Received: 19 June 2021 | Revised: 28 January 2022 | Accepted: 1 March 2022

DOI: 10.1002/ptr.7441

RESEARCH ARTICLE

WILEY

The effect of *Nigella sativa* oil on vascular dysfunction assessed by flow-mediated dilation and vascular-related biomarkers in subject with cardiovascular disease risk factors: A randomized controlled trial

Hadi Emamat[1] | Seyyed Hossein Mousavi[2] | Jalal Kargar Shouraki[3] | Ebrahim Hazrati[4] | Sayid Mahdi Mirghazanfari[5] | Esmaeil Samizadeh[6] | Maryam Hosseini[7] | Vahid Hadi[1] | Saeid Hadi[1]

[1]Department of Health, School of Medicine, AJA University of Medical Sciences, Tehran, Iran

[2]Department of Cardiology, School of Medicine, AJA University of Medical Sciences, Tehran, Iran

[3]Department of Radiology, School of Medicine, AJA University of Medical Sciences, Tehran, Iran

[4]Trauma Research Center, AJA University of Medical Sciences, Tehran, Iran

[5]Department of Physiology and Iranian Medicine, School of Medicine, AJA University of Medical Sciences

[6]Department of Pathology, School of Medicine, AJA University of Medical Sciences, Tehran, Iran

[7]Nutritionist, Emam Reza Hospital, AJA University of Medical Sciences, Tehran, Iran

Correspondence
Saeid Hadi, Department of Health, School of Medicine, AJA University of Medical Sciences, Tehran, Iran.
Email: s.hadinu@yahoo.com

Funding information
This work was supported by the Vice-Chancellor for Research at the AJA University of Medical Sciences, Tehran, Iran.

Abstract

Cardiovascular diseases (CVD) are the leading causes of mortality worldwide. Flow-mediated dilation (FMD) is a marker of vascular function. Beneficial cardiometabolic effects of *Nigella sativa* (*N. sativa*) have been observed. We evaluated the effect of *N. sativa* oil on FMD, plasma nitrite, and nitrate (NOx) as nitric oxide (NO) metabolites, and inflammatory markers in subjects with CVD risk factors. Fifty participants were randomly assigned to either the *N. sativa* (two capsules of 500 mg *N. sativa* oil) or the placebo group (two capsules of 500 mg mineral oil), for 2 months. The brachial FMD, plasma NOx, vascular cellular adhesion molecule-1 (VCAM-1), and intracellular adhesion molecule-1 (ICAM-1) were measured. FMD and plasma NOx levels was significantly increased in the *N. sativa* group compared to the placebo group (changes: 2.97 ± 2.11% vs. 0.71 ± 3.19%, $p < 0.001$ for FMD and 4.73 ± 7.25 μmol/L vs. 0.99 ± 5.37 μmol/L, $p = 0.036$ for plasma NOx). However, there was no significant difference in ICAM-1 and VCAM-1 levels between groups. Therefore, *N. sativa* oil improves vascular NO and FMD in subjects with cardiovascular risk factors. However, more studies are warranted to confirm the beneficial impacts of the *N. sativa* oil on vascular inflammation.

KEYWORDS
CVD, FMD, inflammation, *Nigella sativa*, vascular function

1 | INTRODUCTION

Cardiovascular diseases (CVD) are the leading cause of morbidity and mortality worldwide, with the lifetime risk exceeding 60% (Mozaffarian et al., 2016). CVD account for about 30% of mortality worldwide (Tarride et al., 2009). In line with the global trend, CVDs cause 46% of all deaths and 20%–23% of the burden of disease in Iran (Sarrafzadegan & Mohammmadifard, 2019). Diabetes, dyslipidemia, hypertension, and smoking are known to be the most important modifiable risk factors for CVDs (Romero, Romero, Shlay, Ogden, & Dabelea, 2012). Atherosclerosis is a serious inflammatory condition in which plaques made up of lipids, foams cells (cholesterol-rich macrophages), fibroblasts, calcium, and other substances build up in the intima of arteries, making them narrower and harder which can restrict blood flow (Libby et al., 2019). This disorder is the underlying cause of most CVDs; including myocardial infarction (MI), heart

failure, stroke, and claudication (Frostegård, 2013). Vascular function play an important role in pathogenesis of atherogenesis and is regulated by many components, including endothelial cells (ECs), vascular smooth muscle cells (VSMCs), inflammatory cells, autonomic nervous system, etc. (Shimokawa & Satoh, 2014). Damage to the endothelium upsets the balance between vasodilation and vasoconstriction and trigger events that lead to atherosclerosis (Davignon & Ganz, 2004). Endothelial function can be readily evaluated by several methods and is a prognostic predictor of future CVDs (McMackin & Vita, 2005; Vita & Keaney Jr., 2002). Flow-mediated dilation (FMD), that is, the measurement of the brachial artery diameter before and after a raise in shear stress-induced by reactive hyperemia, is a non-invasive, fast and valid method for clinical measurement of endothelial function (Ghiadoni, Salvetti, Muiesan, & Taddei, 2015). Nitric oxide (NO) is known as endothelium-derived relaxing factor that modulates vascular tone. The nitrite, and nitrate (NOx) is usually used as an indicator of NO release (Levine, Punihaole, & Levine, 2012).

Inflammation plays a significant role in progression of endothelial dysfunction that is a key player in the development of atherosclerosis (De Ciuceis et al., 2005; Ong et al., 2012). Adhesion molecules, such as vascular cellular adhesion molecule-1 (VCAM-1) and intracellular adhesion molecule-1 (ICAM-1) are expressed on activated endothelial cells, up-regulated in atherosclerosis-prone sites, and contribute in the attraction of inflammatory monocytes into the endothelial layer (Libby, 2006). Previous studies have shown an inverse association between the brachial artery vasodilation and circulating ICAM-1 (Vita Joseph et al., 2004) and VCAM-1 (Chen et al., 2015).

Studies indicate that nutritional factors can affect vascular endothelial function (Emamat, Asadian, Zahedmehr, & Ghanavati, 2020; Emamat, Tangestani, Totmaj, Ghalandari, & Nasrollahzadeh, 2020; Vamvakis et al., 2020). Antioxidants have potential effects on improving the endothelial function (Varadharaj et al., 2017). *Nigella sativa* (*N. sativa*) as a seed rich in antioxidants and bioactive compounds such as thymoquinone (TQ), alkaloids, saponins, flavonoids, proteins, fatty acids, and so forth, has been found beneficial in the treatment of various diseases (Butt & Sultan, 2010; Shafiq, Ahmad, Masud, & Kaleem, 2014; Tavakkoli, Mahdian, Razavi, & Hosseinzadeh, 2017). *N. sativa* and its constituents have demonstrated some protective properties against metabolic syndrome (Razavi & Hosseinzadeh, 2014), hypertension (Dehkordi & Kamkhah, 2008; Fallah Huseini et al., 2013), obesity (Mahdavi, Namazi, Alizadeh, & Farajnia, 2015), dyslipidemia (Ibrahim et al., 2014; Tasawar, Siraj, Ahmad, & Lashari, 2011), diabetes (Hadi, Mirmiran, Hosseinpour-Niazi, Hedayati, & Azizi, 2015; Heshmati, Namazi, Memarzadeh, Taghizadeh, & Kolahdooz, 2015), and inflammation (Majdalawieh & Fayyad, 2015). On the other hand, evidence shows that these disorders are associated with vascular dysfunction and so it is possible that improvement in any of these disorders also improves vascular function (Brook, 2006; Dharmashankar & Widlansky, 2010; Lorenzo Ghiadoni et al., 2008; Teixeira et al., 2014). A number of experimental studies have shown that *N. sativa* can increase the production and bioavailability of NO (Purnamayanti, Windu, & Poeranto, 2018; Sayed, El-Latif, Eid, Elsayed, & El-Kader, 2009; Tasar, Sehirli, Yigner, Leymanoglu, & Yegen, 2012). To the best of our knowledge, so far only a few animal studies have been conducted on the effects of *N. sativa* on vascular function (Idris-Khodja & Schini-Kerth, 2012; Niazmand, Fereidouni, Mahmoudabady, & Mousavi, 2014; Suddek, 2010) and vascular inflammation (Abbasnezhad et al., 2019). Therefore, in the present study we decided to evaluate the effect of *N. sativa* oil on vascular dysfunction in a clinical trial setting, using the FMD technique and ICAM-1 and VCAM-1 as vascular inflammatory factors and in subjects with at least one risk factor for cardiovascular disease.

2 | MATERIALS AND METHODS

2.1 | Participants

In the current clinical trial, we recruited subjects with at least one risk factor for cardiovascular disease. The announcement as to how the volunteers could participate in the study and the necessary inclusion criteria was put on display in the hospital. If they met the eligibility criteria, volunteers among AJA personnel who had a medical record in the academic hospital (Emam Reza Hospital, AJA University of Medical Sciences, Tehran, Iran) were recruited to participate in the trial.

The inclusion criteria were having at least one CVD risk factor (including diabetes, hypertension, dyslipidemia or smoking), willingness to participate, an age range between 20 and 65 years, BMI < 30, and no vitamins or minerals supplementation in the last 2 weeks. Exclusion criteria were reluctance to continue, changes in medication, pregnancy, inflammatory or autoimmune diseases, chronic kidney disease (stages 4 or 5), and taking nitrate-containing medications. Participants were able to leave the trial at any time.

The study protocol was according to the Helsinki Declaration. The trial has received ethical approval from the Ethics Committee of AJA University of Medical Sciences, Tehran, Iran (The ethical committee code was IR.AJAUMS.REC.1399.167). All participants signed written informed consent. Present clinical trial was registered at the Iranian Registry of Clinical Trial (IRCT) with number IRCT20201126049495N1.

2.2 | Study design

This was a 2-month, single center, single-blinded, parallel assigned, randomized controlled clinical trial (RCT). Participants were blinded to the type of products, but the main investigator was not blinded. The cardiologist and study staff confirmed that the subjects were eligible according to the inclusion criteria. At the first session, the procedure and the goals of the study were explained to participants, and then they signed a written consent. A questionnaire was filled out regarding some data related to the participants' anthropometric measurements, demographic status, disease history, drug use, smoking status, and physical activity.

The participants were randomly assigned to the *N. sativa* (intervention) or the placebo group. The intervention group received two capsules containing 500 mg of *N. sativa* oil and the placebo group

received two capsules containing 500 mg of mineral oil daily, 30 min before mealtime, during a 2-month period. Soft gel capsules with identical appearance were filled with *N. sativa* and placebos to ensure participants blindness. The cold pressing method is used to extract oils from the *N. sativa* seeds. The *N. sativa* oil standardized based on the presence of at least 6.5 mg TQ and 495 to 605 mg linoleic acid per soft capsule. All products were purchased from Barij Essence Pharmaceutical Company, Kashan, Iran. Participants received 120 capsules in four packs, each containing *N. sativa* oil or mineral oil for daily use. Participants' adherence to supplementation was monitored every 2 weeks by phone call. Also, for each participant the remaining capsules counted to check the adherence to study medication. Participants were asked not to change their current dietary habits, physical activity, and medication regimen during the study period. In case of significant change in these variables, the person in question was excluded from the study. Participants did not participate in another parallel study but continued their previously prescribed medication.

2.3 | Randomization

Patients were randomly allocated to the *N. sativa* oil or the placebo group. Stratified block randomization was employed to assign each subject to either of the groups. The randomization was performed with the aid of a sequence generator by using a random number table and by opening consecutively numbered, sealed, and opaque envelopes. A block size of four was considered, with a 1:1 randomization ratio between the two groups.

2.4 | Biochemical assays

A 10-ml venous blood was obtained following a 12-hr fasting period by a hospital lab technician. The blood samples were centrifuged (4,000 rpm for 20 min); plasma was separated and stored at -80°C. Afterwards, samples were thawed at room temperature and the inflammatory markers were measured using commercially ELISA kits (R&D Systems for ICAM-1 and VCAM-1). Plasma NO-derived end products (NOx) levels were determined using a colorimetric assay kit (Cayman Inc., USA).

2.5 | Flow-mediated dilation

The radiologist in the research team (J.K.) performed the brachial artery FMD measurements using the method previously described (Khandouzi, Zahedmehr, Mohammadzadeh, Sanati, & Nasrollahzadeh, 2019), in radiology section of Emam Reza Hospital. Pre-measurement, the participants were told to keep an overnight fasting state and a 10-min rest. Afterwards, the brachial artery was imaged while the patients were laid in a supine position in a quiet, temperature-controlled room. The measurement procedure included the ultrasound imaging of the brachial artery by the use of a non-automatic (manual) device using the iU22 xMATRIX color Doppler system with a 12-MHz linear array transducer (Philips Medical Systems, Canada, Product number: 795050). A 3.5 MHz linear transducer was used to perform the scans. The brachial artery scan was conducted in a longitudinal section 2 cm above the antecubital fossa. The research team measured the diameters of the vessel from the anterior to the posterior interface, between media and adventitia, at a fixed distance from an anatomical marker. We also obtained a rest image and measured the arterial diameter at the baseline. To perform a second scan, a forearm blood-pressure cuff was placed distal to the antecubital fossa and inflated to at least 50 mmHg above systolic pressure for 5 min, followed by release; the scan was then conducted 90 s after deflation of the cuff. The following formula was used to calculate the percentage FMD (%FMD): $[(d_2 - d_1)/d_1] \times 100$ (d_1: the brachial artery diameter at baseline; d_2: brachial artery diameter after 90 s of cuff release). The radiologist was blinded to the patients' treatment group.

2.6 | Statistical analysis

The study sample size was determined using FMD as the primary outcome variable. By assuming probability of a type I error of 5% ($\alpha = 0.05$) and a type II error of 20% ($\beta = 0.2$; power = 80%) and the Cohen standardized effect value of 0.62, plus considering up to 10% chance of withdraw, the sample size was calculated 25 participants for each group to detect the desired effect.

All statistical analyses were performed using SPSS software (version 21.0, SPSS Inc., Chicago, IL). The Shapiro–Wilk test was used to verify the normality of the data. Mean and standard deviations or frequency and percent were used to express quantitative and qualitative variables, respectively. Between-group differences were analyzed using independent *t* test and Chi-square (χ^2) test for quantitative and qualitative data, respectively. Within-group changes were tested using paired *t* test. In final analysis, the 2-month values of FMD and inflammatory biomarkers were compared between the two groups using ANCOVA test, adjusted for baseline values. We performed an intention-to-treat analysis in this study. Baseline-carry-forward was used to impute missing data.

3 | RESULTS

Fifty subjects were eligible to participate in study. Three subjects were unable to finish the project (one subject due to refusing to continue weeks after consumption in placebo group and two subjects due to refusing to continue weeks after consumption ($n = 1$) and missing telephone responses ($n = 1$) in *N. sativa* group). Finally, 47 participants (23 in the *N. sativa* group and 24 in the placebo group) completed the study (Figure 1). Participant compliance was acceptable throughout the treatment period, and they consumed more than 80% of the capsules delivered to them. No adverse effects were reported.

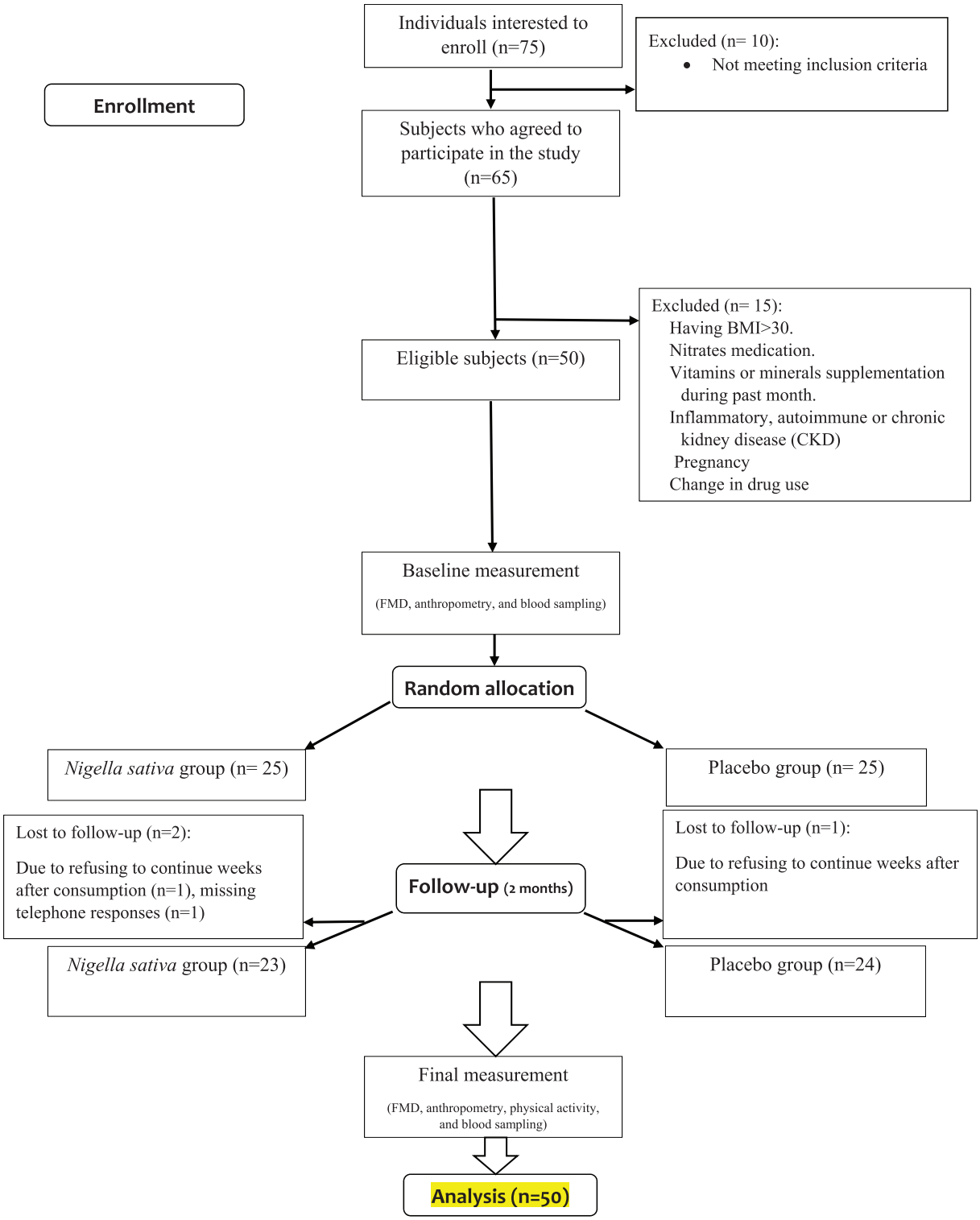

FIGURE 1 CONSORT flow diagram

Baseline characteristics of the participants are shown in Table 1. There was no significant difference between the two groups in terms of the variables of interest. The mean ± SD of age was 43.54 ± 9.63 and ranged between 25 and 64 years. About 84% of the participants were male. Twenty four percent of the participants had diabetes, 42% had high blood pressure, 60% had dyslipidemia, and 30% were smokers.

The mean ± SD of baseline weight, body mass index (BMI), and physical activity (PA) of the participants were 74.81 ± 8.41 kg, 25.85 ± 2.73 kg/m^2, and 30.07 ± 3.49 MET-hr/d; respectively. There was no

TABLE 1 Baseline characteristics of the patients

Variable	*Nigella sativa* group ($n = 25$)	Placebo group ($n = 25$)	p value[a]
Age (years)	44.28 ± 11.29	42.80 ± 7.79	.59
Sex (female)	4 (16%)	4 (16%)	1
Diabetes mellitus	6 (24%)	6 (24%)	1
Hypertension	11 (44%)	10 (40%)	.50
Dyslipidemia	14 (56%)	16 (64%)	.38
Smoking	8 (32%)	7 (28%)	.50

Note: Data represented as mean ± SD or frequency (percent).
[a]Values were compared using independent t test for quantitative and Chi-Square (χ^2) test for qualitative data.

TABLE 2 Body weight, BMI and physical activity of the participant at the baseline and after 2 months

Variable	Time	*Nigella sativa* group (n = 25)	Placebo group ($n = 25$)	p value[a]
Weight (kg)	Baseline	73.63 ± 6.78	75.99 ± 9.77	.32
	After 2 months	73.45 ± 6.74	76.11 ± 9.34	.25
BMI (kg/m^2)	Baseline	25.53 ± 2.85	26.16 ± 2.62	.42
	After 2 months	25.47 ± 2.80	26.21 ± 2.49	.33
Physical activity (MET-h/d)	Baseline	29.52 ± 3.25	30.62 ± 3.70	.26
	After 2 months	29.43 ± 3.61	31.04 ± 3.43	.11

Note: All values are means ± SD.
[a]Values were compared using independent t test.

TABLE 3 Measures of vascular flow-mediated dilation (FMD) in participant at the baseline and after 2 months

Variable	Time	*Nigella sativa* group ($n = 25$)	Placebo group ($n = 25$)	p value[a]	p value[b]
Brachial artery diameter (mm)	Baseline	4.45 ± 0.56	4.39 ± 0.63	0.72	.68
	After 2 months	4.47 ± 0.52	4.40 ± 0.55	0.63	
	p value[c]	.62	.85		
FMD (mm)	Baseline	0.14 ± 0.11	0.13 ± 0.07	0.61	<.001
	After 2 months	0.27 ± 0.11	0.15 ± 0.09	<0.001	
	p value[c]	<.001	.36		
FMD (%)	Baseline	3.44 ± 2.67	3.09 ± 1.94	0.60	.001
	After 2 months	6.41 ± 2.96	3.80 ± 2.56	0.002	
	p-value[c]	<.001	.27		

Note: All values are means ± SD.
[a]Values were compared using independent t test.
[b]Values were analyzed using ANCOVA test with baseline values as a covariate.
[c]Values were compared using paired t test.

difference between the two groups in terms of weight, BMI and PA at the baseline and at the end of the study (Table 2).

The mean ± SD of baseline brachial artery diameter of participants was 4.42 ± 0.59 mm. There was no significant difference in the basal brachial artery diameter between two groups at the baseline and after 2 months. FMD values were similar between groups at baseline, but were significantly higher in *N. sativa* group after 2 months (0.27 ± 0.11 mm or 6.41 ± 2.96% and 0.15 ± 0.09 mm or 3.80 ± 2.56% for *N. sativa* and placebo group; respectively). Intragroup comparisons revealed that FMD values increased significantly in the *N. sativa* group ($p < .001$). After adjusting for baseline values, a significant increase was observed in FMD values in the *N. sativa* group when compared to the placebo group ($p < .001$ and $p = .001$ for FMD (mm) and FMD (%), respectively) (Table 3). The intra-observer variability of FMD was determined by measuring FMD of six patients at two different times. The mean ± SD of repeated measurements were 3.10 ± 0.51 and 3.29 ± 0.73 with correlation coefficient of 0.95 ($p < 0.001$) and the mean difference of 0.19 ± 0.21. This intra-observer variation was less than our observed effect of *N. sativa* on FMD.

In intra-group analysis the plasma levels of ICAM-1 and VCAM-1 were significantly decreased in the *N. sativa* group after 2 months of

TABLE 4 Measures of inflammatory markers in participant at the baseline and after 2 months

Variable	Time	*Nigella sativa* group ($n = 25$)	Placebo group ($n = 25$)	*p* value[a]	*p* value[b]
ICAM-1 (ng/ml)	Baseline	28.32 ± 6.48	29.14 ± 4.57	.60	.07
	After 2 months	26.69 ± 6.87	28.67 ± 4.54	.23	
	p value[c]	.008	.17		
VCAM-1 (ng/ml)	Baseline	21.28 ± 2.19	22.55 ± 1.55	.022	.08
	After 2 months	20.34 ± 2.47	22.24 ± 1.93	.004	
	p value[c]	.019	.26		
NOx (μmol/L)	Baseline	42.63 ± 12.66	37.39 ± 11.08	.12	.036
	After 2 months	47.37 ± 13.99	38.38 ± 11.84	.018	
	p value[c]	.003	.36		

Note: All values are means ± SD.
[a]Values were compared using independent *t* test.
[b]Values were analyzed using ANCOVA test with baseline values as a covariate.
[c]Values were compared using paired *t* test.

intervention (28.32 ± 6.48 to 26.69 ± 6.87 ng/ml for ICAM-1, $p = .008$ and 21.28 ± 2.19 to 20.34 ± 2.47 ng/ml for VCAM-1, $p = .019$, respectively) (Table 4). Moreover, VCAM-1 was significantly higher in placebo compared to the *N. sativa* group at baseline (22.55 ± 1.55 and 21.28 ± 2.19 ng/ml, $p = .022$, respectively) and after 2 months (22.24 ± 1.93 and 20.34 ± 2.47 ng/ml, $p = .004$, respectively). However, after adjusting for baseline values, there was no significant difference in ICAM-1 and VCAM-1 levels between two groups ($p = .07$ and $p = .08$, respectively).

As shown in Table 4, plasma levels of NOx were significantly increased in *N. sativa* group after 2 months (42.63 ± 12.66 to 47.37 ± 13.99 μmol/L, $p = .003$). In placebo group the plasma levels of NOx did not change significantly after 2 months (37.39 ± 11.08 to 38.38 ± 11.84 μmol/L, $p = .36$). After adjusting for baseline values, the plasma levels of NOx were significantly higher in the *N. sativa* group compared to the control group after 2 months ($p = .036$).

4 | DISCUSSION

In the present study, we examined the effect of *N. sativa* oil supplementation on FMD, plasma NOx and vascular inflammatory biomarkers in subject with CVD risk factors. Our results suggest that *N. sativa* oil supplementation significantly improves vascular function when assessed by NOx and FMD. We also observed that *N. sativa* oil supplementation might not be able to improve circulating level of vascular inflammatory biomarkers of ICAM-1 and VCAM-1 compared to the control group; considering the issues of dose and duration.

Previous studies highlights the potential benefits of *N. sativa* or its components on CVD biomarkers (Shabana, El-Menyar, Asim, Al-Azzeh, & Al Thani, 2013; Shakeri, Khazaei, & Boskabady, 2018). The potentially beneficial effects of *N. sativa* on cardio-toxicity (Ahmed & Hassanein, 2013; Nagi, Al-Shabanah, Hafez, & Sayed-Ahmed, 2011), blood pressure (Leong, Rais Mustafa, & Jaarin, 2013; Sayed et al., 2009), endothelial dysfunction (Idris-Khodja & Schini-Kerth, 2012; Niazmand et al., 2014) dyslipidemia (Ali et al., 2013; El-Dakhakhny, Mady, & Halim, 2000), and atherogenesis (Al-Naqeep, Al-Zubairi, Ismail, Amom, & Esa, 2011) are indicated in some experimental animal studies. Some clinical trials have also reported the beneficial effects of *N. sativa* supplementation on inflammation (Hadi, Kheirouri, Alizadeh, Khabbazi, & Hosseini, 2016), blood pressure (Dehkordi & Kamkhah, 2008; Fallah Huseini et al., 2013), and dyslipidemia (Amini, Fallah Huseini, Mohtashami, Sadeqhi, & Ghamarchehre, 2011; Sabzghabaee, Dianatkhah, Sarrafzadegan, Asgary, & Ghannadi, 2012); all of which, as we know, might interact with vascular function. Although no clinical trial has directly examined the effect of *N. sativa* consumption on vascular function, studies have evaluated the beneficial effects of this plant on blood pressure as a closely relevant indicator of vascular function (Brandes, 2014; Dharmashankar & Widlansky, 2010). Eight weeks of intervention with *N. sativa* extract in two doses of 100 and 200 mg twice a day could reduce systolic and diastolic blood pressure in a dose-dependent manner in patients with mild hypertension (Dehkordi & Kamkhah, 2008). In another study, daily intake of 2,000 mg of *N. sativa* oil in overweight and obese women for 8 weeks reduced systolic blood pressure but had no effect on diastolic blood pressure (Razmpoosh et al., 2021). In another study, administration of 300 mg of the *N. sativa* extract twice a day with a shorter intervention period (28 days) and in elderly subjects with hypertension did not cause a significant change in blood pressure (Rizka, Setiati, Lydia, & Dewiasty, 2017). It can be said that the differences in study designs, population characteristics and dosages and duration of N.S supplements might cause these discrepancies.

Most of the beneficial medicinal effects of *N. sativa* are attributed to its volatile oil, of which TQ is a major component (30%–60%) (Iqbal, Ahmad, & Pandey, 2018). Previously desirable effects of TQ in cardiovascular disorders, diabetes, inflammation, atherogenesis, and lipid profile dysfunction have been shown with a wide range of safe doses and we speculated that the beneficial effects of *N. sativa* on vascular function are due to TQ (Darakhshan, Bidmeshki Pour, Hosseinzadeh Colagar, & Sisakhtnezhad, 2015; Gholamnezhad, Havakhah, &

Boskabady, 2016). Therefore, in the present study, *N. sativa* oil was used.

In previous study, intervention with *N. sativa* oil for 2 months was able to have beneficial effects on glycemic control, lipid profile, blood pressure and body weight in diabetic patients (Hadi et al., 2021). In another study, 2 months intervention in patients with rheumatoid arthritis increased plasma levels of interleukin-10 as an anti-inflammatory cytokine and also showed antioxidant properties (Hadi et al., 2016). Therefore, the intervention period in this study was considered 2 months.

To the best of our knowledge, no clinical trial has been conducted on the effect of *N. sativa* supplementation on vascular function indices, as of the composition date of this article. The effect of *N. sativa* on vascular function has been investigated in a few experimental studies. Niazmand et al. evaluated the contractile responses of isolated aorta to KCl and phenylephrine in different dosages of *N. sativa* hydro-alcoholic extracts (2–14 mg/ml). They found that the vasorelaxation effect of *N. sativa* was independent of endothelium and mediated mainly through the inhibition of calcium channels and reduction of intracellular calcium release (Niazmand et al., 2014). Another study examined the in vitro TQ-induced relaxation of isolated rat pulmonary arterial cells. They proposed that this observation is probably mediated by activation of ATP-sensitive potassium channels and possibly by non-competitive inhibition of serotonin, α1-adrenergic, and endothelin receptors (Suddek, 2010). Additionally, worthy of note, a review of evidence showed that *N. sativa* and its active phenolic compound, TQ, has the ability to improve vascular dysfunction in diabetes mellitus due to its anti-diabetic, anti-inflammatory, and antioxidant properties (Mohebbati & Abbasnezhad, 2020). Discovering definitive underlying mechanisms requires further experimental and clinical studies.

Gender plays an important role in vascular function (Khalil, 2005). Regarding gender differences between the two groups, although the number of female participants in this study was lower, there was no significant difference between the two groups in terms of gender (Table 1). Also, female participants in both groups were in premenopausal status and then this factor has not distorted the results.

FMD is an acceptable technique to quantify vascular function and has been shown to have prognostic value for future CVDs (Green Daniel, Jones, Thijssen, Cable, & Atkinson, 2011; Tomiyama et al., 2008). The results of a meta-analysis showed that for every 1 % increase in FMD, the risk of CVD decreased by 8%. This reduction in risk is even greater in subjects with CVD risk factors (13% risk reduction) (Ras, Streppel, Draijer, & Zock, 2013). Therefore, in our study an absolute increase of about 3% in FMD in the *N. sativa* group could have potential clinical relevance. Given that NO bioavailability is known as a vascular relaxant, a significant increase in NOx in the *N. sativa* group can be considered as an underlying mechanism for the observed effects of *N. sativa* on FMD. Consistently the beneficial effects of *N. sativa* on NO production and bioavailability have already been demonstrated in animal studies (Purnamayanti et al., 2018; Sayed et al., 2009; Tasar et al., 2012).

In this study, we also assessed vascular inflammatory biomarkers of ICAM-1 and VCAM-1. In-vitro studies show that TQ reduces expression and secretion of some cytokines or enzymes, such as MCP-1, interleukin-1β, TNF-α, NF-κB, and Cox-2, which might in turn further provoke anti-inflammatory pathways in the body (Chehl, Chipitsyna, Gong, Yeo, & Arafat, 2009). We conducted a similar study in which we examined anti-inflammatory effects of *N. sativa* in rheumatoid arthritis patients. Participants received two capsules of 500 mg *N. sativa* oil daily, lasting for 8 weeks (Hadi et al., 2016). We observed that Interleukin-10 increased in the group supplemented with *N. sativa* oil, but TNF-α did not change. The dose and duration of intervention in this study was similar to those of the present study. However, in the current study, even though the intragroup changes of ICAM-1 and VCAM-1 values in the *N. sativa* group were significant, we were not able to detect such significance when compared to the control group. We can attribute the disparities between the two studies to the higher basic level of inflammation in rheumatoid arthritis patients in the former study. In another study (Farhangi & Tajmiri, 2020), supplementation with 2 g of *N. sativa* powder for 8 weeks was able to reduce VCAM-1, but did not change the levels of ICAM-1. Likewise, this study was performed in patients with Hashimoto's thyroiditis who have higher basic levels of inflammation. Due to such ambiguities regarding the mechanisms of action and the disparities in the results of different studies, we recommend that further clinical trials with longer intervention duration and larger sample sizes be conducted to clarify the possible beneficial effects of *N. sativa* on biomarkers of vascular inflammation. Also in future studies, quality control of the herbal preparations is recommended as one of the limitations of former studies.

Several potential mechanisms for the vasodilator effect of the *N. sativa* have been mentioned, including inhibition of voltage-dependent calcium channels (Niazmand et al., 2014), muscarinic receptors, thromboxane B2, leukotriene B4, and prostaglandin D2 (Keyhanmanesh, Gholamnezhad, & Boskabady, 2014); activation of potassium channels, anticholinergic receptors, adrenergic receptors β2 (Niazmand et al., 2014), NO production (Abbasnezhad et al., 2019); and increasing the release of histamine (Keyhanmanesh et al., 2014).

The present study has some limitations. For instance, the duration of the present intervention did not suffice to detect the potential impact of *N. sativa* on anti-inflammatory vascular biomarkers and the sustainability of positive effects on vascular function. As well, to obtain more knowledge regarding the possible mechanisms of action, we could recommend future studies to explore the impact of *N. sativa* supplementation on the absorption and metabolism of its phenolic compounds by measuring *N. sativa* metabolites in plasma and urine. Assessment of other biomarkers related to endothelial function (such as endothelin and MCP-1) and quality control of the herbal preparations are recommended in future studies. Also, we did not have positive control. Our study is the first that clinically examines the effects of *N. sativa* on vascular function and has some notable strength, including proper design, high compliance, and high completion rates.

5 | CONCLUSION

Our results indicated that supplementation with *N. sativa* oil improves vascular NO and FMD in subjects with cardiovascular risk factors. Therefore, daily consumption of two capsules of 500 mg of *N. sativa* oil in subjects with CVD risk factors might prevent the aggravation of vascular dysfunction and the occurrence of CVDs, such as atherosclerosis in the future. However, confirmation of the beneficial effects of *N. sativa* oil supplementation on vascular inflammatory biomarker, such as ICAM-1 and VCAM-1, requires more clinical trials with longer duration of intervention and larger sample sizes.

ACKNOWLEDGEMENTS

The authors would like to thank the AJA University of Medical Sciences for the financial support of this research. We also sincerely appreciate all those who participated in this study.

CONFLICT OF INTEREST

The authors report no conflict of interest.

AUTHOR CONTRIBUTIONS

Saeid Hadi and Hadi Emamat conceptualized the study and wrote the manuscript. Hadi Emamat, Seyyed Hossein Mousavi, Jalal Kargar Shouraki, Esmaeil Samizadeh, Maryam Hosseini, and Vahid Hadi conducted the research. Ebrahim Hazrati and Sayid Mahdi Mirghazanfari contributed to drafting of the manuscript. All authors approved the final version of the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ORCID

Hadi Emamat https://orcid.org/0000-0002-8562-9136

REFERENCES

Abbasnezhad, A., Niazmand, S., Mahmoudabady, M., Rezaee, S. A., Soukhtanloo, M., Mosallanejad, R., & Hayatdavoudi, P. (2019). *Nigella sativa* L. seed regulated eNOS, VCAM-1 and LOX-1 genes expression and improved vasoreactivity in aorta of diabetic rat. *Journal of Ethnopharmacology*, *228*, 142–147. https://doi.org/10.1016/j.jep.2018.09.021

Ahmed, M. A., & Hassanein, K. (2013). Cardio protective effects of *Nigella sativa* oil on lead induced cardio toxicity: Anti inflammatory and antioxidant mechanism. *Journal of Physiology and Pathophysiology*, *4*(5), 72–80.

Ali, Y., Islam, M., Alam, A., Rahman, M., Mamun, A., Hossain, M., … Rashid, M. (2013). Inhibitory effects of *Nigella sativa* seed extract on adrenaline-induced dyslipidemia and left ventricular hypertrophy in rats. *Journal of Scientific Research*, *5*(2), 325–334.

Al-Naqeep, G., Al-Zubairi, A. S., Ismail, M., Amom, Z. H., & Esa, N. M. (2011). Antiatherogenic potential of *Nigella sativa* seeds and oil in diet-induced hypercholesterolemia in rabbits. *Evidence-Based Complementary and Alternative Medicine*, *2011*.

Amini, M., Fallah Huseini, H., Mohtashami, R., Sadeqhi, Z., & Ghamarchehre, M. A. (2011). Hypolipidemic effects of *Nigella sativa* L. seeds oil in healthy volunteers: A randomized, double-blind, placebo-controlled clinical trial. *Journal of Medicinal Plants*, *10*(40), 133–138.

Brandes, R. P. (2014). Endothelial dysfunction and hypertension. *Hypertension*, *64*(5), 924–928. https://doi.org/10.1161/HYPERTENSIONAHA.114.03575

Brook, R. D. (2006). Obesity, weight loss, and vascular function. *Endocrine*, *29*(1), 21–25. https://doi.org/10.1385/ENDO:29:1:21

Butt, M. S., & Sultan, M. T. (2010). *Nigella sativa*: Reduces the risk of various maladies. *Critical Reviews in Food Science and Nutrition*, *50*(7), 654–665.

Chehl, N., Chipitsyna, G., Gong, Q., Yeo, C. J., & Arafat, H. A. (2009). Anti-inflammatory effects of the *Nigella sativa* seed extract, thymoquinone, in pancreatic cancer cells. *HPB: The Official Journal of the International Hepato Pancreato Biliary Association*, *11*(5), 373–381. https://doi.org/10.1111/j.1477-2574.2009.00059.x

Chen, J., Hamm, L. L., Mohler, E. R., Hudaihed, A., Arora, R., Chen, C. S., … He, J. (2015). Interrelationship of multiple endothelial dysfunction biomarkers with chronic kidney disease. *PLoS One*, *10*(7), e0132047. https://doi.org/10.1371/journal.pone.0132047

Darakhshan, S., Bidmeshki Pour, A., Hosseinzadeh Colagar, A., & Sisakhtnezhad, S. (2015). Thymoquinone and its therapeutic potentials. *Pharmacological Research*, *95-96*, 138–158. https://doi.org/10.1016/j.phrs.2015.03.011

Davignon, J., & Ganz, P. (2004). Role of endothelial dysfunction in atherosclerosis. *Circulation*, *109*(23_suppl_1) III-27-III-32. 27–32. https://doi.org/10.1161/01.CIR.0000131515.03336.f8

De Ciuceis, C., Amiri, F., Brassard, P., Endemann, D. H., Touyz, R. M., & Schiffrin, E. L. (2005). Reduced vascular remodeling, endothelial dysfunction, and oxidative stress in resistance arteries of angiotensin II-infused macrophage colony-stimulating factor-deficient mice: Evidence for a role in inflammation in angiotensin-induced vascular injury. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *25*(10), 2106–2113. https://doi.org/10.1161/01.atv.0000181743.28028.57

Dehkordi, F. R., & Kamkhah, A. F. (2008). Antihypertensive effect of *Nigella sativa* seed extract in patients with mild hypertension. *Fundamental & Clinical Pharmacology*, *22*(4), 447–452. https://doi.org/10.1111/j.1472-8206.2008.00607.x

Dharmashankar, K., & Widlansky, M. E. (2010). Vascular endothelial function and hypertension: Insights and directions. *Current Hypertension Reports*, *12*(6), 448–455. https://doi.org/10.1007/s11906-010-0150-2

El-Dakhakhny, M., Mady, N. I., & Halim, M. A. (2000). *Nigella sativa* L. oil protects against induced hepatotoxicity and improves serum lipid profile in rats. *Arzneimittel-Forschung*, *50*(09), 832–836.

Emamat, H., Asadian, S., Zahedmehr, A., & Ghanavati, M. (2020). The effect of barberry (Berberis vulgaris) consumption on flow-mediated dilation and inflammatory biomarkers in patients with hypertension: A randomized controlled trial. *Phytotherapy Research*. *35*, 2607–2615. https://doi.org/10.1002/ptr.7000

Emamat, H., Tangestani, H., Totmaj, A. S., Ghalandari, H., & Nasrollahzadeh, J. (2020). The effect of garlic on vascular function: A systematic review of randomized clinical trials. *Clinical Nutrition*, *39*(12), 3563–3570. https://doi.org/10.1016/j.clnu.2020.02.016

Fallah Huseini, H., Amini, M., Mohtashami, R., Ghamarchehre, M. E., Sadeqhi, Z., Kianbakht, S., & Fallah Huseini, A. (2013). Blood pressure lowering effect of *Nigella sativa* L. seed oil in healthy volunteers: A randomized, double-blind, placebo-controlled clinical trial. *Phytotherapy Research*, *27*(12), 1849–1853. https://doi.org/10.1002/ptr.4944

Farhangi, M. A., & Tajmiri, S. (2020). The effects of powdered black cumin seeds on markers of oxidative stress, intracellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 in patients

with Hashimoto's thyroiditis. *Clinical Nutrition ESPEN*, *37*, 207–212. https://doi.org/10.1016/j.clnesp.2020.02.015

Frostegård, J. (2013). Immunity, atherosclerosis and cardiovascular disease. *BMC Medicine*, *11*, 117. https://doi.org/10.1186/1741-7015-11-117

Ghiadoni, L., Penno, G., Giannarelli, C., Plantinga, Y., Bernardini, M., Pucci, L., … Del Prato, S. (2008). Metabolic syndrome and vascular alterations in normotensive subjects at risk of diabetes mellitus. *Hypertension*, *51*(2), 440–445. https://doi.org/10.1161/HYPERTENSIONAHA.107.103093

Ghiadoni, L., Salvetti, M., Muiesan, M. L., & Taddei, S. (2015). Evaluation of endothelial function by flow mediated dilation: Methodological issues and clinical importance. *High Blood Pressure and Cardiovascular Prevention*, *22*(1), 17–22. https://doi.org/10.1007/s40292-014-0047-2

Gholamnezhad, Z., Havakhah, S., & Boskabady, M. H. (2016). Preclinical and clinical effects of *Nigella sativa* and its constituent, thymoquinone: A review. *Journal of Ethnopharmacology*, *190*, 372–386. https://doi.org/10.1016/j.jep.2016.06.061

Green Daniel, J., Jones, H., Thijssen, D., Cable, N. T., & Atkinson, G. (2011). Flow-mediated dilation and cardiovascular event prediction. *Hypertension*, *57*(3), 363–369. https://doi.org/10.1161/HYPERTENSIONAHA.110.167015

Hadi, S., Daryabeygi-Khotbehsara, R., Mirmiran, P., McVicar, J., Hadi, V., Soleimani, D., & Askari, G. (2021). Effect of *Nigella sativa* oil extract on cardiometabolic risk factors in type 2 diabetes: A randomized, double-blind, placebo-controlled clinical trial. *Phytotherapy Research*, *35*(7), 3747–3755. https://doi.org/10.1002/ptr.6990

Hadi, S., Mirmiran, P., Hosseinpour-Niazi, S., Hedayati, M., & Azizi, F. (2015). Effect of *nigella sativa* oil extract on lipid profiles in type 2 diabetic patients: A randomized, double blind, placebo-controlled clinical trial. *Iranian Journal of Endocrinology and Metabolism*, *16*(6).411–418.

Hadi, V., Kheirouri, S., Alizadeh, M., Khabbazi, A., & Hosseini, H. (2016). Effects of *Nigella sativa* oil extract on inflammatory cytokine response and oxidative stress status in patients with rheumatoid arthritis: A randomized, double-blind, placebo-controlled clinical trial. *Avicenna Journal of Phytomedicine*, *6*(1), 34–43.

Heshmati, J., Namazi, N., Memarzadeh, M.-R., Taghizadeh, M., & Kolahdooz, F. (2015). *Nigella sativa* oil affects glucose metabolism and lipid concentrations in patients with type 2 diabetes: A randomized, double-blind, placebo-controlled trial. *Food Research International*, *70*, 87–93.

Ibrahim, R. M., Hamdan, N. S., Mahmud, R., Imam, M. U., Saini, S. M., Rashid, S. N., … Ismail, M. (2014). A randomised controlled trial on hypolipidemic effects of nigella sativa seeds powder in menopausal women. *Journal of Translational Medicine*, *12*, 82. https://doi.org/10.1186/1479-5876-12-82

Idris-Khodja, N., & Schini-Kerth, V. (2012). Thymoquinone improves aging-related endothelial dysfunction in the rat mesenteric artery. *Naunyn-Schmiedeberg's Archives of Pharmacology*, *385*(7), 749–758. https://doi.org/10.1007/s00210-012-0749-8

Iqbal, M. S., Ahmad, A., & Pandey, B. (2018). Solvent based optimization for extraction and stability of thymoquinone from *Nigella sativa* Linn. And its quantification using RP-HPLC. *Physiology and Molecular Biology of Plants: An International Journal of Functional Plant Biology*, *24*(6), 1209–1219. https://doi.org/10.1007/s12298-018-0593-5

Keyhanmanesh, R., Gholamnezhad, Z., & Boskabady, M. H. (2014). The relaxant effect of *Nigella sativa* on smooth muscles, its possible mechanisms and clinical applications. *Iranian Journal of Basic Medical Sciences*, *17*(12), 939–949. https://doi.org/10.22038/ijbms.2015.3850

Khalil, R. A. (2005). Sex hormones as potential modulators of vascular function in hypertension. *Hypertension*, *46*(2), 249–254. https://doi.org/10.1161/01.HYP.0000172945.06681.a4

Khandouzi, N., Zahedmehr, A., Mohammadzadeh, A., Sanati, H. R., & Nasrollahzadeh, J. (2019). Effect of flaxseed consumption on flow-mediated dilation and inflammatory biomarkers in patients with coronary artery disease: A randomized controlled trial. *European Journal of Clinical Nutrition*, *73*(2), 258–265. https://doi.org/10.1038/s41430-018-0268-x

Leong, X.-F., Rais Mustafa, M., & Jaarin, K. (2013). *Nigella sativa* and its protective role in oxidative stress and hypertension. *Evidence-Based Complementary and Alternative Medicine*, *2013*.

Levine, A. B., Punihaole, D., & Levine, T. B. (2012). Characterization of the role of nitric oxide and its clinical applications. *Cardiology*, *122*(1), 55–68. https://doi.org/10.1159/000338150

Libby, P. (2006). Inflammation and cardiovascular disease mechanisms. *The American Journal of Clinical Nutrition*, *83*(2), 456s–460s. https://doi.org/10.1093/ajcn/83.2.456S

Libby, P., Buring, J. E., Badimon, L., Hansson, G. K., Deanfield, J., Bittencourt, M. S., … Lewis, E. F. (2019). Atherosclerosis. *Nature Reviews Disease Primers*, *5*(1), 56. https://doi.org/10.1038/s41572-019-0106-z

Mahdavi, R., Namazi, N., Alizadeh, M., & Farajnia, S. (2015). Effects of *Nigella sativa* oil with a low-calorie diet on cardiometabolic risk factors in obese women: A randomized controlled clinical trial. *Food & Function*, *6*(6), 2041–2048. https://doi.org/10.1039/c5fo00316d

Majdalawieh, A. F., & Fayyad, M. W. (2015). Immunomodulatory and anti-inflammatory action of *Nigella sativa* and thymoquinone: A comprehensive review. *International Immunopharmacology*, *28*(1), 295–304. https://doi.org/10.1016/j.intimp.2015.06.023

McMackin, C. J., & Vita, J. A. (2005). Update on nitric oxide-dependent vasodilation in human subjects. *Methods in Enzymology*, *396*, 541–553. https://doi.org/10.1016/s0076-6879(05)96046-1

Mohebbati, R., & Abbasnezhad, A. (2020). Effects of *Nigella sativa* on endothelial dysfunction in diabetes mellitus: A review. *Journal of Ethnopharmacology*, *252*, 112585. https://doi.org/10.1016/j.jep.2020.112585

Mozaffarian, D., Benjamin, E. J., Go, A. S., Arnett, D. K., Blaha, M. J., Cushman, M., … Fullerton, H. J. (2016). Heart disease and stroke statistics—2016 update: A report from the American Heart Association. *Circulation*, *133*(4), e38–e360.

Nagi, M. N., Al-Shabanah, O. A., Hafez, M. M., & Sayed-Ahmed, M. M. (2011). Thymoquinone supplementation attenuates cyclophosphamide-induced cardiotoxicity in rats. *Journal of Biochemical and Molecular Toxicology*, *25*(3), 135–142.

Niazmand, S., Fereidouni, E., Mahmoudabady, M., & Mousavi, S. M. (2014). Endothelium-independent vasorelaxant effects of hydroalcoholic extract from *Nigella sativa* seed in rat aorta: The roles of Ca2+ and K+ channels. *BioMed Research International*, *2014*, 247054. https://doi.org/10.1155/2014/247054

Ong, P., Sivanathan, R., Borgulya, G., Bizrah, M., Iqbal, Y., Andoh, J., … Kaski, J. C. (2012). Obesity, inflammation and brachial artery flow-mediated dilatation: Therapeutic targets in patients with microvascular angina (cardiac syndrome X). *Cardiovascular Drugs and Therapy*, *26*(3), 239–244. https://doi.org/10.1007/s10557-012-6382-4

Purnamayanti, N. M. D., Windu, S. C., & Poeranto, S. (2018). Effect of *Nigella sativa* ethanol extract on the nitric oxide content and renal arteriole diameter of a pre-eclampsia mouse model. *Eurasian Journal of Medicine*, *50*(3), 148–151. https://doi.org/10.5152/eurasianjmed.2018.17123

Ras, R. T., Streppel, M. T., Draijer, R., & Zock, P. L. (2013). Flow-mediated dilation and cardiovascular risk prediction: A systematic review with meta-analysis. *International Journal of Cardiology*, *168*(1), 344–351. https://doi.org/10.1016/j.ijcard.2012.09.047

Razavi, B. M., & Hosseinzadeh, H. (2014). A review of the effects of *Nigella sativa* L. and its constituent, thymoquinone, in metabolic syndrome. *Journal of Endocrinological Investigation*, *37*(11), 1031–1040. https://doi.org/10.1007/s40618-014-0150-1

Razmpoosh, E., Safi, S., Nadjarzadeh, A., Fallahzadeh, H., Abdollahi, N., Mazaheri, M., … Salehi-Abargouei, A. (2021). The effect of *Nigella sativa* supplementation on cardiovascular risk factors in obese and

overweight women: A crossover, double-blind, placebo-controlled randomized clinical trial. *European Journal of Nutrition*, *60*(4), 1863–1874. https://doi.org/10.1007/s00394-020-02374-2

Rizka, A., Setiati, S., Lydia, A., & Dewiasty, E. (2017). Effect of *Nigella sativa* seed extract for hypertension in elderly: A double-blind, randomized controlled trial. *Acta Medica Indonesiana*, *49*(4), 307–313.

Romero, C. X., Romero, T. E., Shlay, J. C., Ogden, L. G., & Dabelea, D. (2012). Changing trends in the prevalence and disparities of obesity and other cardiovascular disease risk factors in three racial/ethnic groups of USA adults. *Advances in Preventive Medicine*, *2012*, 172423. https://doi.org/10.1155/2012/172423

Sabzghabaee, A. M., Dianatkhah, M., Sarrafzadegan, N., Asgary, S., & Ghannadi, A. (2012). Clinical evaluation of *Nigella sativa* seeds for the treatment of hyperlipidemia: A randomized, placebo controlled clinical trial. *Medical Archives*, *66*(3), 198–200. https://doi.org/10.5455/medarh.2012.66.198-200

Sarrafzadegan, N., & Mohammmadifard, N. (2019). Cardiovascular disease in Iran in the last 40 years: Prevalence, mortality, morbidity, challenges and strategies for cardiovascular prevention. *Archives of Iranian Medicine*, *22*(4), 204–210.

Sayed, H., El-Latif, H., Eid, N., Elsayed, A., & El-Kader, E. (2009). Potential antihypertensive and antioxidative effects of *Nigella sativa* seeds or biomass and Syzygium aromaticum extracts on L-NAME-induced hypertensive rats. *Egyptian Journal of Pharmaceutical Sciences*, *50*, 127–146.

Shabana, A., El-Menyar, A., Asim, M., Al-Azzeh, H., & Al Thani, H. (2013). Cardiovascular benefits of black cumin (*Nigella sativa*). *Cardiovascular Toxicology*, *13*(1), 9–21.

Shafiq, H., Ahmad, A., Masud, T., & Kaleem, M. (2014). Cardio-protective and anti-cancer therapeutic potential of *Nigella sativa*. *Iranian Journal of Basic Medical Sciences*, *17*(12), 967–979.

Shakeri, F., Khazaei, M., & Boskabady, M. (2018). Cardiovascular effects of *Nigella sativa* L. and its constituents. *Indian Journal of Pharmaceutical Sciences*, *80*(6), 971–983.

Shimokawa, H., & Satoh, K. (2014). Vascular Function. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *34*(11), 2359–2362. https://doi.org/10.1161/ATVBAHA.114.304119

Suddek, G. M. (2010). Thymoquinone-induced relaxation of isolated rat pulmonary artery. *Journal of Ethnopharmacology*, *127*(2), 210–214. https://doi.org/10.1016/j.jep.2009.11.019

Tarride, J. E., Lim, M., DesMeules, M., Luo, W., Burke, N., O'Reilly, D., … Goeree, R. (2009). A review of the cost of cardiovascular disease. *The Canadian Journal of Cardiology*, *25*(6), e195–e202.

Tasar, N., Sehirli, O., Yigner, O., Leymanoglu, S., & Yegen, B. (2012). Protective effects of *Nigella sativa* against hypertension-induced oxidative stress and cardiovascular dysfunction in rats. *Marmara Pharmaceutical Journal*, *16*(2), 141–149.

Tasawar, Z., Siraj, Z., Ahmad, N., & Lashari, M. H. (2011). The effects of *Nigella sativa* (Kalonji) on lipid profile in patients with stable coronary artery disease in Multan, Pakistan. *Pakistan Journal of Nutrition*, *10*, 162–167.

Tavakkoli, A., Mahdian, V., Razavi, B. M., & Hosseinzadeh, H. (2017). Review on clinical trials of black seed (*Nigella sativa*) and its active constituent, Thymoquinone. *Journal of Pharmacopuncture*, *20*(3), 179–193. https://doi.org/10.3831/KPI.2017.20.021

Teixeira, B. C., Lopes, A. L., Macedo, R. C. O., Correa, C. S., Ramis, T. R., Ribeiro, J. L., & Reischak-Oliveira, A. (2014). Inflammatory markers, endothelial function and cardiovascular risk. *Journal Vascular Brasileiro*, *13*, 108–115.

Tomiyama, H., Matsumoto, C., Yamada, J., Teramoto, T., Abe, K., Ohta, H., … Yamashina, A. (2008). The relationships of cardiovascular disease risk factors to flow-mediated dilatation in Japanese subjects free of cardiovascular disease. *Hypertension Research*, *31*(11), 2019–2025. https://doi.org/10.1291/hypres.31.2019

Vamvakis, A., Gkaliagkousi, E., Lazaridis, A., Grammatikopoulou, M. G., Triantafyllou, A., Nikolaidou, B., … Douma, S. (2020). Impact of intensive lifestyle treatment (diet plus exercise) on endothelial and vascular function, arterial stiffness and blood pressure in stage 1 hypertension: Results of the HINTreat randomized controlled trial. *Nutrients*, *12*(5), 1326. https://doi.org/10.3390/nu12051326

Varadharaj, S., Kelly, O. J., Khayat, R. N., Kumar, P. S., Ahmed, N., & Zweier, J. L. (2017). Role of dietary antioxidants in the preservation of vascular function and the modulation of health and disease. *Frontiers in Cardiovascular Medicine*, *4*, 64. https://doi.org/10.3389/fcvm.2017.00064

Vita, J. A., & Keaney, J. F., Jr. (2002). Endothelial function: A barometer for cardiovascular risk? *Circulation*, *106*(6), 640–642. https://doi.org/10.1161/01.cir.0000028581.07992.56

Vita Joseph, A., Keaney John, F., Larson Martin, G., Keyes Michelle, J., Massaro Joseph, M., Lipinska, I., … Benjamin Emelia, J. (2004). Brachial artery vasodilator function and systemic inflammation in the Framingham offspring study. *Circulation*, *110*(23), 3604–3609. https://doi.org/10.1161/01.CIR.0000148821.97162.5E

How to cite this article: Emamat, H., Mousavi, S. H., Kargar Shouraki, J., Hazrati, E., Mirghazanfari, S. M., Samizadeh, E., Hosseini, M., Hadi, V., & Hadi, S. (2022). The effect of *Nigella sativa* oil on vascular dysfunction assessed by flow-mediated dilation and vascular-related biomarkers in subject with cardiovascular disease risk factors: A randomized controlled trial. *Phytotherapy Research*, *36*(5), 2236–2245. https://doi.org/10.1002/ptr.7441