Complementary Therapies in Medicine 44 (2019) 204–209

Contents lists available at ScienceDirect

Complementary Therapies in Medicine

journal homepage: www.elsevier.com/locate/ctim

Nigella sativa and inflammatory biomarkers in patients with non-alcoholic fatty liver disease: Results from a randomized, double-blind, placebo-controlled, clinical trial

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ARTICLE INFO

Keywords:
Nigella sativa
Black seed
Fatty liver
NAFLD

ABSTRACT

Objective: The aim of this study was to assess the effects of *Nigella sativa* consumption on inflammatory biomarkers in patients with Non-alcoholic fatty liver disease (NAFLD).
Methods: This is a randomized, double-blind, placebo-controlled clinical trial. Fifty NAFLD patients were assigned to receive either two gram/day *Nigella sativa* seed as *Nigella sativa* group (NSG), or two gram/day starch as placebo group (PG) for 12 weeks.
Results: At the end of the study, the serum levels of tumor necrosis factor-α (TNF-α) decreased significantly compared with the beginning of the study in both groups, while the levels of high sensitive C reactive protein (hs-CRP) and nuclear factor kappa-B (NF-κB) only decreased significantly in the NSG ($P 0 < 0.05$). Only reduction in the serum levels of TNF-α was significantly more in NSG compared to the PG ($P = 0.001$). After adjusting the effects of confounding factors, the results remained unchanged. According to Fibroscan exam, hepatic steatosis and its percentage decreased significantly only in the NSG ($P 0 < 0.005$); however, the changes were not significantly different between two groups. After adjusting for confounding factors, only steatosis percentage reduction was significantly more in the NSG compared to PG ($P = 0.005$).
Conclusion: Our results have shown that two gram/day consumption of *Nigella sativa* can reduce inflammatory biomarkers in patients with NAFLD. Further studies with different doses are highly recommended to find the optimal dosage.

1. Introduction

The global prevalence of Non-alcoholic fatty liver disease (NAFLD) is currently estimated to be 25.24%, with the highest rates in the Middle East and South America (32, and 31% respectively).[1,2] The disease is related to increased healthcare costs and resource utilization and reduced quality of life.[3] NAFLD may progress to non-alcoholic steatohepatitis (NASH), and cirrhosis if it is not diagnosed and treated.[4]

Despite extensive research in recent years, specific treatment has not yet established to be effective except lifestyle modifications.[5–7] It has been shown that these effects can be augmented when they accompanied with some dietary supplements.[8–10]

Since oxidative stress plays an important role in the progression of NAFLD to hepatocellular carcinoma (HCC),[11] the use of antioxidant agents for treatment of NAFLD has been flourished in recent years.[12]

Nigella sativa (Black seed) is an annual flowering herb which belongs to the Ranunculacea family.[13] Its seeds and oil are widely used in the treatment of various disorders due to its wide range of pharmacological properties.[14] *Nigella sativa* seeds contain protein (26.7%), fat (28.5%), carbohydrates (24.9%), crude fiber (8.4%), and some vitamins and minerals. Among the active ingredients of *Nigella sativa*, thymoquinone (TQ) is one of the most important ingredients, which has anti-oxidant, anti-inflammatory and immunoprotective properties.[15] It seems that these properties make *Nigella sativa* as a good choice for prevention of inflammation progression in NAFLD.[16,17] The aim of this clinical trial was to investigate the effects of *Nigella sativa* seed consumption on inflammatory biomarkers and hepatic features of NAFLD.

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https://doi.org/10.1016/j.ctim.2019.04.014
Received 22 January 2019; Received in revised form 3 April 2019; Accepted 19 April 2019
Available online 22 April 2019
0965-2299/

2. Methods and materials

2.1. Study design

The study protocol was approved by the National Nutrition and Food Technology Research Institute of Shahid Beheshti University of Medical Science ethics committee. The trial was registered at Iranian Registry of Clinical Trials (http://www.irct.ir/IRCT20100524004010N25).

This randomized, double-blind, placebo-controlled clinical trial was performed at a Hepatology clinic, Tehran, Iran. Inclusion criteria included patients who were 18 years or older with evidence of NAFLD, while hepatic steatosis approved in Fibroscan exam with Controlled Attenuation Parameter (CAP) score > 263 (dB/m); no history of alcohol consumption; absence of other liver disorders (hepatitis B or C), biliary, autoimmune, malignancies, cardiovascular, respiratory and kidney disorders; absence of hepatotoxic medication consumption such as phenytoin, lithium and other drugs; absence of *Nigella Sativa* consumption in their daily diet; absence of consumption of dietary supplements in the last three months; absence of pregnancy or lactation. The exclusion criteria were consumption of less than 90% of the *Nigella Sativa* capsules at the end of the sixth and twelfth weeks; more than ten percent weight loss from baseline body weight during the intervention period for any reason; occurrence of any hepatic or inflammatory disease, and unwillingness to continue the study protocol. Written informed consent was signed by all participants after explaining the study protocol for them.

2.2. Sample size

The sample size was calculated for the Fibroscan controlled attenuation parameter (CAP) score, which was based on detection of a 10 unit (dB/m) difference in the mean CAP score with a power of 80% (β = 20%), yielding a sample size of 21 for each group. Given the probability of samples loss, twenty five patients in each group were considered.[18]

2.3. Randomization and supplements

Eligible patients were randomly assigned to receive 4 capsules containing either 500 mg *Nigella sativa* seed powder (2 g per day) or the same amount of starch as placebo.

Randomization was conducted according to random table generated by computer. Both capsules were identical and the cover was dark to hidden the color of content.

Nigella sativa seeds were purchased from a farm in Isfahan, Iran. Seeds were milled and encapsulated in 500 mg capsules and placed in sealed bottles. Bottles were labeled as A or B by a third person. All investigators and participants were blinded to the treatment assignment groups.

2.4. Follow-up assessments

Participants were visited every 4 weeks. Physical activity was evaluated at the baseline and the end of study using the metabolic equivalent of task (MET) questionnaire.[19]

Dietary intakes were evaluated through three 24-hour recalls (two weekdays and one weekend) at baseline and final visits. Nutrients intakes were calculated according to our national food composition tables.[20]

At the first visit, participants received an advice on energy-balanced diet and physical activity recommendations based on the Clinical Guidelines from the National Institutes of Health.[21] Anthropometric measurements were assessed at the baseline and the end of study as described previously.[22]

Probable adverse effects were asked in each visit, and patients' compliance was assessed according to remained capsules count at second and third visits.

At first and third visits, fasting blood samples were taken from each participant and were centrifuged immediately to extract serum and peripheral blood mononuclear cells. The samples were kept in -80°c until the lab exams were done.

2.5. Paraclinical assessments

Biochemical measurements were accomplished in the same lab by an expert technician. Gamma-glutamyltransferase (GGT), alanine aminotransferase, and aspartate aminotransferase (AST) concentrations were measured using photometric assay (Reckon Diagnostics, Vedodara, India). Fasting high-sensitivity C reactive protein (hs-CRP; (Diaclone, france), and tumor necrosis factor α (TNF-α; Zillbio, Germany) concentrations were measured using an enzyme-linked immunosorbent assay. Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) p65 was assessed in peripheral blood mononuclear cells (PBMC) nuclear extracts using ELISA kits (Cell Signaling) according to the manufacturer's protocol. Hepatic steatosis and fibrosis were measured using FibroScan® exam. It was done at the beginning and the end of the intervention by the same equipment and the same hepatologist. CAP score was used to measure the hepatic steatosis. CAP score of more than 263 was considered as inclusion criteria, which means grade two or more of hepatic steatosis.

2.6. Primary and secondary outcomes

The primary outcome measure was a significant reduction in CAP score in Fibroscan. Secondary outcome measures were the hepatic fibrosis, inflammatory biomarkers concentrations in serum and PBMCs, and serum concentrations of ALT, AST, and GGT.

2.7. Statistical analysis

Statistical analyses were performed using SPSS software (version 20). Data were described by mean ± standard deviations (SD) and frequency (percentages) for quantitative and qualitative variables, respectively. Normal distribution of data was evaluated using Kolmogorov-Smirnov test. Comparisons of the mean qualitative and quantitative variables between the two groups of the study were assessed by Chi-Square test and Student's *t*-test. Paired *t*-test was used to compare the mean of normal quantitative variables in each group and 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. Analysis of covariance test was used to eliminate the effects of confounding factors. P-values of less than 0.05 were considered as statistically significant.

3. Results

Totally, seventy patients were assessed for eligibility and 50 patients who met the inclusion criteria, enlisted and underwent randomization. Four patients in the placebo group (PG) and 1 patient in the *Nigella sativa* group (NSG) decided not to continue the study protocol. Also, two patients in NSG consumed less than 90% of the capsules, and were excluded from the study. Finally, 22 patients in the NSG and 21 patients in the PG completed the study protocol (Fig. 1).

Baseline demographic and metabolic profiles of the study groups were similar except for waist circumference (108.09 ± 9.21 versus 102.19 ± 8.66, P value = 0.037) and waist to hip ratio (WHR) (0.97 ± 0.04 versus 0.92 ± 0.06, P value = 0.006) (Table 1).

Weight, body mass index (BMI), hip circumference (HC), and waist to hip ratio (WHR) decreased significantly in NSG (P 0 < 0.05), whereas dietary energy intake decreased in both groups (P 0 < 0.05). There was no significant difference between two groups in any of the

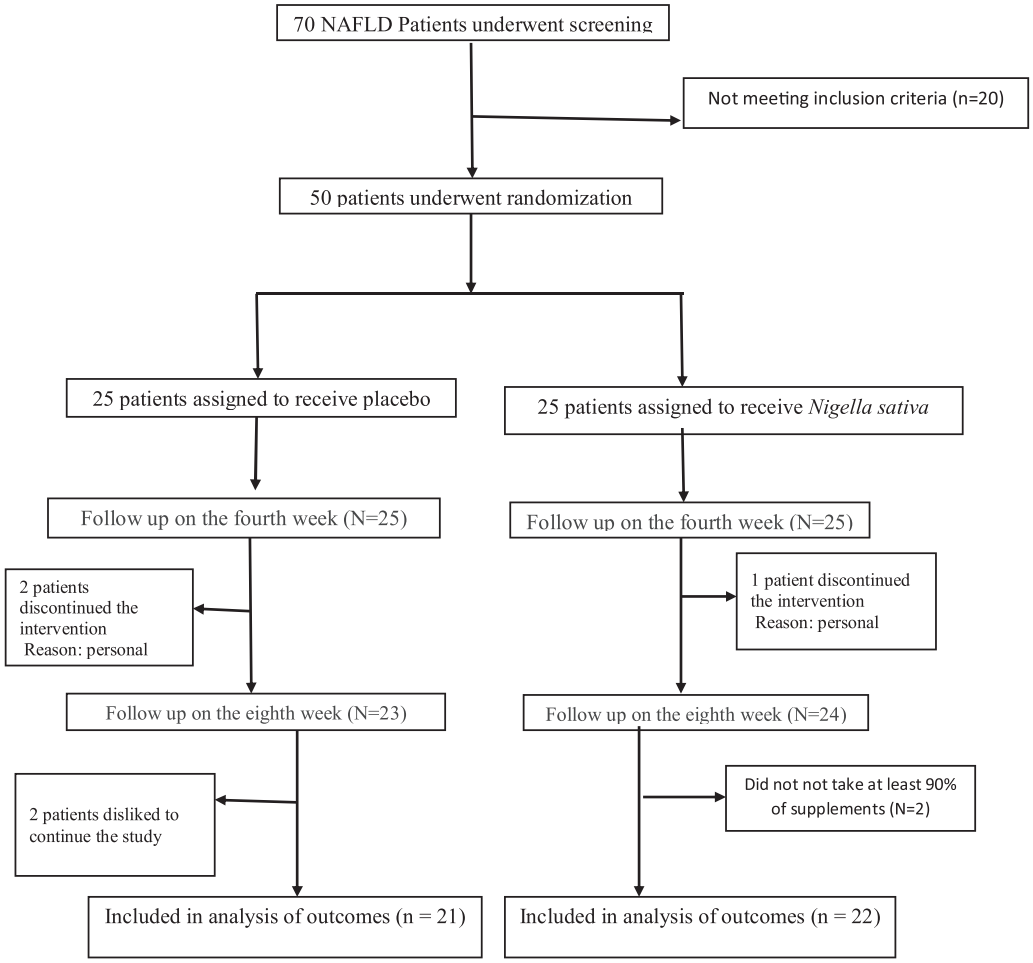

Fig. 1. Participants enrolment diagram.

anthropometric indices and energy intake ($p > 0.05$) (Table 2).

As it is shown in Table 3, there was no significant different between and within groups for hepatic enzymes. At the end of the study, in both intervention and PGs, the serum levels of TNF-α decreased significantly compared with the beginning of the study, while the levels of hs-CRP and NF-κB only decreased significantly in the intervention group ($P 0 < 0.05$). Only, reduction in the serum levels of TNF-α was significantly more in the intervention group compared to the PG ($P = 0.001$). After adjusting the effects of confounding factors, the results remained unchanged (Table 4).

At the end of the study, hepatic steatosis and its percentage decreased significantly only in the NSG ($P 0 < 0.005$); however, the changes were not significantly different between two groups. After adjusting for confounding factors, the reduction in steatosis percentage was significantly more in the NSG compared to PG ($P = 0.005$). There were no significant changes in hepatic fibrosis between and within two groups (Table 5). No participants complained about any side effects of supplements.

4. Discussion

To our knowledge, this study is the first randomized, double-blind, placebo-controlled, clinical trial that investigated the effects of *Nigella sativa* consumption on inflammatory markers in NAFLD patients. Our findings indicated that 12 weeks consumption of two gram/day *Nigella sativa* reduced some inflammatory biomarkers in NAFLD patients.

Although there is no study evaluating the effects of *Nigella sativa* on inflammation in NAFLD patients, there are some studies that assessed the effects of this dietary seed or its extracts on other inflammatory disorders.[23,24] The results of these studies are controversial.[24–26] Only few studies have used *Nigella sativa* seeds, and the majority of studies have focused on the seeds extract, oil, and the active components. Thus, comparing the dosages of *Nigella sativa* in different studies is challenging. Comparing studies using the whole seeds indicate that dosages ≥2 g/day can show anti-inflammatory effects. Salem et al.[27] have evaluated the effect of *Nigella sativa* supplementation on airways inflammation in partly controlled asthma patients. They did not observe any significant difference in inflammatory biomarkers between one and two gram supplementation of total seeds in this study, while Mahdavi et al.[23] reported significant reduction in inflammatory biomarkers after supplementation with 3 g/day of *Nigella sativa* oil plus low calorie diet in obese participants. In our study, two gram supplementation of total seeds plus lifestyle modification reduced all measured inflammatory cytokines significantly; however, this reduction was only significantly more in TNF-α when compared to PG who received only lifestyle modification advice. This might be explained by the pivotal role of lifestyle modification in amelioration of NAFLD features and inflammation.[28]

The mechanism of anti-inflammatory effects of *Nigella sativa* can be partly due to its anti-oxidative properties,[29] which inhibits production of reactive oxygen species (ROS) leading to less cell damages, and consecutively reduces the inflammation. Another mechanism reported by Koshak et al.[30] is that *Nigella sativa* inhibits inflammatory mediators in T-lymphocytes and monocytes, while increases Proglandin (PG) E-2 production leading to suppression of more inflammatory PGs production. Koshak et al have shown that as much as serum TQ levels increase,

Table 1
Baseline characteristics of NAFLD patients.

Characteristics	NSG (n = 22)	PG (n = 21)	P
Sex, n (%)	11 (50)	10 (47)	1.000
Male	11 (50)	11 (53)	
Female			
Smoking	0	2	0.45
Yes	22	19	
No			
Age (y)	48.86 ± 12.74	46.1 ± 10.97	0.45
Male	43.64 ± 12.18	38.4 ± 6.71	0.23
Female	54.1 ± 11.51	53.1 ± 9.33	0.82
Metabolic characteristic			
Height (cm)	168 ± 10.15	165.66 ± 10.71	0.43
Weight (kg)	90.94 ± 15.24	86.9 ± 11.56	0.33
BMI (kg/m2)	32.05 ± 4.17	31.7 ± 3.54	0.77
WC (cm)	108.09 ± 9.21	102.19 ± 8.66	0.03
WHR	0.97 ± 0.04	0.92 ± 0.06	0.007
MET (h/d)	31.57 ± 3.48	30.11 ± 5.31	0.29
Energy intake (kcal)	2644.49 ± 670.60	2437.65 ± 521.63	0.31
Serum biochemistry tests			
ALT (IU/l)	20.06 ± 10.48	23.93 ± 9.20	0.23
AST (IU/l)	15.87 ± 10.66	15.28 ± 4.56	0.83
GGT (IU/l)	26.44 ± 16.8	28.60 ± 8.9	0.63
Inflammatory factors			
hs-CRP (mg/L)	4959.63 ± 3391.89	5057.53 ± 3291.26	0.93
TNFα (pg/mL)	17.71 ± 6.47	18.68 ± 2.18	0.58
NF-κB (ng/mL)	2.08 ± 0.38	2.26 ± 1.09	0.46
Liver characteristics			
Steatosis (dB/m)	319.72 ± 59.39	310.05 ± 36.53	0.526
Fibrosis (KPa)	5.45 ± 1.1	5.23 ± 1.22	0.54

Values are means ± SD, unless otherwise indicated.
P values indicate differences between the Placebo and NSGgroups at baseline. According to Fibroscanassaya.
NSG: Nigella sativa group; PG: placebo group.

inflammatory mediators in T-lymphocytes and monocytes decrease. Moreover, they have reported elevation in PGE_2 production after 4 weeks consumption of one gram/day *Nigella sativa* oil.[30]

Our analysis showed that hepatic steatosis percentage reduced in the NSG significantly more than PG after adjustment for the known confounding factors (BMI, WHR, MET, dietary energy intake and baseline value of the outcome); however, hepatic enzymes, steatosis score, and fibrosis score were not significantly different at the end of the study. Reduction of hepatic steatosis percentage might be explained by the role of TNF-α in insulin resistance.[18] So, reduction in TNF-α might resulted in decrease in insulin resistance and consequently, reduction in hepatic steatosis.

No significant changes in liver enzymes and hepatic fibrosis might be due to the normal level of them at the baseline of the study, which indicates that patients were in primary stages of disease. Moreover, since we used the whole seed, patients received less amounts of total TQ compared to the plant extracts, while they got other beneficial components of it such as fiber and vitamins. Thus, it seems that higher dosages of *Nigella sativa*, and longer duration of intervention may result in more effectiveness of it on liver characteristics.

The current study had several strengths including design of study as a randomized, double- blind, placebo- controlled, clinical trial; a relatively high participation rate (86%); a moderately low drop-out rate; the NF-κB activity measurement in PBMCs; evaluation the effects of

Table 3
The effects of *Nigella sativa* and placebo intake on serum level of liver enzymes after 12-week treatment.

Characteristics	Baseline (means ± SD)	After 12 wk (means ± SD)	P[a]	P[b]	P[c]
ALT (IU/L)					
NSG	20.06 ± 10.48	18.38 ± 16.07	0.64	0.35	0.59
PG	23.93 ± 9.20	22.53 ± 6.87	0.59		
AST (IU/L)					
NSG	15.87 ± 10.66	16.67 ± 13.74	0.75	0.51	0.55
PG	15.28 ± 4.56	14.23 ± 4.74	0.60		
GGT (U/L)					
NSG	26.44 ± 16.80	22.41 ± 14.69	0.99	0.31	0.78
PG	28.60 ± 8.9	26.93 ± 10.60	0.46		

[a] P values indicate comparison within groups.
[b] P values indicate comparison between the variables between 2 goups after 12 wk.
[c] P values indicate comparison between the variables between 2 goups at the end of the study after adjusting the effect of the confounders (BMI,WHR, MET, dietary energy intake and baseline value of the outcome). NSG: Nigella sativa group; PG: placebo group.

Table 2
Metabolic characteristics and energy consumption of patients in two groups at baseline and after 12 weeks.

Characteristics	Baseline (Mean ± SD)	After 12 weeks (Mean ± SD)	*P*[a]	% Change (Mean ± SD)	*P*[b]
Weight (kg)					
NSG	90.94 ± 15.24	88.56 ± 15.03	0.001	−2.3 ± 2.8	0.65
PG	86.9 ± 11.56	86.36 ± 14.32	0.47	−1 ± 5.3	
BMI (kg/m2)					
NSG	32.05 ± 4.17	31.2 ± 4.15	0.001	−.84 ± 1.03	0.83
PG	31.7 ± 3.54	31.52 ± 5.29	0.57	−.27 ± 1.91	
WC (cm)					
NSG	108.09 ± 9.21	106.81 ± 9.65	0.14	−1.27 ± 3.94	0.22
PG	102.19 ± 8.66	104.81 ± 9.36	0.53	−1.62 ± 10.10	
HC (cm)					
NSG	111.9 ± 9.53	108.36 ± 10.08	0.001	−3.54 ± 3.26	0.55
PG	111.23 ± 7.62	108.56 ± 8.04	0.07	−2.68 ± 5.51	
WHR					
NSG	0.97 ± 0.04	0.99 ± 0.05	0.05	.02 ± .04	0.46
PG	0.92 ± 0.06	0.97 ± 0.08	0.15	.03 ± .10	
Physical activity (MET/day)					
NSG	31.57 ± 3.48	33.30 ± 4.99	0.11	1.7 ± 5.01	0.95
PG	30.10 ± 5.31	31.74 ± 5.40	0.30	1.6 ± 7.1	
Energy intake (kcal/d)					
NSG	2644.49 ± 670.60	2125.46 ± 711.81	0.04	−467.26 ± 768.86	0.67
PG	2437.65 ± 521.63	1942.92 ± 625.05	0.005	−582.10 ± 730.47	

WC, waist circumference; HC, hip circumference; WHR, waist:hip ratio; MET, metabolic equivalent of task.
[a] P values indicate comparison within groups.
[b] P values indicate comparison between the changes of each variable between 2 goups. NSG: Nigella sativa group; PG: placebo group.

Table 4
The effects of *Nigella sativa* and placebo intake on inflammatory markers at baseline and after 12-week treatment.

Characteristics	Baseline (means ± SD)	After 12 wk (means ± SD)	P[a]	P[b]	P[c]
hs-CRP (mg/L)					
NSG	4959.63 ± 3391.89	3514.22 ± 3982.89	0.000	0.35	0.40
PG	5057.53 ± 3291.26	4509.86 ± 3288.45	0.48		
TNF-α (pg/mL)					
NSG	17.71 ± 6.47	14.02 ± 1.91	0.01	0.001	0.001
PG	18.68 ± 2.18	17.44 ± 1.77	0.01		
NF-κB (ng/mL)					
NSG	2.48 ± 0.38	2.09 ± 0.68	0.02	0.87	0.88
PG	2.26 ± 1.09	2.54 ± 0.95	0.17		

BMI, body mass index; HC, hip circumference.
[a] P values indicate comparison within groups.
[b] P values indicate comparison between the variables between 2 goups after 12 wk.
[c] P values indicate comparison between the variables between 2 goups at the end of the study after adjusting the effect of the confounders (BMI,WHR, MET, dietary energy intake and baseline value of the outcome). NSG: *Nigella sativa* group; PG: placebo group.

Table 5
The effects of *Nigella sativa* and placebo intake on liver characteristics at baseline and after 12-week treatment.

Characteristics	Baseline (means ± SD)	After 12 wk (means ± SD)	P[a]	P[b]	P[c]
Fibrosis grade(kPa)[d]					
NSG	5.45 ± 1.1	4.97 ± 0.80	0.07	0.71	0.46
PG	5.23 ± 1.22	5.09 ± 1.14	0.75		
Steatosis (dB/m)					
NSG	319.72 ± 59.39	287.12 ± 48.64	0.003	0.835	0.108
PG	310.05 ± 36.53	286.86 ± 54.63	0.071		
Steatosis %					
NSG	79.50 ± 9.94	56.7 ± 28.5	0.00	0.25	0.005
PG	74.42 ± 11.86	66.1 ± 18.3	0.42		

[a] P values indicate comparison within groups.
[b] P values indicate comparison between the variables between 2 goups after 12 wk.
[c] P values indicate comparison between the variables between 2 goups at the end of the study after adjusting the effect of the confounders (BMI,WHR, MET, dietary energy intake and baseline value of the outcome).
[d] According to Fibroscan assay NSG: *Nigella sativa* group; PG: placebo group.

Nigella sativa combined with lifestyle interventions; and the assessment of NAFLD using transient elastography with CAP assessment. This technique easily and noninvasively measures the average stiffness of the liver.[31] Moreover, using the whole seed has the advantage of including fiber and some vitamins and minerals, while its consumption is more convenient and practical than its oil extract products.

Our study had some limitations. Liver biopsy was not done for assessment of liver which is the gold standard for diagnosis of fatty liver. Since liver biopsy is an invasive method, we were not able to perform it; however, we used Fibroscan as a valid test for evaluation of hepatic steatosis and fibrosis.[31,32] Another limitation of this study is that we applied only one dosage of the *Nigella sativa* supplement, which makes it difficult to conclude about the optimum effective dosage of it.

In conclusion, the findings of this randomized, double-blind, placebo-controlled trial showed that 12 weeks supplementation with 2 g/day of *Nigella sativa* plus lifestyle modification might increase the effectiveness of lifestyle modification for treatment of NAFLD, which is probably through the reduction of inflammatory factors. Further clinical trial studies with different doses of *Nigella sativa*, and longer duration of intervention are strongly recommended.

Authors' contributions

MD and AH designed and conducted the study. AH supervised the study and received fund for it. SMA, BHA and AKH recruited the patients. ZY, ZD, MH, and SS were involved in study analysis and paraclinical exams. All authors approved the final manuscript.

The authors declare no competing interests.

Acknowledgement

This study was supported financially by a grant from Shahid Beheshti University of Medical Sciences to AH. Shahid Beheshti University of Medical Sciences, Tehran, Iran

References

1. Younossi Z, Anstee QM, Marietti M, et al. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. *Nat Rev Gastroenterol Hepatol.* 2018;15(1):11.
2. Lankarani KB, Ghaffarpasand F, Mahmoodi M, et al. Non alcoholic fatty liver disease in southern Iran: a population based study. *Hepat Mon.* 2013;13(5).
3. Younossi ZM, Henry L. Economic and quality-of-life implications of non-alcoholic fatty liver disease. *Pharmacoeconomics.* 2015;33(12):1245–1253.
4. Rinella ME. Nonalcoholic fatty liver disease: a systematic review. *Jama.* 2015;313(22):2263–2273.
5. Mokhtari Z, Gibson DL, Hekmatdoost A. Nonalcoholic fatty liver disease, the gut microbiome, and diet. *Adv Nutr (Bethesda, Md).* 2017;8(2):240–252.
6. Eslamparast T, Eghtesad S, Poustchi H, et al. Recent advances in dietary supplementation, in treating non-alcoholic fatty liver disease. *World J Hepatol.* 2015;7(2):204–212.
7. Saadati S, Hatami B, Yari Z, et al. The effects of curcumin supplementation on liver enzymes, lipid profile, glucose homeostasis, and hepatic steatosis and fibrosis in patients with non-alcoholic fatty liver disease. *Eur J Clin Nutr.* 2019;73(3):441–449.
8. Mofidi F, Yari Z, Poustchi H, et al. Effects of synbiotics supplementation in lean patients with nonalcoholic fatty liver disease: study protocol of a pilot randomized double-blind clinical trial. *Arch Iran Med.* 2016;19(4):282–284.
9. Rahimlou M, Yari Z, Hekmatdoost A, et al. Ginger supplementation in nonalcoholic fatty liver disease: a randomized, double-blind, placebo-controlled pilot study. *Hepat Mon.* 2016;16(1):e34897.
10. Yari Z, Rahimlou M, Eslamparast T, et al. Flaxseed supplementation in non-alcoholic fatty liver disease: a pilot randomized, open labeled, controlled study. *Int J Food Sci Nutr.* 2016;67(4):461–469.
11. Madan K, Bhardwaj P, Thareja S, et al. Oxidant stress and antioxidant status among patients with nonalcoholic fatty liver disease (NAFLD). *J Clin Gastroenterol.* 2006;40(10):930–935.
12. Singal AK, Jampana SC, Weinman SA. Antioxidants as therapeutic agents for liver disease. *Liver Int.* 2011;31(10):1432–1448.
13. Sultana S, Asif HM, Akhtar N, et al. Nigella sativa: monograph. *J Pharmacogn Phytochem.* 2015;4(4):103–106.
14. Ahmad A, Husain A, Mujeeb M, et al. A review on therapeutic potential of Nigella sativa: a miracle herb. *Asian Pac J Trop Biomed.* 2013;3(5):337–352.
15. Tavakkoli A, Mahdian V, Razavi BM, et al. Review on clinical trials of black seed (Nigella sativa) and its active constituent, thymoquinone. *J Pharmacopuncture.* 2017;20(3):179–193.
16. Asrih M, Jornayvaz FR. Inflammation as a potential link between nonalcoholic fatty liver disease and insulin resistance. *J Endocrinol.* 2013;218(3):R25–R36.
17. Darand M, Alavian SM, Hekmatdoost A. Nigella sativa and non-alcoholic fatty liver disease: a review of the current evidence. *Hepat Mon.* 2018;18(10).
18. Eslamparast T, Poustchi H, Zamani F, et al. Synbiotic supplementation in nonalcoholic fatty liver disease: a randomized, double-blind, placebo-controlled pilot study. *Am J Clin Nutr.* 2014;99(3):535–542.
19. Ainsworth BE, Haskell WL, Whitt MC, et al. Compendium of physical activities: an

update of activity codes and MET intensities. *Med Sci Sports Exerc.* 2000;32(9; SUPP/1):S498–S504.
20. 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.
21. Initiative NOE, Heart N, Lung, et al. *The practical guide: identification, evaluation, and treatment of overweight and obesity in adults.* The Institute; 2000.
22. Faghihzadeh F, Adibi P, Hekmatdoost A. The effects of resveratrol supplementation on cardiovascular risk factors in patients with non-alcoholic fatty liver disease: a randomised, double-blind, placebo-controlled study. *Br J Nutr.* 2015;114(5):796–803.
23. Mahdavi R, Namazi N, Alizadeh M, et al. Nigella sativa oil with a calorie-restricted diet can improve biomarkers of systemic inflammation in obese women: a randomized double-blind, placebo-controlled clinical trial. *J Clin Lipidol.* 2016;10(5):1203–1211.
24. Gholamnezhad Z, Havakhah S, Boskabady MH. Preclinical and clinical effects of Nigella sativa and its constituent, thymoquinone: a review. *J Ethnopharmacol.* 2016;190:372–386.
25. Gholamnezhad Z, Boskabady MH, Hosseini M. Effect of Nigella sativa on immune response in treadmill exercised rat. *BMC Complement Altern Med.* 2014;14:437.
26. Noor NA, Fahmy HM, Mohammed FF, et al. Nigella sativa amliorates inflammation and demyelination in the experimental autoimmune encephalomyelitis-induced Wistar rats. *Int J Clin Exp Pathol.* 2015;8(6):6269–6286.
27. Salem AM, Bamosa AO, Qutub HO, et al. Effect of Nigella sativa supplementation on lung function and inflammatory mediatorsin partly controlled asthma: a randomized controlled trial. *Ann Saudi Med.* 2017;37(1):64–71.
28. Ghaemi A, Taleban FA, Hekmatdoost A, et al. How much weight loss is effective on nonalcoholic fatty liver disease? *Hepat Mon.* 2013;13(12).
29. Namazi N, Mahdavi R, Alizadeh M, et al. Oxidative stress responses to Nigella sativa oil concurrent with a low-calorie diet in obese women: a randomized, double-blind controlled clinical trial. *Phytother Res.* 2015;29(11):1722–1728.
30. Koshak A, Wei L, Koshak E, et al. Nigella sativa supplementation improves asthma control and biomarkers: a randomized, double-blind, placebo-controlled trial. *Phytother Res: PTR.* 2017;31(3):403–409.
31. Malekzadeh R, Poustchi H. Fibroscan for assessing liver fibrosis: an acceptable alternative for liver biopsy: fibroscan: an acceptable alternative for liver biopsy. *Hepat Mon.* 2011;11(3):157.
32. Saadati S, Hekmatdoost A, Hatami B, et al. Comparing different non-invasive methods in assessment of the effects of curcumin on hepatic fibrosis in patients with non-alcoholic fatty liver disease. *Gastroenterol Hepatol Bed Bench.* 2018;11(Suppl. 1):S8–s13.