Article

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Effects of Combined Live *Bifidobacterium*, *Lactobacillus*, *Enterococcus* and *Bacillus Cereus* Tablets on Post-Stroke Depression and Serum Inflammatory Factorse

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Objectives: Although probiotics have been shown to improve several mental-related diseases, their association with post-stroke depression (PSD) remains unclear. This research aimed to investigate the effect of combined live *Bifidobacterium*, *Lactobacillus, Enterococcus* and *Bacillus cereus* tablets on PSD and serum inflammatory factors.

Methods: A total of 400 stroke patients treated from January 2020 to March 2022 in Shuyang Hospital were included and randomly divided into two groups: The observation group (n = 200) and the control group (n = 200). The control group was treated with conventional therapy, while the observation group was given combined live *Bifidobacterium*, *Lactobacillus, Enterococcus* and *Bacillus cereus* tablets on the basis of conventional therapy. The Hamilton Depression Scale score was used to assess the degree and incidence of depression in the two groups. Recurrence and complications of PSD in stroke patients after treatment were followed up. In addition, ELISA (enzyme-linked immunosorbent assay) was employed for the detection of serum levels of nuclear factor kappa light chain enhancer of activated B cells (NF-κB), interleukin (IL)-1β and tumor necrosis factor (TNF)-α, and logistics regression analysis was also performed for the correlation between the occurrence of PSD and NF-κB, IL-1β, and TNF-α levels.

Results: The analysis of clinical baseline data showed that the two groups of patients were comparable. After treatment, the Hamilton Depression Scale score in the observation group was significantly lower than in the control group ($p < 0.05$), and the observation group had a lower incidence rate of PSD, follow-up recurrence rate and complications ($p < 0.05$). In addition, the observation group showed a significant decrease in the serum levels of NF-κB, IL-1β and TNF-α compared with the control group ($p < 0.05$). Further logistics regression analysis indicated that the levels of NF-κB (OR (odds ratio) = 3.337, $p < 0.001$), IL-1β (OR = 2.411, $p < 0.001$) and TNF-α (OR = 1.557, $p < 0.001$) were risk factors for the development of PSD.

Conclusions: Combined live *Bifidobacterium*, *Lactobacillus, Enterococcus* and *Bacillus cereus* tablets can promote neurological recovery and remission of depression in stroke patients. Such effects may be achieved by regulating the levels of NF-κB, IL-1β and TNF-α.

Keywords: post-stroke depression (PSD); combined live *Bifidobacterium*, *Lactobacillus*, *Enterococcus* and *Bacillus cereus* tablets; inflammatory factors

Introduction

Post-stroke depression (PSD) is a common complication that appears after a stroke, with depressive symptoms as the main clinical feature. Its average incidence is about 31%, and a meta-analysis including 15,573 patients found that the prevalence of depression was as high as 33.5% from 2 weeks to 7 years after stroke [1,2]. Individual patients with PSD experience diverse clinical symptoms, such as mood swings, retardation, agitation, apathy, and cognitive dysfunction, which bring great psychological pain and physical dysfunction to the patients. Additionally, the disease can delay the recovery of neurological and motor functions, increase the disability rate and mortality and affects the quality of life and rehabilitation of the patients [3–6]. Xie *et al*. [7] suggested that patients after stroke presented with different degrees of cognitive impairment and depressive symptoms, and that the two effects interacted.

Presently, it is generally accepted that PSD results from a combination of social-psychological and biological factors. With the gradual increase in reports on genetic testing, model prediction and antidepressant treatment, the inflammatory theory of depression has become a hot spot in the study of the pathophysiological mechanism of PSD in recent years. This theory believes that the differential expression of inflammatory and anti-inflammatory cytokines in the early and chronic phases of PSD is closely related to the development of PSD [8,9].

A review of inflammatory factors and PSD by Tang *et al.* [10] pointed out that pro-inflammatory factors (interleukin [IL]-1β, IL-6, IL-8, tumor necrosis factor [TNF]-α, anti-inflammatory cytokines [IL-4, IL-10], transforming growth factor [TGF]-β1) and other non-specific factors (C-reactive protein, neopterin, nucleotide-binding oligomerization domain [NOD] like receptor thermal protein domain associated protein 3 [NLRP3], matrix metalloproteinase 9, growth differentiation factor 15 and serum amyloid A) were all associated with PSD, especially TNF-α and IL-1β [11,12]. Inflammatory factors were shown to be activated in patients with depression and enter the central nervous system to mediate and participate in the damage of the central nervous system. Consequently, these factors affect the occurrence and development of depression accompanied by cognitive decline [13]. Some anti-inflammatory drugs can effectively relieve stroke and improve depressive symptoms, and some antidepressants can reduce the level of inflammation in patients with depression. Therefore, reducing neuroinflammation might be a potential target for therapeutic intervention in PSD.

Stroke stress can cause the transfer of intestinal microbiota from the gastrointestinal tract to the blood, thus causing the occurrence of immune inflammation, resulting in the aggravation of neuroinflammation [14]. Therefore, improving intestinal microbiota may improve the neurological repair of stroke patients in clinical practice. Several biological agents have been used to regulate intestinal flora, including prebiotics, probiotics and synbiotics [15]. Studies have shown that probiotic supplementation can relieve depressive symptoms, might achieve similar effects to traditional antidepressant drugs, and improve cognition and metabolism [16]. Probiotics can also change depressive-like behaviors by altering inflammatory signaling pathways of the gut microbiome [17]. Lin *et al.* [18] found that prescribing probiotic supplementation to stroke patients improved their intestinal function, depressive symptoms, immune function and disease recovery. Combined live *Bifidobacterium*, *Lactobacillus*, *Enterococcus* and *Bacillus cereus* tablets is a common probiotic drug widely used in China, which is developed and produced by Hangzhou Grand Biologic Pharmaceutical INC (patent number: ZL01108353.0; International Patent Classification: A61K 35/74, Hangzhou, China). *Bifidobacterium, Lactobacillus* and *Enterococcus* are the normal intestinal microbiota in the healthy human intestine, which participate in the maintenance of intestinal peristalsis, directly inhibiting some pathogenic bacteria and maintaining the balance of intestinal microecology [19,20]. *Bacillus cereus* can be colonized in the intestinal lumen, which can create an anaerobic environment by consuming the oxygen in the intestinal lumen, and promote the growth and reproduction of anaerobic bacteria, such as *Bifidobacterium* [21]. With the deepening of research, it has recently proved to be possible to effectively regulate the intestinal microbiota of critically ill patients and improve their immune functions and intestinal mucosal barrier functions [22]. However, it is still unclear whether there is a clinical effect on PSD.

In this study, we assumed that combined live *Bifidobacterium*, *Lactobacillus*, *Enterococcus* and *Bacillus cereus* tablets may play an important role in PSD by regulating inflammatory factors. To verify this hypothesis, we investigated the effect of combined live *Bifidobacterium*, *Lactobacillus*, *Enterococcus* and *Bacillus cereus* tablets on the occurrence of PSD in stroke patients and on inflammation-related factors, such as nuclear factor (NF)-κB, IL-1β and TNF-α, and then explored the possible mechanism of its preventive and therapeutic effects. The aim of this study was to provide a potential alternative for the prevention and treatment of PSD.

Materials and Methods

Clinical Data

A total of 400 patients diagnosed with stroke in Shuyang Hospital from January 2020 to March 2022 were included and randomly divided into two groups: The observation group (n = 200) and the control group (n = 200).

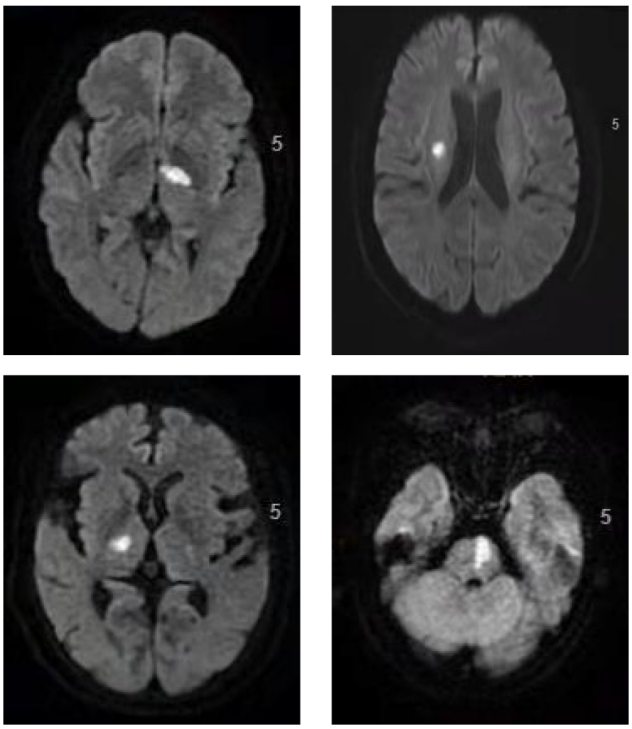

Fig. 1. Imaging of patients with ischemic stroke. Magnetic resonance imaging showed the left lateral thalamic lacunar infarction, right lateral paraventricular lacunar infarction, right basal segment infarction and left cerebral bridge infarction.

Inclusion Criteria

The study inclusion criteria were: (1) Patients diagnosed with ischemic stroke by computed tomography or magnetic resonance imaging at short notice after attack (Fig. 1); (2) Met the diagnostic criteria of stroke; (3) Aged 18 to 80 years; (4) Were conscious, had no cognitive impairment, and could complete the depression questionnaire; (5) Had a stable condition and acute stage duration $<$7 days; And (6) received no antipsychotic drugs, antidepressants, benzodiazepines and other psychotropic drugs in the past 1 year.

Exclusion Criteria

Exclusion criteria were: (1) The disease was combined with other malignant tumors or important organ dysfunction; (2) Combined with severe systemic infection; (3) Had a history or presence of other mental diseases, such as depression and anxiety disorders or schizophrenia; (4) Had a history of epilepsy, traumatic brain injury or organic brain injury; (5) Had a history of electroconvulsive therapy; (6) Had dementia, aphasia or with disturbance of consciousness; (7) Was diagnosed with immune diseases before enrollment; (8) Had abnormal liver and kidney function; And (9) combined with severe wasting, metabolic, endocrine diseases.

Treatment Methods

First, the two groups were treated with conventional drugs for cerebral infarction (antihypertensives, antiplatelet agents, and statins) following the guidelines of the American Heart Association. On the basis of conventional treatment, the observation group was additionally given combined live *Bifidobacterium*, *Lactobacillus*, *Enterococcus* and *Bacillus cereus* tablets (CGE Healthcare, Beijing, China) with a dosage of 3 tablets/time, 3 times/day for 2 weeks. Finally, the two groups of patients were followed up for 6 months, and the relevant indicators were detected.

Baseline Clinical Data

We recorded the basic information of patients, such as age, gender, body mass index, onset symptoms and signs, type and location of infarction, onset time, complications, disease history (hypertension, diabetes, hyperlipidemia, coronary heart disease, stroke, atrial fibrillation, smoking history, and drinking history), and National Institutes of Health (NIH) Stroke Scale (NIHSS) score on admission [23].

Laboratory test results of the following indicators were also obtained: Blood glucose, blood pressure, hemoglobin, urine protein, fibrinogen, C-reactive protein, triglyceride, total cholesterol, high-density lipoprotein and low-density lipoprotein on admission.

Determination of Serum Inflammation-Related Factors

The levels of NF-κB (nuclear factor kappa light chain enhancer of activated B cells) (E-EL-H1386c), IL-1β (E-EL-H0149c), and TNF-α (E-EL-H2305c) in the serum of patients were measured using ELISA (enzyme-linked immunosorbent assay) kits (Elabscience, Wuhai, Hubei, China). Venous blood samples were taken from patients in both groups in the morning on days 1, 7, 14, 30, 60, and 90 after treatment. The samples were processed following the ELISA kit instructions, and the concentrations of the above inflammation-related factors in the serum samples were measured by an automatic microplate reader (BIO-RAD, Hercules, CA, USA).

Comparison of Neuropsychological Assessment and Degree of Post-Stroke Depression

The diagnosis of PSD was made according to the Chinese Classification of mental disorders (CCMD-3) and the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) [24,25]. Subsequently, 24 items introduced in the Hamilton Depression Rating Scale (HDRS) of patients were scored by a well-trained physician, with a score $\geq$8 rated as depression: No depression (0–7); Mild depression (8–16); Moderate depression (17–23); And severe depression ($\geq$24) [26,27]. The HDRS score and the degree of PSD were compared between the two groups on days 1, 7, 14, 30, 60 and 90 after treatment.

Comparison of Recovery and Adverse Reactions

The patients in the two groups were followed up for 6 months. The recurrence rate and complications of the two groups were compared at 1, 3 and 6 months after treatment, and the incidence of adverse reactions was also collected.

Statistical Analysis

The data were analyzed using the Statistical Product and Service Solutions (SPSS, version 19.0, IBM Corporation, Armonk, New York, NY, USA) software. Assessment of data conforming to the normal distribution is expressed as mean $\pm$ standard deviation (SD) and compared using the *t*-test. Data measured at different time points were analyzed by an analysis of variance using repeated measures. Enumeration data were analyzed using the chi-square test. The association between influencing factors and the risks of developing PSD were assessed using multivariate logistic regression analysis. $p < 0.05$ was considered statistically significant.

Results

Clinical Baseline Characteristics

The baseline clinical characteristics of the two groups are displayed in Table 1. The results showed no significant difference in the baseline clinical characteristics, such

Table 1. Comparison of the clinical baseline characteristics of the study cohort.

Variables	Observation group (n = 200)	Control group (n = 200)	χ^2/t	*p*
Age (years)	63.66 ± 6.75	63.60 ± 8.49	0.078	0.938
Gender (n)			0.180	0.672
Male	135	131		
Female	65	69		
Body mass index	27.19 ± 2.97	27.46 ± 3.15	0.884	0.377
Type of infarction (n)			2.373	0.667
Large artery disease	54	49		
Cardioembolism	45	48		
Small vessel occlusion	60	71		
Other determined etiology	27	21		
Undetermined etiology	14	11		
Location of infarction (n)			0.475	0.924
Total anterior circulation infarction	67	64		
Partial anterior circulation infarction	66	64		
Posterior circulation infarction	44	46		
Lacunar infarction	22	26		
Onset time (n)			1.111	0.774
Morning	39	39		
Afternoon	32	25		
Evening	58	59		
Before dawn	71	77		
History of diseases (n)			1.517	0.911
Hypertension	21	24		
Diabetes	19	21		
Hyperlipidemia	26	24		
Coronary heart disease	25	29		
Stroke	34	37		
Atrial fibrillation	75	65		
Complications (n)			1.181	0.991
Focal neurological deficits	34	30		
Hemiplegia	27	28		
Sensory disturbances	19	16		
Aphasia	25	25		
Ataxia	13	15		
Headache	24	22		
Vomiting	30	31		
Coma	28	33		
NIH Stroke Scale (NIHSS) score	12.49 ± 6.22	12.46 ± 2.34	0.055	0.956
Blood glucose (mmol/L)	4.59 ± 0.95	4.56 ± 0.57	0.392	0.696
Diastolic blood pressure (mmHg)	79.56 ± 8.47	80.28 ± 5.93	0.988	0.324
Systolic blood pressure (mmHg)	123.45 ± 11.73	123.72 ± 9.89	0.248	0.804
Hemoglobin (g/L)	144.23 ± 17.59	145.36 ± 20.58	0.586	0.558
Urine protein (n)			1.702	0.192
Positive	102	115		
Negative	98	85		
Fibrinogen (g/L)	4.89 ± 0.88	4.81 ± 0.82	0.900	0.369
C-reactive protein (mg/L)	5.04 ± 1.48	5.09 ± 0.60	0.399	0.690
Triglyceride (mmol/L)	4.00 ± 0.86	3.96 ± 1.01	0.387	0.699
Total cholesterol (mmol/L)	6.39 ± 0.59	6.35 ± 1.00	0.454	0.650
High-density lipoprotein (mmol/L)	1.65 ± 0.22	1.68 ± 0.49	0.823	0.411
Low-density lipoprotein (mmol/L)	3.71 ± 0.79	3.63 ± 0.63	1.211	0.227

Table 2. Hamilton Depression Scale score of patients in the two groups.

Days after treatment	Day 1	Day 7	Day 14	Day 30	Day 60	Day 90	F	p	η^2
Observation group (n = 200)	6.04 ± 2.65	6.61 ± 3.50	7.05 ± 5.22	7.86 ± 6.94	8.63 ± 7.65	11.24 ± 10.61			
Control group (n = 200)	6.63 ± 3.99	8.32 ± 6.17	9.35 ± 9.42	10.49 ± 11.05	11.91 ± 11.12	14.25 ± 13.28			
Groups main effect							10.802	0.001	0.026
Main effect of test times							77.516	0.001	0.163
Sum							3.644	0.027	0.009

as age, gender, type and location of infarction between the two groups ($p > 0.05$), suggesting that the two groups were comparable.

Assessment of Depression after Treatment in Both Groups

We evaluated the occurrence of depression in patients from the two groups on the 1st, 7th, 14th, 30th, 60th and 90th days after treatment using the HDRS score (Table 2). With the treatment from the 1st day to the 90th day, the overall score of the observation group was significantly lower than the control group ($p < 0.05$). In addition, we also clinically assessed the incidence and the degree of depression in the two groups at different times after treatment; The incidence and the degree of depression in the observation group were significantly lower than in the control group from day 1 to day 90 after treatment (Table 3). These results suggest that the treatment with combined live *Bifidobacterium*, *Lactobacillus*, *Enterococcus* and *Bacillus cereus* tablets significantly improved the depressive status of stroke patients.

Comparison of Serum Inflammation-Related Factor Levels between the Two Groups

To clarify the effects of combined live *Bifidobacterium*, *Lactobacillus*, *Enterococcus* and *Bacillus cereus* tablets on the inflammatory response in stroke patients, we measured the serum levels of inflammatory factors using ELISA in the two groups on days 1, 7, 14, 30, 60 and 90 after treatment. With the treatment from the 1st day to the 90th day, their levels in the observation group were gradually lower than those in the control group ($p < 0.01$) (Table 4). The relationship between the levels of NF-κB, IL-1β, and TNF-α in serum and the development of PSD was analyzed by logistic regression analysis, and the results indicated that NF-κB (OR (odds ratio) = 3.337, $p < 0.001$), IL-1β (OR = 2.411, $p < 0.001$), and TNF-α (OR = 1.557, $p < 0.001$) factors in the serum of patients were risk factors for the development of PSD (Table 5).

Recurrence and Complications at 1, 3, and 6 Months after Treatment

Finally, this study followed up on the recurrence rate, complications and adverse reactions of the two groups at 1, 3 and 6 months after treatment. All the patients had finished the follow-up. The follow-up results showed that the recurrence rate at 3 and 6 months after treatment in the observation group was significantly lower than in the control group ($p < 0.05$). In addition, the complications such as headache, dizziness, and limb numbness in the observation group were significantly improved after treatment ($p < 0.05$) (Table 6), suggesting that combined live *Bifidobacterium*, *Lactobacillus*, *Enterococcus* and *Bacillus cereus* tablets could significantly reduce the recurrence rate of PSD in stroke patients and improve the complications.

Discussion

PSD is a common complication in stroke patients, accompanied by cognitive impairment, which greatly affects the recovery after stroke and the quality of the patient's life [28]. Therefore, it is of great significance to improve PSD in stroke patients. Combined live *Bifidobacterium*, *Lactobacillus*, *Enterococcus* and *Bacillus cereus* tablets, which belong to probiotics, is a common drug to regulate the intestinal microbiota. Studies have shown that probiotic supplementation can relieve depressive symptoms. So far, little research on combined live *Bifidobacterium*, *Lactobacillus*, *Enterococcus* and *Bacillus cereus* tablets focuses on mental diseases, and whether it plays a role in PSD is not yet clear. This study demonstrated that combined live *Bifidobacterium*, *Lactobacillus*, *Enterococcus* and *Bacillus cereus* tablets can significantly reduce the incidence, recurrence rate and complications of PSD in stroke patients and promote neurological recovery and remission of depression. Such effects may be achieved by regulating the levels of NF-κB, IL-1β and TNF-α. These findings fill the gap in the research of combined live *Bifidobacterium*, *Lactobacillus*, *Enterococcus* and *Bacillus cereus* tablets on PSD and provide strong evidence for the prevention and treatment of PSD in the future.

The pathophysiological mechanism of PSD is complex, and the theories that have been formed include monoamine neurotransmitters, neurotrophic factors, inflammatory factors, hypothalamic-pituitary-adrenal axis, neuroplasticity and genetic inheritance [29]. Increased levels of inflammatory cytokines in the acute phase of cerebral infarction may be a risk factor for the development of PSD, and pro-inflammatory factors may interfere with the synthesis of neurotransmitters to trigger PSD [30]. IL-1β expression in the hippocampus of the rat model of depression was significantly increased compared with healthy controls

Table 3. Degree of depression in the two groups.

Variables	Degree	Day 1	Day 7	Day 14	Day 30	Day 60	Day 90
Observation group (n = 200)	Mild	5	10	10	15	20	14
	Moderate	0	3	8	9	15	20
	Severe	0	0	2	6	6	19
	Total	5 (2.5%)	13 (6.5%)	20 (10%)	30 (15%)	41 (20.5%)	53 (26.5%)
Control group (n = 200)	Mild	9	11	7	8	10	10
	Moderate	6	9	13	14	20	14
	Severe	0	6	16	25	30	48
	Total	15 (7.5%)	26 (13%)	37 (18.5%)	47 (23.5%)	60 (30%)	72 (36%)
χ^2		7.406	9.516	13.353	15.757	21.255	15.590
p		0.025	0.023	0.004	0.001	<0.001	<0.001

Table 4. Levels of inflammation-related factors in the serum of patients from the two groups.

Variables	Degree	Day 1	Day 7	Day 14	Day 30	Day 60	Day 90	F	p	η^2
Observation group (n = 200)	NF-κB (ng/L)	0.30 ± 0.06	0.34 ± 0.07	0.37 ± 0.08	0.43 ± 0.07	0.41 ± 0.09	0.42 ± 0.10			
	IL-1β (ng/L)	23.14 ± 3.32	26.84 ± 4.47	28.95 ± 5.36	31.97 ± 7.40	35.86 ± 8.36	39.61 ± 9.79			
	TNF-α (ng/L)	21.97 ± 2.78	25.35 ± 3.34	30.85 ± 5.82	34.99 ± 6.49	36.15 ± 6.04	38.64 ± 6.72			
Control group (n = 200)	NF-κB (ng/L)	0.30 ± 0.07	0.36 ± 0.06	0.40 ± 0.07	0.45 ± 0.09	0.47 ± 0.08	0.50 ± 0.09			
	IL-1β (ng/L)	23.13 ± 4.17	28.71 ± 4.47	32.67 ± 7.10	34.79 ± 5.72	37.41 ± 5.50	44.67 ± 8.57			
	TNF-α (ng/L)	22.38 ± 3.77	27.42 ± 5.10	33.36 ± 4.93	37.10 ± 4.49	39.03 ± 4.11	42.44 ± 6.48			
NF-κB	Groups main effect							42.593	<0.001	0.097
	Main effect of test times							32.518	<0.001	0.076
	Sum							38.592	<0.001	0.088
IL-1β	Groups main effect							362.472	<0.001	0.477
	Main effect of test times							654.782	<0.001	0.622
	Sum							1267.082	<0.001	0.761
TNF-α	Groups main effect							17.149	<0.001	0.041
	Main effect of test times							11.537	<0.001	0.028
	Sum							8.061	<0.001	0.020

Table 5. Logistics regression analysis of the relationship between the levels of NF-κB, IL-1β and TNF-α and the development of post-stroke depression.

Variables	B	S.E	Wald	OR	*p*	95% CI
NF-κB	1.205	0.174	48.031	3.337	<0.001	2.373–4.692
IL-1β	1.213	0.170	51.014	2.411	<0.001	2.411–4.691
TNF-α	0.733	0.148	24.506	1.557	<0.001	1.557–2.782

Note: Data collected on day 90 after treatment were selected for analysis. CI, confidence interval.

[31]. In addition, Mu *et al*. [32] found that the serum level of TNF-α in patients with PSD was significantly higher than that in non-stroke patients, but its inhibitor infliximab alleviated depression-like behaviors in mice [33]. NF-κB is also a typical mechanism regulating neuroinflammation [34]. In our study, the levels of inflammatory factors (NF-κB, IL-1β and TNF-α) in the serum of patients in both groups were significantly reduced after treatment, and their levels in the observation group were lower than those in the control group on the 90th day after treatment, suggesting that combined live *Bifidobacterium*, *Lactobacillus*, *Enterococcus* and *Bacillus cereus* tablets could significantly reduce the expression of inflammatory factors in the blood to improve inflammation and had certain promoting effects on the treatment of PSD. Logistics regression analysis further showed that NF-κB, IL-1β and TNF-α were independent risk factors for the development of PSD; Thus, detecting their levels in the serum of patients is essential for predicting PSD occurrences.

Previously, Fan *et al*. [35] investigated the changes in the population and distribution of intestinal microflora and their relationship with depression in post-stroke patients, using the fecal samples from 32 patients with PSD and comparing them with 30 healthy adult patients. Based on gene sequencing of the 16S RNA V3 region of the intestinal microorganism, they found that genus and species of intestinal bacteria showed significant differences between the post-stroke patients and healthy adults, indicating that significant changes in the structure of intestinal flora occur in patients with post-stroke depression. Further, Kang *et al*. [12] conducted an observational study to investigate the correlation between the intestinal flora and the serum inflammatory factors IL-1, IL-2, IL-6 and hs-CRP in 163 ischemic stroke patients, of whom 67 had PSD and 96 did not have PSD. They found intestinal flora imbalance and *Bifidobacterium* undergrowth in patients with PSD, which led to overexpression of serum inflammatory factors (i.e., IL-1, IL-2, IL-6). It concluded that they were both involved in the occurrence and progress of PSD in patients with ischemic stroke. In contrast, in this present study, the observation group had a significantly lower HDRS score than the control group, indicating that the tablets contributed to the improvement of neurological recovery and activities of daily living. In addition, the observation group had a significantly lower incidence of PSD after treatment. The follow-up results indicated that the recurrence rate and complications at 1, 3 and 6 months after treatment in the observation group were significantly lower than in the control group. Collectively, combined live *Bifidobacterium*, *Lactobacillus, Enterococcus* and *Bacillus cereus* tablets reduce the risk of recurrence, complications and adverse reactions; they can thus contribute to controlling the occurrence of PSD and improving the quality of life of the patients. Thereby, we can deduce that intestinal flora may significantly impact the gut-microbiota axis. However, considering that there is still limited research on PSD patients, deeper translational studies are required to further confirm these observations and clarify the underlying mechanism of actions of the gut microbiota and probiotics on the brain.

Despite the interesting findings, there were still some limitations worth describing. In this study, no standardized animal model of PSD was used to further understand the specific mechanism of inflammatory factors in PSD. In addition, the relevant research on serum inflammatory markers, which have vital predictive value for diagnosing PSD, is still insufficient, with a lack of systematic, multi-level and high-quality research. Our study indicated that a combination of combined live *Bifidobacterium*, *Lactobacillus, Enterococcus* and *Bacillus cereus* tablets and conventional drug treatment of cerebral infarction and cerebral hemorrhage could effectively improve the incidence of PSD in stroke patients, inflammatory response, and body function. Such combined treatment demonstrated good efficacy and safety, providing a new alternative and clinical research basis for the clinical prevention and treatment of PSD.

Conclusions

In summary, combined live *Bifidobacterium*, *Lactobacillus, Enterococcus* and *Bacillus cereus* tablets can not only significantly reduce the incidence of PSD, recurrence rate and complications, but also improve the nerve injury and depression of stroke patients. Its effects were associated with reduced levels of NF-κB, IL-1β and TNF-α in the patients, which were also identified as risk factors for PSD occurrence.

Table 6. Analysis of recurrence and complications.

Variables	Observation group (n = 200)	Control group (n = 200)	χ^2	p
Recurrence rate				
At 1 month after treatment	1	6	3.635	0.057
At 3 months after treatment	11	23	4.629	0.031
At 6 months after treatment	21	38	5.746	0.017
Complications				
At 1 month after treatment				
Headache	2	12	7.402	0.007
Dizziness	3	16	9.338	0.002
Limb numbness	1	19	17.053	≤0.001
At 3 months after treatment				
Headache	8	26	10.415	≤0.001
Dizziness	7	31	16.749	≤0.001
Limb numbness	5	26	15.421	≤0.001
Hemiplegia	6	13	2.708	0.100
Aphasia	2	5	1.309	0.253
Epilepsy	2	6	2.041	0.153
At 6 months after treatment				
Headache	13	35	11.458	0.001
Dizziness	12	36	13.636	≤0.001
Limb numbness	11	31	10.641	0.001
Hemiplegia	8	16	2.837	0.092
Aphasia	3	11	4.737	0.030
Epilepsy	3	7	1.641	0.200
Disturbance of consciousness	2	7	2.842	0.092

Availability of Data and Materials

Data involved in the present work are available from corresponding author upon request. All data generated or analysed during this study are included in this published article.

Author Contributions

YWW and LD—contributions to conception and design; YWW and LD—been involved in drafting the manuscript and revising it critically for important intellectual content; XZ, YW and LWL—made substantial contributions to acquisition of data; RW, BCX, XJH and XJ—analysis and interpretation of data. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

This study was approved by the Shuyang Hospital Ethics Committee (SYXRMYY2020KY005) and conducted following the approved guidelines. All participating patients signed an informed consent.

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Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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