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Original Research Article

Probiotic Strains Alleviate Lipopolysaccharide-Induced Depressive-Like Symptoms via Attenuation of Neuroinflammatory Signaling in Rats

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ABSTRACT

A growing body of evidence has linked mental health to the gut microbiome. This has led to the investigation of the gastrointestinal tract as a possible source of novel treatments and probiotic supplements for depressive disorders. Thus, this study aims to evaluate the antidepressant effect of a multi-strain probiotics against lipopolysaccharide (LPS)-induced depression in rats. Twenty-four male Wistar rats were randomly grouped into four groups of 6 animals each. Groups 1 and 2 received vehicle (distilled water, 10 mL/kg), group 3 received a probiotic cocktail (10 mL/kg), while group 4 received fluoxetine (10 mg/kg). All treatments were administered orally for seven consecutive days. One hour after treatment on day seven, LPS (0.85 mg/kg, i.p.) was given to all the animals except group 1 animals. Twenty-four hours later, all the animals were subjected to behavioural tests [Forced Swim Test (FST), Sucrose Splash Test (SST), and Open Field Test (OFT)]. Thereafter, the animals were sacrificed, and brain samples were collected for bioassay of central pro-inflammatory mediators; tumour necrosis factor-alpha (TNF-α), interleukin-6, and interleukin-17 (IL-6 and IL-17) using enzyme-linked immunosorbent assay. The LPS significantly increased immobility of rats in the FST and decreased grooming in the SST which is indicative of depressive-like behaviours. These behaviours were significantly attenuated by probiotics compared to control. LPS caused marked increase in TNF-α, IL-6, and IL-17 concentration in the hippocampus. The elevated cytokine levels were attenuated by pretreatment with probiotics. Therefore, probiotics exhibited antidepressant-like activity which may be due to the inhibition of neuroinflammatory signalling pathways.

Keywords: Depression, Probiotics, Gut microbiome, Neuroinflammation, Animal behaviour.

Introduction

Depression is a common mental illness that can be chronic or recurrent, severely limiting a person's capacity to perform normal daily function. Feelings of sadness, worry, emptiness, hopelessness, powerlessness, worthlessness, guilt, anger, humiliation, or restlessness are common in people who are depressed.[1] They may lose interest in physical activities, lose their appetite, or overeat, have difficulty concentrating, remembering facts, or making judgments, and, in the worst-case scenario, attempt or commit suicide. It is becoming more accepted that healthy people might have subclinical levels of depressive symptoms. Depressive symptoms are a major public health concern due to their effect and widespread incidence. Depression affects about 5% of the global adult population.[2] This prevalence is on a steady increase while affecting various age groups. As a result, research into the prevention and treatment of depression is paramount.

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Over the years, there has been increasing evidence suggesting that neuroinflammation plays a key role in the pathophysiology of depression.[3,4]

Neuroinflammation in individuals with depression was recently shown to be characterized by the release of proinflammatory cytokines, notably the interleukins (e.g. IL-1β, IL-6) and the tumor necrosis factor-alpha (TNF-α), as well as the activation of nuclear factor-kappa B (NF-κB).[5,6] Furthermore, certain animal studies had similar results. For example, the prefrontal cortex (PFC) and hippocampus (HIPP) of depressed mice showed elevated expression of inflammatory mediators and elevated nitric oxide synthase (iNOS) levels.[7,8] In addition, the LPS model of neuroinflammation resulted in depressive-like behaviour as well as cognitive impairment which were related to microglia activation in mice.[9] Therefore, preventing inflammatory signaling has been identified as a potential therapeutic option for depression.

Probiotics are live microorganisms which can be sourced from food or dietary supplements and consumed for their acclaimed health benefit. Lactobacillus genus is one of the most common strains found in commercial probiotics. Studies have shown that probiotics possess immunomodulatory[10] as well as anti-inflammatory property based on their ability to reduce serum concentrations of proinflammatory cytokines and simultaneously increase serum concentrations of anti-inflammatory cytokines.[10-11] In addition to these, more studies have described a link between depression and the gut microbiota, leading to the therapeutic use of probiotics for stress-related diseases.[12] As a matter of fact, a recent study stated that gut dysbiosis alters mood and predisposes an individual to the development of neuropsychiatric disorders by evoking chronic systemic inflammation which will in turn

increase the permeability of the blood-brain barrier and cytokine translocation.[13]

Though the effect of probiotics on neuroinflammation-linked depression and its underlying mechanism has not been comprehensively studied, there is an urgent need to elucidate the potential anti-depressive role and mechanism of action of probiotics against neuroinflammation-induced depression. The study therefore investigated the antidepressant efficacy of a multi-strain probiotics treatment in an LPS-induced rat model of depression.

Materials and Methods

Experimental animals

Twenty-four (24) male Wistar rats weighing between 150 - 170 g were procured, and kept in well ventilated cages and acclimatized for one week under 12 h light and 12 h dark cycle with 24 h access to standard rodent feed and water. All experiments were carried out in compliance with the National Institute of Health Guide for Care and Use of Laboratory Animals.[14]

Experimental design

At the end of one week acclimatization, the twenty-four (24) male rats were randomly grouped into 4 groups of 6 animals each.

Group 1 which served as negative control received vehicle (10 mL/kg),

Group 2 which served as model control received vehicle (10 mL/kg),

Group 3 received probiotics (10 mL/kg),

Bacteria species and strain used include the following:

Lactobacillus; *Lb. pentosus A028, Lb. pentosus B1b, Lb. pentosus A4C, Lb. rhamnosus A012, Lb. rhamnosus A072, Lb. plantarum A011, Lb. plantarum A014, Lb. plantarum A077, Lb. paraplantarum A017; Pediococcus pentosaceus A074;*

Leuconostoc*; Lc. pseudomensenteriod A064, Lc. pseudomensenteriod A044*.

They were obtained from Sigma and prepared as a cocktail following previously described protocol.[15]

Group 4 which served as positive control received fluoxetine (10 mg/kg). Fluoxetine was chosen because of its established efficacy in the treatment of human depression.[16]

All groups were pretreated with their respective agents (vehicle or probiotics or fluoxetine) for seven (7) days. One hour after treatment with the respective agents on day seven, LPS (Sigma; 0.85 mg/kg, i.p.) was given to all the animals except group 1 animals. Twenty-four hours later, all animals were subjected to testing for behavioural changes.

Three (3) standardized behavioural tests were used to assess the antidepressant potential of the probiotic strains. The forced swim test measured despair-like behaviour, the sucrose splash test measured anhedonia, while the open field test measured general locomotor activity.

Sucrose splash test (SST)

A sucrose solution was prepared and administered to the dorsal coat of each animal. Afterwards, the time spent grooming was recorded for a period of 4 minutes as an index of self-care and motivational behaviour. The splash test was carried out 24 hours after administration of LPS.[17]

Forced swim test (FST)

Each animal was carefully dropped into a bucket of water. Afterwards, the mobility and immobility time was recorded for a period of 6 minutes, but the first 2 minutes was disregarded as the active period where the animal is struggling.[18]

Open field test (OFT)

The open field is an arena with walls to prevent escape; the field was painted black and marked with white marker into squares of 15 cm x 15 cm to form 60 squares. The rats were placed in the middle square and the number of squares crossed within 5 minutes was recorded.[17] The field was cleaned with 70% ethanol before another rat was introduced.

Animal sacrifice

At the end of the experiment, the rats were sacrificed under ether anaesthesia: balls of cotton wool were soaked in diethyl ether and placed in a desiccator, then each animal was shut in the same desiccator to allow complete anaesthesia. Once fully anaesthetized, the animals were removed from the desiccator and their whole brains were harvested following established ethical standards of the institution (AB/EC/20/03/105).

Biochemical/Cytokine assay

The hippocampus and prefrontal cortex were carefully dissected from each brain and homogenized in iced cold phosphate buffer. The homogenate was centrifuged for 10 min at 10,000 rpm. The supernatant obtained from the homogenized organs was stored at -20ºC and used to assay for the concentrations of IL- 6, IL-17 and TNF-α.

The concentrations of IL-6, IL-17 and TNF-α in the supernatants of the hippocampus and prefrontal cortex were determined according to the manufacturer's instructions (Melsin, China). All measurements were performed at room temperature using a microplate reader with a 450 nm filter. The concentrations of IL-6, IL-17 and TNF-α in the tissues were extrapolated from the standard curve of IL-6, IL-17 and TNF-α standards included in the assay kits. The levels of IL-6, IL-17 and TNF-α in the specified brain regions were expressed as pg/mL.[19]

Statistical analysis

Statistical analyses were done using one-way analysis of variance (ANOVA). Differences between means were evaluated by Tukey's multiple comparison test using the GraphPad Prism 8 Software. The outcome of the statistical analysis was represented in graphs and bar charts with error bars representing the mean ± SD (standard deviation). The level of significance was set at $p < 0.05$.

Results and Discussion

The gut microbiome has been consistently implicated in the aetiology of neuropsychiatric disorders, especially depression and anxiety.[20] These disorders are commonly considered gut-brain axis disorders which can be treated by targeting the gut microbiota.[21] For example, Lactobacillus and Bifidobacterium species were found to be capable of reducing depression to a large extent.[22] For this reason, the current study sought to evaluate the effect of probiotic bacteria strains on neuroinflammatory mediators resulting from LPS stimulation in rats.[23]

LPS induced a significant alteration in the behavioural pattern of rodents by significantly ($p < 0.05$) increasing the duration of immobility in the FST (Figure 1) and significantly ($p < 0.05$) reducing rodent locomotor activity in an OFT (Figure 2) as well as reducing animal grooming time (i.e. index of anhedonia) in the SST (Figure 3). These behavioural changes are usually associated with psychological disorders in rodents, especially depression. These behaviours were significantly ($p < 0.05$) attenuated by the administration of probiotics in a similar fashion as the positive control (fluoxetine) in the majority of the tests. This is in agreement with the findings from previous studies.[24,25]

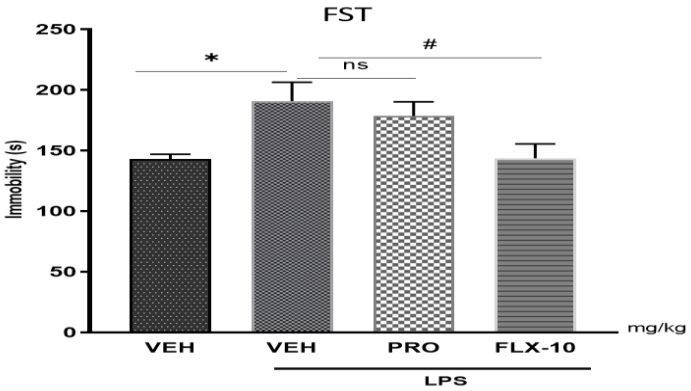

Figure 1: Effect of probiotic on mobility in Forced Swim Test (FST)

Values represent the mean ±SD (n = 6). *$p < 0.05$ compared to control (vehicle-only group 1), # $p < 0.05$ compared to group 2.

VEH = Vehicle; PRO = Probiotics; FLX-10 = Fluoxetine 10 mg/kg; LPS = Lipopolysaccharides.

To further support these results, biochemical analysis of specific brain regions were carried out and cytokine levels were measured. Neuroinflammation has been linked to the chronic overexpression of pro-inflammatory over anti-inflammatory cytokines. This study evaluated the effect of probiotics on key pro-inflammatory cytokines or mediators. All pro-inflammatory cytokine levels measured were significantly ($p < 0.05$) increased in the hippocampus and prefrontal cortex of LPS-treated rats (Figures 4-6). The increased expression of these cytokines in the brain are crucial contributors to neuroinflammation which has been associated with depression.[26,27] However, probiotics administration resulted in a significant ($p < 0.05$) clearance of the excess cytokines in the rat brains in a similar manner as fluoxetine. The findings from this study is corroborated by studies which have reviewed the numerous benefits of probiotics on neuroinflammation as well as disorders linked to neuroinflammation such as depression.[23,25] This positive effect of probiotics in LPS-induced animal model of depression has been linked to its ability to inhibit the binding of LPS to the CD14 receptor, thereby reducing the overall activation of NF-κB and the subsequent production of pro-inflammatory cytokines.[10]

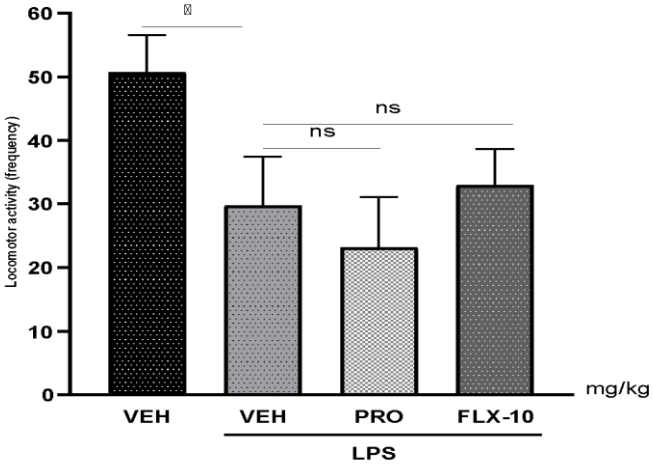

Figure 2: Effect of probiotics on locomotor activity in Open Field Test
Values represent the mean ± SD (n = 6). *p < 0.05 compared to control (vehicle-only group 1), # p < 0.05 compared to group 2.
VEH = Vehicle; PRO = Probiotics; FLX-10 = Fluoxetine 10 mg/kg; LPS = Lipopolysaccharides.

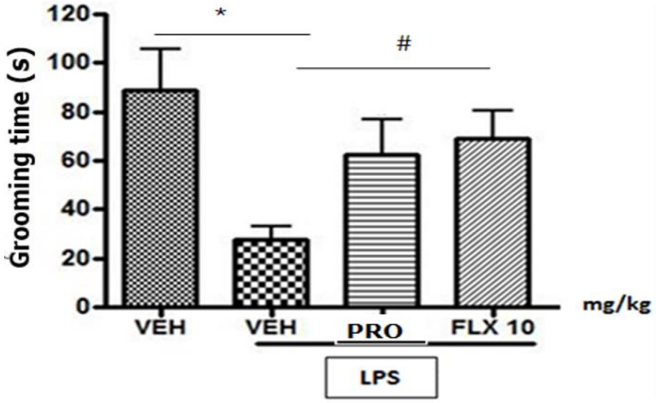

Figure 3: Effect of probiotics on sucrose preference in Sucrose Splash Test
Values represent the mean ± SD (n = 6). *p < 0.05 compared to control (vehicle-only group 1), # p < 0.05 compared to group 2.
VEH = Vehicle; PR = Probiotics; FLX-10 = Fluoxetine 10 mg/kg; LPS = Lipopolysaccharides.

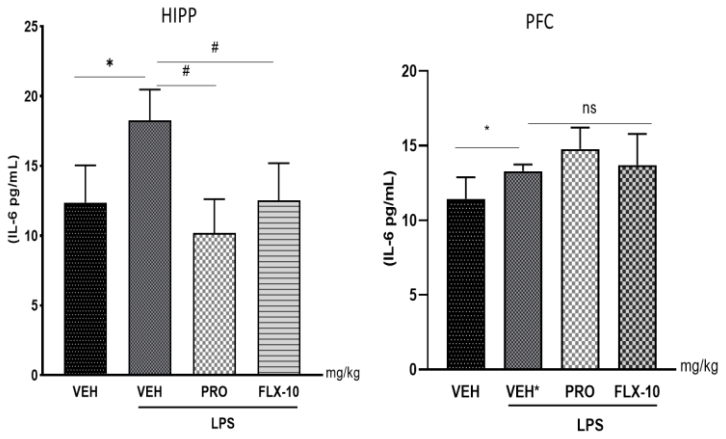

Figure 4: Effect of probiotics on IL-6 levels
Values represent the mean ± SD (n = 6). *p < 0.05 compared to control (vehicle-only group 1), # p < 0.05 compared to group 2.
VEH = Vehicle; PRO = Probiotics; FLX-10 = Fluoxetine 10 mg/kg; LPS = Lipopolysaccharides; HIPP = Hippocampus; PFC = Prefrontal cortex; IL-6 = Interleukin-6.

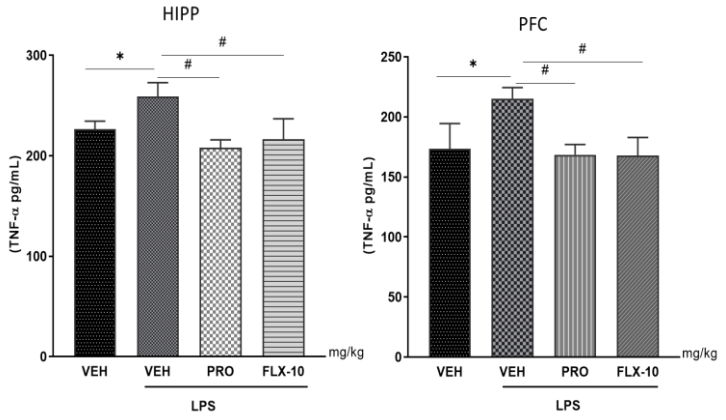

Figure 5: Effect of probiotics on TNF-α levels
Values represent the mean ± SD (n = 6). *p < 0.05 compared to control (vehicle-only group 1), # p < 0.05 compared to group 2.
VEH = Vehicle; PRO = Probiotics; FLX-10 = Fluoxetine 10 mg/kg; LPS = Lipopolysaccharides; HIPP = Hippocampus; PFC = Prefrontal cortex; TNF-α = Tumor necrosis factor-alpha

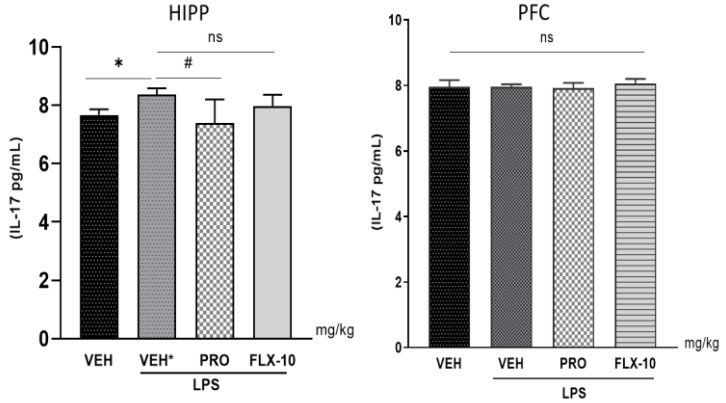

Figure 6: Effect of probiotics on IL-17 levels
Values represent the mean ± SD (n = 6). *p < 0.05 compared to control (vehicle-only group 1), # p < 0.05 compared to group 2.
VEH = Vehicle; PRO = Probiotics; FLX-10 = Fluoxetine 10 mg/kg; LPS = Lipopolysaccharides; HIPP = Hippocampus; PFC = Prefrontal cortex; IL-17 = Interleukin-17.

Conclusion

The health benefits of probiotics are not limited to the gut. Supported from the results of the present study, these live microorganisms are believed to possess potential in modulating the gut-brain axis, alleviating neuroinflammation and improving behaviour. Future research could investigate how best humans can incorporate probiotics into daily diet as a way to prevent inflammatory responses which are commonly triggered by stressful conditions.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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