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The effect of probiotic-fortified kefir on depression, appetite, oxidative stress, and inflammatory parameters in Iranian overweight and obese elderly: a randomized, double-blind, placebo-controlled clinical trial

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Abstract

Background It has been shown that the microflora of the gastrointestinal tract undergoes changes in obese individuals. The present study aimed to investigate the effect of kefir fortified with two strains, Lactobacillus helveticus and Bifidobacterium longum, on depression, appetite, oxidative stress, and inflammatory parameters in overweight and obese elderly individuals.

Methods This study was a double-blind, randomized, and placebo-controlled clinical trial conducted on 67 elderly men aged over 65, who were randomly divided into two groups. One group ($n=35$) received one bottle (240 cc) of regular kefir as a placebo, while the intervention group ($n=32$) received one bottle of probiotic-fortified kefir for eight weeks. Depression and appetite were evaluated using the Geriatric Depression Scale-15 (GDS-15) and a validated Visual Analogue Scale (VAS), respectively. Oxidative stress parameters were assessed using the standard calorimetric method, and inflammatory parameters were measured via the enzyme-linked immunosorbent assay method (ELISA). The differences between the two groups were compared using the independent samples T-test.

Results The median age of participant in both groups was 65 years. A significant difference in depression scores and the mean change between the two groups was observed after eight weeks ($p=0.001$ and $p=0.042$, respectively). Within-group comparison revealed a significant increase in appetite scores in both groups ($p<0.05$ for both). Moreover, a significant difference in the changes in total antioxidant capacity (TAC) was noted ($p=0.009$). However, no significant differences were observed in other oxidative and inflammatory parameters between the two groups ($p>0.05$ for all).

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Conclusions The results demonstrated the positive impact of two specific strains of Bifidobacterium and Lactobacillus on improving depression in the elderly. However, when comparing the two groups, no significant effects were observed on appetite, inflammation, and oxidative stress parameters, except for TAC.

Keywords Probiotics, Kefir, Lactobacillus, Bifidobacterium, Depression, Oxidative stress, Inflammation

Introduction

According to the World Health Organization (WHO), the elderly individuals are defined as those aged 65 years or older [1]. Several factors, including reduced nutritional needs, changes in basal metabolism, poor eating habits, and inactivity, have contributed to an increased prevalence of overweight and obesity in this population [2]. Obesity is recognized as a risk factor for various health conditions, including diabetes [3], heart failure [4], hypertension [5], and psychological issues such as mood disorders and depression [6]. Additionally, being overweight may further elevate the risk of depression in the elderly [7].

Numerous inflammatory markers are associated with obesity and its related adverse outcomes [8]. Research has shown that changes in the gastrointestinal microflora occur in obese individuals, leading to increased permeability of intestinal epithelial cells and endotoxemia. This, in turn, contributes to insulin resistance and obesity through inflammation pathways [9, 10]. Moreover, substantial evidence suggests that gut microbiota play a critical role in energy regulation, nutrient absorption, fat storage, and the alleviation of anxiety and depression by producing tryptophan, a precursor to serotonin [11–13]. In addition, studies indicate that treatment with prebiotics and probiotics may counteract many of the metabolic effects caused by alterations in the gut microflora of obese individuals [10]. As a result, managing obesity remains a key concern, with dietary modification widely recognized as having the most significant impact on addressing this growing epidemic [14].

Evidence suggests that dairy products may contribute to a more favorable body composition [15, 16]. Kefir, a traditional drink produced by fermenting kefir grains with water or milk [17], recognized as a probiotic product [18]. Numerous studies have demonstrated that kefir offers a range of nutritional benefits, including anticancer, antidiabetic, antioxidative, anti-inflammatory, and immunomodulatory properties [19, 20].

Over the past decade, inflammation has been recognized as a significant factor in mood disorders [21]. Clinical studies have also shown that various psychological and physiological stressors can disrupt normal intestinal microbiota, with the most notable impact being a stress-induced reduction in Lactobacillus and Bifidobacterium species [22, 23]. Moreover, a trial involving 55 healthy individuals demonstrated that a combination of Lactobacillus helveticus and Bifidobacterium longum improved mood and anxiety symptoms within 30 days [23]. Additionally, both Lactobacillus helveticus and Bifidobacterium longum play crucial roles in modulating gut-brain communication and systemic inflammation, albeit through distinct pathways [23–25].

The consumption of probiotic microorganisms has significantly increased in recent years, particularly through probiotic dairy products [26]. In today's modern world, people are becoming more health-conscious, prompting changes in diets and lifestyles. These changes often include the incorporation of probiotics and probiotic-based products [27]. Numerous researchers have highlighted the importance of probiotics, leading to extensive investigations into their clinical health benefits [28]. While some studies have demonstrated the positive effects of probiotics on gut microbiota and immune function in adults, there is limited research specifically exploring the impact of probiotic-fortified kefir on appetite regulation, oxidative stress, and inflammatory parameters in elderly men. To our knowledge, no study has examined the effects of probiotic-fortified kefir on depression, oxidative stress, and inflammatory parameters in the elderly. Therefore, this study aimed to evaluate whether kefir enriched with two strains, Lactobacillus helveticus and Bifidobacterium longum, could influence depression, oxidative stress, and inflammatory parameters in overweight and obese elderly individuals.

Methods

Design and setting

This double-blind, parallel, randomized controlled trial was conducted at the Motahari Clinic in Shiraz, Iran. The study's sample size was calculated based on the previous study [29] with an effect size of $d = 0.75$, $\alpha = 0.05$, and $\beta = 80\%$ using G*Power software based on the total antioxidant capacity (TAC) variable. After considering a 20% removal or violation of protocols, 36 participants were included in each of the fortified kefir group and the regular kefir group. Additional details about the study design are provided in a prior publication related to this project [30].

Participants

In this randomized controlled trial, participants were selected based on the following criteria: male gender, a body mass index (BMI) greater than 25, age over 65, willingness to participate, no history of cardiovascular diseases, diabetes, kidney or liver diseases, chronic

infectious diseases, digestive disorders, psychological or neurological disorders, no use of antidepressant drugs, no recent use of antibiotics (within the last three months), probiotics (within the last two months), or antioxidant supplements, and not consuming alcohol regularly (more than three units per week). Participants were excluded if they experienced any of the following during the study: changes in medication or diet, concurrent use of other probiotic supplements, unwillingness to continue participation, or intolerance to the intervention.

Randomization

Permuted-block randomization with a fixed block size of four (2:2 ratio) was used to randomize participants via a computer method. Both participants and researchers were blinded to the allocation and randomization process. A trained assistant was responsible for randomly assigning subjects to either the fortified or regular kefir groups.

Blinding

Both participants and investigators involved in outcome assessments were blinded to the group allocation. The appearance (shape, size, and packaging) of the fortified and regular kefir was identical. To maintain blinding, packaging and coding were handled by the company. Additionally, the randomization sequence was kept confidential until the study was completed.

Intervention

In the fortified kefir group, participants consumed one bottle (240 cc) of fortified kefir containing *Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175 (dosage 3×10^9 colony-forming units (CFU) for each of them). The kefir starter culture included LAF4 and *Kluyveromyces marxianus*). Participants were instructed to consume a bottle of kefir daily with lunch or dinner for eight weeks. In the placebo group, participants received 240 cc of regular kefir under the same schedule. Both the fortified and regular kefir were produced by Pegah Company, Fars, Iran and were identical in shape, size, and packaging.

During the randomized clinical trial, participants were asked not to modify their regular dietary intake and physical activity. Daily reminder messages were sent to encourage adherence to the kefir consumption schedule. Participants also documented their daily kefir intake on a checklist. After two weeks, participants visited the Motahari Clinic to receive their next batch of kefir and return any uneaten bottles. Based on the evaluation of uneaten kefir and the daily checklists, participants who consumed less than 80% of their kefir bottles were excluded from the study.

Outcomes

The primary outcomes of the study were appetite, depression, oxidative stress, and inflammatory markers. Outcome assessments were conducted at baseline and after 8 weeks of intervention. The parameters and their corresponding evaluation methods are outlined below.

Assessment of baseline features

At baseline and after eight weeks, weight was measured using a Seca device (Germany) while participants wore minimal clothing. Height was assessed in a standing position using a non-stretchable, fixed tape. BMI was then calculated using the formula: weight (kg) / height (m^2). Waist circumference (WC) and hip circumference (HC) were measured with non-stretchable tape at the level of the iliac crest.

Physical activity was assessed using the International Physical Activity Questionnaire (IPAQ) at baseline and at the end of the study [31]. The IPAQ evaluates various aspects of physical activity, including vigorous-intensity activity, moderate-intensity activity, and walking, across different domains such as work, transportation, leisure, and household activities [32]. Based on the metabolic equivalent of task (MET), participants were categorized into three groups: low activity (less than 600 MET-minutes/week), moderate activity (600–3000 MET-minutes/week), and high activity (more than 3000 MET-minutes/week).

At baseline and after eight weeks of intervention, dietary intake was assessed using a 3-day dietary record (one weekend and two weekdays). Initially, all food item portion sizes were converted to grams based on Iranian household measurement guidelines [33]. Then, Nutritionist IV was used to calculate energy, macro-, and micronutrient intake [34].

Biochemical assessment

To measure oxidative and inflammatory markers at the beginning and end of the study, 10 mL fasting blood samples were collected using tubes containing ethylenediaminetetraacetic acid (EDTA). The blood samples were then centrifuged at 3000 revolutions per minute (rpm) for 7 min. The isolated serums were subsequently frozen at -76 °C for future biochemical analysis.

TAC, superoxide dismutase (SOD) activity, catalase, glutathione peroxidase (GPx) activity, and malondialdehyde (MDA) levels were assessed using standard calorimetric methods with a Zelbio (Germany) kit and a microplate reader. Additionally, interleukin 6 (IL-6) and C-reactive protein (CRP) levels were determined using the enzyme-linked immunosorbent assay (ELISA) method with an LDN (Germany) kit.

Depression and appetite assessment

Depression was evaluated using the Geriatric Depression Scale-15 (GDS-15) questionnaire. The GDS-15 was developed by Yesavage and Brink in 1983 and later validated for the Iranian population by Malakouti et al. [35]. Each question on the GDS-15 was scored as either 0 or 1, with the total score for each participant ranging from 0 to 15.

Appetite was assessed using a validated Visual Analogue Scale (VAS) at baseline and at the end of the intervention. The VAS is a 100 mm horizontal line with endpoints representing the most positive and negative ratings of desire to eat, hunger, fullness, and satiety.

Statistical analysis

The normality of the study variables was assessed using the Kolmogorov-Smirnov test. Baseline categorical demographic variables, such as physical activity and education level, were compared between the fortified and regular kefir groups using the chi-square test. Quantitative baseline variables were compared using either the Mann-Whitney U test or the independent samples t-test, depending on their normal distribution. Depression and appetite scores, as well as oxidative and inflammatory markers, were evaluated as outcomes, with differences between the two groups compared using the independent samples t-test. Within-group analysis for both groups was conducted using the paired-sample t-test. All analyses were performed using SPSS version 23 (SPSS Inc., Chicago, Illinois). Statistical significance was set at $p < 0.05$.

Ethical considerations

This clinical trial was conducted in accordance with the Declaration of Helsinki guidelines, and all participants provided written informed consent. The study was approved by the ethics committee of Baqiyatallah Hospital (ethical approval number: IR.BMSU.BAQ.REC.1401.113) and registered with the Iranian Registry of Clinical Trials (IRCT20130227012628N3; first registration date: 21/02/2023).

Results

In the current study, 72 eligible elderly participants were randomly assigned to either the fortified kefir group ($n = 36$) or the regular kefir group ($n = 36$) for eight weeks. After eight weeks of supplementation, 67 participants completed the trial: 32 in the fortified kefir group (2 participants were excluded due to digestive issues, and 2 were excluded for consuming less than 80% of the supplement) and 35 in the regular kefir group (1 participant was excluded for non-compliance with the 80% consumption requirement) (Fig. 1). As shown in Table 1, there were no significant differences in anthropometric, demographic characteristics, or nutrient intakes between the two groups at baseline ($p > 0.05$ for all).

The depression and appetite scores of elderly participants in both groups are presented in Table 2. At baseline, there were no significant differences in depression and appetite scores between the fortified and regular kefir groups ($p > 0.05$ for both). However, after eight weeks of supplementation, a significant difference was observed in depression scores and the mean change between the two groups ($p = 0.001$ and $p = 0.042$, respectively). Additionally, within-group comparisons showed a significant increase in appetite scores for both groups ($p < 0.001$ for the fortified kefir group and $p = 0.001$ for the regular kefir group). Nevertheless, there was no significant difference in appetite scores between the two groups ($p > 0.05$).

The effect of fortified and regular kefir on oxidative and inflammatory markers in elderly participants is presented in Table 3. After eight weeks of supplementation, a significant difference was observed in the changes in total antioxidant capacity (TAC) levels ($p = 0.009$). However, no significant differences were found in IL-6, CRP, and MDA levels, nor in the activity of SOD, catalase, and GPx between the two groups ($p > 0.05$ for all). Additionally, within-group analyses revealed a significant difference in TAC levels for both the fortified and regular kefir groups ($p = 0.033$ and $p = 0.035$, respectively).

Discussion

In the present study, a significant reduction in depression was observed in the fortified kefir group compared to the regular kefir group. Additionally, a significant change in TAC was noted in the intervention group compared to the placebo group. However, no significant differences were found between the two groups for the other variables.

The number of individuals suffering from depression and other mood disorders is rapidly increasing. According to the WHO, approximately 300 million people worldwide are affected by depression [36]. Therefore, it is crucial to find new antidepressant treatments to address the issues with current medications, including their high incidence of unwanted side effects, the frequent lack of response, and the prolonged onset of therapeutic effects [37].

As mentioned above, the findings of the current study showed that kefir fortified with probiotics can significantly reduce depression. Our findings are in line with the other studies. In a randomized clinical trial on the effect of Bifidobacterium longum on 44 people with irritable bowel syndrome)IBS(and mild to moderate anxiety or depression for six weeks, it was shown that depression was reduced by supplementing with this probiotic [38]. Also, a study by Soltanmoradi et al. revealed that kefir and Lactobacillus rhamnosus GG could decrease the

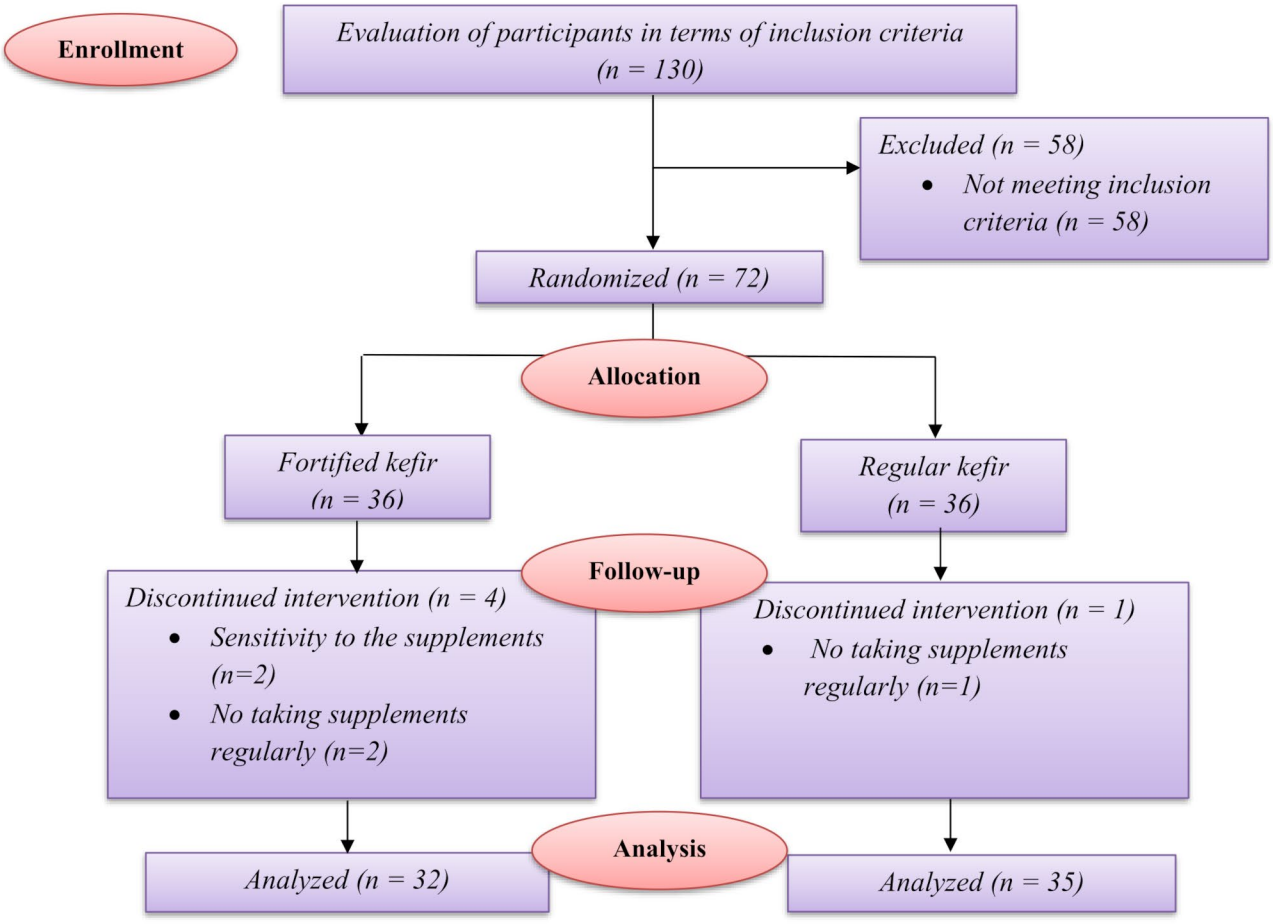

Fig. 1 Flowchart of the study

duration of depression-like activities and increase antidepressant properties in a mouse model [39]. In a cross-sectional study of 26,118 people, the effect of consuming foods containing probiotics on depression was investigated. The results showed that the consumption of probiotic foods may have beneficial effects on depression, especially in men [40]. However, a systematic review and meta-analysis of randomized clinical trials demonstrated that probiotics have a significant effect on reducing depression in the population under 60 years of age and have no effect on the population over 65 [41]. Since there was only one study with people older than 65 years in this meta-analysis, no strong conclusions can be drawn. In general, studies show the important role of probiotics in reducing depression in non-depressed people and patients with depression [41].

The mechanism of probiotics' effect on depression has been investigated in some studies. Bifidobacterium has been shown to reduce pro-inflammatory immune responses and increase tryptophan, a precursor to serotonin synthesis [42]. Studies have shown that bifidobacteria increase the expression of inflammatory cytokines, such as IL-1 and TNF-α, while also enhancing the activity of natural killer cells [43]. It has also been shown that the consumption of probiotics plays an important role in the levels of brain-derived neurotrophic factor [44, 45] and γ-aminobutyric acid, an important neurotransmitter in depression [46]. Moreover, probiotics can be involved in decreasing depression by reducing pro-inflammatory cytokines that contribute to depression [23, 47, 48]. In addition, gut microbiota may play an essential role in modulating behavior and mood disorders through various mechanisms [49]. Improving gut dysbiosis reduces inflammation, resulting in changes in brain function, mood, and behavior [50].

The findings of the present study indicated the positive effect of kefir fortified with probiotics and regular kefir on the appetite of the elderly, and the improvement of appetite was greater in the intervention group compared to placebo. However, it was not statistically significant. A post hoc analysis of randomized clinical trials showed that supplementation with probiotics (Bifidobacterium longum and Lactobacillus helveticus) in patients with major depressive disorder is related to improved appetite

Table 1 Anthropometric and demographic characteristics and nutrient intakes of the study participants at the baseline ($n=67$)

Variables	Fortified kefir group ($n=32$)	Regular kefir group ($n=35$)	*P*-value
Age (year) [1]	65.00 (65.00–66.00)	65.00 (65.00–65.00)	0.375
Weight (kg) [1]	77.00 (73.00–85.00)	77.00 (72.00–85.00)	0.930
Height (cm) [1]	170.00 (165.00-174.50)	170.00 (165.00-173.00)	0.924
BMI (kg/m^2) [1]	26.44 (25.31–28.63)	27.28 (25.30-29.38)	0.935
Waist circumference (cm) [1]	99.00 (97.00-105.75)	99.00 (97.00-107.00)	0.915
Hip circumference (cm) [1]	103.00 (101.00-109.75)	103.00 (100.00-113.00)	0.910
WHR [1]	0.96 (0.96–0.97)	0.96 (0.96–0.97)	0.860
Energy intake (kcal/day) [1]	2087.88 (1857.31-2247.46)	2001.87 (1852.87-2350.85)	0.925
Carbohydrate intake (gr/day) [2]	270.04 ± 69.72	268.03 ± 58.23	0.898
Protein intake (gr/day) [2]	81.35 ± 22.17	86.96 ± 29.28	0.383
Fat intake (gr/day) [2]	81.20 ± 25.00	78.62 ± 20.22	0.642
Fiber intake (gr/day) [2]	27.96 ± 13.77	27.36 ± 12.09	0.848
Calcium (mg/day) [1]	794.59 (526.68-1030.33)	827.60 (483.21-1301.43)	0.543
Magnesium (mg/day) [2]	344.91 ± 123.89	330.19 ± 114.65	0.615
Zinc (mg/day) [1]	9.33 (6.87–12.04)	11.34 (7.63–13.80)	0.156
Selenium (mcg/day) [2]	0.11 ± 0.05	0.10 ± 0.05	0.341
Vitamin C (mg/day) [1]	82.89 (26.60-171.62)	145.69 (43.32-205.93)	0.253
Physical activity, % [3]			0.806
Low	19 (59.40)	22 (62.90)	
Moderate	13 (40.60)	13 (37.10)	
Education Level, %			0.771
Less than diploma	8 (25.00)	7 (20.00)	
Diploma and higher	24 (75.00)	28 (80.00)	
Smoking history, % [3]			0.584
Yes	7 (21.90)	10 (28.60)	
No	25 (78.10)	25 (71.40)	
Diseases history, % [3]			0.787
Yes	10 (31.30)	9 (25.70)	
No	22 (68.80)	26 (74.30)	
Medication, % [3]			1.000
Yes	10 (31.30)	11 (31.40)	
No	22 (68.70)	24 (68.60)	

- kg: kilogram, cm: centimeter, kg/m: kilogram / meter, gr: gram, mg: milligram, mcg: microgram, BMI: body mass index, WHR: waist-to-hip ratio

- Using Mann-Whitney or independent samples T-test for continuous and chi-square test for categorical variables

[1] Values are median (25th -75th)

[2] Values are mean ± SD

[3] Values are number (percent)

[51]. Moreover, a study by Saito et al. illustrated that Lactobacillus brevis can increase appetite by increasing the production of ghrelin and serotonin [52]. In contrast, some studies have shown that some probiotic strains are related to appetite suppression and reduced food intake [53, 54]. However, the presence of probiotics in kefir may be the reason for the lack of significant change in appetite levels when comparing the two groups in the present study. Also, the difference in species compared to those investigated in the present study may account for the observed variation.

Beneficial effects mediated by probiotics on human health can be caused by changes in gut microbiota [55]. Gut microbiota may affect eating behavior, but the mechanism remains unclear [56]. The appetite system and gut microbiota seem to be related, and microbial metabolites and energy metabolism may be potential mechanisms. Metabolites from microbiota may affect appetite by regulating immune system function and hormone secretion [56]. Also, probiotics may increase appetite by reducing leptin levels, but the exact mechanism is unknown [57]. However, it has been shown that probiotic bacteria such as Bifidobacterium and Lactobacillus can cause a decrease in enterohepatic leptin by reducing the hydrolysis of conjugated hormone in the colon [58].

Table 2 The effect of the fortified and regular kefir on depression and appetite scores in elderly participants ($n=67$)

Variables	Fortified kefir group ($n=32$)	Regular kefir group ($n=35$)	*P*-value [1]
GDS-15 Score			
Before	8.91 ± 1.69	9.17 ± 1.68	0.526
After	8.03 ± 1.12	8.94 ± 1.02	**0.001**
Change	-0.87 ± 1.26	-0.22 ± 1.28	**0.042**
P-value [2]	<**0.001**	0.300	
Appetite Score			
Before	5.69 ± 0.95	5.85 ± 1.08	0.522
After	6.40 ± 0.55	6.31 ± 0.67	0.549
Change	0.71 ± 0.88	0.45 ± 0.74	0.194
P-value [2]	<**0.001**	**0.001**	

[1] Using independent samples T-test

[2] Using paired-sample T-test

- Values are mean ± SD

Table 3 The effect of the fortified and regular kefir on oxidative and inflammatory markers in elderly participants ($n=67$)

Variables	Fortified kefir group ($n=32$)	Regular kefir group ($n=35$)	*P*-value [1]
TAC (mmol/L)			
Before	1.41 ± 0.30	1.51 ± 0.23	0.144
After	1.57 ± 0.35	1.47 ± 0.21	0.108
Change	0.12 ± 0.35	-0.09 ± 0.26	**0.009**
P-value [2]	**0.033**	**0.035**	
SOD (U/mL)			
Before	24.99 ± 4.82	24.73 ± 4.40	0.814
After	26.50 ± 4.65	25.49 ± 5.47	0.421
Change	1.50 ± 5.91	0.76 ± 4.34	0.560
P-value [2]	0.146	0.321	
Catalase (U/mL)			
Before	26.40 ± 5.61	25.30 ± 4.93	0.389
After	26.68 ± 5.34	25.53 ± 6.36	0.427
Change	0.27 ± 4.06	0.22 ± 4.85	0.966
P-value [2]	0.696	0.789	
GPx activity (U/mL)			
Before	13.24 ± 4.01	13.00 ± 4.38	0.809
After	13.63 ± 4.14	13.41 ± 4.29	0.830
Change	0.38 ± 3.97	0.25 ± 3.22	0.882
P-value [2]	0.572	0.650	
IL-6 (pg/mL)			
Before	4.58 ± 2.05	4.75 ± 1.92	0.719
After	4.22 ± 1.55	4.32 ± 1.52	0.784
Change	-0.35 ± 1.12	-0.31 ± 1.37	0.882
P-value [2]	0.071	0.198	
CRP (ng/mL)			
Before	4706.02 ± 1235.87	4852.91 ± 1417.63	0.648
After	4642.00 ± 1435.99	4784.93 ± 1545.46	0.696
Change	-64.02 ± 1958.38	-104.00 ± 2301.82	0.939
P-value [2]	0.850	0.797	
MDA (μmol/L)			
Before	2.15 ± 0.40	2.18 ± 0.45	0.813
After	2.04 ± 0.49	1.97 ± 0.49	0.597
Change	-0.11 ± 0.34	-0.20 ± 0.64	0.481
P-value [2]	0.067	0.068	

TAC, total antioxidant capacity; SOD, superoxide dismutase, GPx, glutathione peroxidase; IL-6, interleukin-6; CRP, C-reactive protein; MDA, malondialdehyde

[1] Using independent samples T-test

[2] Using paired-sample T-test

- Values are mean ± SD

Our findings indicated that supplementation with probiotics had no significant effect on inflammatory and oxidative stress parameters except TAC. Mixed evidence is available regarding the anti-inflammatory and antioxidant properties of probiotics. While some showed no effect, other studies reported beneficial effects [59–62]. A meta-analysis study on the effect of probiotics in people with diabetes illustrated that probiotics did not significantly affect CRP levels [63]. Also, in a clinical trial aimed at investigating the effect of Lactobacillus probiotics on inflammatory and oxidative markers in thirty-four subjects with type 2 diabetes, it was shown that Lactobacillus did not exert a significant effect on MDA and IL-6 levels after six weeks of intervention [61]. In contrast, a study by Harisa et al. revealed that Lactobacillus acidophilus could decrease MDA in diabetic rats [64]. Also, in a double-blind, randomized, placebo-controlled study on the effect of probiotics in patients under methadone maintenance treatment programs, it was shown that supplementation with probiotics caused a significant increase in total glutathione and TAC [65].

Although the anti-inflammatory mechanisms of probiotics have not yet been determined, it has been suggested that probiotics play a role in reducing inflammation by producing short-chain fatty acids (SCFAs) [66, 67]. Also, some bacteria (such as Lactobacillus species) reduce inflammation by repairing and maintaining epithelial barriers, thereby reducing the effect of pro-inflammatory stimuli such as lipopolysaccharides [68]. In addition, probiotics can decrease inflammation by increasing the synthesis of antimicrobial peptides [68]. Probiotics can influence the expression of inflammatory markers. For example, certain probiotics can reduce levels of pro-inflammatory cytokines like TNF-α and IL-6 while promoting anti-inflammatory cytokines such as IL-10 [69]. Furthermore, the effect of probiotics on oxidative stress can be attributed to the production of butyrate, which can act as an antioxidant by affecting the levels of non-enzymatic and enzymatic antioxidants and deoxyribonucleic acid (DNA) repair systems [70].

The current research had strengths and limitations. Due to financial constraints, we were unable to examine the participants' stools, as the actual effects of probiotics on intestinal ecology remain a topic of ongoing debate. Additionally, our study sample consisted solely of men over 65 years old. Given that age and gender are

significant factors influencing the gut microbiome, the results may not be applicable to women or individuals under 65. Moreover, since the elderly participants in this study did not experience depression, the findings cannot be generalized to those with depression. Furthermore, the small sample size limits the ability to extrapolate the results to the broader elderly population. Another limitation of this study was controlling for confounding variables, such as diet, physical activity, medication use, and psychosocial factors, all of which can significantly influence appetite, inflammation, oxidative stress, and depression. However, to our knowledge, this is the first study to examine the effects of two specific probiotic strains on oxidative stress, inflammation, appetite, and depression in the elderly. A key strength of the study is its double-blind, placebo-controlled design. Additional strengths include the specific focus on the elderly population, the use of two probiotic strains to allow a more comprehensive evaluation of their effects, and the assessment of multiple health parameters, which may offer insights into synergistic or differential effects on health outcomes.

Conclusions

This study is the first to evaluate the effects of kefir fortified with two bacterial strains on depression, oxidative stress, and inflammatory parameters in overweight and obese elderly individuals in Iran. The findings suggest a positive effect of two specific strains of Bifidobacterium and Lactobacillus on improving depression in this population. Therefore, consumption of kefir fortified with these strains may help alleviate depression in elderly individuals. However, no significant effects were found on appetite, inflammation, or oxidative stress parameters, except for TAC when comparing the two groups. If the results of this study are confirmed by future clinical trials involving depressed individuals, probiotics may serve as an adjunctive treatment for depression, positively influencing the disease process. Further research is required to confirm these findings and explore the mechanisms by which probiotics affect the evaluated parameters.

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Author contributions
M.N and Z.S; Contributed to writing the first draft. M.H.E, S.B and M.S; Contributed to all data and statistical analysis and interpretation of data. M.S, K.P, H.G and H.A.; Contributed to the research concept, supervised the work, and revised the manuscript. All authors read and approved the final manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval
The current research was approved by the Medical Research and Ethics Committee of Baqiyatallah Hospital (IR.BMSU.BAQ.REC.1401.113) and registered at the Iranian Registry of Clinical Trials (IRCT20130227012628N3) at 21/02/2023 and was carried out in accordance with the ethical standards of the Declaration of Helsinki.

Consent to participate
The informed consents were completed by all participants.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Conflict of interest
Not applicable.

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