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The Effects of pomegranate Peel Supplementation on Depression, Anxiety, and Stress Symptoms of Patients with Non-alcoholic Fatty Liver: A Randomized Clinical Trial

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ABSTRACT

Introduction: Nowadays, improving anxiety, depression, and stress is important in managing non-alcoholic fatty liver (NAFLD). Thus, this study aimed to evaluate eight weeks of pomegranate peel (PP) supplementation on depression, anxiety, and stress scale changes among NAFLD patients.

Methods: This randomized clinical trial was conducted on 76 NAFLD patients assigned to the PP (n=39) or placebo (n=37) groups. Participants received the pomegranate peel (1500 mg/day) or placebo for eight weeks. PP capsules were prepared fromdry extract of PP by soaking. A diet with reduced calorie intake and healthy recommendations was given to all participants. The status of NAFLD was checked with two-dimensional elastography. Mental health was evaluated using depression, anxiety, and stress scale, and dietary intake was assessed by 3-day recall before and after the intervention.

Results: The average age of the participants was 43.1±8.6 years, of whom 51.3% were women. In the PP group, weight, liver stiffness, and hepatorenal sonography index changes significantly differed from the placebo group before and after adjusting potential covariates, including weight and physical activity (P< 0.001). Depression and stress scores changed significantly in the PP group during the study before and after adjusting potential covariates (P= 0.002, 0.05, respectively). Anxiety score changes were insignificant between the two groups (P= 0.1).

Conclusion: Based on the results, eight-week supplementation of pomegranate peel ameliorated depression and stress symptoms among NAFLD patients.

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Introduction

Non-alcoholic fatty liver disease (NAFLD) is one of the most common chronic liver diseases worldwide (1). The causes of non-alcoholic fatty liver range from simple steatosis to non-alcoholic steatohepatitis, which may progress to fibrosis, cirrhosis, or even liver cancer (2, 3). According to studies, the prevalence of this disease has been estimated at 25.24% of the adult population and 7.6% of children worldwide (3, 4). The majority of non-alcoholic fatty liver disease in Iran was estimated at 33.9% based on 23 studies with 25865 participants in 2016, much higher than the global average.

Several studies have reported a significant association between metabolic syndrome and anxiety, depression, and stress. A study reported a positive association between depression and hepatocyte ballooning (5). The subclinical depression and anxiety were in 53 and 45% of 567 study patients, 14 and 25% of whom suffered clinical depression and anxiety. Also, a

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significant association was between NAFLD and anti-depressant prescriptions (6). After assessing animal and human studies, a review study suggested direct relationships between chronic psychological stress and NAFLD (5). Moreover, NAFLD patients had poorer health-related quality of life compared to healthy people, following an investigation of 14 relevant studies (7). All mentioned studies supported a link between NAFLD and anxiety, depression, and stress. In this regard, improving and controlling these psychological concerns are important in managing NAFLD.

Many clinical studies have investigated the effectiveness of drugs and supplements in treating NAFLD and its side effects. Meanwhile, special attention has been paid to supplements effective in oxidative stress due to their role in improving liver lipotoxicity and inflammation as the main causes of the pathogenesis of the disease. However, there is still a need for complementary therapy to reduce the inflammatory state and oxidative stress and improve the neuropsychological health of these patients. The red peel of the pomegranate fruit contains the highest number of phytochemicals (8-10). About 48 phenolic compounds (flavonoids, hydrolyzed tannins such as ellagitannins and galagyl esters, anthocyanin, gallotannins, hydroxycinnamic acid, and hydroxybenzoic acid) were identified in the skin and other parts of pomegranate (8). There is considerable evidence to suggest that hydrolyzable polyphenols, especially ellagitannins, are among the most powerful antioxidants in pomegranate peel. In addition, these compounds (ellagic acid, punicalagin, panicalin, and gallic acid) have antioxidant and pleiotropic biological activities and especially act synergistically (11, 12). However, in vivo studies have shown that the antioxidant properties of dietary absorbed polyphenols are related to their metabolized compounds, for example, urolithins (13). Recent studies have indicated that pomegranate skin, in addition to its antioxidant properties, can be a promising candidate for treating non-alcoholic fatty liver by maintaining the microbiome balance of the digestive system and influencing the expression of key genes in the pathways of inflammation and liver lipogenesis and inhibiting the signaling pathways in liver fibrogenesis (14, 15). Moreover, studies have reported PP polyphenols and phytoestrogens' preventive and therapeutic effects in neurological conditions (16-18). However, the effectiveness of PP supplementation on mental health and life improvement of NAFLD patients has not been investigated yet. Thus, the present study aimed to evaluate depression, anxiety, and stress scale (DASS-21) to assess different areas of psychological function in NAFLD patients following eight weeks of pomegranate peel supplementation.

Methods

Study Design and Patients Selection

The present randomized clinical trials were carried out in 2022 on 76 NAFLD patients who participated based on the sampling process. The inclusion criteria were adults (18 to 60 years) admitted directly or transferred to a nutrition clinic diagnosed with hepatic steatosis with or without fibrosis via sonography, fibroscan, or elastography. The non-entry criteria were pregnancy and lactation, morbid obesity, consumption of alcohol (more than 20g for women or 30g for men), suffering or having a history of cancer and any liver or renal failure, or autoimmune disorders and HIV/AIDS, having a history of known food allergies to pomegranate or any herbal supplementation, receiving hepatotoxic medications, including sodium valproate, and a history of bariatric surgeries for weight loss. All participants provided informed written consent. The Ethics Committee of MUMS (Mashhad University of Medical Sciences), Mashhad, Iran, approved the trial (Ethic Number: IRCT20210726051988N1).

Animal studies regarding PP and fatty liver are not suitable for calculating the sample size of human studies. Thus, the sample size calculations were based on Soleimani et al. (19), using the formula for comparing two proportions of a qualitative attribute from two independent statistical societies. Thus, 32 individuals in each group (α= 0.05, β= 0.2, the power of the study is 80%) were determined. In this regard, 39 patients in each group were considered for probable drop out of samples.

General and Clinical Characteristics

Demographics and clinical characteristics, including age, smoking status, and medical history, were collected from each participant at the baseline by trained interviewers.

Randomization and Blinding

All participants were allocated to a placebo or intervention group by classification based on age (18 to 40 and 40 to 60 years old) and gender (male/female) using quadruple blocks. The other nutritionist was responsible for opening the sealed opaque envelope containing the patient's unique identification and the study group assignment in the nutrition cabinet. In addition, investigators, all study staff, clinical teams, and patients were masked to the study group allocation, and 39 patients participated in each study group. Moreover, the placebo and pomegranate peel capsules were the same regarding smell, shape, color, and other physical features. The placebo and pomegranate peel containers had the same labels with different codes, including A or B that only the study pharmacologist knew about until the end of analysis.

Dose Selection

In this study, the dose of pomegranate peel supplement, according to Wei X. et al. (20) and Wei X.L (21), showed the effectiveness of the supplement at a dose of 150mg/kg on the liver condition in rats. Based on the following formula (22) for converting animal doses to human doses without adverse effects, the optimum dose was determined at 1500mg.

$$Human\ eivalent\ dose\ (mg/kg) = \frac{\text{Animal dose (mg/kg)}}{\text{(Animal Km /Human Km)}}$$

Preparation of Pomegranate Peel and Placebo Capsules

The supplement was prepared in the Department of Pharmaceutical Sciences in Iranian Medicine, School of Persian and Complementary Medicine, Mashhad University of Medical Sciences, Mashhad, Iran. About 750Kg of pomegranates of one variety were purchased from the local market in Mashhad, Iran. Without chemical, physical, or microbial damage, pomegranates were washed and dried before being separated. Pomegranates were peeled in a clinical pharmacology laboratory. Afterward, they were dried in the shade and at controlled room temperature in an air-conditioned oven. Then, the dried powder prepare with a 50% hydro-ethanolic extract. The powder was mixed with ethanol (50 % v/v) and shaken at 37°C for 72-96 hours to separate the active ingredients from the pomegranate peel powder. Finally, after 96 hours, the obtained liquid was concentrated using a rotary-evaporator device and then dried using a freeze-dryer. Weighing the dry powder of the extract and measuring its yield were the next steps. Total phenol was measured by the Folin Miran method to standardize. Four capsules of 500 mg were used to provide a daily dose of 1500 mg of pomegranate peel capsules required for the study. Thus, 375mg of each capsule consists of dried extract and 125mg of microcrystalline cellulose (Avicel®). Therefore, the obtained extract was mixed with Avicel® at 66.6% PP extract and 33.3% Avicel. Finally, a capsule-filling machine will prepare pomegranate peel capsules for the intervention group. Avicel, food coloring, and special essence were used for the placebo group, which will be similar in color, scent, and texture to pomegranate peel extract. Capsules of this group will be filled with 500mg of placebo. The pharmacologist pours the capsules into a special medicine container to comply with blinding. Each group's contents were the same, named group A or B, and contained 120 placebo or intervention capsules. No one knew which jars had placebos or pomegranate peel capsules until the end of the study.

Intervention

The intervention group received 1500mg of pomegranate peel per day (two capsules after breakfast and two capsules after dinner) for eight weeks. The placebo group received 1500mg placebo daily (two capsules after breakfast and two capsules after dinner) for eight weeks. All participants received a 500kcal reduced diet with 53% carbohydrate, 30% fat, 17% protein, a Mediterranean diet, and physical activity advice. Follow-ups were conducted through messages each day and telephone each week.

Outcome Measurements

Elastography

In the present study, liver tissue was imaged with a two-dimensional elastography technique and using an Aixplorer supersonic device (Aixplorer; SuperSonic Imagine S.A., Aix-en-Provence, France) before and at the end of the study. Two-dimensional elastography is a new and non-invasive method with a very high inter- and intra-individual agreement in diagnosing fatty

liver. All participants were evaluated by a skilled radiologist using the SXC6-1 wide convex probe according to the manufacturer's instructions. All the evaluations were done in the intercostal spaces and after 4-6 hours of fasting while the patient was lying in an arched position and the patient's right arm was fully extended. According to the guidelines, reliable measurements are considered only if at least ten valid consecutive measurements of LSMS with the ratio of the interquartile range to the median (IQRLSM/Median LSM) less than 0.3 for each individual. The elastography results of liver tissue were reported as a median of LSMS in kilopascals. In addition, the supersonic device can evaluate the severity of hepatic steatosis by quantifying hepatic echogenicity through a hepatorenal ultrasound index. In this study, the numerical value of the hepatorenal ultrasound index is 1.49, 1.86, and 2.23, respectively, based on the analysis of Webb et al., and the ideal cut-off point for determining mild (grade one), moderate (grade two), and severe (grade three) steatosis are considered (23).

Anthropometric and Physical Activity Measurement

Height and weight were measured by a trained dietitian using a standard stadiometer and a clinical scale (SECA), respectively, before, in the middle, and at the end of the study.

The subjects' physical activity was assessed at the beginning and end of the study using the International Physical Activity Questionnaire (IPAQ), designed for people aged 18-65. The study population was classified into three groups using the MET calculation of each item. Patients whose total activity was less than 600 MET-minutes/week, between 600 and 3000, and above 3000 were considered low, moderate, and very active, respectively.

Dietary Intake

Participants were asked to complete a three-day food record at the first visit, including two weekdays and one weekend. A trained dietitian instructed the patients on writing the information on the amount and quality of the foods they consumed. The food records were evaluated and analyzed by Nutritionist IV software (Version 3.5.2, First Databank® Inc., Hearst Corp., San Bruno, CA, USA). Moreover, the total daily intake of calories and micro-and macro-nutrients was obtained. The three-day food record evaluation was repeated on the second visit and at the end of the study.

Depression Anxiety Stress Scales (DASS)

A valid 21-item questionnaire for evaluating mood status called depression anxiety stress scales (DASS) (24, 25) consists of three subscales, including seven questions. Each question is on a four-point (0-3) Likert scale to identify the severity of depression, anxiety, and stress. Lower scores represent a lower degree of negative mood and vice versa. Two classifications are reported, including no or minimal scores and some degree of mood disorder. Therefore, scales less than or equal to 9 indicate No, higher than 9 suggest some degree of depression, and less than or equal to 7 indicate No. Higher than 7 means anxiety, and less than or equal to 14. No, and higher than 14 suggests some stress.

Statistical Analysis

The data were analyzed by SPSS v13 (SPSS, Inc., Chicago, IL). The normality of variables was analyzed using the Shapiro test. Descriptive statistics, including mean and standard deviation (SD), were determined for all variables and expressed as mean ± SD for normally distributed variables and as the median and interquartile range (IQR) for non-normally distributed variables. In addition, categorical indices were demonstrated by number (%). Paired t-test was performed to determine changes in the group. The chi-square and independent sample t-test were applied to compare the numbers and percentages of categorical variables and means±SDs of continuous variables between groups. Finally, covariance (ANCOVA) was analyzed to control covariates. All analyses were considered bilateral, and a p-value of <0.05 was considered significant.

Results

Demographic and Clinical Characteristics of the Population

In this study, the average age of the participants was 43.1±8.6 years, which was 42.8±7.2 years in the pomegranate peel group and 43.3±10.13 years in the placebo group. No significant difference was observed (P=0.8). A total of 37 men (48.7%) and 39 women (51.3%) participated. Among the participants assigned to the pomegranate peel group, 2.6% were normal, 35.9% were overweight, and 61.5% were obese.

In the placebo group, 2.7, 59.5, and 37.8% of the participants were underweight, overweight, and obese, respectively. No statistically significant difference was observed between the two groups (P=0.15). In addition, the amount of physical activity of the studied subjects was classified and analyzed based on low (less than 600), medium (600 to 3000), and high (more than 3000), which were 48.7, 48.7, and 2.6%, respectively. The two groups had no statistically significant differences in physical activity (P=0.99). In addition, there were no significant differences in educational status, hyperlipidemia, hypertension, diabetes, and cardiovascular disease between the two groups (P>0.05). Table 1 presents the demographic and clinical characteristics of the study population.

Table 1. Demographic and clinical characteristics of study population

Variable		All	Pomegranate Peel	Placebo	P value
Age (years)		43.1 ± 8.6	42.8 ± 7.2	43.3 ± 10.1	0.81 *
Women		39 (51.3)	21 (53.8)	18 (48.6)	0.65
Type 2 diabetes		6 (7.9)	3 (7.7)	3 (8.1)	0.99
Cardiovascular disease		8 (10.5)	5 (12.8)	3 (8.1)	0.71
Hyperlipidemia		25 (32.9)	13 (33.3)	12 (32.4)	0.62
Hypertension		12 (15.8)	6 (15.4)	6 (16.2)	0.99
Smoking and others		11 (14.5)	5 (12.8)	6 (16.2)	0.67
Married		70 (92.1)	36 (92.3)	34 (91.89)	0.81
Job Status	Student	3 (4)	0 (0)	3 (8.3)	0.24
	Working	58 (77.3)	31 (79.5)	2 (75)	
	Retired	4 (5.3)	3 (7.7)	1 (2.8)	
	Housekeeper	10 (13.3)	5 (12.8)	5 (13.9)	
	Jobless	0 (0)	0 (0)	0 (0)	
Educational status	Primary	2 (2.6)	1 (2.6)	1 (2.7)	0.85
	Diploma	12 (15.8)	6 (15.4)	6 (16.2)	
	Associate degree	4 (5.3)	2 (5.1)	2 (5.4)	
	BSc or MSc	49 (64.5)	27 (69.2)	22 (59.5)	
	PhD or higher	8 (10.5)	3 (7.7)	5 (13.5)	
	Religious	1 (1.3)	0 (0)	1 (2.7)	
Physical Activity	Low	37 (48.7)	19 (48.7)	18 (48.6)	0.99
	Medium	37 (48.7)	19 (48.7)	18 (48.6)	
	High	2 (2.6)	1 (2.6)	1 (2.7)	
BMI Classification (kg/m²)	Underweight	1 (1.3)	0 (0)	1 (2.7)	0.15
	Normal	1 (1.3)	1 (2.6)	0 (0)	
	Overweight	36 (47.4)	14 (35.9)	22 (59.5)	
	Obese	38 (50)	24 (61.5)	14 (37.8)	

Data is presented as Mean ± SD or Number (percent).
P extracted of Chi Square except (*), which is obtained from 2 independent sample t test.
P < 0.05 is considered as statistical significant.

Table 2. Dietary macronutrient intake of two groups before and after the intervention.

Variable	Pomegranate peel		P-value*	Placebo		P-value*	P-value #
	Before	After		Before	After		
Energy (Kcal)	2463.74 ± 294.10	1820.51 ± 264.75	<0.001	2524.83 ± 318.82	1899.27 ± 301.46	<0.001	0.56
Carbohydrate (g/day)	374.5 ±52.6	341.5 ±61.1	0.001	352.2 ± 59.3	317.9 ± 61.4	0.01	0.92
Protein (g/day)	89 ± 24.1	82.6 ± 16.5	0.22	87.9 ± 18	82 ± 21.1	0.26	0.94
Fat (g/day)	73.8 ± 22	67.3 ± 19.7	0.20	71.09 ± 25.9	66.45 ± 23.3	0.38	0.80
Weight (kg)	89.56 ± 12.06	84.46 ± 12.91	<0.001	90 ± 14.18	89.38 ± 14.32	0.38	<0.001

Data is presented as Mean ± SD.
P * is extracted from Paired t test and P # is extracted from 2 independent sample t test.
P < 0.05 is considered as statistical significant.

Dietary Intake and Weight Changes Before and After Intervention

Table 2 presents the dietary macronutrient intake of the two groups before and after the intervention. Daily energy and carbohydrate intake in the placebo and PP groups decreased significantly before and after the study (P< 0.01). However, daily grams of protein and fat intake

did not change significantly before and after the study (P> 0.1). The Energy, grams of carbohydrate and protein, and fat intake did not change significantly between PP and placebo groups following the survey (P= 0.56, 0.92, 0.94, and 0.80, respectively). In addition, both groups lost weight, but the PP group's changes compared to the placebo group were statistically significant (P<0.001).

Elastography Changes Before and After Intervention

In this study, liver stiffness of the PP group decreased significantly during the study (P<0.001), but liver stiffness increased in the placebo group (P<0.001). In the PP group, liver stiffness changes significantly differed from the placebo group before and after adjusting potential covariates, including weight and physical activity (P<0.001). Table 3 shows the fatty liver status of the two groups before and after the intervention.

Table 3. Fatty liver status of two groups before and after the intervention.

Variable	Pomegranate peel		P-value*	Placebo		P-value*	P-value#	P-value&
	Before	After		Before	After			
Liver stiffness (KP)	5.43 ± 0.39	4.71 ± 0.47	<0.001	5.1 ± 0.56	5.24 ±0.55	<0.001	<0.001	<0.001
Hepatorenal sonography index	1.98 ± 0.25	1.66 ± 0.19	<0.001	1.82 ± 0.24	1.9 ± 0.27	0.03	<0.001	<0.001

Data is presented as Mean ± SD.
P * is extracted from Paired t test and P # is extracted from 2 independent sample t test.
P & is presented adjusted model by weight and physical activity, with ANCOVA.
P < 0.05 is considered as statistical significant.

In addition, the hepatorenal sonography index of the PP group decreased significantly during the study (P<0.001), but this index increased in the placebo group (P<0.001). The hepatorenal sonography index changes in the PP group were significantly different compared to the placebo group before and after adjusting potential covariates, including weight and physical activity (P<0.001).

Depression, Anxiety, and Stress Scale Before and After Intervention

In the present study, depression scores decreased in both groups, which were significant only in the PP group (P=0.02). Depression score changes significantly in the PP group in contrast to the placebo group during the study before and after adjustment of potential covariates (P=0.002, <0.001). Anxiety scores decreased in both groups, but these changes were insignificant in PP and Placebo groups (P=0.06 and 0.08, respectively). Anxiety score changes were insignificant between the two groups before and even after adjustment of potential covariates, including weight and physical activity (P= 0.1 and 0.12, respectively). Moreover, the reduction in stress score in the PP group was significant (P=0.01), but this reduction was not significant in the placebo group (P=0.1). Stress score changes were significant between the two study groups before and after adjusting potential covariates, including weight and physical activity (P=0.05). Table 4 presents the depression, anxiety, and stress scale of the two groups before and after the intervention.

Table 4. Depression, anxiety and stress scale of two groups before and after intervention.

Variable	Pomegranate peel		P-value*	Placebo		P-value*	P-value#	P-value&
	Before	After		Before	After			
Depression	11.1 + 1.1	9.8 + 0.8	0.02	10.9 + 1.2	10.5 + 0.9	0.1	0.002	<0.001
Anxiety	8.8 + 0.7	8.2 + 1.3	0.06	8.9 + 1.1	8.7 + 0.8	0.08	0.1	0.12
Stress	12.02 + 0.5	11.1 + 1.1	0.01	12 + 1.1	11.7 + 2.1	0.1	0.05	0.05

Data is presented as Mean ± SD.
P * is extracted from Paired t test and P # is extracted from 2 independent sample t test.
P & is presented adjusted model by weight and physical activity, with ANCOVA.
P < 0.05 is considered as statistical significant.

Discussion

This study aimed to evaluate the effects of eight-week PP supplementation on the psychological health of NAFLD patients. The study showed that weight loss was significantly different between PP and placebo groups, despite no significant differences in energy, carbohydrate, protein, or fat intake between the two groups. Liver stiffness

and hepatorenal sonography index decreased significantly following the supplementation in the PP group compared to the placebo group in the crude and adjusted model. In addition, DASS demonstrated that depression and stress symptoms decreased significantly in the PP group compared to the placebo group after intervention. However, the reduction in anxiety score was not significant between the two groups.

Metabolic syndrome and NAFLD are potential risk factors for depression and anxiety. The underlying mechanism remains unclear. However, lifestyle variables, including body mass index and hypertension, had a significant association with the severity of depression in NAFLD patients (26). Thus, NAFLD treatment and weight loss may benefit the management of NAFLD-related depression. Moreover, a study reported differences between genders in this regard. Women patients with NAFLD had about 44% more risk of depression (27). In the present study, reducing depression and stress following the intervention did not vary between men and women, and their response to treatment is probably the same. Another important point is that the results reported better responses to therapy in patients with reduced depression symptoms simultaneously. A 48-week lifestyle intervention study reported poor treatment response in NAFLD patients with and without major depressive disorders (28). Memory and self-efficacy disorders in depressive NAFLD patients were mentioned for their poor response to treatment. Therefore, treating depression in NAFLD patients with depressive symptoms simultaneously may cause a better prognosis. In 2020, a study reported an increased risk of NAFLD by 1.3-fold in patients with higher stress symptoms (29). Thus, stress management is important in preventing and treating NAFLD. In this regard, 8-week supplementation in our study reduced stress levels in the PP group and led to better respond to treatment. Increased hepatic disease mortality in patients with psychological distress (30) proved the importance of stress management in NAFLD treatment.

Multiple underlying mechanisms are responsible for the association between NAFLD and mental health disorders, including depression, stress, and anxiety. Hypothalamic-pituitary-adrenal axis dysregulation, obesity-related inflammation, insulin resistance, and gut microbiome dysbiosis are probable mechanisms (5). Hypothalamic-pituitary-adrenal axis leads to stress management via the regulation of glucocorticoids. Stress prolongation leads to hyperactivation of the sympathetic nervous system and the hypothalamic-pituitary-adrenal axis. In this situation, high levels and inappropriate timing of glucocorticoid secretions cause different metabolic disorders, including visceral fat accumulation, high lipid profile, and NAFLD (31, 32). Further, the activation of proinflammatory processes of the liver is promoted.

Pomegranate peel is a rich source of polyphenols with anti-inflammatory and antioxidant properties, which have benefits in controlling NAFLD-related depression and stress (33-35). The gut-liver axis causes an inappropriate inflammatory response by displacing some bacterial products. This action promotes positive regulation of pro-inflammatory pathways and the NADPH oxidase system (36). PP reduces inflammatory pathways in the onset and progression of fatty liver by suppressing the activation of the TLR4/NF-kβ pathway and its prebiotic properties by increasing the level of Bifidobacterium, reducing the firmicute ratio to Bacteroides bacteria, increasing mucin secretion, and gene expression of proteins responsible for tight junctions such as occludin, and improving the integrity of the mucosal barrier of the digestive system, preventing bacteria from settling in the epithelial layer of the gastrointestinal tract, reducing the level of bacterial lipopolysaccharides in the blood circulation and reducing the expression of the TLR4 gene. Thus, PP supplementation helps manage mental health, including depression, stress symptoms, and fatty liver status, by improving gut microbiome dysbiosis.

Moreover, as mentioned earlier, inflammation and oxidative stress are major underlying effects of NAFLD and mental disorders in these patients. The possible mechanism of the anti-inflammatory effects of pomegranate peel is related to its active phenolic compounds, such as hydrolyzable tannins and ellagic acid, which are effective in the expression of genes or the signaling pathways of inflammatory cascades in the body, especially in adipocytes, plays an important role in promoting steatosis and liver fibrosis (37). Under the influence of oxidative

stress and hypoxia, adipocytes secrete pro-inflammatory cytokines, increase insulin resistance, and subsequently increase the flow of fatty acids from adipocytes to the liver (38, 39). In total, these factors reduce liver and systemic inflammation. Thus, PP supplementation decreases proinflammatory processes and prevents mental disorders and stress onset.

The imbalance between free radicals and the body's antioxidant system causes oxidative stress, contributing to steatosis, liver fibrosis, and neuropsychological disorders. Free radicals reduce beta-oxidation of free fatty acids in hepatocytes by damaging the mitochondrial structure and causing insulin resistance by interfering with insulin signaling pathways and activating inflammatory cascades in the body and subsequently increasing the flow of free fatty acids to the liver (40, 41). Pomegranate peel prevents oxidative damage and destruction of lipids, proteins, and DNA, which improves the vicious cycle of oxidative stress in the body and reduces the expression of caspase 9 and 3 mRNA and TNF-α, and anti-apoptotic effects to reduce oxidative stress and free radical levels (41-45). Thus, pomegranate peel inhibits free radicals in the body with its direct impact and strengthens the body's antioxidant system by influencing the expression of related genes. As a result, PP can reduce underlying mechanisms of NAFLD-related depression, anxiety, and stress by improving obesity-related insulin resistance, gut microbiome dysbiosis, and oxidative stress. However, future studies should evaluate the tools behind treating mental disorders in NAFLD management. The present study had limitations. The golden standard of fatty liver determination and changes is a biopsy, but a safer and less expensive method (elastography) was used to avoid the potential risks of a biopsy. In addition, patients' diet adherence cannot be controlled in lifestyle-related studies. In this study, the weight loss slope in PP and placebo groups was significantly different, which should be investigated in future studies focusing on patients' adherence to weight loss programs. On the other hand, this study had strengths. The DASS questionnaire was to assess the depression, anxiety, and stress degree of patients with multilateral questions. Further, mental problems were investigated as one of NAFLD patients' important but less noticed symptoms. The results suggested that a natural, market-value and available supplement was clinically effective in reducing NAFLD's fatty liver stage and mental problems.

Conclusion

To conclude, our results shows that 8-week supplementation of pomegranate peel had ameliorative effects on the depression and stress symptoms of NAFLD patients.

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Conflicts of Interest

The authors declare that they have no conflict of interest

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