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Resveratrol supplementation improves inflammatory biomarkers in patients with nonalcoholic fatty liver disease☆

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ABSTRACT

Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in the world. Resveratrol is a polyphenolic compound with antioxidant capacity that shows beneficial effects on down-regulation of inflammatory mediators and metabolic disorders. We hypothesized that supplementation with resveratrol can further improve the efficacy of lifestyle modifications in the management of NAFLD. In this randomized, double-blinded, controlled clinical trial, 50 NAFLD patients were supplemented with either a 500-mg resveratrol capsule or a placebo capsule for 12 weeks. Both groups were advised to follow an energy-balanced diet and received physical activity recommendations. Serum liver enzymes, inflammatory markers, hepatic steatosis and fibrosis, dietary intake, anthropometric measurements, and physical activity were assessed at both baseline and the end of the study. In both groups, anthropometric measurements (weight, body mass index, waist circumference), liver enzymes, and steatosis grade improved ($P < 005$). Resveratrol supplementation was associated with a significant reduction in liver enzyme alanine aminotransferase, inflammatory cytokines, nuclear factor κB activity, serum cytokeratin-18, and hepatic steatosis grade, as compared with placebo supplementation ($P < .05$). For the treatment of NAFLD, our results showed that 12 weeks of supplementation of 500 mg resveratrol, along with lifestyle modification, is superior to lifestyle modification alone. This is at least partially due to the attenuation of inflammatory markers and hepatocellular apoptosis. More studies are needed to confirm and increase the clinical application of the present results.

Abbreviations: NAFLD, nonalcoholic fatty liver disease; MET, metabolic equivalent of task; PBMC, peripheral blood mononuclear cells; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyltransferase; hs-CRP, high-sensitivity C-reactive protein; IL, interleukin; NF-κB, nuclear factor κB; TNF-α, tumor necrosis factor α; WC, waist circumference; WHR, waist-to-hip ratio.

☆ This trial was registered at clinicaltrials.gov as NCT02030977.

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1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is one of the most common worldwide chronic liver diseases and represents a spectrum of disorders, which are characterized by predominantly macrovesicular hepatic steatosis that occurs in individuals in the absence of significant alcohol consumption [1,2]. Currently, there is no effective pharmacologic treatment of NAFLD. Because oxidative stress plays a pivotal role in the development and consequences of NAFLD, the effectiveness of several antioxidant agents has been evaluated [3–6]. However, the efficacy of such treatments has been equivocal [6,7]. Lifestyle modifications are considered beneficial for management of NAFLD [8–10], although the combination of it with anti-inflammatory agents showed significantly more improvement in NAFLD characteristics [11,12].

Resveratrol (*trans*-3,5,40-trihydroxystilbene) is a polyphenol that is found in a wide variety of plant species. Studies show that it exerts beneficial effects across different species and various disease models; it can prevent or slow down the progression of a wide variety of illnesses such as cancer, cardiovascular disorders, diabetes, and metabolic diseases [13–17]. Resveratrol supplementation improved health and survival in mice with obesity [18] and chronic liver diseases [19]. The mechanisms underlying the beneficial effects of resveratrol are not completely elucidated; however, it has been mainly related to its antioxidant and anti-inflammatory activities that protected tissues, such as the liver, kidney, and brain, against a variety of damage caused by oxidative stress and inflammation [20].

Because oxidative stress and inflammation are involved in NAFLD pathogenesis and because of the antioxidant and anti-inflammatory properties of resveratrol, we assumed that resveratrol supplementation might be effective in managing NAFLD and restraining its progression. Thus, we designed this placebo-controlled, double-blind, randomized clinical trial to evaluate the effects of resveratrol supplementation on liver enzymes, inflammatory indices, hepatic elasticity, and echogenicity in patients with NAFLD.

2. Methods and materials

2.1. Study design

This was a placebo-controlled, double-blind, randomized clinical trial. We recruited adult patients who were 18 years or older and who exhibited the following inclusion criteria: evidence of NAFLD with a steatosis grade higher or equal to 2 in ultrasonography, elastometry value greater than 4 kPa in Fibroscan (Echosens, Paris, France), and serum alanine aminotransferase (ALT) greater than 30 IU/L for men and greater than 19 IU/L for women; no history of alcohol consumption or consumption of less than 10 g of alcohol per day in women and less than 20 g/d in men; absence of other liver disorders, malignancies, cardiovascular, respiratory, and kidney disorders; absence of pregnancy or lactation; absence of medication consumption in the previous 3 months; absence of weight loss in the previous 3 months; and absence of endocrine and metabolism disorders. The exclusion criteria involved weight loss of more than 10% of baseline body weight during the intervention period, pregnancy, and/or preference to not continue the study. Informed consent was obtained from each patient who was included in the trial. The study protocol was approved by the National Nutrition and Food Technology Research Institute, Tehran, Iran.

2.2. Randomization and treatment

Patients who fulfilled all eligibility criteria were randomly assigned to receive a capsule containing either 500 mg *trans*-resveratrol or the same amount of medium-chain triglyceride as placebo, once a day for 12 weeks. Both groups were advised to follow an energy-balanced diet and physical activity recommendations based on the Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults from the National Institutes of Health and the North American Association for the Study of Obesity [21]. The distribution of nutrients in relation to the total caloric value was as follows: total fat ≤30% of total caloric value, saturated fatty acids 10%, monounsaturated fatty acids 15%, polyunsaturated fatty acids 5%, protein 15% to 18%, carbohydrates 52% to 55%, dietary cholesterol <300 mg/d, and 20 to 30 g of fiber per day. Patients were also advised to exercise at least 30 minutes, 3 times per week.

The resveratrol used in this study was pure *trans*-resveratrol, which was produced by Sumabe, NSW, Australia. Study medications were stored in consecutively numbered, sealed bottles, and the preparation was completed at a separate office. Afterward, the study medications were transferred to the study clinic and dispensed by a registered nurse during each clinic visit. The hepatologist, radiologist, nutritionist, and patients were all blinded to the treatment assignment.

2.3. Follow-up assessments

After randomization, the patients were followed up at weeks 4, 8, and 12. At baseline, the patients received a standard 20-minute talk on lifestyle management for NAFLD. A healthy diet and regular exercise, for at least 30 minutes/3 times per week, were encouraged. Each visit documented adverse events, concomitant medications, and alcohol consumption. Adherence to study medications was ascertained by counting the remaining capsules, whereas a loss of more than 10% of the capsules was considered incompliance and, therefore, excluded from the study. Clinical, paraclinical, and dietary intakes were assessed at baseline and final visits (weeks 0 and 12).

2.4. Clinical, paraclinical, and dietary intake assessments

All patients underwent measurements of weight, height, and waist and hip circumferences. Weight was recorded to the nearest 100 g, using digital scales with the participants wearing minimal clothing and no shoes. Height was measured using a tape measure while the participants were standing in a normal position with no shoes. Body mass index was calculated as weight (in kilograms) divided by the square of height (in meters). Waist circumference (WC) was measured at the narrowest level, and hip circumference was measured at the maximum level over light clothing. Both WC

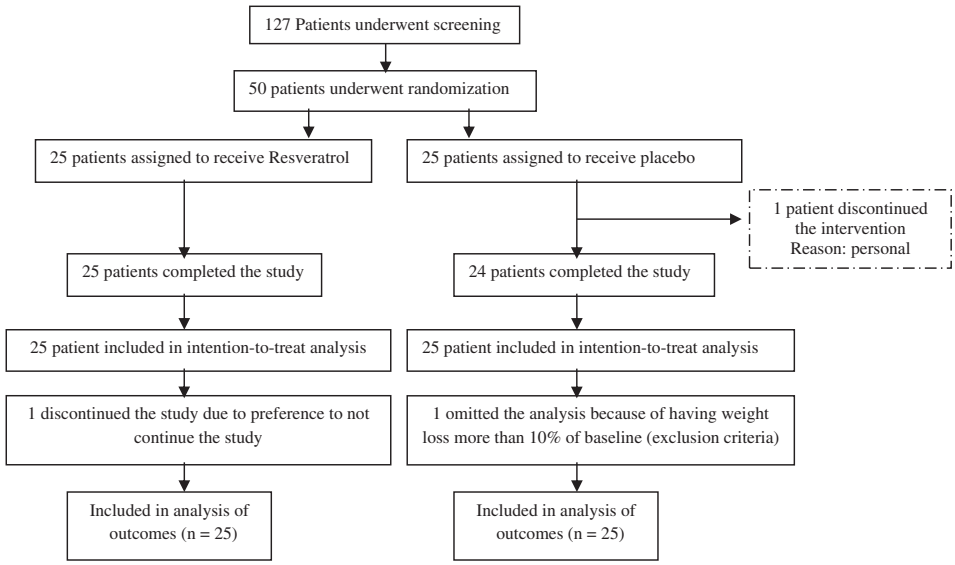

Figure – Flowchart of study participants.

and hip circumference were measured using a nonstretchable tape, without any pressure applied to the surface of the body and recorded to the nearest 0.1 cm. Whenever the narrowest area of the waist could not be defined because of a large amount of abdominal fat or extreme thinness, WC was measured just below the end of the lowest rib. Waist-to-hip ratio (WHR) was measured, according to World Health Organization recommendations [22].

Based on standard protocol, fasting blood samples were collected at baseline and week 12, while subjects were sitting. All biochemical assessments were performed in the same laboratory and using standard laboratory methods. Total bilirubin and γ-glutamyltransferase (GGT) were measured by enzymatic colorimetric assay (Parsazmoun, Tehran, Iran). Alanine aminotransferase, aspartate aminotransferase (AST), and alkaline phosphatase concentrations were measured by photometric assay (Reckon Diagnostics, Vedodara, India). Fasting high-sensitivity C-reactive protein (hs-CRP; Bionik, Tehram, Iran), tumor necrosis factor α (TNF-α; BOSTER, Pleasanton, CA, USA), interleukin (IL)-6 (Boster), and cytokeratin-18 M30 (Glory Science, Hangzhou, China) concentrations were measured using an enzyme-linked immunosorbent assay. By using the enzyme-linked immunosorbent assay kit (Cell Signaling ,Danvers, MA, USA), nuclear factor κB (NF-κB) p65 was measured in peripheral blood mononuclear cell (PBMC) nuclear extracts, according to the manufacturer's protocol.

For the assessment of nutrient intakes, patients were instructed to record their daily dietary intakes for 3 days, including a weekend day at baseline and week 12. Dietary intakes were then analyzed by using the US Department of Agriculture and national food composition tables [23]. Physical activity was also assessed by using the metabolic equivalent of task (MET) questionnaire [24], at weeks 0 and 12. Liver fibrosis was assessed by transient elastography, at baseline and at the end of the study. Transient elastography was performed using the same equipment and by the same operator who was blinded to the study randomization and unaware of the clinical and laboratory results.

2.5. *Primary and secondary outcomes*

The primary outcome measure was a significant reduction in ALT concentration. Secondary outcome measures were the transient elastography score, inflammatory factor concentrations in serum and PBMCs, anthropometric variables, and serum concentrations of AST, GGT, total bilirubin, and alkaline phosphatase.

2.6. *Statistical analyses*

Data were analyzed using SPSS software (version 16; SPSS, Chicago, IL, USA). For all analyses, a *P* value of .05 was considered statistically significant. Continuous and categorical data were presented as means ± SD and frequency, respectively. Demographic variables were analyzed by using a χ^2 or *t* test, as appropriate. The data were analyzed according to the intention-to-treat principle. To compare variables within and between groups, paired *t* test and Student *t* test were used, respectively. If the distribution of variables was not normal, Wilcoxon and Mann-Whitney *U* tests were used to compare quantitative variables within and between groups, respectively. In order to eliminate the effects of confounding factors, either in the beginning or during the study, the analysis of covariance test was used.

3. Results

From June 2012 to December 2012, 50 patients were enrolled and underwent randomization (Figure). Only 1 (4%) patient in the resveratrol group discontinued the study medication after the second week because of personal reasons. All of the remaining 49 patients took at least 90% of the study medications. One patient (4%) in the placebo group had more than 10% weight loss and was excluded from the study. During the treatment period, no patient reported any

Table 1 – Baseline characteristics of NAFLD patients

Characteristics	Resveratrol group (n = 25)	Placebo group (n = 25)	P
Sex, n (%)			
Male	18 (72)	17 (68)	0.86
Female	7 (28)	8 (32)	.79
Smoking			
Yes	2	2	
No	23	23	
Age (y)	44.04 ± 10.10	46.28 ± 9.52	.42
Male	43.00 ± 9.87	45.29 ± 10.91	.51
Female	46.71 ± 10.99	48.38 ± 5.55	.71
Metabolic characteristics			
Height (cm)	167.14 ± 9.85	167.82 ± 10.56	.81
Weight (kg)	79.11 ± 10.94	80.63 ± 9.008	.59
Body mass index (kg/m^2)	28.35 ± 3.49	28.75 ± 3.50	.69
WC (cm)	95.46 ± 7.78	96.24 ± 7.82	.72
WHR	0.94 ± 0.04	0.95 ± 0.07	.52
MET (h/d)	30.82 ± 2.88	30.83 ± 1.42	.99
Energy (kcal)	2265 ± 376.46	2251 ± 317.17	.89
Serum biochemistry tests			
ALT (IU/L)	61.12 ± 39.08	45.08 ± 21.70	.081
AST (IU/L)	33.28 ± 14.24	28.72 ± 9.47	.189
GGT (IU/L)	35.16 ± 14.77	47.79 ± 38.90	.147
Bilirubin direct (mg/dL)	0.24 ± 0.07	0.21 ± 0.06	.210
Bilirubin total (mg/dL)	0.94 ± 0.37	0.79 ± 0.22	.091
Inflammatory factors			
hs-CRP (mg/L)	3.02 ± 1.44	3.20 ± 1.85	.701
Il-6 (pg/dL)	5.49 ± 2.64	7.22 ± 4.73	.14
TNFα (pg/mL)	19.96 ± 18.80	16.55 ± 4.79	.36
NF-κB (ng/mL)	3.60 ± 4.11	2.50 ± 2.40	.291
Cytokeratin-18 (U/L)	457.72 ± 436.18	395.06 ± 317.74	.58
Liver histology			
Steatosis grade (0/1/2/3) [a]	0/8/12/5	0/14/9/2	
Fibrosis (kPa) [b]	6.88 ± 2.35	5.84 ± 1.002	.05

Values are means ± SD, unless otherwise indicated.
[a] Number of patients in each grade according to ultrasound assay.
[b] According to Fibroscan assay.

significant adverse effects. The demographic and metabolic profiles of the 2 groups were well matched (Table 1).

Within both groups, we found a significant decrease in the weight and WC ($P < .05$); however, no significant differences were observed between the 2 groups ($P > .05$). There were no significant changes in daily energy intake and physical activity between and within groups ($P > .05$; Table 2). Serum ALT and AST were significantly decreased in both groups, after the 12-week intervention period. Compared with the placebo group, patients undertaking resveratrol had a significantly greater decrease in serum ALT enzyme (–32.38% ± 25.63% vs –13.54% ± 45.00%; Table 3). Significant changes were also observed in inflammatory markers in the resveratrol group but not the control group. Inflammatory factors (hs-CRP, IL-6, and NF-κB) decreased significantly in the resveratrol group, as compared with the baseline and the placebo group ($P < .05$). Moreover, cytokeratin-18 M30, as a biomarker of hepatocellular apoptosis, decreased significantly in the resveratrol group, when compared with the baseline and the placebo groups ($P < .05$; Table 4). At the end of the study, transient elastography showed a reduction of hepatic fibrosis grade in the resveratrol group; however, this reduction was not significant ($P > .05$). Hepatic tissue echogenicity in the ultrasound assay decreased significantly in both groups ($P < .05$); however, this reduction was significantly more in the resveratrol group compared with the placebo group ($P < .05$; Table 5).

4. Discussion

To our knowledge, this is the first randomized, double-blind, clinical trial that evaluates the affectivity of resveratrol supplementation on NAFLD characteristics and that addresses some of its mechanisms of action. Our results confirmed our assumption and showed that coadministration of resveratrol with lifestyle modification improves conditions by reducing ALT, liver steatosis, markers of inflammation, and hepatocellular apoptosis in patients with NAFLD.

Previous animal studies show that resveratrol protects the liver against steatosis and fibrosis via activation of Sirt1, leading to inactivation of NF-κB and inhibition of TNF-α and resulting in prevention of fibrosis [25–28]. Moreover, resveratrol could improve hepatic metaflammation, fat storage, and oxidative stress in animal models of NAFLD [29–31]. These animal studies are supported by our results in patients with NAFLD.

Few clinical trials have applied resveratrol supplementation to evaluate the metabolic and oxidative stress parameters, and the results are controversial. Some show therapeutic effects of resveratrol in metabolic disorders and oxidative stress. Timmer et al [32] showed that 30 days of 150 mg/d resveratrol supplementation induces metabolic changes in obese humans, mimicking the effects of calorie restriction. Similarly, Bo et al [33] evaluated the effect of 500 mg resveratrol in healthy smokers in a comparable duration and confirmed the anti-inflammatory and antioxidant effects. Bhatt et al [34] also reported that 250 mg/d of resveratrol supplementation for 3 months improved glycemic control in type 2 diabetes mellitus. Moreover, Tomé-Carneiro et al [35] reported that grape resveratrol increases serum adiponectin and down-regulates inflammatory genes in PBMCs in patients with stable coronary artery disease.

There are a meager amount of studies that do not show beneficial metabolic effects of resveratrol. Yoshino et al [36] demonstrated that 12 weeks of supplementation with 75 mg/d of resveratrol did not change body composition, resting metabolic rate, plasma lipids, inflammatory markers, or liver, skeletal muscle, and adipose tissue insulin sensitivity in nonobese, postmenopausal women with normal glucose tolerance. A recent study also showed that an 8-week administration of 3000 mg/d of resveratrol did not significantly improve any features of NAFLD, when compared with placebo; however, it increased hepatic stress, based on increased levels of liver enzymes [37]. These results differ greatly from our study results, but this could be related to different dosages of resveratrol. Other studies with large doses of resveratrol did not find any beneficial metabolic effects [38], or they found a

Table 2 – Metabolic characteristics and energy consumption of patients at baseline and after 12 weeks

Characteristics	Baseline (means ± SD)	After 12 wk (means ± SD)	P [a]	% Change (means ± SD)	P [b]
Weight (kg)					
Resveratrol	79.11 ± 10.94	78.26 ± 11.06	.006	−1.07 ± 1.69	.53
Placebo	80.63 ± 9.00	79.51 ± 9.49	.005	−1.24 ± 2.24	
BMI (kg/m^2)					
Resveratrol	28.35 ± 3.49	27.96 ± 3.39	.06	−0.76 ± 2.04	.48
Placebo	28.75 ± 3.5	28.40 ± 3.60	.01	−1.23 ± 2.34	
WC (cm)					
Resveratrol	95.46 ± 7.78	94.16 ± 7.39	.01	−7.50 ± 2.65	.58
Placebo	96.24 ± 7.82	95.38 ± 7/86	.04	−0.7 ± 1.70	
HC (cm)					
Resveratrol	101.13 ± 5.77	100.80 ± 7.67	.79	−0.17 ± 3.69	.75
Placebo	100.65 ± 5.97	100.49 ± 5.49	.81	0.36 ± 1.05	
WHR					
Resveratrol	0.94 ± 0.04	0.93 ± 0.04	.059	−1.26 ± 2.89	.31
Placebo	0.95 ± 0.07	0.95 ± 0.07	.36	−0.32 ± 1.86	
MET (MET · h/d)					
Resveratrol	30.82 ± 2.88	30.68 ± 3.11	.60	−0.6 ± 5.59	.93
Placebo	30.83 ± 1.42	30.65 ± 1.34	.80	−0.64 ± 2.5	
Energy intake (kcal/d)					
Resveratrol	2265 ± 376.46	2049.3 ± 402.48	.062	−9.58 ± 10.30	.68
Placebo	2251 ± 317.17	2136.5 ± 307.59	.276	−3.77 ± 11.50	

BMI, body mass index; HC, hip circumference.
[a] *P* values indicate comparison within groups.
[b] *P* values indicate comparison between the changes of each variable between 2 groups.

dose-responsive effect [35]. Thus, more studies with different dosages of resveratrol might be useful to find the optimal dose for supplementation.

The current study had several strengths including a high participation rate (>90%); a moderately low dropout rate; successful blinding; the double-blind, placebo-controlled design; the evaluation of cytoketratin-18 as a specific biomarker of hepatocellular apoptosis [39]; and the assessment of NF-κB activity in PBMCs to find a mechanism of action for resveratrol effects on inflammatory cytokines in the body.

However, there were also several limitations. First, because our study was based on a convenient sample, the possibility of voluntary bias cannot be entirely ruled out. Second, the sample size of the current study was relatively small and the study was only conducted on people living in Isfahan city. This could limit the generalizability of the study findings; nonetheless, our sample size was higher than similar previous studies. Third, we could not precisely evaluate the adherence of the participants to the treatments, although we attempted to control this by repeated follow-up

Table 3 – The effects of resveratrol and placebo intake on serum level of liver enzymes and bilirubin at baseline and after 12-week treatment

Characteristics	Baseline (means ± SD)	After 12 wk (means ± SD)	P [a]	% Change (means ± SD)	P [b]
ALT (IU/L)					
Resveratrol	61.12 ± 39.08	36.67 ± 15.56	.005	−32.38 ± 25.63	.03
Placebo	45.08 ± 21.70	33.92 ± 16.43	.041	−13.54 ± 45.00	
AST (IU/L)					
Resveratrol	33.28 ± 14.24	22.62 ± 6.41	.002	−27.48 ± 20.69	.065
Placebo	28.72 ± 9.47	21.04 ± 5.53	.002	−21/23 ± 33.48	
GGT (U/L)					
Resveratrol	35.16 ± 14.47	28.33 ± 11.18	.07	−16.13 ± 27.82	.653
Placebo	47.79 ± 38.90	42.08 ± 39.51	.059	−18.23 ± 22.41	
Bilirubin direct (mg/dL)					
Resveratrol	0.24 ± 0.07	0.24 ± 0.06	.763	7.29 ± 34.69	.530
Placebo	0.21 ± 0.06	0.23 ± 0.17	.978	22.91 ± 100.69	
Bilirubin total (mg/dL)					
Resveratrol	0.94 ± 0.37	0.83 ± 0.25	.026	−8.14 ± 23.45	.440
Placebo	0.79 ± 0.22	0.63 ± 0.20	.025	−13.39 ± 34.15	

[a] *P* values indicate comparison within groups.
[b] *P* values indicate comparison between the changes of each variable between 2 groups.

Table 4 – The effects of resveratrol and placebo intake on inflammatory markers at baseline and after 12-week treatment

Characteristics	Baseline (means ± SD)	After 12 wk (means ± SD)	P [a]	% Change (means ± SD)	P [b]
hs-CRP (mg/L)					
Resveratrol	3.02 ± 1.44	2.11 ± 0.96	.011	−25.20 ± 31.31	.029
Placebo	3.20 ± 1.85	3.40 ± 2.13	.861	−2.00 ± 25.61	
TNF-α (pg/mL)					
Resveratrol	19.96 ± 18.80	16.25 ± 5.47	.317	−3.59 ± 37.07	.016
Placebo	16.55 ± 4.79	20.62 ± 10.88	.094	32.03 ± 68.56	
IL-6 (pg/dL)					
Resveratrol	5.49 ± 2.64	3.96 ± 1.91	.034	−26.60 ± 22.14	.004
Placebo	7.22 ± 4.73	6.65 ± 4.23	.677	−3.99 ± 30.90	
Cytokeratin-18 M30 (U/L)					
Resveratrol	457.72 ± 436.18	287.42 ± 172.22	.004	−17.69 ± 30.22	.047
Placebo	395.06 ± 317.74	343.75 ± 285.68	.902	23.75 ± 95.77	
NF-κB (ng/mL)					
Resveratrol	3.60 ± 4.11	2.74 ± 3.45	.000	−18.90 ± 12.35	.003
Placebo	2.50 ± 2.40	2.41 ± 2.25	.555	−0.81 ± 17.66	

[a] P values indicate comparison within groups.
[b] P values indicate comparison between the changes of each variable between 2 groups.

visits and a capsule count, which showed a compliance rate of over 90% in all of the study groups. Last, because of ethical considerations, we could not conduct liver biopsies, which is a fairly accurate diagnostic tool from which to derive a pathology score of disease. However, we did use transient elastography, which provides a quantitative, noninvasive evaluation of NAFLD by measuring hepatic fibrosis [40]. This technique is proven to be a reliable, noninvasive method for identifying patients with significant hepatic fibrosis. It is readily reproducible, and its score has low interobserver and intraobserver variability [40].

In conclusion, this randomized, double-blind, placebo-controlled trial found some evidence indicating that 500 mg/d of resveratrol supplementation, in addition to lifestyle modification, is superior to lifestyle modification alone for the treatment of NAFLD. This is at least partially due to attenuation of inflammatory markers and hepatocellular apoptosis in the body. More studies with longer durations and different dosages of supplementation are needed to confirm and increase the clinical application of the present results.

Table 5 – The effects of resveratrol and placebo intake on liver histology at baseline and after 12-week treatment

Characteristics	Baseline	After 12 wk	P [a]	P [b]
Steatosis (0/1/2/3) [c]				
Resveratrol	0/8/12/5	12/10/3/0	.000	.02
Placebo	0/14/9/2	8/13/3/0	.000	
Fibrosis (kPa) [d]				
Resveratrol	6.88 ± 2.35	6.04 ± 1.35	.247	.09
Placebo	5.84 ± 1.002	5.90 ± 1.14	.650	

[a] P values indicate comparison within groups.
[b] P values indicate comparison between the variables between 2 groups.
[c] Number of patients in each grade according to ultrasound assay.
[d] According to Fibroscan assay.

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