

## Research Article

# The Potential Protective Effect of Nigella Sativa Oil on Patients with Non-Alcoholic Fatty Liver Disease

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## Abstract

**Background:** Non-alcoholic fatty liver disease (NAFLD) is one of the metabolic disturbances associated with inflammation. Nigella Sativa (NS) seed oil has different chemical compounds including Thymoquinone (TQ), unsaturated fatty acids, and flavonoids. NSs are used as anti-inflammatory and antioxidants in medical sciences. This study aimed to investigate the effect of NS oil on several parameters in serum levels of patients with NAFLD.

**Methods:** Forty-four patients diagnosed with NAFLD participated in a randomized, double-blind, placebo-controlled clinical trial. Patients were randomly assigned into two groups; one receiving NS oil and the other receiving placebo (paraffin oil), for 8 weeks. Blood samples were taken from the patients at the beginning and the end of the study. Afterwards, liver enzymes (ALT, AST, and GGT), inflammatory markers (Hs-CRP, TNF- $\alpha$ , and IL-6), insulin, lipid profiles (total cholesterol, triglyceride, VLDL, LDL-C, and HDL-C), FBS, and blood pressure were measured.

**Results:** Consumption of NS seed oil as supplement decreased the FBS level, lipid profiles (TG, TC, LDL, VLDL), liver enzymes (AST and ALT), hs-CRP inflammatory marker, IL-6, TNF- $\alpha$ , while it increased the HDL-C levels, compared to the placebo group ( $P < 0.05$ ). Receiving NS oil had no significant effect on serum levels of insulin, blood pressure, and GGT in comparison with the beginning of the study ( $P < 0.05$ ).

**Conclusion:** NS seed oil supplements may decrease the liver enzymes and lipid profiles in the patients with NAFLD and play a protective role in the liver via reducing the inflammation in this group of patients.

**Keywords:** Nigella sativa; NAFLD; Insulin; Liver enzymes; Inflammation

## Abbreviations

NS: Nigella Sativa; WC: Waist Circumference; HC: Hip Circumference; WHR: Waist-to-Hip Ratio; BMI: Body Mass Index; TC: Total Cholesterol; TG: Triglyceride; HDL-C: High Density Lipoprotein Cholesterol; LDL-C: Low-Density Lipoprotein Cholesterol; VLDL-C: Very Low-Density Lipoprotein Cholesterol; AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase; GGT: Gammaglutamyltransferase; TNF- $\alpha$ : Tumor Necrosis Factor- $\alpha$ ; IL-6: Interleukin-6, hs-CRP: High Sensitivity C-Reactive Protein

## Introduction

Non-alcoholic fatty liver disease (NAFLD) is characterized by increased lipid accumulation in the liver [1]. NAFLD is derived from simple steatosis, and includes a wide range of liver diseases, from fibrosis to non-alcoholic steatohepatitis with varying degrees [2]. Liver fibrosis by itself is the best predictor of liver cirrhosis [3], NAFLD is considered as the liver manifestation of a metabolic syndrome and is associated with its clinical manifestations such as type-2 diabetes, obesity, dyslipidemia, and hypertension [4]. The prevalence of NAFLD in the world is 25.24%, the highest levels of it have been reported in the Middle East (31.8%) and South America

(30.5%) [5]. It is possible that the lesion progresses to fibrosis and liver failure; therefore, it is necessary to use laboratory tests and liver biopsy for timely diagnosis and evaluation of the severity of the disease and post-treatment follow-ups [6].

The cause of NAFLD is not fully known [7]. An accepted explanation is that the pathogenicity of NAFLD is a two-hit hypothesis, suggesting that insulin resistance, as the first blow, leads to fat accumulation in the liver, followed by the liver toward the second blow, which is the occurrence of oxidative stress indifferent organs [8]. An increase in the prevalence of NAFLD has been reported due to epidemiologic and pathophysiological communication with type-2 diabetes and obesity [9]. When glucose levels increase in diabetes or pre-diabetes, more substrate for triglyceride production is provided. In addition, this deficiency causes the secretion of very low-density lipoprotein and insulin resistance, and this process provides the conditions for fat accumulation in the liver. Insulin resistance is not only a factor in obesity and diabetes, but may also activate some cellular mechanisms of NAFLD, even in non-diabetic lean subjects [10]. Recently, weight loss and lifestyle modification have been suggested as the first lines of treatment used to treat NAFLD. Omega-3 fatty acid may help to treat NAFLD, and bariatric surgery in obese patients can also be helpful [11]. So far, no specific therapeutic

**Table 1:** Anthropometric characteristics of participants.

Variables	NS oil group				Placebo group				P-value <sup>*</sup>	p-value <sup>**</sup>	p-value <sup>***</sup>
	before	After	Changes	p-value <sup>#</sup>	Before	after	changes	p-value <sup>#</sup>			
Weight (kg)	82.59±11.48	82.63±11.09	0.04±1.16	0.85	79.72±15.24	79.56±15.40	-1.15±1.33	0.58	0.48	0.45	0.59
BMI(Kg/m <sup>2</sup> )	27.59±2.83	27.51±2.83	.08±0.27	0.14	27.67±4.37	27.53±4.33	-.4±0.52	0.2	0.94	0.96	0.64
WC (cm)	90.86±9.38	90.70±9.17	.15±1.24	0.55	96.18±2.96	96.77±2.68	0.59±1.9	0.163	0.12	0.07	0.13
WHR	0.93 ± 0.05	0.93 ± 0.05	.00±0.01	0.48	0.91 ± 0.06	0.91±0.06	.00±0.02	0.73	0.15	0.23	0.51

NS: Nigella Sativa; WC: Waist Circumference; HC: Hip Circumference; WHR: Waist-to-Hip Ratio; BMI: Body Mass Index

<sup>\*</sup>Comparison between values before the placebo consumption and NS oil consumption. <sup>\*\*</sup>Comparison between values after placebo consumption and after NS oil consumption. <sup>\*\*\*</sup>Comparison between the changes before and after intervention during placebo and NS group. #: were resulted from paired sample t-test.

**Table 2:** Comparison of biochemical parameters and blood pressure between Nigella Sativa oil group and placebo at baseline and after the intervention.

Variables	NS oil group				Placebo group				P-value <sup>*</sup>	p-value <sup>**</sup>	p-value <sup>***</sup>
	before	after	changes	p-value <sup>#</sup>	before	after	changes	p-value <sup>#</sup>			
SBP (mmHg)	121.4±17.61	121.22±17.36	-0.18±3.77	0.82	125.45±18.18	127.77±18.68	2.31 ± 7.89	0.18	0.45	0.23	0.18
DBP (mmHg)	80.54±11.53	81.04±11.10	0.5±2.61	0.38	79.45±10.22	77.59±13.28	-1.86±9.04	0.34	0.74	0.35	0.24
FBS (mg/dl)	101.13±8.71	94.09±7.41	-7.04±4.14	<0.01	101.40 ±7.13	100.09 ± 7.97	-1.31±2.69	0.06	0.91	0.01	<0.01
TG (mg/dl)	158.55±73.01	129.09±56.61	29.46±38.67	<0.01	137.31±25.48	135.95±34.26	1.36±18.47	0.73	0.20	0.62	<0.01
TC (mg/dl)	204.27±30.54	176.27±27.84	28.00±14.15	<0.01	191.45±19.06	192.22±22.20	0.77±14.41	0.96	0.10	0.04	<0.01
HDL (mg/dl)	34.54±7.55	45.13±4.63	10.59±6.91	<0.01	37.45±4.74	38.09±6.43	0.63±3.52	0.40	0.13	<0.01	<0.01
LDL (mg/dl)	131.18±32.74	107.81±26.67	23.36±16.96	<0.01	123.22±15.90	122.00±16.66	-1.22±8.62	0.51	0.31	0.04	<0.01
VLDL (mg/dl)	43.22±9.89	38.31±10.64	-4.90±2.86	<0.01	40.86±5.43	40.72±6.48	-0.13±2.73	0.81	0.33	0.37	<0.01
Insulin (MU/L)	16.44±5.64	17.23±7.55	0.78±3.37	0.28	14.48±3.7	14.8±3.59	0.32±2.72	0.58	0.18	0.18	0.61

TC: Total Cholesterol; TG: Triglyceride; HDL-C: High Density Lipoprotein Cholesterol; LDL-C: Low-Density Lipoprotein Cholesterol; VLDL-C: Very Low-Density Lipoprotein Cholesterol

<sup>\*</sup>Comparison between values before the placebo consumption and NS oil consumption. <sup>\*\*</sup>Comparison between values after placebo consumption and after NS oil consumption. <sup>\*\*\*</sup>Comparison between the changes before and after intervention during placebo and NS group. #: were resulted from paired sample t-test.

agent has been found for the treatment or prevention of NAFLD, so complementary and alternative therapies are required.

Nigella Sativa (NS), belonging to the family Ranunculaceae, is known as black seed or Cumin [12]. Seed components include carbohydrates 33-34%, protein 19-19.9%, fat 35.5%, fiber 5-6.5%, and saponin 0.013% [13]. The seed oil consists of certain chemicals including Thymoquinone (TQ)30-48%, linoleic acid 44.7-56%, oleic acid 20.7-24.6%, and linolenic acid 0.6 - 1.8% [14,15]. NS oil has been widely studied for its biological and therapeutic potential. It also acts as a diuretic, antihypertensive, anti-diabetes, anticancer, and immunosuppressive agent, liver protector, kidneys and stomach protectors, analgesic, bronchodilator, and antioxidant [16]. TQ has been used as an antioxidant as well as a protective agent against carbon tetrachloride-induced liver toxicity [17]. It also improves hepatic steatosis, prevents hepatic fibrosis, and is used as an inhibitor of the onset of NAFLD in mice with this disease [18]. The oil was very safe even in oral administration to mice [19].

The present study aimed to investigate the effects of this supplement (NS oil) on the improvement of inflammatory marker levels, liver enzymes, lipid profiles and insulin, and lowering blood glucose levels in the patients with NAFLD. Therefore, the supplement could be used as a complementary and alternative treatment which is cost-effective, and at the same time, has the least side effects for the patient.

## Materials and Methods

This clinical trial study was performed on the patients with

NAFLD referred to Golestan Hospital of Ahvaz from March 2017 to June 2017. The study was performed on 44 patients with NAFLD (22 patients in the intervention group and 22 patients in the control group). The research process was explained to all the participants, and the consent forms were signed by them. Patients were assured that all information obtained would be completely confidential. Of 44 patients, 29 were male and 15 were female. Ethical approval was obtained from the Institutional Ethics Committee.

### Inclusion criteria

Patients aged 20-60 years, diagnosed with NAFLD via ultrasonography, and certified by a gastroenterologist were included in the study.

### Exclusion criteria

Pregnant and lactating patients, those with liver transplantation, smokers, alcohol drinkers and drug users, patients taking medications such as corticosteroids, amiodarone, tamoxifen, methotrexate, those with rapid weight loss and diabetes mellitus, heart failure, renal diseases, hereditary hemochromatosis and Wilson, positive hepatitis C virus infection, and autoimmune hepatitis were excluded from the study. Patients were divided into two groups based on the therapeutic interventions.

### Groups

Group 1: (NS oil group) patients with NAFLD, who consumed 1g of NS oil, once a day, orally for 8 weeks

Group 2: (placebo group) patients with NAFLD, who consumed

**Table 3:** Comparison of liver enzymes between NS oil group and placebo at baseline and after the intervention.

Variables	NS oil group				Placebo group				P-value*	p-value**	p-value***
	before	after	changes	p-value#	before	after	changes	p-value#			
AST (u/l)	52.50 ± 9.49	36.90±12.72	-15.59±13.75	<0.01	49.54 ± 4.75	48.54 ± 5.69	-1.00 ± 4.02	0.25	0.19	<0.01	<0.01
ALT (u/l)	29.77 ± 3.39	24.18 ± 3.48	-5.59 ± 2.83	<0.01	28.27 ± 2.84	28.04 ± 7.26	-0.22 ± 6.45	0.87	0.12	0.03	<0.01
GGT (u/l)	40.27 ± 15.92	40.13 ± 20.79	-0.13 ± 7.49	0.93	32.04 ± 9.46	31.86 ± 9.35	-0.18 ± 3.21	0.79	0.12	0.21	0.97

AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase; GGT gammaglutamyltranspeptidase.

\*Comparison between values before the placebo consumption and NS oil consumption. \*\*Comparison between values after placebo consumption and after NS oil consumption. \*\*\*Comparison between the changes before and after intervention during placebo and NS group. #: were resulted from paired sample t-test.

**Table 4:** Comparison of inflammation factors between NS oil group and placebo at baseline and after the intervention.

Variables	NS oil group				Placebo group				P-value*	p-value**	p-value***
	Before	after	changes	p-value#	before	after	changes	p-value#			
HS-CRP (mg/l)	2.91 ± 0.73	2.52 ± 0.58	-0.38 ± 0.32	<0.01	2.70 ± 0.51	2.67 ± 0.81	-0.03 ± 0.65	0.79	0.28	0.49	0.02
TNF-α (pg/ml)	2.09 ± 0.46	1.65 ± 0.42	-0.43 ± 0.26	<0.01	1.93 ± 0.30	1.84 ± 0.43	-0.09 ± 0.29	0.13	0.20	0.15	<0.01
IL-6 (pg/ml)	2.67 ± 0.55	2.05 ± 0.74	-0.62 ± 0.51	<0.01	2.38 ± 0.65	2.29 ± 0.64	-0.08 ± 0.26	0.14	0.11	0.25	<0.01

TNF-α: Tumor Necrosis Factor-α, IL-6: Interleukin-6; hs-CRP: High Sensitivity C-Reactive Protein

\*Comparison between values before the placebo consumption and NS oil consumption. \*\*Comparison between values after placebo consumption and after NS oil consumption. \*\*\*Comparison between the changes before and after intervention during placebo and NS group. #: were resulted from paired sample t-test.

1g of paraffin oil once a day, for 8 weeks

NS oil was purchased from Barij Essence Co., Iran, and provided in the form of capsules. Patients in each group could receive medications prescribed by their physicians. Delivery of capsules (NS oil or placebo) to the patients and follow-ups were performed monthly. Each capsule contained 1000 mg of ground NS.

### Anthropometric measurements

While the participants were standing upright and without shoes, using their height transitions, a digital scale was used to measure their weight with a precision of 0.1 kg and their height in centimeter. Body mass index (BMI) was calculated by dividing the kilogram of body weight by the square of the height in meter. Waist circumference (WC) and hip circumference (HC) were measured with a measuring tape of 0.1 cm with no pressure on the body. WC and HC were measured with the lowest clothing and the closest surface of the skin.

### Biochemical analysis

Ten millilitre of venous blood was obtained from each patient before and after intervention. Samples were collected in dry tubes, centrifuged (3000 RPM, and 15 min), and then stored at -70°C by a trained engineer. Hematologic factors including fasting blood sugar (FBS), insulin, liver enzymes, and lipid profiles were assessed by an analyzer6a, and inflammatory factors by the ECL-e411 luminescence apparatus.

### Statistical analysis

All statistical analyses were performed with the SPSS version 22.0 for Windows. First the normal distribution of all variables was evaluated using the Kolmogorov-Smirnov test. For comparison of quantitative variables in two groups, independent t-test was used, and for before and after the t-test, and in case of non-normal variables, non-parametric equation was used.

## Results

Forty-four patients with NAFLD were divided into two groups: NS (n=22) and placebo (n=22). The mean age in the NS group

and placebo group were 39±5.37 and 42.22±8.85, respectively. Anthropometric parameters, and systolic and diastolic blood pressure of the two groups are provided in Table 1.

At the end of the intervention, the serum levels of FBS in the NS group significantly decreased compared to the placebo group (P<0.01). Intragroup changes in the NS group showed a significant decrease (P<0.01) (Table 2). Serum levels of insulin in the NS and placebo groups did not change significantly (P=0.61). In addition, there was a significant decrease in serum TG levels in the NS group compared to the control group (P<0.01). There was also a significant decrease in intragroup changes in the NS group (P<0.01). After eight weeks of intervention, serum HDL levels in the NS group were significantly higher than those in the placebo group (P<0.01), and intragroup changes in the NS group had a significant increase (P<0.01). Moreover, in the NS group, a significant decrease was observed in TC compared to the placebo group (P<0.01), and within the NS group, there was a significant decrease (P<0.01). There was a significant decrease in serum VLDL levels in the NS group (P<0.01), and intragroup changes were also significantly lower (P<0.01).

Serum AST (P<0.01) and ALT levels (P<0.01) significantly decreased in the intragroup variables. Additionally, there was a significant difference in the levels of AST (P<0.01) and ALT (P<0.01) between the NS and placebo groups, but the serum levels of GGT in the NS and placebo groups did not change significantly (P=0.97) (Table 3).

In the inflammatory factors, the difference was significant between the NS group and the placebo group: Hs-CRP (P=0.02), TNF-α (P<0.01), and IL-6 (P<0.01)(Table 4). Intragroup significant changes were observed in Hs-CRP (P<0.01), TNF-α (P<0.01), and IL-6 (P<0.01).

## Discussion

The results revealed that the consumption of NS oil (1000 mg/day) for 8 weeks could have beneficial effects on serum levels of FBS,

lipid profile, AST, ALT, TNF- $\alpha$ , Hs-CRP, and IL-6 in the patients with NAFLD; however, the levels of insulin and GGT did not change significantly.

Although the effects of NS oil on systemic inflammation in NAFLD and other chronic diseases have been studied in animals, conflicting results have been obtained. This is the first known study on the effect of NS oil on FBS, lipid profiles, inflammatory and infectious factors, and insulin in the patients with NAFLD.

Hussain, et al. showed a significant reduction in the body weight, BMI, AST, and ALT after 12 weeks, in the patients taking 1g of NS oil twice a day [20]. In another study, Najmi, et al. administered 2.5 mL NS oil twice a day for 6 weeks and showed a significant decrease in TC, LDL, and FBS [21]. Sabzeghabaee, et al. observed a significant decrease in TC, LDL, and TG levels in the hyperlipidemic subjects who used NS oil for 4 weeks at a dose of 2 g/day, while no beneficial effect was observed on FBS and HDL level [22]. In another study, Mahdavi, et al. demonstrated a decrease in insulin levels with consumption of 3 g NS oil daily for 8 weeks in obese women with no significant changes in liver enzymes [23]. Differences in the disease, the type and content of the NS oil, and the duration of the flavonoid are probably the reasons for these contradictory results. TQ, the active ingredient in NS oil, can up-regulate LDL receptors [24], inhibit 3-hydroxymethylglutaryl coenzyme reductase gene [24], and down-regulate APO-B100 [25]. It also reduces clearance and decreases the synthesis of LDL.

There are also many phytochemicals in NS oil that can affect hypocholesterolemia. Beta-cytosterol reduces the intestinal absorption of cholesterol [26,27] and is rich in unsaturated fatty acids which reduce and inhibit the oxidation of cholesterol [28]. Possible factors in reducing TG may be its compounds. High concentration of unsaturated fatty acids may also be effective in the synthesis and catabolism of TG rich lipoproteins [29]. TQ is a key ingredient that protects the fatty liver due to its antioxidant and anti-inflammatory activity (30-35). TQ may decrease by reducing oxidative stress via inhibition of cyclooxygenase 1 and 2 [36] and preventing the anti-inflammatory activity of 5-lipoxygenase [37]. TQ improves energy production in rat liver mitochondria [38]. Accordingly,  $\beta$ -oxidation of fatty acids can increase, and fat accumulation in the liver can be reduced. Heshmati, et al. found that NS (3 g/day) decreased FBS, insulin levels, and insulin resistance in type-2 diabetic patients after three months [39]. Bamaso, et al. showed that NS oil (2 g/day) reduced FBS and insulin resistance after 3 months in type-2 diabetic patients [40]. It is reported that NS phosphorylation stimulates acetyl coA carboxylase, involved in signalling AMPK, and acts as an agent in increased insulin sensitivity in the muscle and liver, and increases the gene expression of GLU T4 in the muscle [41]. Awad, et al. showed that TQ reduced the levels of AST and ALT in mice at high and low doses [42]. In one study, TQ and P-cymene extract from NS induced a significant increase in LFT serum levels by reducing the malondialdehyde (MDA) and tumor necrosis factor (TNF- $\alpha$ ) in mice with fatty liver [43]. In another study, TQ not only suppressed oxidative stress, but also reduced inflammation and improved conditions for fibrosis in the NAFLD [42].

## Conclusion

Overall, the results showed that administration of 1000 mg

of NS oil as a supplement for 8 weeks could decrease serum levels of FBS, lipid profiles (TG, TC, LDL, VLDL), liver enzymes (AST, ALT) and inflammatory factors (hs-CRP, TNF- $\alpha$ , and IL-6) and increase serum HDL levels in the patients with NAFLD. However, there was no significant effect on insulin and GGT. Therefore, NS oil supplement can be used as an adjuvant treatment for reducing systemic inflammation. Although this study confirmed the current hypothesis, studies with longer duration of therapy and higher doses, explaining the mechanisms of NS oil for the treatment of patients in general, are recommended to confirm our findings.

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