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Original Article

The Effect of Hempseed Oil on Markers of Systemic Inflammation in Hemodialysis Patients: a single- blind randomized trial

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ABSTRACT

Background: Hempseed oil is a suitable source of alpha-linolenic acid, an omega-3 polyunsaturated fatty acid. In this study, we aimed to evaluate the effects of hempseed oil on the plasma levels of inflammation markers interleukin-6 and tumor necrosis factor-α in maintenance hemodialysis patients.

Methods: This 8-week single-blind randomized study was conducted on 97 hemodialysis patients. Patients were randomly assigned to the hempseed oil (receiving 20 ml of hempseed oil per day) or control (receiving no intervention) group. The plasma concentrations of interleukin-6 and tumor necrosis factor-α were measured at baseline and at the end of the study.

Results: The plasma concentrations of interleukin-6 and tumor necrosis factor- α changed significantly in neither of the group. Furthermore, the comparison of changes in the concentrations of IL-6 and TNF-α throughout the study showed no significant difference between the hempseed oil and control groups.

Conclusion: Hempseed oil consumption did not decrease inflammation in the maintenance hemodialysis patients.

Keywords: Hemodialysis, Hempseed oil, Cannabis oil, Inflammation, Interleukin-6, Tumor necrosis factor-α

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Introduction

Many patients undergoing maintenance hemodialysis (MHD) treatment have chronic systemic inflammation. Elevated levels of proinflammatory cytokines are associated with the increased risk of cardiovascular disease (CVD), protein-energy wasting, erythropoietin resistance and mortality in MHD patients (1). Therefore, the reduction of inflammation in this patient population has considerable beneficial outcomes.

Several clinical studies have shown that omega-3 polyunsaturated fatty acids (PUFAs) including eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and alpha-linolenic acid (ALA) have anti-inflammatory effects in hemodialysis (HD) patients (2).

Some vegetable oils like hempseed oil are rich sources of ALA (3). One of the important characteristics of the vegetable oils is omega-6 to omega-3 PUFAs (n6: n3) ratio. High n6: n3 ratio induces the production of proinflammatory mediators (4). Noori and colleagues have reported that increased dietary n6: n3 ratio increases serum C-reactive protein (CRP) concentration and the risk of death in HD patients. Moreover, n6: n3 ratio $\leq$ 6 was associated with decreased inflammation and all-cause mortality in this study (5).

Cannabis sativa L. is an annual plant. The seeds of nondrug type of the cannabis (industrial hemp), containing no psychoactive compounds, are rich sources of amino acids, vitamins, minerals**,** and fatty acids such as linoleic acid (LA), an omega-6 PUFA, and ALA (6). Furthermore, hempseed oil has an appropriate n6: n3 ratio which is between 2:1 and 3:1 (3, 6).

To the best of our knowledge, there has been no clinical study evaluating the anti-inflammatory effects of hempseed oil in the MHD patients. Therefore, a prospective randomized controlled trial was designed to test the hypothesis that hempseed oil consumption can decrease the plasma concentrations of the markers of systemic inflammation in the MHD patients.

Materials and Methods

Study Design

This study was an eight-week single-blind randomized controlled trial conducted in two HD centers affiliated to Kerman University of Medical Sciences. The researchers who measured the concentrations of mediators and statistically analyzed the results had been blinded to the groups of patients. The protocol of the study was reviewed and approved by the Ethics Committee of Kerman University of Medical Sciences (reference number: IR.KMU.REC.1395.864). Written informed consent was obtained from all participants.

Study population

Patients aged 18 to 70 years who were on regular three times per week HD for at least three months were screened for inclusion in the study. Patients with any of the following conditions were excluded: having active or chronic infections, acute myocardial infarction, autoimmune diseases, temporary vascular access for hemodialysis, hyperkalemia and hypermagnesemia, receiving omega-3 or fish oil supplements, consuming immunosuppressive drugs, pregnancy, and lactation.

During this study, all patients underwent the same hemodialysis procedure with the same type of a biocompatible polysulfone hollow-fiber dialyzer.

Study protocol

Eligible patients were randomly assigned to the hempseed oil or control group using block randomization with randomly selected blocks of size four, six and eight.

Hempseed oil was cold pressed from hempseed and was bottled without any additives in 150-ml brown glass containers. The hempseed used in this study contained 30% w/w oil. ALA constitutes 18.4% of the hempseed oil.

In the hempseed oil group, the patients were instructed to consume 20 ml hempseed oil, containing 3.68 g of ALA, per day. In the control group, the patients received no intervention. The patients were recommended to add hempseed oil to food after cooking or to salad. Furthermore, the patients were asked to have a constant food schedule, especially regarding the intake of seafood and nuts. Moreover, all participants were recommended to avoid consumption of hemp seeds, and the patients in the control group were also recommended to avoid consumption of hempseed oil during the study.

At baseline and the end of week eight of the study, a 5-ml fasting blood sample was drawn from each patient immediately before the initiation of HD and was taken in vacutainer tubes containing EDTA. The plasma was separated from blood by centrifugation at 3000 rpm for 10 minutes and then was stored at -70 °C until further analysis.

Measurement of mediators' concentration

The plasma levels of IL-6, the primary outcome measure, and TNF-α, the secondary outcome measure, were measured at the baseline and end of the study.

The concentrations of IL-6 and TNF-α were determined by enzyme linked immunosorbent assay kits (Karmania Pars Gene, Kerman, Iran), according to the manufacturers' recommended procedure.

Sample size calculation

The calculated sample size at a power $(1 - \beta)$ of 90% and α error of 5% to detect a difference of 3 pg/ml in plasma concentration of IL-6 with standard deviation of 4 pg/ml (7, 8) was 37 patients in each group.

Statistical analysis

Statistical analyses were performed using SPSS version 21 (SPSS Inc., Chicago, IL). Baseline characteristics of the patients were compared between the groups using independent t-test or Chi square test. The analysis of pairwise data was done by paired t-test. Repeated measure analysis of variance (RMANOVA) has been used to compare changes in the concentrations of mediators from baseline to the end of the study between the two groups. The level of significance was considered < 0.05.

Results

Of 150 patients screened for eligibility, 97 patients fulfilled the entrance criteria and participated in the study. Twenty-five patients left the study (13 in the hempseed oil group and 12 in the control group); therefore, 72 patients (38 in the hempseed oil group and 34 in the control group) completed the study, and their data were analyzed (figure 1).

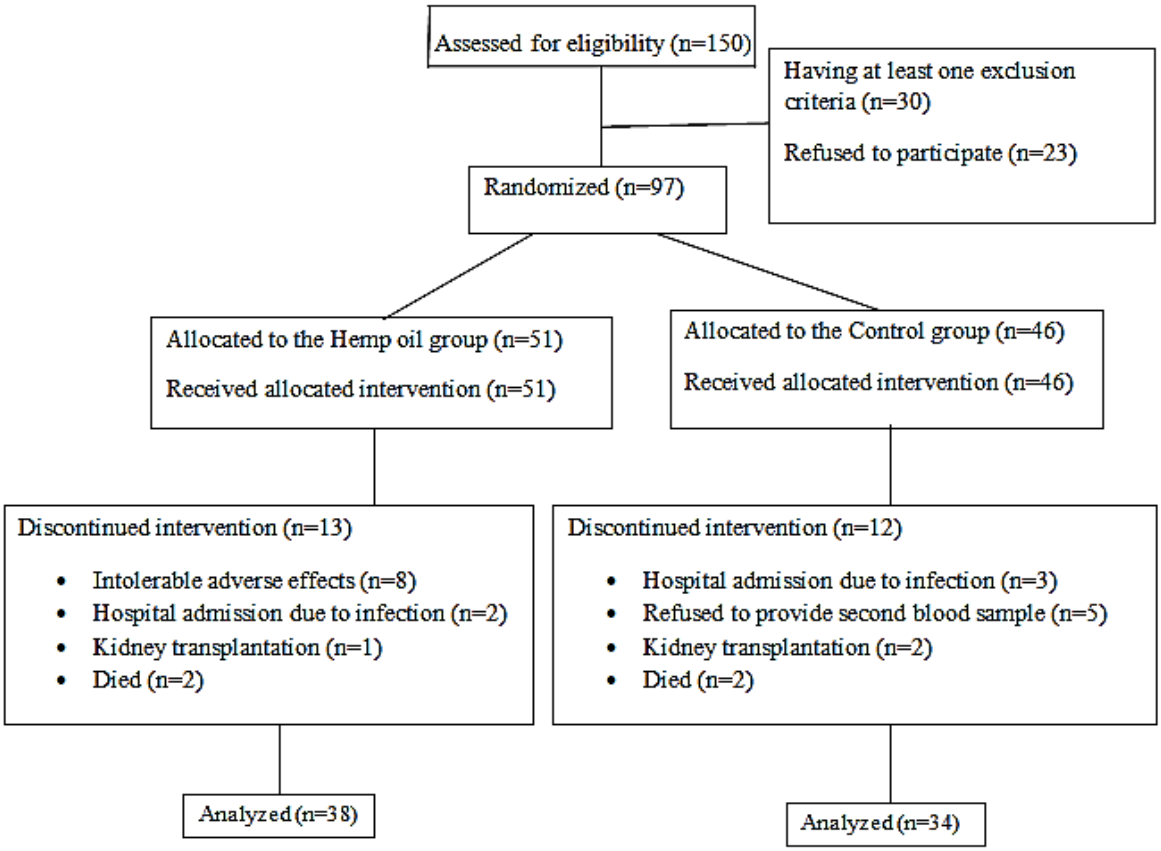

Figure 1. Flow diagram of participants in the study

At baseline, there were no significant differences in the demographic characteristics and laboratory data of participants between the two groups (table 1).

Table 1. Baseline characteristics of participants

	Hempseed oil (n=38)	Control (n=34)	P †
Male number, n (%)	26 (68.42)	16 (47.05)	0.07
Age (year),	61.08 ± 13.5	63.38 ± 13.25	0.46
Dialysis vintage (month)	30.21 ± 18.46	31.35 ± 22.5	0.82
Diabetes diagnosis, (n)	12	15	0.56
Tobacco use, (n)	8	6	0.59
Kt/v ‡	1.38 ± 0.26	1.44 ± 0.31	0.46
URR	0.67 ± 0.09	0.68 ± 0.10	0.64
Sodium (mEq/l)	135.97 ± 3.89	136.00 ± 2.62	0.97
Potassium (mEq/l)	5.25 ± 0.65	5.05 ± 0.69	0.19
Calcium (mg/dl)	8.30 ± 0.81	8.14 ± 1.10	0.5
Phosphate (mg/dl)	5.29 ± 1.07	5.15 ± 1.29	0.6
iPTH (ng/l)	462.78 ± 306.21	420.90 ± 291.36	0.55
Alkaline phosphatase (IU/l)	378.16 ± 329.02	523.47 ± 441.99	0.12
Hemoglobin (g/dl)	10.86 ± 1.79	10.86 ± 1.68	0.99
Tsat (%)	37 ± 72.42	28.82 ± 31.6	0.53
Ferritin (ng/ml)	228.15 ± 181.32	189.14 ± 175.33	0.35
Albumin (g/dl)	3.23 ± 0.5	3.04 ± 0.57	0.15
Creatinine	9.26 ± 2.8	9.54 ± 2.25	0.64

iPTH: intact parathyroid hormone, URR: Urea reduction ratio, Tsat: Transferrin saturation
†Determined by independent t-test or Chi square test
‡Representative of dialysis adequacy

Changes in the plasma concentration of IL-6 in the hempseed oil and control groups were negligible throughout the study. Moreover, the comparison of the two groups showed no significant group × time interaction. Similarly, the plasma concentration of TNF-α did not change significantly in any group, and the difference between the two groups was also insignificant (insignificant group × time interaction) (table 2).

Table 2. Changes in the concentration of inflammation mediators and nutrition indices from baseline to the end of the study

	Hemp seed oil (n=38)			Control (n=34)			$F_{\text{group × time interaction}}$ †*
	Baseline	**Month 2**	**Change***	**Baseline**	**Month 2**	**Change***	
IL6 (pg/ml)	3.84 ± 0.97	3.86 ± 0.85	+ 0.017 ± 1.29	4.81 ± 2.55	4.91 ± 2.16	+ 0.1 ± 1.28	0.07
TNF-α (pg/ml)	4.65 ± 0.77	5.46 ± 1.42	+ 0.81 ± 3.94	6.64 ± 3.69	7.15 ± 4.11	+ 0.51 ± 3.96	0.1

†Repeated measure analysis of variance (ANOVA) * All *P* values > 0.05

Adverse effects

Mild diarrhea and abdominal pain were the most common adverse effects in the hempseed oil group (table 3). No adverse effects were reported in the control group.

Table 3. Frequency of adverse effects in the hempseed oil group

Adverse effect	Number (%)	Continued/Left the study
Mild diarrhea	4 (10.5%)	Four patients continued the study
Abdominal pain	4 (10.5%)	Four patients left the study
Exacerbation of redness and warmness of nail ulcers†	3 (7.8%)	One patient left the study
Headache	2 (5.2%)	Two patients left the study
Drop in blood pressure‡	1 (2.63%)	The patient continued the study
Somnolence	1 (2.63%)	The patient left the study

†Three patients with nail ulcer had diabetes mellitus, and two of them, who continued the consumption of hempseed oil, experienced no worsening of the ulcers.
‡The patient continued the study, and her blood pressure returned to its baseline values.

Discussion

The results of the present study showed that 8 weeks consumption of hempseed oil did not decrease the plasma concentrations of IL-6 and TNF-α in the group of MHD patients.

Some clinical studies have demonstrated that Omega-3 PUFAs have anti-inflammatory effects in HD patients (2). The main mechanism of anti-inflammatory action of omega-3 PUFAs is the competitive inhibition of the conversion of arachidonic acid to inflammatory mediators (9). Vegetable oils have different quantities of omega-3 (e.g. ALA) and omega-6 (e.g. LA) PUFAs (3). It has been well established that the n6: n3 ratio plays an important role in the extent of beneficial effects of PUFAs because a high n6: n3 ratio (e.g. more than 5:1) increases the conversion of arachidonic acid to proinflammatory eicosanoids (4). Hempseed oil is a rich source of fatty acids and has one of the suggested best ratios of n6: n3 which is between 2:1 and 3:1 (3).

To the best of our knowledge, there has been no clinical study evaluating the anti-inflammatory effects of solitary supplementation with hempseed oil in patients with renal failure or other disorders. In Kaul *et al*. study (10), the consumption of 2 g fish oil, flaxseed oil or hempseed oil for 12 weeks caused no significant changes in the levels of CRP and TNF-α in healthy volunteers.

There are a limited number of studies examining the effects of other herbal sources of ALA on the inflammation markers in HD patients. Three studies evaluated the effects of flax seed oil, which approximately contains 60% w/w ALA (3, 11), on the serum CRP concentration in HD patients. Lemos *et al.* found a significant reduction in CRP level following daily consumption of two grams flaxseed oil for four months in HD patients (12). Mirfatahi *et al*. reported that the administration of six grams flaxseed oil per day for eight weeks to patients undergoing HD significantly reduced the serum concentrations of high sensitivity CRP (hs-CRP), and soluble vascular cell adhesion molecule type 1 (sVCAM-1) (11). Khalatbari-Soltani *et al.* demonstrated that daily consumption of 40 g ground flaxseed (containing 13.5 g fat) for eight weeks considerably reduced the serum level of CRP in HD patients (13).

It is worth mentioning that hempseed oil has other constituents such as myrcene (160 mg/L), β-caryophyllene (740 mg/L), β -sitosterol (100-148 g/L), α-tocopherol (7-136 ppm), ϒ-tocopherol (468-870 mg/L) (14-16), and a trace amount of methyl salicylate (14) in addition to fatty acids. However, the anti-inflammatory effects of the most of these ingredients have not been extensively investigated either in in vitro or in vivo studies. β-sitosterol has shown anti-inflammatory and immunomodulatory activities in in vitro (17) and animal studies (18-20) and a clinical study in marathon runners (21). The anti-inflammatory effects of β-caryophyllene (22, 23) and myrcene (24) have only been demonstrated in in vitro and animal studies. ϒ-tocopherol is the major tocopherol in hempseed oil. In a study, the anti-inflammatory effect of α-tocopherol (300 mg/day) has been compared with that of gamma-enriched tocopherols (300 mg/day, consisting of 60% ϒ-tocopherol, 28% δ-tocopherol, and 10% α-tocopherol) in 15 HD patients. The duration of the study was 14 days. At the end of the study, the plasma concentration of CRP was significantly reduced in the gamma-enriched tocopherol group in comparison to the α-tocopherol group. However, the level of IL-6 did not change significantly in any group (25). Himmelfarb et al. reported that oral supplementation with ϒ-tocopherol (308 mg/day) and DHA (800 mg/day) for 8 weeks significantly decreased the plasma concentration of IL-6 but not that of CRP in HD patients (26).

We believe that we did not observe any anti-inflammatory effect in the present study due to the following reasons:

Firstly, different studies that showed significant anti-inflammatory effects of ALA used different dosages of ALA (1.2 g/day to 8 g/day) for different durations (2 to 16 weeks). Although in our study, the dosage of ALA (3.6 g/day) and the length of the study (8 weeks) were in the middle of these ranges, supplementation with higher dosages of ALA for a longer duration may have anti-inflammatory effects in MHD patients.

Secondly, at baseline, the concentrations of IL-6 and TNF-α were not high in the both groups of our study. Therefore, if we had enrolled patients with higher levels of inflammatory markers, we might have detected more profound effects of hemp seed oil on the levels of these mediators. Consistent with this deduction, Lemos et al. reported that in their study, 61% of recruited HD patients had inflammation, and subgroup analysis showed that flax seed oil significantly reduced the level of CRP only in these patients (12).

Thirdly, vegetable oils have several constituents (such as phytosterols and phenolic compounds) other than ALA, which may have anti-inflammatory effects (27, 28). Different concentrations and compositions of these agents may be responsible for divergent anti-inflammatory effects of different vegetable oils.

The main limitations of the present study are the single-blind design without any placebo comparator, low dosage of hempseed oil, and short duration of the study.

In conclusion, this study showed that the consumption of 20 ml hempseed oil per day for eight weeks did not decrease inflammation in a group of MHD patients. Large-scale double-blind placebo-controlled trials are required to conclude whether or not hempseed oil has anti-inflammatory effects in MHD patients.

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