The effects of co-administration of probiotics and prebiotics on chronic inflammation, and depression symptoms in patients with coronary artery diseases: a randomized clinical tria...

Article *in* Nutritional Neuroscience · February 2021

DOI: 10.1080/1028415X.2021.1889451

CITATIONS
19

READS
215

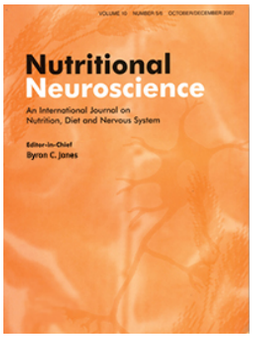
6 authors, including:

Jalal Moludi
Kermanshah University of Medical Sciences
73 PUBLICATIONS **527** CITATIONS
SEE PROFILE

Hamed Khedmatgozar
Texas Tech University Health Sciences Center
22 PUBLICATIONS **67** CITATIONS
SEE PROFILE

Hadi Abdollahzad
Kermanshah University of Medical Sciences
47 PUBLICATIONS **365** CITATIONS
SEE PROFILE

Mehdi Moradinazar
Kermanshah University of Medical Sciences
116 PUBLICATIONS **816** CITATIONS
SEE PROFILE



Taylor & Francis
Taylor & Francis Group

Nutritional Neuroscience

An International Journal on Nutrition, Diet and Nervous System

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/ynns20

The effects of co-administration of probiotics and prebiotics on chronic inflammation, and depression symptoms in patients with coronary artery diseases: a randomized clinical trial

Jalal Moludi, Hamed Khedmatgozar, Seyed Mostafa Nachvak, Hadi Abdollahzad, Mehdi Moradinazar & Ali Sadeghpour tabaei

To cite this article: Jalal Moludi, Hamed Khedmatgozar, Seyed Mostafa Nachvak, Hadi Abdollahzad, Mehdi Moradinazar & Ali Sadeghpour tabaei (2021): The effects of co-administration of probiotics and prebiotics on chronic inflammation, and depression symptoms in patients with coronary artery diseases: a randomized clinical trial, Nutritional Neuroscience, DOI: 10.1080/1028415X.2021.1889451

To link to this article: https://doi.org/10.1080/1028415X.2021.1889451

Published online: 28 Feb 2021.

NUTRITIONAL NEUROSCIENCE
https://doi.org/10.1080/1028415X.2021.1889451

The effects of co-administration of probiotics and prebiotics on chronic inflammation, and depression symptoms in patients with coronary artery diseases: a randomized clinical trial

Jalal Moludi [a,b,c], Hamed Khedmatgozar[d], Seyed Mostafa Nachvak[b], Hadi Abdollahzad[b], Mehdi Moradinazar[c] and Ali Sadeghpour tabaei[e]

[a]Student Research Committee, School of Nutritional Sciences and Food Technology, Kermanshah University of Medical Sciences, Kermanshah, Iran; [b]Department of Nutritional Sciences, School of Nutritional Sciences and Food Technology, Kermanshah University of Medical Sciences, Kermanshah, Iran; [c]Research Center for Environmental Determinants of Health (RCEDH)s, Kermanshah University of Medical Sciences, Kermanshah, Iran; [d]Center for Biotechnology and Genomics, Texas Tech University, Lubbock, TX, USA; [e]Department of Cardiac Surgery, Rajaie Cardiovascular Medical and Research Center, Iran University of Medical Sciences, Tehran, Iran

ABSTRACT

Background: It has been shown that dysbiosis might have a role in developing of chronic inflammation and depression. In this study, we are interested in exploring of anti-inflammatory and anti-depressant effects of *Lactobacillus Rhamnosus* G (LGG), a probiotic strain, alone or in combination with a prebiotic, Inulin, in patients with coronary artery disease (CAD).

Methods: This randomized, double-blind clinical trial was held on 96 patients with CAD. Patients were randomly allocated into four different groups: LGG [a capsule/day, contained 1.9×10^9 colony-forming unit of Lactobacillus Rhamnosus G], inulin (15 g/day), co-supplemented (LGG and inulin), and placebo. Participants consumed the supplements for two months. Beck Depression Inventory (BDI), MacNew questionnaire and Spielberger state-trait anxiety inventory (STAI-Y) were used to assess depression, quality of life and anxiety, respectively. Serum levels of C-reactive protein (hs-CRP), lipopolysaccharide (LPS), tumor necrosis factor (TNF)-α, and Interleukin (IL)-10 were also measured.

Results: Probiotic-Inulin Co-supplementation significantly decreased BDI ($-11.52 \pm 0+3.20$ vs. $+2.97 \pm 0.39$, $P = 0.001$), STAI-state (-17.63 ± 3.22 vs. -0.60 ± 0.33, $P = 0.021$), and STAI-trait (-24.31 ± 7.41 vs. -1.45 ± 0.66, $P = 0.020$) scores, hs-CRP ($-1.69 \pm 0+66$ vs. $+0.82 \pm 0.39$ mg/dL, $P = 0.020$), LPS (-22.02 ± 5.40 vs. $+0.31 \pm 0.18$ (EU/L), $P = 0.047$), and TNF-α (-25.05 ± 7.41 vs. $+0.79 \pm 0.71$ (ng/L), $P = 0.032$) in comparison to placebo.

Conclusion: Co-supplementation of probiotics and inulin in CAD subjects for eight weeks had beneficial effects on depression, anxiety, and inflammatory biomarkers. Adding inulin to probiotic supplements improved psychological outcomes and inflammatory biomarkers more effectively than two supplements separately.

Trial registration: Iranian Registry of Clinical Trials identifier: IRCT20180712040438N4..

KEYWORDS
Coronary artery disease; inflammation; depression; gut microbiota; probiotic; probiotics

Introduction

Depressive symptoms may unfavorably affect the cardiovascular disease (CVD) prognosis and increase the risk of morbidity and mortality [1]. Pooled estimates suggested that the prevalence of depression symptoms ranged from 17% to 27% among coronary artery patients [2]. Depression is a risk factor for myocardial infarction and cardiac mortality [3]. Another psychological factor related to CVD is anxiety, which might be alongside depression symptoms or just by itself [4]. Whatsoever its severity, depression is concomitant with low quality of life (QOL), a significant risk factor for poor medicine adherence [2,3]. However, there is no appropriate treatment for this circumstance. Although antidepressants effectively improve depressive symptoms in subjects with coronary artery disease (CAD), various side effects and drug interactions significantly limited their clinical prescriptions [5].

In recent years, it has been shown that gut microbiota status would be a modifiable risk factor for depression, and some clinical and epidemiologic studies have determined a link between gut microbiota and mental health, especially depression [6]. One of the major topics in this field is probiotics, which would positively affect CVD and depression [7,8].

CONTACT Jalal Moludi jmoludi@yahoo.com School of Nutrition Sciences and Food Technology, Kermanshah University of Medical Sciences, Kermanshah, 5166614711, Iran

Chronic inflammation has been determined as a critical risk factor for CVDs and depression [9]. The link between inflammation and CVD development is well-established, and clinical trials propose that this relationship is imminent [10–12]. Researchers focused on the role of gut microbiota alteration (dysbiosis) as one of the major etiological factors that are complicated in developing many inflammatory diseases such as CVD and depression [13]. Gut barrier breakdown leads to intestinal permeability increase, bacteria and microbiome-derived lipopolysaccharide (LPS), a component in the outer membrane of gram-negative bacteria, translocation into the systemic circulation, and immune-inflammatory system activation [14,15].

Probiotics are defined as the live microorganisms that confer a beneficial effect on the host's health [16]. Probiotics preserve the integration of gut barrier function and decrease intestinal permeability, which consequently declines endotoxin levels [17]. Probiotics demonstrate anti-obesity and anti-inflammatory activities [7,18]. Recent evidence has proposed that *lactobacillus rhamnosus* HN001 as a probiotic improves the psychological state of clinical populations with altered microbiota balance [19]. Prebiotics, like short-chain carbohydrates and inulin, in the colon act as substrate for the endogenous colonic bacteria such as *bifidobacterium and lactobacillus*. Consumption of prebiotics increases the proportion of beneficial bacteria in the gut microbiome [20].

Previous studies have revealed the positive effect of probiotics in the treatment and control of depression. However, to the best of our knowledge, it might be small or even no randomized controlled trials regarding co-supplementation of inulin as prebiotic with *lactobacillus rhamnosus* as probiotic on chronic inflammation, level of microbial translocation, depression symptoms and anxiety in patients with CAD. Therefore, the current study aimed to determine the effects of inulin, the probiotic, and their co-administration on the beck depression inventory (BDI) score, anxiety, and QOL as a primary outcome, and the chronic inflammation, endotoxemia (as indexed by high LPS) as secondary outcomes in adults with CAD.

Materials and methods

Subjects

In this trial, we selected 116 eligible subjects referred to the Imam Ali Cardiovascular Hospital in Kermanshah University of Medical Sciences, Kermanshah, Iran. Subjects were 18–85 years old patients with CAD who agreed to participate in the study. The subjects with end-stage renal disease, undergoing corticosteroid, immunosuppressive, anti-inflammatory, or anti-depressant drugs; a history of dietary supplements including Pre/Pro-biotics, antioxidants, or vitamins at least two months prior were excluded from the study. For ethical reasons, participants were allowed to take routine medications, i.e. Cholesterol-modifying medications, Aspirin, beta-blockers, Plavix, and Angiotensin-converting enzyme. However, they were not permitted to take antioxidants and/or vitamin supplements throughout the study. Participants signed an informed consent form before the study. Our study was in arrangement with the Helsinki Declaration of the World Medical Association and was confirmed by our local ethics committee of Kermanshah University of Medical Sciences (IR.KUMS.REC.1398.1065) and verified in the Iranian Registry of Clinical Trials (IRCT20180712040438N4).

We calculated the sample size according to mean reduction in BDI score, a 10% plausible for loss of follow up, and power of 80%. The calculated sample size was 24 subjects for each of the four groups; overall, 96 participants were included.

Randomization and intervention

This study was a double-blind, four-arm parallel randomized controlled trial. We used random allocation software to randomize participants in groups. The random sequence was preserved and managed by an independent third party who was not aware of the clinical process until the end of the study.

Patients assigned in four groups; (1) prebiotic group (one sachet containing 15 g inulin per day), (2) probiotic group (one capsule contained 1.9×10^9 colony-forming unit (CFU) of *L. rhamnosus* per day), (3) co-supplemented group (both inulin and *L. rhamnosus*), (4) placebo group (a sachet and a capsule filled with maltodextrin). The intervention has been taken for two months. Phone contacts were made every two weeks to check adherence of the study. Compliance with capsules was checked by requesting participants to return the medication containers. The patients have the right to stop the trial if they feel inconvenient, are unwilling to complete the study, or experience any adverse effect during the supplementation.

Dietary intakes

Dietary consumption was accomplished using a 24 recall at weeks 0 and 8 of intervention. The Nutritionist IV software synced for x diets used to obtain nutrients and calorie intake of participants.

Physical activity assessment

The physical activity level was measured using the validated short-form International Physical Activity Questionnaire (IPAQ) and classified as low, moderate, and high.

Assessment of anthropometric indices

A scale measured bodyweight with a minimum dress and without shoes with 0.1 Kg accuracy (Seca, Hamburg, Germany). Height was measured without shoes by a tape with 0.5 cm accuracy. BMI was calculated by dividing weight (Kg) by height^2 (m).

Biochemical variables

After 12 h of fasting, blood was collected and centrifuged at 2500 rpm for 10 min. Interleukin (IL)-10, tumor necrosis factor-alpha (TNF-α), and lipopolysaccharides [(LPS), a marker of gut permeability] have been measured using enzyme-linked immunosorbent assay (ELISA) kits. High sensitivity C-reactive protein (hs-CRP) levels in serum were evaluated by immunoturbidimetry. Lipid profile enclosing total cholesterol (TC), triglyceride (TG), and high-density lipoprotein cholesterol (HDL-C) levels were assessed by enzymatic kits (Pars Azmun, x), and low-density lipoprotein cholesterol (LDL-C) was calculated by Friedewald formula. Fasting blood sugar (FBS) level was measured via the glucose oxidize technique (Pars Azmun, x). These measures were obtained at baseline and after eight weeks' follow-up.

Blood pressure

Diastolic blood pressure (DBP) and systolic blood pressure (SBP) were assessed after 5 min of rest in a sitting position by an automatic oscillometric maneuver (Omron Healthcare Co, Ltd) before and after the intervention.

Psychological assessment

The beck depression inventory-II (BDI-II) is used in most clinical conditions for identifying depression. The BDI-II contains 21 questions; each question can take a score from 0 to 3 so that the higher total scores display severe depressive symptoms. The standardized cut-off points are as follows: 0–13: minimal depression, 14–19: mild depression, 20–28: moderate depression, 29–63: severe depression [21]. In the present study, based on the BDI-II scores, patients were categorized into two groups: depressed or non-depressed.

The QOL was measured using the MacNew questionnaire consisting of 27 questions in three domains: physical, social, and emotional. Items were scored on a 7-point scale so that the higher scores indicate the better QOL. The MacNew total score is calculated by submitting each subscale score; total scores ranged from 27 to 189 [22].

Anxiety was measured by Spielberger state-trait anxiety inventory form Y (STAI-Y). This scale contains two units for each collection of 20 questions. The first part measures state anxiety (i.e. how patients feel when completing the questionnaire), and the second part assesses trait (habitual) anxiety. The answers are counted based on the Likert scale ranged from 1 to 4. Total scores are the sum of the two sections. The validity and reliability among Iranian populations have been approved. Anxiety is defined as a feeling of stress, worry, discomfort by CAD patients [23].

Statistical analysis

We analyzed data via SPSS software (version 21; SPSS Inc., Chicago, IL), and the outcomes were stated as mean ± SD. For all statistical tests, a P value less than 0.05 was interpreted as statistically significant. To find out the normal distribution of variables, we utilized the skewness and kurtosis test. The log-transformation was used to deal with skewed data. Mean imputation was used for missing values [24]. The analyses were conducted using an intention-to-treat approach [25]. In this study, we used one-way ANOVA for comparison between groups in the baseline phase, and post hoc was done with LSD methods. Paired samples t-test applied for within-group comparisons (endpoint vs. baseline). Also, pairwise comparisons with adjustment for multiple comparisons (Bonferroni) were made if the one-way ANOVA was significant. After the intervention, we used the ANCOVA analysis to compare the four groups' differences and adjusted confounders and baseline biomarkers/psychological questionnaires using Sidak's method. For evaluating the clinical importance of probiotic administration, the number needed to treat (NNT) was calculated via the inverse of the Absolute Risk Reduction (ARR and according to ≥3 points reduction in BDI score [26]).

Result

Baseline

Figure 1 summarizes the findings and contributions. From a total 96 patients, 88 of them completed the

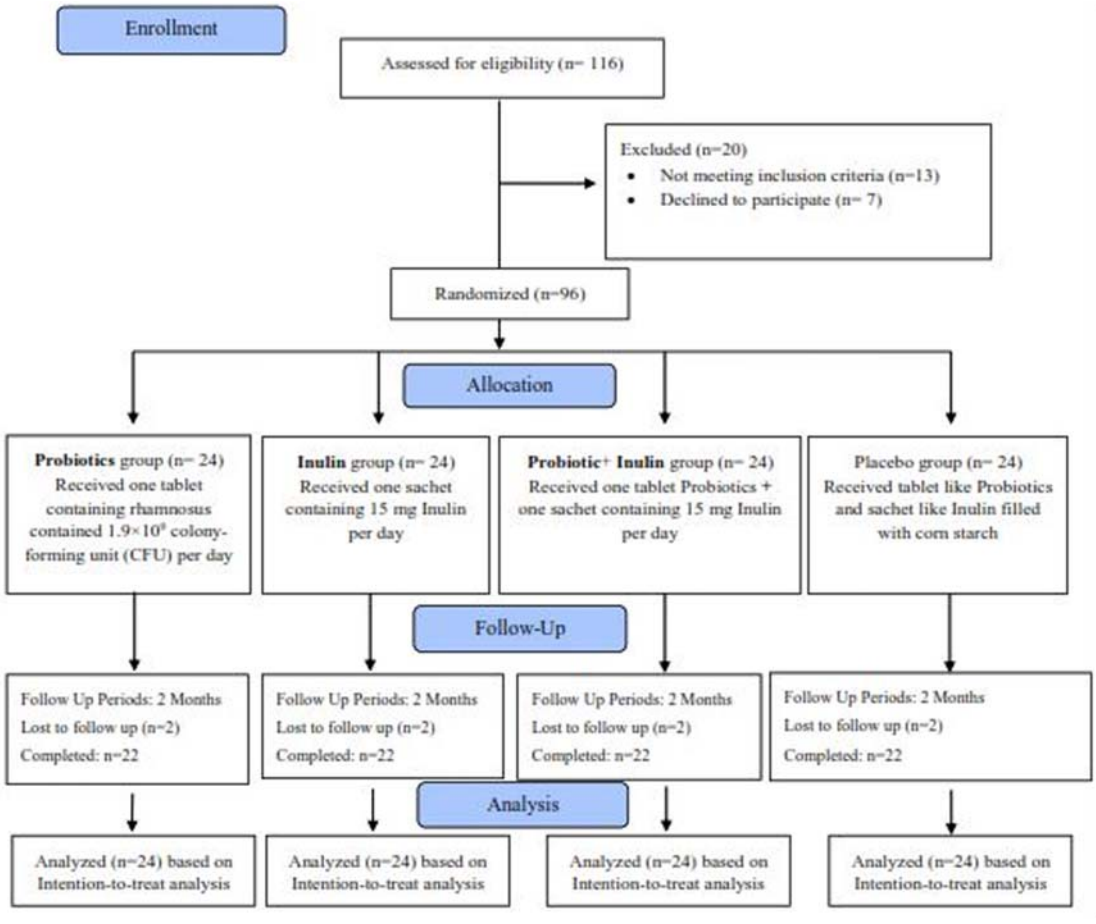


Figure 1. Flowchart of study.

trial (Probiotics group, $n = 22$; Inulin group, $n = 22$; Probiotics plus Inulin group, $n = 22$; placebo group, $n = 22$). Based on the intention-to-treat (ITT) analyses included all participants ($n = 96$). Table 1 represents the patients' baseline characteristics. Participants' mean age was 51.25 (13.22) years, and there was no significant difference in age between the four groups ($p = 0.153$). Baseline parameters were normally distributed throughout the study population, and there was no statically significant difference in the history of depression, sex, physical activity and education levels in four study groups ($p > 0.05$). Based on the residual tablets in the bottle, contributors have 80% compliance.

Table 1. Baseline characteristics of the study subjects.

Variable[a]	Probiotic group ($n = 24$)	Inulin group ($n = 24$)	Probiotic+ Inulin group ($n = 24$)	Placebo group ($n = 24$)	*P*-value[d]
Age (years)[b]	51.25 ±12.66	52.18 (12.78)	49.12 (11.22)	51.82 (12.22)	**0.153**
Men, *n* (%)	15 (62)	12 (50)	15 (62)	16 (66)	**0.891**
Family history of depression[c]	2 (1.00–4)	1 (1.00–5)	3 (1.00–5)	3 (1.00–7)	**0.586**
Smoking	5 (20)	4 (16)	7 (28)	3 (12)	**0.624**
Physical activity N (%)					
Low	18 (81)	16 (72)	17 (77)	17 (77)	**0.473**
Moderate	3(13)	5 (20)	4(18)	3(13)	
High	1(4)	1(4)	2(8)	2(8)	
Education N (%)					
Illiterate	0 (0)	3 (13.5)	1 (4.5)	1 (4.5)	**0.498**
Diploma and lower	20 (90)	18 (72)	18 (72)	21 (94.5)	
Bachelors and higher	2 (9)	1(4.5)	3 (13.5)	0 (0)	
Compliance Rate (%)	91	80	75	90	**0.565**

[a]Values are expressed as frequency (%).
[b]Values are expressed as mean (SD) and *P*-value based on One-Way ANOVA.
[c]Values are expressed as median (percentile 25–75) and *P*-value based on Kruskal-Wallis H test.
[d]Chi-square test.

Anthropometric indices and dietary intakes

Table 2 presents the data about dietary intakes and anthropometric indices. At the end of the study, there was no statistically significant difference between the groups for all anthropometric indices ($p > 0.05$). Similarly, the weight changes were not statistically significant comparing the four groups ($P = 0.792$).

Based on the 3-day dietary records, there were no significant changes in dietary macro- and micro-nutrient intakes. On the other hand, total energy and fat intakes decreased by the end of the intervention in the Probiotics + Inulin combination vs. placebo (−66.02 ± 20.66 vs. −58.5 ± 35.39 calorie, $P = 0.150$), while it was not significant compared to the baseline ($P > 0.05$).

Fasting blood sugar, lipid profile and blood pressure

After supplementation, there were no significant changes in FBS, total cholesterol, LDL-C, TG, HDL-C, DBP and SBP within groups. After adjusting for the cofounders (Adjusted for baseline values, age, and sex), we did not see any significant change in all groups' lipid profiles. Probiotic-Inulin co-supplementation decreased SBP (−7.42 vs. −4.45 mm/Hg, $P = 0.473$), TG (−38.80 ± 5.40 vs. +3.22 ± 1.18 mg/dl, $P = 0.269$), and Cholesterol (−26.14 ± 9.21 vs. +16.22 ± 7.81 mg/dL, $P = 0.358$) compared with the placebo. So, taking Probiotics and /or prebiotic did not significantly affect metabolic indices compared with the placebo (Table 3).

Inflammatory markers

Baselines levels of inflammatory biomarkers and LPS are presented in Table 4. We perceived no significant differences in serum concentrations of hs-CRP, LPS, TNF-α, and IL-10, between four intervention groups at the starting point of the study. After adjustment for baseline levels and confounding factors (age and sex), we determined a significant reduction in LPS, and TNF-α levels in the probiotic compared with placebo ($p = 0.014$, $p = 0.030$, respectively). Moreover, in the Probiotic-Inulin group compared to baseline, hs-CRP, LPS, and TNF-α levels have significantly changed ($p = 0.002$, $p = 0.003$, $p = 0.002$, respectively). After intervention, Probiotic-Inulin co-supplementation significantly decreased serum hs-CRP (−1.69 ± 0.66 vs. +0.82 ± 0.39 mg/dL, $P = 0.020$), LPS (−22.02 ± 5.40 vs. +0.31 ± 0.18 (EU/L), $P = 0.047$), and TNF-α (−25.05 ± 7.41 vs. +0.79 ± 0.71 (ng/L), $P = 0.032$) concentrations compared with the placebo. We did not perceive any significant change in IL-10 concentration in Probiotic-Inulin supplementation (10.10 ± 3.45 vs. −0.59 ± 0.66, $P = 0.453$). From the results, the Probiotics + Inulin combination could be a possible approach in ameliorating chronic inflammation and endotoxemia (LPS).

Psychological outcomes

Table 5 represents the results for psychological outcomes: anxiety, depression, and QOL. The BDI and STAI-trait scores significantly decreased in probiotics groups ($p = 0.001$, $p = 0.006$, respectively) compared to the baseline. We also have found that adding Inulin to probiotic amplified the improvement of psychological outcomes more than either Inulin or probiotics alone. Probiotic-Inulin co-supplementation significantly decreased BDI (−11.52 ± 0+3.2 vs. +2.97 ± 0.39, $P = 0.001$), STAI-state (−17.63 ± 3.22 vs. −0.60 ± 0.33 mmol/L, $P = 0.021$), and STAI-trait (−24.31 ± 7.41 vs. −1.45 ± 0.66, $P = 0.020$) scores compared to placebo, while there were no significant effects for MacNew score.

NNT was calculated via a standard method, concerning ≥3 points reduction in BDI score. Accordingly, for Probiotics, Inulin, and probiotics + Inulin, the calculated NNT for 8-weeks supplementation to reach a minimum decrease of three points on BDI score were 7, 10, and 5 respectively ($p = 0.044$) (Figure 2).

Discussion

The current study demonstrated that supplementation of Inulin with the *Lactobacillus Rhamnosus* decreases inflammation, depression, and anxiety in CAD patients compared to other study groups. In comparison, no changes have been seen in QOL after the intervention. Furthermore, the supplementation of *Lactobacillus Rhamnosus* alone leads to decrease scores of BDI and STAI-trait. Also, among measured inflammatory markers, hs-CRP, LPS, and TNF-α were decreased significantly after administration of Inulin with the *Lactobacillus Rhamnosus*.

To best our knowledge, the present randomized trial study was the first study that evaluated the effects of Inulin and the *Lactobacillus Rhamnosus* on inflammatory markers, depression, and anxiety in CAD patients. Previous studies have confirmed that CAD patients experience some degree of depression and anxiety so that the conditions can reduce the QOL of the patients [3,27,28]. Since depression and anxiety can increase the risk of sudden death, stroke, and overall mortality [27,29], dietary interventions reduced depression and anxiety, especially in CVDs patients.

Table 2. The effect of Probiotic and Inulin supplementation on the some anthropometric and dietary indices in this study.

Variable	Probiotic group ($n = 24$)	Inulin group ($n = 24$)	Probiotic+ Inulin group ($n = 24$)	Placebo group ($n = 24$)	*P*-value
Weight (kg)					
Before	81.27 (12.96)	79.22 (11.42)	78.48 (9.96)	80.46 (10.70)	0.844[a]
After	77.25 (11.86)*	77.36 (13.06)**	78.89.12 (12.86)	79.10 (11.65)	0.792[b]
MD, *p*[c]	−4.02, 0.555	−1.86, 0.117	0.35, 0.656	−1.54, 0.064	
BMI(kg m^{-2})					
Before	28.55 (6.90)	27.64 (4.46)	27.59 (5.00)	26.84 (3.11)	0.998[a]
After	26.88 (7.17)	27.18 (7.96)	27.79 (8.63)	26.12 (4.89)	0.664[b]
MD, *p*[c]	1.67, 0.080-	−0.48, 0.145	0.20, 0.250	−0.72, 0.094	
WC (cm)					
Before	96.33 (6.51)	94.10 (8.18)	96.55 (8.53)	95.73 (4.68)	0.998[a]
After	93.66 (6.91)	92.22 (7.66)	95.14 (8.15)	94.10 (4.45)	0.857[b]
MD, *p*[c]	−2.71, 0.065	−1.90, 0.158	1.45, 0.182-	−1.63, 0.189	
Energy(kcal/day)					
Before	1820.39 (210.94)	1803.05 (290.52)	1685.75 (343.96)	1783.58 (173.154)	0.345
After	1726.48 (188.57)	1654.44 (157.48)	1619.70 (194.10)	1724.71 (188.56)	0.150
MD, *p*[c]	−93.90, 0.200	−148.61, 0.047	−66.05, 0.454	−58.87, 0.388	
Carbohydrate (g)					
Before	292.42 (65)	286.38 (56.13)	272.21 (52.59)	295.85 (69.16)	0.041[a]
After	279.29 (44.30)	262.58 (47.53)	245.02 (55.50)	283.96 (45.75)	0.604[b]
MD, *P*-value[c]	−13.12, 0.446	−23.80, 0.023	−27.19, 0.015	−11.89, 0.483	
Protein (g)					
Before	63.92 (20.36)	62.85 (19.75)	60.01 (21.61)	64.48 (20.22)	0.895
After	67.45 (19.64)	67.84 (17.61)	68.64 (17.77)	66.92 (19.81)	0.992
MD, *P*-value[c]	3.52, 0.311	4.98, 0.090	8.63, 0.09	2.44, 0.482	
Fat (g)					
Before	67.23 (25.63)	70.96 (22.01)	67.81 (23.53)	65.78 (25.59)	0.807[a]
After	65.56 (23.78)	68.80 (21.58)	63.15 (23.08)	63.15 (23.08)	0.911[b]
MD, *P*-value[c]	−1.66, 0.791	−2.16, 0.667	−4.98, 0.348	−2.63, 0.674	
Dietary Fiber(g)					
Before	18.86 (7.66)	19.33 (6.91)	20.91 (7.44)	18.88 (7.75)	0.654
After	21.30 (11.07)	22.10 (8.93)	22.95 (9.35)	20.16 (11.04)	0.556
MD, *P*-value[c]	1.46, 0.321	2.43, 0.124	2.05, 0.156	1.28, 0.375	
Magnesium (mg)					
Before	220.04 (73.92)	222.15 (63.32)	213.57 (72.63)	219.85 (73.96)	0.982
After	215.50 (58.06)	219.75 (51.97)	221.92 (56.90)	215.75 (58.01)	0.977
MD, *P*-value[c]	−4.54, 0.771	−2.40, 0.848	8.34, 0.582	−4.09, 0.793	
Zinc (mg)					
Before	7.72 (2.52)	7.66 (2.21)	7.35 (2.39)	7.83 (2.49)	0.926
After	7.47 (2.52)	7.78 (2.25)	7.47 (2.52)	7.92 (2.24)	0.901
MD, *P*-value[c]	−0.24, 0.613	0.11, 0.769	0.57, 0.207	−0.35, 0.472	

MD: mean difference, Values are expressed as mean (SD).
In each row, mean value with different superscript letters are significantly different ($P < 0.05$).
[a]One-Way ANOVA.
[b]Adjusted for baseline values, age and sex using the analysis of covariance (ANCOVA) test.
[c]Paired-samples t-test.

Our findings indicated that co-administration of Inulin with the *Lactobacillus Rhamnosus* significantly decreases depression and anxiety in CAD patients. Akkasheh et al. [30] reported that probiotic supplementation in patients with severe depression decreased scores of BDI ($P = 0.001$). Another clinical trial study by Slykerman et al. [19] showed that probiotics (*Lactobacillus Rhamnosus* HN001) supplementation decreased depression ($P = 0.037$) and anxiety ($P = 0.014$) in postpartum. Similar to our findings, Haghighat et al. [31] reported that synbiotics supplementation had a significant effect on reducing depression and anxiety symptoms compared to probiotics supplementation in patients undergoing hemodialysis. Also, Ghorbani et al. [32] observed a significant decrease in depression symptoms in depressed patients treated by synbiotic for ten weeks ($P = 0.013$). Nevertheless, results from a systematic review and meta-analysis of 12 randomized controlled trials in 2019 showed that probiotic supplementation did not change the score of BDI (WMD: −11.17; 95% CI: −24.99, 2.65) [33]. However, another systematic review and meta-analysis on 34 randomized controlled trials in 2019 regarding the effects of prebiotics and probiotics on depression anxiety [11] showed that prebiotics had no effects on depression ($P = 0.51$) and anxiety ($P = 0.11$), while probiotics supplementation improved depression ($P = 0.01$) and anxiety ($P = 0.03$). A meta-analysis of the effects of synbiotics on depression showed no evidence, and more clinical trials are needed in this field [7]. Compared with placebo, probiotics + Inulin showed greater improvement in depression scores (number needed to treat [NNT] = 5; 95% confidence interval [CI], 3–7). After calculation of the NNT, we also have found that adding Inulin to

Table 3. Comparison of FBS, lipid profiles and blood pressure within and between groups.

Variable	Probiotic group ($n = 24$)	Inulin group ($n = 24$)	Probiotic+ Inulin group ($n = 24$)	Placebo group ($n = 24$)	*P*-value
SBP (mmHg)					
Before	127.86 (19.34)	125.68 (18.98)	121.90 (17.42)	127.41 (18.65)	0.717[b]
After	120.23 (13.66)	118.36 (15.03)	114.48 (25.70)	122.95 (15.09)	0.473[c]
GMD, *p*[a]	−7.63, 0.015	−7.31, 0.061	−7.42, 0.287	−4.45, 0.164	
DBP (mmHg)					
Before	81.86 (11.72)	84 (13.43)	80.33 (13.66)	81.77 (17.66)	0.867[b]
After	77.64 (8.42)	81.32 (12.40)	79.71 (13.76)	83.41 (24.36)	0.670[c]
GMD, *p*[a]	−4.22, 0.051	−2.68, 0.246	−0.61, 0.00	1.63, 0.568	
FBS (mg/dl)					
Before	119.09 (58.52)	121.77 (62.01)	108 .76 (28.32)	132.59 (79.73)	0.639[b]
After	12 (14.40)	99.23 (24.15)	99.95 (30.26)	125.91 (57.02)	0.360[c]
GMD, *p*[a]	−17.09, 0.194	−22.54, 0.099	−8.81, 0.158	−6.68, 0.454	
LDL (mg/dl)					
Before	91.54 (32.18)	98.25 (33.50)	91.18 (48.49)	101.53 (50.29)	0.808[c]
After	70.05 (41.15)	87.01 (46.52)	67.61 (44.37)	76.31 (35.76)	0.436[c]
GMD, *p*[a]	−21.49, 0.076	−11.23, 0.411	−23.56, 0.140	−25.21, 0.081	
TG (mg/dl)					
Before	173.40 (50.39)	167.59 (47.93)	162.61 (55.16)	172.95 (55.64)	0.894[b]
After	154.86 (54.97)	149.13 (53.09)	123.80 (90.96)	169.72 (97.30)	0.269[c]
GMD, *p*[a]	−18.54, 0.090	−18.45, 0.173	−38.80, 0.081	−3.22, 0.872	
Total Cholesterol(mg/dl)					
Before	167.50 (27.52)	173.32 (30.28)	176.19 (41.52)	164.14 (29.45)	0.611[b]
After	139.89 (42.84)	158.41 (50.83)	150.05 (94.19)	180.36 (103.60)	0.358[c]
GMD, *p*[a]	−27.63, 0.018	−14.90, 0.279	−26.14, 0.233	16.22, 0.501	
HDL (mg/dl)					
Before	41.27 (5)	41.54 (7.17)	41.90 (7.16)	41.77 (5.53)	0.988[b]
After	42.77 (4.84)	44.54 (8.45)	45.23 (9.12)	41.81 (8.83)	0.476[c]
GMD, *p*[a]	1.50, 0.278	3, 0.176	3.33, 0.167	0.045, 0.985	

Values are expressed as geometric mean (minimum, maximum) and the *p*-values are estimated after log-transformation. In each row, mean value with different superscript letters are significantly different ($P < 0.05$).
[a]Paired-samples t-test.
[b]One-Way ANOVA.
[c]Adjusted for baseline values, age and sex using the analysis of covariance (ANCOVA) test.

probiotic amplified the improvement of psychological outcomes more than either Inulin or probiotics alone.

There is a connection between the brain and the gut microbiota through the microbiome-gut-brain axis that the immune system, neuroendocrine, Short-chain fatty acids (SCFAs), tryptophan, the enteric nervous system, the vagus nerves, and the gut microbiota contribute in this pathway [19]. Shreds of evidence showed that prebiotics and probiotics administration have an effect on mood and psychological disorders [18,30]. Gut microbiota dysbiosis also may contribute to depression. Dysbiosis disturbs blood–brain-barrier function and

Table 4. Comparison of inflammatory markers, and level of microbial translocation (LPS) within and between groups.

Variable	Probiotic group ($n = 24$)	Inulin group ($n = 24$)	Probiotic+ Inulin group ($n = 24$)	Placebo group ($n = 24$)	*P*-value
Hs- CRP (mg/dL)					
Before	3.20 (1.68)	3.30 (2.03)	3.00 (1.82)	2.73 (1.81)	0.746[a]
After	2.90 (2.93)	3.75(3.25)	1.31(0.98)	3.55(3.34)	0.020[b]
GMD, *p*[c]	−0.30, 0.662	0.45, 0.572	−1.69, 0.002	0.82, 0.315	
IL-10 (ng/L)					
Before	92.61 (24.73)	92.40 (25.04)	90.63 (19.48)	93.00 (11.36)	0.983[a]
After	95.33 (16.44)	92.78(21.85)	100.74(24.88)	92.41(10.11)	0.453[b]
GMD, *p*[c]	2.71, 0.54	0.37, 0.962	10.10, 0.146	−0.59, 0.711	
LPS (EU/L)					
Before	24.05 (10.64)	25.54 (16.95)	33.35(25.58)	26.96 (20.10)	0.407[a]
After	18.27 (5.88)	23.68(11.91)	11.33 (4.97)	27.27(30.06)	0.047[b]
GMD, *p*[c]	−5.78, 0.014	−1.86, 0.656	−22.02, 0.003	0.31, 0.603	
TNF-alpha (ng/L)					
Before	28.60 (20.82)	29.41 (23.58)	36.70 (29.10)	30.53 (25.55)	0.6980[a]
After	18.27 (5.88)	23.68(11.91)	11.65(11.34)	31.33(41.12)	0.032[b]
GMD, *p*[c]	−10.33, 0.030	−5.73, 0.314	−25.05, 0.002	0.79, 0.942	

GMD: Geometric mean difference, LPS: Lipopolysaccharides, Hs-CRP: high-sensitivity C-reactive protein, TNF- alpha: tumor necrosis factor alpha, Values are expressed as geometric mean (minimum, maximum) and the *p*-values are estimated after log-transformation.
In each row, mean value with different superscript letters are significantly different ($P < 0.05$).
[a]One-Way ANOVA.
[b]Adjusted for baseline values, age and sex using the analysis of covariance (ANCOVA) test.
[c]Paired-samples t-test.

Table 5. Effect of probiotic supplementation on quality of life, BDI score and STAI-Y scales within and between groups.

Variable	Probiotic group ($n = 24$)	Inulin group ($n = 24$)	Probiotic+Inulin group ($n = 24$)	Placebo group ($n = 24$)	*P*-value
BDI					
Before	20 (7.56)	18.70 (6.68)	24.18 (9.59)	20.89 (10.35)	0.196[a]
After	13.37 (7.14)	18.25 (10.59)	12.65 (6.21)	23.87 (13.71)	0.001[b]
GMD, p^c	−6.62, 0.001	−0.45, 0.857	−11.52, 0.00	2.97, 0.446	
Quality of life					
Before	131.09 (49.79)	124.09 (52.52)	119.27 (56.44)	120.95 (50.43)	0.883[a]
After	138.63 (44.61)	137.09(39.58)	158.09(62.70)	117.54(48.75)	0.069[b]
GMD, p^c	7.54, 0.614	13, 0.303	38.81, 0.083	−3.40, 0.843	
STAI-state					
Before	31.99 (17)	33.04 (17)	34.28 (18.22)	38.58 (20)	0.659[a]
After	26.76 (19.41)	32.22(38.25)	16.65 (6.18)	37.95(15.59)	0.021[b]
GMD, p^c	−5.23, 0.313	−0.818, 0.913	−17.63, 0.001	−0.60, 0.903	
STAI-trait					
Before	36.96 (21.47)	40.13 (43.54)	39.91 (21.71)	36.77 (12.58)	0.659
After	26.08 (19.43)	35.50 (38.29)	15.60 (5.90)	38.22 (38.91)	0.020
GMD, p^c	−10.88, 0.006	−4.63, 0.163	−24.31, 0.001	1.45, 0.851	

MD: mean difference, GMD: geometric mean difference, STAI: State-trait anxiety inventory, BDI: Beck depression inventory; Values are expressed as geometric mean (minimum, maximum) and the *p*-values are estimated after log-transformation.
In each row, mean value with different superscript letters are significantly different ($P < 0.05$).
[a]One-Way ANOVA.
[b]Adjusted for baseline values, age and sex using the analysis of covariance (ANCOVA) test.
[c]Paired-samples t-test.

induces hyperactivity of hypothalamic–pituitary–adrenal (HPA) axis, which is implicated in depression pathophysiology. It is proposed that through restoring gut microbiota balance and reversing the consequences of gut dysbiosis, prebiotics and/or might alleviate depressive symptoms [31]. Probiotics intake also has beneficial effects on decreasing inflammatory cytokines and oxidative stress [31,33,34]. This neuro-protein is involved in the survival of neurons and affects the pathophysiology of psychological disorders such as depression and anxiety [31]. In this study, CRP, LPS, and TNF-α were decreased after prebiotics and probiotics administration. However, none of the prebiotics or probiotics had any effects on IL- 10. LPS secreted from the gram-negative bacterial membrane leads to systemic inflammation [35], and toll-like receptor 4 (TLR4) is a primary receptor for LPS; interestingly, probiotics intake suppress TLR4 activity in the paraventricular nucleus of the hypothalamus in response to LPS-induced anxiety and prevented stressfully and depression behaviors [36]. Besides, probiotics' administration might improve symptoms of depression using decreased serotonin concentrations in the cortex and dopamine metabolite levels in the amygdaloid, and increased plasma tryptophan levels, Prebiotics/probiotics through modulating intestinal barrier enhance the immune system function and decrease inflammation. This leads to decreased glucocorticoid resistance, lower kynurenine production from tryptophan, and improved brain-derived neurotrophic factor (BDNF) expression; these together might be partly responsible for alleviation of depression [37,38].

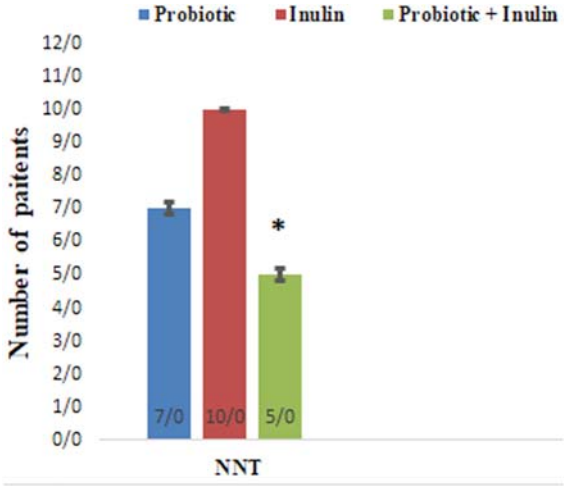


Figure 2. Number needed to treat (NNT) Response based on BDI total score.

Prebiotic intake increases the production of SCFAs and stimulates intestinal probiotic microorganisms' growth and activity [11]. SCFAs in gut microbiota by decreased expression of inflammatory cytokines mediate anti-inflammatory effects [10]. The combination of probiotics and prebiotics and synbiotics have antidepressant effects by affecting the innate immune system to reduce inflammation [39]. Therefore, prebiotics and probiotics supplementation reduce inflammation and increase expression of BDNF, synergistically improve psychological disorders such as depression and anxiety caused by gut dysbiosis.

The current study suffered from some limitations. The intervention period was relatively short, which we did not observe any changes in the QOL of participants. Therefore, well-designed clinical trials are required to evaluate the QOL in CAD patients by modulating inflammation and oxidative stress. Like all

questionnaires, the BDI suffers from the same problems as other self-reported inventories; those scores can be easily exaggerated or minimized by personal answers. To fix this defect, we used another psychological scale, i.e. the STAI-Y. Regardless of those, most investigators agreed to recommend the BDI-II as a valuable tool in CAD patients. Furthermore, funding constraints did not allow gut microbiota analysis, which is a key variable in identifying the effect of probiotics or probiotics. One of our study's strengths is the use of the strains of probiotics and the prebiotic that has been shown in the prior studies to affect mood complaints. Although synbiotics' relative effects have been shown to control chronic inflammation and depression, the dose of inulin is not sufficient in those studies.

In conclusion, co-administration of Inulin with the *Lactobacillus Rhamnosus* had synergically effects on decreasing symptoms of depression and anxiety. Therefore, the current trial supported the beneficial effects of prebiotics and probiotics supplementation to improve depression and anxiety in CAD patients. Also, inflammatory cytokines decreased in both prebiotics and probiotics groups compared to the placebo. These results propose that probiotic plus prebiotic may exert at least part of their effects on depression through the inflammatory cytokines and LPS.

Availability of data and materials

All data generated and analyzed during this study are included in the manuscript.

Consent for publication

All authors support the submission to this journal.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Informed consent

Informed consent was obtained from all individual participants included in the study.

ORCID

Jalal Moludi http://orcid.org/0000-0002-8333-414X

References

[1] Bush DE, Ziegelstein RC, Patel UV, Thombs BD, Ford DE, Fauerbach JA, et al. Post-myocardial infarction depression: summary. AHRQ Evidence Report Summaries: Agency for Healthcare Research and Quality (US); 2005.

[2] Rudisch B, Nemeroff CB. Epidemiology of comorbid coronary artery disease and depression. Biol Psychiatry. 2003;54(3):227–40.

[3] Rutledge T, Gould HM. Epidemiological evidence linking stress and depression with CVD. Cardiovascular implications of stress and depression. Academic Press; 2020. p. 15–34.

[4] Roest AM, Martens EJ, Denollet J, De Jonge P. Prognostic association of anxiety post myocardial infarction with mortality and new cardiac events: a meta-analysis. Psychosom Med. 2010;72(6):563–9.

[5] Nezafati MH, Vojdanparast M, Nezafati P. Antidepressants and cardiovascular adverse events: a narrative review. ARYA Atheroscler. 2015;11(5):295.

[6] Liang S, Wu X, Jin F. Gut-brain psychology: rethinking psychology from the microbiota–gut–brain axis. Front Integr Neurosci. 2018;12:33.

[7] Vaghef-Mehrabany E, Maleki V, Behrooz M, Ranjbar F, Ebrahimi-Mameghani M. Can psychobiotics "mood" ify gut? An update systematic review of randomized controlled trials in healthy and clinical subjects, on antidepressant effects of probiotics, prebiotics, and synbiotics. Clin Nutr. 2020;39(5):1395–410.

[8] Moludi J, Alizadeh M, Mohammadzad MHS, Davari M. The effect of probiotic supplementation on depressive symptoms and quality of life in patients after myocardial infarction: results of a preliminary double-blind clinical trial. Psychosom Med. 2019;81(9):770–7.

[9] Halaris A. Inflammation, heart disease, and depression. Curr Psychiatry Rep. 2013;15(10):400.

[10] Karamese M, Aydin H, Gelen V, Sengul E, Karamese SA. The anti-inflammatory, anti-oxidant and protective effects of probiotic mixture on organ toxicity in a rat model. Future Microbiol. 2020;15:401–12.

[11] Liu RT, Walsh RF, Sheehan AE. Prebiotics and probiotics for depression and anxiety: a systematic review and meta-analysis of controlled clinical trials. Neurosci Biobehav Rev. 2019;102:13–23.

[12] Vale S. Psychosocial stress and cardiovascular diseases. Postgrad Med J. 2005;81(957):429–35.

[13] Slyepchenko A, Maes M, Machado-Vieira R, Anderson G, Solmi M, Sanz Y, et al. Intestinal dysbiosis, gut hyperpermeability and bacterial translocation: missing links between depression, obesity and type 2 diabetes. Curr Pharm Des. 2016;22(40):6087–106.

[14] Singh RK, Chang H-W, Yan D, Lee KM, Ucmak D, Wong K, et al. Influence of diet on the gut microbiome and implications for human health. J Transl Med. 2017;15(1):73.

[15] Park C, Brietzke E, Rosenblat JD, Musial N, Zuckerman H, Ragguett R-M, et al. Probiotics for the treatment of depressive symptoms: an anti-inflammatory mechanism? Brain Behav Immun. 2018;73:115–24.

[16] Slyepchenko A, Carvalho A F, Cha D S, Kasper S, McIntyre R S. Gut emotions-mechanisms of action of probiotics as novel therapeutic targets for depression and anxiety disorders. CNS Neurol Disord Drug Targets. 2014;13(10):1770–86.

[17] Kazemi A, Noorbala AA, Azam K, Eskandari MH, Djafarian K. Effect of probiotic and prebiotic vs placebo on psychological outcomes in patients with major depressive disorder: A randomized clinical trial. Clin Nutr. 2019;38(2):522–8.
[18] Talbott SM, Talbott JA, Stephens BJ, Oddou MP. Effect of coordinated probiotic/prebiotic/phytobiotic supplementation on microbiome balance and psychological mood state in healthy stressed adults. Funct Foods Health Dis. 2019;9(4):265–75.
[19] Slykerman R, Hood F, Wickens K, Thompson J, Barthow C, Murphy R, et al. Effect of Lactobacillus rhamnosus HN001 in pregnancy on postpartum symptoms of depression and anxiety: a randomised double-blind placebo-controlled trial. EBioMedicine. 2017;24:159–65.
[20] Saavedra J, Tschernia A. Human studies with probiotics and prebiotics: clinical implications. Br J Nutr. 2002;87 (S2):S241–6.
[21] Ghassemzadeh H, Mojtabai R, Karamghadiri N, Ebrahimkhani N. Psychometric properties of a Persian-language version of the beck depression inventory-second edition: BDI-II-PERSIAN. Depress Anxiety. 2005;21(4):185–92.
[22] Naderi N, Bakhshandeh H, Amin A, Taghavi S, Dadashi M, Maleki M. Development and validation of the first Iranian questionnaire to assess quality of life in patients with heart failure: IHF-QoL. Res Cardiovasc Med. 2012;1(1):10.
[23] Bados A, Gómez-Benito J, Balaguer G. The state-trait anxiety inventory, trait version: does it really measure anxiety? J Pers Assess. 2010;92(6):560–7.
[24] White IR, Royston P, Wood AM. Multiple imputation using chained equations: issues and guidance for practice. Stat Med. 2011;30(4):377–99.
[25] Wright C, Sim J. Intention-to-treat approach to data from randomized controlled trials: a sensitivity analysis. J Clin Epidemiol. 2003;56(9):833–42.
[26] Masson SC, Tejani AM. Minimum clinically important differences identified for commonly used depression rating scales. J Clin Epidemiol. 2013;66(7):805–7.
[27] Hiriscau EI, Bodolea C. The role of depression and anxiety in frail patients with heart failure. Diseases. 2019;7(2):45.
[28] Westas M, Lundgren J, Mourad G, Neher M, Johansson P. How healthcare professionals in cardiac care address depressive symptoms: experiences of patients with cardiovascular disease. J Cardiovasc Nurs. 2020.
[29] Pasdar Y, Hamzeh B, Moludi J, Mehaki B, Darbandi M, Moradi S. Dietary intake and risk of depression among male and female with HIV/AIDS. Eating and Weight Disorders-Studies on Anorexia, Bulimia and Obesity. 2019:1–10.
[30] Akkasheh G, Kashani-Poor Z, Tajabadi-Ebrahimi M, Jafari P, Akbari H, Taghizadeh M, et al. Clinical and metabolic response to probiotic administration in patients with major depressive disorder: a randomized, double-blind, placebo-controlled trial. Nutrition. 2016;32(3):315–20.
[31] Haghighat N, Rajabi S, Mohammadshahi M. Effect of synbiotic and probiotic supplementation on serum brain-derived neurotrophic factor level, depression and anxiety symptoms in hemodialysis patients: a randomized, double-blinded, clinical trial. Nutr Neurosci. 2019: 1–10.
[32] Ghorbani Z, Nazari S, Etesam F, Nourimajd S, Ahmadpanah M, Jahromi SR. The effect of synbiotic as an adjuvant therapy to fluoxetine in moderate depression: a randomized multicenter trial. Arch Neurosci. 2018;5(2).
[33] Amirani E, Milajerdi A, Mirzaei H, Jamilian H, Mansournia MA, Hallajzadeh J, et al. The effects of probiotic supplementation on mental health, biomarkers of inflammation and oxidative stress in patients with psychiatric disorders: a systematic review and meta-analysis of randomized controlled trials. Complement Ther Med. 2020:49:102361.
[34] Venturini L, Bacchi S, Capelli E, Lorusso L, Ricevuti G, Cusa C. Modification of immunological parameters, oxidative stress markers, mood symptoms, and well-being status in CFS patients after probiotic intake: observations from a pilot study. Oxid Med Cell Longevity. 2019;2019:1–10.
[35] Zhang H, Qi R, Zeng Y, Tsao R, Mine Y. Chinese Sweet Leaf Tea (Rubus suavissimus) mitigates LPS-induced low-grade chronic inflammation and reduces the risk of metabolic disorders in a C57BL/6J mouse model. J Agric Food Chem. 2019;68(1):138–46.
[36] Murray E, Sharma R, Smith KB, Mar KD, Barve R, Lukasik M, et al. Probiotic consumption during puberty mitigates LPS-induced immune responses and protects against stress-induced depression-and anxiety-like behaviors in adulthood in a sex-specific manner. Brain Behav Immun. 2019;81:198–212.
[37] Wang H, Lee IS, Braun C, Enck P. Effect of probiotics on central nervous system functions in animals and humans: a systematic review. J Neurogastroenterol Motil. 2016;22(4):589.
[38] Liu L, Zhu G. Gut-brain axis and mood disorder. Front Psychiat. 2018;9:223.
[39] Westfall S, Pasinetti G. Design of a novel synbiotic formulation to optimize gut-derived phenolic acid mediated gut-brain axis Signals for the treatment of stress-induced depression and anxiety (OR23-03-19). Curr Dev Nutr. 2019;3(Supplement_1):nzz040. OR23-03-19.