ORIGINAL ARTICLE

Probiotics Supplementation Alleviates Endotoxemia-induced Behavioral Deficits in Mice

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Abstract

Objective: The gut microbiota plays a critical role in maintaining normal mental health. Studies have highlighted the role of probiotics in monitoring the bacterial population and modulating the gut-brain axis. Probiotic supplementation has received considerable attention as a potential therapeutic regime for the treatment of behavioral abnormalities. The present study elucidated the modulatory role of probiotics alone or as adjuncts with an agonist of the antipsychotic peptide neurotensin (NTS_1) on behavioral deficits induced by endotoxin, bacterial lipopolysaccharide (LPS). **Materials and Methods:** Five groups of mice were maintained: control, LPS, LPS + probiotics (Pro), LPS + NTS agonist (PD), and LPS + pro + PD. Mice were challenged with LPS (1 mg/kg bw) for 5 days, followed by supplementation of probiotics (0.6 g/kg bw), PD (50 µg/kg bw), and a combination of pro and PD for 4 weeks. **Results:** LPS-exposed mice showed sickness behavior (decrease in food and water intake), anxiety behavior (reduction in time duration, entries in the central square in the open-filed arena, and entries in the open arm in the elevated plus maze), and depressive behavior (increment in immobility time in the force swimming test). A significant reduction in sickness, anxiety, and depressive behaviors as compared to their LPS counterparts on supplementation of probiotics alone or in combination withNTS_1 agonist; the efficacy was enhanced in the latter. Probiotics supplementation alone or in combination with NTS_1 agonist, reduced the plasma levels of tumor necrosis factor-α, interleukin-6 (IL-6), and corticosterone, and increased plasma levels of IL-10 might be due to increased beneficial bacteria *Bifidobacteria* and a decreased population of harmful bacteria *Clostridia.* **Conclusion:** Probiotic supplementation has the potential to prevent behavioral abnormalities, restore the homeostasis of the microbiota, and may be used as a therapeutics adjunct along with other anti-psychotic drugs for mental illness.

Key words: Endotoxemia, inflammation, lipopolysaccharide, multi-stain probiotics, pro-inflammatory cytokines

INTRODUCTION

The gastrointestinal tract (GI tract) harbors trillions of microbes that form a complex and dynamic system that plays a crucial role in maintaining the homeostasis of the host.[1] The GI tract and the central nervous system (CNS), under the influence of the gut microbiota, participate in reciprocal communication. In this regard, the emerging gut-brain axis concept suggests a bidirectional interaction between the GI tract and the CNS involved in the modulation of neural, immune, and endocrine functions.[2] In view of the fact that the gut microbiome has been linked to anxiety and cognitive functioning in both humans and rodent models.[3,4] Perturbations of the gut microbiota homeostasis are linked to anxiety and depressive-like behavior.[4] Gut microbiota disturbances due to certain circumstances, such as infection, food-borne antigens, cause the production of harmful bacterial products, including lipopolysaccharide (LPS).[5] LPS is involved in the innate immune system and is detected by receptors on immune cells such as macrophages, dendritic cells, and endothelial cells. LPS signals activate the biosynthesis of inflammation mediators (such as tumor necrosis factor [TNF-α] and interleukin-6 [IL-6] through the toll-like receptor 4-linked nuclear factor-κB signaling pathway) to cause inflammation.[6] The overproduction

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of LPS in the gut microbiota increases blood LPS levels through gut inflammation.[7] In addition to this, LPS, through systemic circulation, crosses the blood-brain barrier and induces the activation of the hypothalamic-pituitary adrenal (HPA) axis, as well as inflammation and oxidative stress in the brain, ultimately causes mental comorbidities like anxiety and depression.[2,8] A study has reported that patients with chronic gut disorders such as inflammatory bowel disease commonly exhibit psychiatric comorbidities such as anxiety and depression.[9,10]

Probiotics are live microorganisms whose ingestion in the proper amount benefits the overall health of the host.[11] There is a growing body of evidence that probiotic interventions benefit mental health, cognition, and other behavioral outcomes.[12,13] Treatment of mental illness through the intervention of probiotics is an emerging therapeutic strategy that is clinically practiced by doctors. The preferably used probiotic bacterial species are *Lactobacillus*, *Bifidobacteria,* and other lactic acid bacteria alone or in multistrain form for the prevention of human and animal diseases.[14] Several reports have revealed the psychiatric symptom attenuation effects of the administration of probiotics.[15] Administration of the probiotic *Lactobacillus casei* strain Shirota alleviates stress-associated symptoms by modulating the gut-brain axis in human and animal models.[16] Probiotic *Lactobacillus paracasei HT6* administration showed beneficial effects on anxiety and depressive behavior by improving plasma levels of corticosterone (CORT) and adrenocorticotropic hormone in rats.[17] Modulation of the gut microbiota with multi-strains probiotics, including *Lactobacillus*, *Bifidobacterium,* and *Pediococcus,* reduced the depressive symptoms in mice.[18] Administration of three probiotic strains, *Lactobacillus helveticus, Lactobacillus plantarum*, and *Bifidobacterium longum,* has demonstrated that chronic mild stress-induced anxiety and depressive-like behaviors were ameliorated.[19] The treatment of probiotic *L. plantarum* PS128 has shown psychophysiological effects on major depressive disorder.[20] Probiotic NVP1704, a mixture of *Limosilactobacillus reuteri* NK33 and *Bifidobacterium adolescentis* NK98 ingestion reduced the symptoms of depression and improved sleep quality.[21] Moreover, clinical studies have also suggested that probiotics exert anti-depressive and anxiolytic effects.[22] Although there are animal and human model reports on the attenuation of anxiety and depression by the intervention of probiotics, chronic stress-induced anxiety and depression line of study is limited.

Recently, it has been reported that probiotics modulate the brain level of neuropetides, including neurotensin (NTS). The NTS level in the brain was elevated on supplementation with single or mixed probiotics to prevent the neurotoxic effect induced by propionic acid.[23] NTS is a tridecpetide present in the gut and brain. The NTS1 agonist PD149163 is an analog of NTS.[8-13] It is a brain-penetrant NTS1 agonist that demonstrated its antipsychotic,[24] anxiolytic,[25] and anti-depressant effects.[26]

The present study was carried out to explore the efficacy of the multistrain probiotics (a mixture of *Lactobacillus*, *Bifidobacterium*, and *Streptococcus* containing four strains of *Lactobacillus*, three strains of *Bifidobacterium*, and one strain of *Streptococcus*) administration in ameliorating the LPS-induced behavioral deficits. A comparative assessment was done with the co-administration of the probiotics and the NTS receptor agonist to depict the use of probiotics as a therapeutic adjunct.

MATERIALS AND METHODS

Animals

Swiss albino female mice (25 ± 2 g) of 7 weeks of age purchased from the Central Drug Research Institute (Lucknow, India) were housed in polyvinyl chloride cages and under controlled laboratory conditions (12:12 h light/dark cycle, temperature 23 ± 2°C, and humidity 55 ± 5%). The animals had free access to food (pellets) and water. Before being subjected to experimentation, all the mice were acclimatized for 1 week.

Required chemicals and doses

LPS (*Escherichia coli* O26:B6), NTS_1 agonist PD149163, and the enzyme-linked immunosorbent assay (ELISA) kits for mouse TNF-α (catalogue: RAB0477) and IL-6 (catalogue: RAB0308) were procured from Sigma Aldrich (Bengaluru, India). Probiotics are sold as Visbiome in India (Zydus Heptiza A div. of Cadila Healthcare Ltd., India). Others required chemicals procured from Himedia, Mumbai, India. The dose of LPS (1 mg/kg bw i.p.) and the NTS_1 agonist PD149163 (50 µg/kg bw) was decided from our previous study.[27] NTS_1 agonist PD149163 (50 µg/kg bw) is the half dose of our previous study. The dose of visbiome (0.6 g/kg bw) was suspended in 200 µL of saline and given to mice for a period of 28 days by oral gavageg.[28]

Experimental design

Mice (age: 8 weeks; sex: female; body weight/bw: 25 ± 2 g; total number: 30) were maintained in five groups (6 mice/group) as follows: Group I was the control (0.9% saline), and Group II-Group V mice were exposed to LPS (1 mg/kg bw) intraperitoneally (i.p.) for 5 days. Thereafter, probiotics (0.6 g/kg bw) were orally gavaged to LPS-exposed Group III (LPS + Pro), NTS_1 agonist PD149163 (50 µg/kg bw) was treated to Group IV (LPS + PD), and combined treatment of probiotics (0.6 g/kg bw) and NTS_1 agonist PD149163 (50 µg/kg bw) was given to Group V (LPS + Pro + PD) for 4 weeks. Mice from all groups were maintained for 4 weeks. Every day, the food and water intake of mice was noted. On the day of experiment termination, mice were euthanized with pentobarbital (100 mg/kg bw).

Sickness behavior

Sickness behavior induced by LPS exposure was assessed by measuring body weight loss and food and water intake inhibition. Body weight was measured on a daily basis after LPS treatment until the end of the experiment.

Anxiety-related behavior

Open field test (OFT)

An assessment of anxiety-like behavior (OFT) was performed. The apparatus, an open black wooden box (81 cm × 81 cm interior area) with a 28 cm wall height, was used. The box floor was painted with white lines 3 mm wide to form 16 equal squares (20 cm × 20 cm; 12 outer and 4 inner). The test was initiated by placing a mouse in the center of the box and allowing it to explore freely for 5 min. The total number of entries and time spent in each of the four central squares were recorded (entry of all four paws is considered a single entry). It decreases in time spent (inner zone spent time: IZT) and number of entries (inner zone entry: IZE) are considered as indices of anxiogenic behavior.[29]

Elevated plus maze (EPM)

The EPM test assessed the anxiety-like behavior by measuring the number of entries and time spent in the open arms. EPM is a wooden, plus-shaped apparatus, 80 cm elevated above the ground. The maze is composed of two open arms (30 cm × 5 cm) and two closed arms (30 cm × 5 cm × 15 cm), with each arm having an open roof. A mouse was placed on a central arena (5 cm × 5 cm) facing an open arm and observed for 5 min. The total number of entries into the open arms and time spent in the open arms were recorded. Entrance to an arm was counted where the animal's all four paws were in the arm.[29]

Depressive behavior

Forced swimming test (FST)

The FST is widely used for assessing despair behavior. The following procedure was applied for FST.[29] In brief, each mouse was placed individually into the transparent glass cylinder (height: 25 cm, diameter: 10 cm) filled with water at a height of 15 cm and maintained at a temperature of 25 ± 1°C. After each test, the water in the cylinder was replaced with fresh water to prevent alteration in the behavior of animals due to used water. After 1 min of acclimatization, the immobility time (sec.) of the mouse was scored for another 5 min. Immobility was considered when the mouse ceased struggling and remained motionless, floating in the water, and only necessary movements took place to keep their head above the water. A state of despair behavior was considered when animals lacked struggling.

Cytokine assays

The blood was collected via cardiac puncture in heparinized tubes, and the plasma was separated by centrifugation at 3000 rpm for 15 min and stored at −20°C until use. The plasma levels of TNF-α and IL-6 were determined using ELISA kits following the manufacturer's protocols (Sigma Aldrich). The level of IL-10, the anti-inflammatory cytokine, was assessed using the ELISA kit following the manufacturer's protocol (Krishgen Biosystems, Mumbai, India).

CORT assay

The CORT assay was done following the manufacturer's protocols (Krishgen Biosystems, Mumbai, India). Samples from the control and experimental groups were pooled and assayed in duplicate. The intra-assay and inter-assay coefficients of variation were <15% and <18% of the kit, respectively. For the estimation of CORT level, optical density was measured in an iMark Microplate Reader (Bio-Rad Laboratories, CA, USA) at 450 ± 10 nm.

Enumeration of *Bifidobacteria* and *Clostridia* using fluorescent *in situ* hybridization

The *Bifidobacteria* and *Clostridia* bacteria populations were enumerated using fluorescent *in situ* hybridization.[30] The 16S rRNA-targeted probes (Bif164 and His150, specific for *Bifidobacteria* and *Clostridia,* respectively) were used in the study. The probes were commercially synthesized with 5'-labelled fluorescent dye Cy3 (Sigma, India). Bacterial numbers per g of feces (B) were calculated as B = (N/X) (A/B) (1/S), and the values were given in $\log_{10}$.[31] (N, the number of bacteria counted; X, the number of fields of view [grids]; A, the area of the slide covered by sample; B, the area of the field of view to be measured with an object stage micrometer; and S, the amount of sample on the slide).

Data analysis

All the data were analyzed by using Graph Pad Prism 5.0 (Graph Pad Software Inc., USA). The data were presented as mean ± standard error of the mean (SEM). The intergroup variation was statistically analyzed by one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test, and a two-way ANOVA followed by a Bonferroni post-test was applied for intergroup and week variations. The values were considered statistically significant at $P < 0.05$.

RESULTS

Effect on body weight

The body weight decreased significantly in LPS-exposed mice from day 7 ($P < 0.01$) to become highly significant on

day 14 ($P < 0.001$) and remained until day 28 as compared to the control. In probiotic-co-treated (LPS + Pro) mice, the body weight significantly increased from day 14 onward until day 28 from that of the LPS group ($P < 0.001$) but remained less ($P < 0.05$) as compared to the control. In NTS_1 agonist PD149163 co-treated mice (LPS+ PD), body weight significantly increased from day 14 onward until day 28 ($P < 0.001$) as compared to LPS but remained significantly less ($P < 0.01$) as compared to the control. On combined treatment of the probiotics along with the NTS_1 agonist PD149163 (LPS + Pro + PD), the body weight increased significantly consistently from day 7 ($P < 0.01$) onward to become highly significant on day 14 ($P < 0.001$) and remained so until day 28 from that of LPS and became comparable to the control [Figure 1a].

Effect on food and water intake

In LPS-exposed mice, food and water intake decreased significantly from day 7 ($P < 0.01$) and became highly significant on day 14 ($P < 0.001$) onward and persisted until day 28 as compared to the control. In probiotic-co-treated (LPS + Pro) mice, the food intake was significantly increased from day 14 ($P < 0.001$) onward as compared to the LPS group but remained less ($P < 0.05$) from that of the control group. The water intake in probiotics-co-treated mice significantly increased from day 21 ($P < 0.01$) onward from that of the LPS group, but remained less ($P < 0.05$) as compared to the control. In NTS_1-agonist PD149163-agonist co-treated mice (LPS + PD), food ($P < 0.01$) and water intake ($P < 0.05$) were significantly increased from day 21 onward until day 28 as compared to LPS but remained less ($P < 0.01$) as compared to the control. On combined treatments of probiotics and NTS1 agonist PD149163 (LPS + Pro + PD), the food and water intake increased significantly ($P < 0.001$) from day 14 onward until day 28 as compared to the LPS and became comparable to the control mice [Figure 1b and c].

Effect on pro-inflammatory cytokines

The plasma levels of both the pro-inflammatory cytokines TNF-α and IL-6 were significantly increased in LPS-treated mice ($P < 0.001$) as compared to the control. On probiotic-co-treatment (LPS + Pro), both TNF-α and IL-6 significantly decreased ($P < 0.01$) compared to the LPS group but still remained higher ($P < 0.05$) than the control. In NTS_1-agonist PD149163-co-treated mice (LPS + PD), the plasma levels of both TNF-α and IL-6 decreased significantly ($P < 0.05$) than the LPS but remained significantly higher ($P < 0.01$) than those of the control. On combined treatments of the probiotics along with NTS1 agonist PD149163 (LPS + Pro + PD), both TNF-α and IL-6 increased significantly ($P < 0.001$) from that of the LPS and became comparable to the control [Figure 2a and b].

Effect on anti-inflammatory cytokines

The plasma level of the anti-inflammatory cytokine IL-10 was decreased significantly ($P < 0.001$) in LPS-exposed mice as compared to the control mice. In probiotic-co-treated mice (LPS + Pro), the IL-10 level increased significantly ($P < 0.001$) from that of LPS mice but remained less ($P < 0.05$) than the control. In NTS_1-agonist PD149163-co-treated mice (LPS + PD), the plasma level of IL-10 increased significantly ($P < 0.01$) from that of the LPS, but, remained less ($P < 0.01$) from that of the control. On administration of probiotics together with the NTS_1 agonist PD149163 (LPS + Pro + PD), the IL-10 increased significantly as compared to LPS ($P < 0.001$) and became comparable to the control [Figure 2c].

Effect on plasma CORT

Plasma CORT increased significantly ($P < 0.01$) in LPS-exposure mice. On probiotic administration to LPS-exposed mice, there was a significant decrease ($P < 0.05$) in the plasma

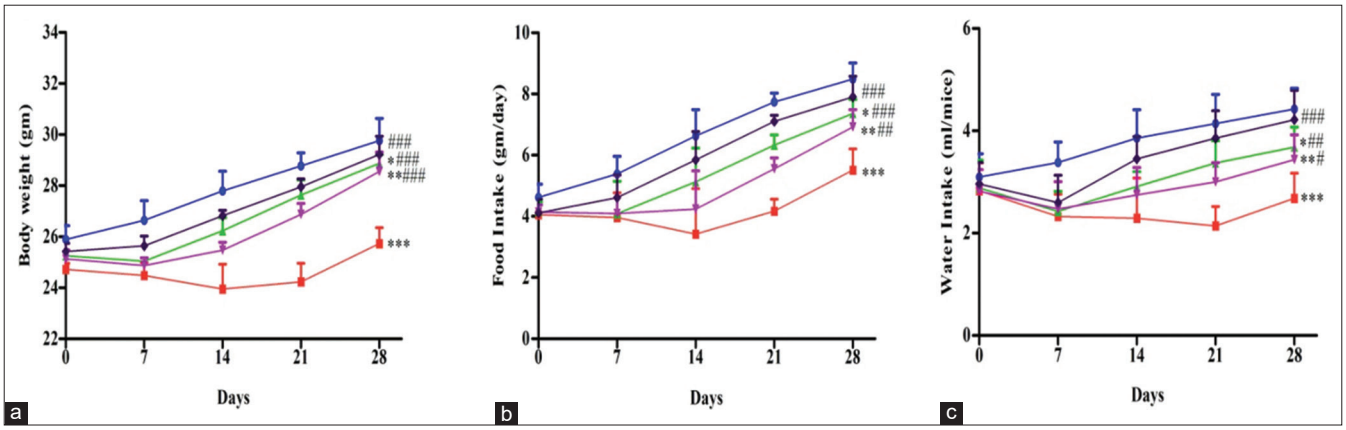

Figure 1: Body weight (a), Food intake (b) and Water intake (c) in different groups. The values were represented as the Mean ± standard error of the mean. Statistical significance was subjected as control versus other groups $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$; lipopolysaccharide versus other groups $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$

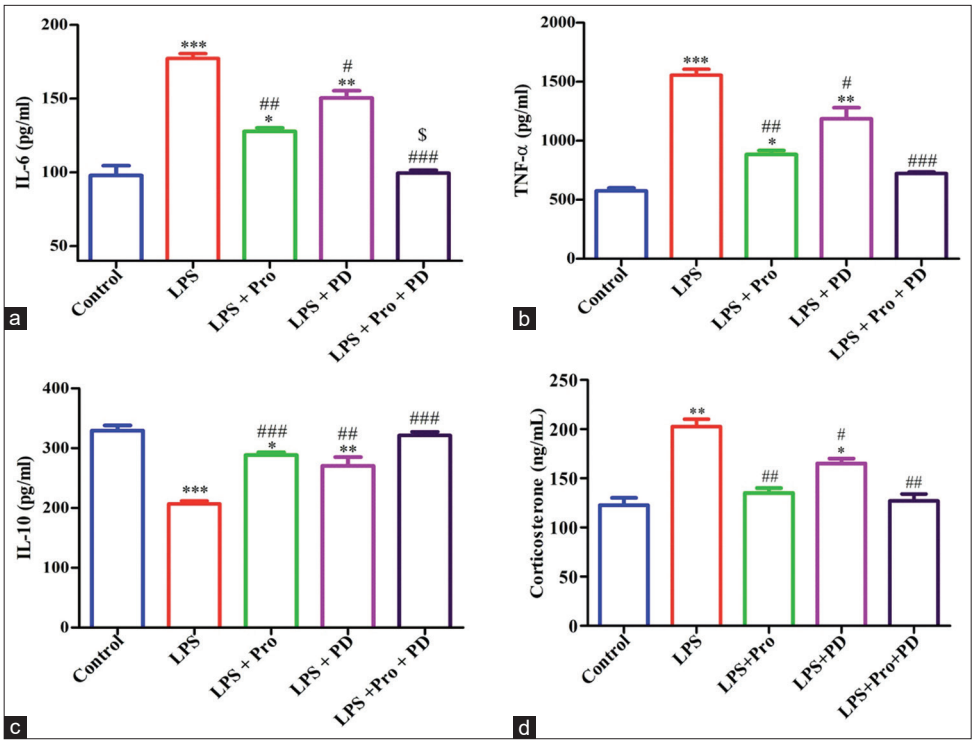

Figure 2: Showing plasma level of IL-6 (a), TNF-α (b), IL-10 (c) and plasma level of corticosterone (d) in different groups. The values were given as the Mean ± standard error of the mean. Statistical significance was subjected as Control vs. other groups $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$; LPS versus other groups $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$; LPS + Pro versus other group $^{\$}P < 0.05$. IL: Interleukin, LPS: lipopolysaccharide

level of CORT as compared to the LPS mice. On NTS_1 agonist PD149163 (LPS + PD) treatment, the plasma level of CORT non-significantly decrease as compared to LPS, but as compared to control, it remained elevated ($P < 0.05$) significantly. On combinatorial treatment of the probiotic and PD, the plasma level of CORT decreased significantly ($P < 0.01$) and became equivalent to the control [Figure 2d].

Effect on anxiety-rerated behavior

Open field test

In the open field arena test, there was a significant decrease in central square entry ($P < 0.01$) and spend time ($P < 0.001$) on LPS exposure to mice. Treatments of probiotics to LPS-exposed increased mice the central square entry ($P < 0.05$) and spend time ($P < 0.01$) were increased significantly as compared to LPS mice. In the treatment of NTS_1 agonist PD149163 in LPS mice, though, the central square entry and spend time increased non-significantly compared to LPS but remained less ($P < 0.01$) as compared to the control. Supplementation of probiotics along with PD increased both the central square entry ($P < 0.01$) and spend time ($P < 0.001$) significantly became comparable to control [Figure 3a and b].

Elevated plus maze test

In the elevated plus maze test, the entry and spend time in the open arms were significantly decreased ($P < 0.001$) in LPS-exposed mice as compared to the control. On probiotic supplementation, the entry and spending time in the open arms were significantly increased ($P < 0.01$) as compared to LPS. On the treatment of the peptide NTS_1 agonist PD149163, there was a non-significant decrease in both the entry and spend time in the open arms as compared to LPS, but it remained less ($P < 0.01$) as compared to the control. On combinatorial treatment of both the probiotic and peptide NTS_1 agonist PD149163, the entry and spend time in the open arms significantly increased ($P < 0.001$) and became comparable to the control [Figure 3c and d].

Effect on depressive behavior

Force swimming test

In the force swimming test, both immobility ($P < 0.01$) and immobility time ($P < 0.001$) were significantly increased in LPS-exposed mice. Supplementation of probiotics to LPS-exposed mice the immobility ($P < 0.05$) and immobility time ($P < 0.01$) were decreased significantly as compared to LPS. Treatment of NTS_1 agonist PD149163 in LPS-exposed mice there was a non-significant decreased in immobility and immobility time from that of LPS, but it remained elevated the immobility ($P < 0.05$) and immobility time ($P < 0.01$) as compared to control. Combinatorial treatment of the probiotics and NTS_1 agonist PD149163 significantly decreased immobility ($P < 0.01$) and immobility time ($P < 0.001$) as compared to LPS and became equivalent to the control [Figure 3e and f].

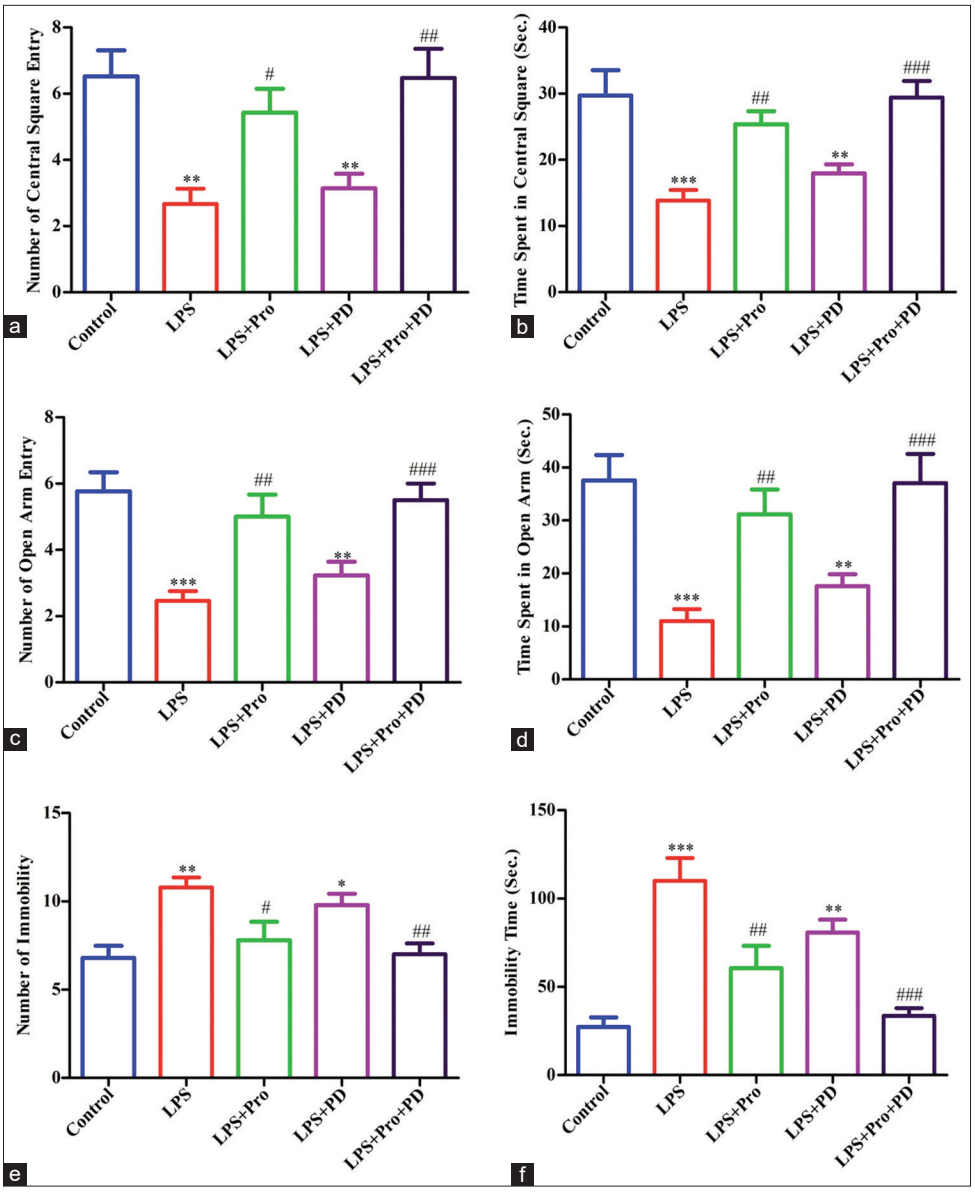

Figure 3: Number of central square entry (a), central square spent time (b) in open field test; number of open arm entry (c), open arm spent time (d) in elevated plus maze; number of immobility (e), immobility time (f) in forced swimming test in different groups. The values were given as the Mean ± standard error of the mean. Statistical significance was subjected as control versus other groups $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$; lipopolysaccharide versus other groups $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$

Effect on beneficial and harmful bacteria populations

There was a significant decrease in the population of the *Bifidobacteria* in LPS-challenged mice on day 14 ($P < 0.01$) that persisted until 28 days as compared to that of the control mice. On the contrary, the population of the *Clostridia* increased significantly from day 7 ($P < 0.01$) onward, and a further increase on day 21 ($P < 0.001$) persisted until day 28 as compared to the control group. Probiotics supplementation significantly increased the population of *Bifidobacteria* on day 14 ($P < 0.05$) and further increased from day 21 ($P < 0.01$), persisted until day 28, and decreased the population of *Clostridia* on days 21 ($P < 0.05$) and 28 ($P < 0.01$) compared to the LPS group, but remained higher ($P < 0.05$) compared to the control by day 28. On NTS_1 agonist PD149163 administration, there was a significant decrease in *Bifidobacteria* from day 14 ($P < 0.01$) and day 21 ($P < 0.05$) that remained until day 28, but the decrease was not significant until day 7 as compared to that of the control mice. However, as compared to LPS, there was a significant increase ($P < 0.05$) on day 28. In contrast, the population of *Clostridia* remained significantly higher ($P < 0.01$) from day 7 onward as compared to control; however, there was a significant decrease on day 28 as compared to LPS. In combined treatments of both the probiotics and the NTS_1 agonist PD149163 in LPS-exposed mice, the population of *Bifidobacteria* increased significantly from day 14 onward (day 14 $P < 0.05$; day 21 $P < 0.01$; day 28 $P < 0.01$) and became comparable to the control on day 28. On the contrary,

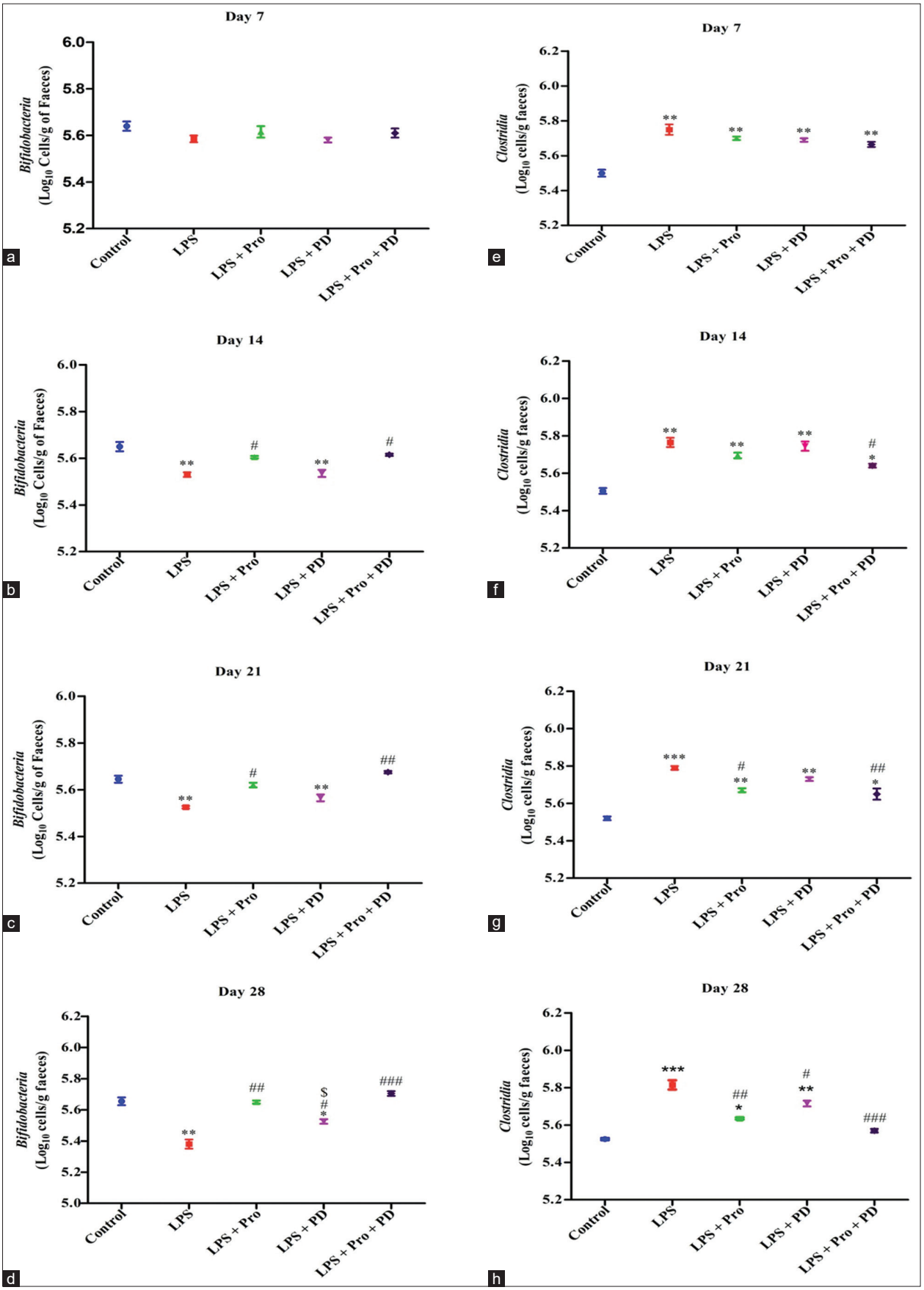

Figure 4: Populations of *Bifidobacteria* (Day 7 [a]; Day 14 [b]; Day 21 [c]; Days 28 [d] and *Clostridia* (Day 7 [e]; Day 14 [f]; Day 21 [g]; Day 28 [h]) determined by Fluorescent *in situ* hybridization. Statistical significance was subjected as Control versus other groups $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$; lipopolysaccharide versus other groups $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$

the population of *Clostridia* significantly decreased from day 14 onward (day 14 $P < 0.05$; day 21 $P < 0.01$; day 28 $P < 0.001$) as compared to LPS and became comparable to the control group [Figure 4a-h].

DISCUSSION

The present study has evaluated the modulatory role of probiotics along with the NTS_1 agonist PD149163 in endotoxemia-induced behavioral deficits in mice. Inflammation is the leading cause of depression and anxiety.[32] Systemic exposure of LPS activates the inflammatory cascade and recruits pro-inflammatory mediators (Interferon-γ, IL-1β, IL-6, and IL-6 and TNF-α) that cause inflammation.[33] In the present study, LPS-exposed mice showed increased plasma pro-inflammatory cytokines IL-6 and TNF-α. The pro-inflammatory factors cross the blood-brain barrier into the brain through blood circulation, activating microglia and releasing reactive oxygen and nitrogen, leading to neuroinflammation and ultimately mental illness.[3,34] In addition to this, activating the HPA axis results in an elevation of CORT, as reported in the present study.[35] The increase in pro-inflammatory and decrease in anti-inflammatory cytokines are linked to depressive behavior, as shown in the present investigation.[36] In this study, LPS-exposed mice exhibit anxiety-like behavior (reduction in time duration, entries in the central square in OFT, and entries in the open arm in EPM) and depressive-like behavior (increment in immobility time in FST), which is consistent with other reports.[37,38] This study has also shown loss of body weight, inhibition of food and water intake, and indicated sickness behavior. The systemic immune response causes symptoms of weight loss, decreased eating and drinking, and motor activity, as reported before.[8,39]

In the present study, administration of multistrain probiotics has shown modulating effects on behavioral abnormalities such as sickness behavior, anxiety-like behavior, and depressive-like behavior. A number of studies have demonstrated that probiotics reduce inflammation and improve depressive symptoms.[40-42] In the present investigation, administration of the multistrain probiotics reduced the pro-inflammatory cytokines (TNF-α and IL-6) and increased the anti-inflammatory cytokine (IL-10). Ingestion of probiotics reduced depressive behavior (as measured by FST) through down-regulating pro-inflammatory cytokines (TNF-a), as demonstrated in the present study.[43] In an early-life stress-induced mice model, the administration of probiotic *L. plantarum* PS128 reduced serum pro-inflammatory cytokines as well as CORT levels in both basal and stressed states.[44] Ingestion of *B. longum* subspecies infant is strain CCFM687 alleviated the hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis response and improved stress-induced dysbiosis of the gut microbiome through increased alpha diversity and abundance of butyrate-producing bacteria.[45] This study has depicted an increase in *Bifidobacterium* and a decrease in the *Clostria* bacterial population on the ingestion of probiotics. Guo and coworkers reported that restoration of the gut microbiota by ingestion of *B. adolescentis* associated with a reduction in inflammatory cytokines and exhibits antidepressant and anxiolytic effects.[46] Another study found that reshaping the gut microbiota by *Bifidobacterium breves* CCFM1025 exerts an anti-depressant effect by decreasing the production of beneficial metabolites and attenuating the HPA axis and inflammation in the brain.[47] Treatment of *L. casei* significantly increased the growth rate, sucrose preference, and decrease immobility time in FST by amending the gut microbiota structure changes in rats.[48] Administration of *B. adolescentis* attenuates anxiety and depressive by increasing Lactobacillus and decreasing bacteroids in mice.[46]

On combinatorial supplementation of probiotics and NTS_1 agonist PD149163 (50 μg/kg bw.), the effectiveness of amelioration of the behavioral abnormalities was enhanced more significantly than in the alone probiotic-co-administrated groups. All the inflammatory mediators (IL-6, TNF-α) were normalized. Miyaoka and coworkers have demonstrated that a combination of antidepressants and probiotics is more effective in treating drug-resistant depression.[49]

Treatment of NTS_1 agonist PD149163 (50 μg/kg bw.) to LPS-exposed mice has shown partial efficacy in reducing the behavioral deficits (sickness, anxiety, and depressive behavior), the inflammatory markers (IL-6, TNF-α, CORT), and the significant increase of the anti-inflammatory cytokines IL-10 as compared to the LPS-exposed mice, but still remained a significant difference from the control group. The anti-psychotic effect of this agonist is suggested to be through a central anti-inflammatory effect.[24] Recently, we have reported that treatment with the higher dose (100 μg/kg bw) of PD14963 has shown a significant reduction in pro-inflammatory cytokines and oxidative stress in the gut and liver.[27] Hence, the lower dose (50 μg/kg bw) of PD14963 could not exhibit a significant ameliorative effect.

CONCLUSION

The present investigation has shown that treatment with multistrain probiotics ameliorates the behavioral abnormalities by reducing inflammatory markers and enhancing the beneficial bacterial population. In addition, combinatorial supplementation of probiotics and the NTS_1 agonist PD149163 has shown better efficacy in ameliorating of behavioral deficits. Hence, probiotics may be used as an adjunct with other antipsychotic drugs in pharmaceutical strategies for the treatment of mental illness.

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EHTICAL STATMENET

The handling and caring of the animals followed the guidelines of the Committee for the Purpose of Control and Supervision of Experimental Animals, Ministry of Environment and Forest, Government of India. The experimental protocols were approved by the Institutional Animal Ethical Committee of the University, University of Allahabad, Prayagraj (Approval No. IAEC/AU/2019(1)/04).

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