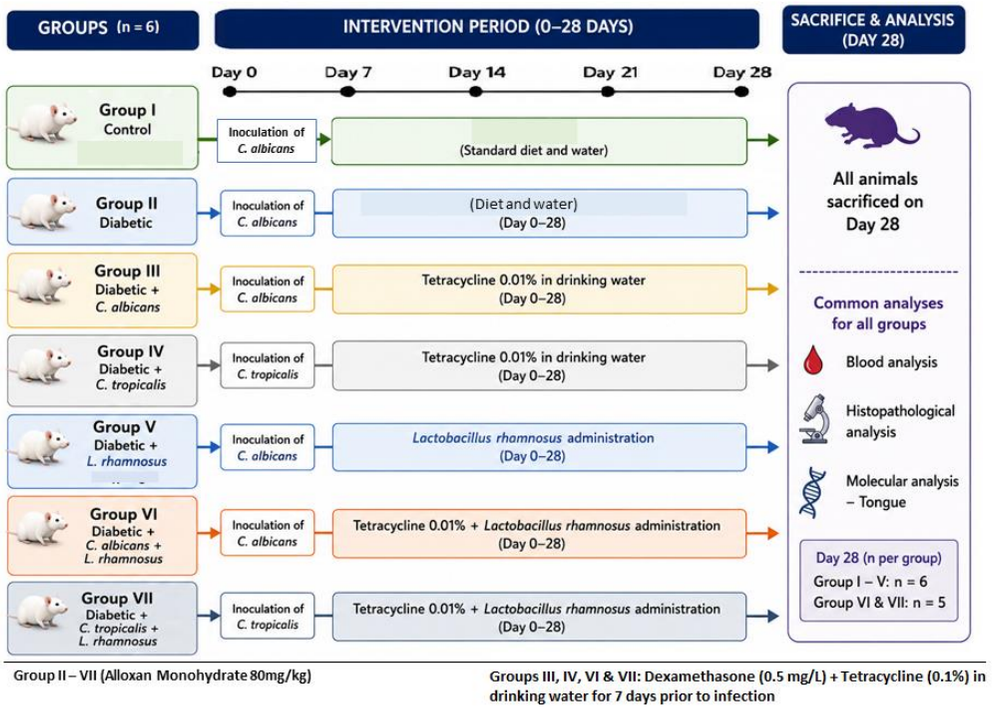

Fig. 1. Flowchart of the experimental design.

eters such as glucose, total protein, albumin, globulin, total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT), urea, uric acid, creatinine, calcium, corrected calcium and phosphate, total cholesterol (TC), triglycerides (TGL), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and non-HDL cholesterol. The TC/HDL ratio was calculated mathematically.

Histopathological examination. At the end of the study, all experimental rats were euthanized under diethyl ether anaesthesia. The tongue samples were processed for histopathological examination at EMAN Bio Discovery Sdn. Bhd., Malaysia. The tongue tissues were fixed in 10% formalin, dehydrated for 16 h in an automatic tissue processor (Thermo Scientific Excelsior AS, USA), and embedded in paraffin wax using a paraffin embedding system (Sakura Tissue Tek Embedder, USA). Each sample was sliced into 5 µm-thick sections using a rotary microtome (Leica RM 2235, Japan). The sections were mounted on glass slides, stained with hematoxylin and eosin (H&E), and examined under a light microscope.

Immunohistochemistry. Rat tongue tissues were fixed overnight in 10% neutral buffered formalin, dehydrated through graded ethanol, cleared in chloroform, and embedded in paraffin wax. Sections of 3 µm thickness were prepared, deparaffinized in xylene, rehydrated through decreasing ethanol concentrations, and mounted on poly-L-lysine-coated slides (Thermo) for immunohistochemical (IHC) analysis.

Antigen retrieval was performed using a microwave method, followed by blocking of endogenous peroxidase activity with hydrogen peroxide for 10 min and 5% Bovine Serum Albumin (BSA) (Sigma, USA) for another 10 minutes. Slides were then incubated overnight at 4°C with primary antibodies against Msp1/p75, TLR2, and TLR4 (AB108319) (Abcam – USA), followed by Phosphate Buffer Saline (PBS) washes. Sections were incubated with a rabbit-specific Horseradish peroxidase/ 3,3´-Diaminobenzidine (HRP-DAB) secondary antibody for 10 min at room temperature. After final PBS washes, slides were counterstained with hematoxylin, covered with a mounting medium, and examined under a light microscope.

Western blot analysis. Tongue tissue samples (40–50 mg), previously stored at -80°C, were transferred to Eppendorf tubes and homogenized in ice-cold 1× RIPA buffer (Sigma-Aldrich, USA) at a 1:3 ratio (w/v). Following the manufacturer's instructions, 300

µL of buffer was used per 100 mg of tissue (Abcam kit, ab270054). The tissues were then homogenized in an electric homogenizer and centrifuged thoroughly. Concentration of the proteins was determined using the Bradford assay method (14). Forty micrograms (40 µg) of protein, mixed with loading dye, were loaded onto a Tris-Bis gel, transferred to nitrocellulose membranes, and then incubated with 3% BSA for 60 min. Membranes were incubated with anti-Msp1, anti-TLR2, and anti-TLR4 primary antibodies (Abcam, USA) at a dilution of 1/500 overnight at 4°C.

Membranes were then washed three times in 1× TBST buffer for 5 min each, followed by incubation with anti-rabbit secondary antibody (Abcam-USA) at a dilution of 1:2500 for 1 h. Membranes were then washed and subjected to a mixture of enhanced chemiluminescence (ECL) (Bio-Rad) and western clarity solution (Biorad) (1:1) to visualize the protein bands using FluorChem E System (Bio-Techne). Images of the bands were captured, and the density of each band was determined by using *ImageJ* software. The ratio of the density of each target protein band to that of β-actin was calculated and considered as the expression level of the targets proteins and plotted as appropriate graphs.

Statistical analysis. The data were presented as mean ± standard error mean (SEM). The data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test. A value of $P < 0.05$ was considered to be statistically significant. The Unpaired t-test with Welch Correction was used to determine the significance of microbial growth.

RESULTS

Effect of LGG on the growth of *C. albicans* and *C. tropicalis* in rats. *Candida* counts remained stable in healthy controls throughout the study. Diabetic control rats showed a gradual increase in *Candida* counts, although this increase did not reach statistical significance. However, immunosuppressed diabetic rats infected with *C. albicans* or *C. tropicalis* showed a statistically significant increase in the *Candida* growth as compared with both healthy and diabetic controls. In diabetic rats infected with *C. albicans* or *C. tropicalis*, *Candida* counts increased from day 1 to day 28, reflecting disease progression. However, LGG supplementation reduced *C. albicans* levels to 1.80×10^7 CFU/mL on day 21 and 1.66×10^7 CFU/mL on day 28, while *C. tropicalis* counts decreased to 1.85×10^7 CFU/mL on day 21 and 1.70×10^7 CFU/mL on day 28, respectively (Table 1).

Effect of LGG on the haematological parameters of rats with oral candidiasis. RBC parameters across all experimental groups in the present study showed minor variations compared with controls, with slight decreases observed in all diabetic rats. In this study, alloxan-induced diabetic rats exhibited a marked reduction in WBC counts compared with controls, while lymphocyte levels remained largely unchanged.

Platelet counts remained within the normal range across all experimental groups, with no significant changes in diabetic rats. Overall, these findings suggest that LGG may help stabilize haematological indices under these conditions (Table 2).

Effect of LGG on the liver profile in rats with oral candidiasis. In alloxan-induced diabetic rats, significant elevations in liver enzymes (ALP and ALT) and a slight increase in total bilirubin indicated hepatic dysfunction and metabolic disturbance. In diabetic rats with oral candidiasis, LGG supplementation further improved liver function, particularly in *C. albicans* infections. Overall, LGG supplementation may alleviate liver stress and support hepatic metabolism under diabetic and infectious conditions (Table 3).

Effect of LGG on the renal profile in rats with oral candidiasis. Urea levels were significantly elevated in diabetic rats and those with *C. albicans* infection, while LGG supplementation reduced these elevations, suggesting a possible protective effect (Table 4).

Effect of LGG on the lipid profile of rats with oral candidiasis. Total cholesterol, triglycerides, and non-HDL cholesterol largely remained unchanged, while LDL levels trended higher in diabetic rats. HDL significantly increased in diabetic rats after LGG administration, especially in those with *C. tropicalis* infection, suggesting improved lipid metabolism (Table 5).

Histopathological examination. Control rats showed normal tongue architecture, while diabetic rats exhibited mild inflammatory cell infiltration without structural disruption. Diabetic rats administered LGG alone showed a keratinized stratified squamous epi-

Table 1. Effect of LGG on the growth of *C. albicans* and *C. tropicalis* in rats.

Group	Day 1 (CFU/mL)	Day 7 (CFU/mL)	Day 14 (CFU/mL)	Day 21 (CFU/mL)	Day 28 (CFU/mL)
Control	$1.78 \times 10^5 \pm 7.58 \times 10^3$	$1.78 \times 10^5 \pm 3.94 \times 10^3$	$1.68 \times 10^5 \pm 5.13 \times 10^3$	$1.75 \times 10^5 \pm 5.43 \times 10^3$	$1.80 \times 10^5 \pm 8.16 \times 10^3$
DM Control	$1.93 \times 10^5 \pm 7.46 \times 10^3$	$1.98 \times 10^5 \pm 3.29 \times 10^3$	$1.85 \times 10^5 \pm 4.02 \times 10^3$	$1.96 \times 10^5 \pm 3.08 \times 10^3$	$1.99 \times 10^5 \pm 3.27 \times 10^3$
DM + *C. albicans*	$1.71 \times 10^7 \pm 3.92 \times 10^{5***@@@}$	$1.86 \times 10^7 \pm 9.33 \times 10^{4***@@@}$	$1.89 \times 10^7 \pm 1.73 \times 10^{5***@@@}$	$1.92 \times 10^7 \pm 1.41 \times 10^{5***@@@}$	$1.96 \times 10^7 \pm 7.50 \times 10^{4***@@@}$
DM + *C. tropicalis*	$1.75 \times 10^7 \pm 3.55 \times 10^{5***@@@}$	$1.91 \times 10^7 \pm 2.18 \times 10^{5***@@@}$	$1.94 \times 10^7 \pm 1.00 \times 10^{5***@@@}$	$1.95 \times 10^7 \pm 8.93 \times 10^{4***@@@}$	$1.97 \times 10^7 \pm 7.63 \times 10^{4***@@@}$
DM + LGG	$1.94 \times 10^5 \pm 1.75 \times 10^3$	$1.61 \times 10^5 \pm 6.35 \times 10^3$	$1.49 \times 10^5 \pm 3.25 \times 10^3$	$1.44 \times 10^5 \pm 5.33 \times 10^3$	$1.32 \times 10^5 \pm 2.93 \times 10^3$
DM + *C. albicans* + LGG	$1.72 \times 10^7 \pm 2.82 \times 10^5$	$1.87 \times 10^7 \pm 2.13 \times 10^5$	$1.91 \times 10^7 \pm 2.30 \times 10^5$	$1.80 \times 10^7 \pm 3.31 \times 10^{5###}$	$1.66 \times 10^7 \pm 1.81 \times 10^{5###}$
DM + *C. tropicalis* + LGG	$1.77 \times 10^7 \pm 3.19 \times 10^5$	$1.91 \times 10^7 \pm 1.80 \times 10^5$	$1.93 \times 10^7 \pm 9.61 \times 10^4$	$1.85 \times 10^7 \pm 3.35 \times 10^{5\&\&}$	$1.70 \times 10^7 \pm 6.23 \times 10^{5\&\&\&}$

CFU/mL: Colony Forming Units/ Milliliter. All the values are mean ± SEM (n = 6 except DM + *C. albicans* + LGG and DM + *C. tropicalis* + LGG [n = 5]). ***P<0.001 compared with that of control, $^{@@@}$P<0.001 compared with that of diabetic control, $^{###}$P<0.001 compared with that of *C. albicans* control, $^{\&\&}$P<0.01 and $^{\&\&\&}$P<0.001 compared with that of *C. tropicalis* control. (unpaired t-test with Welch Correction).

Table 2. Effect of LGG on the haematological parameters of rats with oral candidiasis induced by *C. albicans* and *C. tropicalis.*

Parameters	Control	DM Control	DM + *C. albicans*	DM + *C. tropicalis*	DM + LGG	DM + *C. albicans* + LGG	DM + *C. tropicalis* + LGG
Hb (g/L)	130.83 ± 6.44	123.33 ± 8.69	127 ± 0.97	130.33 ± 2.93	139.00 ± 2.22	138.00 ±3.99	135.40 ± 1.99
RBC (/L)	$8.01 \times 10^{12} \pm 1.41 \times 10^{11}$	$7.91 \times 10^{12} \pm 2.73 \times 10^{11}$	$8.21 \times 10^{12} \pm 4.06 \times 10^{11}$	$7.97 \times 10^{12} \pm 4.18 \times 10^{11}$	$8.23 \times 10^{12} \pm 3.23 \times 10^{11}$	$8.36 \times 10^{12} \pm 5.30 \times 10^{11}$	$8.64 \times 10^{12} \pm 1.94 \times 10^{11}$
PCV (L/L)	0.57 ± 0.03	0.58 ± 0.03	0.58 ± 0.05	0.58 ± 0.06	0.58 ± 0.04	0.58 ± 0.03	0.59 ± 0.06
MCV (fL)	64.67 ± 1.67	59.00 ± 3.92	60.67 ± 1.86	60.33 ± 1.33	65.83 ± 2.52	60.80 ± 4.85	60.40 ± 2.11
MCH (pg)	19.00 ± 0.82	17.50 ± 0.56	18.67 ± 0.67	17.50 ± 0.43	18.50 ± 0.34	17.40 ± 0.24	18.20 ± 0.37
MCHC (g/L)	276.17 ± 5.62	275.50 ± 6.58	266.17 ± 6.75	258.67 ± 6.60	291.17 ± 2.10	256.60 ±8.08	252.00 ± 6.57
RDW (%)	17.57 ± 0.34	17.20 ± 0.89	18.05 ± 0.64	17.42 ± 0.80	18.63 ± 0.26	19.52 ± 0.62	19.48 ± 0.20
WBC (/L)	$9.01 \times 10^9 \pm 1.28 \times 10^8$	$5.79 \times 10^9 \pm 5.56 \times 10^{8***}$	$6.94 \times 10^9 \pm 7.40 \times 10^{8*}$	$5.82 \times 10^9 \pm 2.54 \times 10^{8***}$	$7.23 \times 109 \pm 5.03 \times 10^8$	$6.15 \times 10^9 \pm 3.38 \times 10^{8**}$	$6.05 \times 10^9 \pm 4.37 \times 10^{8**}$
Neutrophils (%)	27.50 ± 1.84	22.50 ± 1.23	25.67 ± 0.71	25.33 ± 0.61	25.17 ± 1.54	28.00 ± 2.28	25.20 ± 1.11
Lymphocytes (%)	64.00 ±1.98	69.17 ± 1.64	66.83 ± 0.91	66.67 ± 0.92	65.33 ± 1.45	64.00 ± 2.35	66.00 ± 1.22
Monocytes (%)	5.67 ± 0.49	5.33 ± 0.21	4.83 ± 0.40	5.17 ± 0.31	6.50 ± 0.22	5.20 ± 0.49	6.00 ± 0.45
Eosinophils (%)	2.83 ± 0.31	3.00 ± 0.58	2.67 ± 0.21	2.83 ± 0.31	3.00 ± 0.37	2.80 ± 0.37	2.80 ± 0.37
Basophils (%)	0	0	0	0	0	0	0
Platelets (/L)	$862 \times 10^9 \pm 160 \times 10^9$	$838 \times 10^9 \pm 251 \times 10^8$	$838 \times 10^9 \pm 251 \times 10^8$	$838 \times 10^9 \pm 251 \times 10^8$	$815 \times 10^9 \pm 867 \times 10^8$	$820 \times 10^9 \pm 781 \times 10^8$	$833 \times 10^9 \pm 135 \times 10^9$

All the values are mean ± SEM (n = 6 except DM + *C. albicans* + LGG and DM + *C. tropicalis* + LGG [n = 5]). *P<0.05, **P<0.01, ***P<0.001 compared with that of control. One-way ANOVA followed by Tukey *post-hoc* test.

thelium with epithelial hyperplasia, congested blood vessels, and chronic inflammatory infiltrate, reflecting an immune response to the LGG. Infection with *C. albicans* or *C. tropicalis* led to epithelial changes consistent with mucosal fungal invasion. Diabetic rats administered LGG following infection displayed a keratinized epithelium with pseudohyphal invasion but reduced inflammatory infiltration, suggesting partial modulation of the immune response.

Msp1/p75 in the tongue tissues of rats. Molecular analysis revealed positive expression of the major secreted protein Msp1/p75 across all experimental groups (Figs. 2A, 2B and 3). A significant decrease in Msp1/p75 expression was observed in *C. albicans*-infected rats treated with LGG compared with the *C. albicans* control, as shown by immunohistochemistry. In contrast, *C. tropicalis*-infected rats treated with the probiotic showed little change in Msp1/p75 expression relative to their controls. Western blot analysis further confirmed a significant decrease in Msp1/p75 band intensity, normalized against β-actin, in LGG-treated rats compared with untreated infected controls.

TLR2 and TLR4 expression in the tongue tissues of rats. In the rat oral candidiasis model, LGG modulated the expression of critical immune receptors involved in fungal recognition in a species-specific manner. In *C. albicans* infections, LGG administration significantly decreased TLR2 expression while altering TLR4 protein levels (Figs. 2C, 2D and 3). Conversely, in *C. tropicalis*-infected rats, TLR2 expression altered with no significant change in TLR4, indicating differential modulation of the host's innate immune response (Figs. 2E and F).

DISCUSSION

Administration of LGG showed a specific growth pattern for *C. albicans* and *C. tropicalis* in rats. This finding supports previous reports showing higher oral *Candida* carriage in diabetic individuals due to increased epithelial binding, reduced tissue resistance, and altered salivary glucose and pH levels (15). Elevated salivary glucose during hyperglycemia promotes the accumulation of glycosylation products on buccal cells, enhancing *Candida* adherence and infection risk (16). Administration of LGG resulted in a steady decline in *Candida* growth from day 1 to

Table 3. Effect of LGG on the liver profile in rats with oral candidiasis induced by *C. albicans* and *C. tropicalis*.

Group	Glucose (mg/dl)	Total Protein (g/L)	Albumin (Alb) (g/L)	Globulin (Glo) (g/L)	Alb/ Glo ratio	ALP (U/L)	Total Bilirubin (umol/L)	GGT (U/L)	AST (U/L)	ALT (U/L)
Control	70.57 ±3.56	65.33 ± 2.12	46.67 ± 2.04	24.17 ± 1.35	1.97 ± 0.16	398.67 ± 13.31	2.00 ± 0.00	3.83 ± 0.40	225.00 ± 28.96	106.83 ± 4.64
DM Control	200.45 ± 3.26***	35.00 ± 1.29***	33.67 ± 1.63**	21.33 ± 0.71	1.58 ± 0.07	493.17 ± 5.59***	2.67 ± 0.21	3.83 ± 0.40	265.67 ± 29.87	180.67 ± 7.24***
DM + *C. albicans*	205.41 ± 3.81***	36.17 ± 2.21***	31.67 ± 1.52***	20.50 ± 0.73	1.55 ± 0.09	493.67 ± 4.75***	2.50 ± 0.22	3.67 ± 0.42	231.00 ± 6.25	183.83 ±11.73***
DM + *C. tropicalis*	196.43 ± 2.62***	32.82 ± 1.12***	32.75 ± 1.63***	21.33 ± 0.71	1.54 ± 0.06	466.33 ± 15.97*	2.33 ± 0.21	4.17 ± 0.48	229.67 ± 13.82	184.67 ± 3.50***
DM + LGG	143.84 ±5.45 $^{***@@@}$	58.17 ±0.70$^{@@@}$	44.50 ± 2.67$^{@}$	21.00 ± 2.66	2.35 ± 0.38	480.50 ± 20.77**	2.33 ± 0.21	3.33 ± 0.42	203.00 ± 10.69	132.67 ± 5.93$^{@@}$
DM + *C. albicans* + LGG	152.79 ±7.06$^{***@@@###}$	60.20 ± 1.93$^{###}$	47.60 ± 2.94$^{###}$	23.00 ± 1.82	2.15 ± 0.26	407.40 ± 17.87$^{##}$	2.40 ± 0.24	3.20 ± 0.37	226.80 ± 12.88	123.00 ±16.40$^{###}$
DM + *C. tropicalis* +LGG	161.44 ± 2.71$^{***@@&&&}$	59.40 ± 2.38$^{&&&}$	41.80 ± 1.85	23.00 ± 2.17	1.89 ± 0.22	395.80 ± 3.26$^{&}$	2.20 ± 0.20	3.60 ± 0.40	200.20 ± 6.68	128.40 ± 7.05$^{&&}$

All the values are mean ± SEM (n = 6 except DM + *C. albicans* + LGG and DM + *C. tropicalis* + LGG [n = 5]). *P<0.05, **P<0.01 and ***P<0.001 compared with that of control, @P<0.05, @@P<0.01 and @@@P<0.001 compared with that of diabetic control. ##P<0.01 and ###P<0.001 compared with that of *C. albicans* control, &P<0.05, &&P<0.01 and &&& P<0.001 compared with that of *C. tropicalis* control. One-way ANOVA followed by Tukey *post-hoc* test.

Table 4. Effect of LGG on the renal profile of rats with oral candidiasis induced by *C. albicans* and *C. tropicalis.*

Group	Urea (mmol/L)	Creatinine (umol/L)	Uric acid (mmol/L)	Calcium (mmol/L)	Corrected calcium (mmol/L)	Phosphate (mmol/L)
Control	5.78 ± 0.21	58.50 ± 3.66	0.24 ± 0.02	2.39 ± 0.14	2.09 ± 0.15	2.25 ± 0.12
DM Control	6.98 ± 0.29**	57.83 ± 2.68	0.28 ± 0.02	2.41 ± 0.12	2.31 ± 0.12	2.18 ± 0.11
DM + *C. albicans*	6.87 ± 0.10**	57.17 ± 1.58	0.34 ± 0.05	2.43 ± 0.16	2.02 ± 0.10	2.18 ± 0.09
DM + *C. tropicalis*	6.53 ± 0.20	57.67 ± 2.43	0.26 ± 0.04	2.38 ± 0.16	2.29 ± 0.20	2.14 ± 0.16
DM + LGG	6.12 ± 0.17@	58.00 ± 0.86	0.24 ± 0.02	2.35 ± 0.18	2.31 ± 0.11	2.21 ± 0.13
DM + *C. albicans* + LGG	6.62 ± 0.17	47.80 ± 3.50	0.28 ± 0.03	2.21 ± 0.10	2.01 ± 0.08	2.31 ± 0.18
DM + *C. tropicalis*+ LGG	6.14 ± 0.17	63.80 ± 2.76	0.28 ± 0.03	2.34 ± 0.05	2.49 ± 0.02	2.25 ± 0.16

All the values are mean ± SEM (n = 6 except DM + *C. albicans* + LGG and DM + *C. tropicalis* + LGG [n = 5]). **P<0.01 compared with that of control, @P<0.05 compared with that of diabetic control. One-way ANOVA followed by Tukey post-hoc test.

Table 5. Effect of LGG on the lipid profile of rats with oral candidiasis induced by *C. albicans* and *C. tropicalis.*

Group	Total cholesterol (mmol/L)	Triglyceride (mmol/L)	HDL (mmol/L)	LDL (mmol/L)	Non-HDL Cholesterol (mmol/L)	Total Cholesterol/ HDL ratio
Control	2.36 ± 0.10	1.50 ± 0.09	1.44 ± 0.03	0.62 ± 0.07	0.92 ± 0.08	1.64 ± 0.05
DM Control	2.29 ± 0.14	1.49 ± 0.06	1.04 ± 0.06***	0.95 ± 0.11	1.25 ± 0.12*	2.22 ± 0.13***
DM + *C. albicans*	2.53 ± 0.06	1.65 ± 0.08	1.33 ± 0.04@@@	0.87 ± 0.08	1.40 ± 0.08	1.92 ± 0.08
DM + *C. tropicalis*	2.41 ± 0.11	1.61 ± 0.04	1.15 ± 0.05***	0.94 ± 0.14	1.26 ± 0.15	2.13 ± 0.18*
DM + LGG	2.27 ± 0.10	1.54 ± 0.06	1.22 ± 0.04*	0.74 ± 0.09	1.04 ± 0.09	1.86 ± 0.09
DM + *C. albicans* + LGG	2.38 ± 0.02	1.77 ± 0.08	1.40 ± 0.04@@@	0.62 ± 0.04	0.98 ± 0.03	1.70 ± 0.04@
DM + *C. tropicalis* + LGG	2.30 ± 0.03	1.60 ± 0.13	1.38 ±0.04@@@&	0.60 ± 0.04	0.92 ± 0.06	1.68 ± 0.06@

All the values are mean ± SEM (n = 6 except DM + *C. albicans* + LGG and DM + *C. tropicalis* + LGG [n = 5]). *P<0.05 and ***P<0.001 compared with that of control, @P<0.05 and @@@P<0.001 compared with that of diabetic control, &P<0.05 compared with that of *C. tropicalis* control. One-way ANOVA followed by Tukey *post-hoc* test.

day 28, despite the diabetic state, indicating its beneficial probiotic effect. Similar in vivo studies have demonstrated that LGG significantly reduces *C. albicans* growth in both diabetic and non-diabetic hosts (17). The higher proliferation of *Candida* in diabetes may be related to reversible glycosylation products, oxidative stress, and weakened antioxidant defenses, while probiotics mitigate these effects by scavenging reactive oxygen species and reducing inflammation. These differences in the candidal count between species may also be due to species-specific enzymatic activities, including phospholipase, esterase, and hemolysin, as well as biofilm-forming capacities (18). An initial increase in oral *Candida* carriage was observed in diabetic rats, likely due to hyperglycemia, followed by a gradual decline after LGG administration, confirming its therapeutic potential against oral candidiasis.

In accordance with our study, several studies have reported reduced RBC indices (Hb, PCV, RBC count, MCV, MCH and MCHC) in alloxan-induced diabetic rats, consistent with anemia (19). This decline is linked to increased protein oxidation and hyperglycemia, which promote lipid peroxidation and RBC hemolysis (20). Additional contributing factors include abnormal hemoglobin synthesis, altered osmoregulation, and alloxan-induced cytotoxicity mediated by reactive oxygen species (21). These minor variations in RBC parameters may be attributed to factors such as the alloxan dose-dependent administration and the short duration of the study. Some studies report variable WBC and lymphocyte responses in diabetic models, including decreased WBC counts with increased lymphocyte counts (22) or elevated WBC

Molecular analysis

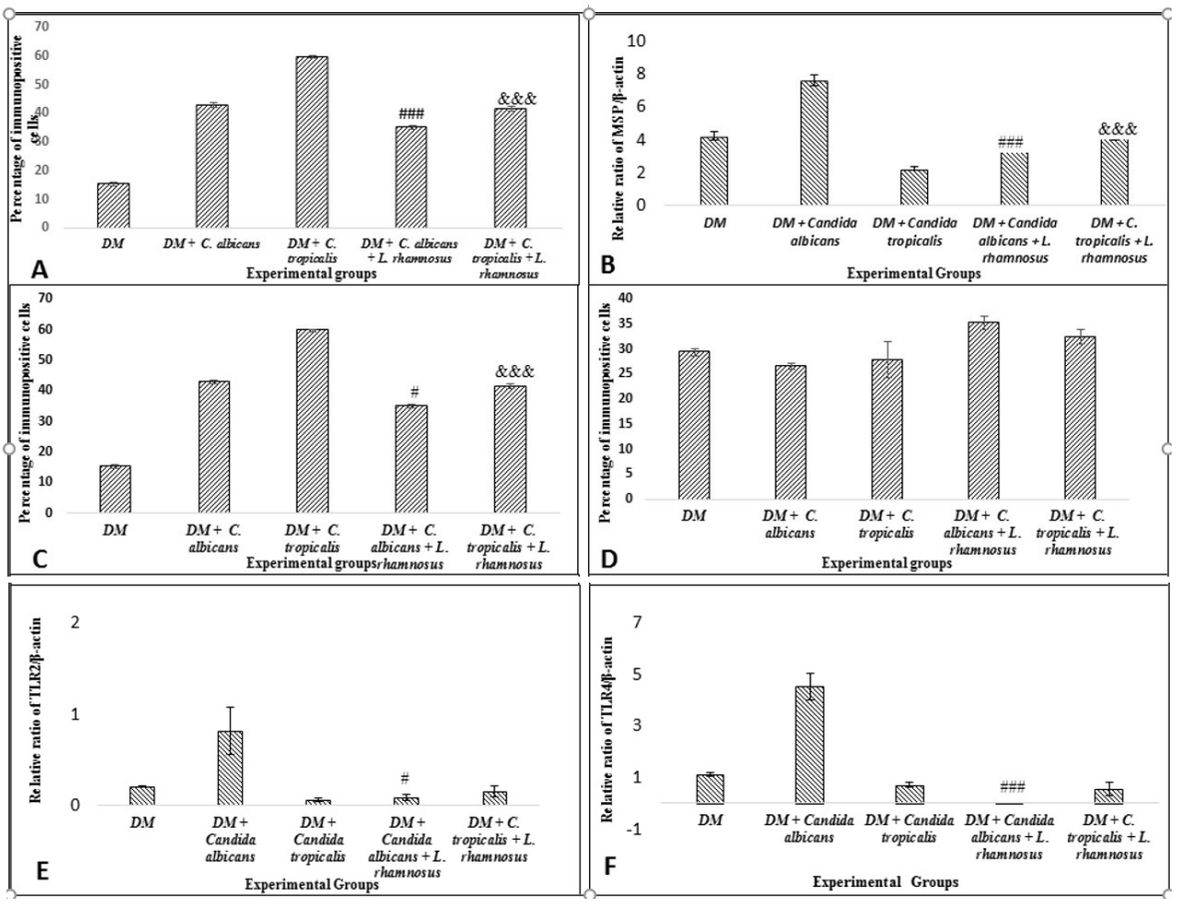

Fig. 2. A) Percentage of immunopositive cells of Msp1/p75 expression in diabetic rats. All the values are mean ± SEM (n = 3) ###P<0.001 compared with that of DM + *C. albicans*; &&&P<0.001 compared with that of DM + *C. tropicalis* (Unpaired t-test with Welch Correction). B) Relative ratio of Msp1/p75 band intensity normalized against ß-Actin protein using Western blot analysis. All the values are mean ± SEM (n = 3) ###P<0.001 compared with that of *C. albicans*; &&&P<0.001 compared with that of *C. tropicalis* (One-way ANOVA followed by Tukey's test); C) Percentage of Immunopositive Cells of TLR2 expression in diabetic rats. All the values are mean ± SEM (n = 3) #P<0.05 compared with that of DM + *C. albicans*; &&&P<0.001 compared with that of DM + *C. tropicalis* (Unpaired t-test with Welch Correction). D) Percentage of immunopositive cells of TLR4 expression in diabetic rats. All the values are mean ± SEM (n = 3); E) Relative Ratio of TLR2 band intensity normalized against ß-Actin protein using Western blot analysis. All the values are Mean ± SEM (n = 3) #P<0.05 compared with that of *C. albicans* (One-way ANOVA followed by Tukey's test); F) Relative ratio of TLR4 band intensity normalized against ß-Actin protein using Western blot analysis. All the values are Mean ± SEM (n = 3). ###P<0.001 compared with that of *C. albicans* (One-way ANOVA followed by Tukey's test). DM: Diabetes mellitus.

and lymphocyte counts, suggesting an enhanced immune response. Other studies have shown increased total WBC counts with reduced lymphocyte counts. These discrepancies are attributed to factors such as insulin resistance, oxidative stress, and accumulation of advanced glycation end-products (AGEs), which influence immune system behavior in diabetes (23).

This study observed no significant changes in neutrophil or lymphocyte counts in diabetic rats compared with controls, though slight alterations in differential counts suggest a modulated immune response to infection, inflammation, or stress. Previous studies, however, reported increased neutrophil counts in alloxan-induced diabetic rats (24). These findings likely reflect the complex interaction between diabetes and oral candidiasis, with the effect of LGG potentially helping to stabilize immune parameters despite the co-existing conditions. Contrast-

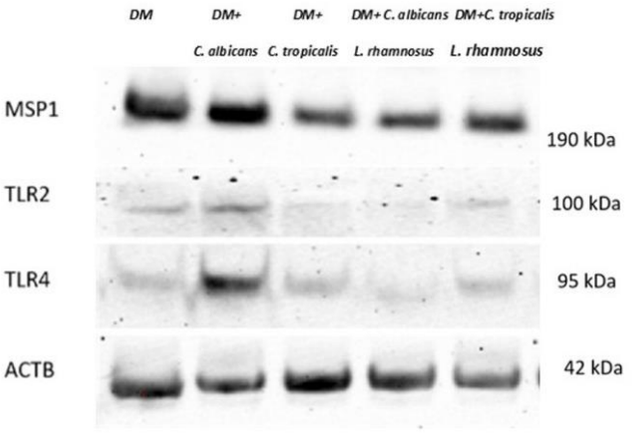

Fig. 3. Representative Western blot images of Msp1/p75, TLR2 and TLR4.

ingly, prior studies have reported elevated platelet levels in alloxan-induced diabetic rats, possibly due to acute infection, inflammation, and free radical-induced thrombocytosis. LGG administration appeared to modulate haematological parameters, potentially reducing the alterations typically observed in alloxan-induced diabetes. Despite the presence of diabetes and oral candidiasis, changes in RBC and platelet counts were minimal and remained within physiological limits. Variations in WBC counts reflect differing immune responses across experimental groups, likely influenced by diabetes, infection, and LGG administration.

Changes in the liver profile of rats are linked to altered lipid metabolism, fatty liver, and oxidative stress associated with diabetes (25, 26). The findings suggest that elevated ALP may reflect cholestasis and membrane damage rather than direct hepatic injury. Administration of LGG reduced ALP, ALT, and bilirubin levels compared with diabetic controls, demonstrating hepatoprotective potential, possibly through modulation of hepatic biomarkers and β-glucoronidase inhibition (27). Although the mechanism by which LGG affects the renal profile remains unclear, its reported anti-hyperuricemic activity may contribute to these changes. However, all renal parameters remained within normal limits (28).

Dyslipidaemia in diabetes is linked to altered carbohydrate metabolism and elevated free fatty acids, increasing cardiovascular risk. The observed effects of LGG, including higher HDL and reduced LDL and triglycerides, indicate a potential cardioprotective and therapeutic role, even in the presence of oral candidiasis.

Histopathological analysis of diabetic rat tongues treated with LGG for oral candidiasis assessed parameters including yeast and hyphal invasion, epithelial hyperplasia, hyperkeratosis, inflammatory infiltration, exocytosis, loss of filiform papillae, microabscesses, basal layer disorganization, and spongiosis (29, 30). Overall, histopathology indicated that LGG may enhance immune activity in diabetic rats; however, its effectiveness in limiting fungal invasion varies depending on the *Candida* species. LGG appears to mitigate excessive inflammation while allowing an appropriate host immune response.

Studies have shown that LGG supernatants contain Msp1/p75, which inhibits hyphal morphogenesis, disrupts chitin in fungal cell walls, and impedes biofilm formation (31). The protein's profile resembles that of cell wall hydrolases, suggesting enzymatic and probiotic functions, although its exact role remains unclear. At the molecular level, Msp1/p75 interacts with host cells through NF-κB, Akt, and MAPK signaling pathways, contributing to immune regulation, cytokine-mediated cell death, barrier preservation, increased mucus production, immune tolerance, and IgA stimulation. Variations in observed expression likely reflect differences in sample type, detection sensitivity, and methodological factors such as antibody specificity and tissue preparation.

In the rat oral candidiasis model, LGG modulated the expression of critical immune receptors involved in fungal recognition in a species-specific manner. This differential expression of TLR4 indicates a strong inflammatory response that contributed to tissue destruction in the context of chronic diabetes. However, further studies are needed to elucidate the exact mechanism underlying these effects.

The interaction between pathogen-associated mo-

lecular patterns (PAMPs) and pattern recognition receptors (PRRs) is crucial for host defense, orchestrating dendritic cell-mediated cytokine signaling that polarizes immune cells into pro- and anti-inflammatory states, thereby maintaining immune homeostasis during infection (32). LGG influences this process through its microbial-associated molecular patterns (MAMPs), which engage PRRs on macrophages and epithelial cells. Initial activation triggers NF-κB and MAPK pathways to induce pro-inflammatory responses, while prolonged exposure induces tolerance via negative TLR regulators, structural variations in MAMPs, and upregulation of inhibitory molecules such as Toll-interacting protein (Tollip), promoting immune homeostasis.

Specifically, LGG stimulates macrophages via TLR2, activating NF-κB and IL-8 for pro-inflammatory signaling, while concurrently triggering ERK-dependent TLR2 pathways to enhance IL-10 production, exerting anti-inflammatory effects (33). In epithelial cells, peptidoglycan and lipoteichoic acid from the probiotic downregulate TLR2/4-MyD88-NF-κB signaling, induces regulatory T cells (34) and promote IgA-mediated mucosal immunity. Repeated TLR4 stimulation also limits excessive inflammation, preventing tissue damage. In vivo studies with *C. albicans*-infected mice demonstrated that LGG enhanced TLR2 and TLR4 expression in tongue tissues, promoting neutrophil recruitment, phagocytosis, and cytokine production, facilitating fungal clearance. A subsequent decline in PRR and chemokine expression indicated resolution of inflammation and protective immune modulation.

Overall, LGG exhibits dual antifungal and immunomodulatory effects by fine-tuning TLR2 and TLR4 signaling during *C. albicans* and *C. tropicalis* infections. These effects, combined with stable haematological and biochemical profiles and significant inhibition of fungal growth, underscore its potential as a supportive adjunct in antifungal therapy to enhance host immunity, accelerate recovery, and minimize adverse effects. These findings highlight the LGG's ability to support immune balance and enhance antifungal defense, suggesting potential as an adjunctive therapy for fungal infections.

CONCLUSION

This study investigated the antifungal efficacy of LGG against *C. albicans* and *C. tropicalis* in diabetic rat models of oral candidiasis. Across all experimental groups, microbiological, haematological, biochemical, and histopathological parameters remained largely within normal physiological ranges despite immune-mediated changes. Administration of LGG led to a marked reduction in *Candida* colonization, highlighting its potential as an adjunctive antifungal therapy.

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