



The effect of probiotic supplementation on constipation in patients with Parkinson's disease: A randomized controlled trial

Received: 09 Mar. 2025
Accepted: 05 May 2025

Saeedeh Zareie¹, Maziar Emamikhah², Mohammad Rohani², Ahmad Saedisomeolia^{3,4}

¹ Ward of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran

² Department of Neurology, Hazrat Rasool Medical Complex, Iran University of Medical Sciences, Tehran, Iran

³ Higher Education College of Health Sciences, Education Centre of Australia, Parramatta, NSW, Australia

⁴ School of Human Nutrition, Macdonald Campus of McGill University, Quebec, Canada

Keywords

Parkinson's Disease; Constipation; Probiotics; Gut Microbiota; Randomized Controlled Trial

Abstract

Background: Constipation is a common non-motor symptom (NMS) in Parkinson's disease (PD), affecting up to 70% of patients and reducing quality of life (QOL). Probiotics may improve bowel function via gut microbiota modulation and gut-brain axis regulation. This randomized, double-blind, placebo-controlled trial evaluated the efficacy of six-week multi-strain probiotic supplementation on constipation in Iranian patients with PD.

Methods: Seventy-two patients with PD (aged 50-80 years, functional constipation per Rome IV criteria) were randomized 1:1 to probiotics or placebo. The probiotic capsule contained *Lactobacillus acidophilus* (L. acidophilus), *Lactobacillus casei* (L. casei), *Lactobacillus*

rhamnosus (L. rhamnosus), *Bifidobacterium lactis* (B. lactis), *Bifidobacterium longum* (B. longum), and *Bifidobacterium breve* (B. breve) [1×10^9 colony-forming unit (CFU) each, total 12×10^9 CFU/day]. Dietary intake and physical activity were assessed and included as covariates in analysis of covariance (ANCOVA) models. Primary outcome was weekly spontaneous bowel movements (SBMs); secondary outcomes included Patient Assessment of Constipation-Quality of Life (PAC-QOL) and Unified Parkinson's Disease Rating Scale (UPDRS) Part III. Baseline demographic, clinical, dietary, and physical activity characteristics were comparable between groups.

How to cite this article: Zareie S, Emamikhah M, Rohani M, Saedisomeolia A. The effect of probiotic supplementation on constipation in patients with Parkinson's disease: A randomized controlled trial. Curr J Neurol 2025; 24(3): 195-203.

Results: The probiotic group showed a mean SBM increase of 3.41 versus 0.44 in placebo [between-group difference = 2.97, 95% confidence interval (CI): 2.46-3.53, $P < 0.001$]. PAC-QOL improved significantly (adjusted mean difference = -15.22, 95% CI: -22.32 to -8.11, $P < 0.001$). No significant changes were observed in UPDRS Part III.

Conclusion: Six-week probiotic supplementation increased bowel movement frequency and improved constipation-related QOL in patients with PD, with good tolerability. Larger, multicenter trials are warranted to confirm efficacy and explore potential effects on systemic PD progression.

Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder affecting over 11.7 million people worldwide,¹ with incidence steadily rising in both developed and developing countries, including Iran.² Patients with PD were identified using the United Kingdom (UK) Parkinson's Disease Society Brain Bank criteria, a widely accepted standard for accurate clinical diagnosis, ensuring a well-defined and homogeneous study population.³

According to the Global Burden of Disease (GBD) Study, the age-standardized prevalence and incidence of PD in Iran increased by 23.1% and 33.2%, respectively, between 1999 and 2021, with significant regional disparities.¹ While PD is traditionally characterized by motor symptoms such as tremor, bradykinesia, rigidity, and postural instability, non-motor symptoms (NMSs) are increasingly recognized as major contributors to disease burden, progression, and reduced quality of life (QOL).⁴

Gastrointestinal (GI) disturbances, particularly chronic constipation, are among the most prevalent NMSs and often precede motor symptoms by several years, suggesting an important role in early disease pathophysiology.⁵ Constipation affects over 60% of patients with PD and is associated with reduced medication efficacy, abdominal discomfort, and psychological distress.⁵ Constipation in PD is multifactorial, involving abnormal aggregation of α -synuclein, a neuronal protein implicated in neurodegeneration and impaired gut motility, along with autonomic dysfunction, reduced physical activity, and dietary alterations.⁶

Additionally, intestinal dysbiosis and increased gut permeability may exacerbate systemic inflammation and disrupt the gut-brain

axis, further contributing to GI and neurological dysfunction.⁷

Functional constipation is commonly defined according to the Rome IV criteria, which require at least two of the following during the previous three months: fewer than three spontaneous bowel movements (SBMs) per week, excessive straining, sensation of incomplete evacuation, or the need for manual maneuvers to facilitate defecation.⁸

Gut microbiota modulation has emerged as a promising therapeutic approach in PD.⁹ Probiotics, live microorganisms conferring health benefits, may improve bowel function by restoring microbial balance, enhancing short-chain fatty acid (SCFA) production, reducing local and systemic inflammation, and modulating gut-brain signaling.¹⁰ Systematic reviews and meta-analyses indicate that probiotic supplementation increases stool frequency and alleviates constipation in patients with PD compared with placebo,¹¹ though heterogeneity in strains, doses, duration, and outcome definitions remains a limitation.

Beyond stool frequency, constipation significantly impairs health-related QOL, emphasizing the use of validated instruments such as the Patient Assessment of Constipation-Quality of Life (PAC-QOL) questionnaire.¹²

Physical activity also affects GI motility and should be considered as a potential confounder. Although not PD-specific, the International Physical Activity Questionnaire (IPAQ) provides a standardized framework to quantify activity in elderly or mobility impaired population.¹³

However, evidence from Middle Eastern populations, where dietary habits, gut microbiota composition, and genetic backgrounds differ from Western cohorts, remains scarce. In particular, randomized controlled trials (RCTs) from Iran that concurrently evaluate GI outcomes, motor symptoms, and lifestyle-related confounders are limited. To address this gap, we conducted a randomized, double-blind, placebo-controlled trial to examine the effects of a 6-week multi-strain probiotic supplementation on Iranian patients with PD. The primary objective was to determine whether probiotics increased weekly SBMs compared to placebo. Secondary outcomes included changes in PAC-QOL, Unified Parkinson's Disease Rating Scale (UPDRS) Part III, and physical activity levels. We hypothesized that probiotic supplementation would significantly improve constipation-related outcomes and overall QOL in this population.

Materials and Methods

Study design and ethics approval: This study was a randomized, double-blind, placebo-controlled, parallel-group clinical trial conducted at a tertiary referral hospital in Tehran City, Iran. Recruitment occurred from May to August 2021, with follow-up completed in October 2021. The trial was approved by the Institutional Ethics Committee (IR.IAU.SRB.REC.1400.011) and registered prior to participant enrollment. Written informed consent was obtained from all participants. The trial adhered to the principles of the Declaration of Helsinki and followed Consolidated Standards of Reporting Trials (CONSORT) guidelines. No changes were made to the protocol after trial initiation.

Participants: Eligible participants were adults aged 50-80 years diagnosed with idiopathic PD according to the UK Parkinson's Disease Society Brain Bank criteria. Functional constipation was defined based on Rome IV criteria, requiring at least two criteria (e.g., straining, lumpy or hard stools, sensation of incomplete evacuation) consistently over the previous three months. Only participants with persistent, clinically relevant symptoms were included.

Exclusion criteria were:

- GI diseases [e.g., inflammatory bowel disease (IBD), irritable bowel syndrome (IBS)]
- Antibiotic or probiotic use within 4 weeks prior to enrollment
- Cognitive impairment interfering with informed consent
- Known allergy to probiotic components
- Non-adherence (missing > 30% of doses).

Participants were instructed to maintain their usual diet and physical activity and avoid new supplements or traditional medicines that could influence bowel function.

Randomization and blinding: A total of 72 participants were randomized in a 1:1 ratio to the probiotic or placebo group using permuted block randomization (block size = 4) generated in R software. Allocation concealment was ensured using sequentially numbered, opaque, sealed envelopes (SNOSE) prepared by an independent staff member not involved in enrollment or outcome assessment.

Blinding was maintained for participants, caregivers, and outcome assessors. Probiotic and placebo capsules were identical in size, color, and packaging. At study completion, participants and assessors were asked to guess group allocation; the

proportion of correct guesses did not differ from chance, confirming successful blinding.

Intervention: The intervention group received a multi-strain probiotic capsule containing:

- *Lactobacillus acidophilus* (*L. acidophilus*) [1×10^9 colony-forming unit (CFU)]
- *Lactobacillus casei* (*L. casei*) (1×10^9 CFU)
- *Lactobacillus rhamnosus* (*L. rhamnosus*) (1×10^9 CFU)
- *Bifidobacterium lactis* (*B. lactis*) (1×10^9 CFU)
- *Bifidobacterium longum* (*B. longum*) (1×10^9 CFU)
- *Bifidobacterium breve* (*B. breve*) (1×10^9 CFU).

Total daily dose was 12×10^9 CFU, administered orally twice daily for 6 weeks. Capsules were stored at 2°-8° C, with manufacturer confirmation of CFU stability throughout the study.

The placebo group received visually identical capsules containing starch. Adherence was monitored via weekly phone calls and capsule counts at follow-up visits; participants with < 70% adherence were excluded from per-protocol analyses. Concomitant anti-parkinsonian medications (e.g., levodopa, dopamine agonists) were recorded throughout the study.

Outcome measures: The primary outcome of this study was the change in weekly SBMs, recorded using patient diaries. Secondary outcomes included GI-related QOL and motor function. GI-related QOL was assessed using the PAC-QOL questionnaire, a validated tool with an available Persian version,¹⁴ which evaluates physical discomfort, psychosocial discomfort, worries, and satisfaction related to constipation. Motor function was evaluated using Part III of the UPDRS, which assesses the severity of motor symptoms in patients with PD. These measures were selected to provide a comprehensive assessment of the effects of probiotic supplementation on both constipation-related QOL and PD motor symptoms.

Outcomes were assessed at baseline and at the end of the 6-week intervention. Dietary intake and physical activity were recorded and included as covariates in the analysis to adjust for potential confounding effects.

Sample size calculation: Based on previous literature, assuming a between-group difference of 1.3 SBMs/week [standard deviation (SD) = 1.5], two-sided $\alpha = 0.05$, and 80% power, 65 participants were required. Accounting for a 10% dropout rate, 72 participants were recruited.

Baseline comparability between groups was assessed for total energy intake (kcal/day), macronutrients, fiber intake (g/day), and physical activity level (assessed using the IPAQ) using independent t-tests. No significant differences were observed (all P s > 0.05). Despite baseline comparability, pre-specified covariates (baseline SBM count, total energy intake, fiber intake, and physical activity) were included in analysis of covariance (ANCOVA) models for primary and key secondary outcomes to adjust for potential residual confounding. Sensitivity analyses, including additional adjustment for age, sex, and disease duration, confirmed the robustness of the primary outcome. Normality of data was assessed using the Shapiro-Wilk test, and categorical variables were compared using chi-square tests. All analyses were conducted using SPSS software (version 27, IBM Corporation, Armonk, NY, USA), with $P < 0.05$ considered statistically significant. Both intention-to-treat (ITT) and per-protocol analyses were performed.

Results

Participant flow: A total of 80 patients with PD and chronic constipation were assessed for eligibility. Eight were excluded (5 did not meet inclusion criteria, 3 declined participation). Seventy-two participants were randomized equally into probiotic ($n = 36$) and placebo ($n = 36$) groups. During the 6-week intervention, one

participant from the placebo group and one from the probiotic group were lost to follow-up. Thus, 70 participants completed the study and were included in the per-protocol analysis. All 72 participants were included in the ITT analysis. Missing data were minimal (< 5%) and handled using the last observation carried forward (LOCF) method. A CONSORT flow diagram is presented in figure 1.

Baseline characteristics: The demographic and clinical characteristics of the participants are summarized in table 1. There were no significant differences between the two groups in terms of age, sex, or body mass index (BMI) (all P s > 0.05). Comparisons of dietary intake, including total energy, total fiber, and physical activity, are presented in table 2. No significant differences were found between the groups for any of these variables (all P s > 0.05). Mean age was 64.72 ± 7.02 years in the placebo group and 65.00 ± 6.82 years in the probiotic group, with 47.2% male participants. Mean BMI, PD duration, levodopa dose, PAC-QOL and UPDRS III scores, total energy intake, macronutrient and fiber intake, and physical activity levels (assessed using the IPAQ) did not differ significantly between groups (all P s > 0.05). Adjusted analyses using ANCOVA, including baseline SBM count, energy intake, fiber intake, and physical activity as covariates to control for potential residual confounding, confirmed the primary outcome results.

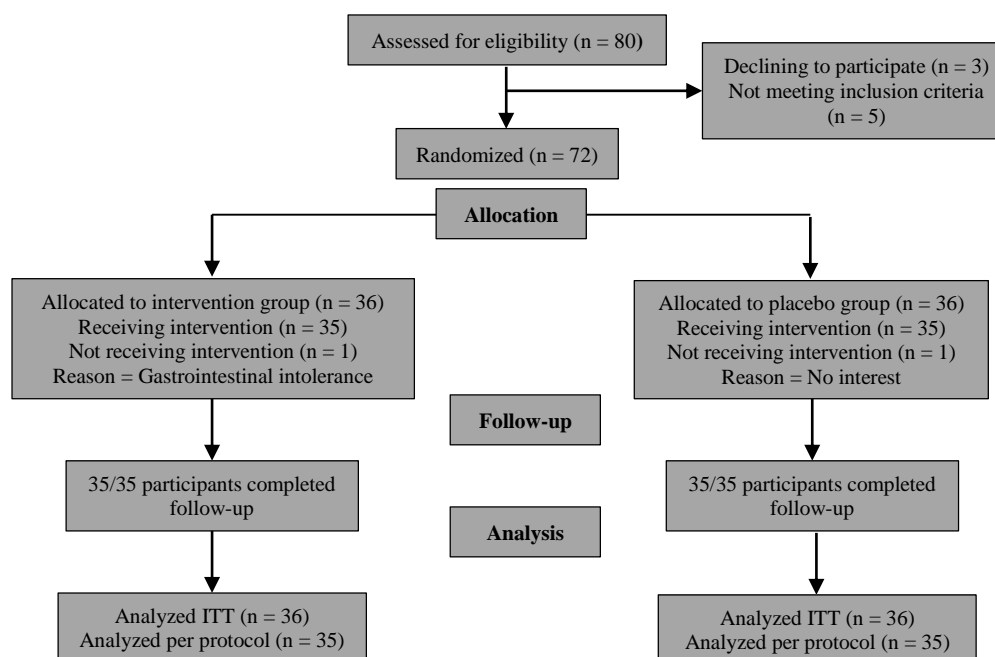


Figure 1. Consolidated Standards of Reporting Trials (CONSORT) flow diagram of study participants (ITT: Intention-to-treat)

Table 1. General demographic and clinical characteristics of study participants in the probiotic and control groups at baseline

	Placebo group (n = 36)	Probiotic group (n = 36)	P*
Gender			
Men	18 (50)	16 (45)	0.63**
Women	18 (50)	20 (55)	
Duration of PD (year)	6.15 ± 3.69	5.85 ± 4.37	0.75
Age (year)	64.72 ± 7.02	65.00 ± 6.82	0.86
Dosage of the drug (mg/day)	733.67 ± 390.34	656.58 ± 373.45	0.39
Height (cm)	167.11 ± 7.26	165.97 ± 6.73	0.49
Weight at study baseline (kg)	69.96 ± 11.39	69.85 ± 10.32	0.96
Weight at end of the trial (kg)	70.55 ± 11.73	71.18 ± 10.33	0.82
BMI at study baseline (kg/m ²)	24.99 ± 3.20	25.42 ± 3.90	0.61
BMI at end of the trial (kg/m ²)	25.11 ± 3.31	25.54 ± 3.94	0.63

Data are presented as mean ± standard deviation (SD) for continuous variables and number (%) for categorical variables.

*Obtained from an independent t-test; **Obtained from the chi-square test

PD: Parkinson's disease; BMI: Body mass index

Sensitivity analyses, including additional adjustment for age, sex, and disease duration, yielded similar findings, confirming the robustness of the primary and key secondary outcomes.

Primary outcome: As presented in table 3, although no significant differences were observed between the groups at baseline, the probiotic group demonstrated significantly greater improvements in both SBM and QOL compared with the placebo group at the end of the trial ($P = 0.001$ for both). After 6 weeks, the probiotic group showed a significant increase in SBMs, from 2.42 ± 0.65 at

baseline to 5.83 ± 1.46 at week 6, compared to 2.39 ± 0.60 to 2.83 ± 0.65 in the placebo group. The mean increase in SBM frequency was 3.41 in the probiotic group versus 0.44 in the placebo group, with an adjusted mean difference of 2.97 [95% confidence interval (CI): 2.46-3.53, $P < 0.001$] after controlling for baseline dietary intake and physical activity. The proportion of participants achieving ≥ 3 SBMs/week post-intervention was 80% in the probiotic group versus 36.2% in the placebo group ($P < 0.001$). These findings were consistent in both ITT and per-protocol analyses.

Table 2. Comparison of dietary intake and physical activity between baseline and after 6 weeks in probiotic and control groups

	Placebo group (n = 36)			Probiotic group (n = 36)		
	Week 0	Week 6	P	Week 0	Week 6	P*
Food groups (servings/day)						
Dairy	1.21 ± 1.12	0.96 ± 1.12	0.09	0.79 ± 0.96	0.78 ± 1.01	0.74
Fruits	3.50 ± 2.22	2.99 ± 1.87	0.59	3.20 ± 2.52	3.47 ± 2.39	0.72
Vegetables	1.51 ± 1.37	1.41 ± 1.42	0.43	1.74 ± 1.10	1.62 ± 1.05	0.28
Grains	10.98 ± 5.37	9.98 ± 4.67	0.68	10.46 ± 5.39	10.39 ± 4.74	0.79
Meats	5.36 ± 2.81	5.56 ± 2.88	0.55	4.97 ± 2.73	5.34 ± 2.78	0.83
Fat and oils	10.89 ± 4.71	11.39 ± 4.53	0.51	10.07 ± 5.88	10.12 ± 5.46	0.87
Total energy intake (kcal/day)	2122.62 ± 560.00	2027.33 ± 510.00	0.17	1938.06 ± 582.00	1994.05 ± 562.00	0.16
Water intake (ml/day)	1260.00 ± 417.94	1240.00 ± 410.00	0.85	1277.78 ± 397.47	1257.70 ± 390.00	0.89
Soluble fiber (g/day)	0.71 ± 0.48	0.50 ± 0.39	0.40	0.62 ± 0.46	0.47 ± 0.46	0.26
Insoluble fiber (g/day)	3.71 ± 2.63	3.41 ± 2.77	0.15	2.90 ± 2.11	2.46 ± 1.98	0.62
Crude fiber (g/day)	8.53 ± 4.54	7.98 ± 3.99	0.36	7.62 ± 3.86	8.76 ± 4.33	0.17
Total fiber intake (g/day)	21.49 ± 13.72	20.32 ± 13.21	0.86	19.46 ± 9.77	20.87 ± 9.92	0.86
MET-hour/day	34.98 ± 3.59	34.98 ± 3.59	> 0.99	38.45 ± 4.01	38.45 ± 4.01	> 0.99

Data are presented as mean ± standard deviation (SD).

*Obtained from paired sample t-test

MET: Metabolic equivalent; 1 MET \approx 3.5 ml O₂/kg/minute

Table 3. Change in the mean number of spontaneous bowel movements (SBMs) and quality of life (QOL) related to constipation and Unified Parkinson's Disease Rating Scale-Part III (UPDRS III) between baseline and end of the trial in the probiotic and control groups

	Placebo group (n = 36)	Probiotic group (n = 36)	P*
SBM per week at the study baseline	2.39 ± 0.60	2.42 ± 0.65	0.850
SBM per week at end of the trial	2.83 ± 0.65	5.83 ± 1.46	0.001
QOL score at study baseline	32.44 ± 17.93	38.58 ± 19.98	0.170
QOL score at end of the trial	30.97 ± 17.31	15.75 ± 12.54	0.001
UPDRS III at study baseline	26.97 ± 10.10	27.89 ± 8.80	0.680
UPDRS III at end of the trial	25.97 ± 9.64	27.97 ± 8.99	0.400

Data are presented as mean ± standard deviation (SD).

*Obtained from an independent t-test; P-values < 0.05 were considered statistically significant; statistical power was calculated using G*Power version 3.1.

SBM: Spontaneous bowel movement; QOL: Quality of life; UPDRS III: Unified Parkinson's Disease Rating Scale-Part III (motor examination)

Secondary outcomes

- **PAC-QOL:** The probiotic group showed a significant reduction in total PAC-QOL scores, from 38.58 ± 19.89 at baseline to 15.75 ± 12.54 post-intervention. The placebo group showed minimal reduction, from 32.44 ± 17.93 to 30.97 ± 17.31. The adjusted mean difference in score changes between groups was -15.22 points (95% CI: -22.32 to -8.11, P < 0.001), indicating a significant improvement in constipation-related QOL among participants receiving probiotics.
- **UPDRS III:** No significant changes were observed in motor function scores. In the probiotic group, the mean UPDRS III score slightly increased from 27.89 ± 8.80 to 27.97 ± 8.99, while the placebo group decreased from 26.97 ± 10.10 to 25.97 ± 9.64. The adjusted mean difference in change between groups was 2.04 points (95% CI: -2.73 to 6.73, P = 0.40), indicating no statistically significant difference. These results suggest that probiotics improved bowel function without affecting motor symptoms over the 6-week period.

Adverse events and safety: No serious adverse events were reported. Mild bloating occurred in one participant in the probiotic group and resolved spontaneously. No participants discontinued the intervention due to adverse events, confirming good tolerability.

Sensitivity analysis: Analyses using both ITT and per-protocol datasets produced consistent results for the primary outcome. The robustness of the findings was confirmed after adjusting for baseline dietary intake and physical activity.

Discussion

Principal findings: Our randomized, double-blind, placebo-controlled trial evaluated the efficacy of a

six-week multi-strain probiotic supplementation on constipation in patients with PD. The results demonstrated a statistically and clinically significant increase in SBMs per week in the probiotic group compared to placebo (mean increase: 3.41 vs. 0.44, mean difference: 2.97, 95% CI: 2.46-3.53, P < 0.05). Additionally, participants receiving probiotics showed marked improvements in QOL related to bowel function as measured by PAC-QOL (adjusted mean difference: -15.22 points, 95% CI: -22.32 to -8.11, P < 0.05). No significant changes were observed in UPDRS Part III motor scores, indicating that GI improvements occurred independently of motor function.

Comparison with previous studies: Recent years have seen growing interest in the role of probiotics for managing constipation in PD. A 2023 meta-analysis by Xie et al. reported that probiotic supplementation significantly improved bowel movement frequency and stool consistency, and reduced laxative use in patients with PD.¹⁵ Similarly, an RCT by Ibrahim et al. demonstrated that a multi-strain probiotic (Hexbio) improved bowel function and gut motility in patients with PD.¹⁶ Consistent findings were also observed in other clinical trials including Barichella et al.¹⁷ Notably, Barichella et al. demonstrated that a fermented milk containing multiple probiotic strains significantly improved stool frequency and consistency in PD patients with constipation. Furthermore, a systematic review by Yin and Zhu concluded that probiotic supplementation could improve gut motility and reduce constipation severity in neurodegenerative disorders, particularly PD.¹⁸ Despite these encouraging results, heterogeneity in strains, dosage, treatment duration, and outcome measures across studies underscores the need for standardized protocols and multicenter trials.

Several mechanistic pathways, including SCFA production, modulation of gut permeability, and anti-inflammatory effects, may underlie the observed benefits, although these remain hypothetical without direct metabolomic or multi-omics data. A comprehensive meta-analysis by Xie et al., including 12 RCTs with a total of 818 patients with PD, reported that probiotics significantly increased stool frequency compared with controls, with a weighted mean difference (WMD) of 0.94 bowel movements per week (95% CI: 0.53-1.34).¹⁵ While these results support the beneficial role of probiotics in alleviating constipation, the authors highlighted substantial heterogeneity related to probiotic strains, treatment duration, and outcome definitions. In comparison, our trial demonstrated a larger effect size (mean difference of 2.97 SBMs/week), which may reflect differences in probiotic formulation, baseline severity of constipation, and the relatively homogeneous Iranian cohort. Importantly, our study strengthens the existing body of evidence by using standardized Rome IV criteria and validated QOL assessments, thereby addressing some of the methodological limitations identified in previous trials.

Mechanisms underlying probiotic effects:

Several biological mechanisms likely contribute to the observed benefits:

1. **SCFAs:** Probiotic strains such as *L. acidophilus* and *B. breve* produce SCFAs, particularly butyrate, which enhance enteric nervous system activity, promote smooth muscle contractility, increase mucus secretion, and reduce intestinal inflammation.¹⁰
2. **Intestinal barrier function:** Probiotics strengthen tight junction integrity, reducing intestinal permeability and systemic exposure to pro-inflammatory molecules, which may exacerbate PD pathology.¹⁹
3. **Gut-brain axis modulation:** Probiotics modulate vagal and serotonergic pathways, influencing gut motility and central nervous system (CNS) signaling.²⁰
4. **Immunomodulation:** Probiotic supplementation reduces pro-inflammatory cytokines [interleukin (IL)-6, tumor necrosis factor-alpha (TNF-α)] and increases anti-inflammatory cytokines (IL-10), supporting both gut and systemic health.¹⁹
5. **Microbiota composition and diversity:** Multi-strain probiotics increase beneficial taxa (*Lactobacillus*, *Bifidobacterium*) and decrease potentially pathogenic bacteria

(*Enterobacteriaceae*), improving gut microbiota diversity and correlating with motility improvement.²¹

Although we did not perform microbial profiling, these mechanisms provide a plausible explanation for the increased SBMs and improved PAC-QOL scores observed in our study. Future incorporation of microbiome sequencing and metabolomic analyses will be essential to confirm these mechanisms.

ANCOVA using baseline UPDRS Part III scores as a covariate revealed no significant influence of motor severity on GI outcomes (adjusted $P = 0.635$). Given the short intervention duration (6 weeks) and the predominantly peripheral target of probiotic treatment, large clinically meaningful improvements in motor function were not expected. Mechanistic pathways such as the gut-brain axis, modulation of systemic inflammation, and SCFA production could plausibly influence neurodegeneration over longer periods. Preclinical and early clinical studies suggest that microbiome modulation may have delayed or cumulative effects on neuroinflammation and motor progression. Additionally, UPDRS measurement variability and floor/ceiling effects can limit the detection of small changes in short-term trials. Therefore, longer, adequately powered longitudinal studies incorporating clinical motor endpoints and objective biomarkers are warranted to evaluate potential disease-modifying effects of probiotics in PD. These findings highlight that the current intervention primarily targeted GI symptoms, and the lack of motor improvement should not be interpreted as evidence against potential long-term neuroprotective effects of probiotics.

Safety and adherence: The probiotic supplement was well tolerated. Mild and transient bloating was reported in a single participant. No serious adverse events occurred, and adherence exceeded 90%, demonstrating the feasibility and safety of multi-strain probiotics in PD populations.

Limitations: Key limitations include the single-center design, short intervention duration, absence of microbial profiling, and potential recall bias from 24-hour dietary recalls, particularly in cognitively impaired patients. Another limitation is that the primary outcome, weekly SBMs, was recorded using patient-reported daily diaries. Although diaries were collected prospectively with weekly telephone reminders and in-clinic checks to maximize

completeness, patient-reported data remain susceptible to reporting errors and recall bias. To mitigate this, participants were trained on accurate diary recording, reminder calls were conducted, and diaries were checked for completeness during clinic visits. Future studies may consider the use of electronic real-time recording systems or objective stool frequency monitoring to further reduce reporting bias.

Future directions: Future research should focus on multicenter, long-term trials with microbial profiling to clarify the mechanisms of probiotic action. Investigating effects on other NMSs (e.g., depression, cognitive outcomes) and evaluating combined interventions (dietary modifications, pharmacologic therapy) could further optimize constipation management in PD. While our study demonstrates beneficial effects of probiotics on GI outcomes, these results should be interpreted with caution given the limited sample size, short intervention duration, and single-center design. Further well-powered multicenter RCTs are required to confirm these findings and to assess potential long-term effects on PD progression.

Conclusion

This study demonstrates that six-week supplementation with a multi-strain probiotic significantly improved SBMs and constipation-related QOL in patients with PD, without causing

adverse effects. These findings add to the growing evidence that modulation of gut microbiota may represent a safe and effective adjunctive therapy for NMSs in PD, particularly GI dysfunction. Although no changes in motor function were observed, the favorable safety profile and clinical efficacy of probiotics justify their consideration in supportive care strategies. Nevertheless, the single-center design, relatively short intervention duration, and absence of microbiota profiling limit the generalizability and mechanistic insights of our findings. Confirmation in larger, multi-center and longer-term studies, integrating microbiota and metabolomic analyses, is warranted before recommending routine clinical use.

Conflict of Interests

The authors declare no conflict of interest in this study.

Acknowledgments

The authors would like to express their sincere gratitude to all the patients who participated in this study, as well as to the clinical staff and neurologists who supported patient recruitment and assessments. The authors also appreciate the Ethics Committee of Islamic Azad University, Science and Research Branch, for reviewing and approving the study protocol. This study received no external funding.

References

- Luo Y, Qiao L, Li M, Wen X, Zhang W, Li X. Global, regional, national epidemiology and trends of parkinson's disease from 1990 to 2021: findings from the global burden of disease study 2021. *Front Aging Neurosci* 2024; 16: 1498756.
- Sarmadi M, Rezaei M, Poursadeghiyan M, Soltaninejad M, Moradi S, Ahangarzadeh M, et al. Burden of parkinson's disease in Iran: disparities, trends, and the impact of social development indicators. *Neurol Sci* 2025; 46(8): 3651-62.
- Di Biase L, Pecoraro PM, Di Lazzaro V. Validating the accuracy of parkinson's disease clinical diagnosis: A UK brain bank case-control study. *Ann Neurol* 2025; 97(6): 1110-21.
- Yao L, Liang W, Chen J, Wang Q, Huang X. Constipation in parkinson's disease: A systematic review and meta-analysis. *Eur Neurol* 2023; 86(1): 34-44.
- Ivan IF, Irincu VL, Diaconu S, Falup-Pecurariu O, Ciopleiaș B, Falup-Pecurariu C. Gastro-intestinal dysfunctions in Parkinson's disease (Review). *Exp Ther Med* 2021; 22(4): 1083.
- Shin C, Kim SI, Park SH, Kim JM, Lee JY, Chung SJ, et al. Diagnostic accuracy and predictors of alpha-synuclein accumulation in the gastrointestinal tract of Parkinson's disease. *NPJ Parkinsons Dis* 2024; 10(1): 155.
- Zhai J, Zhang Y, Ma S, Zhang Y, Jin M, Yan H, et al. The role of gut microbiota dysbiosis in drug-induced brain injury: mechanisms and therapeutic implications. *Front Cell Dev Biol* 2025; 13: 1604539.
- Tai YC, Liao PH, Leta V, Lin CH, Chaudhuri KR. Irritable bowel syndrome based on Rome IV diagnostic criteria associates with non-motor symptoms of Parkinson's disease. *Parkinsonism Relat Disord* 2023; 113: 105496.
- Gao A, Lv J, Su Y. The Inflammatory Mechanism of Parkinson's Disease: Gut Microbiota Metabolites Affect the Development of the Disease Through the Gut-Brain Axis. *Brain Sci* 2025; 15(2): 159.
- Kalyanaraman B, Cheng G, Hardy M. Gut microbiome, short-chain fatty acids, alpha-synuclein, neuroinflammation, and ROS/RNS: Relevance to Parkinson's disease and therapeutic implications. *Redox Biol* 2024; 71: 103092.
- Xiang S, Ji JL, Li S, Cao XP, Xu W, Tan L, et al. Efficacy and Safety of Probiotics for the Treatment of Alzheimer's Disease, Mild Cognitive Impairment, and Parkinson's Disease: A Systematic Review and Meta-Analysis. *Front Aging Neurosci* 2022; 14: 730036.
- Leshchysyn IM, Markulan LY, Okhotska OI, Susak YM, Byk PL. Quality of life in patients with chronic slow-transit constipation according to the PAC-QOL scale one year after surgical treatment: comparison with preoperative data and reference values. *Wiad Lek* 2025; 78(4): 726-34.
- Clina JG, Sayer RD, Friedman JE, Chui TK, Mehta T, Rimmer JH, et al. Reliability and Validity of the International Physical Activity Questionnaire Adapted to Include Adults With Physical Disability. *J Phys Act Health* 2024; 21(2): 189-96.
- Nikjooy AP, Jafari HP, Saba MAMP, Ebrahimi NMP, Mirzaei RM. Patient Assessment of Constipation Quality of Life Questionnaire: Translation, Cultural Adaptation, Reliability, and Validity of the Persian Version. *Iran J Med Sci* 2018; 43(3): 261-8.
- Xie L, Chen D, Zhu X, Cheng C. Efficacy and safety of probiotics in Parkinson's

- constipation: A systematic review and meta-analysis. *Front Pharmacol* 2022; 13: 1007654.
16. Ibrahim A, Ali RAR, Manaf MRA, Ahmad N, Tajurruddin FW, Qin WZ, et al. Multi-strain probiotics (Hexbio) containing MCP BCMC strains improved constipation and gut motility in Parkinson's disease: A randomised controlled trial. *PLoS One* 2020; 15(12): e0244680.
 17. Barichella M, Pacchetti C, Bolliri C, Cassani E, Iorio L, Pusani C, et al. Probiotics and prebiotic fiber for constipation associated with Parkinson disease: An RCT. *Neurology* 2016; 87(12): 1274-80.
 18. Yin S, Zhu F. Probiotics for constipation in Parkinson's: A systematic review and meta-analysis of randomized controlled trials. *Front Cell Infect Microbiol* 2022; 12: 1038928.
 19. Zheng Y, Zhang Z, Tang P, Wu Y, Zhang A, Li D, et al. Probiotics fortify intestinal barrier function: a systematic review and meta-analysis of randomized trials. *Front Immunol* 2023; 14: 1143548.
 20. Nápoles-Medina AY, Aguilar-Uscanga BR, Nápoles-Medina BG, Brand-Rubalcava PA, Tejeda-Martínez AR, Flores-Soto ME. Bridging the gap: Unveiling the gut's influence on Parkinson's disease through probiotic interventions. *Neurol Perspect* 2025; 5(4): 100206.
 21. Bedarf JR, Romano S, Heinzmann SS, Duncan A, Traka MH, Ng D, et al. A prebiotic dietary pilot intervention restores faecal metabolites and may be neuroprotective in Parkinson's Disease. *NPJ Parkinsons Dis* 2025; 11(1): 66.