Effectiveness of Nigella sativa oil on patients with non-alcoholic fatty liver: A randomized double-blind placebo-controlled trial

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ABSTRACT

Non-Alcoholic Fatty Liver Disease (NAFLD) is the most common chronic liver disorder in the western countries. This clinical trial was carried out on the effects of Nigella sativa in the treatment of NAFLD. In this clinical trial, 147 patients were randomly selected. The necessary tests including ALT and AST were measured and liver ultrasonography performed. Data were analyzed by SPSS 18 and chi-square and independent t-tests conducted. Out of 120 patients, 53% (64) were males, while 47% (56) were females and the age range was 22 to 67 years old and the mean BMI 27.27 (2.24). After the three-month period, the levels of AST, ALT, LDL and TG in both intervention and placebo groups were significantly lower than the beginning of the study (P-value =0.001). Ultrasound results after the three months of follow-up showed that 18 patients in the intervention group and only four in the control group had normal liver ultrasonography and the difference was significant. Results showed that the consumption of Nigella sativa oil in patients with NAFLD significantly decreased the aminotransferases and improved the lipid profile and ultrasound findings in the intervention group as compared to the control group.

Key words: Nigella sativa, non-alcoholic fatty liver disease, aminotransferase.

INTRODUCTION

Non-Alcoholic Fatty Liver Disease (NAFLD) with a 20 to 30% prevalence is the most common chronic liver disorder in the western world (Angulo, 2002). NAFLD consists of two steps: silent liver disease where simple steatosis is only histologic finding and benign; non-alcoholic steatohepatitis characterized by liver cell damage and inflammation with or without fibrosis that may lead to liver cirrhosis and its complications and hepatocellular carcinoma (Sanyal, 2002). When cirrhosis is associated with NAFLD, the risk of an initial liver cancer is 1% annually. There are several non-invasive methods for detecting and tracking the disease, but liver biopsy is gold standard. The most important treatment for this disease is lifestyle changes, including changing dietary habits in order to reduce weight and insulin resistance. Pharmacotherapy is the best option for some patients with NAFLD/non-alcoholic steatohepatitis (Hernaez et al., 2011).

Effective treatments for NAFLD should aim at reducing insulin resistance and metabolic risk factors (Meral et al., 2001). Patients should be encouraged with a strong incentive for a major change in their previous harmful habits. Histologic improvement in patients with NAFLD that lose weight has been reported and the complications of metabolic syndrome often disappear after obesity surgery or lifestyle changes; however, unfortunately, most patients with non-fatty liver cannot lose weight or sustain weight loss. Although several medical treatments in clinical and research conditions have been tested and no drug has been approved for the treatment of NAFLD, so pharmacotherapy can be considered for further research (Ashtari et al., 2015; Petersen et al., 2010).

On the other hand, it should be noted that mild non-
alcoholic fatty liver disease may progress in a number of patients which may result in cirrhosis and related complications. Therefore, treatment of patients is very important even for a relatively insignificant NFL because cirrhosis is associated with cardiovascular death (Fallah et al., 2013). Pharmacotherapy is mainly focused on treatment of metabolic abnormalities, including the use of statin and anti-diabetes medications (biguanides, thiazolidinedione, incretin agonists) (de Alwis and Day, 2008; Matteoni et al., 1999). Ursodeoxycholic acid is not superior to placebo in terms of improvement of histological findings. Information on the efficacy and safety of specific drugs for the treatment of NAFLD is incomplete (Ludwig et al., 1980).

The clinical trial of pioglitazone and vitamin E for non-alcoholic steatohepatitis has promising results. As few other studies have confirmed, pioglitazone steatosis improves inflammation and biochemical parameters, however, its effect on fibrosis is not clear (Poynard et al., 2006; Wong et al., 2010). Nevertheless, pioglitazone is associated with significant side effects, particularly weight gain, fluid retention, heart failure and osteopenia. Vitamin E is superior to placebo for the improvement of non-electrolyte steatohepatitis. There are concerns about the safety of long-term intake of vitamin E in high doses (400 units per day) for the treatment of NAFLD, since vitamin E has been shown to increase the risk of death due to all causes. Weight loss is the only factor that can improve liver tissue changes in non-fatal fatty liver disease (Fallah et al., 2013; Ishak et al., 1995).

Seeds of Nigella sativa have been used for centuries in traditional medicine in the Middle East, North Africa and Southeast Asia to promote health and treat various diseases such as diabetes, dyslipidemia, obesity and hypertension (Kaatabi et al., 2012). Several studies have been conducted on this plant. The Nigella sativa extract has a PPAR gamma agonist effect and may therefore be useful in the treatment of diabetes, metabolic syndrome and obesity (Slentz et al., 2011). The Nigella sativa extract reduces systolic and diastolic blood pressure in hypertensive patients. The Nigella sativa oil has reduced the body weight and fasting blood levels of total cholesterol, LDL-C and glucose in diabetic patients and patients with metabolic syndrome, thereby improving direct and indirect effects on the hepatic status of patients (Fallah et al., 2013).

In addition, Nigella sativa has anti-oxidant and anti-inflammatory effects and prevents liver damage in animal models, including alcohol-induced liver damage, which could affect the oxidative and inflammatory damage in this disease. The common drugs used to treat NAFLD have limited effect and safety, therefore finding new drugs with efficacy and safety is required. However, due to the lack of confirmation of existing drugs and the adverse consequences of the disease, as well as taking into account the properties of Nigella sativa and limited human studies in this field, a clinical trial was conducted to investigate the effectiveness of Nigella sativa on the treatment of NAFLD.

MATERIALS AND METHODS

In this clinical trial study, 147 patients with NAFLD who were referred to Baqiyatallah Hospital were divided into control and intervention groups in order to determine the effect of Nigella sativa oil on biochemical and sonographic parameters of liver as criteria for fatty liver. The code of ethics was registered by the Ethics Committee of the Baqiyatallah University and the study was approved by the committee. The code of ethics for this study is ir.bmsurec.1395.350. Inclusion criteria were age group of 20 to 70 years old, liver ultrasound showing fatty liver, patients with liver disease other than non-alcoholic liver, signing patients consent form; not taking other effective medicines on treatment of NAFLD prior to entering the study. Exclusion criteria were Child Pugh Score above 7 (Salerno et al., 2002), other liver disease after entering the study, taking other potentially effective drugs on the treatment of fatty liver, patients who have not completed the course of the drug intake, following no diet or changing diet or training program given on the basis of patient’s self-report or confirmation of nutritionist and doctor, complications, exacerbations, and unwillingness to continue the study. All patients completed the consent form of the clinical trial and the patient information was kept confidential by the researcher.

Patients were examined at the start of the study. People with liver ultrasonography with liver hyper echogenicity and liver (Grade 1 to 3), or elevated levels of serum aminotransferases more than 1.5 times higher than normal were suspected of NAFLD. Patients were classified based on ultrasound findings using the following criteria into three groups (Dasarathy et al., 2009):

Grade 0: This involves a situation where there is no fatty liver.
Grade 1 (mild): This involves mild increase in liver parenchyma echogenicity or hepatoportal contrast enhancement with irregular margin of intrahepatic vessels and diaphragm.
Grade 2 (moderate): This simply refers to moderate increase in liver parenchyma echogenicity and hepatoportal contrast enhancement with an irregular margin of intrahepatic arteries and diaphragm.
Grade 3 (severe): In addition to moderate stenosis, the posterior segment of the right lobe of the liver, the margins of the intrahepatic vessels and the diaphragm are not observed.

Patients were asked about the history of drug and alcohol consumption, so that alcohol-related liver disease could be excluded. In that case, to reject other liver diseases that can mimic NAFLD, the necessary tests include hepatitis B virus surface antigens, hepatitis C virus antibodies, serum ferritin, serum iron, serum ceruloplasmin, ANA and serum protein electrophoresis for patients were requested. In the
case of refusing these cases and having ultrasound and biochemical conditions of the liver, the patient was diagnosed with NAFLD (Dowman et al., 2009; Hernaez et al., 2011; Kleiner et al., 2005). With other conditions of entry, the patient was enrolled in the study.

The formulation of the Nigella sativa oil syrup containing 2.5 ml of Nigella sativa oil was considered in every 5 ml of Nigella sativa oil, and the placebo syrup contained all the syrup additives except Nigella sativa oil. The appearance and taste of Nigella sativa syrup and placebo were identical. Patients were selected by specialist physician and biochemical tests and liver ultrasonography. If necessary, they were selected according to the inclusion and exclusion criteria, and for each of them a special checklist was completed. Eventually, 147 patients were randomly assigned to two groups through a random number table. Dietary consumption was calculated based on basal metabolism and daily activity level equivalent to 25 kg per kilogram of body weight per day, such that the daily diet program contains 500 calories less energy than the calculated energy, hence, fats (30%), carbohydrates (55%) and proteins (15%) contribute to the energy supply of this diet; all food groups, fruits and vegetables were used and consumption of salt and sugar-based foods reduced. All patients with inclusion criteria were referred to a nutrition dietitian before receiving a Nigella sativa oil syrup or placebo to receive the diet.

The diet program was administered on a weekly basis on the basis of patients’ self-report on phone and administered monthly by a nutritionist and diet therapy. Aerobic exercise program with three successive 45-minute sessions per week was performed on walking and running smoothly during the study period and its observance assessed on the basis of patients’ self-reports on phone weekly. The first group were patients who, along with their diet and exercise program, consumed 1 teaspoon Nigella sativa syrup daily containing 2.5 ml of Nigella sativa oil every 12 h for oral administration, while the second group were patients who had a diet with a training program as well as, daily intake of 1 teaspoon of placebo containing 2.5 ml every 12 h daily for oral administration.

The patients were visited every month during the three-month diet exercise program and Nigella sativa syrup or placebo was used to ensure that no possible complications were observed. At the beginning and after three months of taking the Nigella sativa syrup or placebo, the necessary tests including ALT and AST were measured and assessed by one experienced liver sonographer to evaluate the effect of treatment on the level of liver aminotransferases and ultrasound findings of the liver. Biosystems kit and the IFCC method were used to measure the aminotransferases (Uesugi et al., 2002).

It is worth noting that in the event of non-observance or modification of the diet or exercise program for more than 1 week, the patients were excluded from the study based on the patient’s self-report or dietitian’s approval and the patient’s physician. Regarding the beneficial effect of Nigella sativa syrup on patients with NAFLD, improvement of liver ultrasound grading after three months was considered with the return of serum aminotransferase levels to normal levels. Data were analyzed by SPSS 18 and chi-square and independent t-tests used for this purpose. A significant level of less than 0.05 was also considered.

RESULTS

The aim of this study was to evaluate the effect of Nigella sativa oil on NAFLD. At the beginning, 147 patients were enrolled in the study, of which, 27 patients did not participate in the follow-up and were excluded. Of the 10 patients in the intervention group, eight were absent from the follow-up and two patients had mild abdominal pain and of 17 patients in the control group, 13 cases were absent from the follow-up, two cases had nausea, and two were suffering from pain. Among the complaints of the intervention group were nausea (4), mild abdominal pain (2), and bitter taste of the drug (4). Among the complaints of patients in the control group were the bitter taste of the drug (10), nausea (8), and abdominal cramp (9). The bitter taste of the drug was due to its low paraffin content. Of the 120 cases that completed the three-month follow-up period, 53% were men (64 cases) and 47% women (56) and the age range of patients were 22 to 67 years old. The mean BMI was not significantly different between the two groups before the study. Table 1 shows the baseline characteristics of the patients in the two groups. Baseline values of biochemical variables including AST, ALT, LDL, and HDL, other than triglyceride, were not significantly different between the two groups (P-value > 0.05).

After the three-month period, the levels of AST, ALT, LDL and TG in both intervention and placebo groups were significantly lower than the beginning of the study (P-value = 0.001). HDL also significantly increased in both groups after the follow-up period (P-value = 0.001). Table 2 shows patients’ characteristics after the three-month follow-up period. The levels of AST, ALT, and LDL in the intervention group were lower than the control group and with the exception of LDL the observed difference between the two groups was significant. HDL was significantly higher in the intervention group than in the control group (P-value = 0.001). The level of triglyceride was significantly different between the two groups at the beginning of the study. There was also a significant difference after the three-month period of the follow-up (P-value = 0.001), such that triglyceride was lower than the control group after the intervention. BMI was not significantly different between the two groups after three-month follow-up between the two groups (P-value = 0.11).

The results of sonography after the three-month period of the follow-up revealed that 18 patients in the intervention group and only 4 in the control group had

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Table 1: Baseline characteristics of patients compared between control and intervention groups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Drug (n=60)</th>
<th>Control (n=60)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>47.92±12.02</td>
<td>45.92±12.3</td>
<td>0.25</td>
</tr>
<tr>
<td>(Mean ± SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex N (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>34(56.7%)</td>
<td>30(50%)</td>
<td>0.29</td>
</tr>
<tr>
<td>Female</td>
<td>26(43.3%)</td>
<td>30(50%)</td>
<td></td>
</tr>
<tr>
<td>BMI (Mean ± SD)</td>
<td>27.88±2.69</td>
<td>26.66±1.47</td>
<td>0.1</td>
</tr>
<tr>
<td>Fatty liver N (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 1</td>
<td>24(40%)</td>
<td>22(36.7%)</td>
<td>0.19</td>
</tr>
<tr>
<td>Grade 2</td>
<td>31(51.7%)</td>
<td>61.7%(37)</td>
<td></td>
</tr>
<tr>
<td>Grade 3</td>
<td>5(8.3%)</td>
<td>1(1.7%)</td>
<td></td>
</tr>
<tr>
<td>AST (mg/dl) (Mean ± SD)</td>
<td>66.45±8.96</td>
<td>68.45±4.9</td>
<td>0.13</td>
</tr>
<tr>
<td>ALT (mg/dl) (Mean ± SD)</td>
<td>72.32±8</td>
<td>74.28±5.66</td>
<td>0.12</td>
</tr>
<tr>
<td>LDL (mg/dl) (Mean ± SD)</td>
<td>118.77±23.38</td>
<td>115.8±23.63</td>
<td>0.49</td>
</tr>
<tr>
<td>HDL (mg/dl) (Mean ± SD)</td>
<td>39.82±5.1</td>
<td>38.55±3.11</td>
<td>0.1</td>
</tr>
<tr>
<td>Triglycerides (mg/dl) (Mean ± SD)</td>
<td>121.97±38.57</td>
<td>144.58±38.92</td>
<td>0.002</td>
</tr>
</tbody>
</table>

AST: Aspartate transaminase; ALT: Alanine transaminase; LDL: Low-density lipoprotein; HDL: High-density lipoproteins.

Table 2: Between group comparison after three months.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Drug (n=60)</th>
<th>Control (n=60)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (mg/dl) (Mean ± SD)</td>
<td>46.57±11.87</td>
<td>59.98±12.4</td>
<td>0.001</td>
</tr>
<tr>
<td>ALT (mg/dl) (Mean ± SD)</td>
<td>48.50±12.63</td>
<td>63.65±15.25</td>
<td>0.001</td>
</tr>
<tr>
<td>LDL (mg/dl) (Mean ± SD)</td>
<td>101.17±20.75</td>
<td>103.88±19.15</td>
<td>0.45</td>
</tr>
<tr>
<td>HDL (mg/dl) (Mean ± SD)</td>
<td>43.53±5.7</td>
<td>40.37±3.47</td>
<td>0.001</td>
</tr>
<tr>
<td>Triglycerides (mg/dl) (Mean ± SD)</td>
<td>101.83±28.43</td>
<td>127.65±37.06</td>
<td>0.001</td>
</tr>
</tbody>
</table>

normal liver ultrasonography and the difference was significant (P-value = 0.004) (Figure 1). Improvement of sonographic findings after the quarterly follow-up period was 45% (27) in the intervention group and 20% (12 patients) in the control group. The observed difference was significant (P-value = 0.003).

DISCUSSION

The results show that the consumption of Nigella sativa oil in patients with NAFLD significantly decreased the aminotransferases and improved the lipid profile in the intervention group as compared to the control group. In
addition, a three-month ultrasound examination demonstrated that the liver status of the intervention group was significantly better compared to the control group. Although, ultrasound for the NAFLD is not a gold standard, its sensitivity has been reported between 60 and 80% (Hernaez et al., 2011). Since it is not invasive as compared to liver biopsy, this method was used to determine the grade of fatty liver of patients.

Non-alcoholic fatty liver disease (NAFLD), known as inflammatory disease causes elevated levels of aminotransferase through oxidative stress and inflammation, and drugs such as vitamin E and silymarin that have been proven to have anti-oxidant and anti-inflammatory effects are effective for these patients (Aller et al., 2015).

In a study, thymoquinone and p-cymene, taken from Nigella sativa oil due to their antioxidant effects improved LFT by reducing the accumulation of malondialdehyde (MDA) and tumor necrosis factor (TNF-α) in mice with fatty liver (Prabhakar et al., 2015). In another study, thymoquinone, in addition to inhibiting oxidative stress, reduced inflammation, apoptosis and fibrosis in NAFLD mice (Vanamala et al., 2012). Chakravarty (1993) revealed that Nigellone (polythymoquinone), the active ingredient of a Nigella sativa plant, reduced intracellular concentration and calcium transmission, and it seems it is done by inhibiting oxidative energy metabolism and protein kinase c. It was also argued that Nigella sativa oil contributes to oxidative stress in liver and kidney toxicity induced by cisplatin in mice and decreases lipid peroxidation in brain phospholiposomes by reducing the synthesis of inflammatory mediators, leukotrienes and prostaglandins by inhibiting lipoxygenase and cyclooxygenase enzymes (Develi et al., 2014; Leong et al., 2013; Takaki et al., 2013). These studies showed that Nigella sativa oil against free radicals may have a protective effect and also inhibit, liberate and synthesize inflammatory mediators.

The results of the present study on liver enzymes showed a significant difference between the two groups after the three-month period of treatment, which is line with the study of Kanter et al. (2005). They administered Nigella sativa oil for 6 days to mice that used CCl₄ and had fibrosis and cirrhosis of the liver. They showed that the level of liver enzymes was significantly reduced due to the antioxidant effect of Nigella sativa oil on liver cells.

El-Dakhkhny et al. (2000) studied a group of mice that used CCl₄ for 4 weeks at a dose of 800 mg/kg of Nigella sativa oil, and found that AST and ALT had a significant decrease as compared to those who consumed only CCl₄. This was due to the role of Nigella sativa in the activation of cytoprotective mechanisms. It was also shown in this study that the use of Nigella sativa oil for 4 weeks reduced serum total cholesterol, LDL and triglyceride levels in mice and increased HDL. Hussain et al. (2007) carried out a research on the effect of Nigella sativa oil on the NAFLD. In this study, patients received 1 g of Nigella sativa capsules twice daily for three months and the placebo was given to the control group. The results of this study showed that the intervention group had a significant decrease in BMI, liver enzymes and improvement of liver ultrasonography as compared to the control group. One of the differences in
this study with our study is the practice of treatment for groups, higher sample size (120 versus 70), lipid profile and expression of patient’s complications.

Limitations of this study include limited access to eligible non-alcoholic fatty liver patients, drop-out of patients during the study and patients’ lack of consent for participation in the project. Also, with proper randomization, we found that TG levels were significantly different between the two groups before the study. Given that low-cost herbal medicines are available with fewer complications, it is suggested that extensive clinical trials with longer colonoscopy and the administration of different doses of Nigella sativa oil should be considered in order to evaluate the exact mechanism of NAFLD and it should be added on the treatment of patients with NAFLD.

REFERENCES


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