The Effects of Nigella Sativa Extract Oil Supplementation on Plasma Level of ICAM-1, VCAM-1, Metabolic and Oxidative Parameters and Nutritional Status in Patients With Coronary Artery Disease: Study Protocol for A Double-Blind, Randomized Controlled Trial

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Abstract

Background: endothelial and metabolic dysfunction are the main risk factors for atherosclerosis, which eventually leads to cardiovascular diseases, such as coronary artery disease (CAD). However, it has been shown that increased inflammation and oxidative stress play an essential role in this process. *Nigella sativa* may affect declining atherosclerosis and CAD by reducing inflammation and improving vascular function.

Methods: Two sections that are presented in this article include; first, a review of recent findings related to cardiovascular disease, CAD, and their association with *N. sativa* supplementation. Secondly, we will illustrate a randomized, double-blind clinical trial in 46 Iranian adults with CAD. The subjects (age: 35-65, proof of 50% stenosis in at least one of their major coronary arteries in angiography) will be selected by a simple random sampling method and will be assigned to two months supplementation of *N. sativa* extract oil or placebo group. The primary outcome is the inflammatory and oxidative variables. The secondary outcomes measures include lipid profile, quality of life, depression, and anthropometric indices. The data will be equated within and between groups using appropriate statistical methods.

Discussion: The result of this trial will offer evidence about the efficacy of *N. sativa* supplementation by improving risk factors related to atherosclerosis and coronary artery disease.

Trial registration: Iranian clinical trial registry: [IRCT20190506043494N1](#), Date of registration: November 8, 2019.

Background

cardiovascular disease (CVD), with an estimated 17.9 million death around the world, is the leading cause of bereavement, and it is predicted to increase more than 23 million death by 2030[1, 2]. CVD includes different disorders of the heart or the blood vessels, such as peripheral artery disease (PAD), coronary artery disease (CAD), cerebrovascular disease, and congenital heart disease[3]. Obesity, dyslipidemia, blood pressure is among the risk factors to accelerate the CVD through impaired metabolism[4]. These unfavorable changes in blood biomarkers of glucose[5], the balance of adipokines, and cholesterol homeostasis, along with chronic inflammation and oxidative stress, are the critical factors associated with endothelial dysfunction and atherosclerosis[6]. Also, Increased biomarkers of endothelial dysfunction such as adhesion molecules (ICAM-1, VCAM-1) and oxidative markers are conventional in the process of atherosclerosis. Despite considerable pharmacotherapy, the survival rate is still low, and quality of life and depression as an important factor in CAD patients has not been enhanced by current therapeutic modalities[7, 8]. Therefore, elucidating a novel intervention with anti-inflammatory, antioxidant, and lipid-lowering effects alongside improving life quality and depression may modify the endothelial function, and overall health status and can be used as a clinical implication to reduce CVD.

Atherosclerosis is a chronic inflammatory process, responsible for numerous adverse vascular events, including stroke, myocardial infarction, coronary artery disease, and peripheral artery disease. Endothelial

dysfunction, inflammation, oxidative stress, vascular proliferation, thrombosis, hyperlipidemia, insulin resistance, and obesity are risk factors related to the initiation and progression of atherogenesis[9, 10]. Oxidative stress plays a pivotal role in the pathophysiological mechanism of atherosclerosis. When the balance between total antioxidant capacity and activity species such as reactive oxygen (ROS) and free radical species is disturbed, it leads to cell damage by direct lipid, protein, and DNA oxidation[11, 12]. Oxidative stress triggers the secretion of chemokines and cytokines, which gather the inflammatory cells to the inflammation site, leading to increased ROS production in a vicious feed-forward cycle [13–15]. Adhesion molecules, including ICAM-1 and VCAM-1, are crucial inflammatory mediators that trigger a signaling cascade in the endothelial cell, resulting in the increased intercellular ROS[16, 17]. Increased ROS enables the leukocytes and monocytes transmigration from the vessel lumen to the intima by restructuring of actins and disruption of endothelial junctions[7, 18–20]. The monocytes differentiate into macrophages and then changing into foam cells by internalizing lipoproteins. The presence of these foam cells is the hallmark of early atherosclerosis lesions. This process continues with smooth muscle cell migration and deposition of collagen, which eventually leads to the development of atherosclerotic plaque[21, 22].

Various types of plants and nutrients with health-improving properties have been identified due to their anti-inflammatory, lipid-lowering, anti-hypertensive and antioxidant properties, which can be potential interventions for cardiovascular disease[3, 23, 24]. *Nigella sativa*, which is also recognized as Black seed, is one of the medicinal plants that was used traditionally in the treatment of a wide range of conditions such as asthma, diarrhea, bloating[25–27]. Numerous studies have been carried out to evaluate the therapeutic effects of this plant. Improving the components of metabolic syndrome and lipid profile, lowering blood pressure, alleviating glycemic indexes, as well as reducing inflammation, have been among the properties discovered in recent years[28–34]. *N. sativa* is shown to improve the endothelial inflammation and atherosclerosis by reducing the gene expression of Vascular cell adhesion molecule (VCAM-1) and oxidized low-density lipoprotein (LOX-1) in endothelial cell of diabetic rats[35]. Also, in another study conducted on patients with Hashimoto's thyroiditis, *N. sativa* caused a significant reduction in VCAM-1[36].

Although previous studies have shown a significant potential effect of *N. sativa* on various conditions related to cardiovascular disease risk factors, whether *N. sativa* is an effective intervention in patients with CAD is still unknown. Consequently, the goal of this survey is to assess the possible effect of *N. sativa* on cardiac and metabolic associated CVD risk factors, which may lead to an improvement in endothelial function in patients with CAD. To test this hypothesis, an eight-week placebo-controlled, double-blinded, randomized clinical trial will be conducted to examine the efficacy of *Nigella sativa* intervention on CVD risk factors compared with placebo.

The general aim of this survey is to assess the effect of two-months *N. sativa* supplementation on controlling risk factors of CAD and preventing its progression through a double-blind, randomized, placebo-control, clinical trial in subjects with CAD. To determine whether *N. sativa* supplementation will improve endothelial function and inhibit atherosclerosis progression through enhancement of

inflammatory and oxidative stress indices, and lipid profile in patients with CAD. We hypothesize that *N. sativa* supplementation will reduce systolic and diastolic blood pressure, intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), malondialdehyde (MDA), low-density lipoprotein (LDL), total cholesterol, and triglyceride (TG) serum level; besides an improvement in high-density lipoprotein (HDL), superoxide dismutase (SOD), and total antioxidant capacity (TAC) level. Moreover, we aim to evaluate the effect of *N. sativa* on anthropometric indexes of these patients. Weight, waist circumference, hip circumference, neck circumference, and body composition, including fat mass and lean mass, are expected to change after supplementation. We also hypothesize that *N. sativa* will improve the quality of life and depression in patients with CAD.

Methods

Study design

The present study is an eight weeks double-blind, placebo-controlled clinical trial. Eligible subjects will be randomly assigned to 2 groups of placebo or intervention (2 soft gel containing 1 g of *N. sativa* extract oil each). The projected RCT will be conducted at the nutrition department of Tabriz medical university to evaluate the possible effect of *Nigella sativa* extract oil on patients with coronary artery disease. The Standard Protocol Items: Recommendations for Interventional Trials 2013 (SPIRIT) checklist is provided as an additional file.

Study population

Patients with coronary artery disease (CAD) will be recruited from Shahid Madani Heart center in Tabriz, Iran. Participants will be screened for the eligibility criteria by a cardiologist and based on their medical records. Then the included patients are informed by their physician about the trial protocol and routine appointments. After declaring their interest in the trial, they will be asked to sign a flyer with additional information about the study.

Inclusion criteria

Subjects will be recruited from Shahid Madani Heart center affiliated to Tabriz Medical Science, and those who meet the following criteria can participate in this project: a proof of 50% stenosis in at least one of their major coronary arteries in angiography, the ability and willingness to collaborate in the project, aged between 35-65 years, body mass index (BMI) of 18.5- 35.

Exclusion criteria

Exclusion criteria will include pregnancy or lactation, smoking cigarettes or opiates, cancer, acute or chronic renal failure, any hepatic or thyroid disorder, diabetes, any changes in medication, taking herbal supplementations, or any weight-loss drugs, having a history of food allergy.

Randomization and blinding

Eligible subjects will be randomly assigned in either control (taking placebo, n=30) or intervention (nigella sativa supplementation, n=30) group. A third party will use a computerize-generated random number to allocate the participant into the intervention and placebo groups. The company that produces the supplements will label the boxes with unique alphabetic codes based on the generated allocation sequence. Furthermore, the company will prepare two identical sealed boxes, each containing 30 soft gels inside. Every participant will receive four boxes during the supplementation. All of the investigators, participants, medical staff, and statisticians will be blind to the intervention, allocation, and study treatment until the final analysis. Any unintended unblinding will be reported to the main researcher. In such cases, applicant ID, date, and unblinding circumstances will be documented for inner control.

Sample size calculation

The sample size calculation was based on N. sativa supplementation on changes in total antioxidant capacity (TAC), which was conducted by Hadi et al. [37] it was computed by considering 95% confidence interval and 90% power. We also consider a 15% attrition rate, which at least 23 subjects are considered for each group.

Study procedure

At the baseline, a questionnaire, including past medical history, demographic information, drug history, and medication, will be filled. All the data will be confidential and accesable only by the investigators. Dietary intake will be assessed by 3-day questionnaires (two weekdays and one weekend) at the onset, internal, and the end of the study. Total calories, micro, and macro-nutrients intake will be calculated by NUT IV (the Hearst Corporation, San Bruno, CA, USA). Also, the physical activity level will be evaluated at the beginning and the end of the study by the international physical activity questionnaire (IPAQ). **Table 1** shows the timetable for enrollment, involvement, and evaluation based on the Standard Protocol Items; Recommendations for Interventional Trials (SPIRIT). The anthropometric measures will be assessed with light clothes and without shoes. The height will be measured by stadiometer with the accuracy of 0.1-cm. Actual body weight, total fat mass, fat-free mass, visceral fat, and body muscle along with their percentage will be measured by the [Tanita MC-780MA S](#) body analyzer. Besides, waist/hip/neck circumferences and blood pressure will be assessed at each visit. Compliance will be assessed based on returned capsule count; those who miss more than ten percent of supplementation dosage will be excluded from the trial. At the start and end of the study, 10 milliliters of blood will be collected. Blood samples will be taken after 10-12 hours of fasting and will be stored at -80 C till the end of the interventions. Enzyme-linked immunosorbent assay (ELISA) kits will be used to evaluate the serum ICAM-1, VCAM-1, and insulin concentration. Other biochemical analyses such as high-density lipoprotein (HDL-C), triglyceride (TG), total cholesterol (TC), fasting blood sugar (FBS), superoxide dismutase (SOD), malondialdehyde (MDA), and total antioxidant capacity (TAC) will be measured. Fried Ewald equation will be used to estimate the low-density lipoprotein (LDL-C) concentration. Homeostasis model assessment – insulin resistance (HOMA-IR) will be calculated. Also, the atherogenic index of plasma will be assessed by

the formula. Beck's Depression Inventory (BDI-II) and MacNew quality of life questionnaire related to heart disease will be used as the secondary outcomes.

Frequency of analyses

Only at the final analysis, outcome data will be analyzed, although statistical monitoring of safety data will be conducted throughout the study and reported at agreed intervals. Final analysis will take place eight weeks after the last patient is randomized.

Statistical analysis

We will use the SPSS program version 16.0 to analyze all the data (SPSS Inc., Chicago, IL), and P-value < 0.05 will be considered statistically significant. The efficacy of this trial will be assessed by both the per-protocol approach, and the intention-to-treat principle will be analyzed [38]. Missing or dropout data will be analyzed by modern imputation methods.

Subgroup analysis

Subgroup analysis are not planned yet.

Monitoring

Data monitoring

A clinical trial monitor occasionally supervises the study progress in each to ensure patient rights and well-being are safeguarded, that the protocol, ethical requirements, standards, and regulations are being followed, that the essential documentation is available and that collected data are accurate as there were recorded. Any change or amendments in the protocol will be shared.

Harm

There is no side effect reported after 2-g/day *Nigella sativa* supplementation. However, this clinical trial will be monitored by the data monitoring committee (DMC). Also, any possible side effects such as shortness of breath, allergy reaction, stomach disorders, muscular pain, and serious adverse events (SAEs) will be reported to the ethics committee of the Tabriz University of Medical Science.

Discussion

The objective of this trial is to appraise the effect of 2-g daily supplementation of N. sativa extract oil on cardiometabolic risk factors. The results of this study will demonstrate significant evidence of N. sativa value as an intervention in patients with coronary artery disease.

Despite the widespread role of drugs in the management of heart disease, including coronary artery disease, the incidence is significantly high. Also, these therapeutic strategies cause enormous economic

pressure on the patients and the healthcare system. Atherosclerosis involves increased expression of adhesion molecule, cell migration, and fibrous tissue proliferation that results in partial or total obstruction of blood flow. Due to the stages of atherogenesis, inflammation, and oxidative stress play a significant role in this phenomenon. Thus, performing strategies to control or alleviate these factors would be of interest. Complementary therapies are increasingly accepted as a possible promising strategy to optimize treatment outcome, as clinical studies indicated its effectiveness in various articles. Adjuvant therapy not only may increase medication treatment efficacy but also, it may cause a reduction in medication dosage, which leads to fewer side effects and increased medication adherence.

Previous evidence suggests that the prevention and treatment of atherosclerosis might be achieved by managing the risk factors. *N. sativa* supplementation has been considered as an effective and safe approach for the treatment of a wide range of diseases. *N. sativa* may affect CVD through a variety of mechanisms, including immune mediators, modulating glycemic status, by anti-inflammatory and antioxidant effects[39].

Studies suggest a beneficial effect of *N. sativa* supplementation on glycemic status, especially fasting blood glucose and HbA1c [24, 28]. Also, a hypolipidemic effect of *N. sativa* was shown by reducing very-low-density lipoprotein, triglyceride, total cholesterol, and low-density lipoprotein level[28, 40, 41]. Moreover, *N. sativa* extract consumption showed a cardioprotective effect against isoproterenol-induced MI by restoring cardiac markers and antioxidant status[42]. On the other hand, *N. sativa* had a significant clinical effect on patients with rheumatoid arthritis pains by alleviating inflammation and oxidative stress[37]. These results trigger a hypothesis that inflammatory and oxidative modulation with *N. sativa* supplementation might act as a substitute method in the treatment of the atherogenesis process in patients with coronary artery disease.

In conclusion, this is the first study that is designed to evaluate the effect of *N. sativa* supplementation on CAD patients by measuring significant inflammatory and oxidative biomarkers. The underlying pathway for these effects has not been understood yet; however, the preliminary evidence suggests that decreased inflammation and oxidative stress along with the hypolipidemic effect of *N. sativa* could attenuate these processes. This trial may play a vital role in providing primary clinical data and indicating mechanisms related to CAD. Besides, using an oral administration of *N. sativa* offers a new approach in the management of patients with CAD. We also anticipate that *N. sativa* supplementation will improve cardiovascular health by improving lipid profile, anthropometric indices, inflammatory and glycemic status. We hope that this trial will provide scientific evidence in supporting *N. sativa* intervention for preventing and treatment of CAD.

Trial status

Currently, recruitment is ongoing. The recruitment has started on 22nd November 2019, and it is predicted to be completed in October 2020. The current protocol is version 1.0, dated 17 August 2020.

List Of Abbreviations

BDI: Beck's Depression Inventory, **BMI**: body mass index, **CAD**: coronary artery disease, **CVD**: cardiovascular disease, **DMC**: data monitoring committee, **ELISA**: Enzyme-linked immunosorbent assay, **FBS**: fasting blood sugar, **HDL**: high-density lipoprotein, **HOMA-IR**: Homeostasis model assessment – insulin resistance, **ICAM-1**: intercellular adhesion molecule-1, **IPAQ**: international physical activity questionnaire, **LDL**: low-density lipoprotein, **LOX-1**: oxidized low-density lipoprotein, **MDA**: malondialdehyde, **PAD**: peripheral artery disease, **SAEs**: serious adverse events, **SOD**: superoxide dismutase, **TAC**: total antioxidant capacity, **TC**: total cholesterol, **TG**: triglyceride, **VCAM-1**: vascular cell adhesion molecule-1.

Declarations

Ethics approval and consent to participate

This protocol is approved by the ethics committee of the Tabriz University of Medical Science, which is conducted in accordance with the most recent version of the declaration of Helsinki (approval number: IR.TBZMED.REC.1398.687). Also, it is registered in the Iranian Registry of Clinical Trials (registration number: IRCT20190506043494N1) and is available at <https://irct.ir/trial/39337>. All patients will be given written, informed consent before the procedure and gave consent for personal information to be used in this study.

Consent to publication

Not applicable. This manuscript does not comprise any person's data in any for publication.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Comparing interests

The authors declare no competing interests.

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Authors' contribution

OMTR, MA, MA were involved in the conception and trial design. OMTR, MA, VM designed the sampling plan, data collection, and analysis plan. OMTR, MA, MA, VM, AR were involved in writing the manuscript. All authors have read and also approved the final version of the document.

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Tables

Table 1
Timetable of planned activities during the study directly related to participants

	Study period				
	Enrolment	Allocation	Post allocation		
Time Point	T= -4 weeks	T = 0	T = 0	T = 4	T = 8
Enrolment:					
Eligibility screen	*				
Informed consent		*			
allocation		*			
Intervention:					
Two placeboes soft gel daily			*	*	*
Two <i>nigella sativa</i> soft gel daily			*	*	*
Assessments:					
Blood collection			*		*
Anthropometric measurements			*	*	*
Questionnaires			*		*

Figures

Figure 1

flow diagram of the study protocol

Supplementary Files

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- [SPIRITchecklist.doc](#)