Research Article

Evaluation of the antidepressant effect of *Lactobacillus casei* strain shirota in an ethanol-induced depression mouse model through behavioral, biochemical, neuroinflammatory, and histopathological assessment

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How to cite this article: Tarale P, Burakle PV, Ande SN. Evaluation of the antidepressant effect of *Lactobacillus casei* strain shirota in an ethanol-induced depression mouse model through behavioral, biochemical, neuroinflammatory, and histopathological assessment. Innov Pharm Pharmacother 2026;14(3):40-47.

Source of Support: Nil
Conflicts of Interest: None declared.

ABSTRACT

Introduction: Depression is a complex neuropsychiatric disorder associated with behavioral abnormalities, neurotransmitter imbalance, oxidative stress, and neuroinflammation. Chronic alcohol exposure and withdrawal contribute to depressive-like behavior through disruption of neuronal function. The gut-brain axis has gained attention as a potential therapeutic target, with probiotics showing promising neuroprotective effects. **Aims:** This study evaluated the antidepressant-like effects of *Lactobacillus casei* strain Shirota LcS () in an ethanol-induced depression mouse model by assessing behavioral, biochemical, inflammatory, and histopathological changes. **Materials and Methods:** Male Swiss albino mice were divided into five groups ($n = 5$). Depression-like behavior was induced by gradual ethanol exposure for 14 days, followed by withdrawal. LcS (0.5×10^9 and 1×10^9 colony forming unit [CFU]) was administered orally for 14 days. Behavioral assessments included the open field test, elevated plus maze, sucrose preference test, tail suspension test, and light/dark box test. Brain tissues were analyzed for γ-aminobutyric acid (GABA), malondialdehyde, tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1β, and IL-6 levels, along with hippocampal histopathological evaluation. **Results:** Ethanol exposure significantly induced depressive-like behavior, decreased GABA levels, increased lipid peroxidation, elevated pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6), and caused hippocampal neuronal damage ($P < 0.001$). LcS treatment, particularly at 1×10^9 CFU, significantly improved behavioral abnormalities, partially restored GABA levels, reduced oxidative stress and proinflammatory cytokines without completely normalizing them, and preserved hippocampal neuronal architecture. The observed findings are consistent with modulation of the microbiota-gut-brain axis, although this mechanism was not directly investigated. **Conclusion:** LcS demonstrated antidepressant-like activity in this preclinical model by improving behavioral outcomes, partially restoring neurotransmitter balance, reducing oxidative stress and neuroinflammation, and preserving hippocampal integrity. These findings are consistent with a potential role of the microbiota-gut-brain axis, although this mechanism remains to be confirmed. LcS warrants further mechanistic studies and clinical evaluation as a potential psychobiotic intervention for alcohol-associated depression.

Keywords: Depression, ethanol withdrawal, gut-brain axis, *Lactobacillus casei* strain Shirota, probiotic

Introduction

Depression is one of the most prevalent neuropsychiatric disorders and is characterized by persistent sadness, anhedonia, impaired cognition, and reduced quality of life. It affects more than 280 million people worldwide and is a major contributor to disability and disease burden. Although conventional antidepressants such as selective serotonin reuptake inhibitors and benzodiazepines are widely used, their clinical utility is often limited by delayed onset of action, adverse effects, and incomplete therapeutic response. Therefore, the development of safer and more effective therapeutic approaches remains an important research priority.[1,2]

Access this article online	
Website: www.innpharmacotherapy.com	**e-ISSN:** 2321-323X
DOI: 10.31690/ipp.2026.v014i03.006	**p-ISSN:** 2395-0781

Chronic alcohol consumption is an important risk factor for the development of depressive disorders. Ethanol exposure and subsequent withdrawal disrupt neurotransmitter systems, including γ-aminobutyric acid (GABA), dopamine, serotonin, and glutamate, while simultaneously increasing oxidative stress, neuroinflammation, and neuronal damage, particularly in the hippocampus. These pathological changes contribute to depressive- and anxiety-like behaviors, making ethanol withdrawal animal models valuable tools for evaluating novel antidepressant therapies.[3]

Recent research has highlighted the crucial role of the microbiota-gut-brain axis in regulating brain function and emotional behavior. Alterations in gut microbiota composition have been associated with depression and other neuropsychiatric disorders. Consequently, probiotics have emerged as promising psychobiotic agents capable of influencing central nervous system function through immune, endocrine, metabolic, and neural pathways.[4]

Among probiotics, *Lactobacillus casei* strain Shirota (LcS) is one of the most extensively studied strains due to its antioxidant, anti-inflammatory, and immunomodulatory properties.[5] Previous studies have shown that LcS can improve intestinal barrier function, regulate cytokine production, reduce systemic inflammation, and influence neurotransmitter synthesis. Since neuroinflammation, oxidative stress, and impaired GABAergic neurotransmission are key mechanisms underlying alcohol-induced depression, LcS may provide neuroprotective and antidepressant benefits through modulation of the gut-brain axis.[6]

Therefore, the present study was undertaken to evaluate the antidepressant effect of LcS in an ethanol-induced depression mouse model. Behavioral assessments were complemented by biochemical estimation of GABA, oxidative stress marker malondialdehyde (MDA), pro-inflammatory cytokines (tumor necrosis factor-alpha [TNF-α], interleukin [IL]-1β, and IL-6), and histopathological examination of the hippocampus to comprehensively investigate the neuroprotective mechanisms of probiotic treatment.

Materials and Methods

Chemicals and reagents

Commercial Yakult® (Yakult Danone India Pvt. Ltd., Sonipat, Haryana, India) containing LcS (6.5×10^9 colony-forming unit [CFU]/65 mL) was used as the probiotic source. Analytical-grade chemicals, including ethanol, dimethyl sulfoxide, hydrochloric acid, sodium hydroxide, hydrogen peroxide, trichloroacetic acid, and thiobarbituric acid, were obtained from standard laboratory suppliers. Commercial enzyme-linked immunosorbent assay (ELISA) kits for GABA, TNF-α, IL-1β, and IL-6 were purchased from KRISHGEN Biosystems (Mumbai, India) [Figure 1].

Experimental animals[7]

Adult male Swiss albino mice (7–8 weeks old, 22–25 g) were obtained from the institutional animal facility and housed under standard laboratory conditions (25 ± 2°C, 60–65% humidity, 12 h light/dark cycle) with free access to food and water. Animals were acclimatized for 1 week before the study. All experimental procedures were approved by the Institutional Animal Ethics Committee (Approval No. 2240/PO/Re/S/23/CCSEA) and conducted according to CPCSEA guidelines.

Preparation of LcS suspension

Yakult® was centrifuged at 4000 × g for 10 min at 4°C to obtain the bacterial pellet. The pellet was washed twice with sterile phosphate-buffered saline (PBS) and resuspended in PBS. Fresh suspensions containing 0.5×10^9 CFU and 1×10^9 CFU were prepared daily for oral administration.

Experimental design

Twenty-five mice were randomly divided into five groups ($n = 5$):

- Group I: Normal control (normal saline).
- Group II: Disease control (ethanol withdrawal).
- Group III: Standard treatment (diazepam, 1 mg/kg, i.p.).
- Group IV: LcS low dose (0.5×10^9 CFU/day, p.o.).
- Group V: LcS high dose (1×10^9 CFU/day, p.o.).

Induction of ethanol withdrawal-induced depression

Depression-like behavior was induced by administering progressively increasing concentrations of ethanol (2%, 5%, 10%, 15%, and 20% v/v) in drinking water for 14 consecutive days, followed by a 4-day withdrawal period. During withdrawal, animals underwent the sucrose preference test (SPT). After withdrawal, diazepam or probiotic treatment was administered daily for 14 days.

Behavioral assessment[8]

Behavioral tests were conducted between 09:00 and 15:00 h under identical environmental conditions.

Open field test (OFT)

Each mouse was placed in the center of a 60 × 60 cm open field arena for 5 min. Time spent in the central and peripheral zones was recorded to assess locomotor activity and anxiety-like behavior.

Elevated plus maze (EPM)

Animals were placed at the center of the maze facing an open arm and observed for 5 min. Time spent and entries into the open and closed arms were recorded.

SPT

Anhedonia was assessed using a two-bottle choice test (1% sucrose solution and water). Sucrose preference (%) was calculated as:

$$\text{Sucrose preference } (\%) = \frac{\text{Sucrose intake}}{\text{Total fluid intake}} \times 100$$

Tail suspension test (TST)

Mice were suspended individually by the tail for 6 min, and total immobility time was recorded as an indicator of depressive-like behavior.

Light/dark box test

Animals were placed in the dark compartment of the apparatus and allowed to explore freely for 5 min. Time spent in the dark compartment was recorded as a measure of anxiety-like behavior.

Biochemical analysis[9]

Following behavioral studies, mice were euthanized, and brains were rapidly excised and homogenized (10% w/v) in phosphate buffer (0.1 M, pH 7.4). The homogenates were centrifuged, and the supernatants were used for biochemical estimations.

Estimation of GABA

Brain GABA levels were measured using a commercial ELISA kit according to the manufacturer's instructions.

Lipid peroxidation assay

Lipid peroxidation was determined by estimating MDA using the thiobarbituric acid reactive substances method, and absorbance was measured at 532 nm.

Estimation of pro-inflammatory cytokines

Brain concentrations of TNF-α, IL-1β, and IL-6 were quantified using commercial sandwich ELISA kits following the manufacturer's protocol.

Histopathological examination[10]

Brain tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 μm, stained with hematoxylin and eosin, and examined microscopically for hippocampal neuronal degeneration and structural alterations.

Statistical analysis

Data are presented as mean ± standard error of the mean. Statistical analysis was performed using GraphPad Prism software. One-way analysis of variance followed by Dunnett's multiple comparison test was used to compare groups. A value of $P < 0.05$ was considered statistically significant.

Results

Effect of LcS on behavioral parameters

OFT

Ethanol withdrawal significantly reduced the time spent in the center zone (13.3 ± 0.33 s) compared with the normal control group (40.0 ± 0.67 s; ***$P < 0.001$; $F(4,10) = 128$) and increased peripheral activity (156.33 ± 1.95 s vs. 119.30 ± 2.14 s; **$P < 0.002$). Treatment with LcS improved exploratory behavior in a dose-dependent manner. The higher dose (1×10^9 CFU) significantly increased center-zone time (32.0 ± 0.53 s) and reduced peripheral activity (132.67 ± 3.35 s), while the lower dose showed moderate improvement [Table 1 and Figure 2].

Table 1: Time Spent at center zone and periphery zone (sec) in OFT

S. No.	Groups	Time spent at center zone (s) Mean±SEM	Time spent at periphery zone (s) Mean±SEM
1.	Control	40±0.67	119.3±2.14
2.	Disease	13.3±0.33	156.33±1.95
3.	Standard	34.67±0.55	129.3±2.21
4.	Treatment 1 (0.5×10^9 CFU)	27.33±0.51	144.3±3.50
5.	Treatment 2 (1×10^9 CFU)	32±0.53	132.67±3.35

OFT: Open field test, SEM: Standard error of the mean, CFU: Colony forming unit

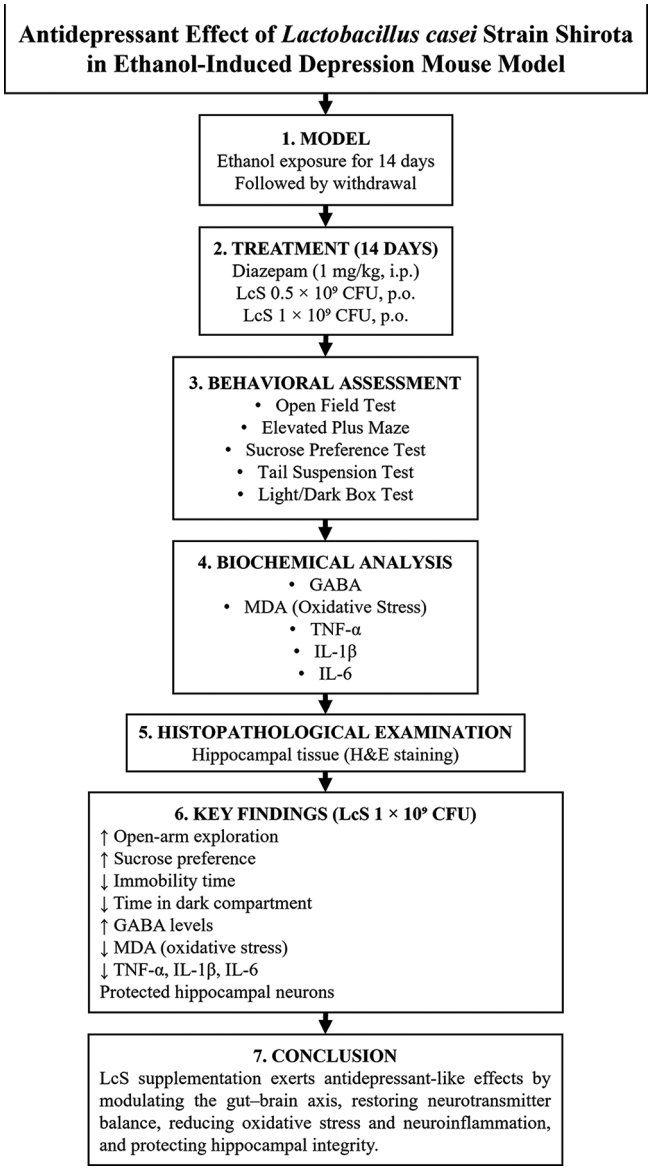

Figure 1: Schematic representation of the experimental design and antidepressant effects of *Lactobacillus casei* strain Shirota in an ethanol-induced depression mouse model

EPM test

Ethanol withdrawal significantly decreased time spent in the open arms (13.87 ± 0.46%) and increased time in the closed arms (82.27

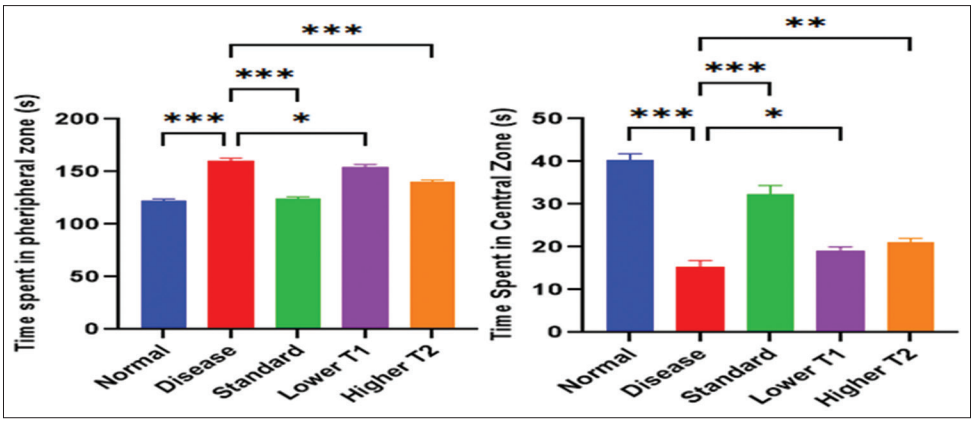

Figure 2: Effect of *Lactobacillus casei* strain shirota on time spent in the center zone and periphery zone (sec) in the open field test Note: $p < 0.05$, $p < 0.001$.

± 0.33%) compared with normal controls (***$P < 0.001$). Treatment with the higher probiotic dose significantly increased open-arm time (24.45 ± 1.08%) and open-arm entries (57.33 ± 1.01%) while reducing closed-arm time (66.70 ± 0.63%), indicating reduced anxiety-like behavior [Table 2].

SPT

Sucrose preference was significantly reduced in the disease control group (52.75 ± 0.52%) compared with normal controls (85.0 ± 0.73%; ***$P < 0.001$; $F(4,15) = 84.5$). The higher probiotic dose restored sucrose preference to 64.45 ± 0.80%, whereas the standard treatment showed 71.0 ± 0.69% [Table 3].

TST

Immobility time increased significantly following ethanol withdrawal (194.60 ± 1.71 s) compared with normal controls (110.60 ± 2.33 s; ***$P < 0.001$; $F(4,10) = 142$). Treatment with the higher probiotic dose reduced immobility time to 121.0 ± 0.33 s, comparable to the standard treatment (116.30 ± 2.77 s) [Table 3].

Light/dark box test

The disease control group spent significantly more time in the dark compartment (128.67 ± 1.50 s) than normal controls (65.67 ± 0.69 s; ***$P < 0.001$; $F(4,10) = 602$). Treatment with LcS significantly reduced dark-compartment time, with the higher dose (80.30 ± 0.91 s) showing greater efficacy than the lower dose [Table 3].

Table 2: Percentage time spent at open arm, closed arm, and No. of entry in open arm in EPM

S. No.	Groups	Percentage time spent at open arm Mean±SEM	Percentage time spent at closed arm Mean±SEM	Percentage No. of entry in open arm Mean±SEM
1.	Control	40.6±0.83	54±0.88	84.67±1.34
2.	Disease	13.87±0.46	82.27±0.33	33±0.88
3.	Standard	33.70±1.33	55.37±0.80	73.33±1.18
4.	Treatment 1 (0.5×10^9 CFU)	21.36±0.65	70.11±0.64	41.33±0.83
5.	Treatment 2 (1×10^9 CFU)	24.45±1.08	66.7±0.63	57.33±1.01

EPM: Elevated plus maze, SEM: Standard error of the mean, CFU: Colony forming unit

Table 3: Percentage sucrose intake, percentage immobility time, and time spent in dark compartment

S. No.	Groups	Percentage sucrose intake Mean±SEM	Percentage immobility time Mean±SEM	Time spent in dark compartment Mean±SEM
1.	Control	85±0.73	110.6±2.33	65.67±0.69
2.	Disease	52.75±0.52	194.6±1.71	128.67±1.50
3.	Standard	71±0.69	116.3±2.77	73.67±0.70
4.	Treatment 1 (0.5×10^9 CFU)	52±0.77	185.6±0.69	120.67±1.01
5.	Treatment 2 (1×10^9 CFU)	64.45±0.80	121±0.33	80.3±0.91

SEM: Standard error of the mean, CFU: Colony forming unit

Effect on neurotransmitter and oxidative stress markers

Brain GABA levels

Brain GABA levels were significantly reduced after ethanol withdrawal (102.46 ± 2.04 pg/mL) compared with controls (248.26 ± 0.87 pg/mL; ***$P < 0.001$; $F(4,10) = 398$). The higher probiotic dose significantly increased GABA levels to 162.70 ± 0.88 pg/mL [Table 4 and Figure 3].

Lipid peroxidation

MDA levels increased significantly in the disease control group (5.50 ± 0.10 nmol/mL) compared with normal controls (1.90 ± 0.03 nmol/mL; ***$P < 0.001$; $F(4,10) = 156$). Treatment with the higher probiotic dose reduced MDA levels to 4.00 ± 0.06 nmol/mL [Table 4 and Figure 3].

Effect on neuroinflammatory cytokines

TNF-α

TNF-α levels were significantly elevated following ethanol withdrawal (432.10 ± 0.82 pg/mL) compared with controls (285.70 ± 1.21 pg/mL; ***$P < 0.001$; $F(4,10) = 743$). The higher probiotic dose reduced TNF-α to 331.50 ± 0.74 pg/mL [Table 5 and Figure 4].

IL-1β

IL-1β concentrations increased significantly after ethanol withdrawal (50.67 ± 0.42 pg/mL) compared with controls

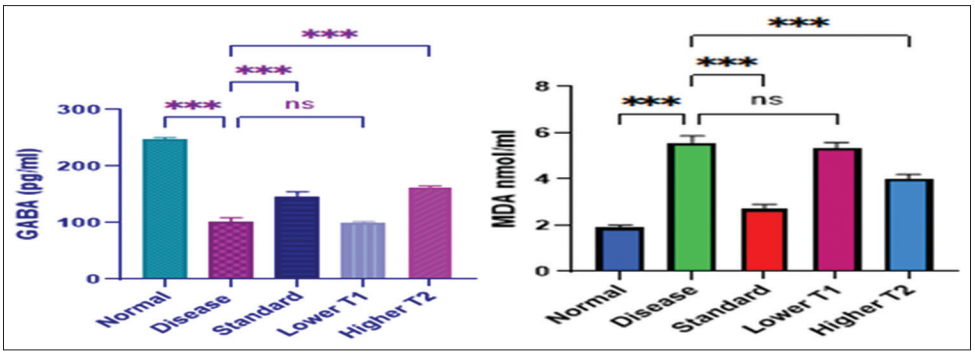

Figure 3: Effect on neurotransmitter and oxidative stress markers Note: $p < 0.001$.

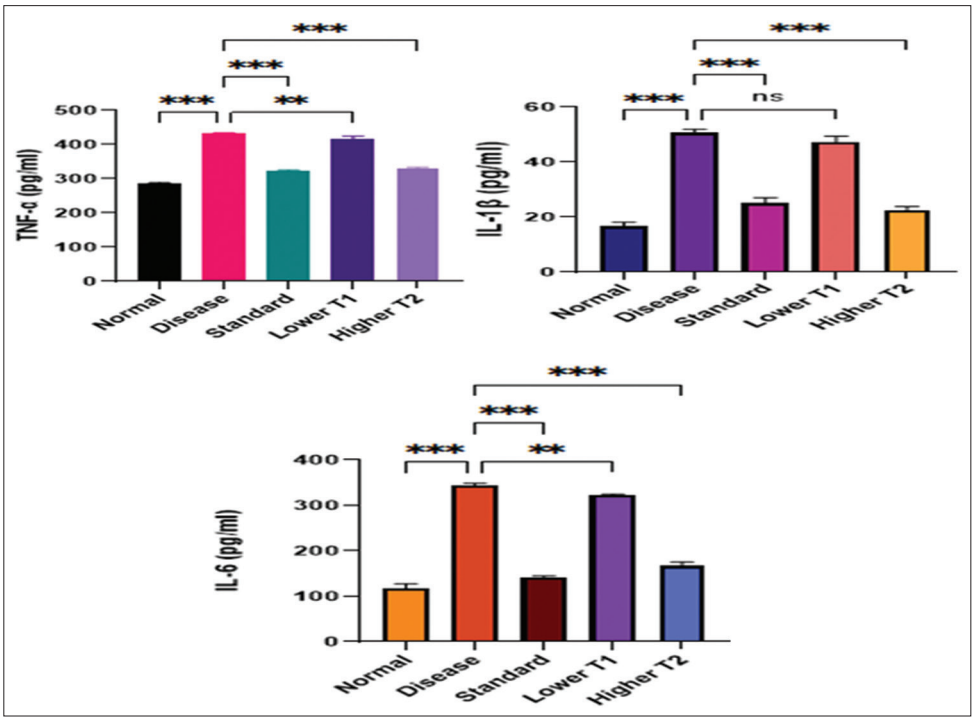

Figure 4: Concentration of neuroinflammatory cytokines in brain tissue Note: $p < 0.01$, $p < 0.001$.

Table 4: Effect on neurotransmitter and oxidative stress markers

S. No.	Groups	GABA neurotransmitter	Lipid peroxide
		Concentration (pg/mL) Mean±SEM	Mean of Concentration (nmol/mL)
1.	Control	248.26±0.87	1.9±0.03
2.	Disease	102.46±2.04	5.5±0.10
3.	Standard	112.3±3.03	2.7±0.06
4.	Treatment 1 (0.5×10^9 CFU)	100.5±0.55	5.2±0.08
5.	Treatment 2 (1×10^9 CFU)	162.7±0.88	4.0±0.06

SEM: Standard error of the mean, GABA: γ-aminobutyric acid, CFU: Colony forming unit

Table 5: Concentration of neuroinflammatory cytokines in brain tissue

S. No.	Groups	TNF-α	IL-1β	IL-6
		Mean of concentration (pg/mL)	Mean of concentration (pg/mL)	Mean of concentration (pg/mL)
1.	Control	285.7±1.21	16.67±0.50	117±3.4
2.	Disease	432.1±0.82	50.67±0.42	345.3±1.2
3.	Standard	323.1±0.93	25±0.66	140.9±1.4
4.	Treatment 1 (0.5×10^9 CFU)	417.3±2.33	47.33±0.69	322.1±1.0
5.	Treatment 2 (1×10^9 CFU)	331.5±0.74	22±0.53	167.2±2.7

CFU: Colony forming unit, TNF-α: Tumor necrosis factor-alpha, IL: Interleukin

(16.67 ± 0.50 pg/mL; ***$P < 0.001$; $F(4,10) = 246$). Treatment with the higher probiotic dose reduced IL-1β to 22.00 ± 0.53 pg/mL [Table 5 and Figure 4].

IL-6

IL-6 levels were significantly elevated in the disease control group (345.30 ± 1.20 pg/mL) compared with controls (117.00 ±

3.40 pg/mL; ***$P < 0.001$; $F(4,10) = 776$). Administration of the higher probiotic dose reduced IL-6 to 167.20 ± 2.70 pg/mL [Table 5 and Figure 4].

Histopathological evaluation

Histological examination of the hippocampus showed normal neuronal architecture in the control group, whereas ethanol withdrawal caused neuronal degeneration, vacuolization, and reduced neuronal density. Standard treatment and LcS markedly improved hippocampal morphology in a dose-dependent manner, with the higher dose showing near-normal neuronal architecture and minimal tissue damage [Figure 5].

Discussion

The present study evaluated the antidepressant-like activity of LcS in a chronic ethanol withdrawal-induced neurobehavioral dysfunction model in mice. Progressive ethanol concentrations (2%, 5%, 10%, 15%, and 20% v/v) were employed to facilitate gradual adaptation to chronic ethanol exposure while minimizing ethanol aversion, dehydration, and acute toxic effects. This dose-escalation strategy closely resembles the progressive pattern of alcohol consumption observed in humans and promotes the development of physiological dependence through sustained neuroadaptive changes. Chronic ethanol exposure is known to disrupt GABAergic and glutamatergic neurotransmission, increase oxidative stress, and activate neuroinflammatory pathways, thereby establishing a reproducible model of alcohol dependence and withdrawal.

A 14-day ethanol exposure period was selected based on previous experimental evidence demonstrating that continuous ethanol administration for 2 weeks is sufficient to induce alcohol dependence, neurochemical adaptations, hippocampal dysfunction, oxidative stress, and neuroinflammation without causing excessive mortality or severe systemic toxicity. This duration reliably produces behavioral abnormalities resembling anxiety- and depressive-like phenotypes, making it a well-established model for investigating alcohol-induced neurobehavioral dysfunction. Following ethanol exposure, mice underwent a 4-day withdrawal period to permit the manifestation of withdrawal-associated behavioral and biochemical disturbances. During withdrawal, compensatory neuroadaptations developed during prolonged ethanol exposure become unmasked, resulting in reduced GABAergic inhibitory tone, enhanced glutamatergic excitability, increased oxidative stress, activation of pro-inflammatory signaling pathways, and the emergence of anxiety- and depressive-like behaviors. Previous studies have shown that these neurobiological alterations are most prominent during the early withdrawal phase, supporting the selection of a 4-day withdrawal period for evaluating therapeutic interventions.[11]

Diazepam (1 mg/kg, i.p.) was selected as the standard drug because it remains the first-line pharmacological treatment for alcohol withdrawal syndrome. As a positive allosteric modulator of GABAA receptors, diazepam enhances inhibitory neurotransmission, thereby reducing withdrawal-induced neuronal hyperexcitability, anxiety, and behavioral disturbances. Although selective serotonin reuptake inhibitors (SSRIs), such as fluoxetine, are frequently used as reference drugs in conventional depression models, the present study employed an ethanol withdrawal-induced neurobehavioral model in which restoration of GABAergic neurotransmission represents the primary therapeutic target. Therefore, diazepam served as an appropriate positive control for validating the experimental model and comparing the efficacy of the probiotic intervention.[12,13]

The successful establishment of the ethanol withdrawal-induced neurobehavioral dysfunction model was confirmed using complementary behavioral, biochemical, and histopathological assessments. Behavioral validation was demonstrated by reduced locomotor exploration in the OFT, decreased open-arm exploration in the EPM, reduced sucrose preference, prolonged immobility in the TST, and increased time spent in the dark compartment of the Light/Dark Box Test, collectively indicating anxiety- and depressive-like behaviors. Biochemical analyses further confirmed the model through

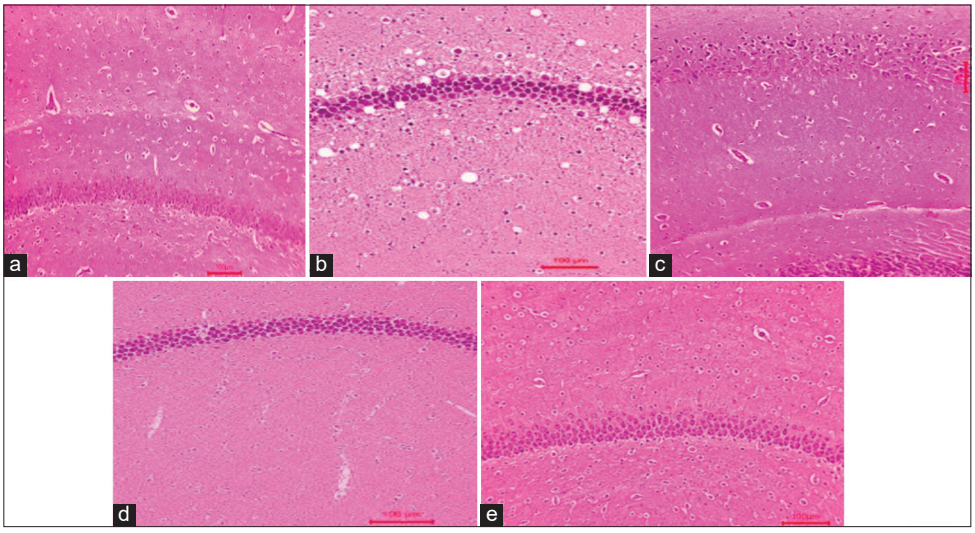

Figure 5: Histopathological changes in the ethanol withdrawal mice compared the control mice. (a) Control group, (b) disease control group, (c) standard group, (d) treatment group (0.5×10^9 colony-forming unit [CFU]), and (e) treatment group (1×10^9 CFU)

significantly reduced brain GABA concentrations together with increased MDA, TNF-α, IL-1β, and IL-6 levels, reflecting oxidative stress and neuroinflammation. Histopathological examination revealed marked hippocampal neuronal degeneration, vacuolization, and disruption of normal neuronal architecture. Collectively, these findings confirm the successful induction of ethanol withdrawal-associated neurobehavioral dysfunction.[14,15]

It is important to recognize that chronic ethanol withdrawal produces a spectrum of emotional disturbances encompassing both anxiety-like and depressive-like behaviors, which share common underlying mechanisms, including impaired GABAergic neurotransmission, oxidative stress, neuroinflammation, and hippocampal dysfunction. Consequently, several behavioral paradigms employed in the present study assess overlapping behavioral domains. The OFT, EPM, and light/dark box test primarily evaluate anxiety-related behavior by measuring exploratory activity and aversion to anxiogenic environments, whereas the SPT assesses anhedonia, a core symptom of depression, and the TST measures behavioral despair, a widely accepted indicator of depressive-like behavior. Integration of these complementary behavioral paradigms with biochemical and histopathological analyses provides a comprehensive assessment of ethanol withdrawal-induced neurobehavioral dysfunction and strengthens the interpretation of the antidepressant-like activity of LcS.[16]

LcS supplementation significantly improved behavioral performance, with the higher dose (1×10^9 CFU) producing greater effects than the lower dose. These findings are consistent with previous reports demonstrating beneficial effects of *Lactobacillus* species on stress- and depression-related behaviors. The present study also demonstrated that ethanol withdrawal markedly reduced brain GABA concentrations, whereas LcS treatment partially restored GABA levels. As GABA is the principal inhibitory neurotransmitter regulating emotional behavior, partial restoration of GABAergic signaling may have contributed to the observed behavioral improvements. Previous studies have suggested that probiotics can influence neurotransmitter metabolism through microbial, neural, endocrine, and immune-mediated pathways, although these mechanisms were not directly investigated in the present study.[17]

Oxidative stress represents another major consequence of chronic ethanol exposure. The marked elevation in brain MDA levels observed in ethanol-withdrawn mice indicated enhanced lipid peroxidation, whereas LcS treatment significantly reduced MDA concentrations, suggesting attenuation of oxidative damage. Similarly, ethanol withdrawal significantly increased brain TNF-α, IL-1β, and IL-6 levels, while LcS treatment significantly reduced these proinflammatory cytokines, although their concentrations were not completely normalized. These findings indicate that LcS attenuated, but did not fully reverse, ethanol-induced oxidative stress and neuroinflammation, consistent with previous reports describing the antioxidant and immunomodulatory properties of probiotic supplementation.

Histopathological examination further supported the neuroprotective effects of LcS. Ethanol withdrawal produced neuronal degeneration, vacuolization, and disruption of hippocampal architecture, whereas probiotic treatment preserved neuronal morphology and improved hippocampal integrity in a dose-dependent manner. These morphological improvements are consistent with the observed behavioral and biochemical changes and suggest that attenuation of oxidative stress and inflammatory injury contributed to preservation of hippocampal structure.[16,17]

Overall, the progressive ethanol exposure protocol followed by a defined withdrawal period effectively reproduced neurobehavioral alterations associated with chronic alcohol abuse. The significant behavioral impairments, reduced GABA levels, elevated oxidative stress and inflammatory markers, together with hippocampal neuronal damage observed in the disease control group, validate the reliability of the experimental model and support its suitability for evaluating psychobiotic interventions. LcS demonstrated antidepressant-like activity by improving behavioral outcomes, partially restoring GABAergic neurotransmission, reducing oxidative stress and neuroinflammation, and preserving hippocampal architecture. Although these findings are consistent with a potential role of the microbiota–gut–brain axis, this mechanism was not directly examined in the present study and therefore requires further mechanistic investigation. Additional preclinical and clinical studies are warranted to establish the therapeutic potential of LcS for alcohol withdrawal-associated depression.

Conclusion

The present study concludes that LcS significantly attenuates ethanol withdrawal-induced depressive-like behavior and neurobiological alterations in mice. LcS restored GABA levels, reduced oxidative stress and neuroinflammation, and protected hippocampal neurons. These findings suggest that LcS is a promising psychobiotic for the management of alcohol-related depression and warrants further clinical investigation.

Acknowledgments

The authors would like to thank the management of Dr. Rajendra Gode Institute of Pharmacy, Amravati, for their administrative support, continuous encouragement, and for providing the necessary facilities to conduct this study.

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