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The effects of probiotic supplementation on anxiety, depressive symptoms, and inflammatory markers

Submission date
30/05/2026

Recruitment status
No longer recruiting

Registration date
03/08/2026

Overall study status
Completed

Last Edited
03/08/2026

Condition category
Mental and Behavioural Disorders

[ ] Prospectively registered
[X] Protocol
[ ] Statistical analysis plan
[ ] Results
[ ] Individual participant data
[X] Record updated in last year

Plain English summary of protocol

Background and study aims
Recent research suggests that the bacteria naturally living in the human gut (the gut microbiome) may influence brain function, mood, and emotional wellbeing through what is known as the “gut–brain axis.” Changes in gut bacteria have been linked to symptoms of anxiety and depression, possibly through effects on inflammation, immune function, and the nervous system.

Probiotics are live microorganisms that may help restore healthy gut balance. Some studies suggest that probiotics could improve symptoms of anxiety and depression, but the evidence in humans remains limited and inconsistent.

This pilot study aims to investigate whether a multi-strain probiotic supplement can improve symptoms of anxiety and depression in adults, and whether any psychological changes are associated with changes in inflammatory markers measured in blood samples.

Who can participate?
Adults aged 18–85 years attending the Cardiology, Gastroenterology, or Dermatology Outpatient Clinics of the University General Hospital “Attikon” may participate if they experience symptoms of anxiety and/or depression based on a screening questionnaire.

What does the study involve?
Participants first attend a screening visit where they complete a psychiatric interview and a questionnaire measuring anxiety and depressive symptoms (Hospital Anxiety and Depression Scale – HADS). A blood sample is also collected to measure inflammatory and neurotrophic biomarkers.

Participants are then randomly assigned to receive either:
- A probiotic supplement containing Lactobacillus and Bifidobacterium strains, or
- A placebo preparation without active probiotic ingredients.

Neither the participants nor the researchers know which treatment each participant receives during the study.

The study treatment is taken orally once daily for 3 months. At the end of the study period, participants repeat the questionnaire and provide a second blood sample for follow-up measurements.

What are the possible benefits and risks of participating?
Participants may or may not experience improvement in symptoms of anxiety or depression. The study may also help researchers better understand the relationship between probiotics, inflammation, and mental health.

Possible risks are minimal and may include mild gastrointestinal discomfort related to probiotic use or temporary discomfort, bruising, or dizziness during blood collection.

Where is the study run from?
Attikon University Hospital, in association with the National and Kapodistrian University of Athens, Greece.

When is the study starting and how long is it expected to run for?
Participant recruitment began in June 2023. Each participant takes part in the study for approximately 3 months.

Who is funding the study?
UNI-PHARMA Pharmaceutical Laboratories S.A.

Who is the main contact?
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Public, Scientific

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Study information

Scientific Title
The effects of probiotic supplementation on anxiety, depressive symptoms, and inflammatory markers: a double-blind, randomized, placebo-controlled pilot study

Study objectives

Ethics approval required
Ethics approval required

Ethics approval(s)
Approved 06/03/2023, Hospital Scientific Committee - University General Hospital ''Attikon'' (Rimini Street 1, Chaidari, Athens, 12462, Greece; +302105831081; greps@attikonhospital.gr), ref: ΒΨ/Κ,ΕΒΔΙ 16/21-02-2023

Primary study design
Interventional

Allocation
Randomized controlled trial

Masking
Blinded (masking used)

Control
Placebo

Assignment
Parallel

Purpose
Treatment

Study type(s)

Health condition(s) or problem(s) studied
Symptoms of anxiety and/or depression

Interventions

At baseline (t0), participants will undergo a clinical psychiatric interview to determine eligibility for inclusion in the study according to predefined inclusion and exclusion criteria. Demographic and clinical data will also be collected to assess for additional psychopathology. Participants will subsequently complete the Hospital Anxiety and Depression Scale (HADS) questionnaire for the qualitative and quantitative assessment of depressive and anxiety symptoms.

At the same time point (t0), a blood sample will be collected and analyzed either as whole blood, centrifuged serum, or both, immediately or following storage at -20°C. The following laboratory parameters will be measured: C-reactive protein (CRP), brain-derived neurotrophic factor (BDNF), tumour necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6) levels.

Following the provision of informed consent, participants will be randomized using a computer-generated randomisation sequence in a double-blind manner to either an intervention group or a control group. Randomisation was performed in a 1:1 ratio using block randomisation to ensure balanced allocation between study arms. The allocation sequence was generated by an independent researcher who was not involved in participant recruitment, assessment, intervention delivery, or data analysis. Each study product package was labelled with a unique identification number corresponding to the randomisation schedule. Allocation concealment was maintained throughout the study, and both participants and study personnel remained blinded to treatment assignment until completion of the final analyses.

Participants in the experimental group will receive a psychobiotic preparation, while participants in the control group will receive a placebo preparation containing identical excipients but no active nutritional ingredients.

Intervention group: Participants received an oral probiotic formulation administered once daily for 3 months. The formulation was provided as granules for suspension and contained Lactobacillus rhamnosus, Bifidobacterium animalis subsp. lactis, Bifidobacterium breve, and Bifidobacterium longum (not less than 1×10^9 CFU per sachet), together with magnesium (75 mg), fructooligosaccharides (100 mg), and saffron extract (28 mg). Participants self-administered one sachet daily throughout the intervention period.

Control group: Participants received a placebo formulation identical in appearance, packaging, taste, consistency, and method of administration to the probiotic product. The placebo contained the same excipients but did not contain the active probiotic strains. Participants self-administered one sachet daily for 3 months.

Participants were assessed at baseline (t0) and again after 3 months (t1). Assessments included administration of the Hospital Anxiety and Depression Scale (HADS) and collection of blood samples for measurement of inflammatory biomarkers (IL-6, TNF-α, CRP) and brain-derived neurotrophic factor (BDNF).

Intervention Type

Supplement

Primary outcome(s)

1. Anxiety symptomatology measured using the Hospital Anxiety and Depression Scale–Anxiety (HADS-A) at t0 (baseline) and t1 (3 months)

2. Depressive symptomatology measured using the Hospital Anxiety and Depression Scale–Depression (HADS-D) at t0 (baseline) and t1 (3 months)

Key secondary outcome(s)
1. Whole blood/serum concentrations of inflammatory and neurotrophic biomarkers, including C-reactive protein (CRP), brain-derived neurotrophic factor (BDNF), tumour necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6), measured using enzyme-linked immunosorbent assay (ELISA) at t0 (baseline) and t1 (3 months)

Completion date
05/06/2024

Eligibility

Key inclusion criteria
1. A score ≥8 on the Hospital Anxiety and Depression Scale–Depression (HADS-D) and/or the Hospital Anxiety and Depression Scale–Anxiety (HADS-A)
2. Ages 18-85
3. Patients who attended the Cardiology, Gastroenterology, or Dermatology Outpatient Clinics of the University General Hospital “Attikon”

Healthy volunteers allowed
No

Age group
Mixed

Lower age limit
18 Years

Upper age limit
85 Years

Sex
All

Total final enrolment
19

Key exclusion criteria
1. Patients were excluded if they did not meet the symptom severity threshold (HADS-D <8 and HADS-A <8)
2. Younger than 18 or older than 85 years
3. Current or past psychiatric disorder diagnosis, including schizophrenia spectrum and other psychotic disorders, bipolar and related disorders, other mood disorders, eating disorders, or substance-related and addictive disorders, as defined by the DSM-5
4. Presence of major gastrointestinal disorders at enrollment according to ICD-10 codes K50–K52 and K55–K64 (16,17)
5. Pregnancy within two months prior to enrollment
6. Lactation at the time of enrollment

7. Exposure within the preceding six months to psychotropic medications (ATC codes N03, N05, N06), immunomodulatory or antineoplastic agents (ATC codes L01, L03, L04), or within the preceding three months to antibiotics, antifungal, antimycobacterial, or antiviral agents, immune sera, or immunoglobulins (ATC codes J01, J02, J04, J05, J06)
8. Diagnosis of any active infection within one month prior to enrollment, a confirmed SARS-CoV-2 infection within 35 days prior to enrollment
9. Patients who had a clinical indication for initiation of antidepressant or anxiolytic pharmacotherapy at the time of enrollment or had initiated psychotherapy within the 12 weeks preceding or at the time of study entry

Date of first enrolment
07/04/2023

Date of final enrolment
12/03/2024

Locations

Countries of recruitment
Greece

Sponsor information

Organisation
University General Hospital Attikon

ROR
https://ror.org/03gb7n667

Funder(s)

Funder type

Funder Name
UNI-PHARMA Pharmaceutical Laboratories S.A.

Results and Publications

Individual participant data (IPD) sharing plan

IPD sharing plan summary
Not expected to be made available

Study outputs

Output type	Details	Date created	Date added	Peer reviewed?	Patient-facing?
Participant information sheet			02/06/2026	No	Yes
Protocol file			01/06/2026	No	No