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The Effects of Nigella Sativa Oil on Serum Levels Inflammatory Markers, Oxidative Stress Markers, and Lipid Profile in Dialysis Patients: A Double-Blind Clinical Trail

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ABSTRACT

Background: High concentrations of serum inflammatory and oxidative stress markers and lipid abnormities are important risk factors for cardiovascular diseases in hemodialysis patients. This study aims to investigate the effects of Nigella sativa oil on serum levels inflammation and oxidative stress markers and lipid profile in hemodialysis patients. **Methods**: Fifty hemodialysis patients participated in this randomized, double-blind, placebo-controlled clinical trial. The patients were randomly assigned into two groups, including receiving 2 tablets of Nigella sativa oil (1000 mg/day, NG) and receiving placebo (PG), for 8 weeks. Blood samples were taken from the patients at the beginning and the end of the study after 12 to 14 hour fasting for measuring serum markers. Then, levels of triglycerides (TG), total cholesterol (TC), LDL-c, HDL-c, high sensitivity C-reactive protein (hs-CRP), and malondialdehyde (MDA) were measured. **Results**: Consumption of Nigella sativa oil as a supplement decreased levels of TC (P = 0.0002), LDL-c (P = 0.001), mean serum hs-CRP, and MDA compared to the PG (P = 0.001). **Conclusion**: The Nigella sativa oil supplement may reduce serum hs-CRP and MDA, TC, LDL-c, and risk factors for cardiovascular diseases in hemodialysis patients.

Keywords: *Nigella sativa; Oxidative stress; Inflammation; Lipids*

Introduction

Cardiovascular diseases are the most important cause of mortality in patients with chronic renal failure (Jungers *et al.*, 1999). Some studies have indicated that high concentrations of serum inflammation markers, especially vascular inflammation markers, are an important risk factor for cardiovascular disease in hemodialysis patients (Bolton *et al.*, 2001, Kooshki *et al.*, 2019, Musiał *et al.*, 2004a, Musiał *et al.*, 2004b, 2005, Stenvinkel, 2002).

Nigella sativa (NS) is a small shrub and annual flowering plant which belongs to the family Ranunculaceae. It bears white, yellow, pink, and purplish delicate flowers containing 5 to10 petals

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(Forouzanfar *et al.*, 2014). The fruit is an inflated large capsule which bears large number of black seeds when ripped. NS is commonly grown in Middle Eastern and Western Asian countries, including Syria, Lebanon, Pakistan, India, and Afghanistan (Gilani *et al.*, 2004, Randhawa and Alghamdi, 2011, Zohary *et al.*, 2012).

NS oil and seeds have been widely used to treat different diseases within centuries and regarded as important drug in traditional medical system in Asian and Middle East countries (Rashidmayvan *et al.*, 2019). NS seed components include carbohydrates 33-34%, protein 19-19.9%, fat 35.5%, fiber 5-6.5%, and saponin 0.013% (Zohary *et al.*, 2012). The seed oil consists of certain chemicals, including thymoquinone (TQ) 30-48%, linoleic acid 44.7-56%, oleic acid 20.7-24.6%, and linolenic acid 0.6-1.8% (Mohtashami *et al.*, 2016). So far, limited studies have been done on the effects of NS oil supplement on serum systemic inflammation markers, such as C-reactive protein (CRP) that were controversy (Kheirouri *et al.*, 2016, Tavakoly *et al.*, 2019). NS seed and seed oil supplementation can significantly reduce serum CRP level. However, RCTs with a larger sample size and longer follow-up periods should be conducted for future investigations to confirm the veracity of these results (Kheirouri *et al.*, 2016, Le *et al.*, 2004, Mohtashami *et al.*, 2016, Tavakoly *et al.*, 2019).

The hypolipidemic effect of NS has been demonstrated, earlier, in experimental animals. These studies have reported that NS has a favorable effect on serum lipid profiles in normal rats (Dahri *et al.*, 2005, Kocyigit *et al.*, 2009, Nader *et al.*, 2010). Similar data were shown by the administration of TQ, the active ingredient of NS, to rabbits fed cholesterol enriched diet (Ismail *et al.*, 2010) and hypercholesterolemic rats (Abdullah O *et al.*, 1997).

Therefore, this study aims to investigate the effects of NS oil on serum levels inflammation and oxidative stress markers and lipid profile in hemodialysis patients.

Materials and Methods

Study design and pupulation: The samples were selected based on sample size formula and were divided to small groups (25 patients in each group). Due to likelihood of loss, 5 participants were added to this study and total sample size was 55 patients (Kooshki *et al.*, 2011).

Fifty patients were selected using convenience sampling from the hemodialysis unit of Vaseii Hospital in Sabzevar, Iran. The inclusion criteria consisted of patients aged 45 to 75 years, being dialyzed with polysulfone capillary dialyzers 3 times a week for 4 h per session, the hemodialysis procedure and type of dialyzer do not change, and being able to swallow capsules. The exclusion criteria included those with chronic inflammatory diseases, including inflammatory bowel diseases and infectious (like hepatitis) diseases, receiving any steroidal or non-steroidal anti-inflammatory drugs, including NS oil, omega-3 fatty acid supplements, L-carnitine, vitamin E, and/or vitamin C supplements. The drugs used in both groups were similar (Iron, Erythropoietin, Calcium carbonate, vitamin B, Folic acid, and Calcitriol).

In the present study, the participants and researchers during the study did not know whether each individual would be in the NS (NG) or placebo group (PG). For blinding, NS oil and placebo capsules were similar in appearance, packaging, and labeling. For this double blind study, at the start of the study, a set of bottles containing capsule was encoded A and B, by a person other than the researcher, so that the researcher was not aware of the type of capsules received by each group.

The patients were allocated to either the NG or the PG by block randomization (Manubhai *et al.*, 2014). The participates in the NG received 2 capsules (1000 mg NS oil) daily for 8 weeks, whereas the PG received 2 placebo capsules containing medium-chain triglyceride oils. NS oil and placebo capsules were prepared by Barij Essence Kashan Company and all capsules had similar appearance. Both the researchers and patients were unaware of the type of supplement received.

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The participants were advised not to change their dietary habits, physical activities, and drug regimens during the study period. Changes in these items were evaluated by 24-hour food recall and physical activity questionnaires.

The degree of compliance was determined for each patient based on the number of capsules returned. The compliance of all patients was more than 90% and no adverse events were reported for the patients.

Measurements: At the beginning and the end of week 8, 5 ml blood was collected from each patient after fasting for 12 to 14 hours before hemodialysis. Blood samples after clotting at room temperature (20–25 °C) were centrifuged at 2,000 rpm for 10 min. The sera were separated into small aliquots and frozen at 70 °C until they were used.

The serum hs-CRP concentration was determined using enzyme linked immune sorbent assay (ELISA) kits (Monobind, Inc., Lake Forest, Calif., USSA) with a coefficient of variation (CV) of 2.9%. The serum malondialdehyde (MDA) concentration was assessed using commercial colorimetric kits (Cayman Chemical, Ann Arbor, Mich., USSA) with a CV of 6.3%. Concentrations of triglycerides (TG), total cholesterol (TC), and serum high density lipoprotein-cholesterol (HDL-c) were measured enzymatically using commercial kits (Pars Azemoon Co., Tehran, Iran), with the aid of a Hitachi 717 auto-analyzer (Boehringer Mannheim Diagnostics, Mannheim, Germany). The CV for serum lipids was less than 5%. Given serum TG in all participating patients was less than 400 mg/dl, serum low-density lipoprotein cholesterol (LDL-c) was estimated using the Friedewald equation (Manubhai *et al.*, 2014).

The patients were weighed after hemodialysis to determine dry body weight (or post dialysis weight), at baseline and at the end of week 8. In addition, the dietary intakes of the participants were assessed using a 2-day dietary recall (1 dialysis day and 1 non-dialysis day) at the beginning and at the end of the study.

Dialysis adequacy based on the Kt/V index was determined for each patient at baseline by Kt/V calculator software using information recorded in patients' files, including pre-dialysis and post-dialysis blood urea nitrogen concentration, dialysis session length, post dialysis weight and ultra-filtration volume (Kooshki *et al.*, 2011).

Ethical considerations: The study protocol was approved by the Ethics Committee of Sabzevar University of Medical Sciences and Iranian Registry of Clinical Trials (Number IRCT20180122038472N2). Written informed consent was obtained from each of the participants.

Data analysis: Patients' diets were analyzed by Nutritionist-IV software (N-Squared Computing, San Bruno, Calif., USA). Statistical analysis of the data was performed using SPSS 15 (SPSS, Inc., Chicago, Ill., USA). All quantitative parameters were normally distributed according to the Kolmogorov-Smirnov test. Repeated measures were used to compare parameters between and within groups for all quantitative data. The results were expressed as Mean ± SD and differences were considered significant at $P < 0.05$.

Results

Sample size was 55 people, but three of them were subsequently excluded from the intervention group and two from PG due to lack of cooperation (**Figure 1**). Twenty-eight (56%) patients were female and 22 (44%) patients were male. The participants' age ranged 45-75 years. The mean age of the patients was 60.67 ± 13.81 years and mean duration of diabetes was 31.5 ± 11.0 months. Their mean body mass index (BMI) was 22.86 ± 5.75 kg/m^2. The patients' baseline characteristics were not different significantly between the two groups (**Table 1**).

Anthropometric and dietary factors were not significantly different between the two groups at baseline and at the end of week 8. In addition, these factors did not significantly change within each group during the study (**Table 2**).

Serum hs-CRP concentration decreased significantly in the NG at the end of week 8 compared to baseline (P = 0.001), whereas no

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significant change was observed in the PG. The reduction in hs-CRP concentration in the NG was significant in comparison with the PG (*P* = 0.001; **Table 3**). Serum MDA concentration decreased in the NG at the end of week 8 compared to baseline. The reduction in serum MDA concentration in the NG was significant in compared to the placebo group (*P* = 0.001; **Table 3**).

The results showed that NS oil supplement caused significant reduction in serum TC, LDL-c in NG, while these differences were not significant in the PG compared to baseline (**Table 3**). In between groups' comparison, the results showed that the observed difference in serum TC and LDL-c was significant between the two groups (**Table 3**).

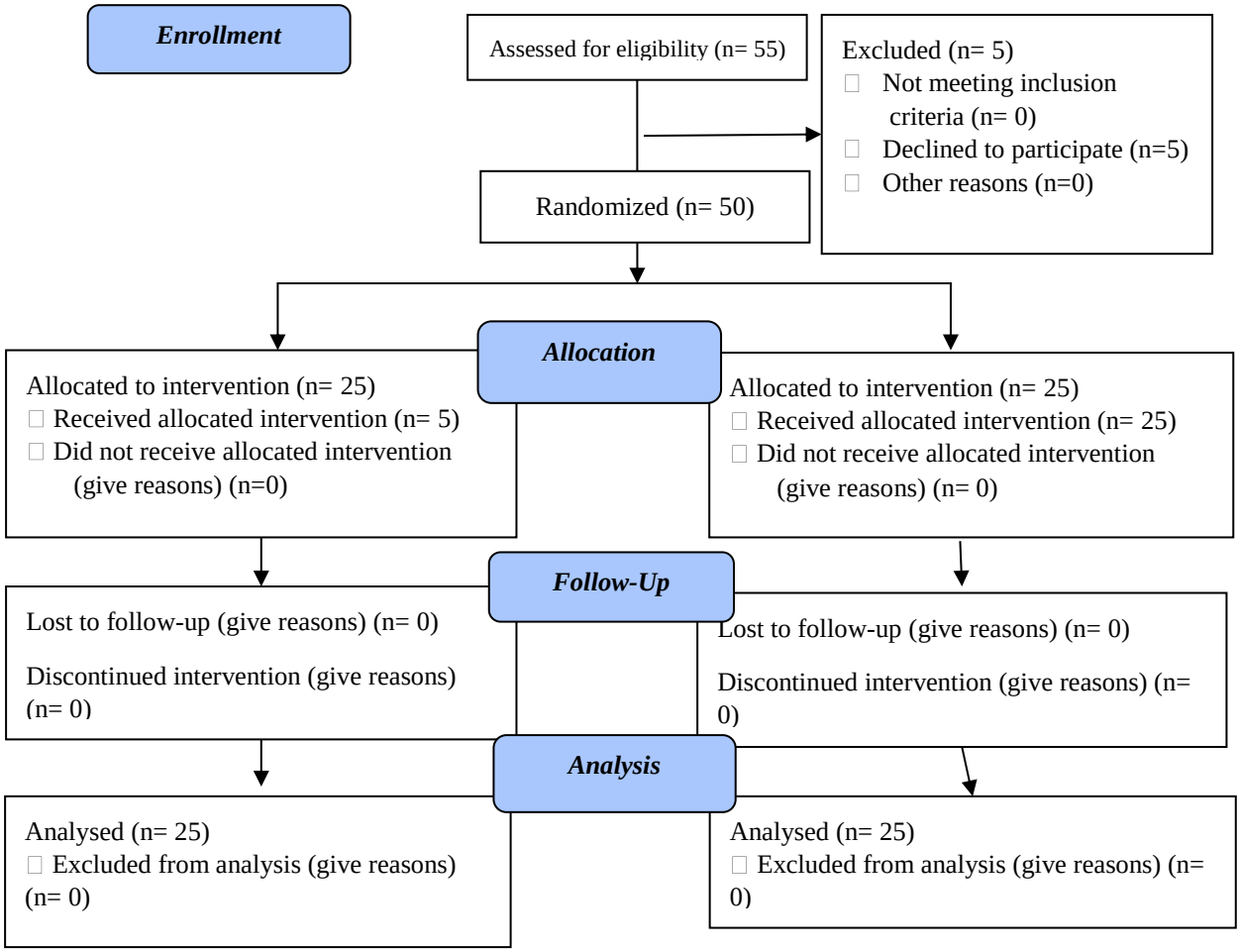


Figure 1. Flow chart of study.

Table 1. Baseline some characteristics of dialysis patients in the Nigella sativa and placebo groups.

Characteristics	Nigella sativa group (N= 25)	Placebo group (n=25)	P-value
Age (year)	60.04 ±14.83	61.30 ± 12.80	0.78[c]
Dialysis duration (month)	33.0 ± 16.0	30.0 ± 6.0	0.07[c]
Body mass index (kg/m^2)	33.12 ± 5.20	22.60 ± 6.30	0.33[c]
Sex			
Male	11(44)[a]	11(44)	1[b]
Female	14(56)	14(56)	

[a]: n (%); [b]: Chi-square tests; [c]: Independent t-test.

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Table 2. Mean (± SD) of anthropometric and dietary data in Nigella sativa and placebo groups.

Variables	Baseline	Week 4	Week 8	P-value [a]
Weight (kg)				
Nigella sativa	62.8 ± 17.0	62.6 ± 17.0	63.5 ± 17.0	0.126
Placebo	61.5 ± 11.0	61.5 ± 17.0	61.7 ± 10.0	0.298
P-value[b]	0.088	0.980	0.085	
Body mass index (kg/m^2)				
Nigella sativa	22.4 ± 3.0	22.3 ± 3.0	22.3 ± 3.0	0.176
Placebo	22.7 ± 3.5	22.7 ± 3.5	22.8 ± 3.5	0.078
P-value	0.076	0.084	0.073	
Energy (Kcal/day)				
Nigella sativa	1823.0 ± 502.0	1854.0 ± 500.0	1812.0 ± 505 .0	0.162
Placebo	1835.0 ± 495.0	1622 .0 ± 504.0	1853.0 ± 500.0	0.236
P-value	0.112	0.101	0.098	
Protein (g/day)				
Nigella sativa	62.0 ± 17.0	65.0 ± 15.0	60.0 ± 15.0	0.143
Placebo	58.0± 20.0	63.0 ± 23.0	61.0 ± 21.0	0.164
P-value	0.082	0.126	0.142	
Carbohydrate(g/day)				
Nigella sativa	305.0 ± 110.0	330.0 ± 105.0	295.0 ± 95.0	0.133
Placebo	302.0 ± 103.0	301.0 ± 96.0	310.0 ± 110.0	0.203
P-value	0.242	0.186	0.282	
Total fat (g/day)				
Nigella sativa	39.0 ± 15.0	43.0 ± 18.0	38.0 ± 15.5	0.143
Placebo	44.0 ± 15.0	43.0 ± 18.0	48 .0 ± 18 .0	0.164
P-value	0.418	0.990	0.549	
Cholesterol (mg/day)				
Nigella sativa	110.0 ± 45.0	120.0 ± 55.0	115.0 ± 75.0	0.385
Placebo	113.0 ± 50.0	115.0 ± 73.0	113.0 ± 62.0	0.413
P-value	0.286	0.202	0.189	
Fiber (g/day)				
Nigella sativa	13.5 ± 3.0	13.0 ± 4.0	13.0 ± 4.0	0.618
Placebo	11.0 ± 4.0	10.5 ± 4.0	11 .0 ± 4.0	0.546
P-value	0.389	0.456	0.402	

[a]: Repeated measures test; [b]:Independent t-test.

Table 3. Mean (± SD) of serum parameters concentration in the Nigella sativa and placebo groups (n =25 for all values).

Variables	Baseline	Week 8	P-value[a]
hs-CRP(mg/l)			
Nigella sativa	23.5 ± 3.2	13.2 ± 2.8	0.001
Placebo	24.3 ± 3.1	27.0 ± 3.3	
P-value[b]	0.90	0.001	
Malondialdehyde (µmol/l)			
Nigella sativa	2.6 ± 0.9	1.25 ± 0.7	0.001
Placebo	2.5 ± 0.5	2.75 ± 0.3	
P-value	0.95	0.001	
Triglyceride(mg/dl)			
Nigella sativa	119.5 ± 57.4	106.6 ± 61.2	0.810
Placebo	109.0 ± 19.0	115.0± 17.0	
P-value	0.84	0.81	

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Table 3. Mean (± SD) of serum parameters concentration in the Nigella sativa and placebo groups (n =25 for all values).

Variables	Baseline	Week 8	P-value[a]
Total cholesterol (mg/dl)			
Nigella sativa	142.9 ± 20.4	128.4 ± 23.1	0.001
Placebo	123.0 ± 15.0	131.0 ± 16.0	
P-value	0.01	0.001	
HDL-c (mg/dl)			
Nigella sativa	38.5 ± 26.8	34.0 ± 6.7	0.07
Placebo	42.0 ± 3.5	41.0 ± 5.0	
P-value	0.86	0.07	
LDL-c (mg/dl)			
Nigella sativa	86.5 ± 18.0	74.10 ± 21.1	0.001
Placebo	58.0 ± 13.0	64.00 ± 16.0	
P-value	0.9	0.001	

[a]: Paired t-test; [b]: Independent t-test?; HDL-c: High density lipoprotein cholesterol; LDL-c: Low density lipoprotein cholesterol

Discussion

In this study, the administration of NS oil had significant effect on hs-CRP as serum systemic inflammation marker. No study was found in the available literature on the effect of NS oil on serum hs-CRP and MDA in hemodialysis patients. However, similar studies have been performed on non-renal-failure patients. The results of the present study are not in line with the report of Mohtashami et al. and Datau et al. They showed that NS supplementation could not reduce significantly serum hs-CRP in people with syndrome metabolic and obesity (Datau *et al.*, 2010, Mohtashami *et al.*, 2016). However, this result is similar to a study on El-deep which showed NS supplementation reduced serum CRP in patients with coronary artery diseases (Al-Naggar *et al.*, 2017). The anti-inflammatory effect of NS is associated with its inhibitory effects on cyclooxygenase and 5-lipoxygenasse pathways. NS can inhibit inflammation by reducing the nitric oxide production and inhibiting cytokines interleukin-1 and 6 and the transcription factor βK inflammation. It also reduced the synthesis of MCP-1, TNF-α, and IL-β1 and inhibited the histone deacetylases Cox-2 by induction of histone hyper acetylation. NS oil has a mild effect on the expression of Cox-1 and PGE-2 in animal models of respiratory allergies (Al-Naggar *et al.*, 2017).

Hammad et al. have demonstrated that administration of tTQ prior to and following renal ischemia-reperfusion injury has specifically resulted in a significant improvement in the hemodynamic and tubular renal functional parameters and in attenuation of the gene expression of some of the pro-inflammatory and pro-fibrotic cytokines, namely TNF-α, TGF-β1, and PAI (Hammad and Lubbad, 2016). They also investigated the effect of TQ on TNF-α, TGF-β1 and PAI in IRI. TNF-α is a pro-inflammatory cytokine produced by several cells, including renal cells and TGF-β is a pro-fibrotic cytokine stimulating renal cells to produce extracellular matrix proteins leading to glomerulo-sclerosis and tubule interstitial fibrosis. PAI-1 is also a pro-fibrotic cytokine as it is considered as the major inhibitor of fibrinolysis. All these cyto-kines get up-regulated in renal diseases and the protective effect of TQ on these cytokines in Ischemia-reperfusion injury (IRI), appears to be similar to its action in other conditions (Hammad and Lubbad, 2016).

Oxidative stress is also a common complication in hemodialysis patients and can result in various complications, such as cardiovascular diseases (Canaud *et al.*, 1999, Galli and Ronco, 2000, Tetta *et al.*, 1999). In the current study, serum MDA concentration as a marker of oxidative stress

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decreased significantly in NG. This result is similar to the report of Mohebbati et al. which indicated that the administration of NS decreased tissue MDA level but increased total thiol and superoxide dismutase (SOD) activity in the kidney tissue (Mohebbati *et al.*, 2016). Cascurlu et al. also reported that treatment of renal reperfusion injury with NS decreased the elevated tissue MDA levels and increased activities of the enzymatic antioxidants glutathione peroxidase and catalase (Caskurlu *et al.*, 2016). It was opposite to a study by Heshmati al. which showed NS oil concurrent with a low-calorie diet that decreased weight and increased SOD levels in obese women. However, no significant changes were observed in lipid peroxidation, glutathione peroxidase, and total antioxidant capacity concentrations (Heshmati and Namazi, 2015).

Several studies have demonstrated that TQ efficiently scavenge free radicals and provides defense against lipid peroxidation (Al-Naggar *et al.*, 2017, Nagi *et al.*, 2011). The antioxidant and free radical scavenging activity of TQ revealed in the present study, was corroborated with these reports.

The decrease in MDA level following treatment with TQ can be ascribed to the enhanced activities of antioxidant status. The antioxidant activity could be explained by its conversion of TQ to thymohydroquinone. TQ (oxidized form) possesses low antioxidant activity, while its reduced form (thymohydroquinone) exerts a high radical scavenging capacity (Staniek and Gille, 2010).

Hayatdavoudi et al. reported that NS and its main component, TQ, had positive effects on prevention or treatment of kidney stones and renal failure through various mechanism, such as anti-oxidative, anti-inflammatory, anti-eicosanoid, and immune modulatory effects (Hayatdavoudi *et al.*, 2016).

In this study, NS oil supplement decreased serum TC and LDL-c concentration during 8 weeks in hemodialysis patients and this reduction was significant compared to the PG. Interestingly, the effect of NS on lipid profile in humans is controversial. Earlier study on healthy volunteers reported that intake of powdered NS for 2 weeks caused a decrease in plasma cholesterol level in the first week (Dehkordi and Kamkhah, 2008), while another study indicated that oral NS seed extract supplement showed significant reduction in blood pressure and TC and LDL-c in patients with mild hypertension within 8 weeks (Hebi *et al.*, 2016, Kooshki *et al.*, 2011). However, the literature indicates information on lipid lowering effect of NS on hemodialysis patients.

Kaatabi et al. showed that NS supplementation at a dose of 2 g/day for 12 weeks may improve the dyslipidemia associated with type 2 diabetic patients (Kaatabi *et al.*, 2012). A systematic review and meta-analysis of randomized controlled trials (RCTs) showed that NS had a significant impact on plasma lipid concentrations, decreasing TC, LDL-c, and TG levels, while increased HDL-c and it was only related to receiving NS powder (Sahebkar *et al.*, 2016). Several clinical trials have shown that NS reduced plasma levels of TC, low-LDL-c, and TG, but its effect on HDL-c was not significant (Heshmati and Namazi, 2015). The findings of the present study are not consistent with other studies on the effect of serum TG and HDL-c. It might be due to the duration and dosage of NS and type of supplementation, as well as differences in baseline serum levels.

Lipid-modifying effects of NS could be attributed to the inhibition of intestinal cholesterol absorption, decreased hepatic cholesterol synthesis, and up-regulation of LDL receptors (Heshmati and Namazi, 2015). Kaleem et al. also confirmed that the activity of NS in controlling the dyslipidemia may be due to its antioxidant effects (Kaleem *et al.*, 2006).

The present study increases the reliability of a growing number of studies conducted on the beneficial effects of NS oil supplement on serum systemic inflammation and oxidative stress and lipids profiles in hemodialysis patients. However, the study has quite some limitations, such as the low number of patients participated in this study. Moreover, the effect of NS oil was not determined on vascular inflammation markers and apo-lipoproteins, which should be considered in future

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studies. However, further well-planned studies in clinical settings may be needed to assess the effects of NS on vascular inflammation markers and the mechanisms of its effects.

Conclusion

The results of the present study indicate that NS oil supplement may reduce serum hs-CRP and MDA, total cholesterol and LDL-c, and risk factors for cardiovascular diseases in hemodialysis patients.

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Conflict interest

The authors declare that they have no conflict of interest.

Authors' contributions

Kooshki A, Taghizadeh M, and Akbarzadeh R designed the research; Kooshki A, and Akbarzadeh R, conducted the research; Kooshki A analyzed the data; and Kooshki A, Taghizadeh M, and Akbarzadeh R wrote the paper. Kooshki A had primary responsibility for final content. All authors read and approved the final manuscript.

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