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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

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Abstract

Background: Inflammation is one of the primary mechanisms in the development of metabolic complications. Although anti-inflammatory characteristics of *Nigella sativa* (NS) have been indicated in animal models, clinical trials related to the effects of NS on inflammatory parameters are relatively scarce.

Objective: The aim of the present study was to determine the effects of NS oil combined with a calorie-restricted diet on systemic inflammatory biomarkers in obese women.

Methods: In this double-blind placebo-controlled randomized clinical trial, 90 volunteer obese (body mass index =30-34.9 kg/m²) women aged 25-50 years were recruited. Participants were randomly divided into two groups, an intervention group (n=45) and a placebo group (n=45). Each group received either: 1) a low-calorie diet with 3g/day of NS oil, or 2) a low-calorie diet with 3g/day placebo for eight weeks.

Results: A total of 84 females (intervention group= 43; placebo group= 41) completed the trial. Subjects in the intervention group did not report any side effects with the NS oil supplementation. NS oil decreased serum levels of tumor necrosis factor-alpha (-40.8 vs. -16.1%, \(p=0.04\)) and high-sensitivity C-reactive protein (-54.5 vs. -21.4%, \(p=0.01\)) compared to the placebo group. However, there were no significant changes in interleukin-6 levels (-8.6 vs.-2.4%, \(p=0.6\)) in the NS group compared to the placebo group.

Conclusion: NS oil supplementation combined with a calorie-restricted diet may modulate systemic inflammatory biomarkers in obese women. However, more studies are needed to clarify the efficacy of NS oil as an adjunct therapy to improve inflammatory parameters in obese subjects.
**Keywords:** Nigella sativa, Inflammation, Obesity, Weight loss
Introduction

Inflammation is one of the primary mechanisms in the development of metabolic disorders. Increased pro-inflammatory factors (Interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and high-sensitivity C-reactive protein (hs-CRP)), are involved in the development of hyperglycemia, dyslipidemia, atherosclerosis, and cardiovascular diseases (1). In obesity, the blood supply to adipose tissue may decrease. Necrosis and macrophage infiltration into adipose tissue are followed by hypoxia, leading to an over-production of pro-inflammatory factors which results in localized and systemic inflammation (2).

Losing weight can modulate the inflammatory markers and prevent obesity-related complications (3). However, due to difficulties in adhering to dietary recommendations for weight loss, obese subjects often turn to anti-obesity medications and supplements (4). More recently, there has been an increased tendency to use medicinal plants to assist weight loss (5). Previous studies have provided evidence on the anti-obesity properties of some medicinal herbs such as green tea, pepper, Camellia sinensis (6) and Nigella sativa (7,8).

The genus Nigella belongs to the Buttercup or Ranunculaceae family, and contains several different species. Nigella sativa Linn. seed, or black seed, is a species common in the Middle East. Its seeds are black and taste bitter (9). NS has various chemical ingredients, including thymoquinone (TQ) (2-isopropyl-5-methyl-1,4-benzoquinone), dithyoquinone, thymol, essential fatty acids (particularly linoleic and oleic acid), nigellicine, trans-Anethole, limonene, and carvacrol (10-12). It has been used in Iranian traditional medicine in the treatment of several diseases such as rheumatoid arthritis, diabetes, digestive disorders, and dyslipidemia (10, 11). There were no reported toxic effects of NS in animal models (13) and no serious side effects in clinical trials (14). TQ is the main pharmacologically active component of NS and is responsible
for many of the therapeutic properties of NS (anti-inflammatory, antioxidant, anti-cancer, anti-
hyperglycemic, anti-histaminic, etc.). It is also fat-soluble, meaning that NS oil may be more
effective than aqueous extract for therapeutic purposes (11).

There is evidence showing the positive effects of NS on the inflammation status of animal and in
vitro models (15-17), but there are limited clinical trials evaluating the anti-inflammatory effects
of NS. Hadi et al. reported that 1g/day NS oil increased Interleukin-10 (IL-10) with no changes
in serum levels of TNF-α in patients with rheumatoid arthritis (18). Yusof et al. indicated that a
50 mg/kg/day mixture of honey with NS powder did not change IL-6 levels after eight weeks
(19). There are also limited studies showing discrepant results regarding the effects of NS on
obesity (8, 20-22). It seems that no clinical trials have determined the efficacy of NS in
combination with a calorie-restricted diet versus calorie-restricted diet alone in obese women.
Therefore, we conducted the present study as the first clinical trial to determine the effects of NS
combined with moderate-fat, balanced-nutrient weight reduction diet on inflammatory
parameters in obese women.

Materials and Methods

Participants

In this double-blind placebo-controlled randomized clinical trial, volunteer obese women (n=90)
were recruited at the Obesity Clinic affiliated with Tabriz University of Medical Sciences,
Tabriz, Iran, from April through July 2014. Inclusion criteria were as follows: women between
the ages of 25-50 with a body mass index (BMI) greater than 30 and less than 35 kg/m². Subjects
were excluded if they had a history of cardiovascular, renal, hepatic, or pancreatic diseases,
diabetes, recent, active, and debilitating infectious diseases, or using antibiotics a month prior to
the intervention. If they were following a weight-loss diet or had been taking any anti-obesity
medications in the previous six months, smoked, were pregnant or lactating, were taking herbal
drugs, medicinal herbs, alcohol, antioxidant supplements, aspirin, vitamin E, or any other
anticoagulant medications. At the beginning of the trial, information was collected via
comprehensive interviews on the general characteristics of the subjects, including age, family
history of obesity, disease history, and current medications. The trial was conducted according to
the guidelines established in the Declaration of Helsinki, and approved by the Ethics Committee
of Tabriz University of Medical Sciences. Informed written consent was obtained from the
participants. The trial was registered on the Iranian registry of clinical trials (www.irct.ir/,
IRCT201106191197N10).

**Study design**

Participants were randomly divided into two groups based on age and BMI, using a block
randomization procedure. In every permuted block, two subjects were allocated to each arm of
the trial. The allocation sequence was randomly generated by random allocation software (RAS).
To maintain blinding, the allocation was performed by an investigator with no clinical
involvement in the study, and the investigators and participants remained blind for
randomization and group assignment until data analysis were completed. The sample size was
calculated based on the previous study (8). A minimum sample size of 37 participants was
calculated with a confidence level of 95% and a power of 90% per treatment group. Considering
the 20% dropout, the sample size was increased to 45 in each group. All the participants received
a calorie-restricted diet. A dietitian designed an individualized moderate fat, balanced-nutrient
reduction diet to reduce energy intake. Resting energy expenditure was calculated based on the
Mifflin equation (23). After calculating the required energy for each subject, a weight loss diet
plan was provided based on 500 kcal less than calculated energy. It contains 30% of calories
from fat, 15% from protein and 55% of calories from carbohydrate. The participants were also
advised to follow healthy eating recommendations (24). These healthy eating recommendations
included limiting sugar, sweets, sugar-sweetened beverages, saturated fats, high-fat foods, and
fast foods and increasing consumption of whole grains, a variety of fruits and vegetables, low-fat
meats and dairy products. Compliance with the recommended diet was evaluated using 24-hour
dietary recall (one weekend day and two weekdays).

The intervention group received 3 g/d NS oil soft gel capsules (one capsule, three times a day, 30
minutes before each main meal), and the placebo group received similar amounts of sunflower
oil as a placebo for eight weeks. Both NS oil and sunflower oil capsules were provided for
subjects in similar opaque bottles. Participants received half the bottles at the beginning of the
trial and the remainder in the middle of the trial. Supplements were distributed among the
volunteers based on the allocation code after randomization. In order to minimize drop-out rate
and ensure that supplements were consumed, subjects received a phone call every week. Subjects
were advised to contact research staff immediately if they suspected a reaction to the
supplements. The participants were asked not to change their usual physical activity, and were
advised to contact the research staff if they took any medications or changed their physical
activity during the trial. To determine compliance with the supplements, the participants were
asked to return the bottles (empty or full) at each visit. Therefore, compliance could be estimated
by counting the remaining capsules. The participants were excluded if they had taken less than
95% of the supplements in each visit.
Characteristics of supplements

NS oil and sunflower oil soft gel capsules were prepared by the Dana Company (Tabriz, Iran) in the same size and color. NS oil was prepared using the cold press procedure with a yield of 30% as explained elsewhere (25). Findings from our previous pilot study revealed that most subjects used sunflower oil in food preparation in Tabriz. Thus it was chosen as a suitable placebo. The type of intervention was concealed from researchers and participants, and capsule bottles were coded as A or B by an individual with no involvement in the trial.

Fatty acid concentrations of NS and sunflower oil were determined by the gas chromatography–mass spectrometry (GC–MS) technique (26), using the Buck Scientific model 610 system. Fatty acid measurement was explained in our previous study (25). The fatty acid content of the two oil supplements was presented in Table 1. For TQ measurement, the distillate was diluted using n-hexane and then analyzed by the GC–MS method (27). Based on an analysis available from the manufacturer, TQ, a major phytochemical bioactive component of NS oil, was 1.25mg in each 100 g of NS oil.

Anthropometric indices, physical activity and dietary intake assessment

Anthropometric indices, physical activity, and dietary intake were evaluated at baseline and at the end of the study. Body weight and height were measured by trained staff. Weight was measured using a weighing scale (Seca, Hamburg, Germany) with minimal clothing and without shoes to the nearest 0.1 kg. Height was measured without shoes using the SECA stadiometer (Seca, Hamburg, Germany) to the nearest 0.1 cm. BMI was calculated as weight in kilograms divided by the squared height in meters.
The International Physical Activity Questionnaire (IPAQ) (28) was administered using face to face interviews in order to estimate physical activity levels. Based on calculated scores, subjects were classified as being sedentary, moderately active, or highly active. Dietary intakes were evaluated using a three-day food diary (two weekdays and one weekend day) and the subjects recorded their food intake daily. Before the intervention, all subjects were provided with instructions on how to use a food scale and record their food intake. Dietary intakes were analyzed using the Nutritionist IV (First Databank Inc., Hearst Corp., San Bruno, CA) modified for Iranian foods, to calculate total energy, macronutrients and total dietary fiber.

**Blood sampling and biochemical measurements**

After 12-14 hours of overnight fasting, 5ml venous blood samples were collected and transferred into Venoject glass tubes to measure inflammatory parameters (IL-6, TNF-alpha, hs-CRP) at baseline and at the end of the trial. The serum samples were separated from whole blood by centrifugation at 2,500 rpm for 10 minutes (Beckman Avanti J-25; Beckman Coulter, Brea, CA) at room temperature. Serums were stored at -80°C until assay time. IL-6 and TNF-alpha concentrations were measured using the ELISA method with commercial kits (Orgenium, Finland). Inter-assay coefficients of variability (CV) for IL-6 and TNF were less than 10% for both, and their intra-assay coefficients were 5.5 and 4.5%, respectively. hs-CRP measurement was performed using COBAS 6000 autoanalyser (Roche, Germany) with a kit (BioSystems SA, Costa Brava 30, Barcelona, Spain). The inter and intra-assay CV for hs-CRP were 3.6 and 1.8%, respectively.

**Statistical analysis**

The analyses were done based on the intention-to-treat principle. Results were reported as mean and standard deviations. The normality of data distribution was evaluated by the one-sample
Kolmogorov-Smirnov test. For data with normal distribution, the independent t-test and paired t-test were used for inter and intra-comparison of quantitative variables, respectively. Data with non-normal distribution were converted to data with normal distribution using logarithmic conversion. Parametric tests were then used for inter and intra-group comparisons. To avoid potential bias, analysis of covariance (ANCOVA) was used for adjusting known confounding factors (body weight changes, dietary intake changes, and baseline values). In order to calculate the percentage of mean changes in markers, at the beginning and end of the study (after eight weeks) mean changes in markers from the baseline were calculated in each group by [(eight weeks values - baseline values) / baseline values) × 100. All data were analyzed using SPSS software version 16.0 (SPSS, Chicago, IL, USA). *P*-value <0.05 was considered significant.

Results

A total of 90 participants were recruited for the study, and 84 of these completed the trial. Two subjects in the NS oil group were excluded, one because of pregnancy (n=1), and one because of stomach ache (n=1). In the placebo group, four subjects were excluded: participants who did not adhere to the trial procedures (n =3) and with stomach ache (n =1) (Figure 1). Since serum levels of hs-CRP is readily affected by infection, hs-CRP ≥10 mg/dL (29) was used to screen participants for major infection (n=0) at baseline and at the end of the study. Data analysis was based on the intention-to-treat principle, therefore all the participants (n=90) were considered in the final analysis. The capsule counts indicated that all the participants who completed the study had high compliance (>95%) with the supplementation. Subjects reported no side effects during the intervention except mild gastrointestinal problems. The primary outcome of the present study was the effect of NS oil supplementation with a low-calorie diet on weight, dietary antioxidant intake and serum levels of inflammatory parameters (IL-6, TNF-alpha, hs-CRP) in obese
women. The secondary outcome was the effect of NS oil supplementation with a low-calorie diet on energy, macronutrient and fiber intake in obese women.

All the study participants were pre-menopausal females with a mean BMI and age 32.4± 2.2 kg/m^2 and 40.5±10.3 years, respectively. The intervention and placebo groups were similar in their baseline characteristics (Table 2). Although no criteria were considered for lipid profile and dyslipidemia, only 15.6% of subjects (NS group= 6.7% and placebo group= 8.9%) took anti-hyperlipidemic medications (statin, 20 mg/day) at the baseline.

Table 3 shows anthropometric indices and dietary intake in the intervention and placebo groups at baseline and after eight weeks of the intervention. At baseline, there were no significant differences in body weight and BMI between the two groups (p>0.05 in both variables). After the intervention, weight (-6.0 % vs.-3.5) and BMI (-3.9% vs. -1.5%) decreased significantly compared to the baseline in NS and placebo groups, respectively. Intra-group comparison revealed a significant reduction in weight in the intervention group compared to the placebo group (p=0.03) with no significant changes in BMI (p=0.2) at the end of the study.

Based on the three-day food diaries, no significant differences were observed between two groups in dietary intake at baseline except for total fat intake (p<0.01). Since both groups adhered to a low-calorie diet throughout the study, total energy, carbohydrate, and fat intake decreased significantly in both groups (p<0.05 in all variables) compared to the beginning of the trial. But no significant reduction in protein intake was observed. (Table 3). Reduction in energy intake of NS group was more than the placebo group at the end of the trial, but it was not significant (-782 vs.-530 kcal/day; p=0.1). Besides, comparison of the two groups indicated no significant differences in macronutrient intake at the end of the trial (p>0.05 for all variables, adjusted for baseline values) (Table 3).
As presented in Table 3, after eight weeks of the intervention, no significant differences were observed in main vitamins and mineral intake with antioxidant properties (vitamin E, vitamin C, and selenium). In addition, no significant differences in fiber intake were identified between the intervention and placebo groups at the end of the study (p=0.3).

Inflammatory parameters before and after the intervention are presented in Table 4. At baseline, there were no significant differences between the NS group and the placebo group for TNF-alpha, hs-CRP, and IL-6 concentrations (p>0.05 for all variables). The comparison groups revealed that TNF-alpha levels (-40.8 vs. -16.1%, p=0.04) and hs-CRP concentrations (-54.5 vs.-21.4%, p=0.01) significantly decreased in the NS group compared to the placebo group, but an insignificant decline in IL-6 concentrations (-8.6 vs. -2.4%, p=0.6) was observed (Fig 2). NS oil in combination with a calorie-restricted diet led to a significant decrease in serum levels of TNF-alpha (p=0.03) and hs-CRP (p<0.01) compared to the placebo group at the end of the study (ANCOVA adjusted for weight changes, energy, macronutrients, dietary antioxidant and fiber intake changes), and baseline values).

Discussion

Numerous studies have evaluated the effects of medicinal herbs on inflammatory cytokines, which are involved in metabolic complications (30). In the present study, consumption of 3 g/day NS oil concurrent with a calorie-restricted diet decreased body weight and serum levels of TNF-alpha and hs-CRP in the study subjects after eight weeks. Thus it seems that NS oil may help better manage weight and inflammatory status in obese women. To the best of our knowledge, no clinical trials have evaluated the effects of NS with calorie restriction on inflammatory parameters in obese subjects and clinical trials on the anti-inflammatory effects of NS are limited.
It seems that only two clinical trials have investigated anti-inflammatory effects of NS (18, 19). Our findings were in line with Yusof et al.’s study. They found that a 50 mg/kg/day mixture of honey with NS powder did not reduce IL-6 levels in healthy adults after eight weeks (19). But Hadi et al. reported that 1 g/day NS oil did not change serum levels of TNF-α in patients with rheumatoid arthritis after two months (18). Differences in prescribed dosages of NS oil, baseline inflammatory parameters, disease background, dietary intake and physical activity may lead to different results.

The anti-inflammatory effects of NS and its constituents have been indicated in cell line and animal models (15-17). Beneficial effects of NS on systemic inflammation may be due to several ingredients found in black seed, including TQ and its carbonyl polymer (nigellone) (16), thymol, thymohydroquinone, alpha-Hederin, limonene and polyphenols (15, 31, 32). TQ is a primary bioactive constituent of NS and is found in the volatile oil of black cumin. Anti-inflammatory properties of TQ were mainly reported in some inflammation-based models including colitis, rheumatoid arthritis, asthma, and cancer (11, 17, 33, 34). Umar et al. reported that 5mg/kg/day TQ reduced the level of IL-6 and TNF-alpha in Wistar rats with arthritis after 21 days. They found that TQ suppressed nitric oxide (NO) production and maintained homeostasis in the inflammatory cytokine imbalance (35). Mahmood et al.’s also evaluated the in vitro effect of NS extract on NO production. They reported that the aqueous extract of NS can inhibit NO production in murine macrophages. Reducing NO via the suppression of inducible nitric oxide synthase (iNOS) can reduce inflammation symptoms (36). Based on the Kanter et al. study, NS seed extract inhibited the inflammatory responses in rats with lung tissue damage after seven days. They also indicated that NS can decrease iNOS activity in lung tissue and reduce inflammatory responses (37). Ammar et al. reported that 10mg/kg/day TQ reduced TNF mRNA
levels in the lung cells of asthmatic mice. In Ammar’s study, TQ indicated superior inhibitory
effects on iNOS production after eight days of treatment. It has also been demonstrated that
production of IL-6 is decreased in response to TQ in cell line studies (38). El Gazzar et al.
reported that TQ affects TNF-alpha in rat basophil cells. Hence, TNF-alpha is involved in the
activation of the nuclear factor-kB (NF-kB), and treatment with TQ indicated a negative effect
on transactivation of the NF-kB pathway (39, 40). Other potential anti-inflammatory effects of
NS and derived TQ are related to their inhibitory role in eicosanoid generation. They can inhibit
both pathways (lipooxygenese and cyclooxygenase) of arachidonate metabolism, which are
involved in the generation of inflammatory mediators (33).

In obesity, hypoxia disturbs the balance between pro- and anti-inflammatory activities in adipose
tissue, and it activates nuclear factor-kappa B (NF-kB) in adipocytes and macrophages (41).
Therefore, losing weight may increase the level of adiponectin, an anti-inflammatory and anti-
atherosclerotic hormone, which can modulate the inflammation profile (42). Other possible anti-
inflammatory mechanism of NS oil may be related to its anti-obesity effects. In the present
study, despite insignificant changes in the energy intake between the two groups, body weight
decreased in the NS oil group compared to the placebo group (6.0% vs.-3.5%; p<0.01). As
discussed in our previous study (25), evidence indicated discrepant results for the effects of NS
on obesity. Le et al reported that intragastric gavage with a petroleum ether extract of NS seeds,
had an anorexic effect and decreased food intake and weight in rats after 4 weeks (22). Our
findings are in line with Datau et al.’s study. They indicated that 3g/day of NS powder reduced
weight in men with abdominal obesity after 3 months (8). But Dehkordi et al. reported that 100
and 200 mg/day of NS extract did not change body weight after 8 weeks in men with mild
hypertension (43). The diversity in the findings may be due to differences in dosage and type of
NS, duration of intervention, disease background, adipose tissue distribution, lifestyle and race.

Moreover, in the previous studies the participants did not receive a low-calorie diet in the previous studies, and only the effects of supplementation were evaluated. In the current study, a weight loss diet was recommended for all the participants, and thus a reduction in body weight was observed in both groups. Anti-obesity mechanisms of NS are not clear. Previous studies reported that bioactive components of NS such as TQ and thymol may be involved in anti-obesity effects of NS (7, 8, 22). Recently, it has been reported that TQ can act as a ligand of the PPAR-γ gene and increase PPAR-γ activity (44). PPAR-γ regulates energy homeostasis and adipose tissue differentiation (45).

In the current study, although intergroup comparison indicated reduction in weight in the placebo group (-3.5%), changes in the inflammatory parameters were not significant. Our findings in the placebo group were in line with the study by Imayama et al., who indicated that a calorie-restricted diet did not decrease the hs-CRP and IL-6 levels in overweight and obese women with <5% weight loss (46). Based on the Madsen et al. study, weight reduction of >10% is needed to significantly improve the inflammatory cytokine levels in obese subjects (42). Based on our findings, it seems that synergetic effects between the anti-inflammatory/antioxidative constituents of NS and a weight loss diet may modulate cytokine inflammatory parameters (47). Since the confounding factors (weight changes, dietary intake changes and baseline values) were controlled using the ANCOVA analysis for comparison of the two study groups at the end of the study, the between group differences in inflammatory parameters may be attributed to the intervention in the NS group.

The present study had some limitations: 1) only female subjects were included, (2 the intervention was limited to eight weeks and its effect over longer periods of time is not clear, and
3) the pure effect of NS oil (NS oil supplementation without a low-calorie diet was not evaluated. The strengths were that double-blinded and biochemical parameters were adjusted for some of the known confounding factors. Further studies are recommend 1) using a cross-over design to determine the efficacy of NS oil against a placebo, 2) adding a third arm to the study that includes encapsulated TQ, 3) measuring serum levels of TQ and its metabolites and 4) evaluating the effects of NS in different dosages and forms (extract, oil, powder) with and without a low-calorie diet.

**Conclusion**

NS oil concurrent with a calorie-restricted diet is more efficient than a calorie-restricted diet alone in reducing systemic inflammation in obese women. However, more studies are suggested clarifying efficacy of NS oil as a complementary therapy modulating inflammatory parameters for women who adhere to a weight loss diet.

**Conflict of Interest**

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

**Acknowledgment**

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Table 1- Percentage of fatty acid content of NS and sunflower oils

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Nigella sativa oil</th>
<th>Sunflower oil</th>
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<tr>
<td>Linoleic acid (18:2)</td>
<td>56.84</td>
<td>56.79</td>
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<tr>
<td>Oleic acid (18:1c)</td>
<td>24.74</td>
<td>26.67</td>
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<tr>
<td>Palmitic acid (16:0)</td>
<td>16.59</td>
<td>12.41</td>
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<td>Myristic acid (14:0)</td>
<td>1.40</td>
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<tr>
<td>Stearic acid (18:0)</td>
<td>0.21</td>
<td>0.95</td>
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<tr>
<td>Cis palmitoleic acid (16:1c)</td>
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<td>0.33</td>
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<tr>
<td>Arachidic acid (20:0)</td>
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<td>0.21</td>
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<td><strong>Total</strong></td>
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<td><strong>99.99</strong></td>
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<tr>
<td>Variables</td>
<td>Intervention group (n=45)</td>
<td>Placebo group (n=45)</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>---------------------------</td>
<td>----------------------</td>
</tr>
<tr>
<td>Age (year)</td>
<td>41.0±11.8*</td>
<td>39.5±9.8</td>
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<tr>
<td>Weight (kg)</td>
<td>81.8±9.5</td>
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<tr>
<td>Height (cm)</td>
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<td>157.5±6.9</td>
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<tr>
<td>Body Mass Index (kg/m²)</td>
<td>32.5±1.5</td>
<td>31.6±1.5</td>
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<tr>
<td>Physical activity (%)</td>
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<td>Sedentary</td>
<td>73.4</td>
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<tr>
<td>Moderate</td>
<td>26.6</td>
<td>24.4</td>
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* Mean±SD
Table 3. Comparison of body weight and dietary intakes between NS oil group and placebo group at baseline and after the intervention.

<table>
<thead>
<tr>
<th>Variable</th>
<th>NS oil group (n=45)</th>
<th>Placebo group (n=45)</th>
<th>P-value&lt;sup&gt;c&lt;/sup&gt;</th>
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</thead>
<tbody>
<tr>
<td>Weight (Kg)</td>
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<td></td>
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<tr>
<td>Baseline</td>
<td>81.8±9.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>80.7±10.5</td>
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<td>End</td>
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<td>Pre to post P-value&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>&lt;0.01*</td>
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</tr>
<tr>
<td>BMI(Kg/m&lt;sup&gt;2&lt;/sup&gt;)</td>
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<td></td>
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<td>Baseline</td>
<td>32.5±1.5</td>
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<tr>
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<td>Pre to post P-value&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>&lt;0.01*</td>
<td></td>
</tr>
<tr>
<td>Energy (kcal/day)</td>
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</tr>
<tr>
<td>Baseline</td>
<td>2643±289</td>
<td>2484±369</td>
<td>0.05</td>
</tr>
<tr>
<td>End</td>
<td>1861±163</td>
<td>1954±280</td>
<td>0.2&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pre to post P-value&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.01*</td>
<td>&lt;0.01*</td>
<td></td>
</tr>
<tr>
<td>Carbohydrate (g/day)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>340.8±54.7</td>
<td>343.6±68.6</td>
<td>0.8</td>
</tr>
<tr>
<td>End</td>
<td>227.3±36.3</td>
<td>254.9±53.7</td>
<td>0.1&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pre to post P-value&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.01*</td>
<td>&lt;0.01*</td>
<td></td>
</tr>
<tr>
<td>Protein (g/day)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>98.8±23.9</td>
<td>87.5±22.5</td>
<td>0.05</td>
</tr>
<tr>
<td>End</td>
<td>79±17.7</td>
<td>77±18.9</td>
<td>0.7&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pre to post P-value&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.07</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>Total fat (g/day)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Baseline</td>
<td>102.4±19.7</td>
<td>88.6±20.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>End</td>
<td>71.1±11.9</td>
<td>72.1±19.8</td>
<td>0.8&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pre to post P-value&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.01*</td>
<td>0.04&lt;sup&gt;e&lt;/sup&gt;</td>
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</tr>
<tr>
<td></td>
<td>Baseline</td>
<td>End</td>
<td>Pre to post P-value</td>
</tr>
<tr>
<td>----------------------</td>
<td>--------------</td>
<td>--------------</td>
<td>---------------------</td>
</tr>
<tr>
<td><strong>Dietary fiber (g/day)</strong></td>
<td>12.1±4.7</td>
<td>15.2±6.5</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>12.9±4.6</td>
<td>15.4±4.7</td>
<td>0.3*</td>
</tr>
<tr>
<td><strong>Vitamin E (mg/day)</strong></td>
<td>11.5±8.0</td>
<td>7.5±6.7</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>10.4±6.9</td>
<td>7.6±5.7</td>
<td>0.2*</td>
</tr>
<tr>
<td><strong>Vitamin C (mg/day)</strong></td>
<td>124.5±64.3</td>
<td>122.8±69.6</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>132.6±73.7</td>
<td>107.8±61.9</td>
<td>0.1*</td>
</tr>
<tr>
<td><strong>Selenium (mg/day)</strong></td>
<td>0.06±0.02</td>
<td>0.05±0.02</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>0.05±0.01</td>
<td>0.05±0.02</td>
<td>0.2*</td>
</tr>
</tbody>
</table>

BMI; Body Mass Index

*Mean±SD

bPaired t-test

Independent t-test

cAnalysis of covariance (adjusted for dietary intake changes and baseline values)

dAnalysis of covariance (adjusted for baseline values)

*p<0.05 considered significant
Table 4- Comparison of inflammatory parameters between NS oil group and placebo group at baseline and after the intervention.

<table>
<thead>
<tr>
<th>Variable</th>
<th>NS oil group (n=45)</th>
<th>Placebo group (n=45)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TNF-alpha (pg/mL)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>24.0±17.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.7±15.2</td>
<td>0.5&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>End</td>
<td>14.2±10.9</td>
<td>18.2±13.5</td>
<td>0.03&lt;sup&gt;b,#&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Pre to post P-value&lt;sup&gt;c&lt;/sup&gt;</strong></td>
<td>&lt;0.01&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td><strong>IL-6 (pg/mL)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>12.7±5.0</td>
<td>12.3±5.2</td>
<td>0.8&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>End</td>
<td>11.6±4.8</td>
<td>12.0±5.3</td>
<td>0.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Pre to post P-value&lt;sup&gt;c&lt;/sup&gt;</strong></td>
<td>0.7</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td><strong>hs-CRP (pg/mL)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>3.3±2.4</td>
<td>2.8±1.8</td>
<td>0.3&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>End</td>
<td>1.5±1.3</td>
<td>2.2±1.6</td>
<td>0.03&lt;sup&gt;b,#&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Pre to post P-value&lt;sup&gt;c&lt;/sup&gt;</strong></td>
<td>&lt;0.01&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.3</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Mean±SD
<sup>b</sup>Analysis of covariance (adjusted for weight changes, dietary intake changes and baseline values)
<sup>c</sup>Paired t-test
<sup>d</sup>Independent t-test

* P<0.05 considered significant
Figure 1- Summary of the study participants flow diagram
**Figure 2** - Percentage changes of inflammatory parameters in the intervention and placebo groups

- TNF-α: p=0.04
- IL-6: p=0.6
- hs-CRP: p=0.01
Highlights

- *Nigella sativa* with a low-calorie diet decreased serum levels of TNF-alpha and hs-CRP in obese women after 8 weeks.
- *Nigella sativa* with a low-calorie diet did not change serum levels of IL-6 in obese women after 8 weeks.
- *Nigella sativa* with a low-calorie diet decreased body weight with no changes in BMI after 8 weeks.