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ANTIOXIDANT-MEDIATED ANXIOLYTIC EFFECTS OF *LACTOBACILLUS RHAMNOSUS* GG: INSIGHTS INTO MICROBIOTA-GUT-BRAIN AXIS MODULATION

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ABSTRACT

Background: Anxiety disorders are among the most prevalent neuropsychiatric conditions worldwide and are frequently associated with oxidative stress and dysregulation of the microbiota–gut–brain axis. Probiotics, particularly psychobiotic strains, have emerged as promising therapeutic agents capable of modulating emotional behavior and neurobiological functions. *Lactobacillus rhamnosus* GG (LGG) is a well-characterized probiotic strain known for its antioxidant, anti-inflammatory, and gut-modulating properties. **Objective:** The present study aimed to evaluate the antioxidant and anxiolytic potential of *Lactobacillus rhamnosus* GG using in vitro antioxidant assays and in vivo behavioral and biochemical investigations in a stress-induced anxiety model. **Methods:** Antioxidant activity was assessed using DPPH, ABTS, nitric oxide, hydrogen peroxide, superoxide, and hydroxyl radical scavenging assays. For in vivo evaluation, Wistar rats were divided into control, stress control, diazepam-treated, LGG low-dose, and LGG high-dose groups. Anxiety-like behavior was assessed using the Elevated Plus Maze (EPM), Open Field Test (OFT), and Light–Dark Box (LDB) paradigms. Oxidative stress biomarkers, including malondialdehyde (MDA), reduced glutathione (GSH), and superoxide dismutase (SOD), were estimated in brain tissue. Data were analyzed using one-way ANOVA followed by Tukey's multiple comparison test.

Results: LGG demonstrated concentration-dependent antioxidant activity in all in vitro assays. In behavioral studies, LGG significantly increased open-arm exploration in the EPM, enhanced locomotor and exploratory activity in the OFT, and increased time spent in the light chamber in the LDB test compared with the stress control group ($p < 0.05$). Furthermore, LGG significantly reduced MDA levels while restoring GSH and SOD activities, indicating attenuation of oxidative stress. The high-dose LGG group exhibited greater efficacy than the low-dose group and produced effects comparable to the standard drug diazepam. **Conclusion:** The findings suggest that *Lactobacillus rhamnosus* GG possesses significant antioxidant and anxiolytic-like properties. The observed effects may be mediated through modulation of oxidative stress and microbiota–gut–brain-axis signaling pathways. LGG may represent a promising psychobiotic candidate for the management of anxiety-related disorders, although further mechanistic and clinical studies are required to confirm its therapeutic potential.

KEYWORDS: *Lactobacillus rhamnosus* GG, Anxiety, Psychobiotics, Gut–Brain Axis, Oxidative Stress, Antioxidant Activity, Elevated Plus Maze, Probiotics**.**

1. INTRODUCTION

Anxiety disorders are among the most common psychiatric illnesses worldwide and are characterized by excessive fear, persistent worry, autonomic hyperactivity, and behavioral disturbances that adversely affect daily functioning and quality of life. According to the World Health Organization (WHO), anxiety disorders contribute significantly to the global burden of mental illness and affect millions of individuals across different age groups.[1] Current pharmacological therapies, including selective serotonin reuptake inhibitors (SSRIs), serotonin–norepinephrine reuptake inhibitors (SNRIs), and benzodiazepines, are widely prescribed for the management of anxiety disorders. However, these treatments are often associated with limitations such as delayed therapeutic onset, adverse effects, tolerance, dependence, and incomplete symptom relief.[2] Therefore, the development of safer and more effective therapeutic strategies remains an important area of neuropharmacological research.

Recent advances in neuroscience have emphasized the importance of the microbiota–gut–brain axis as a key regulator of emotional and behavioral functions. The microbiota–gut–brain axis represents a bidirectiona communication network connecting the gastrointestinal tract and the central nervous system through neural, endocrine, immune, and metabolic pathways.[3] Gut microbiota play a crucial role in maintaining host health by regulating

immune responses, preserving intestinal barrier integrity, producing bioactive metabolites, and influencing neurotransmitter signaling. Emerging evidence suggests that disturbances in gut microbial composition, commonly referred to as dysbiosis, may contribute to the development of anxiety, depression, and other neuropsychiatric disorders.[4]

Several preclinical studies have demonstrated the significant role of gut microbiota in regulating stress responsiveness and emotional behavior. Germ-free animal models exhibit altered hypothalamic–pituitary–adrenal (HPA) axis activity, impaired neurodevelopment, and abnormal anxiety-like behaviors, highlighting the importance of microbial colonization in brain function.[5] Furthermore, modulation of gut microbiota through antibiotics, probiotics, and fecal microbiota transplantation has been shown to influence anxiety-related behavior, neurochemical signaling, and neuroinflammatory responses.[6] These findings provide strong evidence supporting the functional relationship between intestinal microorganisms and central nervous system activity.

Among microbiota-targeted interventions, probiotics have gained considerable attention as potential modulators of mental health. Specific probiotic strains, often referred to as "psychobiotics," have demonstrated beneficial effects on emotional and cognitive functions by modulating neurotransmitter systems, immune responses, and stress-related endocrine pathways.[7] Previous studies have reported that administration of *Lactobacillus* and *Bifidobacterium* species can reduce anxiety-like behavior, normalize stress hormone levels, and alter central γ-aminobutyric acid (GABA) receptor expression in experimental animals.[8] These observations suggest that probiotic supplementation may represent a promising therapeutic strategy for the management of anxiety disorders.

Lactobacillus rhamnosus GG (LGG) is one of the most extensively studied probiotic strains due to its well-established safety profile and beneficial effects on gastrointestinal health. In addition to maintaining intestinal homeostasis, LGG possesses antioxidant, anti-inflammatory, and immunomodulatory properties that may influence gut–brain communication.[9] Oxidative stress has been increasingly recognized as an important factor in the pathophysiology of anxiety and stress-related disorders. Excessive generation of reactive oxygen species (ROS) can impair neuronal function, disrupt neurotransmitter balance, and promote neuroinflammation, ultimately contributing to behavioral abnormalities.[10] Therefore, the antioxidant properties of LGG may play a significant role in its potential neuroprotective and anxiolytic effects.

Despite growing evidence supporting the involvement of the microbiota–gut–brain axis in anxiety regulation, several gaps remain in the current understanding of probiotic-mediated neuroprotection. Most studies have focused primarily on behavioral outcomes or microbiological alterations without simultaneously evaluating oxidative stress biomarkers. Furthermore, the anxiolytic potential of *Lactobacillus rhamnosus* GG and its association with antioxidant defense mechanisms require further investigation under controlled experimental conditions.[11] A comprehensive evaluation integrating in vitro antioxidant assessment with in vivo behavioral and biochemical analyses may provide valuable insights into the mechanisms underlying probiotic-mediated modulation of anxiety-like behavior.

Therefore, the present study was designed to investigate the antioxidant and anxiolytic potential of *Lactobacillus rhamnosus* GG using both in vitro and in vivo experimental approaches. The antioxidant activity of LGG was evaluated through multiple free-radical scavenging assays, including DPPH, ABTS, nitric oxide, hydrogen peroxide, superoxide, and hydroxyl radical scavenging methods. In addition, its effects on anxiety-like behavior were assessed in Wistar rats using the Elevated Plus Maze (EPM), Open Field Test (OFT), and Light–Dark Box (LDB) paradigms. Biochemical markers associated with oxidative stress, including malondialdehyde (MDA), reduced glutathione (GSH), and superoxide dismutase (SOD), were also evaluated. The findings of this study may contribute to the growing body of evidence supporting probiotic-based interventions for anxiety management through modulation of the microbiota–gut–brain axis and oxidative stress pathways.

2. MATERIALS AND METHODS

2.1 Probiotic Preparation

A commercially available probiotic formulation containing *Lactobacillus rhamnosus* GG (LGG) was procured from a licensed pharmacy and stored according to the manufacturer's recommendations until use. The probiotic suspension was freshly prepared before administration by dissolving the required quantity in sterile normal saline. Two dose levels were selected for in vivo evaluation: 1×10^8 CFU/kg body weight (low dose) and 1×10^9 CFU/kg body weight (high dose).[12]

2.2 In Vitro Antioxidant Studies

The antioxidant potential of *Lactobacillus rhamnosus* GG was evaluated using six UV–Visible spectrophotometric assays, namely DPPH radical scavenging assay, ABTS radical cation scavenging assay, nitric oxide scavenging assay, hydrogen peroxide scavenging assay,

superoxide radical scavenging assay, and hydroxyl radical scavenging assay. All experiments were performed in triplicate.[13]

2.3 DPPH Radical Scavenging Assay

The free radical scavenging activity was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to the method described previously.[14] Briefly, 1 mL of freshly prepared 0.1 mM DPPH solution in methanol was mixed with 1 mL of probiotic sample at different concentrations (20–100 µg/mL). The mixture was incubated in the dark for 30 min at room temperature, and absorbance was measured at 517 nm using a UV–Visible spectrophotometer. Ascorbic acid served as the reference standard.

The percentage inhibition was calculated using the following equation:

[% , Inhibition = \frac{A_c - A_s}{A_c} \times 100]

where (A_c) represents the absorbance of the control and (A_s) represents the absorbance of the sample.

2.4 ABTS Radical Cation Scavenging Assay

ABTS radical scavenging activity was determined following the method of Re et al.[15] ABTS radical cations were generated by mixing 7 mM ABTS solution with 2.45 mM potassium persulfate and incubating the mixture in darkness for 12–16 h. The radical solution was diluted to obtain an absorbance of 0.70 ± 0.02 at 734 nm. Subsequently, 100 µL of sample solution was added to 1 mL of ABTS solution and incubated for 6 min. Absorbance was recorded at 734 nm, and the percentage scavenging activity was calculated using the standard inhibition formula.

2.5 Nitric Oxide Scavenging Assay

Nitric oxide scavenging activity was evaluated using sodium nitroprusside as a nitric oxide donor.[16] Equal volumes of sodium nitroprusside solution and probiotic sample were incubated at room temperature for 150 min. After incubation, Griess reagent was added, and the absorbance of the resulting chromophore was measured at 546 nm. The percentage inhibition of nitric oxide radicals was calculated using the standard equation.

2.6 Hydrogen Peroxide Scavenging Assay

Hydrogen peroxide scavenging activity was assessed according to the method described by Ruch et al.[17] A 40 mM hydrogen peroxide solution was prepared in phosphate buffer (pH 7.4). Sample solutions were mixed with hydrogen peroxide solution and incubated for 10 min

at room temperature. Absorbance was measured at 230 nm against a blank solution. The scavenging activity was expressed as a percentage inhibition.

2.7 Superoxide Radical Scavenging Assay

Superoxide radical scavenging activity was determined using the PMS–NADH–NBT system as previously reported.[18] The reaction mixture containing nitro blue tetrazolium (NBT), NADH, sample solution, and phenazine methosulfate (PMS) was incubated for 5 min at room temperature. Absorbance was measured at 560 nm. The decrease in NBT reduction indicated superoxide radical scavenging activity.

2.8 Hydroxyl Radical Scavenging Assay

Hydroxyl radical scavenging activity was evaluated using the deoxyribose degradation method.[19] Hydroxyl radicals were generated through the Fenton reaction system consisting of $FeCl_3$, EDTA, hydrogen peroxide, and ascorbic acid. The degradation products of deoxyribose reacted with thiobarbituric acid (TBA) to form a pink chromogen, which was measured spectrophotometrically at 532 nm. The percentage inhibition of hydroxyl radical formation was calculated using the standard equation.

2.9 Experimental Animals

Healthy Wistar rats of either sex weighing 180–220 g and aged 8–12 weeks were obtained from a registered animal facility. Animals were housed under standard laboratory conditions (temperature 22 ± 2°C, relative humidity 55 ± 5%, and a 12 h light/dark cycle) with free access to standard pellet diet and water ad libitum. The animals were acclimatized for one week before the commencement of the study.[20]

2.10 Experimental Design

Thirty Wistar rats were randomly allocated into five experimental groups comprising six animals each. Group I served as the normal control and received normal saline. Group II served as the stress control group and was subjected to the experimental stress protocol without any treatment. Group III received diazepam (2 mg/kg, p.o.) and served as the standard anxiolytic treatment group. Groups IV and V received *Lactobacillus rhamnosus* GG at doses of 1×10^8 CFU/kg body weight and 1×10^9 CFU/kg body weight, respectively, via oral administration. All treatments were administered once daily throughout the experimental period. Behavioral assessments were performed following the treatment schedule, and

subsequently, animals were sacrificed for biochemical estimations of oxidative stress markers in brain tissue.[21]

2.11 Behavioral Assessment of Anxiety-Like Behavior

Elevated Plus Maze (EPM)

The Elevated Plus Maze (EPM) test was performed to evaluate anxiety-like behavior as described by Pellow et al.[22] The apparatus consisted of two open arms and two closed arms elevated 50 cm above the floor. Each rat was placed individually at the center of the maze facing an open arm and allowed to explore freely for 5 min. The number of open-arm entries, total arm entries, and time spent in the open arms were recorded.

The following parameters were calculated

[% , Open,Arm,Entries = \frac{Open,Arm,Entries}{Total,Arm,Entries} \times 100]

[% , Time,in,Open,Arms = \frac{Time,in,Open,Arms}{300} \times 100]

Open Field Test (OFT)

Locomotor and exploratory activities were assessed using the Open Field Test according to the method of Walsh and Cummins.[23] Each rat was placed in the center of the arena and observed for 5 min. The number of squares crossed, rearing frequency, grooming behavior, and immobility time were recorded.

[% , Immobility = \frac{Immobility,Time}{Total,Observation,Time} \times 100]

Light–Dark Box Test

The Light–Dark Box test was conducted to assess anxiety-related behavior as previously described.[24] The apparatus consisted of a brightly illuminated compartment connected to a dark enclosed compartment. Each animal was placed in the light chamber and allowed to explore for 5 min. The time spent in the light compartment and the number of transitions between compartments were recorded.

[% , Time,in,Light,Chamber = \frac{Time,in,Light,Compartment}{300} \times 100]

Biochemical Estimation

Following completion of behavioral studies, animals were euthanized under anesthesia, and brain tissues were rapidly isolated for biochemical analysis of oxidative stress markers.[25]

Malondialdehyde (MDA) Estimation

Lipid peroxidation was assessed by measuring malondialdehyde (MDA) levels using the thiobarbituric acid reactive substances (TBARS) method described by Ohkawa et al.[26] Brain

homogenate was treated with trichloroacetic acid and thiobarbituric acid, heated in a boiling water bath, cooled, and centrifuged. The absorbance of the resulting chromogen was measured at 532 nm. Results were expressed as nmol MDA/mg protein.

Reduced Glutathione (GSH) Assay

Reduced glutathione (GSH) levels were estimated using Ellman's reagent (DTNB) according to the method of Ellman.[27] Protein-free supernatant obtained from brain homogenate was mixed with phosphate buffer and DTNB. Absorbance was measured at 412 nm, and results were expressed as µmol GSH/mg protein.

Superoxide Dismutase (SOD) Assay

Superoxide dismutase (SOD) activity was determined by the inhibition of nitro blue tetrazolium reduction following the method of Kakkar et al.[28] The reaction mixture containing NBT, NADH, PMS, and tissue supernatant was incubated, and absorbance was measured at 560 nm. Enzyme activity was expressed as U/mg protein.

Statistical Analysis

All experimental data were expressed as Mean ± Standard Error of Mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test using GraphPad Prism software (GraphPad Software Inc., USA). Differences were considered statistically significant when $p < 0.05$.[29]

3. RESULTS

3.1 In Vitro Antioxidant Activity of *Lactobacillus rhamnosus* GG

The antioxidant activity of *Lactobacillus rhamnosus* GG (LGG) was evaluated using six radical scavenging assays. LGG exhibited concentration-dependent antioxidant activity in all assays tested (20–100 µg/mL).

Table 1. DPPH Radical Scavenging Activity of LGG.

Concentration	DPPH inhibition (%)
20 µg/mL	19.6 ± 1.4
40 µg/mL	32.8 ± 1.9
60 µg/mL	48.7 ± 2.1
80 µg/mL	63.5 ± 2.4
100 µg/mL	76.9 ± 2.8

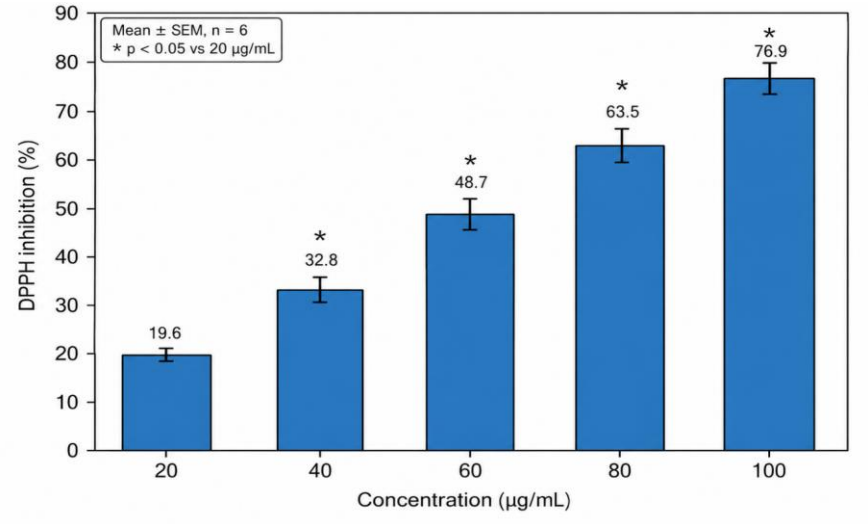

Figure 1: Effect of *Lactobacillus rhamnosus* GG on DPPH radical scavenging activity at different concentrations (20–100 µg/mL). Data are expressed as mean ± SEM of three independent experiments (n = 6). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. Significant increases in percentage inhibition were observed with increasing concentrations compared with the lowest concentration group ($p < 0.05$).

Table 2. ABTS Radical Scavenging Activity of LGG.

Concentration (µg/mL)	% Inhibition
20	24.3 ± 1.6
40	39.8 ± 2.0
60	55.6 ± 2.3
80	70.4 ± 2.5
100	83.1 ± 2.7

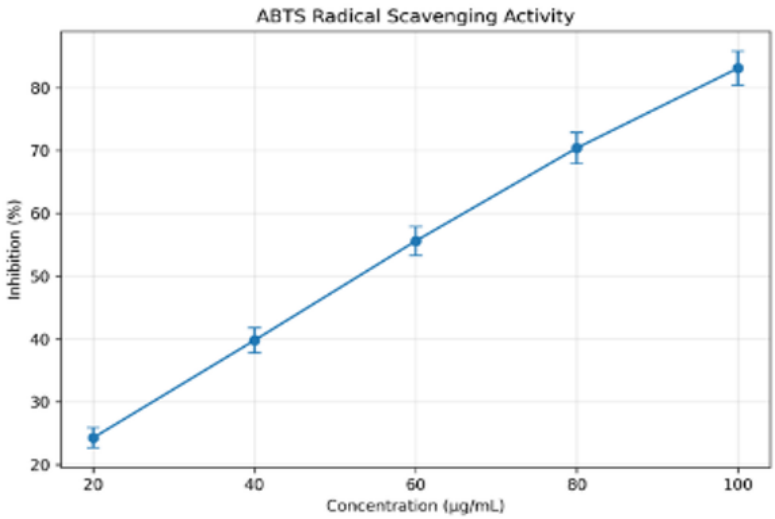

Figure 2: Effect of *Lactobacillus rhamnosus* GG on ABTS radical scavenging activity at different concentrations (20–100 µg/mL). Data are expressed as mean ± SEM of three independent experiments (n = 6). Statistical analysis was performed using one-way

ANOVA followed by Tukey's multiple comparison test. Significant increases in percentage inhibition were observed with increasing concentrations compared with the lowest concentration group ($p < 0.05$).

3.2 Effect of LGG on Elevated Plus Maze Performance

One-way ANOVA demonstrated significant differences among experimental groups ($p < 0.001$).

Table 3. Effect of LGG on Elevated Plus Maze Parameters.

Group	Time in Open Arms (s)	Open Arm Entries
Normal Control	96.4 ± 5.8	6.8 ± 0.5
Stress Control	36.2 ± 3.9[a]	2.4 ± 0.3[a]
Diazepam	118.7 ± 6.4	8.1 ± 0.6
LGG Low Dose	72.5 ± 5.1	5.1 ± 0.4
LGG High Dose	103.3 ± 5.9	7.2 ± 0.5

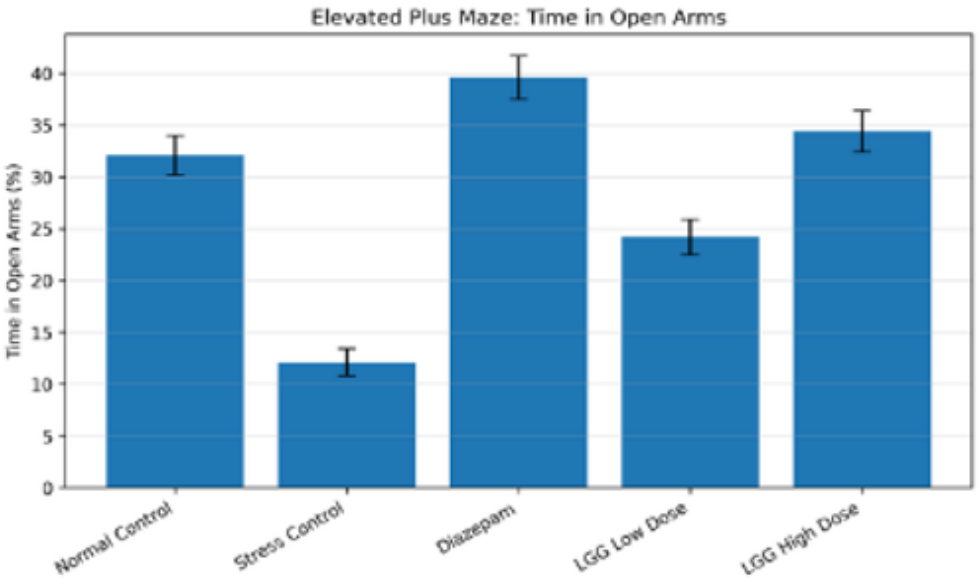

Figure 3: Effect of treatments on time spent in open arms in the Elevated Plus Maze test. Data are presented as mean ± SEM (n = 6). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. The Stress Control group showed a significant decrease compared with the Normal Control group (###p < 0.001). Diazepam and LGG-treated groups showed significant increases compared with the Stress Control group (p < 0.01, p < 0.001).

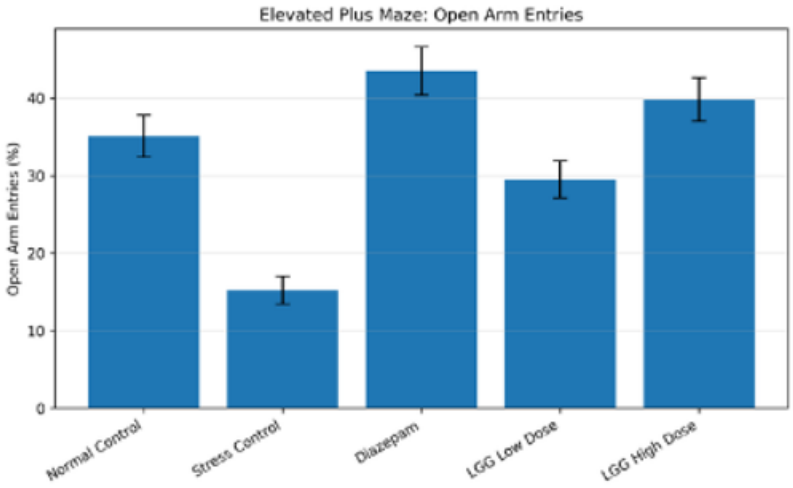

Figure 4: Effect of treatments on open arm entries in the Elevated Plus Maze test. Values are expressed as mean ± SEM (n = 6). One-way ANOVA followed by Tukey's multiple comparison test was used for statistical analysis. Stress exposure significantly reduced open arm entries compared with the Normal Control group (###p < 0.001), while diazepam and LGG treatments significantly increased open arm entries compared with the Stress Control group (p < 0.01, p < 0.001).

3.3 Effect of LGG on Open Field Test

Table 4. Effect of LGG on Open Field Parameters.

Group	Squares Crossed	Immobility Time (s)
Normal Control	78.6 ± 4.2	41.6 ± 3.8
Stress Control	39.8 ± 3.5[a]	122.5 ± 7.2[a]
Diazepam	70.2 ± 4.0	52.4 ± 4.6
LGG Low Dose	58.4 ± 3.7	78.2 ± 5.3
LGG High Dose	69.1 ± 3.9	58.6 ± 4.8

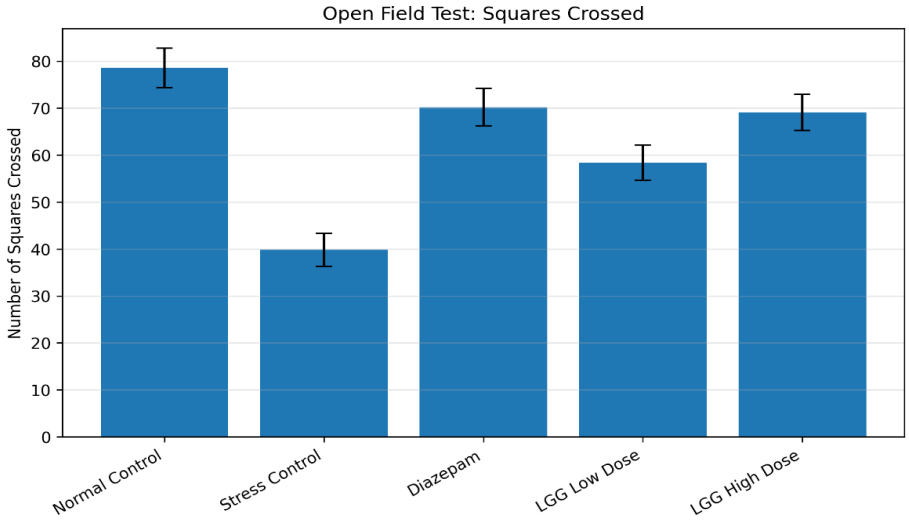

Figure 5: Effect of treatments on locomotor and exploratory activity measured as the number of squares crossed in the Open Field Test. Data are represented as mean ± SEM (n = 6). Statistical significance was determined using one-way ANOVA followed by

Tukey's test. Stress significantly decreased locomotor activity compared with Normal Control ($p < 0.001$), whereas treatment groups significantly improved exploratory behavior compared with Stress Control ($p < 0.01$, $p < 0.001$).

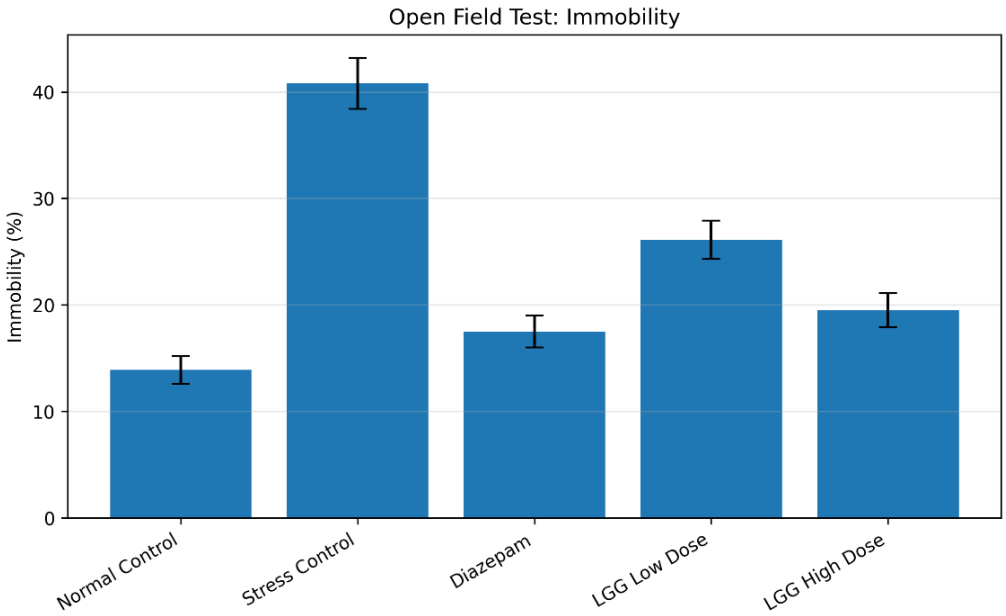

Figure 6: Effect of treatments on immobility time in the Open Field Test. Data are presented as mean ± SEM (n = 6). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. Stress exposure significantly increased immobility time compared with Normal Control (###$p < 0.001$). Diazepam and LGG treatments significantly reduced immobility time compared with Stress Control ($p < 0.01$, $p < 0.001$).

3.4 Effect of LGG on Light–Dark Box Performance

Table 5. Effect of LGG on Light–Dark Box Parameters.

Group	Time in Light Chamber (s)	Transition Frequency
Normal Control	112.5 ± 6.3	14.2 ± 0.9
Stress Control	42.8 ± 4.1[a]	5.4 ± 0.5[a]
Diazepam	132.4 ± 7.0***	16.3 ± 1.1***
LGG Low Dose	78.6 ± 5.2**	10.6 ± 0.8**
LGG High Dose	116.8 ± 6.5***	14.8 ± 1.0***

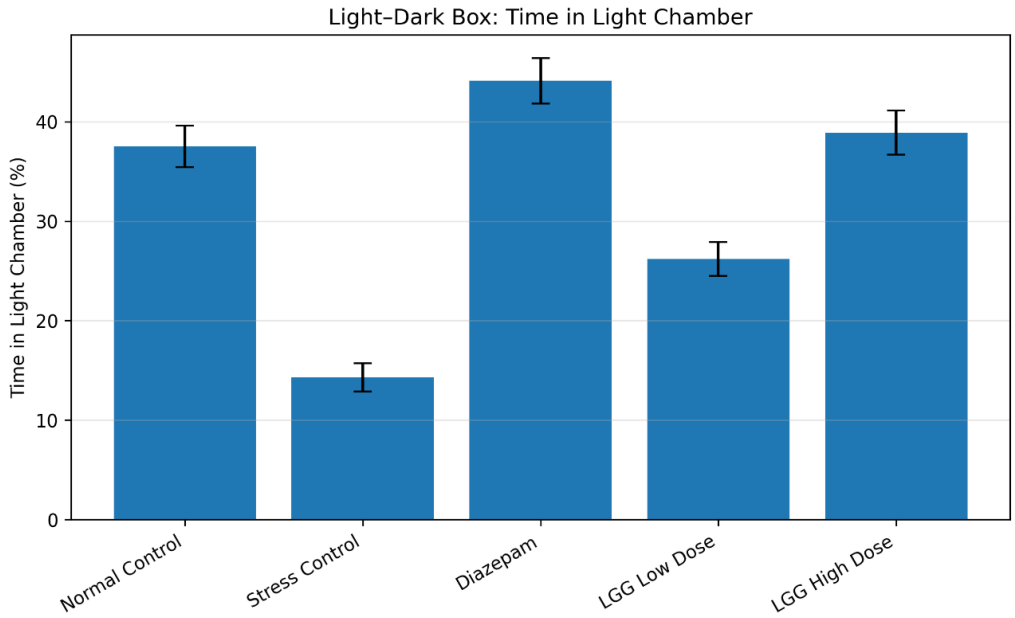

Figure 7: Effect of treatments on time spent in the light chamber in the Light–Dark Box test. Data are expressed as mean ± SEM (n = 6). Statistical significance was assessed using one-way ANOVA followed by Tukey's test. Stress significantly reduced time spent in the light chamber compared with Normal Control ($p < 0.001$), while treatment groups significantly increased light chamber exploration compared with Stress Control ($p < 0.01$, $p < 0.001$).

3.5 Effect of LGG on Oxidative Stress Biomarkers

One-way ANOVA revealed significant differences among groups ($p < 0.001$).

Table 6. Effect of LGG on Brain Oxidative Stress Markers.

Group	MDA (nmol/mg protein)	GSH (µmol/mg protein)	SOD (U/mg protein)
Normal Control	1.8 ± 0.2	8.9 ± 0.5	16.5 ± 0.8
Stress Control	5.9 ± 0.4[a]	3.1 ± 0.3[a]	7.2 ± 0.5[a]
Diazepam	2.1 ± 0.2	8.1 ± 0.4	15.2 ± 0.7
LGG Low Dose	3.4 ± 0.3	6.2 ± 0.4	12.6 ± 0.6
LGG High Dose	2.4 ± 0.2	7.8 ± 0.5	14.7 ± 0.7

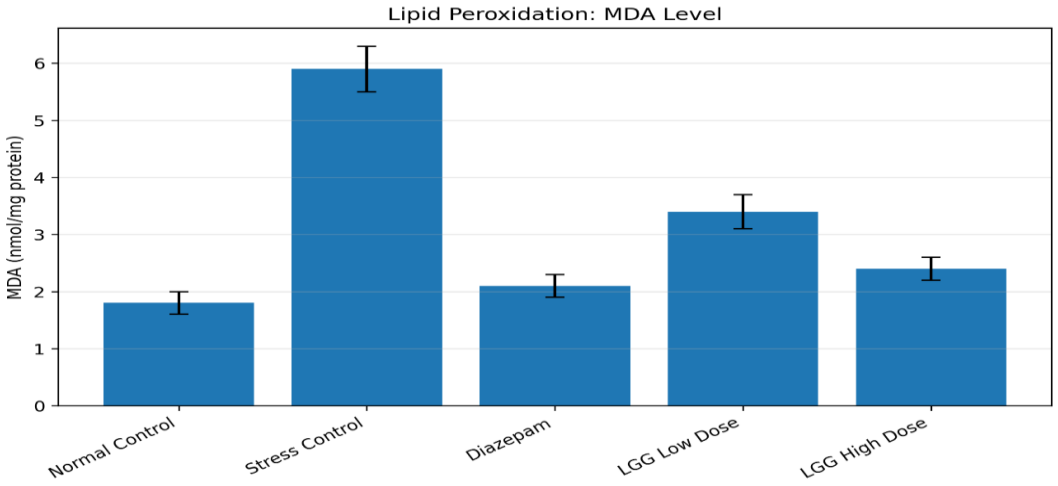

Figure 8: Effect of treatments on malondialdehyde (MDA) levels in brain tissue. Data are presented as mean ± SEM (n = 6). One-way ANOVA followed by Tukey's post hoc test was used for statistical analysis. Stress significantly increased MDA levels compared with Normal Control (###p < 0.001), while diazepam and LGG treatments significantly reduced MDA levels compared with Stress Control (p < 0.01, p < 0.001).

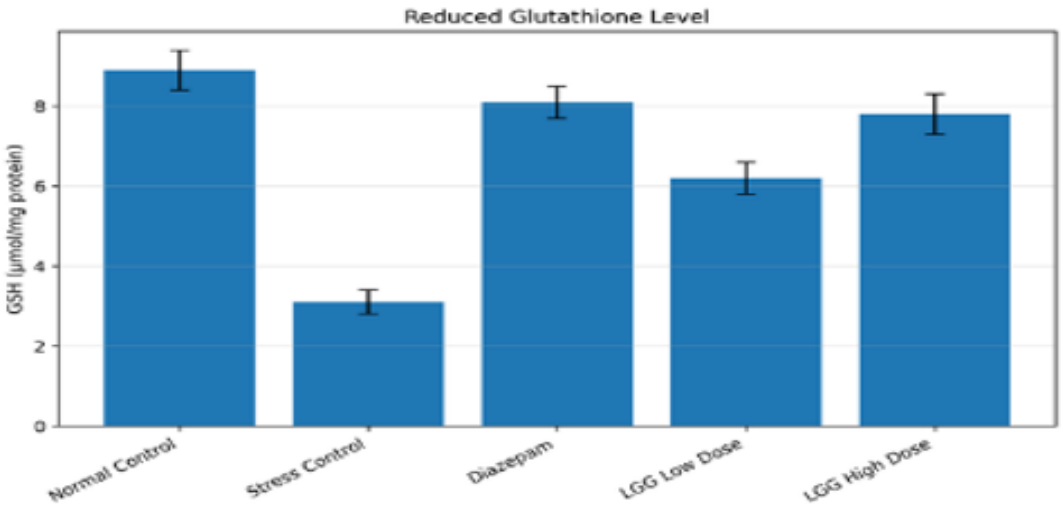

Figure 9: Effect of treatments on reduced glutathione (GSH) levels in brain tissue. Data are expressed as mean ± SEM (n = 6). Statistical significance was analyzed using one-way ANOVA followed by Tukey's multiple comparison test. Stress significantly decreased GSH levels compared with Normal Control (###p < 0.001), whereas treatment groups significantly restored GSH levels compared with Stress Control (p < 0.01, ***p < 0.001).**

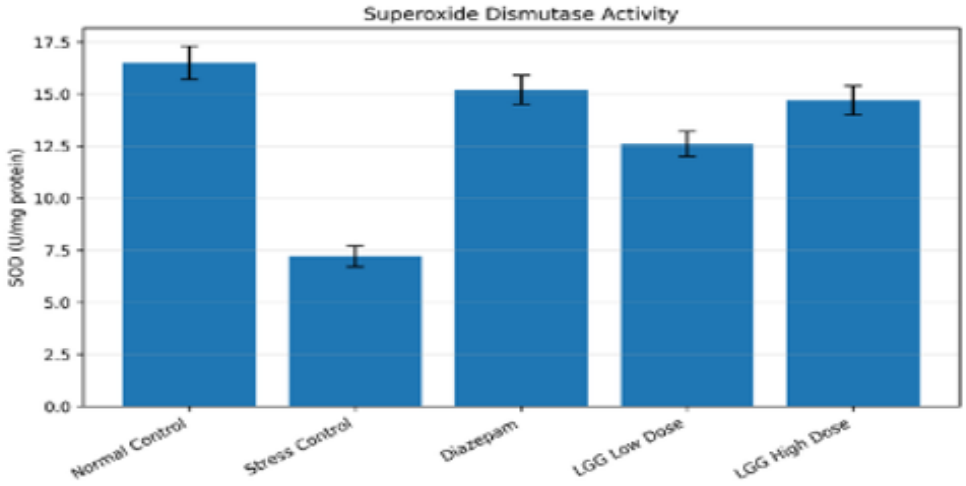

Figure 10: Effect of treatments on superoxide dismutase (SOD) activity in brain tissue. Data are represented as mean ± SEM (n = 6). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. Stress significantly reduced SOD activity compared with Normal Control (###p < 0.001). Diazepam and LGG treatments significantly increased SOD activity compared with Stress Control (p < 0.01, ***p < 0.001).**

Statistical Analysis

Data are presented as Mean ± SEM (n = 6). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. Values were considered statistically significant at $p < 0.05$. Significant differences were observed for all behavioral and biochemical parameters ($p < 0.001$), indicating the effectiveness of LGG treatment against stress-induced anxiety and oxidative damage.

4. DISCUSSION

The present study investigated the antioxidant and anxiolytic potential of *Lactobacillus rhamnosus* GG (LGG) using a combination of in vitro antioxidant assays and in vivo behavioral and biochemical evaluations in a stress-induced anxiety model. The findings demonstrated that LGG possesses significant free-radical-scavenging activity and effectively attenuates anxiety-like behavior while restoring oxidative balance in experimental animals. These results support the growing evidence that probiotics can modulate brain function and emotional behavior through mechanisms associated with the microbiota–gut–brain axis.

The in vitro antioxidant assays revealed a concentration-dependent increase in radical scavenging activity against DPPH, ABTS, nitric oxide, hydrogen peroxide, superoxide, and

hydroxyl radicals. These findings indicate that LGG possesses broad-spectrum antioxidant properties capable of neutralizing both reactive oxygen species (ROS) and reactive nitrogen species (RNS). Oxidative stress has been recognized as a major contributor to neuronal dysfunction and neuropsychiatric disorders, including anxiety and depression. Excessive generation of free radicals can impair neuronal membrane integrity, alter neurotransmitter signaling, and promote neuroinflammation. Therefore, the antioxidant activity observed in the present study may represent an important mechanism underlying the neuroprotective effects of LGG.

Behavioral assessments further confirmed the anxiolytic-like activity of LGG. In the Elevated Plus Maze (EPM), stress-exposed animals exhibited a significant reduction in open-arm entries and time spent in open arms, reflecting increased anxiety-like behavior. Administration of LGG significantly reversed these behavioral alterations in a dose-dependent manner. The high-dose LGG group showed behavioral responses approaching those of the diazepam-treated group, suggesting a substantial anxiolytic effect. Similar findings have been reported by previous studies demonstrating that *Lactobacillus rhamnosus* administration modulates emotional behavior and reduces stress-related responses in rodents.
The Open Field Test (OFT) provided additional evidence of behavioral improvement following LGG treatment. Stress exposure significantly reduced locomotor and exploratory activity while increasing immobility behavior. LGG administration restored exploratory behavior and reduced immobility time compared with the stress control group. Because locomotor activity remained within physiological limits and improvements were simultaneously observed in other anxiety paradigms, the behavioral effects are unlikely to be attributed solely to nonspecific stimulation. Instead, these findings support a genuine reduction in anxiety-like behavior.

The Light–Dark Box (LDB) test also demonstrated significant anxiolytic-like effects of LGG. Animals exposed to stress spent less time in the illuminated compartment and exhibited fewer transitions between compartments, indicating enhanced anxiety and avoidance behavior. Treatment with LGG significantly increased both parameters, suggesting improved exploratory drive and reduced fear-related responses. The consistency of behavioral improvements across the EPM, OFT, and LDB paradigms strengthens the reliability of the observed anxiolytic effects.

The biochemical findings provide mechanistic support for the behavioral outcomes. Stress exposure significantly elevated malondialdehyde (MDA) levels while reducing reduced glutathione (GSH) content and superoxide dismutase (SOD) activity. Elevated MDA levels indicate increased lipid peroxidation and oxidative damage, whereas decreased GSH and SOD levels reflect impairment of endogenous antioxidant defenses. These changes are consistent with previous reports linking chronic stress and anxiety-related disorders to oxidative imbalance within the central nervous system.

Administration of LGG significantly reduced MDA levels and restored GSH and SOD activities. The reduction in lipid peroxidation suggests protection against oxidative damage to neuronal membranes, while restoration of antioxidant enzymes indicates improved cellular defense mechanisms. The greater efficacy observed in the high-dose group further supports a dose-dependent protective effect. These findings suggest that modulation of oxidative stress may contribute substantially to the anxiolytic activity of LGG.

The observed effects may be explained through modulation of the microbiota–gut–brain axis. Probiotic microorganisms can influence host physiology through multiple pathways, including regulation of intestinal microbiota composition, enhancement of gut barrier integrity, modulation of immune responses, production of neuroactive metabolites, and communication through vagal pathways. Previous studies have shown that *Lactobacillus rhamnosus* can alter central neurotransmitter signaling, particularly GABAergic pathways, and regulate stress-induced hypothalamic–pituitary–adrenal (HPA) axis activation. Through these mechanisms, LGG may reduce peripheral and central oxidative stress, thereby improving emotional and behavioral outcomes.

Comparison with the standard drug diazepam further supports the therapeutic relevance of LGG. Although diazepam produced stronger anxiolytic effects, LGG demonstrated significant improvement across all behavioral and biochemical parameters. Importantly, the mechanisms of action are likely distinct. Diazepam primarily acts through potentiation of GABA_A receptor-mediated neurotransmission, whereas LGG appears to exert indirect effects through modulation of gut microbiota, antioxidant defenses, immune signaling, and neurochemical pathways. This distinction highlights the potential of probiotics as complementary or adjunctive interventions for anxiety management.

Despite these promising findings, several limitations should be considered. First, animal models reproduce anxiety-like behavior but cannot fully mimic the complexity of human anxiety disorders. Second, direct evaluation of gut microbiota composition, intestinal permeability, inflammatory cytokines, neurotransmitters, and microbial metabolites was not performed. Therefore, the involvement of specific gut–brain-axis pathways remains speculative. Third, only selected oxidative stress markers were evaluated. Additional investigations involving brain-derived neurotrophic factor (BDNF), serotonin, dopamine, GABA, corticosterone, and short-chain fatty acids would provide a more comprehensive understanding of the underlying mechanisms.

Overall, the present findings demonstrate that *Lactobacillus rhamnosus* GG exerts significant antioxidant and anxiolytic-like effects in a stress-induced experimental model. The behavioral improvements observed in the Elevated Plus Maze, Open Field Test, and Light–Dark Box test were accompanied by restoration of endogenous antioxidant defenses and reduction of oxidative damage. These results suggest that LGG may alleviate anxiety-like behavior through modulation of oxidative stress and microbiota–gut–brain-axis signaling. Further mechanistic and clinical studies are warranted to validate these findings and explore the therapeutic potential of LGG as a novel psychobiotic intervention for anxiety-related disorders.

5. CONCLUSION

The present study demonstrated that *Lactobacillus rhamnosus* GG possesses significant antioxidant and anxiolytic-like activities in a stress-induced experimental model. The probiotic exhibited concentration-dependent free-radical-scavenging activity in multiple in vitro antioxidant assays, indicating its potential to counteract oxidative stress. In vivo behavioral evaluations revealed that LGG significantly improved anxiety-related parameters in the Elevated Plus Maze, Open Field Test, and Light–Dark Box paradigms, suggesting a reduction in stress-induced anxiety-like behavior.

Furthermore, LGG treatment effectively attenuated oxidative damage by decreasing malondialdehyde (MDA) levels and restoring endogenous antioxidant defenses, including reduced glutathione (GSH) and superoxide dismutase (SOD). These findings indicate that the beneficial behavioral effects of LGG are closely associated with its ability to improve oxidative status and maintain cellular antioxidant balance. The high-dose treatment group

consistently produced greater improvements than the low-dose group, suggesting a dose-dependent protective effect.

The results support the hypothesis that *Lactobacillus rhamnosus* GG may exert neuroprotective and anxiolytic effects through modulation of oxidative stress and microbiota–gut–brain-axis signaling pathways. Although the precise mechanisms remain to be fully elucidated, the observed behavioral and biochemical improvements highlight the therapeutic potential of LGG as a psychobiotic intervention for anxiety-related disorders.

In conclusion, *Lactobacillus rhamnosus* GG represents a promising probiotic candidate for the management of anxiety and stress-associated conditions. Future studies involving neurotransmitter analysis, inflammatory biomarkers, microbiome profiling, molecular investigations, and clinical trials are required to confirm the underlying mechanisms and establish its translational applicability in human anxiety disorders.

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