



Grape seed extract supplementation in non-alcoholic fatty liver disease

A randomized controlled trial

Parisa Ghanbari¹, Roghayeh Alboebadi¹, Hadi Bazayr^{2,3}, Davoud Raiesi⁴, Ahmad ZareJavid⁵, Mohammad Karim Azadbakht⁶, Mahdi Karimi¹, and Hamidreza Razmi¹

¹ Student Research Committee, Ahvaz Jundishapur University of Medical Sciences, Iran

² Department of Public Health, Sirjan School of Medical Sciences, Sirjan, Iran

³ Student Research Committee, Sirjan School of Medical Sciences, Sirjan, Iran

⁴ Department of Internal Medicine, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Iran

⁵ Nutrition and Metabolic Diseases Research Center, Clinical Sciences Research Institute, Ahvaz Jundishapur University of Medical Sciences, Iran

⁶ Department of Basic Sciences, Sirjan School of Medical Sciences, Sirjan, Iran

Abstract: *Background:* Despite rising non-alcoholic fatty liver disease (NAFLD) prevalence and its impact on liver health, there's a lack of studies on grape seed extract's (GSE) effect on oxidative stress and quality of life (QoL) in NAFLD patients. This study aims to fill this gap by the potential benefits of GSE in reducing oxidative stress and improving QoL. *Methods:* In this randomized clinical trial study, fifty patients with NAFLD were randomly assigned to receive either 2 tablets of GSE containing 250 mg of proanthocyanidins or placebo (25 participants in each group) for two months. QoL was evaluated using the SF-36 questionnaire, and oxidative stress variables (TAC, MDA, SOD, GPx, CAT, and IL-6) were measured at the beginning and end of the study. *Results:* Compared with the control group, the group supplemented with GSE experienced greater reductions in IL-6 and MDA (3.14 ± 1.43 pg/ml vs. 2.80 ± 0.31 pg/ml; 4.16 ± 2.09 μ M vs. 4.59 ± 1.19 μ M, p for all < 0.05), as well as greater increases in TAC, SOD, and GPx levels (0.18 ± 0.08 mM vs. -0.03 ± 0.09 mM; 10.5 ± 6.69 U/ml vs. 8.93 ± 1.63 U/ml; 14.7 ± 13.4 U/ml vs. 8.24 ± 3.03 U/ml, p for all < 0.05). Furthermore, the QoL questionnaire showed that physical limitations, general health, and total physical health were significantly improved in the GSE group compared with the placebo (17.0 ± 42.0 vs. -12.0 ± 37.5 ; 3.80 ± 14.8 vs. -3.92 ± 9.55 ; 5.08 ± 5.26 vs. -7.01 ± 13.7 , p for all < 0.05). *Conclusions:* GSE can be effective in improving oxidative stress and QoL in patients with NAFLD. More studies are needed to confirm the results of this study.

Keywords: non-alcoholic fatty liver disease, oxidative stress, quality of life, grape seed extract

Introduction

Nonalcoholic fatty liver disease (NAFLD) is characterized by fat accumulation in the liver and lack of other causes, such as chronic alcohol use or medication side effects [1]. According to studies, the prevalence of NAFLD in the world is approximately 32.4%, and in Asia, Iran has the highest rate with a 40% prevalence [2]. Because of the increasing prevalence of metabolic syndrome factors, NAFLD has become one of the main causes of chronic liver disease [3]. Oxidative stress, by disrupting mitochondrial oxidation and increasing the release of inflammatory cytokines, can increase lipid peroxidation, and liver inflammation induces the progression of NAFLD to non-alcoholic steatohepatitis, fibrosis, and cirrhosis [4]. Furthermore, in recent years, there has been a growing interest in better understanding the relationship between NAFLD and patient-reported outcomes such as quality of life (QoL). Studies have shown that

patients with NAFLD have lower health-related QoL and health utility scores than the general population [5]. The association between NAFLD and QoL is complex and multifactorial, with factors such as hepatic inflammation, obesity, type 2 diabetes, and dyslipidemia negatively correlated with QoL [6]. Furthermore, NAFLD patients may experience fatigue and depression, which can further impair their QoL [7]. Therefore, reducing oxidative stress is necessary for the prevention and treatment of patients with NAFLD and for improving their QoL. Grape seed extract (GSE) is a natural polyphenolic compound with antioxidant and anti-inflammatory properties [8, 9, 10]. GSE contains various beneficial compounds that are present in different parts of grapes and have a high antioxidant capacity [11]. Furthermore, GSE has been found to have an antioxidant effect that corrects experimental autoimmune encephalomyelitis behavioral dysfunctions, demyelination, and glial activation [12]. Therefore, in this study, the

hypothesis of a positive effect of GSE on NAFLD was investigated, in contrast to its lack of effect on NAFLD. On the other hand, regarding the key role of oxidative stress and inflammation in the development of NAFLD and the antioxidant and anti-inflammatory potential of GSE as well as the limited number of randomized clinical trial studies on the effect of GSE on QoL and oxidative stress, the present study was conducted with the aim of investigating the effect of GSE on oxidative stress and QoL in patients with NAFLD as a randomized clinical trial.

Material and methods

Study design and participants

The current study is a randomized double-blind placebo-controlled clinical trial that was conducted with the referral of 50 patients with NAFLD to the clinic of Shohadaye Hindijan Hospital from September 2022 to March 2023 (Figure 1). Ultrasound was used to diagnose NAFLD. According to the echogenicity of the liver, hepatorenal echogenicity, bright gallbladder, and vessel walls, hepatic steatosis is defined and divided into four categories: normal, mild, moderate, and severe [13]. Randomization of patients in each of the study groups (supplement or placebo) was done by “Random allocation software” using the stratified block random division method, and a sequentially numbered, opaque, sealed envelope technique was used for allocation concealment [14]. In this technique, each patient was assigned a sealed envelope containing a three-digit code (prepared by the software), which was coded by someone outside the study. The codes were recorded on cards and placed in envelopes in a pre-determined order. The envelope surfaces were numbered in the same order as the random sequence to maintain a consistent order. The envelopes were then placed in a box to be opened in the order of the arrival of eligible participants during the study registration. When opened, the patient received a can of supplement or placebo that corresponded to the 3-digit code on the envelope. The researcher, clinician, and participants were blinded to the treatment allocation. Patients in the intervention group received 263 mg tablets of Shari company’s GSE for 2 months, twice daily (morning and evening), and the placebo group took 2 tablets/day containing 263 mg cellulose, silicon dioxide, magnesium stearate, and starch, which are of similar color, shape, and size and produced by the faculty of pharmacy, Ahvaz Jundishapur University of Medical Sciences, Iran. Before initiating the intervention, informed consent was obtained from all study subjects. Grape (*Vitis vinifera*) seed extract, obtained through hydroalcoholic extraction by Shari Company in Iran, was used in this study. Each tablet contains 250 mg of proanthocyanidin. The

duration of the study and the dosage of GSE were established on the basis of prior studies [15, 16]. Once every two weeks, a phone call was made to check the patients’ tablet consumption. The current study was conducted in agreement with the ethics committee of Ahvaz Jundishapur University of Medical Sciences (IR.AJUMS.REC.1401.183) and was registered in the Iranian Registry of Clinical Trials (IRCT) as IRCT20190731044392N2.

Inclusion and exclusion criteria

Inclusion criteria

All patients with moderate to severe steatosis in the ultrasonography, willingness to participate in the research, age 20–60 years, no history of alcohol consumption, and BMI between 25 and 35 kg/m².

Exclusion criteria

Patients having diseases other than NAFLD (such as chronic liver disease, diabetes, kidney failure, thyroid, and anemia), consumption of food supplements, anti-inflammatory and immunosuppressive drugs, following special diets, smokers, pregnancy, and lactation.

Assessment of anthropometric indices, food intake, and physical activity

Body weight was measured with light clothes and using a Seca scale with an accuracy of 500 g, and height without shoes was measured with an accuracy of 0.5 cm. The BMI of the patients was calculated using the formula [weight (kg)/height² (m²)]. Waist circumference (WC) was measured at the smallest circumference between the edge of the last rib and the iliac crest, and hip circumference was measured with the largest diameter in the hip region with an accuracy of 0.5 cm while standing. WHR was calculated by dividing WC by HC. The assessment of anthropometric indices before and after the intervention was done using the same standardized tools and measured by an experienced nutritionist. In this study, to evaluate the diet of the patients in terms of calories, macronutrients, and micronutrients, at the beginning of the study and the end of the 2 months, a 3-day food intake recall (one day off and two non-off days) was completed through face-to-face interviews. The total frequency of consumption for each food item was calculated by summing up the number of times it was reported across the three days. To obtain the daily intake status, the total frequency of consumption was divided by three (as the data was collected over three days) to estimate the average daily intake. This process was repeated for each food item included in the questionnaire, allowing for the conversion of frequency data into daily intake status. After reviewing the questionnaires, the reported household

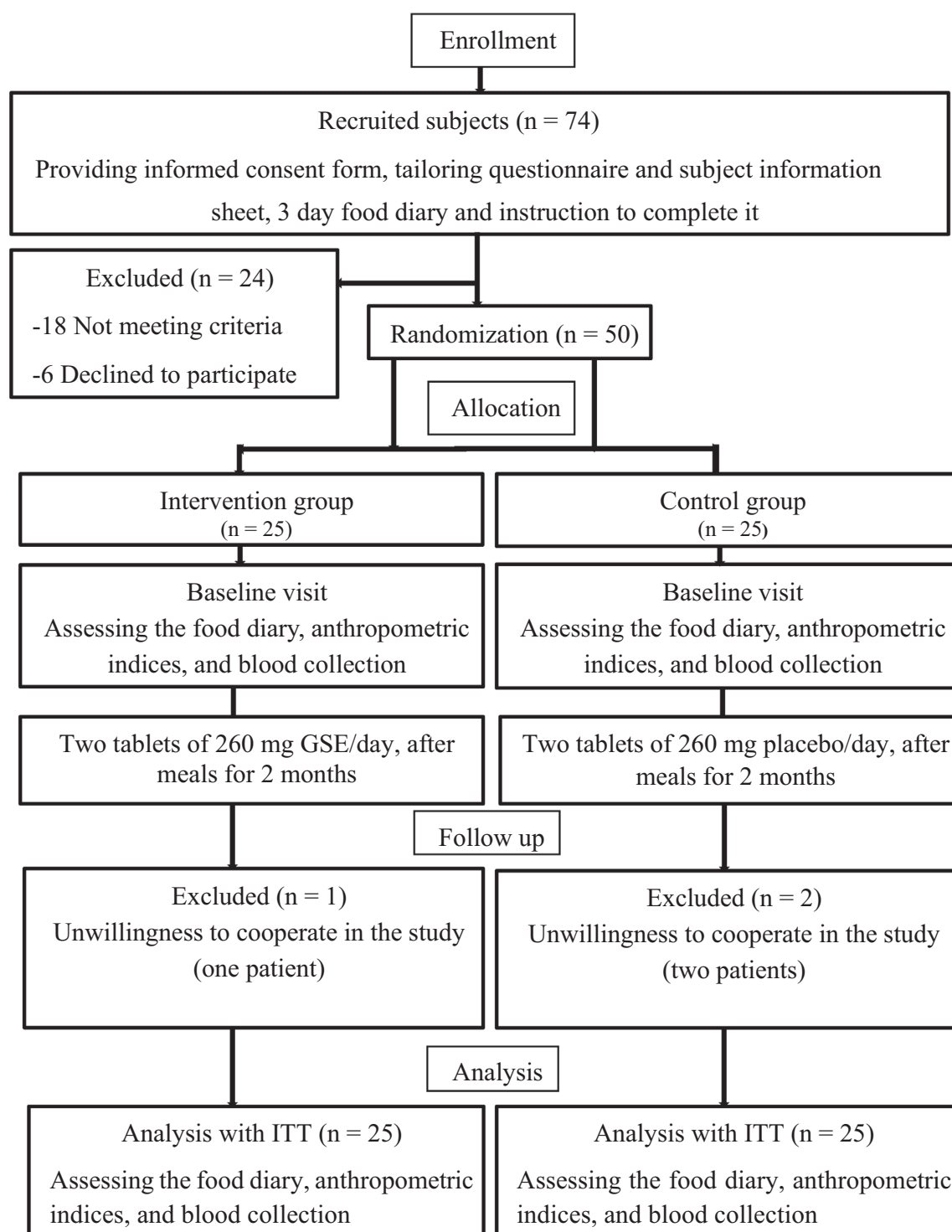


Figure 1. Stages of clinical trial progress.

quantities were converted to grams, coded, and entered into the N4 nutrition software (Nutritionist4). National food composition tables were used as a reference [17]. The 3-day food intake questionnaire is a practical and cost-effective approach that has been used to identify habitual dietary patterns in the Iranian population, such as “Western”,

“Healthy”, “Traditional”, and “Vegetarian” patterns [18]. Furthermore, in Iranian validation studies, the 3-day food intake questionnaire and 24-h recalls are commonly used as reference methods to assess dietary intake [19].

The physical activity of participants was measured at the beginning and end of the 2 months by completing the valid

and reliable International Physical Activity Questionnaire (IPAQ). IPAQ data were converted to MET-min/week according to the IPAQ scoring protocol [20]. In addition, the participants were asked not to change their physical activity level or diet during the study.

Quality of life (QoL) questionnaire

The SF-36 questionnaire was used to evaluate QoL. This tool has 36 questions and examines QoL in 8 domains of health, including physical performance, physical limitations, emotional limitations, energy and freshness, mental health, social performance, physical pain, and general health. Furthermore, the domains of physical pain, physical performance, general health, and physical limitations contribute to the total physical health score. Similarly, the domains of emotional limitations, energy and freshness, mental health, and social performance form the total mental health score. Each of the eight SF-36 domains is scored from zero to 100, with higher scores indicating better QoL [21]. The theoretical model of the sf-36 assumes that physical performance, physical limitations, physical pain, and general health domains strongly correlate with the physical component. In turn, the emotional limitations, energy and freshness, mental health, and social performance domains correlate more strongly with the mental component and its summary measure [22].

QoL data were collected at the beginning and end of the 2-month intervention period. The participants received thorough explanations of the content of the SF-36 questionnaire from an experienced expert. The expert then proceeded to ask questions to the patients. This was done to ensure that the participants understood the questionnaire well. In addition, the participants had the opportunity to seek clarification from the expert if needed.

Biochemical variables

After 10–12 hours of fasting, 5 cc of blood was taken from all participants at the beginning of the study and at the end of the 2 months. The samples were centrifuged for 10 minutes at room temperature at a speed of 5000×g to separate their serum. The separated serum was placed in 1.5-cc microtubes to measure the desired biochemical factors and kept in a freezer at −80°C until the time of the experiments. The total antioxidant capacity (TAC) was determined by enzyme-linked immunosorbent assay (ELISA) using commercial kits according to manufacturer's instructions (ZellBio GmbH, Germany). The assay sensitivity was 0.1 mM, and the diagnostic range was (0.12–2 mM). The final absorbance was read at 490 nm, and unit conversion was performed.

To measure malondialdehyde (MDA) concentration, an MDA assay kit (Zell Bio, Germany) was used according to

the manufacturer's protocol. This test is based on the reaction between MDA and thiobarbituric acid (TBA) at boiling temperature. The concentration of MDA (μmol) was calculated using a standard calibration curve. The absorbance of MDA was measured using an ELISA reader at a wavelength of 535 nm.

The activity of antioxidant enzymes [superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT)] in the serum was measured using commercially available assay kits (ZellBio GmbH, Germany, Cat No. ZB-96A) according to the manufacturer's instructions. The absorbances of SOD, GPx, and CAT were read with an ELISA reader at wavelengths of 420, 412, and 405 nm, respectively. IL-6 was evaluated using an ELISA according to the manufacturer's guidelines (Human IL-6 ELISA kit [LDN, Germany]). In summary, the desired sample is added to microplate wells containing immobilized primary Interleukin 6 (IL-6) antibodies. After incubation for 60 minutes, the plate was washed to remove any unbound reagents. An HRP substrate solution was then added for 15 minutes to produce a colorimetric reaction proportional to the bound IL-6 concentration. The color change of the chromogen is read at a wavelength of 450 nm.

Follow-up

During the study, the participants underwent monitoring at the 4th and 8th weeks to ensure that their progress was tracked effectively. To promote a healthy lifestyle, all participants were initially provided with guidance on adopting habits such as maintaining a nutritious diet and engaging in regular physical activity. In the fourth week, follow-up was conducted, during which participants were contacted to evaluate their adherence to the study protocol. Additionally, at the conclusion of the study, adherence was assessed by examining the number of remaining pills that participants had in their possession. Notably, participants who consumed less than 90% of the supplements were excluded from further analysis, ensuring the integrity and reliability of the study outcomes.

Sample size and statistical analysis

According to the Evans et al. [23], study and considering the 95% CI, 90% power and 25% loss to follow-up, the sample size was calculated 25 subjects in each group according to the following formula. SOD was considered as the main variable ($\sigma_1=0.07$, $\sigma_2=1.21$, $\mu_1=1.59$, $\mu_2=1.65$).

$$n = \frac{\left(Z1 - \frac{\alpha}{2} + Z1 - \beta\right)^2 (\sigma_1^2 + \sigma_2^2)}{(\mu_1 - \mu_2)^2}.$$

Statistical analysis of data was performed using SPSS software version 23. The normality of the distribution of all investigated variables was checked by the Kolmogorov-Smirnov test. The independent t-test was used to compare average data between two groups, and the Paired t-test were used for intergroup analysis. In cases where the data did not follow a normal distribution, appropriate non-parametric tests, such as the Mann-Whitney U test or the Wilcoxon test, were employed to compare average data between two groups or for intergroup analysis, respectively. The analysis of covariance (ANCOVA) test was used to adjust the results according to the baseline levels [24]. The chi-square test was used to compare qualitative variables between two groups. Also, intention-to-treat (ITT) method was used to compensate the missing subjects. In all statistical methods used, a significance level of $P < 0.05$ was considered. In the present study, SOD was considered as the primary outcome and other variables were considered as the secondary outcome.

Results

Participant characteristics

A total of 50 patients with NAFLD were included in the study, meeting the inclusion criteria. They were divided into two groups: the GSE group (15 women and 10 men) and the placebo group (11 women and 14 men). During the 2-month intervention period, one patient from the GSE group and two patients from the placebo group dropped out (unwillingness to cooperate in the study), resulting in 25 patients analyzed in each group using the intention-to-treat (ITT) method. No side effects were observed due to the consumption of GSE supplement or placebo during the intervention period.

Baseline characteristics

There were no significant differences in basic demographic and anthropometric characteristics between the two groups ($P \geq 0.05$). The average age was 44.8 ± 10.1 years in the control group and 43.52 ± 8.12 years in the intervention group. In the GSE group, 32% of the participants were overweight and 68% were obese, with an average BMI of $31.5 \pm 3.58 \text{ kg/m}^2$. The placebo group had 42% overweight and 58% obese participants, with an average BMI of $31.4 \pm 3.63 \text{ kg/m}^2$. The severity of hepatic steatosis and liver enzyme levels did not significantly differ between the two groups at baseline ($P \geq 0.05$) (Table 1).

Nutritional intake

The mean $\pm$ standard deviation of calorie intake, macronutrients, vitamin C, vitamin E, vitamin A, beta-carotene, alpha-tocopherol, and selenium are shown in Table 2. Intra-group and between-group tests did not show significant differences in calorie intake, macronutrients, and micronutrients ($P \geq 0.05$).

GSE and QoL

Physical component summary

Due to the abnormal distribution of QoL-related data, the Wilcoxon test was used to compare the intra-group and the Mann-Whitney U test was used to compare between groups. According to the supplementary table (ESM 1), there was no significant difference in the basic levels of the domains of QoL between the two groups ($P \geq 0.05$). The results of the present study showed that the scores of physical performance and total physical health in the control group were significantly reduced, while these reductions were not seen in the grape seed intervention group (55.0 ± 28.6 vs. 62.6 ± 31.8 , $P = 0.001$; 47.4 ± 22.2 vs. 54.4 ± 24.8 ; $P = 0.01$, respectively). The concepts of physical limitations and total physical health were significantly improved in the GSE group (Figure 2) (-12.0 ± 37.6 vs. 17.0 ± 42.0 , $P = 0.04$; 5.08 ± 16.2 vs. -7.01 ± 13.7 , $P = 0.001$, respectively).

Mental component summary

The results showed that emotional limitations, energy and freshness, and social performance domains had no significant differences compared with the baseline after two months of intervention ($P \geq 0.05$). However, mental health and total mental health scores significantly improved (73.0 ± 33.8 vs. 64.8 ± 12.9 , $P = 0.04$; 72.3 ± 20.3 vs. 67.0 ± 15.4 , $P = 0.002$, respectively). Furthermore, none of the domains related to mental component summary showed significant changes compared with the control group after two months of intervention ($P \geq 0.05$).

GSE and oxidative stress biomarkers

Lipid peroxidation

In the GSE-supplemented group, MDA levels were considerably lower than baseline values (15.5 ± 2.55 vs. $17.0 \pm 1.95 \text{ } \mu\text{M}$) after 2 months of GSE intervention. Additionally, both before and after adjustment, the mean change in MDA levels in the GSE group was notably lower than those in the placebo group ($-1.43 \pm 3.14 \text{ } \mu\text{M/L}$ vs. $0.31 \pm 2.80 \text{ } \mu\text{M/L}$, $P = 0.04$, $P = 0.03$, respectively).

Table 1. The characteristics of subjects at baseline

Variables	Control group (n=25)	Intervention group (n=25)	P-value*
Gender (n) (%)			0.25 ^a
Female	11 (44)	15 (68)	
Male	14 (56)	10 (32)	
Age (years)	44.8±10.1	43.5±8.12	0.60
Height (cm)	168±9.77	166±8.14	0.44
Weight (kg)	87.7±5.77	87.4±5.77	0.84
BMI (kg/m ²)	31.4±3.63	31.5±3.58	0.87
WC (cm)	108±9.21	110±6.38	0.25
HC (cm)	114±6.74	117±7.84	0.40
Race (n) (%)			0.75 ^a
Fars	19 (76)	21 (84)	
Lor	2 (8)	1 (4)	
Arab	4 (16)	3 (12)	
Education (n) (%)			0.13 ^a
Illiterate – elementary	6 (24)	1 (4)	
Middle – school	8 (32)	13 (52)	
High – school	3 (12)	5 (20)	
College	8 (32)	6 (24)	
Job (n) (%)			0.92 ^a
Unemployed			
Labor	9 (36)	8 (32)	
Housekeeper	11 (44)	11 (44)	
Employee	5 (20)	6 (24)	
Physical Activity (met-min/week)	354±169	334±130	0.63

Abbreviations: BMI: body mass index; WC: waist circumference; HC: hip circumference. Values are expressed as means±SD. P<0.05 was considered as significant. *P<0.05 was considered as significant using independent t-test between the two groups at baseline. ^aP<0.05 was considered as significant using Chi-square test.

Total antioxidant capacity

The difference in mean serum TAC levels between the GSE and placebo groups was significant both before and after adjustment (0.08±0.18 vs. −0.03±0.09 mM, P=0.005, P=0.001). Additionally, TAC in the intervention group with GSE from 0.48±0.08 mM reached 0.56±0.14 mM (P=0.02) (Table 2).

Antioxidant enzymes activity

Compared with baseline values, participants in the GSE group had significantly higher SOD (55.0±6.49 U/ml vs. 48.3±7.95 U/mL of serum) and GPx (119±24.04 U/ml vs. 106±20.1 U/mL of serum) activities. Furthermore, there was a significant increase in the level of SOD (6.69±10.5 U/ml vs. 6.69±10.5 U/ml, P<0.001) and GPx changes (13.4±14.7 U/ml vs. 3.03±8.24 U/ml, P=0.003) in the GSE group compared with the placebo group. These results were not seen in the CAT levels (P≥0.05).

IL-6

The level of IL-6 decreased significantly after 2 months of intervention with GSE (27.9±3.85 pg/ml vs. 25.2±3.60 pg/ml, P=0.01). In addition, the between-group results showed

that the levels of IL-6 in the intervention group with GSE improved significantly compared to the control group (−2.09±4.16 pg/ml vs. 1.19±4.59 pg/ml, P=0.01). After adjusting for baseline levels of IL-6, the difference between the two groups was significant, as presented in Table 3 (P=0.01).

Tolerance and side effects

There were no noticeable negative effects in either the GSE or placebo groups during the study. Additionally, several studies have suggested that GSE is well-received and safe [25]. During the use of GSE supplements or the placebo, no adverse effects were observed in the subjects.

Discussion

These findings indicate, for the first time, that GSE supplementation improved some physical components among patients with NAFLD, as determined by the SF-36 assessment. Moreover, GSE supplements demonstrated beneficial effects on markers related to inflammation and oxidative stress in patients with NAFLD.

Table 2. Mean±SD of energy, macronutrients, and micronutrients intake at baseline and post-intervention

Variables	Baseline (n=25)	Post-intervention (n=25)	P-value**
Energy (kcal/d)			
Control group	1995±161	2021±139	0.35
Intervention group	2061±172	2052±164	0.64
P-value*	0.17	0.72	
Carbohydrate (g/d)			
Control group	256±20.8	256±17.9	0.96
Intervention group	263±22.3	260±22.2	0.14
P-value*	0.25	0.60	
Protein (g/d)			
Control group	78.6±7.02	79.4±5.69	0.43
Intervention group	81.5±6.43	80.4±6.28	0.15
P-value*	0.12	0.57	
Fat (g/d)			
Control group	70.8±5.91	71.1±5.20	0.74
Intervention group	72.6±5.45	71.9±5.70	0.23
P-value*	0.26	0.49	
Cholesterol (g/d)			
Control group	167±26.1	163±21.6	0.73
Intervention group	136±24.1	125±34.3	0.81
P-value*			
Vitamin A (mcg/d)			
Control group	369±115	371±74.5	0.64
Intervention group	385±101	383±98.4	0.75
P-value*	0.19	0.35	
Beta-Carotene (mcg/d)			
Control group	4264±1631	4250±1132	0.89
Intervention group	4572±1738	4157±1147	0.37
P-value*	0.44	0.26	
Selenium (mcg /d)			
Control group	48.8±19.3	51.1±14.5	0.81
Intervention group	56.3±18.0	51.7±22.7	0.29
P-value*	0.16	0.90	
Vitamin C (mg/d)			
Control group	100±36.0	95.6±27.6	0.18
Intervention group	89.7±30.4	92.4±29.9	0.27
P-value*	0.28	0.45	
α-tocopherol (mg/d)			
Control group	7.25±1.70	7.37±1.41	0.29
Intervention group	6.70±1.90	7.55±2.21	0.12
P-value*	0.16	0.74	
Vitamin E (mg/d)			
Control group	2.32±0.67	2.27±0.55	0.11
Intervention group	2.26±0.61	2.25±0.52	0.16
P-value*	0.73	0.55	

*P<0.05 was considered as significant at baseline and significant post-intervention using independent t-test between two groups. **P<0.05 was considered as significant using paired t-test.

Studies indicate that NAFLD predominantly impacts QoL concerning physical health [26]. This study showed that GSE is helpful in improving functional limits, general health, and total physical health, similar to other studies

suggesting benefits of grapes and their products for brain function and all-round functioning [27]. Grape products, including freeze-dried grape powder, GSE, and grape juice, appear to improve brain function through antioxidant

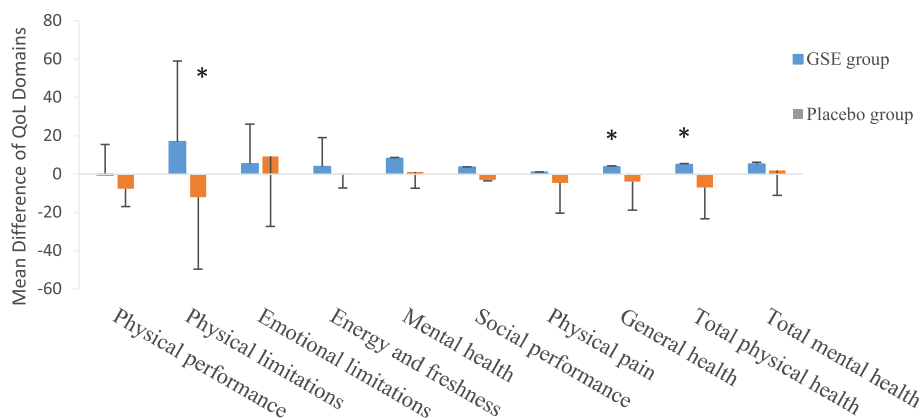


Figure 2. Comparison between the mean differences of QoL domains. Values are expressed as means $\pm$ SD. * $P < 0.05$ was considered as significant using Mann-Whitney U test.

stress, enhancement of trophic factors, reduction in inflammatory factors, and vascular functions [28, 29].

According to Liu Xianchu et al. [30], grape seed proanthocyanidin extract (GSPE) has a fatigue-reducing effect by enhancing antioxidant capacity and resisting oxidative stress. Furthermore, GSPE administration significantly suppressed the activity of inflammatory markers, such as tumor necrosis factor- α and interleukin-1 β , suggesting its potential protective role against fatigue induced by exhaustive exercise through the inhibition of the inflammatory response [30]. Thus, it appears that GSE is an ergogenic nutraceutical that enhances exercise performance [31]. Contrary to our study, in the study of O'Connor et al. [32], there was no effect of grape consumption on the different domains of QoL. In addition, in this study, factors related to physical performance, such as arm muscle inflammation, pain, function (isometric strength and range of motion), and VO₂max, did not change significantly. The effectiveness of grapes in improving physical function may be influenced by the type of intervention used because GSE contains more concentrated active compounds (proanthocyanidins) than whole grapes, thus producing greater effects. Furthermore, although the body can access the polyphenols in grape juice, these compounds may undergo transformations into inactive forms within the intestines or liver. Alternatively, they may interact with other grape constituents, resulting in diminished efficacy [33]. Additionally, our study aimed to investigate the effects of GSE supplementation on QoL related to physical health domains in patients with NAFLD, in contrast to a previous study that focused on recreationally active young adults. Prior research indicates that compared with healthy individuals, NAFLD patients have a lower QoL [7].

Our findings indicated 12% improvement in mental health and 7.8% improvement in total mental health after supplementation with GSE compared to baseline. Grape juices, both organic and conventional, modulate important markers in brain tissue, such as neurotrophic factor, which

could help prevent brain diseases [34]. In addition, supplementation with GSE has been shown to significantly alter the total mental health, social function, and energy/fatigue of patients with multiple sclerosis [27]. However, a randomized, double-blind, placebo-controlled trial by Bell et al. [35] showed that grape seed polyphenol extract intake did not appear to have a beneficial effect on cognitive functions in healthy adults. Proanthocyanidins have bioactivities such as antimicrobial, cardioprotective, and neuroprotective effects, which contribute to overall health improvement [36]. It is interesting to note that proanthocyanidin has prebiotic activities and may be effective in the microbiome-gut-brain axis by stimulating the growth of *Lactobacillus* and *Bifidobacterium* species as well as some butyrate-producing bacteria in the large intestine [37]. However, we did not find significant differences between the groups for the mental component summary after the intervention period. Mental health can vary significantly between individuals based on personality, life circumstances, and coping mechanisms [38, 39]. This variability may have obscured the smaller effects. In addition, disease-specific tools (multiple sclerosis quality of life-54) may be more sensitive to changes than general instruments (SF-36) in measuring mental health-related indicators.

Furthermore, our data showed that after 2 months of GSE supplementation (520 mg/day), the level of TAC increased by 16%, SOD by 13.8%, GPx by 12.7%, and MDA activities decreased by 8.4% and IL-6 by 7.6%. Studies have shown that patients with NAFLD have significantly lower serum TAC levels and higher levels of MDA compared to healthy controls [40].

GSE helps induce the expression of certain genes that control lipid oxidation in the liver, and PPAR β/δ is one of the genes that plays a role in this process [8]. PPAR β/δ shares several functions with PPAR α in terms of improving NAFLD by promoting fatty acid oxidation in various tissues [41]. Numerous animal studies have shown a decrease in MDA levels after supplementation with GSE [42, 43].

Table 3. Serum inflammatory and antioxidant factors at baseline and post-intervention

Variables	Intervention group (n=25)	Control group (n=25)	P-value**	P-value***	P-value****
IL-6 (pg/ml)					
Baseline	27.3±1.87	26.7±1.96	0.25		
After 2 months	25.2±3.60	27.9±3.85	0.01		
P-value*	0.01	0.20			
Difference	−2.09±4.16	1.19±4.59		0.01	0.01
TAC (mM)					
Baseline	0.48±0.08	0.50±0.08	0.28		
After 2 months	0.56±0.14	0.46±0.03	0.001		
P-value*	0.02	0.07			
Difference	0.08±0.18	−0.03±0.09		0.005	0.001
MDA (μM)					
Baseline	17.0±1.95	17.2±2.45	0.66		
After 2 months	15.5±2.55	17.5±2.23	0.004		
P-value*	0.03	0.57			
Difference	−1.43±3.14	0.31±2.80		0.04	0.003
SOD (U/ml)					
Baseline	48.3±7.95	50.5±7.99	0.35		
After 2 months	55.0±6.49	48.8±4.94	<0.001		
P-value*	0.004	0.36			
Difference	6.7±10.5	−1.63±8.93		0.004	<0.001
GPx (U/ml)					
Baseline	106±20.1	111±20.1	0.38		
After 2 months	119±24.0	114±15.6	0.34		
P-value*	<0.001	0.07			
Difference	13.5±14.8	3.03±8.24		0.003	0.003
CAT (U/ml)					
Baseline	8.37±1.20	8.61±1.21	0.48		
After 2 months	8.65±1.60	8.76±1.07	0.78		
P-value*	0.36	0.46			
Difference	0.28±1.56	0.15±1.04		0.72	0.94

Abbreviations. IL-6: interleukin 6; TAC: total antioxidant capacity; MDA: malondialdehyde; SOD: superoxide dismutase; GPx: glutathione peroxidase; CAT: catalase. Values are expressed as mean±SD. *P<0.05 was considered as significant using Paired t-test. **P<0.05 was considered as significant using independent t-test between the two groups at baseline and post-intervention. ***P<0.05 was considered as significant difference using independent t-test between the two groups post-intervention. ****P<0.05 was considered as significant using analysis of covariance (ANCOVA) between the two groups post-intervention after adjusting for confounding factors.

Hasona et al. demonstrated that GSE at a dose of 200 and 400 mg/kg reversal of dexamethasone-induced lipid peroxidation [44]. However, Taghizadeh et al. showed that GSE supplementation for 8 weeks increases GSH and decreases MDA, but has no significant effect on TAC and lipid profile [45]. Measuring TAC in serum provides a comprehensive overview of how the body's antioxidant defenses work together to counteract oxidative stress [46]. Our results corroborate the findings of the prior meta-analysis conducted by Khorasani et al. [9], which illustrated that polyphenol-rich grape products notably elevated serum TAC. Notably, to the best of our knowledge, no clinical trial had assessed the effects of GSE in reducing oxidative stress markers, including MDA, TAC, SOD, GPx, and CAT, in patients with NAFLD, making this study the pioneering work in this area to date. The findings

showed that GSE improved antioxidant enzymes (SOD, GPx) but had no significant effect on CAT. Evidence on the effect of GSE supplementation on antioxidant enzymes has shown inconsistent results. In a study conducted on healthy individuals, organic grape juices increased the activities of SOD by 16.3% and GPx by 7.3% [10]. Therefore, discrepancies between the present findings and a 2-month GSE supplementation trial in adults aged 30–65 years may potentially stem from variations in the flavonoid content of GSE and differences in dosages [47]. Proanthocyanidin, the main effective substance in our intervention, apart from its ability to reduce oxidative stress by suppressing MAPK pathway activity, has the potential to enhance antioxidant enzyme levels through the involvement of ERK, JNK, and p38 MAPK pathways [48]. However, CAT is an abundant and stable enzyme [49], so a stronger or longer antioxidant

intervention may be needed to see the effects on its expression/activity. The dose or duration of GSE supplementation may not have been sufficient to induce changes in CAT levels. Furthermore, Hokayem et al. [50], showed that the intake of grape polyphenols derived from red grape extract had no significant effect on the antioxidant enzymes GPx, SOD, CAT, and inflammatory factors such as IL-1 α , IL-1 β , IL-6, and IL-42. The differences in antioxidant capacity found in grape seeds [11, 51], as well as the sample size and the short intervention period of previous studies, could contribute to the variations in the results. Furthermore, our results have shown that after 8 weeks of GSE supplementation (526 mg/d), the serum concentration of IL-6, an important inflammatory marker [52], showed a significant decrease from 27.3 pg/ml to 25.2 pg/ml and was significantly different from the placebo group. In this study, diet and physical activity levels did not significantly change during the study period. Therefore, the observed effects are thought to be due specifically to GSE supplementation rather than other lifestyle factors. Additionally, numerous reports have advocated that GSE supplementation had a significant effect on inflammatory markers in many animal settings [53, 54].

Overall, there were some strengths and limitations to this study. This study's strength lies in its novelty, being the first to investigate the potential impact of GSE on the QoL and oxidative stress of NAFLD patients. Nevertheless, the limitations of this study, including the small sample size and duration, may have contributed to some unchanging factors. Additionally, while several potential confounding factors were controlled for through exclusion criteria and collecting basic demographic data, the authors acknowledge that other unmeasured factors could also influence QoL. Variables such as socioeconomic status, social support, lifestyle factors, and comorbidities that were not assessed could impact participants' QoL scores. Future studies would benefit from collecting a more comprehensive set of covariates to better account for potential confounders. Nonetheless, this preliminary study provides useful insights into the effects of GSE supplementation on QoL in NAFLD patients. More robust research controlling for additional variables is still warranted. Furthermore, it can be said that GSE can be effective in improving oxidative stress in patients with NAFLD. Therefore, GSE can be considered as an adjuvant treatment to improve the condition of NAFLD patients.

Electronic supplementary material

The following electronic supplementary material is available with this article at <https://doi.org/10.1024/0300-9831/a000805>

ESM 1. The quality of life parameters at baseline and post-intervention (Table E1).

References

- Milić S, Stimac D. Nonalcoholic fatty liver disease/steatohepatitis: epidemiology, pathogenesis, clinical presentation and treatment. *Digestive diseases (Basel, Switzerland)*. 2012;30(2): 158–62.
- Riazi K, Azhari H, Charette JH, Underwood FE, King JA, Afshar EE, et al. The prevalence and incidence of NAFLD worldwide: a systematic review and meta-analysis. *Lancet Gastroenterol Hepatol*. 2022;7(9):851–61.
- Han MAT, Yu Q, Tafesh Z, Pysopopoulos N. Diversity in NAFLD: a review of manifestations of nonalcoholic fatty liver disease in different ethnicities globally. *J Clin Transl Hepatol*. 2021;9(1): 71–80.
- Cichoż-Lach H, Michalak A. Oxidative stress as a crucial factor in liver diseases. *World J Gastroenterol*. 2014;20(25): 8082–91.
- Sayiner M, Stepanova M, Pham H, Noor B, Walters M, Younossi ZM. Assessment of health utilities and quality of life in patients with non-alcoholic fatty liver disease. *BMJ Open Gastroenterology*. 2016;3(1):e000106.
- Huber Y, Boyle M, Hallsworth K, Tiniakos D, Straub B, Labenz C, et al. Health-related quality of life in non-alcoholic fatty liver disease associates with hepatic inflammation. *Clin Gastroenterol Hepatol*. 2019;17(10):2085–92.
- Golubeva JA, Sheptulina AF, Yafarova AA, Mamutova EM, Kiselev AR, Drapkina OM. Reduced quality of life in patients with non-alcoholic fatty liver disease may be associated with depression and fatigue. *Healthcare (Basel)*. 2022;10(9):1699.
- Guisantes-Batan E, Mazuecos L, Rubio B, Pereira-Caro G, Moreno-Rojas JM, Andrés A, et al. Grape seed extract supplementation modulates hepatic lipid metabolism in rats. Implication of PPAR β / δ . *Food & Function*. 2022;13(21):11353–68.
- Sarkhosh-Khorasani S, Sangsefidi ZS, Hosseinzadeh M. The effect of grape products containing polyphenols on oxidative stress: a systematic review and meta-analysis of randomized clinical trials. *Nutr J*. 2021;20(1):25.
- Toaldo IM, Cruz FA, da Silva EL, Bordignon-Luiz MT. Acute consumption of organic and conventional tropical grape juices (*Vitis labrusca* L.) increases antioxidants in plasma and erythrocytes, but not glucose and uric acid levels, in healthy individuals. *Nutr Res*. 2016;36(8):808–17.
- Xia EQ, Deng GF, Guo YJ, Li HB. Biological activities of polyphenols from grapes. *Int J Mol Sci*. 2010;11(2):622–46.
- Mabrouk M, El Ayed M, Démosthènes A, Aissouni Y, Aouani E, Daulhac-Terrail L, et al. Antioxidant effect of grape seed extract corrects experimental autoimmune encephalomyelitis behavioral dysfunctions, demyelination, and glial activation. *Front Immunol*. 2022;13:960355.
- Ferraioli G, Soares Monteiro LB. Ultrasound-based techniques for the diagnosis of liver steatosis. *World J Gastroenterol*. 2019;25(40):6053–62.
- Doig GS, Simpson F. Randomization and allocation concealment: a practical guide for researchers. *J. Critical Care*. 2005;20(2):187–91.
- Odai T, Terauchi M, Kato K, Hirose A, Miyasaka N. Effects of grape seed proanthocyanidin extract on vascular endothelial function in participants with prehypertension: a randomized, double-blind, placebo-controlled study. *Nutrients*. 2019; 11(12):2844.

16. Mojiri-Forushani H, Hemmati A, Khanzadeh A, Zahedi A. Effectiveness of grape seed extract in patients with nonalcoholic fatty liver: a randomized double-blind clinical study. *Hepat Mon.* 2022;22(1):e132309.
17. Ghaffarpour M, Houshiar-Rad A, Kianfar H. The manual for household measures, cooking yields factors and edible portion of foods. Tehran: Nashre Olume Keshavarzy. 1999; 7(213):42–58.
18. Lesani A, Djafarian K, Akbarzade Z, Janbozorgi N, Shab-Bidar S. Meal-specific dietary patterns and their contribution to habitual dietary patterns in the Iranian population. *Br J Nutr.* 2022;1–10. <https://doi.org/10.1017/S0007114521005067>
19. Mohammadifard N, Sajjadi F, Maghroun M, Alikhasi H, Nilforoushzadeh F, Sarrafzadegan N. Validation of a simplified food frequency questionnaire for the assessment of dietary habits in Iranian adults: Isfahan Healthy Heart Program, Iran. *ARYA Atheroscler.* 2015;11(2):139–46.
20. Craig CL, Marshall AL, Sjöström M, Bauman AE, Booth ML, Ainsworth BE, et al. International physical activity questionnaire: 12-country reliability and validity. *Med Sci Sports Exerc.* 2003;35(8):1381–95.
21. Montazeri A, Goshtasebi A, Vahdaninia M, Gandek B. The Short Form Health Survey (SF-36): translation and validation study of the Iranian version. *Qual Life Res.* 2005;14(3):875–82.
22. Montazeri A, Goshtasebi A, Vahdaninia MS. The Short Form Health Survey (SF-36): Translation and validation study of the Iranian version. *Payesh (Health Monitor) Journal.* 2006;5(1): 875–82
23. Evans M, Wilson D, Guthrie N. A randomized, double-blind, placebo-controlled, pilot study to evaluate the effect of whole grape extract on antioxidant status and lipid profile. *J Funct Foods.* 2014;7:680–91.
24. Use CfMPfH. Guideline on adjustment for baseline covariates in clinical trials. London: European Medicines Agency; 2015.
25. Ray S, Bagchi D, Lim PM, Bagchi M, Gross SM, Kothari SC, et al. Acute and long-term safety evaluation of a novel IH636 grape seed proanthocyanidin extract. *Res Commun Mol Pathol Pharmacol.* 2001;109(3–4):165–97.
26. Vachliotis I, Goulas A, Papaioannidou P, Polyzos SA. Nonalcoholic fatty liver disease: lifestyle and quality of life. *Hormones (Athens).* 2022;21(1):41–9.
27. Siahpoosh A, Majdinasab N, Derakhshannezhad N, Khalili HR, Malayeri A. Effect of grape seed on quality of life in multiple sclerosis patients. *J Contemp Med Sci.* 2018;4(3):148–152.
28. Jiang C, Sakakibara E, Lin WJ, Wang J, Pasinetti GM, Salton SR. Grape-derived polyphenols produce antidepressant effects via VGF- and BDNF-dependent mechanisms. *Ann N Y Acad Sci.* 2019;1455(1):196–205.
29. Ashoori M, Soltani S, Kolahtouz-Mohammadi R, Moghtaderi F, Clayton Z, Abdollahi S. The effect of whole grape products on blood pressure and vascular function: A systematic review and meta-analysis of randomized controlled trials. *Nutr Metab Cardiovasc Dis.* 2023;33(10):1836–48.
30. Xianchu L, Ming L, Xiangbin L, Lan Z. Grape seed proanthocyanidin extract supplementation affects exhaustive exercise-induced fatigue in mice. *Food. Nutr Res.* 2018;62. <https://doi.org/10.29219/fnr.v62.1421>
31. Nho H, Kim K-A. Effects of grape seed extract supplementation on endothelial function and endurance performance in basketball players. *Int J Environ Res Public Health.* 2022; 19(21):14223.
32. O'Connor PJ, Carvalho AL, Freese EC, Cureton KJ. Grape consumption's effects on fitness, muscle injury, mood, and perceived health. *Int J Sport Nutr Exerc Metab.* 2013;23(1): 57–64.
33. Richards JC, Lonac MC, Johnson TK, Schweder MM, Bell C. Epigallocatechin-3-gallate increases maximal oxygen uptake in adult humans. *Med Sci Sports Exerc.* 2010;42(4):739–44.
34. Dani C, Andreazza AC, Gonçalves CA, Kapizinski F, Henriques JAP, Salvador M. Grape juice increases the BDNF levels but not alter the S100B levels in hippocampus and frontal cortex from male Wistar Rats. *An Acad Bras Cienc.* 2017;89(1):155–61.
35. Bell L, Whyte AR, Lampert DJ, Spencer JP, Butler LT, Williams CM. Grape seed polyphenol extract and cognitive function in healthy young adults: a randomised, placebo-controlled, parallel-groups acute-on-chronic trial. *Nutr Neurosci.* 2022;25(1):54–63.
36. de Araújo Rodrigues P, de Moraes SM, Pereira JF, de Assis ALC, Alves AA, Benjamin SR, et al. Neuroprotective effects of proanthocyanidins of grape seed extracts against oxidative stress and apoptosis induced by 6-hydroxydopamine in PC12 cells. *Conjecturas.* 2021;21(2):68–86.
37. Cires MJ, Wong X, Carrasco-Pozo C, Gotteland M. The Gastrointestinal tract as a key target organ for the health-promoting effects of dietary proanthocyanidins. *Front Nutr.* 2016;3:57.
38. Fossati A. Towards an approach to mental disorders based on individual differences. *World Psychiatry.* 2011;10(2):115–6.
39. Otten D, Tibubos AN, Schomerus G, Brähler E, Binder H, Kruse J, et al. Similarities and differences of mental health in women and men: a systematic review of findings in three large German cohorts. *Front Public Health.* 2021;9:553071.
40. Asghari S, Hamed-Shahraki S, Amirkhizi F. Systemic redox imbalance in patients with nonalcoholic fatty liver disease. *Eur J Clin Invest.* 2020;50(4):e13211.
41. Hong T, Chen Y, Li X, Lu Y. The role and mechanism of oxidative stress and nuclear receptors in the development of NAFLD. *Oxid Med Cell Longev.* 2021;2021:6889533.
42. Sano A, Uchida R, Saito M, Shioya N, Komori Y, Tho Y, et al. Beneficial effects of grape seed extract on malondialdehyde-modified LDL. *J Nutr Sci Vitaminol (Tokyo).* 2007;53(2):174–82.
43. Farahat MH, Abdallah FM, Ali HA, Hernandez-Santana A. Effect of dietary supplementation of grape seed extract on the growth performance, lipid profile, antioxidant status and immune response of broiler chickens. *Animal.* 2017;11(5): 771–7.
44. Hasona N, Morsi A. Grape seed extract alleviates dexamethasone-induced hyperlipidemia, lipid peroxidation, and hematological alteration in rats. *Indian J Clin Biochem.* 2019;34(2):213–8.
45. Taghizadeh M, Malekian E, Memarzadeh MR, Mohammadi AA, Asemi Z. Grape seed extract supplementation and the effects on the biomarkers of oxidative stress and metabolic profiles in female volleyball players: a randomized, double-blind, placebo-controlled clinical trial. *Iran Red Crescent Med J.* 2016;18(9):e31314.
46. Rodríguez-Carrizalez AD, Castellanos-González JA, Martínez-Romero EC, Miller-Arevillaga G, Pacheco-Moisés FP, Román-Pintos LM, et al. The effect of ubiquinone and combined antioxidant therapy on oxidative stress markers in non-proliferative diabetic retinopathy: A phase IIa, randomized, double-blind, and placebo-controlled study. *Redox Rep.* 2016;21(4):155–63.
47. Pourghassem-Gargari B, Abedini S, Babaei H, Aliasgarzadeh A, Pourabdollahi P. Effect of supplementation with grape seed (*Vitis vinifera*) extract on antioxidant status and lipid peroxidation in patient with type II diabetes. *J Med Plant Res.* 2011;5(10):2029–34.
48. Yang L, Xian D, Xiong X, Lai R, Song J, Zhong J. Proanthocyanidins against oxidative stress: from molecular mechanisms to clinical applications. *Biomed Res Int.* 2018;2018:8584136.

49. van Lith R, Ameer GA. Chapter Ten – Antioxidant Polymers as Biomaterial. In: Dziubla T, Butterfield DA, editors. *Oxidative Stress and Biomaterials* (pp. 251–296). Academic Press; 2016.
50. Hokayem M, Blond E, Vidal H, Lambert K, Meugnier E, Feillet-Coudray C, et al. Grape polyphenols prevent fructose-induced oxidative stress and insulin resistance in first-degree relatives of type 2 diabetic patients. *Diabetes Care*. 2013;36(6): 1454–61.
51. Busserolles J, Gueux E, Balasinska B, Piriou Y, Rock E, Rayssiguier Y, et al. In vivo antioxidant activity of procyanidin-rich extracts from grape seed and pine (*Pinus maritima*) bark in rats. *Int J Vitam Nutr Res*. 2006;76(1):22–7.
52. Gabay C. Interleukin-6 and chronic inflammation. *Arthritis Res Ther*. 2006;8(Suppl 2):S3.
53. Wang Q, Chen Y-y, Yang Z-c, Yuan H-j, Dong Y-w, Miao Q, et al. Grape seed extract attenuates demyelination in experimental autoimmune encephalomyelitis mice by inhibiting inflammatory response of immune cells. *Chin J Integr Med*. 2023;29(5): 394–404.
54. Giribabu N, Karim K, Kilari EK, Kassim NM, Salleh N. Anti-inflammatory, antiapoptotic and proproliferative effects of vitis vinifera seed ethanolic extract in the liver of streptozotocin-nicotinamide-induced type 2 diabetes in male rats. *Can J Diabetes*. 2018;42(2):138–49.

History

Received July 12, 2023

Accepted February 9, 2024

Published online February 29, 2024

Conflict of interest

The authors declare that there are no conflicts of interest.

Funding

The authors acknowledge the contribution of the patients and appreciate the financial support provided by the Student Research Committee, Ahvaz Jundishapur University of Medical Science (registration code: 01s25).

Hamidreza Razmi

Student Research Committee

Ahvaz Jundishapur University of Medical Sciences

Ahvaz, Iran

hamidrezarazmi95@gmail.com