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RESEARCH ARTICLE

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Hesperidin improves hepatic steatosis, hepatic enzymes, and metabolic and inflammatory parameters in patients with nonalcoholic fatty liver disease: A randomized, placebo-controlled, double-blind clinical trial

Makan Cheraghpour[1] | Hossein Imani[2] | Shahrzad Ommi[3] | Seyed Moayed Alavian[4] | Elahe Karimi-Shahrbabak[5] | Mehdi Hedayati[6] | Zahra Yari[7] | Azita Hekmatdoost[7,8]

[1] Cancer Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

[2] Department of Clinical Nutrition, School of Nutritional Sciences and Dietetics, Tehran University of Medical Sciences (TUMS), Tehran, Iran

[3] Department of Dietetics and Nutrition, Florida International University, Miami, Florida

[4] Baqiyatallah Research Center for Gastroenterology and Liver Diseases, Baqiyatallah University of Medical Sciences, Tehran, Iran

[5] Human Nutrition Department, McGill University, Montreal, Quebec, Canada

[6] Cellular and Molecular Endocrine Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[7] Department of Clinical Nutrition and Dietetics, Faculty of Nutrition and Food Technology, National Nutrition and Food Technology Research Institute, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[8] Division of Gastroenterology, BC Children's Hospital, Vancouver, British Columbia, Canada

Correspondence
Zahra Yari and Azita Hekmatdoost, Department of Clinical Nutrition and Dietetics, Faculty of Nutrition and Food Technology, National Nutrition and Food Technology Research Institute, Shahid Beheshti University of Medical Sciences, Tehran 1981619573, Iran.
Email: zahrayari_nut@yahoo.com; a_hekmat2000@yahoo.com

This study aimed to evaluate the effects of hesperidin on nonalcoholic fatty liver disease (NAFLD) characteristics. In this randomized, double-blind, controlled clinical trial, 50 NAFLD patients were supplemented with either 1-g hesperidin capsule or identical placebo capsule for 12 weeks. During the intervention, both groups were advised to follow healthy lifestyle habits including dietary and physical activity recommendations. At the end of the study, hesperidin supplementation, compared with placebo, was associated with a significant reduction in alanine aminotransferase (p = .005), γ-glutamyltransferase (p = .004), total cholesterol (p = .016), triglyceride (p = .049), hepatic steatosis (p = .041), high-sensitivity C-reactive protein (p = .029), tumor necrosis factor-α, and nuclear factor-κB (NF-κB). In conclusion, our results indicate that hesperidin supplementation accompanied with lifestyle modification is superior to lifestyle modification alone in management of NAFLD at least partially through inhibiting NF-κB activation and improving lipid profile. Further studies with higher dose of hesperidin are required to find the optimal dose.

KEYWORDS

hesperidin, inflammation, insulin resistance, liver enzymes, liver steatosis, nonalcoholic fatty liver disease

1 | INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is considered as a main cause of liver-related morbidity and mortality worldwide (Bellentani, 2017; Masarone, Federico, Abenavoli, Loguercio, & Persico, 2014). It represents a wide spectrum of liver disorders, which encompass from simple steatosis to Non-alcoholic Steatohepatitis (NASH), fibrosis, cirrhosis, and hepatocellular carcinoma (Adams, Sanderson, Lindor, & Angulo, 2005). Despite the high prevalence of NAFLD, no effective treatment has been proposed for it up to now. However, lifestyle modifications are considered as the main management strategy for patients with NAFLD (Mokhtari, Gibson, & Hekmatdoost, 2017; Promrat et al., 2010; Yari et al., 2016). One of the most important factors in pathogenesis of NAFLD is oxidative stress (Roskams et al., 2003). Markers and mediators of oxidative stress such as lipid peroxidation products and antioxidant enzyme activities have been proposed as successor indicators of NAFLD (Obika & Noguchi, 2011). Accordingly, previous studies have investigated the effect of antioxidant agents on different aspects of development and treatment of NAFLD (Arendt & Allard, 2011; Faghihzadeh, Adibi, Rafiei, & Hekmatdoost, 2014; Rahimlou, Yari, Hekmatdoost, Alavian, & Keshavarz, 2016; Sanyal et al., 2010). However, the findings of such interventions were controversial (Gonciarz et al., 2012; Rahimlou, Ahmadnia, & Hekmatdoost, 2015; Sanyal et al., 2010).

Flavonoids comprise a large group of polyphenolic compounds that arise naturally in plants (Gentile et al., 2018). Earlier studies have reported that these compounds have a wide variety of therapeutic properties such as anti-inflammatory, antioxidant, and antihypercholesterolemic activities (Elliot-Middleton, Kandaswami, & Theoharides, 2000). Hesperidin (3′,5,7-trihydroxy-4′-methoxy-flavanone-7-rhamnoglucoside) is an abundant flavonoid found in citrus fruits (Shahbazi et al., 2018). Up to now, many in vivo and epidemiologic studies have pointed to the pharmacological properties of hesperidin including its antioxidative, anti-inflammatory, antihyperglycemic, antihypercholesterolemic, and antitumor activity properties (Bok et al., 1999; Shahbazi et al., 2018).

Because oxidative stress and inflammation are implicated in the pathogenesis of NAFLD, and due to antioxidant and anti-inflammatory effects of hesperidin, we designed this placebo-controlled, double-blind, randomized clinical trial to evaluate the effects of hesperidin supplementation on insulin resistance, dyslipidemia, liver enzymes, inflammatory markers, hepatic steatosis, and fibrosis in patients with NAFLD.

2 | METHODS AND MATERIALS

2.1 | Subjects and study protocol

A total of 50 men and women with NAFLD grades 2 and 3 were recruited in this double-blind, randomized, placebo-controlled trial from three health clinics in Tehran, Iran. The selection of patients was based upon steatosis involving at least 35% of hepatocytes (controlled attenuation parameter [CAP] >263) on FibroScan examination. This trial was conducted in patients aged between 18 and 70 years, without any of the following exclusion criteria: (a) evidence of excessive alcohol consumption (>10 g/day); (b) serious clinically diagnosed diseases or history of liver cirrhosis, renal diseases, cardiovascular diseases (CVDs), cancers, and diabetes mellitus; (c) use of any approved therapies known to have potential benefit in NAFLD management (i.e., vitamin E, betaine, pioglitazone, rosiglitazone, pentoxifylline, and gemfibrozil); (d) history of weight loss of >5% of body weight or bariatric surgery within the past 6 months; and (e) pregnancy or breastfeeding.

Written informed consent was obtained from all participants prior to the study that allowed them to withdraw from the study at any time without penalty. The Ethical Committee of Shahid Beheshti University of Medical Sciences approved the study protocol. This trial was registered at ClinicalTrials.gov (registration number: NCT 03377140).

An investigator who had no clinical involvement in the trial numbered bottles containing supplements and placebos and assigned the participants to the trial groups in accordance with the randomization list. All researchers and participants were blinded throughout the study until the end of statistical analysis.

After the patient's eligibility was confirmed, they were randomly assigned into two groups (hesperidin and placebo) for 12 weeks. Randomization lists were computer generated by a statistician and given to the interviewer. Subjects, investigators, and staff were blind to the treatment assignment until the end of the study. The consort diagram of trial is presented in Figure 1. All data of anthropometric measurements, three 24-hr dietary recall, and laboratory tests were

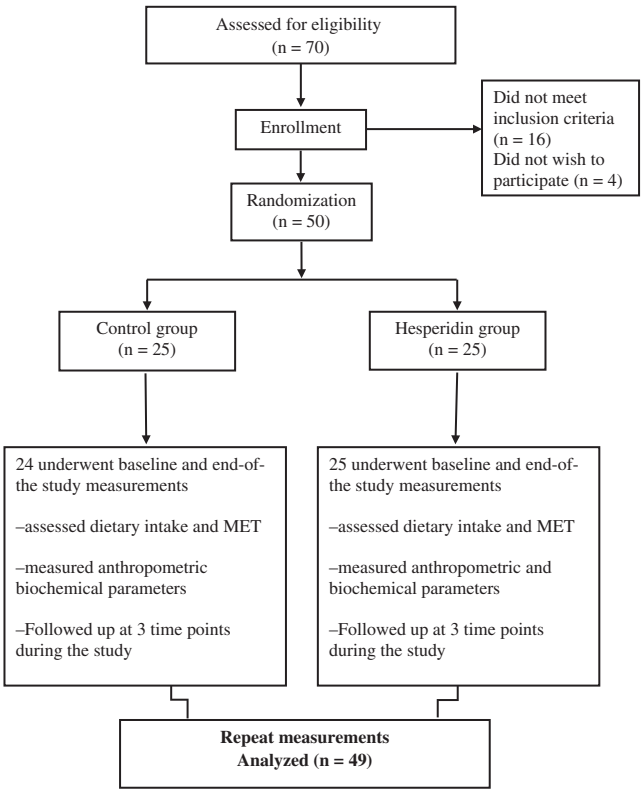

FIGURE 1 Flowchart depicting the study design

collected at baseline and after trial. Patients were provided with a 4-week supply of supplements. Follow-up assessments and monitoring the compliance were performed by the same nutritionist through telephone calls and clinic visits every 4 weeks following initiation of study. Patients were asked to return unused supplements at each follow-up visit to check adherence to the study protocol.

Both groups were advised to follow healthy lifestyle habits including dietary recommendations (based on the *Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults* from the National Institutes of Health [NIH] and the North American Association for the Study of Obesity; NIH, National Heart, Lung, and Blood Institute, North American Association for the Study of Obesity, 2000) and increased physical activity (more than 30 min of moderate intensity physical activity, three times per week). The intervention group was administered orally two capsules of hesperidin (each contains 500 mg), and the control group was given two capsules of placebo (starch) for 12 weeks. The capsules were similar in size and color. A loss of more than 10% of the capsules was considered incompliance and, therefore, excluded from the study.

2.2 | Sample size

The sample size was calculated for the FibroScan CAP score. Determination of the sample size for this study was based on detection of a 20-unit difference in the mean CAP score with a power of 80% (β = 20%), yielding a sample size of 21 for each group (Eslamparast et al., 2014). Due to the potential loss of samples, 25 patients in each group were considered.

2.3 | Anthropometric measurements, dietary intake assessment, and biochemical assays

A general questionnaire was completed during a personal interview for each participant. Height, weight, and waist and hip circumference were measured by an expert nutritionist to calculate body mass index and waist-to-hip ratio at baseline and after 12 weeks of treatment. Calibrated instruments were used.

All the participants of the study completed a 3-day food recall (two weekdays and one weekend) at the beginning and at the end of the study. Dietary intakes were then analyzed using Nutritionist 4 (First Data Bank), incorporating the use of food scales and models to enhance portion size accuracy. The validated semiquantitative questionnaire based on metabolic equivalent of tasks (MET) values was used to assess physical activity at first and the end of Week 12 (Kelishadi et al., 2001).

Venous blood samples were obtained after 12 hr of fasting for measuring glucose homeostasis parameters, lipid profiles, and inflammatory biomarkers. Liver enzyme concentration was measured using enzymatic colorimetric assay for γ-glutamyltransferase, and photometric assay for alanine aminotransferase (ALT) and aspartate aminotransferase (Parsazmoun, Tehran, Iran). Lipid profiles including triglyceride (TG), total cholesterol, low-density lipoprotein (LDL), and high-density lipoprotein were assessed by enzymatic photometric method, using Pars Azmoon test kits (Parsazmoun).

GOD/POD method was used to measuring fasting blood glucose. Fasting insulin concentrations were measured by ELISA (Monobind Inc., Lake Forest, CA). High-sensitivity C-reactive protein (hs-CRP) concentration was measured using ELISA kit (Zellbio, Germany). Serum tumor necrosis factor-α (TNF-α) concentration was measured using a commercial ELISA kit (Diaclone, Inc., Besançon, France). Nuclear factor-κB (NF-κB) p65 was measured in peripheral blood mononuclear cell nuclear extracts by using an ELISA kit (Zellbio) according to the manufacturer's protocol. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the following formulas (Matthews et al., 1985): HOMA − IR = fasting glucose (mg/dl) × fasting insulin (IU/ml)/405.

2.4 | FibroScan evaluation

An expert hepatologist assessed liver steatosis and fibrosis before and after the trial using a transient elastography (FibroScan; Echosens, Paris, France). The CAP above 261 (grade $\geq$ 2) was used as an inclusion criterion.

Fibrosis is classified on a 0 to 4 scale: F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis and a few septa; F3, numerous septa without cirrhosis; and F4, cirrhosis. The categorization of steatosis is based on visual assessment of fat deposition in hepatocytes as S0 (nonsignificant, <5%), S1 (mild, 5–33%), S2 (moderate, 34–66%), and S3 (severe, >66%; Brunt, Janney, Di Bisceglie, Neuschwander-Tetri, & Bacon, 1999; Kleiner et al., 2005).

2.5 | Primary and secondary end points

Liver steatosis reduction was a primary outcome. Besides hepatic enzymes, improvement of metabolic factors including anthropometric measures, glucose homeostasis factors, lipid profiles, and serum concentrations of inflammatory factor were considered as secondary outcomes.

2.6 | Statistical analysis

Data were analyzed using SPSS software (Version 19; SPSS Inc., Chicago, IL). Statistical significance was set at $p < .05$, based on two-sided tests. Normal distribution of all variables was determined with Kolmogorov–Smirnov test. Results of continuous data were presented as means ± *SD*, and categorical findings were shown as frequency.

Student's unpaired *t* test was used to compare the normally distributed pretreatment and posttreatment variables between groups. For within-group comparisons, before and after the dietary intervention, paired *t* test was used.

Eliminating the effects of confounding factors, either in the beginning or during the study, was made using the analysis of covariance test, adjusted for the baseline value of each variable and mean change in body mass index, waist-to-hip ratio, MET, energy intake, and serum concentration of ALT.

3 | RESULTS

From June 2017 to February 2018, 50 patients were recruited and randomly assigned to receive either hesperidin (n = 25) or placebo (n = 25). Only one patient in the placebo group discontinued the study due to personal reasons (Figure 1). We did not find any significant difference between the rates of dropout between the two groups. The overall compliance rate was estimated to be 98% during the 3-month supplementation period. The baseline demographic and metabolic data of both groups are summarized in Table 1. None of the variables were significantly different between the two groups, except for ALT, which was significantly higher in the control group compared with the hesperidin group.

As it is shown in Table 2, at the end of 12 weeks of intervention, all of the inflammatory markers decreased in the hesperidin group significantly more than the placebo group.

Blood glucose and insulin decreased significantly in the hesperidin group (p = .001); however, the changes were not significantly different between the two groups.

There were a significant reduction in serum concentration of TG (p = .003), total cholesterol (p = .017), and LDL cholesterol (p = .005) in the hesperidin group; however, only the reduction in serum level

TABLE 1 Baseline characteristics at enrollment

Characteristic	Total (n = 49)	Hesperidin group (n = 25)	Control group (n = 24)	p value
Age (years)	47.30 ± 12.73	47.32 ± 11.66	47.29 ± 13.76	.995
Sex (M/F)	22/25	10/13	12/12	.772
Smoking				
Yes	8	5	3	.432
No	39	18	21	
Metabolic characteristics				
Height (cm)	165.47 ± 10.14	167.47 ± 8.56	163.88 ± 13.64	.252
Weight (kg)	85.80 ± 12.98	82.61 ± 11.67	88.34 ± 13.63	.153
BMI (kg/m^2)	32.43 ± 5.09	31.70 ± 5.21	33.00 ± 5.03	.412
WHR	0.92 ± 0.05	0.93 ± 0.04	0.91 ± 0.06	.277
MET (hr/day)	32.71 ± 5.40	32.83 ± 4.42	32.62 ± 6.17	.901
Energy (kcal)	2,338.80 ± 492.44	2,408.25 ± 584.99	2,304.08 ± 448.96	.557
Serum biochemistry tests				
ALT (U/L)	26.69 ± 11.38	22.80 ± 10.29	30.95 ± 11.21	.016
AST (U/L)	18.83 ± 5.19	17.06 ± 5.44	20.77 ± 4.23	.054
GGT (U/L)	28.45 ± 13.23	26.47 ± 14.22	30.61 ± 12.04	.307
FBS (mg/dl)	103.99 ± 17.91	107.74 ± 17.87	99.46 ± 17.35	.138
Insulin (mU/L)	13.52 ± 6.67	12.05 ± 4.72	15.31 ± 8.23	.116
HOMA-IR	3.53 ± 1.92	3.29 ± 1.60	3.81 ± 2.27	.402
Triglyceride (mg/dl)	165.14 ± 71.89	175.96 ± 86. 94	152.05 ± 46.93	.289
Total cholesterol	188.54 ± 43.27	185.91 ± 50.16	191.74 ± 34.22	.670
LDL-C (mg/dl)	124.79 ± 37.26	127.78 ± 39.75	121.17 ± 34.74	.574
HDL-C (mg/dl)	35.44 ± 9.76	36.94 ± 11.72	33.63 ± 6.56	.279
Inflammatory factors				
hs-CRP (ng/dl)	3,873.36 ± 2,857.87	4,380.17 ± 2,458.58	3,318.29 ± 3,208.11	.222
TNF-α (pg/ml)	25.08 ± 11.85	28.01 ± 14.97	21.54 ± 4.67	.078
NF-κB (ng/mg protein)	2.63 ± 2.97	2.92 ± 4.15	2.39 ± 1.01	.506
Liver histology (transient elastography)[a]				
Fibrosis score (kPa)	6.50 ± 2.32	5.93 ± 2.00	6.96 ± 2.49	.152
Steatosis score (CAP)	309.30 ± 34.79	309.58 ± 34.22	309.08 ± 35.98	.964

Note. Mean ± *SD* (all such values).

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CAP, controlled attenuation parameter; FBS, fasting blood sugar; GGT, γ-glutamyltransferase; HDL-C, high-density lipoprotein cholesterol; hs-CRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; MET, metabolic equivalent of tasks; NF-κB, nuclear factor-κB; TNF-α, tumor necrosis factor-α; WHR, waist-to-hip ratio.

[a]FibroScan (Echosens).

TABLE 2 Mean changes [95% confidence interval] from baseline in metabolic characteristics by treatment group

Change from baseline	Hesperidin group (n = 25)	Control group (n = 24)	p value[a]
ALT (U/L)	−8.22 [−11.55, −4.90]	−7.91 [−14.94, −0.88]	.005
AST (U/L)	0.84 [−1.77, 3.44]	−2.02 [−5.31, 1.26]	.678
GGT (U/L)	−9.94 [−13.46, −6.42]	1.03 [−4.76, 6.82]	.004
FBS (mg/dl)	−9.11 [−13.94, −4.28]	−4.80 [−9.07, −0.53]	.216
Insulin (mU/L)	−2.27 [−3.38, −1.14]	−3.21 [−5.17, −1.26]	.313
HOMA-IR	−0.82 [−1.14, −0.50]	−0.90 [−1.45, −0.35]	.164
Triglyceride (mg/dl)	−23.27 [−58.14, 11.58]	15.37 [−6.95, 37.69]	.049
Total cholesterol (mg/dl)	−13.76 [−24.67, −2.84]	−2.94 [−15.19, 9.29]	.016
LDL-C (mg/dl)	−18.81 [−30.98, −6.63]	−5.72 [−17.81, 6.37]	.157
HDL-C (mg/dl)	1.52 [−4.26, 7.31]	0.96 [−0.46, 2.39]	.555
hs-CRP (ng/dl)	−968.72 [−1,791.82, −145.62]	137.36 [−1,489.94, 1,764.67]	.029
TNF-α (pg/ml)	−8.52 [−12.60, −4.44]	−2.17 [−3.65, −0.68]	.020
NF-κB (ng/mg protein)	−0.81 [−2.18, 0.55]	0.69 [0.17, 1.22]	.037
Fibrosis score (kPa)	−0.62 [−1.38, 0.14]	−0.66 [−1.22, −0.09]	.790
Steatosis score (CAP)	−51.27 [−76.28, −26.27]	−31 [−45.12, −16.87]	.041

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CAP, controlled attenuation parameter; FBS, fasting blood sugar; GGT, γ-glutamyltransferase; HDL-C, high-density lipoprotein cholesterol; hs-CRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; NF-κB, nuclear factor-κB; TNF-α, tumor necrosis factor-α.

[a]Based on an analysis of covariance model that regressed changes from baseline on treatment group, baseline value of the outcome, age, sex, and mean changes in body mass index, MET, and energy.

of TG and total cholesterol was significantly higher in the hesperidin group compared with the placebo group (p = .049 and p = .016, respectively).

Reduction of hepatic enzymes ALT and γ-glutamyltransferase during the study was significantly higher in the hesperidin group compared with the placebo group (p = .005 and p = .004, respectively). The results of the FibroScan indicated that hepatic steatosis and fibrosis decreased in both groups whereas only the reduction in hepatic steatosis was significantly higher in the hesperidin group compared with the placebo group (p = .058).

Dietary intake and physical activity showed no significant differences between the two groups during the study as is shown in Table 3. No participant complained of any side effects during taking hesperidin or placebo for 12 weeks.

4 | DISCUSSION

To the best of our knowledge, this study is the first randomized, double-blind, placebo-controlled clinical trial designed to evaluate the possible efficacy of hesperidin supplementation in management of NAFLD. We have also clarified some of the mechanisms that underlie observed beneficial effects. The results of the present study showed that coadministration of hesperidin with lifestyle modification improves NAFLD-related risk factors at least partially through inhibiting NF-κB activation and improving lipid profile and insulin sensitivity.

Up to now, few clinical trials have studied the effect of hesperidin supplementation on insulin resistance, dyslipidemia, inflammation, and other risk factors involved in NAFLD development. Dyslipidemia is considered as one of the significant risk factors contributing to CVD (Garber, 2002). Patients with NAFLD also exhibit a higher mortality rate of CVD than the general population (Lonardo et al., 2016). Therefore, NAFLD can be associated with an increased risk of CVD, the leading cause of death in the world (Garber, 2002). Therefore, hesperidin in modifying lipid profiles can be effective as a complementary treatment for both fatty liver and CVDs.

Intestinal cholesterol absorption and endogenous cholesterol synthesis in the liver are two main physiological pathways to determine serum cholesterol concentration (Brown, 2002). Patients with NAFLD experience an imbalance between cholesterol absorption and endogen synthesis of cholesterol, which manifests as lower absorption and higher hepatic cholesterol synthesis (Simonen et al., 2011). In present study, daily intake of 1 g of hesperidin significantly reduced serum levels of total cholesterol and TG. These results are inconsistent with a previous study that has shown that supplementation with 800-mg hesperidin for 4 weeks did not affect lipid profile in moderately hypercholesterolemic men and women (Demonty<!——> et al., 2010). Moreover, Haidari et al. (2015) reported that 600 mg of hesperidin per day for 4 weeks significantly improved only high-density lipoprotein in myocardial infarction patients. It seems that these studies did not observe a significant improvement in lipid profiles due to low dose and short duration of the studies. Hesperidin suppresses Hydroxy methylglutaryl-Coenzyme A (HMG-CoA) reductase (a key enzyme in cholesterol synthesis), acyl-CoA cholesterol acyltransferase (a key

TABLE 3 Mean ± *SD* in dietary components

Variable	Time of study		*p* value[a]
	Baseline	Week 12	
Energy (kcal/day)			
Hesperidin group	2,408.25 ± 584.99	2,078.20 ± 480.16	<.001
Control group	2,304.08 ± 448.96	1,959.30 ± 464.34	.003
p value[b]	.557	.494	
Protein (g/day)			
Hesperidin group	86.23 ± 21.66	86.42 ± 15.67	.955
Control group	83.95 ± 22.28	86.69 ± 16.31	.728
p value[b]	.772	.964	
Total fat (g/day)			
Hesperidin group	92.22 ± 20.94	77.82 ± 15.64	.001
Control group	85.14 ± 19.23	67.94 ± 33.02	.025
p value[b]	.319	.341	
Saturated fatty acids (g/day)			
Hesperidin group	20.92 ± 6.95	18.70 ± 6.41	.058
Control group	24.83 ± 9.36	21.50 ± 10.01	.257
p value[b]	.209	.394	
Monounsaturated fatty acids (g/day)			
Hesperidin group	27.20 ± 9.98	29.28 ± 6.86	.255
Control group	28.18 ± 6.62	22.50 ± 15.76	.161
p value[b]	.725	.170	
n-3 Polyunsaturated fatty acids (g/day)			
Hesperidin group	0.95 ± 0.32	1.03 ± 0.15	.422
Control group	1.40 ± 1.24	1.39 ± 0.33	.988
p value[b]	.230	.407	
n–6 polyunsaturated fatty acids (g/day)			
Hesperidin group	7.32 ± 2.49	8.45 ± 1.511	.055
Control group	9.54 ± 2.45	6.05 ± 2.45	.031
p value[b]	.880	.119	
Cholesterol (mg/day)			
Hesperidin group	240.07 ± 85.29	207.11 ± 72.65	.063
Control group	231.78 ± 131.33	183.58 ± 119.94	.091
p value[b]	.844	.545	
Carbohydrate (g/day)			
Hesperidin group	303.97 ± 97.47	257.23 ± 82.01	.001
Control group	313.05 ± 92.78	242.10 ± 61.36	.001
p value[b]	.787	.556	
Dietary fiber (g/day)			
Hesperidin group	19.71 ± 7.25	21.63 ± 5.52	.054
Control group	24.78 ± 9.26	22.27 ± 8.94	.177
p value[b]	.107	.826	
Vitamin C (mg/day)			
Hesperidin group	87.70 ± 59.26	80.37 ± 60.44	.563
Control group	93.26 ± 39.00	98.10 ± 35.77	.441
p value[b]	.771	.365	
Vitamin E (mg/day)			
Hesperidin group	19.21 ± 6.81	19.23 ± 4.74	.989
Control group	17.33 ± 5.46	14.61 ± 5.31	.051
p value[b]	.377	.670	
Zinc (mg/day)			
Hesperidin group	11.26 ± 4.07	12.45 ± 4.02	.054
Control group	12.28 ± 4.45	11.70 ± 3.52	.441
p value[b]	.510	.585	
Selenium (μg/day)			
Hesperidin group	126.66 ± 55.62	134.58 ± 46.34	.354
Control group	116.32 ± 30.73	106.79 ± 33.85	.396
p value[b]	.475	.087	

[a]Paired *t* test.

[b]Student's *t* test.

cholesterol-regulation enzyme that participates in cholesterol absorption), and sterol-COA desaturase (a key enzyme in lipid synthesis; Nichols, Jackson, Manthey, Shukla, & Holland, 2011; Suckling & Stange, 1985). Moreover, it induces the expression of LDL receptor mRNA and can stimulate biliary cholesterol excretion.

The results of our intervention revealed that 12 weeks of supplementation with 1-g hesperidin improved blood glucose significantly; however, the reduction was not significantly different between groups. These findings are in line with previous study by Homayouni, Haidari, Hedayati, Zakerkish, and Ahmadi (2017); they did not find any significant reduction in blood glucose and insulin resistance after 6 weeks of supplementation with 600-mg hesperidin in type 2 diabetic patients. Experimental studies reported that hesperidin can exert beneficial and therapeutic effects on fatty liver due to several mechanisms including reducing serum glucose level, hepatic TG level, and fatty acid oxidation (Jung, Lee, Park, Kang, & Choi, 2006). Ahmed, Mahmoud, Abdel-Moneim, and Ashour (2012) have shown the antidiabetic effects of hesperidin through stimulating the pancreatic secretion of insulin, glucose transport to peripheral tissues, peripheral glucose uptake, activation of gluconeogenic enzymes in the liver and skeletal muscles, and inhibition of glycogenolysis. It seems that human studies did not find hypoglycemic effects of hesperidin because they used low dosages of it.

NF-κB is a transcription factor that has been proposed to exert a key role in the expression of TNF-α, interleukin-6, and the other inflammatory mediators (Lawrence, Gilroy, Colville-Nash, & Willoughby, 2001). Additionally, some studies reported that TNF-α has a pivotal function in insulin resistance and inflammation (Larter & Farrell, 2006; Tilg & Hotamisligil, 2006). Therefore, it seems logical that reducing inflammation would be an effective step in improving NAFLD. Our results exhibited that 12 weeks of supplementation with

1-g hesperidin significantly improves hs-CRP, TNF-α, and NF-κB. These results are also in good agreement with the study of Rizza et al. (2011), which declared that 3 weeks of supplementation with 500-mg hesperidin significantly reduced hs-CRP concentration in metabolic syndrome patients. Homayouni et al. (2017) also reported a significant reduction in serum hs-CRP, interleukin-6, and TNF-α after 6 weeks of supplementation with 500-mg hesperidin in type 2 diabetic patients. The anti-inflammatory effect of hesperidin can be explained by the ability of this flavonoid to inhibit NF-κB activation through impeding phosphorylation of IκB and upregulating of PPARγ and its antioxidant activity (Mahmoud, 2014).

Our results have shown that hesperidin supplementation reduced hepatic enzymes and liver steatosis significantly. No study has yet evaluated the effects of hesperidin on NAFLD patients. It seems that hesperidin ameliorated hepatic feature of NAFLD due to its effects on reduction of serum lipids, glucose, and inflammation.

This study has some limitations. First, modulations after the 3-month intervention cannot accurately reflect the long-term outcomes of hesperidin consumption in NAFLD patients. Second, due to ethical consideration, it was not possible to perform liver biopsy and histological examination for each patient, which is a gold standard for diagnosing of NAFLD. However, we evaluated the liver by transient elastography with CAP test. This method has proven to be a reliable and noninvasive approach for identifying patients with hepatic steatosis and fibrosis (Malekzadeh & Poustchi, 2011). Moreover, it is accurately reproducible, and its score has low intraobserver- and interobserver variability (Malekzadeh & Poustchi, 2011). Another limitation of this study was that serum ALT was higher in the placebo group compared with the hesperidin group at the baseline; however, we adjusted its effects in our analysis using analysis of covariance test.

The current study has some strengths including assessment of NF-κB p65 activity in peripheral blood mononuclear cells, a high participation rate (>90%), a moderate to low dropout rate, successful blinding, and the double-blind, placebo-controlled design.

In conclusion, this randomized, double-blind, placebo-controlled trial showed some evidences that 1-g hesperidin supplementation accompanied with lifestyle modification (diet and physical activity) could improve the effectiveness of lifestyle intervention alone for treatment of NAFLD. These effects are at least partially due to attenuating inflammatory markers and lipid profile improvement. Although the results of this study confirmed our assumption, further studies with higher dosages and longer intervention period are needed to verify these findings and explore more exact mechanism of action for hesperidin.

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CONFLICT OF INTEREST

There is no conflict of interest.

ORCID

Azita Hekmatdoost https://orcid.org/0000-0002-1944-0052

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