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CLINICAL TRIAL REPORT

Efficacy and Safety of Morinda Officinalis Oligosaccharides Combined with Probiotics as a Therapeutic Strategy for Major Depressive Disorder with Gastrointestinal Symptoms: A Randomized, Double-Blind, Controlled Clinical Trial

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Objective: This study aimed to evaluate the clinical efficacy and safety of Morinda officinalis oligosaccharides (MOO) combined with probiotics compared to placebo in individuals diagnosed with major depressive disorder (MDD) who also presented with gastrointestinal symptoms, a common comorbidity in this population.

Methods: In an 8-week, randomized, double-blind, placebo-controlled trial conducted at Beijing Anding Hospital, 108 patients meeting DSM-5 criteria for MDD were recruited. Participants were randomly assigned to receive either MOO (2 capsules BID) plus probiotics (Lacticaseibacillus paracasei L9, Bifidobacterium animalis subsp. lactis A6, Bifidobacterium bifidum B09, and Lactiplantibacillus plantarum LP06) or MOO plus placebo. The primary outcome was the change in HAMD-17 (17-item Hamilton Rating Scale for Depression) scores from baseline to Week 8. Both intention-to-treat (ITT) and per-protocol (PP) analyses were conducted.

Results: The ITT analysis revealed no significant difference in total HAMD-17 score reduction between groups ($Z = 0.02$, $p = 0.983$). However, the group receiving MOO plus probiotics demonstrated a significantly greater reduction in insomnia-related symptoms at Week 2 ($Z = -2.23$, $p = 0.028$). Although the overall depression severity did not differ, early response (Week 2) and response rates in the probiotics group were 35.19% and 51.85%, respectively, without significant group differences. Logistic regression suggested that female patients and those with a family history of psychiatric disorders were less likely to respond to treatment. Adverse events were mild, mainly gastrointestinal (eg, diarrhea, constipation), and occurred at the same rate (5.56%) in both groups.

Conclusion: Although MOO combined with probiotics did not significantly improve overall depressive symptoms compared to placebo, it showed early and specific benefits for insomnia symptoms. The intervention was safe and well tolerated. These findings highlight the potential role of gut microbiota modulation in treating MDD-related symptoms and suggest further research is needed to explore underlying mechanisms and identify responsive subgroups.

Trial Registration: The study was prospectively registered with the Chinese Clinical Trial Registry (ChiCTR2200055780; https://www.chictr.org.cn/showproj.html?proj=150742) on January 19, 2022.

Keywords: major depressive disorder, probiotics, morinda officinalis oligosaccharides, gastrointestinal, efficacy

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Introduction

Major depressive disorder (MDD) is a widespread and long-standing mental health condition, currently impacting over 280 million individuals globally. It is a major contributor to the global disease burden and is closely associated with suicide, accounting for more than 700,000 deaths each year.[1] Currently, pharmacological intervention remains the primary approach for treating depression; however, approximately 60% of people with MDD experience some degree of nonresponse to first-line treatments, and approximately one-third continue to experience symptoms despite further treatment.[2]

A pivotal role of gut microbiota in the pathogenesis of MDD has long been postulated.[3] The gut microbiome has emerged as a promising target for novel therapeutic strategies, aiming to improve mental health, immunity, and energy balance by modulating microbial metabolism within the gut. Prebiotics and probiotics have gained significant attention in microbiota–gut–brain (MGB) axis research, not only as modulators of the host gut microbiota but also as potential therapeutic agents for mood disorders, including MDD.[4] With a deeper understanding of the role of the MGB axis in the pathophysiology of MDD, regulating gut microbiota through probiotics and other methods has become a promising intervention in the adjunctive treatment of MDD, and its effectiveness and safety have been proven in clinical studies.[5–7] The disruption of gut microbiota observed in patients with irritable bowel syndrome resembles the reduced microbial diversity found in individuals with MDD, suggesting a complex interplay between MDD and gastrointestinal dysfunction.[8] Patients with MDD accompanied by gastrointestinal symptoms may benefit from additional probiotic treatment.

The undesirable side effects of antidepressants often result in poor compliance with treatment.[9] Capsules of Morinda officinalis oligosaccharide (MOO) as a traditional Chinese medicine (TCM) mainly contain inulin-type oligosaccharides extracted from the roots of M. officinalis and have been approved for sale by the Chinese Food and Drug Administration (CFDA) since 2012. Previous randomized controlled trials have suggested that the efficacy of MOO capsules in treating mild to moderate depression might not be inferior to that of conventional antidepressants, which may provide a potential alternative direction for clinical antidepressant therapy.[10] This FAERS-based pharmacovigilance[11] study revealed that probiotics may cause gastrointestinal adverse effects, such as bloating, abdominal pain, and flatulence; however, most studies suggest these reactions are generally mild.

The MOO capsule, a natural prebiotic, may exert its antidepressant effects through the microbiota-gut-brain axis by modulating immune responses, reducing harmful gut microbes, and promoting the growth of beneficial probiotics.[12,13] Research indicates that MOO exerts its antidepressant effect through the regulation of serotonin (5-HT) synthesis within the gut microbiota.[14] The study demonstrated that low molecular weight fructo-oligosaccharides (FOS) derived from Morinda officinalis can mitigate depression-like behavior, with FOS supplementation fostering the growth of beneficial bacterial strains linked to antidepressant effects and helping to restore gut microbiota balance.[12] MOO may exert antidepressant effects by modulating the gut microbiota.

Furthermore, to our knowledge, there are currently no clinical studies investigating the combined use of MOO capsules and probiotics for treating depression, particularly in individuals with concurrent gastrointestinal symptoms. Despite the growing evidence supporting the antidepressant effects of both MOO and probiotics individually, the synergistic potential of combining these two agents to modulate the gut–brain axis remains unexplored. Given the limitations of current antidepressant therapies and the high prevalence of gastrointestinal symptoms among patients with MDD, this study addresses a critical clinical gap by evaluating a novel, low-risk, and potentially synergistic treatment strategy. In this randomized controlled trial, participants were allocated to receive either MOO with probiotics or MOO with placebo to assess the therapeutic efficacy and safety of this combined intervention in a specific and underserved patient population.

Materials and Methods

Study Design

This 8-week, randomized, double-blind clinical trial enrolled 108 participants presenting with major depressive disorder and concurrent gastrointestinal symptoms. The participants were outpatients in Beijing Anding Hospital, affiliated with Capital Medical University. Participants were recruited from July 19, 2022, to December 15, 2023. All participants included in this study gave written informed consent. The study received ethical approval from the Ethics Committee of Beijing Anding Hospital, Capital Medical University (Approval No. [2021] Research No. [202]-202211FS-2/339), and

was prospectively registered in the Chinese clinical trial database (ChiCTR2200055780; https://www.chictr.org.cn/showproj.html?proj=150742) on January 19, 2022.

Participants

An individual must meet all the following inclusion criteria to be included in the study: (1) being diagnosed with MDD without psychotic symptoms, either first or recurrent episodes, according to the Diagnostic and Statistical Manual of Mental Disorders-5 (DSM-5) diagnostic criteria, using the Mini International Neuropsychiatric Interview (M.I.N.I). 7.0.2; (2) aged 18 to 65 years; (3) exhibiting mild to moderate depressive symptoms (reflected by a 17-item Hamilton Rating Scale for Depression [HAMD-17] score of 14 to 24); (4) having a Generalized Anxiety Disorder 7-item (GAD-7) score of 5 or more; (5) concurrently experiencing gastrointestinal symptoms, reflected by having at least three symptoms described in the Gastrointestinal Symptom Rating Scale (GSRS) and having a score of 4 to 6 on that scale; (6) being able to comprehend and willing to comply with the study protocol; being able to comprehend and willing to sign written informed consent forms.

Individuals who met any of the following exclusion criteria would be excluded from the study: (1) being diagnosed, either currently or previously, with any psychiatric disorder other than depressive disorder; (2) current substance abuse (defined as meeting criteria b, c, and d in the M.I.N.I).; (3) having a history of any surgical treatment, vagus nerve stimulation, deep brain stimulation, or stem cell therapy for depression; (4) currently being treated with non-convulsive electroconvulsive therapy (Modified Electroconvulsive Therapy [MECT]) or other physical treatments; (5) being in an unstable condition that may require medication or surgical treatment because of any physical illnesses; (6) having any organic gastrointestinal diseases such as inflammation, infection, tumors, or other structural abnormalities; (7) having used any antibiotics, probiotic products, or laxatives in the month prior to study entry; (8) having a history of allergy or nonresponse to MOO capsules.

Interventions

From the time of enrollment, the patients were asked to take MOO with either probiotics powder or placebo powder. Specifically, individuals in the intervention group were instructed to consume two sachets of probiotic powder each morning, along with two MOO capsules approximately 30 minutes after both breakfast and dinner. Those in the placebo group followed an identical schedule using placebo sachets and MOO capsules. All participants were advised to adhere to a consistent daily dosing routine, ideally within a 30-minute time window.

The specifications and instructions of the medications are as follows:

MOO capsule: The capsules were produced by Beijing Tong Ren Tang Co., Ltd. Each capsule contains 0.3 g of MOO. Take 2 capsules after breakfast and dinner, with a total daily dose of 1.2 g (4 capsules). The capsules should be swallowed whole and should not be chewed, broken, or crushed.

Probiotics powder: The probiotics powder was produced by San he Fu cheng Biotechnology Co., Ltd. The powder is distributed in sachets with 2 g of content in each. The probiotics powder contains *Lacticaseibacillus paracasei* L9, *Bifidobacterium animalis* subsp. *lactis* A6, *Bifidobacterium bifidum* B09, and *Lactiplantibacillus plantarum* LP06. The total bacterial count is 50 billion CFU every 2 g. The powder is to be taken once daily, 2 sachets each time, with warm water.

Placebo powder: The placebo powder was produced by San he Fu cheng Biotechnology Co., Ltd. The powder is distributed in sachets with 2 g of content in each; the packaging is identical to that of the probiotics powder. The placebo powder contains 2 g of freeze-dried corn starch and maltodextrin. The powder is to be taken once daily, 2 sachets each time, with warm water.

Measures

In this study, we designed a questionnaire to collect the participants' demographic information such as age, sex, marital status, education level, height, weight, and history of physical and mental illnesses.

We used the M.I.N.I.[15] to evaluate symptoms of depression, suicide risk, mania, psychosis, and substance abuse during screening. The M.I.N.I. is a structured diagnostic interview developed to identify major psychiatric disorders as classified under Axis I of the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV). The study used the M.I.N.I. 7.0.2, which was developed based on the DSM-5 criteria.

Depressive symptoms were evaluated using the 17-item Hamilton Depression Rating Scale (HAMD-17) at baseline, as well as at Weeks 2 and 8. Items are rated on either a 3-point or 5-point scale, with higher scores reflecting greater symptom severity. The total possible score ranges from 0 to 52. The HAMD-17 is a clinician-administered scale used to assess depressive symptoms; it evaluates functioning with 4 subscales: depression (Items 1, 2, 3, 7, and 8), anxiety (Items 9, 10, 11, 15, and 17), insomnia (Items 4, 5, and 6), and somatic symptoms (Items 12, 13, 14, and 16).[16]

The GAD-7 is effective at detecting changes in anxiety symptoms during treatment, with scores of 5 or higher indicating the presence of anxiety.[17]

The GSRS[18] is a validated instrument for evaluating gastrointestinal symptoms associated with specific diseases. It comprises 15 items grouped into five symptom domains: abdominal discomfort, reflux-related complaints, loose stool patterns, dyspeptic symptoms, and issues related to bowel regularity. Each item is scored on a seven-point Likert scale, where 1 represents the absence of symptoms and 7 reflects extreme severity.

The Constipation Assessment Scale (CAS)[19] is an 8-item self-administered questionnaire that has been used in numerous studies as a primary tool for assessing constipation. It can be completed in a short time (typically two minutes), making it a convenient and efficient assessment tool.

Outcome Measures

Primary Outcome Measure

The primary efficacy endpoint was the change from baseline at Week 8 in the HAMD-17 total score.

Secondary Outcome Measures

Secondary outcome measures included the changes in the scores on the HAMD-17, GAD-7, GSRS, CAS, and Body mass index (BMI) from baseline at Week 8, as well as the response rates, early response, and remission rates at Week 8 between the two groups evaluated with HAMD-17 scores. Treatment response was operationalized as a ≥50% decrease in the HAMD-17 total score from baseline, while remission was defined as achieving a HAMD-17 score ≤7 at Week 8.[20] Early response rate refers to the proportion of patients whose HAMD-17 scores were reduced by 20% or more between baseline and Week 2.[21] Body mass index (BMI) was derived by dividing body weight (kg) by the square of height (m^2).

Safety and tolerability were assessed with adverse events (AEs), vital signs checking, clinical laboratory tests, and electrocardiogram (ECG) parameters. A serious AE[22] is defined as any untoward medical occurrence resulting in persistent or significant disability, posing an immediate threat to life, causing death, necessitating hospitalization, or requiring medical intervention to prevent any unfavorable outcomes.

Randomization and Blinding

The patients were randomized to either the MOO-plus-probiotics group or the MOO-plus-placebo group in a 1:1 ratio using block randomization. The randomized allocation sequence was generated by an off-site statistician using the PLAN procedure in the SAS software (version 9.4). The random number table was kept by nurses and pharmacists outside the research team, who were responsible for packaging the medication used in this study according to the group assignments. Participants were assigned an enrollment number based on their order of the time of enrollment; every number represented the assignment to either the MOO-plus-probiotics group or the MOO-plus-placebo group which had specific medication schedules throughout the research. During the study, the results of group assignments were concealed from the patients, researchers, and evaluators.

Sample Size

The sample size was calculated using PASS 2021, with the HAMD-17 score as the primary efficacy endpoint. We referred to a study conducted by Muhammed Majeed et al,[23] of which the intervention period was 90 days. The score reductions in the intervention and control groups were −7.7 (4.66) and −2 (7.59), respectively, with a combined standard deviation of 6.85. However, considering our intervention and follow-up period would be 56 days in total and after discussions with clinical and methodological experts, we decided to conservatively reduce the score reduction in the intervention group by 10%, setting it at −6.9 (4.66). After calculation with $\alpha = 0.05$ and $\beta = 0.9$, the required sample size would be 54 cases per group (accounting for a 20% dropout rate).

Statistical Analysis

The primary analyses were conducted on an intent-to-treat (ITT) basis, including all randomized patients. Missing HAMD-17 scores were imputed using the last observation carried forward (LOCF) method. For baseline descriptive data, continuous variables were presented as mean (standard deviation) or median with interquartile range (IQR), and categorical variables were presented as count (percentage). The primary endpoint was analyzed using Analysis of Covariance (ANCOVA), with adjustments for baseline score, sex, first episode, and family history of psychiatric disorders, based on LOCF-imputed data. Sensitivity Analysis 1 involved multiple imputations for monotone missing data, in which a regression model incorporating age, sex, baseline score, first episode, and family history was used to generate imputed values. Multiple imputations (PROC MI) were employed to impute 25 values for every missing observation, with combined estimates analyzed using PROC MI ANALYZE in the SAS. Sensitivity Analysis 2, a per-protocol analysis, assessed whether there were differences in the HAMD-17 score reductions and response rates between the groups. Sensitivity Analysis 2 utilized linear mixed modeling (LMM) to evaluate the effect of the intervention on HAMD-17 scores, using all available data without imputation. The model incorporated treatment group, visit, sex, family history of psychiatric disorders, first episode, baseline score and their interaction (group × visit) as fixed effects, and the participant as a random effect. For secondary outcomes, the response rate, was initially assessed using the chi-square test. Subsequently, generalized estimating equations (GEEs) with a Poisson distribution and log-link (using PROC GENMOD) were applied, incorporating treatment group, visit, sex, family history of psychiatric disorders, and their interaction (group × visit), to calculate the relative risk (RR), 95% confidence intervals (CIs), and p-value.[24] Reductions in the scores for the depression factor on the GAD-7, GSRS, and CAS scores, were analyzed using LMM.

All statistical analyses were conducted using SAS software (version 9.4; SAS Institute Inc., Cary, NC, USA) and R (version 4.4.1; R Foundation for Statistical Computing, Vienna, Austria). Two-tailed P values were reported, with statistical significance set at $P < 0.05$.

Results

Participants

A total of 108 eligible participants were enrolled and randomly allocated in a 1:1 ratio to either the intervention group (MOO combined with probiotics, n = 54) or the control group (MOO combined with placebo, n = 54). Two individuals assigned to the intervention arm discontinued participation by Week 2, while five participants in the control arm failed to complete the full duration of the study (see Figure 1 for participant flow). Baseline demographic and clinical data for all enrolled subjects are summarized in Table 1. Females comprised the majority of the sample (67.6%). The participants had a mean age of 33.30 ± 10.22 years, an average of 15.19 ± 2.31 years of education, and a mean BMI of 22.55 ± 3.67 kg/m². Among the participants, 65.7% were experiencing their first episode, and 24.07% had a family history of mental disorders.

Primary Outcomes

In the intention-to-treat analysis, the change from baseline at Week 8 in the HAMD-17 total score between the two groups was not statistically significant (F =0.97, P = 0.326) (Figure 2A and Table S1).

Secondary Outcomes of Primary Outcome

The Per-Protocol (PP) analysis found no significant difference between the two groups at week8 (F =3.58, P = 0.062). The mixed-effects model analysis incorporated the sex, family history of psychiatric disorders, first episode, baseline score, treatment group, visit, and their interaction (group × visit) as fixed effects and the participant as a random effect, and it revealed that the interaction between the treatment group and time was not statistically significant (t=−1.65, P=0.099) (Table 2).

Secondary Outcomes

At Week 2, a significant difference was observed in the means of the reduction of HAMD-17 scores at Week 2 (F =0.88, P = 0.349). Meanwhile, a greater proportion of participants in the intervention group (n = 19/54, 35.19%) achieved a response, defined as a 50% or greater reduction from baseline in the HAMD-17 total score, compared to the control group (n = 10/54,

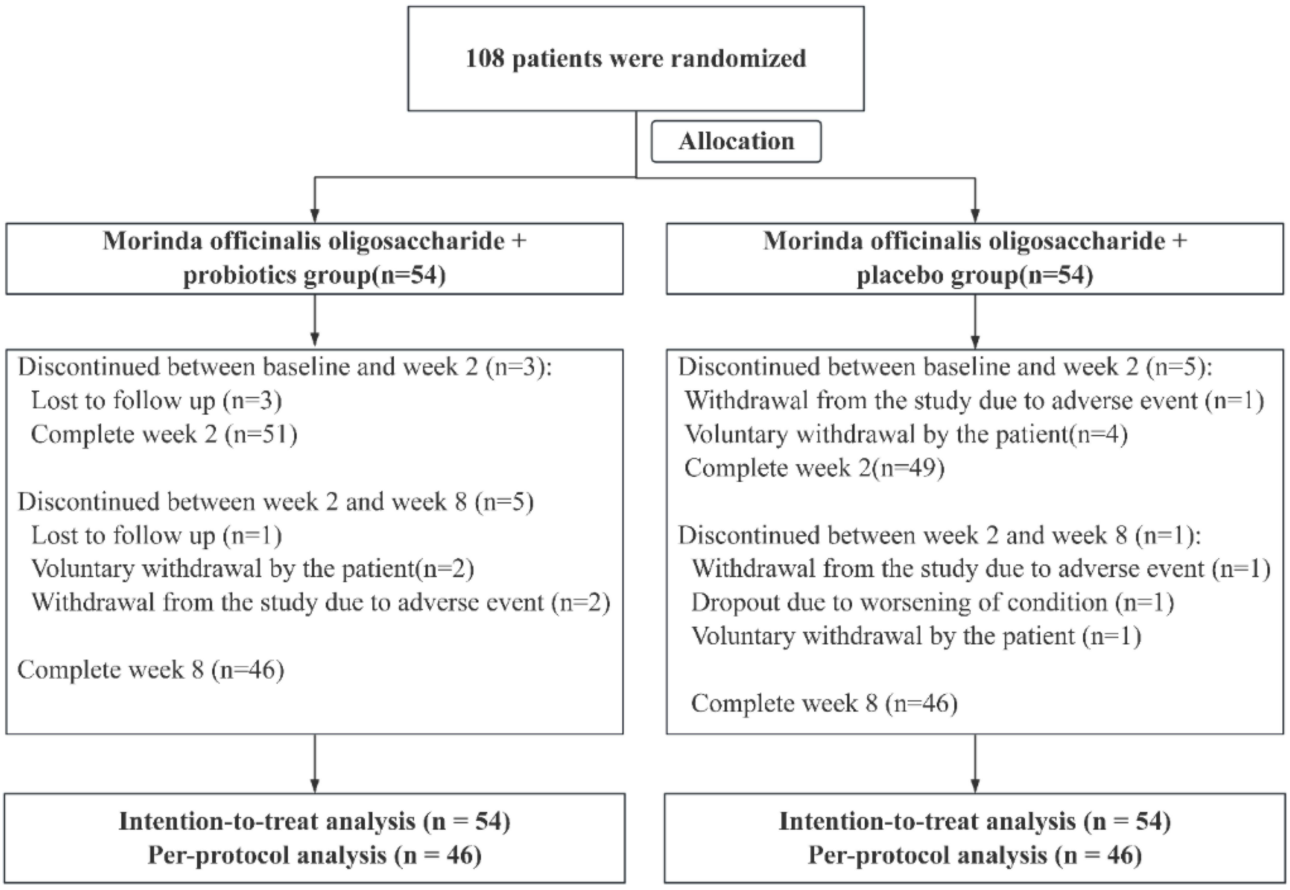

Figure 1 Overview of participant enrollment, group assignment, and follow-up throughout the study.

18.52%). Although this difference suggested the intervention group's superiority, the P-value (χ^2 = 3.82, P = 0.051) was slightly above 0.05, indicating that the result did not reach statistical significance. Moreover, this difference was no longer evident at Week 8 (intervention group: n = 28/54, 51.85%; control group: n = 25/54, 46.30%; χ^2 = 0.33, P = 0.564) (Figure 2B). The adjusted risk ratio for the response rate based on GEEs was 0.740 (95% CI: 0.208–2.632; P =0.608) (Table 3). No statistically significant interaction between the treatment group and time was observed for the GSRS (t = 0.09, P = 0.930), CAS (t = 1.13, P = 0.260), and GAD-7 (t = −0.70, P = 0.486) total scores across visits (Tables S2–S4).

Table 1 Demographic and Clinical Characteristics of Participants at Baseline

Variables	Participants n (%)	
	Intervention Group	Control Group
Age (mean [SD], year)	33.48(10.99)	33.11(9.49)
Sex		
Male	14(25.93)	21(38.89)
Female	40(74.07)	33(61.11)
Educational level (mean [SD], year)	16.00(12.00–16.00)	16.00(16.00–16.00)
Body mass index[a]		
Normal or healthy weight	28(51.85)	25(46.30)
Overweight or obese	17(31.48)	18(33.33)
Underweight	9(16.67)	11(20.37)

(*Continued*)

Table 1 (Continued).

Variables	Participants n (%)	
	Intervention Group	Control Group
First episode		
No	22(40.74)	15(27.78)
Yes	32(59.26)	39(72.22)
Marital status		
Divorced or widowed	5(9.26)	4(7.41)
Married	24(44.44)	22(40.74)
Unmarried	25(46.30)	28(51.85)
Family history of psychiatric illness		
No	46(85.19)	36(66.67)
Yes	8(14.81)	18(33.33)
Total course ≥ 5 years		
No	42(77.78)	44(81.48)
Yes	12(22.22)	10(18.52)
Total score of the HAMD-17 at baseline, median (IQR)	17.50(15.00–20.00)	17.5(16.00–22.00)
Total score of the GAD-7 at baseline, median (IQR)	10.50(7.00–14.00)	11.00(6.00–17.00)
Total score of the GSRS at baseline, median (IQR)	35.50(29.00–40.00)	35.00(31.00–39.00)
Total score of the CAS at baseline, median (IQR)	8.50(4.00–13.00)	9.00(4.00–14.00)

Notes: [a]A body mass index of less than 18.5 is considered indicative of underweight; overweight or obese is defined as body mass index ≥24; normal or healthy weight is defined as body mass index≥18.5 and <24.
Abbreviations: HAMD-17, 17-item Hamilton Rating Scale for Depression; GAD-7, Generalized Anxiety Disorder-7; GSRS, Gastrointestinal Symptom Rating Scale; CAS, Constipation Assessment Scale.

Safety Analysis

In total, 108 participants were included in the safety analysis. During the treatment period, one patient in the intervention group developed severe diarrhea due to intolerance (which resolved after discontinuing the probiotics), and two patients experienced severe constipation. In the control group, one patient had an allergic reaction to morinda officinalis oligosaccharide capsules (the condition improved after discontinuing the medication and receiving symptomatic anti-allergy treatment), one patient was diagnosed with prostatitis, and one patient experienced gum swelling and pain (Table S5). The percentages of adverse events were 5.56% in both the probiotics group and the control group.

Furthermore, no patients exhibited symptoms of mania or hypomania during the study, and no serious adverse events such as self-harm, suicide, or hospitalization were observed.

Discussion

The purpose of this study is to evaluate the effectiveness and safety of a combination of MOO capsules and probiotics powder in patients with MDD accompanied by gastrointestinal symptoms. The ITT analysis did not find the anticipated decrease in depressive symptoms. Furthermore, based on the ITT analyses, no statistically significant difference was observed in the proportion of responders after 8 weeks of treatment between the groups receiving MOO combined with either probiotics or placebo. However, a significant difference was detected in the mean reduction of HAMD-17 scores at Week 2. The MOO plus probiotics group demonstrated notable improvement in sleep disturbances (assessed using the insomnia dimension of the HAMD-17 scale) after 2 weeks of treatment, although no similar improvement was observed at Week 8. We have clarified that these results from the insomnia subscale should be interpreted as exploratory secondary findings, rather than definitive conclusions regarding treatment efficacy. Sensitivity analyses revealed that although the reduction in scores reflecting depressive symptoms was not significant in the PP analysis, a significant reduction in HAMD-17 scores at Week 2 was found using the multiple imputation analysis. The subgroup analysis also found that sex and family history of psychiatric illnesses had an impact on the response to probiotics treatment. Given that these

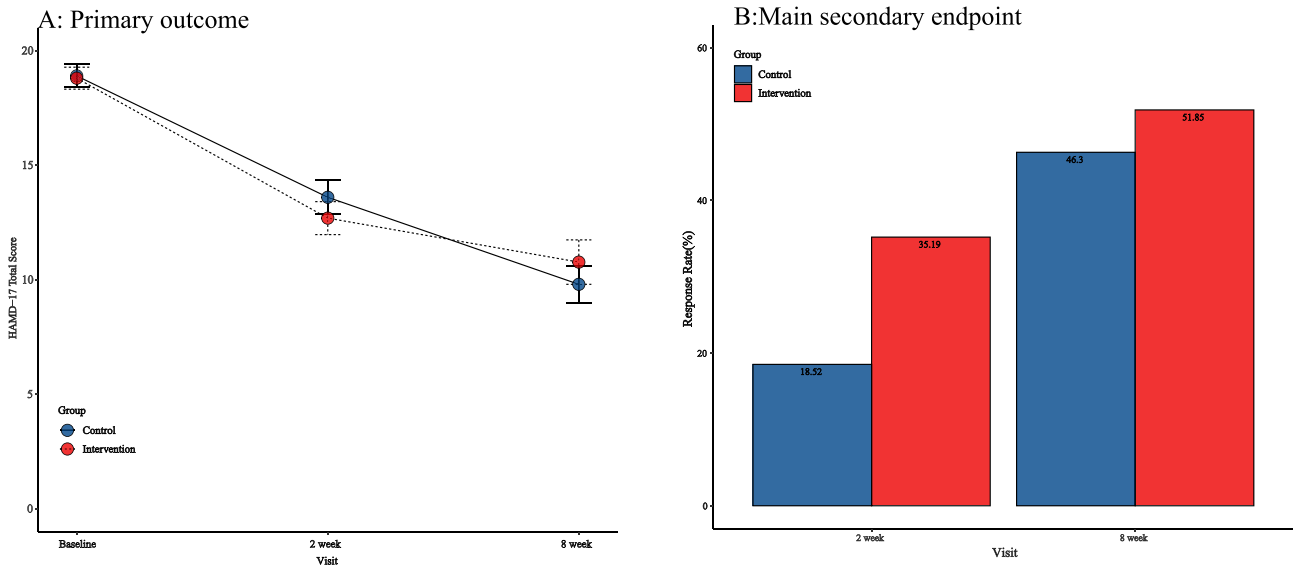

Figure 2 Comparison of treatment effects between the intervention and control groups.
Notes: (**A**) The HAMD-17 total score calculated with the Full Analysis Set (FAS); (**B**) The response rate was defined as the proportion of participants exhibiting a ≥50% reduction from baseline in the total HAMD-17 score; * P <0.05.
Abbreviation: HAMD-17, 17-item Hamilton Rating Scale for Depression.

subgroups analyses were not prespecified and were exploratory in nature, we have highlighted them cautiously and avoided overinterpretation. Throughout the trial, both drug regimens were relatively safe, with only one patient in the intervention group withdrawing from the study due to severe diarrhea; there were no reports of severe adverse reactions or incidents throughout the study.

The primary outcome measure of this trial is the reduction in HAMD-17 scores from baseline to the end of the 8-week treatment period for both groups. At the end of the trial, there was no significant difference observed between the

Table 2 HAMD-17 Total Score Between Intervention and Control Groups Across Different Visits

Parameter	Estimate	Standard Error	t	P
Intercept	8.9184	1.7696	5.04	<0.0001
Baseline HAMD-17 score	0.7210	0.07533	9.57	<0.0001
Time	−4.2724	0.4120	−10.37	<0.0001
Group	1.3970	1.2581	1.11	0.2694
Family History	0.1742	0.6425	0.27	0.7868
Sex	−0.6263	0.5779	−1.08	0.2811
First episode	0.05653	0.5700	0.10	0.9212
Group*Time	−0.9629	0.5823	−1.65	0.0998

Table 3 Intervention Effects on Response Rate Across Visits

Parameter	Estimate	Standard Error	95% CI		Z	P
Intercept	−1.7070	0.4494	−2.5878	−0.8262	−3.80	0.0001
Time	0.6034	0.2229	0.1666	1.0403	2.71	0.0068
Group	−0.3017	0.6477	−1.5711	0.9678	−0.47	0.6414
Family History	−0.3134	0.1286	−0.5654	−0.0614	−2.44	0.0148
Sex	0.2647	0.1378	−0.0054	0.5347	1.92	0.0547
Visit*Group	0.2623	0.3345	−0.3934	0.9179	0.78	0.4330

two groups in the reductions in HAMD-17 scores during the treatment period compared to the baseline. Regarding depressive symptoms, both groups showed significant improvement after treatment compared with baseline, but no significant difference was observed between the two groups in terms of alleviating depressive symptoms. The current results regarding whether probiotics can alleviate depressive symptoms are inconsistent. Rudzki et al[25] conducted a randomized double-blind controlled study, assigning 79 patients with severe depression into two groups: one receiving probiotics (LP299v) combined with selective serotonin reuptake inhibitor (SSRI) (n=40), and the other receiving placebo combined with SSRI (n=39). The types of SSRIs used in that study were not restricted. A total of 60 participants completed the 8-week treatment period and were included in the analysis, with 30 individuals in each group. No significant difference between the two groups in the alleviation of depressive and anxiety symptoms was observed at the end of the intervention, which is in line with our findings. Nikolova et al[25] conducted a randomized controlled trial with 49 patients with MDD who had been on a stable dose of an antidepressant for at least 6 months. In this study, one group received adjunctive probiotics therapy and the other received adjunctive placebo therapy. The assessments were scheduled at baseline, Week 4, and Week 8. The probiotics group received a combination of 14 probiotic strains, including *Bifidobacterium infantis* and *Bifidobacterium longum*. This study found a significant decrease in HAMD-17 scores only at Week 4 in the probiotics group and no significant difference at Week 8. The effects of adding probiotics to different antidepressants are inconsistent, and further elucidating the mechanism of drug interactions may promote related research in the future.

Several factors may explain the absence of significant differences between groups. First, our primary endpoint was assessed at Week 8, whereas previous studies reporting early improvements often measured outcomes at Week 4, suggesting that any transient effects of probiotics may diminish over time. Second, the majority of participants in this study were treatment-naïve, which may have contributed to a stronger placebo or MOO-only response and minimized detectable differences. Third, our trial did not include a MOO-only arm, making it difficult to isolate the specific contribution of probiotics from the combined treatment.

In recent years, there has been a proliferation of studies investigating adjunctive probiotic therapy for depression, yet few have incorporated Chinese herbal antidepressants as the foundational medication. Most studies chose SSRIs as the primary antidepressants and often lack control over the specific types of antidepressants employed. Notably, the trials also had not controlled the types of antidepressants taken post-enrollment. In contrast, our study restricted the antidepressant treatment for both groups at baseline to MOO capsules, thereby reducing potential confounding effects arising from the heterogeneity in antidepressants and enhancing the robustness and reliability of our findings. This condition may have contributed to the absence of significant differences in efficacy outcomes between the two groups. Compared to the typical sample sizes of 47 to 79 participants in previous studies[25,26] on the clinical efficacy of adjunctive probiotic therapy, our study has a relatively large sample size, with 54 participants in each group included in the ITT analysis and 46 participants in each group included in the Per-Protocol (PP) dataset, and most outpatient participants demonstrated good compliance. Additionally, the sensitivity analysis conducted in this study confirmed the reliability of the results, indicating the findings may provide invaluable reference points for future research.

Differences among probiotic strains are also significant causes of the heterogeneity in results, as there are often complex interactions between probiotics and antidepressant medications as well as among different probiotics. Lactobacillus strains, particularly *L. paracasei* CCFM1229 and *L. rhamnosus* CCFM1228, have been shown to alleviate depressive and anxiety symptoms by producing neurotransmitters such as γ-GABA, reducing serum corticosterone levels, and increasing brain serotonin and brain-derived neurotrophic factor (BDNF) levels.[27,28] Probiotics exert antidepressant effects primarily through the gut–brain axis by producing indole-3-lactic acid, which activates aryl hydrocarbon receptor signaling and mitigates neuroinflammation.[29] In a randomized, placebo-controlled clinical trial complemented by stress-induced depressive mouse models, Tian et al[30] demonstrated that multi-strain probiotics ameliorate depressive symptoms and comorbid gastrointestinal dysfunction by modulating the gut–brain serotonergic system and enhancing short-chain fatty acid–mediated signaling. Mechanistically, probiotics may require more sustained colonization or targeted strain combinations to impact gut motility and inflammation. The absence of a MOO-only group further limited our ability to clarify whether observed improvements were attributable to MOO, probiotics, or their combination. Future research should therefore employ multi-arm designs (MOO-only, probiotics-only, and combination), integrate more granular

gastrointestinal assessments, and extend follow-up durations to capture delayed effects. Mechanistic studies using microbiome profiling and metabolomics are also warranted to elucidate how specific strains or strain combinations modulate the gut–brain axis.

In this study, no significant differences between the GAD-7 scores at Week 2 and Week 4 compared to baseline were observed in any group, indicating that the adjunctive use of probiotics did not provide an additional benefit in ameliorating anxiety symptoms. Previous studies have yielded inconsistent results regarding the effectiveness of probiotics in improving anxiety symptoms. A meta-analysis incorporated 10 randomized controlled trials involving 656 participants and concluded that the use of probiotics does not impact symptoms of anxiety.[31] A randomized controlled study recruited 80 postmenopausal women who were then divided into two groups and given either trans-resveratrol (150 mg per day) or placebo for 14 weeks, the results showed that trans-resveratrol supplements could effectively alleviate anxiety but had no effect on regulating depression, anger, fatigue, confusion, or vitality.[32] Another meta-analysis[33] found that while probiotics significantly reduced anxiety-like behaviors in diseased animals, with Lactobacillus rhamnosus being particularly effective, they had no effect on untreated animals, suggesting that the anxiolytic effects of probiotics may be contingent upon underlying pathological conditions and occur only when anxiety reaches a certain threshold. In our study, 77 participants scored 5 to 14 on the GAD scale (indicating mild to moderate anxiety), and 31 scored 15 to 21 (indicating severe anxiety), despite the predominance of mild to moderate anxiety among participants, individuals diagnosed with MDD typically exhibited anxiety symptoms that surpassed the threshold of anxiety arousal, potentially explaining the absence of significant statistical differences between the two groups. The above study found that only *Lactobacillus rhamnosus* could significantly reduce anxiety-like behavior, while in our study we used a combination of several strains, so the interaction between these strains may also be a reason for the inconsistent results. These observations underscore that future studies should predefine anxiety endpoints and use more targeted probiotic strains or combinations to clarify their anxiolytic potential.

The results of the HAMD-17 symptom dimensions showed that after two weeks, the reduction in insomnia scores in the probiotics group was significantly larger than that in the control group, suggesting that probiotic intervention may have beneficial effects on symptoms such as difficulty falling asleep, frequent awakenings, and early awakening, which may explain the higher early onset response rates. The results of this study align with previous findings,[34–36] indicating that probiotics may help improve sleep symptoms; however, efficacy was not observed beyond 8 weeks of treatment, possibly due to factors such as the use of non-specialized sleep assessment scales and differences in participant selection compared to other studies. We emphasized that the observed improvement in insomnia represents an exploratory secondary outcome and should be further confirmed in future studies using mixed-model analyses incorporating treatment-by-time interactions.

In our study, the reductions in the GASRS and CAS scores at Weeks 2 and 8 from baseline showed no significant between-group differences, suggesting that the combination treatment with MOO did not improve gastrointestinal symptoms in patients with depression. Gastrointestinal disturbances, especially disruptions in gut motility, frequently accompany MDD. A study involving 9000 individuals diagnosed with anxiety and depression revealed that constipation affected approximately 29.8% of cases.[37] Previous studies[29] have also demonstrated that when participants maintain stable lifestyle and dietary habits, eight weeks of probiotic supplementation typically produces only minimal changes in gut microbial abundance and related biomarkers, which is consistent with our findings. This absence of intergroup differences may reflect several factors. First, the GSRS and CAS instruments, although widely applied, may not adequately capture nuanced gastrointestinal symptom changes or interactions between co-occurring symptoms such as diarrhea and bloating. Second, the lack of a MOO-only arm in this trial limited our ability to differentiate the specific effects of MOO from those of probiotics, which may have obscured potential benefits attributable to probiotics alone. Third, the gut-brain axis involves intricate pathways including inflammatory signaling, motility regulation, and microbial neurotransmitter synthesis; these mechanisms may require longer interventions or more targeted probiotic formulations to manifest measurable clinical effects. Future research should therefore incorporate more granular gastrointestinal assessments or composite endpoints that better reflect symptom interactions, adopt multi-arm designs to clearly isolate the contributions of each intervention, and extend follow-up durations to determine whether longer interventions yield sustained or delayed improvements in gastrointestinal outcomes.

Our study revealed sex differences in the efficacy of probiotic treatment, with females showing a lower response rate compared to males after probiotic therapy. Growing evidence suggests that the gut microbial ecosystem significantly contributes to the neurobiological basis of psychiatric disorders, exhibiting marked differences between male and female individuals.[38,39] Although there are few studies specifically addressing sex differences in probiotics, some studies on mental disorders suggested that males and females respond differently to probiotics. For instance, the probiotic *Lactobacillus plantarum* strain has been shown to positively impact autism spectrum disorder (ASD) symptoms in male children.[40] This study has also demonstrated that younger children experienced greater benefits than older ones, indicating that there may also be differences depend on the age and health status of the subjects.[40] However, some studies have reported different results. For instance, Tran et al[41] reported a significant decrease in worrying in female participants and a significant decrease in autonomic anxiety in male participants. In a randomized, double-blind, placebo-controlled trial involving healthy individuals aged 65 and older, a 12-week probiotic treatment significantly reduced the phylum Firmicutes in females but not in males, compared to the placebo group.[42] We have explicitly stated that the sex-difference findings in our study are exploratory, require cautious interpretation, and should be confirmed in larger trials designed with sex as a predefined effect modifier.

In our study, patients with a family history of psychiatric illnesses showed a lower response rate than those without. A positive family history of depression may reflect both genetic heritability and common environmental factors.[43] The genetic profile and clinical course of patients with depression and a family history of affective disorders may be different from that of the patients with depression but without a family history.[44] Given these differences, it is reasonable to hypothesize that a family history of affective disorders may be one of the factors that predicts the effectiveness of different antidepressant medications, but previous research on this issue has been inconclusive.[45–47] Future studies with larger samples and predefined subgroup analyses are warranted to clarify whether this represents a true biological difference or a statistical artifact.

In the probiotics group, there were no cases of inflammation or allergic reactions, one case of diarrhea, and two cases of constipation, with only one patient withdrawing from the study due to severe diarrhea. The percentages of adverse events in the probiotics group and the control group were the same (5.56% for both). Conditions of all patients experienced adverse events improved after discontinuation of the medication or symptomatic treatment. Most AEs were mild or moderate in severity, and throughout the study, there were no reports of conversion, suicide, or serious adverse reactions or events. In terms of safety profile, the AEs in this study were generally similar to those observed in previous clinical studies,[48] and occasional reports of bloating or mild abdominal discomfort were usually self-limiting. An 8-week randomized controlled study evaluated the effect of probiotics on improving depressive symptoms and found no side effects or adverse effects associated with the probiotic supplement.[49]

Limitations of This Study

Several limitations of this study should be noted. First, the relatively short follow-up period may not be able to capture the long-term effects or outcomes of the intervention, so studies with longer follow-up are necessary to fully reflect the long-term implications of the probiotics. Second, the study population was drawn from a single hospital (Beijing Anding Hospital), which may introduce selection bias and limit the applicability of our findings to other settings or populations. Future studies should include multiple centers to enhance representativeness. Third, we did not conduct an analysis of the intestinal microbiome, which prevented us from verifying the effective colonization of the gut by the probiotic formulation. Fourth, the study did not include a MOO-only group, making it difficult to distinguish the effects of MOO from those of probiotics; a three-arm design would be more informative in future trials. Finally, the intervention group had a significantly higher incidence of family history of psychiatric illnesses compared to the control group. Despite these limitations, the findings are promising and endorse the potential of probiotics as a therapeutic approach for managing depression.

Conclusion

This randomized controlled trial indicated that combining MOO capsules with probiotics does not significantly improve the treatment of depression compared to combining the MOO capsules with placebo. However, the addition of probiotics

may have a potential advantage in the early alleviation of depressive symptoms as well as insomnia symptoms associated with depression. In terms of safety, the combination of MOO capsules and probiotics was well-tolerated, with no significant adverse effects observed. The study also found that sex and family history of psychiatric illnesses influenced the efficacy of probiotics treatment. Hence, probiotics seem to be a vital part of managing depression in individuals with gastrointestinal symptoms, and further studies should investigate how probiotics achieve their antidepressant effects. Exploring different dosages and types of probiotic supplementation in the future will be worthwhile as probiotic supplementation may be a potential strategy for preventing depression.

Data Sharing Statement

All data can be obtained by the corresponding author, [JY, yangjian@ccmu.edu.cn], upon reasonable request.

Statement of Ethics

All procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. Written informed consent was obtained from all participants. The study protocol was reviewed and approved by the Institutional Review Board (IRB) of Beijing Anding Hospital (certificate number: [2021] Research No. [202]-202211FS-2/339).

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors have no conflicts of interest to declare in this work.

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