Peer-review Comments and Author Responses

Reviewer 1

Dear Reviewer 1,
We would like to thank you for the insightful comments, we believe that the modifications made through your comments improved the clarity of our text. Given word count constraints for this article type, some suggested changes were not possible to follow, otherwise, the maximum word count would be exceeded. Following we explain the changes implemented point-by-point.

1. *"I believe the beginning of the introduction should include epidemiological information about Parkinson's disease, covering its most common symptoms and discussing how it impacts the lives of patients and caregivers.*

Response: A brief mention of epidemiological information and disease impact on the patient's quality of life is described from lines 91 to 96. Unfortunately, the limited word count limit does not allow a more detailed description of those topics.
Lines 91 - 96: "Symptoms of Parkinson's Disease (PD) are progressive and include both motor and non-motor symptoms. Around 38% of patients with PD experience major depressive disorder (MDD) (Cong et al., 2022) which has a significant negative impact on subjective perception of disability, daily functioning, and quality of life (Assogna et al., 2020). MDD increases healthcare consumption; amplifying costs and adding to the burden of disease experienced at both the individual and societal levels (World Health Organization, 2017)."

2. *"Describing the current standard of care for symptomatic control, depression, and treatment of the disease would help convey why there is a gap available for testing other drugs or interventions."*

Response: As this study focuses on major depressive disorder (MDD) in patients with Parkinson's, we agree that these are very important points. We had previously included them briefly in the discussion. However, we agree that it would be better to mention thin on the introduction; thus, we have relocated the following sentence from the discussion to the introduction.
Lines 103 - 105: "Although there are no standard guidelines for the treatment of depression in patients with PD, some studies suggest using SSRIs for treating MDD in this population (Pontone & Mills, 2021)."

3. *"Explain how probiotics could act on the pathophysiology and symptom control of PD."*

Response: Thank you for the insightful comment. We believe that this is an important point in our protocol. However, we believe we have addressed the pathophysiology and symptom control of PD and how probiotics could act on the pathophysiology and symptom control of PD.
Lines 98-103: "In these patients, the blood-brain barrier may be disrupted as a result of gut-derived inflammatory products, allowing dopaminergic loss to occur in the substantia nigra, leading to classic PD motor symptoms (Banks & Erickson, 2010). Serotonergic depletion in the raphe nuclei and noradrenergic loss in the locus coeruleus lead to behavioral symptoms and support the association of PD with MDD (Grosch et al., 2016)."

4. *"Introduce the correlation between probiotics and neuroprotective effects and their relationship with depressive symptoms, and provide a brief overview of existing literature, justifying why to test a probiotic intervention for Parkinson's disease."*

Response: We appreciate this comment. We addressed how probiotics could act on the pathophysiology and symptom control of PD as follows:
Lines 105-112: "Probiotic supplementation has demonstrated neuroprotective effects due to inhibition of pathogen colonization, modulation of the microbiome, immunomodulation, and improved host epithelial barrier function, leading to decreased accumulation of alpha-synuclein in both the enteric and central nervous systems (Tan et al., 2021). While the association between the use of probiotics and improved depression symptoms has been well established (El Dib et al., 2021), to the best of our knowledge, no study has been conducted to investigate the effect of probiotics on MDD in patients with PD."

5. "Regarding the inclusion criteria, I believe it would be ideal to describe the definition of mild, moderate, and severe Parkinson's disease and where this classification can be obtained, including the reference used."

Response: We have addressed this important suggestion by defining mild-to-moderate PD as Hoehn and Yahr stages I-III and including a corresponding reference.
Lines 130-131: "Adults with clinical diagnoses of MDD and mild-to-moderate PD (Hoehn and Yahr stages I-III) (Clark et al., 2016) and stable treatment with SSRI will be included."

6. "The study will include patients with mild to moderate disease without stratification. Could the imbalance in this variable significantly interfere with the study results?"

Response: Thank you for the insightful comment. After careful consideration, we believe that permuted block randomization, along with adequate allocation concealment, coupled with an appropriate sample size, is sufficient to allow for a relatively balanced distribution of this variable between groups.

7. *"Will patients who have previously used probiotics or have any gastrointestinal conditions be included in the study?"*

Response: We have amended the exclusion criteria to explicitly include primary gastrointestinal diseases and previously probiotic use.
Lines 131-134: “Exclusion criteria will be cognitive impairment, neurological disease other than PD, primary gastrointestinal diseases, and recent (within the last 12 weeks) use of probiotics (full inclusion and exclusion criteria in Table S1)."

8. *“The recruitment strategies are not clear. I suggest describing how you will recruit the subjects and in how many centers.”*

Response: We have amended the recruitment strategy section as follows to add further clarity. Under the “Trial designs” heading, we have described the study design as “single center.”
Lines 137-138: “Participants will be recruited via investigator-based subject recruitment from inpatient and outpatient neurology and psychiatry departments at a single university hospital."
Lines 125-126: "We report the design of a single center, phase IIb, randomized, triple-blind, placebo-controlled, parallel-group, superiority trial, with a two-week run-in phase."

9. *“In the blinding section, describe how adverse effects are classified in terms of severity, adding the reference.”*

Response: We have added “see discontinuation section” under the blinding heading. We extended the section to say who can trigger unblinding as well.
Lines 169-171: “If a serious adverse event occurs (see discontinuation section), blinding can be broken by order of the treating physician or data monitoring committee, if deemed necessary, through the e-CRF.”
We have also amended the secondary outcome “Adverse Events” by including classifications of AE severity as follows:
Lines 292-297: “Adverse events will be classified as Grade 1 (mild), 2 (moderate), 3 (severe), 4 (life-threatening), or 5 (lethal) according to the Common Terminology Criteria for Adverse Events (CTCAE) version 5 or the most recent publication (Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0, 2017). The type, grade, and frequency (%) of adverse events will be compared between groups.”

10. *“In the timeline, I believe the appropriate term should be "assess" [SIC] instead of "confirm."“*

Response: We have amended the manuscript as follows:
Line 188: “The study has a two-week run-in period to assess compliance before randomization.”

11. *“The acronym "HIPAA"-telehealth should be described and referenced.”*

Response: We have written the acronym in full as follows:
Lines 195-196: “All assessment visits will be conducted either on-site or remotely via a Health Insurance Portability and Accountability Act (HIPAA)-compliant telehealth platform.”

12. *“In the "data collection" section, you may describe the variables and how they will be measured. One suggestion is to group binary variables (yes/no) and mention them together. Other variables such as "alcohol use" and "comorbidities" should specify the assessment.”*

13. *"The MADRS and BDI-II scores should be described and referenced".*

Response: We have amended the data collection section to clearly describe the variables that will be collected.
Lines 201-208: "The following demographic data will be collected via participant interview and chart review during site visit 2: participant age (years), sex (male or female), ethnicity (Caucasian, Black, Asian, other), dependency on caretaker (yes or no), height (centimeters), weight (kilograms), smoking status (never, former, active), alcohol use (yes or no), comorbidities (yes or no, type), current use of antidepressant medication (total number of medications, type, and schedule), current stage of PD (Hoehn and Yahr scale [Clarke et al., 2016]) , physical activity physical activity (Modified Parkinson Activity [Taniguchi et al., 2021]), time since PD diagnosis (months), and current anti-Parkinson medications (name and dose)".

Response A: MADRS score description has been amended in the manuscript and the supplemental material.
Lines 257-258: "The MADRS is composed of 10 items, which are rated from 0-6, with total scores ranging from 0 to 60 points (Ketharanathan et al., 2016)."
Response B: Additional BDI-II score description has been included in the manuscript and the supplemental material.
Lines 268-271: "Depression will also be evaluated using BDI-II at baseline and 3 months. BDI-II is a 21-item self-reported scale, with a maximum score of 63 points which has been validated for individuals with Parkinson's disease (Steer et al., 1997)."

14. *"In the intervention, you should specify what protocol will be used in terms of dosage, timing, and composition of the intervention."*

Response A: The dose and composition are described.
Lines 217-219: "The intervention group will receive Cerebiome® capsules (Lactobacillus helveticus Rosell®-52 and Bifidobacterium longum Rosell®-175, 3 billion CFU each per capsule) (Wallace & Milev, 2021)."
Response B: The dosage and timing are described.
Lines 228-230: "Participants in both groups will ingest one capsule orally daily one hour before, or two hours after eating. The capsule will be taken 2 hours after scheduled antiparkinson medications to avoid interactions."

15. *"Regarding outcomes, the SSRI outcome should be better explained and detailed."*

Response: The SSRI outcome was better described in the manuscript. Given the short word limit stipulated by the journal for this article category, a brief explanation must be written in the main manuscript. Therefore, to overcome this limitation, we added a full outcomes section in the supplemental material with more detailed information and explanation for each outcome mentioned in this study protocol.
Liness 256-258: "Coprimary outcomes, MADRS and changes in SSRI equivalent doses, will be evaluated at baseline and 3 months. The MADRS is composed of 10 items, which are rated from 0-6, with total scores ranging from 0 to 60 points (Ketharanathan et al., 2016)."

16. *"In the secondary outcome section, using "levodopa dose modification" as a surrogate should be preceded by explaining the drug's usual dosage and how doses are modified. Report how and how often doses can be modified in the study."*

Response: Similar to the previous comment, we improved the description of "levodopa dose modification" in the "disease progression" secondary outcome in the manuscript. Additional detailed information and explanation about this outcome can be found in the supplemental material.
Lines 279-281: "Change in levodopa dose will be reported as the mean variation in milligrams per day (e.g., Δ mg/day3 months = mg/day at 3 months - mg/day at baseline)."

17. *"Will there be standardization of longitudinal care for this control? How will they be reported?"*

Response: Longitudinal care will be provided as per the participants' healthcare provider for PD and MDD control for the control and intervention groups. Both PD and MDD pharmacological treatment modification information is collected as part of the outcomes in this study.

18. *"In the "sample size calculation" section, I believe the word "similar study" should be replaced with "previous study" as the studies have significant methodological differences."*

Response: The authors acknowledge it is more appropriate to use "previous study" instead of "similar study." This was changed in the text. We appreciate this suggestion.
Lines 314-315: "(...) based on a previous study by Majeed et al. (2018)."

19. *"I suggest describing the MADRS scale at some point, including results that can be obtained and how to interpret them. This description would help understand the impact of a 4.5-point change on the scale."*

Response: Given the word count limitation, a brief description of the MADRS scale was included in the main manuscript. A full and detailed description of the MADRS scale was included in the supplemental material, along with the other outcomes.

20. *"Tables need to include a description, including the abbreviations used."*

Response: We appreciate the reviewer's comment and acknowledge the need for a description/legend for each figure and table. A description was included for each figure and table, including the abbreviations used.

21. *"Table 2 might be better represented by swapping columns for rows."*

Response: After extensive and meticulous discussion by the authors, we decided to keep the original format of Table 2. We highly appreciate the reviewer's suggestion.

22. *"The resolution of Figure 1 is low, making it unreadable when zoomed. Perhaps trying to obtain a better resolution could improve visibility."*

Response: We appreciate the reviewer's comment. We have recreated the image with a higher resolution.

23. *"Concerning the discussion, the sensitivity and specificity of MADRS should be referenced."*

Response: The reference has been added.
Lines 351-356: "A major strength of the protocol is the use of co-primary outcomes with joint modeling. The first co-primary outcome is the MADRS, which is widely used in clinical research to assess depression owing to its high specificity and sensitivity (0.995 AUC in PD). The MADRS was selected for this study because it relies on observer ratings and assesses fewer somatic symptoms. This helps avoid overestimating depression in patients with PD (Ketharanathan et al., 2016)."

24. "*The phrase "We believe it is most appropriate..." should be reformulated, specifying what you believe to be more appropriate more directly."*

Response: We appreciate this comment. The sentence has been reformulated for clarity.
Lines 353-356: “The MADRS was selected for this study because it relies on observer ratings and assesses fewer somatic symptoms. This helps avoid overestimating depression in patients with PD (Ketharanathan et al., 2016).”

25. *“Monte Carlo simulation ideally should be referenced. I couldn't comprehend whether you will incorporate it into the statistical analysis of the study or not.”*

Response: Monte Carlo simulation is not part of the statistical analysis proposed for this trial. It was mentioned in the limitations of this study as an analysis that could be somehow beneficial, but is not possible to perform in this study given insufficient data. To avoid confusion, we decided to exclude the mention of Monte Carlo simulation from the manuscript.

Reviewer 2

Dear Reviewer 2,
We would like to thank you for the insightful questions and the suggestions that helped us to improve our manuscript clarity and the methodological aspects of our proposed study. Your comments are addressed as follows.

26. *“Introduction (pag 3 - line 110): Do you find correct to report the 'commercial blend' of the drug, considering that in the probiotic-field many drugs have the same composition?”*

Response: Thank you for the insightful comment. We understand that providing a commercial blend may seem biased. However, after some extensive research, this blend was chosen due to it having shown efficacy in the treatment of MDD. We have written a new sentence, providing an explanation for using this particular commercial blend.
Lines 116-118: “This particular commercial blend was selected because the dose and constituents are standardized and it has demonstrated efficacy in the treatment of MDD (Wallace & Milev, 2021).”

27. “*Introduction (pag 3 – line 113): Why did you choose a so short follow-up time?”*

Response: We believe this is a valid point. We have included an additional sentence explaining the 3-month duration in the “timeline subsection” under the assessment heading.
Lines 189-191: “We selected a 3-month follow-up based on the observed efficacy of probiotics in managing depression across various populations within the same timeframe (Majeed et al., 2018).”

28. *“Methods (pag. 5 - line 189): Do you not think to investigate also the physical activity level at baseline? We have in fact important data that show the importance of this factor for MDD and PD too.”*

Response: Thank you for your suggestion, we have included a baseline assessment and grading of patients’ physical activity based on Modified Parkinson Activity Scale. Proper reference to the scale has also been added.
Line 207: “(...), physical activity (Modified Parkinson Activity [Taniguchi et al., 2021]) (...)”

29. *"Methods (pag. 5 - line 200): Could you explain why you speak about a 1:1 simple randomization, when you spoke before of a 'permuted block randomization' method, please?"*

Response: Thank you for the comment. We will be using a permuted block randomization with a 1:1 ratio.
5. "Outcomes (pag 6-line 243): could you please specify how you measure the 'changes in SSRI equivalent dose'?"

Response: We appreciate this question. We believe that this point was not clear in the text, as more than one reviewer has raised it. Due to the word-count limitation, we have added supplementary material with a detailed explanation about the 'changes in SSRI equivalent dose'.

30. *"Outcomes (pag 7-line 302): could you clarify please how do you figure out 'changes in SSRI equivalent dose' as a categorical variable?"*

Response: "Changes in SSRI dose" are measured as any change in the SSRI prescribed dose between 2 study timepoints. This outcome is measured as a binary outcome in which "yes" represents any increase or decrease in SSRI dose and "no" represents no changes in the prescribed SSRI dose between 2 study timepoints. The magnitude of dose change is not in the scope of this study, therefore, a binary variable for change in SSRI dose was preferred. The SSRI outcome was better described in the manuscript to make it more clear to the readers. Given the short word limit stipulated by the journal for this article category, a brief explanation must be written in the main manuscript. Therefore, to overcome this limitation, we added a full outcomes section in the supplemental material with more detailed information and explanation for each outcome mentioned in this study protocol.

Reviewer 3

Dear Reviewer 3,
We would like to thank you for the careful review of the paper and your kind words.
We believe the modifications have further strengthened our paper and we hope that you will find this revised version acceptable for publication. Thank you in advance for your time and consideration of our manuscript.

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