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RESEARCH ARTICLE

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The effects of black seed supplementation on cardiovascular risk factors in patients with nonalcoholic fatty liver disease: A randomized, double-blind, placebo-controlled clinical trial

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Nonalcoholic fatty liver disease (NAFLD) is highly related to cardiovascular disorders risk factors. This study aimed to evaluate the effects of black seed (*Nigella sativa*) supplementation on cardiovascular disorders risk factors in patients with NAFLD. This randomized, double-blind, placebo-controlled clinical trial was conducted on 50 patients with NAFLD. Participants were assigned to receive a lifestyle modification plus 2 g/day of either *N. sativa* or placebo for 12 weeks. Compared with the placebo, *N. sativa* supplementation led to significant reductions in serum glucose (−7.95 vs. −1.22; p = .041), serum insulin (−3.87 vs. −1.07; p = .027), homeostatic model of assessment for insulin resistance (−1.02 vs. −0.28; p = .021), and a significant increase in quantitative insulin sensitivity check index (0.03 vs. 0.006; p = .002). All of these changes were remained significant after adjusting for known confounding variables; however, there was no significant difference in lipid profile changes between the two groups (p = .05). *N. sativa* supplementation significantly decreased hepatic steatosis percentage compared with the placebo after adjustment for confounding variables (p = .005). In conclusion, our results indicate that daily intake of 2-g *N. sativa* plus lifestyle modification is superior to lifestyle modification alone in amelioration of insulin resistance and hepatic steatosis in patients with NAFLD.

KEYWORDS

black seed, blood glucose, diabetes, insulin resistance, lipid profile, *Nigella sativa*, nonalcoholic fatty liver disease

1 | INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is one of the most common chronic liver diseases in the world that its prevalence is growing fast all over the world (Mokhtari, Gibson, & Hekmatdoost, 2017; Younossi, 2019). NAFLD includes a variety of disorders like nonalcoholic steatohepatitis, liver fibrosis, and liver cirrhosis. The pathogenesis of NAFLD is associated with metabolic diseases such as diabetes mellitus, hyperlipidemia, and obesity, which are also the main risk factors of cardiovascular disorders (Cicero, Colletti, & Bellentani, 2018; Patti et al., 2018). Currently, there is no approved treatment for NAFLD although health promotion strategies and lifestyle modifications are recommended as the primary steps in disease management (Emamat et al., 2015; Hekmatdoost et al., 2016; Mokhtari, Poustchi, Eslamparast, & Hekmatdoost, 2017; Noori, Jafari, & Hekmatdoost, 2017; Rahimlou, Ahmadnia, & Hekmatdoost, 2015; Rahimlou, Yari, Hekmatdoost, Alavian, & Keshavarz, 2016). Over the past few years, numerous studies have been conducted to find plant-based biological

compounds for treatment of NAFLD (Bahrami et al., 2019; Mofidi et al., 2017; Saadati et al., 2019). Black seed (*Nigella sativa*) is one of the plant-based compounds with protecting effects on the liver, kidney, digestive system, nervous system, and cardiovascular system, as well as antiinflammatory and antimalignancy effects (Darand, Alavian, & Hekmatdoost, 2018). Thymoquinone is the main active component of the *N. sativa*. It has been shown that thymoquinone alleviates NAFLD through reduction of oxidative stress, inflammation, and apoptosis in experimental model of disease (Awad, Al Haleem, El-Bakly, & Sherief, 2016). Moreover, it has been shown that *N. sativa* can reduce serum triglycerides (TGs) and low-density lipoprotein cholesterol (LDL-C) level, whereas improve glycemic indices in diabetic patients (Badar et al., 2017; Ibrahim et al., 2014; Kaatabi et al., 2015).

According to the current evidence, we hypothesized that *N. sativa* might be beneficial for management of cardiovascular disorders (CVD) risk factors in NAFLD patients. Therefore, the present study was conducted to determine the effects of *N. sativa* supplementation on CVD risk factors in patients with NAFLD.

2 | MATERIALS AND METHODS

2.1 | Study design

This study was conducted as a randomized, double-blind, placebo-controlled clinical trial. The study was conducted to evaluate the effect of *N. sativa* on glycemic indices, lipid profile, liver enzymes, and liver fibrosis. Samples of the study were selected from the hepatology clinic of Baqiyatullah Hospital.

The inclusion criteria for entering the study consisted of age above 18, evidence of nonalcoholic steatohepatitis in FibroScan (controlled attenuation parameter > 263), negative history of alcohol consumption, no history of chronic liver diseases like hepatitis B and C, no history of gallbladder diseases, no history of any chronic diseases, negative history of any supplement or medication consumption, no history of daily consumption of *N. sativa* in previous 3 months, and negative history for pregnancy and lactating. Consumption of less than 90% of supplements at the end of study, lack of willingness to continue participation (Badar et al., 2017; Ibrahim et al., 2014; Kaatabi et al., 2015) in the study, and more than 10% loss in body weight during the study period were the exclusion criteria of the study.

Participants were selected by convenience sampling and were randomly assigned to receive either four *N. sativa* containing capsules or the same amount of identical placebo capsules for 12 weeks. Each *N. sativa* capsule contained 500-mg–milled edible *N. sativa*, and each placebo capsule was filled by 500-mg rice starch. Capsules' color was dark to make similar appearance for capsules. The seeds were purchased from a farmer all together. They were milled every 4 weeks, placed in the capsules, and delivered to the participants. We chose 2 g/day because previous studies have reported the beneficial effects of this dosage on other health-related outcomes (Badar et al., 2017; Farzaneh, Nia, Mehrtash, Mirmoeini, & Jalilvand, 2014; Hussain, Tunio, Arain, & Shaikh, 2017; Sabzghabaee, Dianatkhah, Sarrafzadegan, Asgary, & Ghannadi, 2012). *N. sativa* seeds contain protein (26.7%), fat (28.5%), carbohydrates (24.9%), crude fiber (8.4%), and total ash (4.8%). *N. sativa* seeds also contain a good amount of various vitamins and minerals like Cu, P, Zn, and Fe. Many active compounds have been identified in *N. sativa*. The most important active compounds of *N. sativa* are thymoquinone (30–48%), thymohydroquinone, dithymoquinone (nigellone), p-cymene (7–15%), carvacrol (6–12%), 4-terpineol (2–7%), t-anethole (1–4%), sesquiterpene longifolene (1–8%), α-pinene, and thymol. The *N. sativa* oil contains fatty oil rich in unsaturated fatty acids, constituting linoleic acid (50–60%), oleic acid (20%), eicosadienoic acid (3%), and dihomolinoleic acid (10%), and saturated fatty acids (palmitic and stearic acids).

Both groups were advised on a balanced diet and moderate physical activity during the study period (Eslamparast et al., 2014).

Supplement boxes were labeled as A or B to blind the investigators and patients to group assignments. Labeling was done by a third person. Boxes were given to the patients at the start of the study and at the end of the fourth and eighth weeks, and they were asked to bring empty boxes or remained capsules back to monitor patients' compliance. The patients who had not consumed more than 10% of the received capsules were excluded from the study. Follow-up assessments were done every 2 weeks in order to control the patients' compliance and adherence to the study protocol.

2.2 | Sample size

Sample size was calculated using the standard formula for clinical trials, considering type 1 error (α) of .05 and type 2 error (β) of .20 (power = 80%). According to a previous published study (Eslamparast et al., 2014), we used 10% as the difference in mean for homeostatic model assessment for insulin resistance (HOMA-IR) as the key variable. On the basis of this information, 21 individuals were required to be included in each treatment group. Considering four probable dropouts in each group, the final sample size was determined as 25 patients in each group.

2.3 | Anthropometric assessment

At the beginning of the study, the weight of each patient was measured with accuracy of 100 g using a Seca® scale, and the height of each patient was measured in a standing position without shoes with accuracy of 0.5 cm. Waist circumference was measured with accuracy of 0.5 cm in standing position and in the narrowest part between the last rib and the iliac bone, and the hip circumference was measured with accuracy of 0.5 cm in standing and in the maximum level of the hip circumference. The body mass index (BMI) of the patients was calculated by dividing the weight (kg) to height (meter), and waist-to-hip ratio (WHR) was calculated by dividing the waist circumference to hip circumference. All of the mentioned indicators remeasured at the end of the fourth, eight, and 12th weeks.

2.4 | Biochemical assessment

In the beginning of the study and at the end of 12th week, after 12–14 hr of fasting, 5 cc of blood was taken from the cubital vein of samples, and their blood serums were centrifuged at 4,000 rpm, and the serum was isolated in 1.5-cc microtubes to measure the biochemical factors and was stored at −80°C. The concentration of liver enzymes (ALT and AST), triglyceride and LDL-C, was measured by colorimetric method using Parsazmun® kits. The concentration of total cholesterol was measured by using Parsazmun® kits using photometric method. Calculation of high-density lipoprotein cholesterol (HDL-C) concentration was also performed using the Friedewald formula (Friedewald, Levy, & Fredrickson, 1972).

Blood glucose was measured using enzyme colorimetric method based on glucose oxidase and an autoanalyzer. The concentration of insulin was determined using an ELISA kit (Monobind, Inc., Lake Forest, CA, USA, catalog no.: 58K1L4). Insulin resistance was calculated using the HOMA-IR formula (Matthews et al., 1985), and insulin sensitivity was also calculated using the quantitative insulin sensitivity check index (QUICKI) formula (Vanhala, Vanhala, Kumpusalo, & Keinanen-Kiukaanniemi, 2002).

2.5 | Liver fibrosis and steatosis assessment

In this study, the degree of steatosis and hepatic fibrosis was measured using Echosense® FibroScan machine, which is an easy and noninvasive technique measuring the stiffness of the liver by using a 3.5-Hz ultrasonic transducers. This probe transmits a vibration with a range of 50 Hz to the liver. This vibration induces an elongated elastic shear wave, which is reflected in the liver. The intensity of the ultrasonic wave is directly related to the degree of stiffness of the liver; for example, in a hard and stiff liver, the waves flow faster. Results of liver fibrosis were reported in the unit of kilopascal (kPa). For measuring the liver steatosis, controlled attenuation parameter was used, and the results were reported in dB/m. All of the FibroScan measurements were done in the initial phase and at the end of 12th week of study.

2.6 | Dietary intake assessment

In this study, to evaluate the diet of patients in terms of energy intake, carbohydrate, protein, total fat, saturated fatty acid, polyunsaturated fatty acid, omega-3, omega-6, monounsaturated fatty acid, cholesterol, fiber, vitamin E, vitamin C, zinc, and selenium, a 24-hr diet recall was used at the beginning and at the end of the 12th week of the study. Three days (one holiday and two nonholidays) of the 24-hr diet recall were completed through face to face and telephone interview. Analysis of 24-hr diet recall questionnaires was done using the nutritional software Nutritionist IV (N4).

2.7 | Physical activity assessment

The physical activity of the samples was measured at the beginning of the study and at the end of the 12th week by completing a valid and reliable questionnaire through interviews with the individuals (Aadahl & JØrgensen, 2003). This questionnaire measured the intensity of physical activity based on the metabolic equivalents.

2.8 | Statistical analysis

The data are presented as "mean ± standard deviation" for quantitative data and frequency (%) for qualitative variables. Normal distribution of data was evaluated using Kolmogorov–Smirnov test to compare the confounding qualitative variables between the two groups. Chi-squared test was used. For comparing the mean of confounding quantitative variables between the two groups, Student's *t* test was used. To compare the mean of quantitative variables in each group, if their distribution was normal, paired *t* test was used, and if their distribution was not normal, Wilcoxon test was used. To compare quantitative variables between the two groups, Mann–Whitney was used. Analysis of covariance test was used to eliminate the effects of confounding factors that had a significant difference between the two groups in the beginning of the study or during the research. In this study, the *p* value less than .05 was considered statistically significant. Statistical analysis was performed using Statistical Package for the Social Sciences software Version 20.

2.9 | Ethical consideration

The study protocol was approved by the Ethics Committee of the Nutrition Institute and Food Research of Shahid Beheshti University of Medical Sciences in Iran (NNFTRI045042). This research was registered at Iranian Clinical Trials Center with registration number: IRCT4197. In order to observe ethical principles, volunteers entered the study after signing an informed consent. In this study, individuals were given complete freedom to leave at any stage of the study if they were not willing to continue to collaborate in the study.

3 | RESULTS

Among the patients referred to the hepatology clinic of Baqiyatullah Hospital, 50 patients who met inclusion criteria of the study were enrolled. They were randomly assigned into two equal groups of 25 patients. At the end of the study, 22 patients (11 males and 11 females; 88%) in intervention group and 21 patients (10 men and 11 women; 84%) in placebo group completed the study (Figure 1). There was no significant difference between the two groups in terms of dropout (*p* value = .69). Participation rate in this study was 86%. All of the findings presented in the results are related to the people who completed the study.

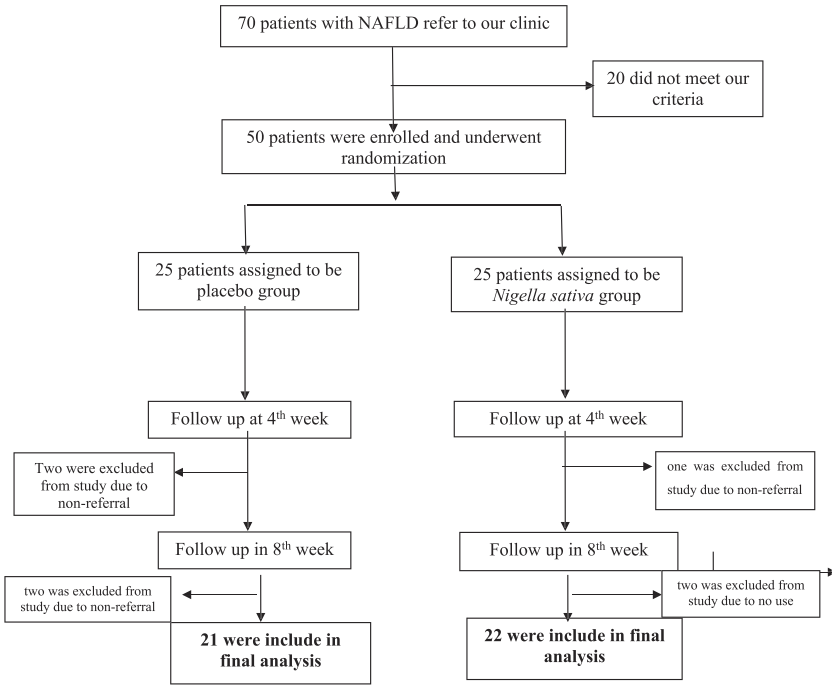

FIGURE 1 Consolidated Standards of Reporting Trials flowchart of the study

3.1 | Demographic and anthropometric factors

There was no significant difference in terms of gender distribution, age, physical activity, and smoking between the two groups at the beginning of the study (Table 1).

The anthropometric indices of patients with NAFLD are shown in Table 2. At the beginning of the study, only waist circumference and WHR were significantly higher in intervention group, and no other significant difference was observed in anthropometric measurements. At the end of the study, body weight, BMI, and hip circumference reduced significantly only in intervention group (p value $\leq$ .001).

Dietary intakes of patients in two groups are shown in Table 3. Diet comparison at the beginning and the end of the study showed

TABLE 1 Baseline characteristics of nonalcoholic fatty liver disease patients

Characteristics	*Nigella sativa* group (n = 22)	Placebo group (n = 21)	p
Age (year)	48.9 ± 12.7	46.2 ± 11.0	.45
Sex (male), n (%)	11 (50)	10 (47)	1.00
Smoking (yes)	0	2	.45
BMI (kg/m^2)	32.05 ± 4.17	31.7 ± 3.54	.77
Weight (kg)	90.94 ± 15.24	86.9 ± 11.56	.33
Energy intake (kcal)	2,644 ± 670	2,437 ± 521	.31
ALT (IU/L)	20.0 ± 10.5	24.0 ± 9.2	.23
Steatosis (dB/m)[a]	319.7 ± 59.4	310.0 ± 36.5	.52

Note. Values are means ± standard deviation, unless otherwise indicated. p values indicate differences between the two groups at baseline.

Abbreviation: BMI, body mass index.

[a]According to FibroScan-controlled attenuation parameter score.

TABLE 2 The anthropometric indices in *Nigella sativa* and placebo groups before and after the study

Variables	*N. sativa* group	Placebo group	p value[b]
Height (cm)	168 ± 10.15	165.66 ± 10.71	.434
Weight (kg)			
Before	90.94 ± 15.24	86.9 ± 11.56	.334
After	88.56 ± 15.03	86.36 ± 14.32	.653
p value[a]	.001	.47	
BMI (kg/m^2)			
Before	32.05 ± 4.17	31.7 ± 3.54	.770
After	31.2 ± 4.15	31.52 ± 5.29	.838
p value[a]	.001	.573	
Waist circumference (cm)			
Before	108.09 ± 9.21	106.81 ± 9.65	.037
After	102.19 ± 8.66	104.81 ± 9.36	.526
p value[a]	.14	.530	
Hip circumference (cm)			
Before	111.9 ± 9.53	111.23 ± 7.62	.801
After	108.36 ± 10.08	108.56 ± 8.04	.948
p value[a]	.001	.07	
Waist-to-hip ratio			
Before	0.97 ± 0.04	0.92 ± 0.06	.006
After	0.99 ± 0.05	0.97 ± 0.08	.378
p value[a]	.05	.15	

Abbreviation: BMI, body mass index.

[a]Within group comparison.[b]Between group comparison.

TABLE 3 Dietary intakes in *Nigella sativa* and placebo groups before and after the study

Variables	Baseline	End of the study	*p* value[a]
Physical activity (MET.hr/day)			
N. sativa group	31.57 ± 3.48	33.30 ± 4.99	.119
Placebo group	30.10 ± 5.31	31.74 ± 5.40	.309
p value[b]	.290	.329	
Total energy (kcal/day)			
N. sativa group	2,644 ± 670	2,125 ± 711	<.041
Placebo group	2,437 ± 521	1,942 ± 625	<.005
p value[b]	.313	.417	
Total carbohydrate (g/day)			
N. sativa group	374 ± 156	246 ± 68	<.017
Placebo group	337 ± 113	217 ± 60	<.001
p value[b]	.419	.191	
Total protein (g/day)			
N. sativa group	111 ± 45	81 ± 35	.110
Placebo group	90 ± 23	68 ± 20	.002
p value[b]	.104	.181	
Total fat (g/day)			
N. sativa group	92 ± 26	75 ± 24	.047
Placebo group	86 ± 17	65 ± 33	.029
p value[b]	.489	.332	
Cholesterol (mg/day)			
N. sativa group	340 ± 216	314 ± 225	.429
Placebo group	361 ± 211	303 ± 128	.212
p value[b]	.782	.858	
Fibers (g/day)			
N. sativa group	28.2 ± 17.0	26.3 ± 11.9	.478
Placebo group	25.8 ± 10.8	23.1 ± 10.3	.182
p value[b]	.622	.398	
Saturated fatty acid (g/day)			
N. sativa group	33.3 ± 17.7	20.5 ± 8.2	.025
Placebo group	30.2 ± 9.8	18.6 ± 8.3	<.001
p value[b]	.510	.485	
MUFA (g/day)			
N. sativa group	31.8 ± 10.9	27.5 ± 8.3	.283
Placebo group	29.3 ± 6.7	22.4 ± 16.9	.141
p value[b]	.411	.299	
PUFA–ω6 (g/day)			
N. sativa group	5.81 ± 6.90	3.54 ± 4.70	.301
Placebo group	5.12 ± 1.94	3.78 ± 2.34	.006
p value[b]	.667	.855	
PUFA–ω3 (g/day)			
N. sativa group	1.38 ± 1.31	0.92 ± 0.94	.310
Placebo group	1.25 ± 1.15	0.98 ± 1.27	.442
p value[b]	.762	.863	
Vitamin E (mg/day)			
N. sativa group	16.14 ± 8.53	13.16 ± 7.57	.366
Placebo group	14.98 ± 3.48	11.79 ± 5.75	.027
p value[b]	.580	.576	
Vitamin C (mg/day)			
N. sativa group	95.8 ± 122.0	116.9 ± 112.9	.894
Placebo group	102.2 ± 66.5	90.1 ± 75.8	.227
p value[b]	.839	.405	
Zinc (mg/day)			
N. sativa group	14.7 ± 5.3	14.4 ± 9.2	.930
Placebo group	13.2 ± 4.9	11.9 ± 4.4	.230
p value[b]	.411	.302	
Selenium (mg/day)			
N. sativa group	116.6 ± 60.9	109.6 ± 70.4	<.335
Placebo group	104.7 ± 50.1	99.9 ± 41.8	.524
p value[b]	.556	.628	

Abbreviations: MET, metabolic equivalents; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

[a]Within group comparison.[b]Between group comparison.

that there was no significant difference between the two groups in terms of energy, carbohydrate, protein, fat, cholesterol, fiber, saturated and unsaturated fatty acids, vitamins E and C, zinc, and selenium. Comparison of dietary intake at the beginning and at the end of the study showed that the intake of energy, carbohydrate, total fat, and saturated fat decreased significantly in both groups whereas the intake of protein and omega-6 fatty acids was significantly decreased only in the placebo group. There were no significant differences in other nutrients during the study period. At the end of the study, the physical activities in both groups were higher than the beginning of the study; however, this change was not significant. Moreover, there was no significant difference between the intervention and placebo groups at the end of the study (Table 3).

Serum lipid profiles of patients are shown at the beginning and at the end of the study in Table 4. At the beginning and at the end of the study, the mean of TG, HDL-C, total cholesterol, and LDL-C concentrations did not differ significantly between the two groups ($p > .05$). At the end of the study, both serum levels of TG and LDL-C decreased, and serum HDL-C concentration increased only in the intervention group nonsignificantly. After the effect of the confounders (BMI, WHR, energy, and physical activity) is adjusted, the results remained unchanged.

Serum glucose, insulin concentrations, insulin resistance index, and insulin sensitivity are shown in Table 5. Serum glucose and insulin concentrations as well as HOMA-IR and QUICKI did not show any significant difference between the two groups at the beginning of the study. *N. sativa* powder could significantly reduce serum glucose

TABLE 4 Serum lipid profiles in *Nigella sativa* and placebo groups before and after the study

Variables	*N. sativa* group			Placebo group			*p* value [a]	*p* value[b]
	Baseline	End of the study	Changes	Baseline	End of the study	Changes		
TG (mg/dl)	140.81 ± 52.16	136.95 ± 65.92	−3.86 ± 57.18	160.06 ± 76.36	152.86 ± 32.77	−7.20 ± 64.47	.780	.535
HDL-C (mg/dl)	37.25 ± 4.43	38.70 ± 9.13	1.45 ± 7.74	34.89 ± 5.01	34.42 ± 4.66	−0.47 ± 2.09	.291	.456
LDL-C (mg/dl)	80.10 ± 17.72	79.85 ± 23.82	−0.24 ± 18.58	86.46 ± 1.52	84.57 ± 17.55	−1.89 ± 28.61	.823	.459
TC (mg/dl)	151.13 ± 32.42	153 ± 27.74	1.86 ± 30.45	160.73 ± 30.59	157.20 ± 36.96	−3.53 ± 42.96	.657	.378

Abbreviations: HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglyceride.

[a]Adjusted for baseline value.[b]Additionally adjusted for weight, body mass index, waist-to-hip ratio, energy, and physical activity.

TABLE 5 Serum glucose, insulin concentrations, homeostatic model assessment for insulin resistance (HOMA-IR), and quantitative insulin sensitivity check index (QUICKI) in *Nigella sativa* and placebo groups before and after the study

Variables	*N. sativa* group			Placebo group			*p* value[a]	*p* value[b]
	Baseline	End of the study	Changes	Baseline	End of the study	Changes		
Serum glucose (mg/dl)	92.95 ± 15.29	85 ± 13.94	−7.95 ± 11.17	95.77 ± 18.29	94.55 ± 15.33	−1.22 ± 7.70	.041	.033
Insulin (mU/L)	12.32 ± 4.61	8.45 ± 4.32	−3.87 ± 3.27	12.46 ± 5.22	11.39 ± 6.07	−1.07 ± 4.11	.027	.041
HOMA-IR	2.82 ± 1.13	1.80 ± 1.08	−1.02 ± 0.74	3 ± 1.44	2.72 ± 1.67	−0.28 ± 1.11	.021	.024
QUICKI	0.332 ± 0.022	0.360 ± 0.032	0.03 ± 0.023	0.331 ± 0.025	0.337 ± 0.024	0.006 ± 0.014	.002	.009

[a]Adjusted for baseline value.[b]Additionally adjusted for weight, body mass index, waist-to-hip ratio, energy, and physical activity.

(p = .041), insulin (p = .027), and HOMA index (p = .021), whereas significantly increased QUICKI (p = .002). This significant difference was maintained between the two groups after adjusting for the known confounders.

Hepatic enzymes, steatosis, and fibrosis in two groups are shown in Table 6. Hepatic enzymes were not significantly different within and between groups. After the effect of the confounders (BMI, WHR, energy, and physical activity) is adjusted, the differences between two groups remained insignificant. At the end of the study, hepatic steatosis score and the percentage of steatosis significantly reduced in both intervention and placebo groups. Hepatic steatosis percentage reduced significantly more in the intervention group compared with placebo group after adjusting for confounding factors (p = .005). No side effects were reported during study from any participant.

4 | DISCUSSION

Our results have shown that 2-g/day *N. sativa* supplementation along with dietary recommendations is superior to dietary recommendation alone in reduction of serum glucose, insulin resistance, and hepatic steatosis. Interestingly, weight and BMI decreased significantly only in *N. sativa* group whereas energy intake reduced significantly in both groups. It is known that weight change is induced through alteration in energy intake or energy expenditure. Because energy intake and physical activity were not different between two groups, it seems that *N. sativa* might decrease body weight through rising basal metabolism. Although no study has yet evaluated the effects of *N. sativa* on basal metabolism, previous studies have shown the beneficial effects of *N. sativa* on metabolic disturbances in patients with diabetes (Kaatabi et al., 2015) and hyperlipidemia (Sabzghabaee et al., 2012).

TABLE 6 Hepatic features in *Nigella sativa* and placebo groups before and after the study

Variables	*N. sativa* group			Placebo group			*p*	*p*
	Baseline	End of the study	*p* value*	Baseline	End of the study	*p* value*	value**	value***
ALT (IU/L)	20.0 ± 10.4	18.3 ± 16	.64	23.9 ± 9.2	22.5 ± 6.8	.59	.35	.59
AST (IU/L)	15.8 ± 10.6	16.6 ± 13.7	.75	15.2 ± 4.5	14.2 ± 4.7	.60	.51	.55
Fibrosis score[a]	1.45 ± 1.1	0.97 ± 0.80	.078	1.23 ± 1.22	1.09 ± 1.14	.75	.15	.46
Steatosis grade[a]	2.77 ± 0.52	2.04 ± 1.04	.005	2.61 ± 0.69	2.25 ± 0.856	.02	.48	.42
Percentage of steatosis[a]	79.50 ± 9.94	56.7 ± 28.5	<.001	74.4 ± 11.8	66.12 ± 18.37	.04	.05	.005

[a]All data are according to FibroScan exam.

*p values indicate comparison within groups.**p values indicate comparison between the variables between two groups after 12 weeks.

***p values indicate comparison between the variables between two groups at the end of the study after adjusting the effect of the confounders (body mass index, waist-to-hip ratio, metabolic equivalents, dietary energy intake, and baseline value of the outcome).

In this study, serum levels of liver enzymes (ALT and AST) did not change significantly in both groups. In both groups, the concentration of liver enzymes decreased due to dietary and physical activity recommendations; however, this decrease was not significantly different between and within groups. It seems that the reason for these results is the normal level of serum liver enzymes at the beginning of the study. Hussain et al. (2017) have reported that administration of 2-g *N. sativa* powder for 12 weeks resulted in a significant reduction in serum levels of ALT and AST enzymes in NAFLD patients. The main difference between these studies is that liver enzymes were in normal range in our study, which might be the reason for detecting no significant reduction after intervention. Thymoquinone as an active ingredient of black seed has protective effects on oxidative stress in the liver cells, and it also improves the fatty acid beta-oxidation and alleviates lipid accumulation in the liver (Balbaa, Abdulmalek, & Khalil, 2017; Hosseinian et al., 2018; Khaldi et al., 2018). One of the other mechanisms of thymoquinone in reducing levels of liver enzymes is improvement in the mitochondrial function and the production of ATP (Sayed-Ahmed & Nagi, 2007).

In the present study, concentration of lipid profiles (TG, LDL-C, and total cholesterol) did not significantly decrease in both groups at the end of the study. It seems that the normal lipid profile of both groups at the beginning of the study is responsible for these results. Previous studies in patients with hyperlipidemia have shown that 2 g/day *N. sativa* supplementation reduces blood lipids significantly (Badar et al., 2017; Farzaneh et al., 2014; Sabzghabaee et al., 2012). The effects of black seed on the lipid profile are due to presence of antioxidants such as thymoquinone, tocopherol, and phytosterol, which can prevent LDL-C oxidation, inhibit intestinal absorption of cholesterol, reduce the production of hydroxyl–methyl–glutaryl coenzyme A reductase (HMGCR), and improve the expression of LDL receptor genes by reducing intracellular cholesterol (Al-Naqeeb & Ismail, 2009; Brufau, Canela, & Rafecas, 2008; Mariod, Ibrahim, Ismail, & Ismail, 2009).

The results of this study showed that receiving black seed powder had a significant reduction in fasting serum glucose and insulin, HOMA-IR, and a significant increase in the QUICKI in the intervention group compared with the placebo group. These results remained significant after adjusting for known confounding variables. The results of this study were consistent with the results of previous studies (Kaleem, Kirmani, Asif, Ahmed, & Bano, 2006; Khan et al., 2015; Meral, Yener, Kahraman, & Mert, 2001). The mechanisms of this effect can be explained by the effects of *N. sativa* on regeneration of beta-pancreatic cells, which leads to insulin secretion, a decrease in liver gluconeogenesis, and stimulation of insulin sensitivity in peripheral tissues (Benhaddou-Andaloussi et al., 2008; Fararh et al., 2004; Kanter, Meral, Yener, Ozbek, & Demir, 2003).

Another finding of this study was that *N. sativa* reduced hepatic steatosis percentage more than placebo. None of the previous studies investigated the steatosis using transient elastography. The possible mechanism for this reduction of steatosis might be related to the inhibition of lipid oxidation and the antioxidants effect by *N. sativa* (R Moschen, Wieser, & Tilg, 2012).

This study has some strengths; it is the first randomized, double-blind, placebo-controlled clinical trial that evaluated the superiority of *N. sativa* plus lifestyle modification to lifestyle modification alone on CVD risk factors in patients with NAFLD. Using FibroScan for assessment of hepatic steatosis and fibrosis is another advantage of this study. Another strength of this study is that we used the edible black seed for intervention, which is available for general population to use it as an additive in their diet.

This study has some limitations. First of all, we did not use liver biopsy because it is an invasive procedure and nonethical; however, we used FibroScan as a valid test for evaluation of hepatic steatosis and fibrosis (Malekzadeh & Poustchi, 2011; Saadati et al., 2018). Another limitation of this study was that we did use only one dosage of black seed, which might be low to affect on some parameters of CVD risk factors.

5 | CONCLUSION

In conclusion, this randomized, double-blind, placebo-controlled clinical trial shows that daily intake of 2-g *N. sativa* plus lifestyle modification is superior to lifestyle modification alone in amelioration of insulin resistance and hepatic steatosis in patients with NAFLD. Further studies with different dosages and duration of *N. sativa* supplementation are required to be able to conclude about the effects of this dietary supplement on cardiovascular risk factors and NAFLD characteristics.

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CONFLICT OF INTEREST

There is no conflict of interest.

AUTHORS' CONTRIBUTIONS

Azita Hekmatdoost, Mina Darand, Zahra Darabi, Behnam Hosseini-Ahangar, and Seyed Moayyed Alavian conceived and designed the study and provided administrative, technical, or material support. Mehdi Hedayati provided lab assessments. Mina Darand and Zahra Yari analyzed and interpreted the data. Mohammad Amin Shahrbaf and Azita Hekmatdoost drafted the manuscript. Azita Hekmatdoost critically revised the manuscript for important intellectual content. And Azita Hekmatdoost obtained funding and supervised the study.

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