

Gaba-Enriched Fermented Germinated Brown Rice Produced By *Lentilactobacillus Parabuchneri* Exhibits Antidepressant Potential Through Monoaminergic Regulation

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ABSTRACT

Depression is a major global health concern, and existing pharmacological treatments are often associated with side effects, highlighting the need for safer, food-based alternatives. This study investigates the potential of *Lentilactobacillus parabuchneri* (MTCC-13315)-mediated fermentation to enhance γ -aminobutyric acid (GABA) production and functional properties of germinated brown rice (GBR). TLC analysis indicated the absence of GABA in unfermented GBR, while fermented GBR (FGBR) and probiotic culture showed positive bands. HPLC confirmed GABA with retention times comparable to the standard (3.263 min), observed at 3.314 min in FGBR and 3.381 min in probiotic supernatant. Quantification revealed higher GABA content in FGBR (22.32 mg/mL) than in probiotic culture (14.23 mg/mL). FGBR also exhibited significant antioxidant, anti-inflammatory, and α -amylase inhibitory activities. In vivo studies demonstrated dose-dependent antidepressant-like effects, supported by increased monoamine neurotransmitter levels. These findings suggest that fermented GBR is a promising functional food for managing depression.

Keyword: Depression, Probiotic, *Lentilactobacillus parabuchneri*, GABA and Germinated Brown Rice.

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INTRODUCTION

Depression is a complex neuropsychiatric condition marked by synaptic dysfunction, disrupted neural networks, and episodic mood changes, and it continues to be a primary cause of disability globally (Lynch et al., 2024). It has a significant impact on suicide rates and disability-adjusted life years, affecting nearly 280 million people worldwide, with a higher incidence in women (WHO, 2025). Clinically, depression manifests as chronic low mood, anhedonia, lack of appetite and sleep disorders, exhaustion, and cognitive impairment that significantly affects day-to-day functioning. In

addition to supportive dietary measures, current management includes advanced neuromodulation techniques like TMS, tDCS, and DBS, medication including SSRIs and SNRIs, and psychotherapy (CBT, IPT). New studies also emphasize the pathogenesis of GABAergic dysfunction and immunological pathways (Bai et al., 2024; Godfrey et al., 2025; Mohan et al., 2026).

Oryza sativa L., particularly its pigmented varieties such as black and red rice, has garnered significant scientific interest due to its rich phytochemical profile and diverse

therapeutic potential (Gunaratne et al., 2013). These rice cultivars are rich in bioactive compounds, including anthocyanins, phenolic acids, and flavonoids, which contribute to their health-promoting properties (Gunaratne et al., 2013). Brown rice, which retains the bran and germ layers after dehiscing, has emerged as a nutritionally superior option, offering higher fibre, vitamins, and phytochemicals. Germinated brown rice (GBR), a bioprocessed form of *O. sativa L.*, is produced by soaking brown rice in water to initiate germination, has gained significant attention in recent years due to its enhanced nutritional and functional value compared to white and normal brown rice (Imam et al., 2014).

Probiotics, defined as live microorganisms that confer health benefits when administered in adequate amounts, have gained significant attention as functional agents influencing host physiology beyond the gastrointestinal tract (Ayyadurai et al., 2026). These beneficial microbes, primarily belonging to genera such as *Lactobacillus* and *Bifidobacterium*, transiently colonize the gut and modulate multiple biological pathways, including immune regulation, metabolic activity, and microbial balance (Sanders et al., 2010). Emerging evidence suggests that probiotics exert systemic effects by enhancing intestinal barrier integrity, reducing pro-inflammatory cytokines, and influencing key biochemical processes. Additionally, probiotics have been shown to produce bioactive compounds, exhibit antioxidant properties, and improve nutrient absorption, collectively contributing to improved host health. Due to their multifaceted mechanisms of action and minimal adverse effects, probiotics are increasingly explored as promising natural interventions for maintaining physiological homeostasis and preventing disease (Mafe et al. 2025). Fermented millet-based probiotic foods have recently emerged as promising natural interventions for managing depressive disorders due to their combined prebiotic and psychobiotic potential. Millets such as foxtail millet provide a rich source of dietary fibres, phenolic compounds, and resistant starch, which support the growth and metabolic activity of beneficial microorganisms during fermentation. Probiotic strains isolated from fermented cereals can produce neuroactive compounds, particularly gamma-aminobutyric acid (GABA), a key inhibitory neurotransmitter involved in mood regulation. Microbially synthesised GABA, along with other metabolites, can modulate the gut-brain axis through neural, endocrine, and immune pathways, thereby influencing emotional and cognitive functions. Recent studies have demonstrated that fermented millet enriched with GABA-producing probiotics significantly improves behavioural parameters and restores neurotransmitter balance, including serotonin,

dopamine, and corticosterone, indicating strong antidepressant-like effects. Additionally, these fermented products exhibit antioxidant and anti-inflammatory properties, further contributing to neuroprotection and stress reduction. Thus, probiotic-fermented millets represent a novel, plant-based psychobiotic approach for the development of functional foods targeting depression and mental health disorders (Sasikumar et al., 2025).

MATERIALS AND METHODS

Collection and Fermentation:

The GBR samples were collected from fields at Thrissur, Kerala, India. Samples were cleaned thoroughly and dried, then ground into powder using a blender to make a thin powder. Germinated brown rice was dried and made into a thin powder by blending and separated by sieving. *Lentilactobacillus parabuchneri* (MTCC-13315) was purchased from MTCC, Pune, India. Then about 5 g of GBR powder was dissolved in 100 mL of water and sterilised. After sterilisation, the probiotic culture was inoculated and incubated at 37°C for 24 hours.

Screening of GABA using TLC

Preliminary GABA screening was conducted using the Ninhydrin-TLC method. The CFS of probiotic, GBR water extract and fermented GBR supernatant were used for the analysis of GABA. The samples were spotted on a TLC plate. It was developed by spraying 0.5% ninhydrin in acetone to detect GABA (red spots) using a solvent mixture of n-butanol, acetic acid, and water (5:3:2). A red band in the TLC plate is an indication of GABA production. High-performance liquid chromatography was used to further confirm the TLC-positive samples (Kanklai et al., 2020).

Determination of GABA using HPLC

The CFS of probiotic, GBR water extract and fermented GBR supernatant were used for the quantification of GABA. Samples and GABA standards were derivatized with phenylisothiocyanate (PITC) to form phenylthiocarbonyl-GABA, and excess reagents were removed using vacuum evaporation. Chromatographic separation was carried out on a reverse phase C18 column (250 mm × 4.6 mm, 5 µm particle size) maintained at 30 °C, using a diode array detector set at 250 nm. The mobile phase consisted of 80% Solution A (1.4 mM sodium acetate, 0.1% triethylamine, 6% acetonitrile) and 20% Solution B (60% acetonitrile) under gradient conditions at a flow rate of 1.0 mL/min. The injection volume was 20 µL, and the total run time was 70 min [Kim and Kim 2012]. The identity and purity of GABA were confirmed by comparison of retention time and chromatographic peak profile with an authenticated reference standard, following the method

of (Defaix et al., 2018) and quantified with this calculation $\text{Sample concentration} = (\text{Sample area} / \text{Standard area}) \times \text{Standard conc.}$

QUANTITATIVE ANALYSIS OF FERMENTED GBR:

Total Phenol Content (TPC):

The TPC content of the fermented GBR was determined by the Folin–Ciocalteu method. Briefly, 0.6 mL of the fermented GBR was mixed with 0.5 mL of Folin–Ciocalteu reagent, followed by the addition of 1.5 mL of sodium carbonate after 4–5 minutes. Then, 2 mL of methanol was added, and the volume was made up to 7 mL with distilled water. The mixture was incubated at room temperature for 30 minutes, and the absorbance was measured at 760 nm. Gallic acid was used to generate a standard calibration curve (20–100 $\mu\text{g}/\text{dL}$), and results were expressed as millimolar gallic acid equivalents ($\mu\text{g}/\text{mL}$ GAE) (Ay et al., 2023).

Total Flavonoid Content (TFC):

The aluminium chloride method was used to determine the total flavonoid content in the fermented GBR. The fermented GBR solution was taken, and to this, 0.1 mL of 1M potassium acetate, 0.1 mL of AlCl_3 (10%), and 2.8 mL distilled water were added sequentially. The test solution was vigorously shaken and incubated for 30 minutes, and the absorbance was measured at 415 nm. A standard calibration plot was generated using a known concentration of quercetin. The concentration of flavonoids in the test samples was calculated from the calibration plot and expressed as $\mu\text{g}/\text{mL}$ QE of sample (Nurcholis et al., 2023).

DPPH radical scavenging assay:

The antioxidant activity of fermented GBR was evaluated by DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. An ethanolic DPPH solution (0.1 mM) was prepared, and 1 mL of the solution was added to the different concentrations (20 μg to 100 μg) of the fermented GBR. The mixture was then incubated in the dark at room temperature for 30 minutes. The absorbance was measured at 517 nm using a UV-visible spectrophotometer (UV-VIS Shimadzu). Ethanol was used as a blank, DPPH with ethanol without fermented GBR served as a positive control, and ascorbic acid served as a standard (Nadiveedhi et al., 2021; Sasikumar et al., 2025). The experiments were done in triplicate, and the percentage of radical scavenging activity was determined by the following formula;

$$\% \text{ of Inhibition} = \frac{\text{Control Abs} - \text{Test Abs}}{\text{Control Abs}} \times 100.$$

Anti-inflammatory analysis:

The anti-inflammatory activity of fermented GBR was performed by using the inhibition of albumin denaturation method. The reaction mixture consists of test fermented GBR and a 1% aqueous solution of bovine albumin fraction. The pH was adjusted with a small amount of 1N HCL, and the sample fermented GBR were incubated at 37°C for 20 min and then heated to 51°C for 20 min. After cooling, the turbidity of the fermented GBR was measured at 660 nm (UV-Visible Spectrophotometer Model 371, Elico India Ltd) (Dharmadeva et al., 2018; Sasikumar et al., 2025). The experiment was performed in triplicate, and the percentage inhibition of protein denaturation was calculated as follows:

$$\text{Percentage inhibition} = \frac{(\text{Abs Control} - \text{Abs Sample}) \times 100}{\text{Abs Control}}$$

Inhibition of α -amylase activity:

The α -amylase inhibition assay was performed using 3, 5-dinitrosalicylic acid (DNSA). The fermented GBR was initially dissolved in a minimum amount of 10% DNSA and then further diluted in buffer Na_2HPO_4 (PH 6.9) to obtain concentrations ranging from 10 to 1000 $\mu\text{g}/\text{mL}$. A volume of 200 μL of the fermented GBR was incubated at 30°C for 10 min. Subsequently, 200 μL of the starch solution was added to each tube and incubated for 3 min. Then 200 μL of DNSA reagent was added, and the mixture was boiled in a water bath at 85 to 90°C for 10 min. After cooling, the mixture was diluted with 5 mL of distilled water, and the absorbance was measured at 540 nm. The blank with 100% enzyme activity was prepared by replacing the fermented GBR with 200 μL of buffer. A blank reaction was similarly prepared using the fermented GBR at each concentration in the absence of the enzyme solution. A positive control sample was prepared by using Acarbose (100 $\mu\text{g}/\text{mL}$ –2 $\mu\text{g}/\text{mL}$), and the reaction was carried out similarly. The α -amylase inhibitory activity was expressed as percent inhibition (Khan et al., 2023).

IN-VIVO ANTIDEPRESSANT STUDY:

Groupings and Dose Schedule:

Swiss albino mice were randomly divided into six groups, each consisting of six animals, and were treated as follows. Group I served as vehicle control, received normal saline (10 mL/kg) through the oral route, Group II served as reference control, administered 20 mg/kg fluoxetine as an antidepressant drug. Groups III to VI received different concentrations of the fermented GBR through the oral route, respectively. All test drugs were administered via gastric intubation. The animals were subjected to exposure in the Open Field Exploratory test, forced swim and tail suspension tests 1 h after test drug treatment.

Open Field Exploratory Test:

The apparatus was made of wood 50 cm in length, 50 cm in width, and 25 cm in height. The plain floor of the box was divided into 8 cm by 8 cm, with 16 squares on it. Sixteen squares were defined as the centre and the others adjacent to the walls as the periphery. A 60 W white bulb illuminated the apparatus. One hour after oral administration vehicle and test drugs each mouse was gently placed at the centre of the open field and number of squares crossed was counted for 5 min. The mouse was taken out of the apparatus after 5 min. and the floor was cleaned with ethanol. A mouse was deemed to have crossed over from one square to another when all four paws had crossed. The number of readings and assisted readings (when the fore paws are placed on a wall of the open field) within the observation period of 5 min. was recorded (Shilpe et al., 2004).

Forced Swimming Test:

The forced swimming test was based on the method of Porsolt *et al.*, 1977. The mice were forced to swim individually in a glass cylinder (20 cm × 14 cm) containing fresh water up to a height of 10 cm at 25 ± 1°C. The mice were individually, forced to swim for a period 6 minutes and the total duration of immobility was recorded during the last 4 minutes. Mice were considered immobile when they floated in the water without struggling and making only those movements necessary to keep their heads above the water.

Tail Suspension Test:

The tail suspension test was conducted as previously described by Steru *et al.*, 1985. The mice were suspended on the edge of the table, 50 cm above the floor, with the help of adhesive tape placed approximately 1 cm from the tip of the tail. The total duration of immobility induced by tail suspension was recorded during a last 4 minutes of the 6 minutes period. Animal was immobile when it did not show any movement of the body, hanged passively and completely motionless (Steru *et al.*, 1985).

Biochemical Estimations:

Whole brain was dissected out and separated the forebrain. Weighed quantity of tissue and was homogenized in 0.1 mL hydrochloric acid -butanol, (0.85 ml of 37% hydrochloric acid in one liter n- butanol for spectroscopy) for 1 min in a cool environment. The sample was then centrifuged for 10 min at 2,000 rpm. 0.08 mL of supernatant phase was removed and added to an Eppendorf reagent tube containing 0.2 mL of heptane (for spectroscopy) and 0.025 mL 0.1 M hydrochloric acid. After 10 min of vigorous shaking, the

tube was centrifuged under same conditions to separate two phases. Upper organic phase was discarded, and the aqueous phase (0.02 mL) was used for estimation of Serotonin, Nor Adrenaline and Dopamine assay (Schlumpf *et al.*, 1974).

Nor adrenaline and Dopamine Assay:

The assay represents a miniaturization of the trihydroxide method. To 0.02ml of HCl phase, 0.05ml 0.4M and 0.01ml EDTA/Sodium acetate buffer (pH 6.9) were added, followed by 0.01ml iodine solution (0.1M in ethanol) for oxidation. The reaction was stored after two minutes by addition of 0.01ml Na₂SO₃ in 5m NaOH. Acetic acid was added 1.5 minutes later. The solution was then heated to 100 for 6 minutes. When the sample again reached room temperature, excitation and emission spectra at 395-485nm for NA and 330-375nm for DA uncorrected instrument values (Schlumpf et al., 1974).

Serotonin Assay:

For 5-HT determination, the O-phthaldialdehyde (OPT) method was employed. From the OPT reagent 0.025ml were added to 0.02ml of the HCl extract. The fluorophore was developed by heating at 100°C for 10 min. After the samples reached equilibrium with the ambient temperature, excitation / estimation spectra or intensity reading at 360-470 nm were taken in the micro cuvette (Schlumpf et al., 1974).

Statistical Analysis:

Results were expressed as mean ± SEM. The data were analysed by using one way analysis of variance (ANOVA) followed by Dunnett's 't' test using GraphPad version 3. The ANOVA was used to determine whether there were any statistically significant differences between the means of three or more independent (unrelated) groups. Dunnett's 't' test compares means from several experimental groups against a control group mean to see, there was a difference. When an ANOVA test has significant findings, it doesn't report which pairs of means was different. Dunnett's can be used after the ANOVA has been run to identify the pairs with significant differences. P values < 0.05 was considered as significant.

RESULTS

Screening of GABA using TLC

Thin-layer chromatography (TLC) analysis was performed to evaluate the presence of γ -aminobutyric acid (GABA) in germinated brown rice (GBR), probiotic culture (LP), and fermented GBR (FGBR). The unfermented GBR extract did not exhibit any detectable bands, indicating the absence or negligible levels of GABA under the experimental conditions. In

contrast, the probiotic sample (LP) displayed distinct bands corresponding to amino-containing compounds. Notably, the fermented GBR (FGBR) sample exhibited bands at similar Rf positions to those observed in the probiotic culture, suggesting the biosynthesis of GABA during fermentation. This observation supports the role of probiotic-mediated decarboxylation of glutamate into GABA.

Determination of GABA using HPLC

High-performance liquid chromatography (HPLC) analysis was performed to confirm and quantify GABA in fermented germinated brown rice (FGBR) and probiotic cell-free supernatant (CFS). The GABA standard exhibited a characteristic peak at a retention time of 3.263 min, which served as the reference for identification. The fermented GBR sample showed a prominent peak at 3.314 min, closely matching the standard retention time. Similarly, the probiotic CFS exhibited a major peak at 3.381 min. These results confirm the presence of GABA in both fermented GBR and probiotic samples. The slight variation in retention

times may be attributed to matrix complexity and differences in injection volume.

Quantitative analysis based on peak area comparison, with correction for injection volume differences, revealed that the fermented GBR sample contained a GABA concentration of 22.32 mg/mL, whereas the probiotic CFS showed a concentration of 14.23 mg/mL. The fermented GBR exhibited approximately 1.57-fold higher GABA levels compared to the probiotic sample. These findings indicate that fermentation not only facilitates GABA biosynthesis by the probiotic strain but also enhances its accumulation within the GBR matrix, resulting in a functionally enriched product.

Quantitative Analysis of total Phenols and Flavonoids content:

Fermented germinated brown rice (GBR) showed a total phenolic content (TPC) of 134.18 ± 12 $\mu\text{g/ml}$ GAE and a higher total flavonoid content (TFC) of 293.78 ± 19 $\mu\text{g/ml}$ QE (Table-1). The elevated TFC indicates that flavonoids are the dominant bioactive compounds, likely enhanced through fermentation-driven release and biotransformation.

Table 1: Table-1: Quantitative Analysis of total Phenols and Flavonoids content

Sample	TPC ($\mu\text{g/ml}$ GAE)	TFC ($\mu\text{g/ml}$ QE)
Fermented GBR	134.18 ± 12	293.78 ± 19

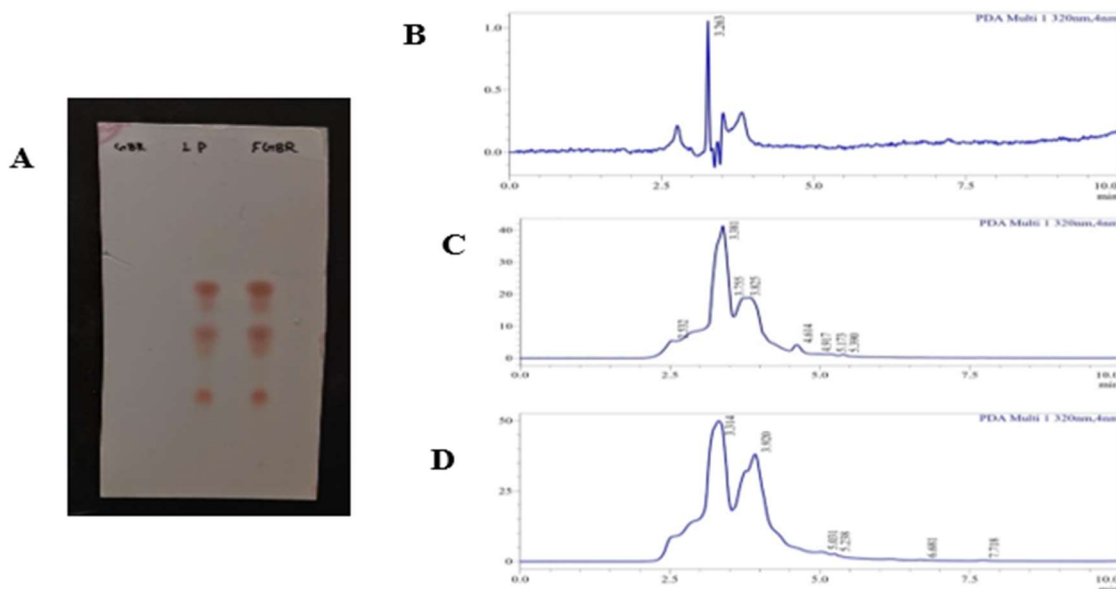


Figure-1: Characterization of GABA. A) TLC, B) HPLC chromatogram of Standard GABA, C) HPLC chromatogram of Probiotic CFS, D) HPLC chromatogram of Fermented GBR

DPPH radical scavenging assay:

The DPPH radical scavenging activity of fermented GBR exhibited a concentration-dependent increase, with % inhibition ranging (Fig. 2A). from 18% to 88% across 20–100 $\mu\text{g/ml}$. The IC_{50} value was approximately 51 $\mu\text{g/ml}$, indicating substantial antioxidant potential. The standard, ascorbic acid showed strong radical scavenging activity with an IC_{50} of approximately 18 $\mu\text{g/ml}$, validating the assay performance. The antioxidant activity of fermented GBR can be attributed to fermentation-induced enhancement and biotransformation of phenolic and flavonoid compounds, which facilitate effective hydrogen atom donation and free radical neutralisation.

Invitro Anti-Inflammatory Assay

The anti-inflammatory activity of the sample, evaluated by the albumin denaturation assay, demonstrated a concentration-dependent increase in percentage inhibition (Fig. 2B), ranging from **25.21% to 52.01%** across 20–100 $\mu\text{g/ml}$. The IC_{50} value was estimated to be **63 $\mu\text{g/ml}$** , indicating moderate protein stabilisation potential. The standard drug, aspirin, exhibited strong inhibition of albumin denaturation with values ranging from **31.42% to 99.43%**, and an IC_{50} of **32 $\mu\text{g/ml}$** , confirming the reliability of the assay. The observed activity of the sample may be attributed to bioactive compounds capable of stabilising protein structure and preventing heat-induced denaturation, a key mechanism associated with anti-inflammatory effects.

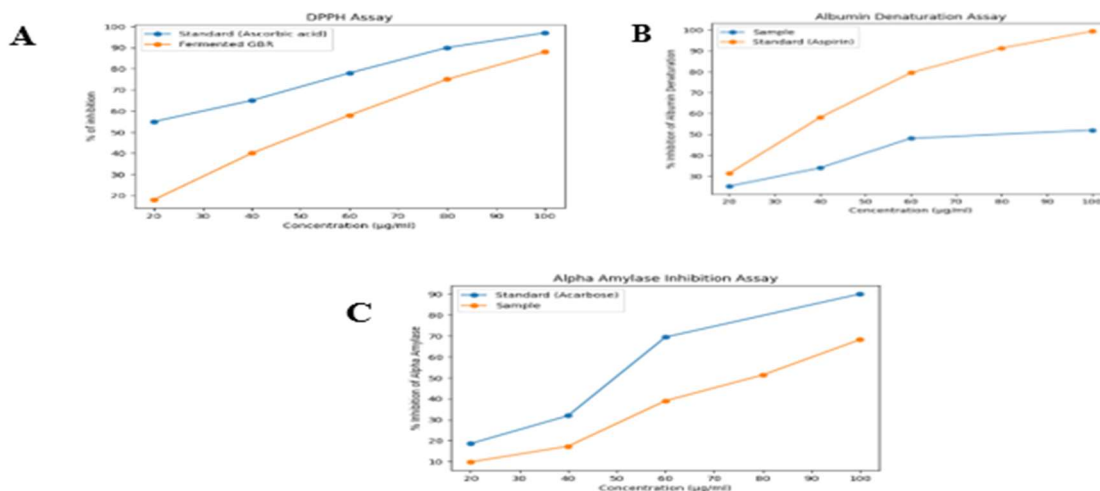


Figure-2: Invitro Pharmaceutical Application. A) DPPH Scavenging Assay, B) Invitro Anti-Inflammatory Assay, C) Invitro Antidiabetic Activity

α -Amylase inhibitory activity:

The α -amylase inhibitory activity of the sample exhibited a concentration-dependent increase, with % inhibition ranging from **9.83% to 68.26%** across 20–100 $\mu\text{g/ml}$ (Fig. 2C). The IC_{50} value was **77 $\mu\text{g/ml}$** , indicating moderate enzyme inhibitory potential. The standard drug, acarbose, demonstrated effective inhibition with an IC_{50} of **50 $\mu\text{g/ml}$** , confirming the validity of the assay. The observed inhibitory activity of the sample may be attributed to the presence of bioactive compounds capable of interacting with the active site of α -amylase, thereby reducing starch hydrolysis through competitive or non-competitive mechanisms.

In-vivo Antidepressant Study:

Open Field Exploratory Test

The open field test demonstrated a dose-dependent increase in No. of Rearing, No. of Assisted Rearing, and No. of Sectional Crossing following administration of fermented germinated brown rice (GBR). The control group showed baseline values of 7.65 ± 0.53 (No. of Rearing), 11.24 ± 1.70 (No. of Assisted Rearing), and 51.35 ± 3.95 (No. of Sectional Crossing). The standard drug, fluoxetine (20 mg/kg), significantly increased these parameters to 15.72 ± 0.55 , 21.70 ± 0.96 , and 85.25 ± 4.05 , respectively.

In the fermented GBR-treated groups, the 50 mg/kg dose showed a slight increase in No. of Rearing (8.25 ± 0.75), No. of Assisted Rearing (12.23 ± 1.27), and No. of Sectional Crossing (61.30 ± 2.95). The 100 mg/kg dose demonstrated moderate improvement with 11.42 ± 0.86 , 14.72 ± 0.65 , and 67.35 ± 2.22 . A more

pronounced increase was observed at 200 mg/kg, with 14.66 ± 1.02 , 18.54 ± 1.15 , and 73.34 ± 2.62 . The highest dose, 400 mg/kg, produced the greatest enhancement in No. of Rearing (16.24 ± 0.67), No. of Assisted Rearing (21.80 ± 1.05), and No. of Sectional Crossing (91.34 ± 2.88).

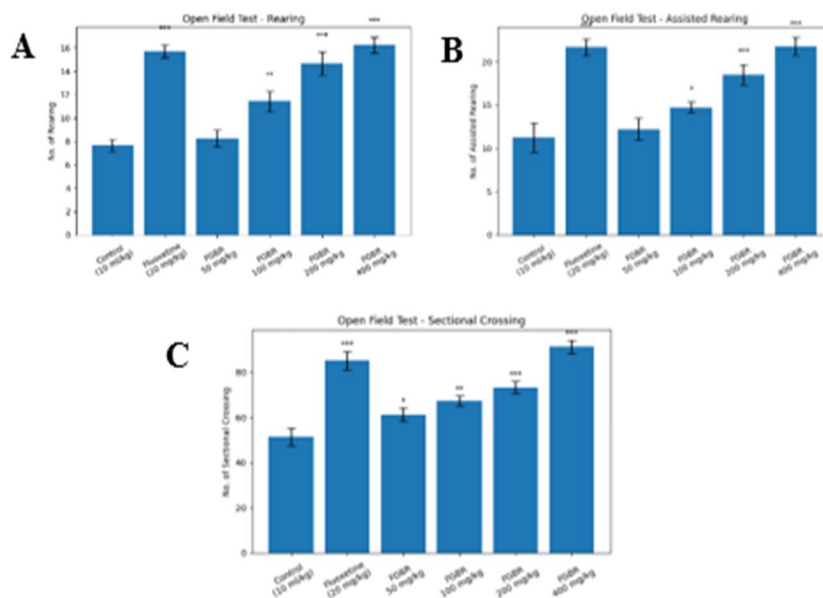


Figure-3: Open Field Exploratory Test. A) Rearing, B) Assisted rearing, C) Sectional Crossing

Forced Swimming Test and Tail Suspension Test

The antidepressant-like activity of fermented GBR was evaluated using the Forced Swim Test (FST) and Tail Suspension Test (TST), where a reduction in immobility time indicates improved behavioural response. In the control group, immobility times were 135.27 ± 6.50 s (FST) and 108.20 ± 4.72 s (TST). The standard drug, fluoxetine (20 mg/kg), significantly reduced immobility to 14.25 ± 0.85 s (FST) and 12.25 ± 0.65 s (TST).

Treatment with fermented GBR resulted in a dose-dependent decrease in immobility time. At 50 mg/kg, immobility was reduced to 107.20 ± 5.85 s (FST) and 83.21 ± 4.57 s (TST). The 100 mg/kg dose showed a greater reduction with 62.24 ± 5.20 s (FST) and 49.82 ± 3.33 s (TST). A marked decrease was observed at 200 mg/kg, with values of 22.60 ± 1.52 s (FST) and 18.53 ± 1.62 s (TST). The highest dose, 400 mg/kg, exhibited the strongest effect, reducing immobility to 21.50 ± 1.08 s (FST) and 16.48 ± 0.83 s (TST).

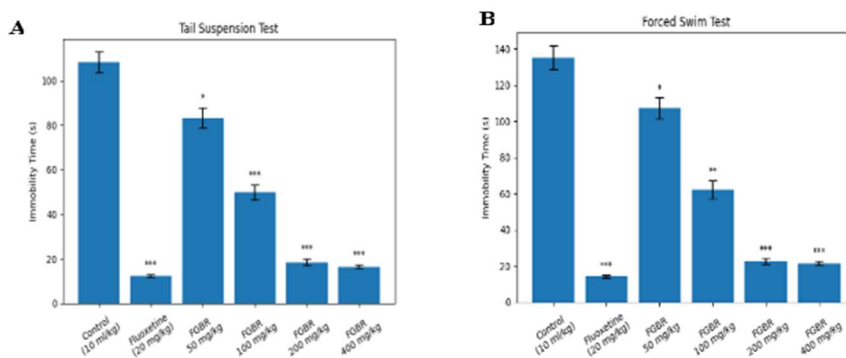


Figure-4: Forced Swimming Test and Tail Suspension Test. A) Tail Suspension Test, B) Forced Swim Test

Biochemical Estimation

The effect of fermented GBR on brain catecholamine levels was evaluated by measuring noradrenaline, dopamine, and serotonin concentrations. The control group showed baseline levels of 18.75 ± 1.22 ng/100 mg (noradrenaline), 3.76 ± 0.21 ng/100 mg (dopamine), and 9.54 ± 0.34 ng/100 mg (serotonin). The standard drug, fluoxetine (20 mg/kg), significantly elevated these levels to 41.67 ± 2.10 , 6.80 ± 0.43 , and 18.34 ± 0.95 ng/100 mg, respectively. Administration of fermented GBR resulted in a dose-dependent increase in all three neurotransmitters. The

50 mg/kg dose showed minimal changes with 19.20 ± 1.36 (noradrenaline), 3.86 ± 0.14 (dopamine), and 10.24 ± 0.80 (serotonin). At 100 mg/kg, moderate increases were observed, reaching 26.90 ± 1.66 , 4.53 ± 0.22 , and 11.30 ± 1.20 ng/100 mg, respectively. A more pronounced elevation was seen at 200 mg/kg, with values of 31.74 ± 1.23 (noradrenaline), 5.34 ± 0.27 (dopamine), and 14.76 ± 1.06 (serotonin). The highest dose, 400 mg/kg, produced the greatest increase, showing 38.08 ± 2.65 , 5.94 ± 0.33 , and 17.50 ± 0.98 ng/100 mg, respectively.

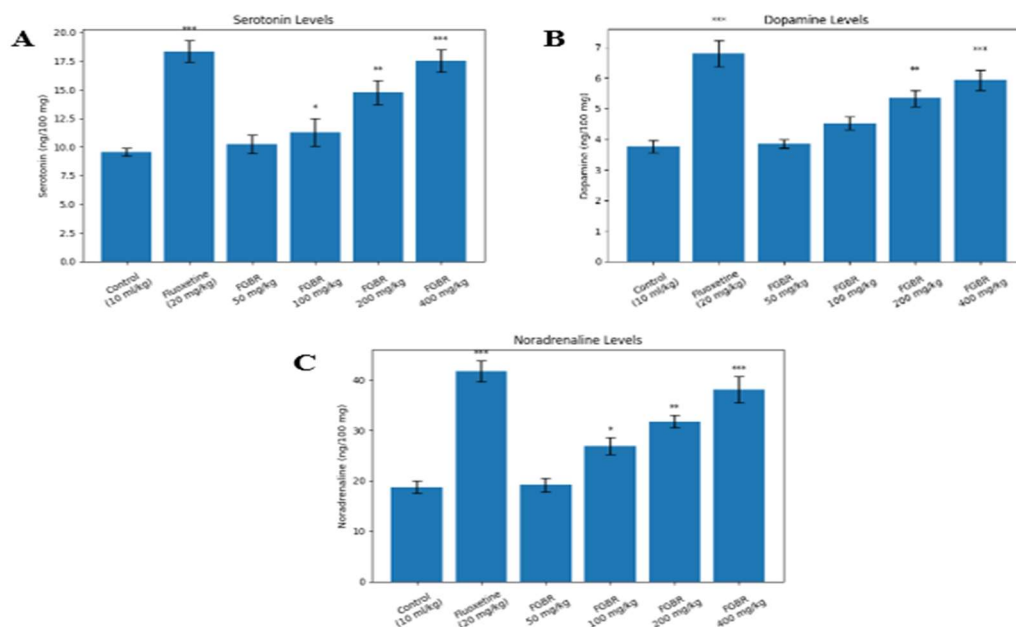


Figure-5: Biochemical Estimation. A) Serotonin, B) Dopamine, C) Noradrenaline

DISCUSSION

The present study demonstrates that fermentation of germinated brown rice (GBR) using *Lentilactobacillus parabuchneri* (MTCC-13315) significantly enhances its functional and neuroactive properties through GABA biosynthesis and enrichment of phenolic compounds (Hur et al., 2014). The multi-level evidence obtained from chromatographic, biochemical, and in vivo analyses provides a coherent mechanistic framework linking microbial metabolism to antidepressant-like effects (Godfrey et al., 2025).

Initial TLC screening indicated the absence of detectable GABA in unfermented GBR, whereas both the probiotic culture and fermented GBR (FGBR) exhibited distinct amino compound bands. This observation was substantiated by HPLC analysis, where the GABA standard showed a retention time of 3.263 min. Corresponding peaks observed in FGBR (3.314 min) and probiotic CFS (3.381 min) confirm

that *L. parabuchneri* actively produces GABA during fermentation (Sasikumar et al., 2026). This is consistent with the known metabolic capability of lactic acid bacteria possessing the glutamate decarboxylase (GAD) system, which catalyses the conversion of glutamate into GABA as part of acid resistance and energy regulation mechanisms (Mafe et al., 2025).

Quantitative analysis revealed that FGBR contained higher GABA levels (22.32 mg/mL) than the probiotic CFS (14.23 mg/mL), indicating that the GBR matrix plays a critical role in enhancing GABA accumulation (Oh et al., 2004). This suggests that *L. parabuchneri* not only synthesizes GABA but also efficiently utilizes glutamate-rich substrates present in GBR, leading to increased metabolite retention. The observed 1.57-fold higher GABA content in FGBR highlights a synergistic interaction between microbial enzymatic activity and substrate composition, which is a key

factor in developing functional fermented foods (Patil et al., 2011).

In addition to GABA production, FGBR exhibited elevated levels of total phenolic content (TPC) and total flavonoid content (TFC), with flavonoids being predominant. Fermentation by *L. parabuchneri* likely facilitates the enzymatic release of bound phenolics through the action of esterases and glycosidases, resulting in enhanced bioavailability and bioactivity (Huihui et al 2024). This biochemical transformation is reflected in the significant antioxidant activity observed in the DPPH assay ($IC_{50} \approx 51 \mu\text{g/mL}$), where the combined effect of GABA and phenolic compounds contributes to effective free radical scavenging (Lobo et al., 2010).

The anti-inflammatory activity of FGBR, demonstrated through inhibition of albumin denaturation, further supports the functional enhancement induced by fermentation (Dharmadeva et al., 2018). Phenolic compounds are known to stabilize protein structures and inhibit inflammatory mediators, while GABA has been reported to modulate immune responses and reduce pro-inflammatory signalling (Chen et al., 2017). The moderate IC_{50} value ($63 \mu\text{g/mL}$) indicates that FGBR possesses biologically relevant anti-inflammatory potential (El Khomsi et al., 2024).

Similarly, the α -amylase inhibitory activity suggests that fermentation enhances the metabolic regulatory properties of GBR. The presence of bioactive compounds capable of interacting with digestive enzymes contributes to reduced carbohydrate hydrolysis, supporting potential applications in glycaemic control (Imam et al., 2012). Although the inhibitory activity was lower than that of acarbose, it remains significant within the context of functional food development.

The in vivo antidepressant studies provide strong evidence linking these biochemical changes to physiological outcomes. Behavioural assessments, including the open field test, forced swim test (FST), and tail suspension test (TST), demonstrated dose-dependent improvements, with higher doses of FGBR (200–400 mg/kg) showing effects comparable to fluoxetine (Machado et al., 2012). These behavioural changes are indicative of reduced depressive-like states and enhanced central nervous system activity (Promtang et al., 2023).

Biochemical analysis of brain neurotransmitters further substantiated these findings, showing significant increases in serotonin, dopamine, and

noradrenaline levels following FGBR administration (Schlumpf et al., 1974). The role of GABA in modulating neuronal excitability and influencing monoaminergic systems provides a plausible mechanistic explanation for these observations (Godfrey et al., 2025). Additionally, the antioxidant and anti-inflammatory properties of phenolic compounds likely contribute to neuroprotection by reducing oxidative stress and neuroinflammation, both of which are closely associated with depression (Bai et al., 2024; Santhiya et al., 2026).

Overall, the findings establish a clear mechanistic pathway in which *Lentilactobacillus parabuchneri* (MTCC-13315) mediates the bioconversion of glutamate into GABA within the GBR matrix, accompanied by the release of phenolic bioactive (Huret al., 2014). This dual enhancement leads to improved antioxidant, anti-inflammatory, and metabolic regulatory properties, ultimately translating into significant antidepressant-like effects in vivo (Mamiya T et al., 2007; Sakthivel et al., 2026). The study highlights the potential of strain-specific fermentation as an effective strategy for developing functional foods with neuroactive benefits (Tyagi et al., 2025).

CONCLUSION

Fermentation of germinated brown rice (GBR) with *Lentilactobacillus parabuchneri* (MTCC-13315) markedly improved its functional and neuroprotective attributes by promoting GABA synthesis and increasing the levels of bioactive phenolic and flavonoid compounds. Analytical investigations confirmed efficient GABA formation in fermented GBR, while functional assays demonstrated enhanced antioxidant, anti-inflammatory, and α -amylase inhibitory properties. In addition, behavioural assessments in experimental models indicated significant antidepressant-like activity, reflected through improved locomotor and stress-response behaviours alongside increased concentrations of key neurotransmitters, including serotonin, dopamine, and noradrenaline.

Collectively, these findings highlight the potential of probiotic fermentation as an effective strategy to enhance the bioactivity of GBR and generate a GABA-rich functional food with possible psychobiotic benefits. The study supports the prospect of fermented GBR as a natural dietary intervention for promoting mental well-being and addressing depressive conditions. Nevertheless, further mechanistic investigations and well-designed clinical studies are necessary to substantiate these findings and determine

their applicability in human health and therapeutic settings.

ETHICS COMMITTEE APPROVAL

The study was approved (Proposal No: SLIMS/01/IAECC2021-22 and Approval No: 932/Po/Pe/S/06/CPCSEA) by IAEC of Sri Lakshmi Narayana Institute of Medical Sciences, Puducherry, India.

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Conflict of interest

The authors have no conflicts of interest to declare.

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