The effect of nigella sativa oil supplementation on serum levels of adiponectin, leptin and blood pressure in patients with non-alcoholic fatty liver. A double blind, placebo, controlled randomized clinical trial

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Abstract

Aim: Non-alcoholic fatty liver disease (NAFLD) is one of the metabolic disorders related with inflammation. Nigella sativa (NS) has various chemical compounds includes thymoquinone (TQ), unsaturated fatty acids and flavonoids. NS is used as anti-inflammatory and anti-oxidant in medical science. The aim of this study was to investigate the effect of NS oil supplementation on levels of adiponectin, leptin and blood pressure in patients with non-alcoholic fatty liver. Methods: This randomized, double-blind, placebo-controlled clinical trial was performed among 44 patients diagnosed with NAFLD. Subjects were randomly assigned to placebo group (n=22) and/or intervention group (n=22), supplemented with 1g/day of NS oil. The duration of the intervention was 8 weeks. Blood samples were collected at baseline and at the end of the intervention and serum levels of adiponectin, leptin and systolic and diastolic blood pressure were measured. Results: NS oil supplementation did not have a significant effect on serum levels of adiponectin and leptin. Also, no significant effects were seen with this supplementation on systolic and diastolic blood pressure among patients with NAFLD. Conclusions: In the current trial, 8 weeks of nigella sativa oil supplementation demonstrated did no significant effects on serum levels of adiponectin, leptin and blood pressure in people with NAFLD.

What's already known about this topic?

Previous studies showed the positive effects of nigella sativa oil.

There have been no human studies to investigate the effect of nigella sativa or its compound on leptin, adiponectin and blood pressure in patients with non-alcoholic fatty liver.

What does this article add?

This study is the first human investigation that was designed to evaluate the effect of nigella sativa supplementation on leptin, adiponectin and blood pressure in patients with NAFLD.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) characterized by increased fatty lipid accumulation in the liver[1]. NAFLD affects all age group but especially in people in their 40s and 50s who are at high risk of heart disease because of such risk factors as obesity and type 2 diabetes[2]. The condition is also closely linked to metabolic syndrome, which is a cluster of abnormalities including increased abdominal fat, poor ability to use the hormone insulin, high blood pressure and high blood levels of triglycerides [3]. The prevalence of NAFLD has been reported about 24% in the world. Reports indicate that the prevalence of metabolic

syndrome was significantly higher in NAFLD than healthy people (47% vs 23%)[4]. There is no effective treatment that completely reverses the effects of the NAFLD however healthy lifestyle such as dietary changes, regular physical activity and gradual weight loss lo that can improve the disease [5-7]. Increased prevalence of NAFLD is due to epidemiologic and pathophysiological communication with type 2 diabetes and obesity[8]. When glucose levels increase in diabetes or pre-diabetes, it provides more substrate for triglyceride production. In addition, deficit in the secretion of very low density lipoprotein (VLDL) is common in insulin resistance, and this process provides conditions for the accumulation of fat in the liver[9]. Insulin resistance is not just a factor in obesity, but it may also lead to NAFLD, even in thin individuals[9]. Adipose tissue is an active tissue that plays an important role in energy homeostasis, hormonal signaling, metabolic balance, and adipokines secretions. Evidence shows that the adipose tissue secretes more than 50 molecules signaling and hormones called adipokine [10]. Adipokines play a role in regulation of thermogenesis, appetite, glucose metabolism and insulin sensitivity[11]. Adiponectin is secreted from adipose tissue as a protein that has anti-inflammatory activity [12, 13]. It also plays a role in the metabolism of glucose and fats and plays an important role in reducing insulin resistance and the risk of cardiovascular disease[14]. Leptin is a hormone secreted from fat cells that helps to regulate body weight[15]. In human liver cells, leptin has some insulin-inducing activity that eventually causes insulin resistance. This insulin resistance, which is a common finding in patients with NAFLD, may be due to this role of leptin[16, 17].

Nigella sativa (NS) has been traditionally used in India, Arab countries, Europe and Iran to treat diseases such as asthma, hypertension, diabetes, inflammation, tumor, cough, bronchitis, headache, eczema, fever, dizziness, gastrointestinal disorders, impotence and Influenza[18] . NS has various chemicals including thymoquinone (TQ), unsaturated fatty acids and flavonoids[19, 20]. NS in medicine as anti-inflammatory and antioxidant used[21, 22]. TQ protects the liver from injury by several mechanisms such as inhibition of iron-dependent lipid peroxidation, elevation in glutathione level and total thiol content, radical scavenger, improving the activity of quinone reductase, catalase, superoxide dismutase and glutathione transferase, inhibition of NF-$\varkappa$B activity and inhibition of both lipoxygenase and cyclooxygenase also improves hepatic steatosis and prevents hepatic fibrosis[23]. Animal studies have shown that TQ diminishes thioacetamide-induced hepatic fibrosis and inflammation via activating LKB1-AMPK signaling pathway in mice[24, 25]. Since there is no study on the effects of NS oil on adiponectin, leptin levels and blood pressure in patients with NAFLD, we decided to perform this study.

2. Material and methods

2.1. Study design

In the present randomized, double-blind, placebo-controlled trial 44 people diagnosed with NAFLD aged 20-60 years participated. Exclusion criteria were: 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.

The target population selected from all adult men and women with non-alcoholic fatty liver referred to gastrointestinal clinic of Golestan hospital in Ahvaz. . This study was approved by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences (*IR.AJUMS.REC.1395.695*) and was registered in the Iranian Registry of Clinical Trials website (IRCT ID: IRCT2017012232109N1). The study goals and protocol were described to the patient and written informed consent was taken before participating in the study. Patients were randomly allocated into two groups by block randomization. Patients and researchers were blinded during the study. The subjects received NS oil (intervention group) or Paraffin Oil (placebo group) for 8 weeks. NS oil and paraffin oil soft gel purchased Barij Essence Company of Kashan. The placebo group received 1gr of paraffin oil soft gel and the intervention group received 1gr of NS oil soft gel for 8 weeks. Each group received the medication prescribed by the physician. Anthropometric measurements, including height, weight, waist and hip circumference were obtained and three days food record was recorded at the beginning and the last week of the study. Dietary intake was analyzed by Nutritionist IV software, modified

for Iranian foods.

2.2. Biochemical assays

Fasting blood samples was taken at the beginning and at the end of the intervention. The serum and plasma were separated by centrifugation at 3000 rpm for 10 min. The serum samples were stored at -70 °C quickly after centrifugation until analysis. Serum leptin level was determined by Enzyme-linked immunosorbent assay kits following the manufacturer's protocols (LDN Co., Nordhorn, Germany) and adiponectin (Booster Biological Technology Co., Wuhan, China).

2.3. Statistical assays

Data was analyzed using the SPSS software, version 22 (SPSS Inc., Chicago, USA). Quantitative data are reported as mean ± standard deviation (SD), and qualitative data are presented as frequency and percent. Normality of data was assessed by Kolmogorov-Smirnov test. Paired t-test was used for comparison before and after the intervention. Paired t-test was used to compare before and after the intervention. Independent-samples t-test was used to identify any differences between two treatment groups. Results were considered statistically significant at p-value < 0.05.

4. Results

The flow chart of participants and their follow-up is shown in Figure 1. A total of 44 cases completed the study. Four patients were excluded from the study because of personal reasons (three patient) and allergy to Nigella sativa oil (one patient). At baseline, there was no significant difference in general characteristics between the groups (Table 1). The results of our study showed that NS oil supplementation had no significant effect on serum levels of leptin and adiponectin ($p > 0.05$). Also, no significant effect were seen with this supplementation on systolic and diastolic blood pressure among patients with NAFLD (Table 2).

5. Discussion

The results of this study showed that NS oil supplementation at 1000 mg/day dosage had no significant effect on serum levels of leptin and adiponectin. Also our finding showed Nigella sativa oil supplementation no significant effect on systolic and diastolic blood pressure among patients with non-alcoholic fatty liver disease. In a study by Dehkordi et al on subjects with mild hypertension at a dose of 100 mg and 200 mg)twice daily(were conducted, a significant reduction in systolic blood pressure and diastolic blood pressure was shown [26]. Jarrin et al in an animal study in rats with induced hypertension NS oil daily at a dose of 2.5 mg / kg body weight Systolic and diastolic blood pressure levels were significantly reduced. This effect being reduced by stimulating nitric oxide synthesis and release from the vascular endothelium. The hypotensive effects may also be based on the diuretic activity [27] Also, in this study, the levels of angiotensin converting enzyme activity in the Nigella sativa oil group were reduced [28]. Bader et al in a study that was conducted in patients with type 2 diabetes, NS oil supplementation significantly reduced systolic and diastolic blood pressure in this patients[29].

Clinical trial studies on the effects of NS on adiponectin levels are limited. Our findings are in agreement with the findings of Datau et al, indicating that NS powder (3g/day) supplementation had no significant effect on adiponectin serum levels in men with abdominal obesity after 12 weeks [30]. In another study by Mahdavi et al, NS oil supplementation concurrent with a low low-calorie diet in obese women showed a significant increase in adiponectin levels [31]. In an animal study on rats with metabolic syndrome, the results showed that administration of NS oil increased adiponectin levels in these rats [32]. It has been shown that adiponectin regulate the interaction between obesity and insulin resistance, and a reduction in weight and body fat mass increases plasma levels of adiponectin [31]. The current study showed that NS oil supplementation for 8 weeks do not a significant effects on serum leptin levels, compared to the placebo group. In animal study by Güllü et al that evaluated the effects of TQ on leptin, a significant decrease in leptin plasma levels was observed [33]. No significant effect of NS oil was observed on SBP and DBP. Similar to the results of our study, Qidwai and Bin Sayeed showed that NS seed supplementation doese not

significant effect on SBP and DBP[34, 35]. In addition, Amin et al in another study have been shown that supplementation with Black seeds (1.5g/day) had no effect on SBP and DBP[36].

Also In contrast with our study, there are some studies that found NS had a significant beneficial effect on blood pressure. In clinical trial study by Dehkordi in patients with mild hypertension, NS seed exctract (two capsules per day containing 100 and/or 200 mg) supplementation for 8 weeks significantly lowered SBP and DBP[26]. Also, Najimi et al in a trial study investigated the effect 500mg/days of NS Oil supplementation on 45 patients with metabolic syndrome. They results after 8 weeks supplementation with of NS Oil have been shown a significant reduction in SBP and DBP[37]. There are several reasons for the inconsistent results between our study and another previous studies. Firstly, lack of hypertension in patients in this study. Secondly, the dosage and duration were not the same. Perhaps a higher dose with longer duration supplementation of NS is needed to produce an effect on leptin, adiponectin and blood pressure in patient with NAFLD. Further studies are needed to investigate the possible effect of black seed on leptin and adiponectin. The feasible mechanisms involved in the improvements in patient with NAFLD have been explained in previous study[38]. This effects may be connected to some factors like diuretic effect and calcium channels blocking feature of NS that are likely related to several components of NS involved in this effect, including TQ, and fatty acids that contain remarkable quantities of linoleic, oleic, and arachidonic acids, nigellicine, flavonoids, transanethole, p-Cymene, a-Pinene, limonene, carvone, and soluble fiber[38, 39]. NS compounds are also reported to have endothelium independent relaxation effects that may be due to suppression of Ca2+ release from the sarcoplasmic reticulum across the smooth muscle cells membrane and decrease of Ca2+ sensitivity and influx.

This is the first clinical trial to evaluate the effect of NS oil on the patient with NAFLD. The main limitations of our study were: short duration of intervention and small sample size.

Conclusion

The current study revealed that supplementation with NS oil after 8 weeks at dose of 1000 mg/day had no significant effects on serum levels of adiponectin, leptin and blood pressure in people with NAFLD**.**

Conflict of interest

The authors declare no conflict of interest.

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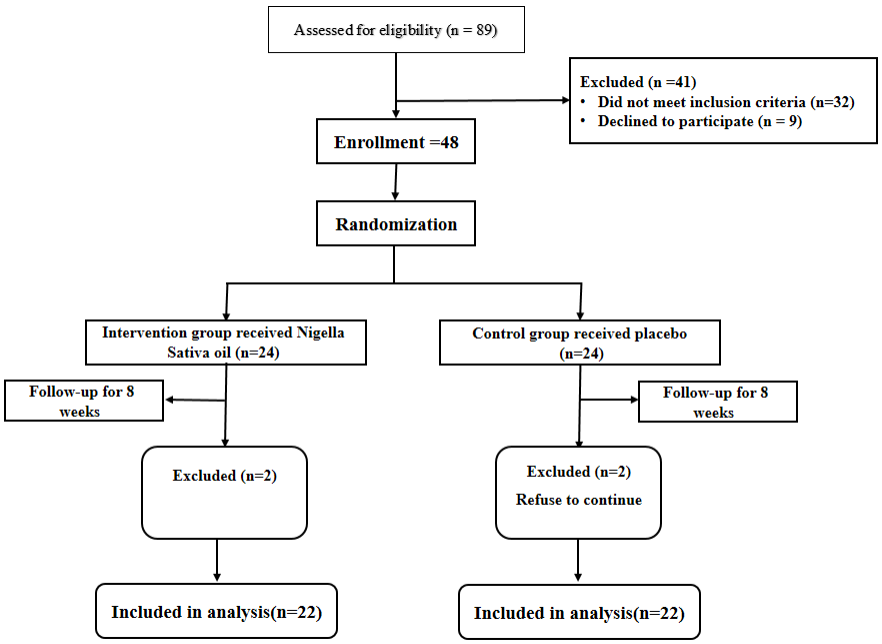

Fig. 1. Flow diagram of study recruitment