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Research Article

Effect of *Juglans regia* L. Ridge on Blood Lipids in Type 2 Diabetic Patients with Dyslipidemia: A Double-blind Placebo-Controlled Randomized Clinical Trial

Mehrzad Mirshekari[1], Arman Zargaran[2], Nooshin Shirzad[3], Mahboobeh Hemmatabadi[3], Mahnaz Khanavi[4], Mahbube Ebrahimpur[3], Fatemeh Afra[1], Soha Namazi[5,1*]

[1]*Clinical Pharmacy Department, School of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.*

[2]*Traditional Pharmacy Department, School of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.*

[3]*Endocrinology and Metabolism Research Center, Endocrinology and Metabolism Clinical Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran.*

[4]*Pharmacognosy Department, School of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.*

[5]*Research Center for Rational Use of Drugs, Tehran University of Medical Science, Tehran, Iran.*

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Abstract

Background: Dyslipidemia and diabetes mellitus are two important risk factors for coronary artery disease and stroke. Traditionally, herbal remedies like walnut were used to treat dyslipidemia. The study aimed to evaluate the effect of *Juglans regia* L. (*J. regia* L.) internal septum extract (ISE) on lipid profile of patients with type 2 diabetes.

Methods: After preparing hydroalcoholic ISE, Folin-Ciocalteau (FC) and AlCl3 colorimetric methods were used to determine total phenolic content (TPC) and total flavonoid content (TFC), respectively. In a randomized, double-blind placebo-controlled trial, 86 diabetic patients with dyslipidemia were randomly divided into equal groups and received ISE or placebo capsules 1500 mg/day for 12 weeks. Lipid profile, LFT, SCr, urea, hemoglobin A1c (HbA1c), blood pressure (BP), weight, waist and waist to hip ratio (WHR) were determined at baseline and after 12 weeks. The paired sample t-test and independent sample t-test were performed to compare the differences within and among the groups, respectively. This study was registered in the Iranian registry of clinical trials (IRCT ID: IRCT20201227049850N1).

Results: The mean (SD) of TPC and TFC were measured based on 74.57 (5.20) milligram gallic acid equivalent/gram of dry extract (mg GAE/g DE) and 14.11 (2.73) mg quercetin equivalent/g of DE (mg QE/g DE), respectively. During the trial, 26 patients lost follow-up, and the study continued with remaining 60 patients. After intervention, there were no significant differences in LDL-C (p=0.44), total cholesterol (TC) (p=0.42), high-density lipoprotein cholesterol (HDL-C) (p=0.99), triglyceride (TG) (p =0.32) and Lp(a) (p=0.55) between two groups. Moreover, no significant (p>0.05) changes were observed in HbA1c, LFT, SCr, urea, BP, weight, waist, and WHR among the groups after 12 weeks.

Conclusion: Our findings showed *J.regia* L. ISE had no significant effect on lipid profile compared to placebo. Moreover, no adverse effect was observed on liver and kidney function tests.

Introduction

Dyslipidemia can cause lipid deposition in the lining of vessels wall, forming plaque and progression of atherosclerosis. so It is one of the most important risk factors for coronary artery disease (CAD) and cerebrovascular disease.[1] Several risk factors can play a role in development of dyslipidemia , like inappropriate life style and some disorders such as uncontrolled diabetes mellitus.[2] In diabetic patients, insulin resistance can change lipid metabolism which leads to high levels of plasma triglycerides (TG), low levels of high-density lipoprotein cholesterol (HDL-C), and increased concentration of low-density lipoprotein cholesterol (LDL-C).[3]

One study from 2015 to 2018 has estimated the prevalence of dyslipidemia in American adults (age≥20 years) as 38.1% and 28.9% for total cholesterol (TC>240) and LDL-C (>130 mg/dl), respectively.[4] In Iran also according to a systematic review and meta-analysis study,

*Corresponding Author: Soha Namazi, E-mail: namazisoha@yahoo.com

the prevalence of high TC (≥200 mg/dl), high LDL-C (≥130 mg/dl), low HDL-C (<40 mg/dl in males, <50 mg/dl in females) and high TG (≥150 mg/dl) was estimated to be 41.6%, 35.5%, 46.0% and 43.9%, respectively.[5]

There are several medications to treat dyslipidemia. Statins are the most commonly used drugs to prevent primary and secondary cardiovascular disease (CVD) by lowering serum LDL-C levels; however, some patients do not respond to treatment with statins or cannot tolerate their side effects.[6,7]

High Lipoprotein(a) [Lp(a)] is another risk factor for CVDs via its prothrombotic properties.[8] One large cohort study in 2017 has shown that 18.4% (n= 9733) of cardiovascular patients had Lp(a) plasma level more than 50 mg/dl.[9] Although statins are one of the most common investigated medications in clinical trials, current evidence indicates that statins have little effect on Lp (a)'s plasma levels or can even cause modest level increase.[10] Niacin, according to results of two large clinical trials, decreased Lp(a) serum levels by approximately 20%, but it did not show any decrease in adverse cardiovascular events. In addition, serious adverse events such as diabetic complications, were observed in niacin treatment groups.[11,12] There are as well other medications which have the modest effect on lowering of Lp(a) such as proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors, cholesteryl ester transfer protein (CETP) inhibitors, antisense oligonucleotides (ASOs), and microsomal triglyceride transfer protein (MTP) inhibitors, but these agents are expensive.[13] Due to the lack of cost-effective drugs and/or drugs with favorable safety profiles which lower Lp(a), studies focus on agents with appropriate efficacy and fewer side effects in comparison, like herbal remedies.[14]

One of the most well-known herbal products to treat dyslipidemia in many countries is the walnut tree with the scientific name *Juglans regia* L. (*J.regia* L.) from the *Juglandaceae* family.[15] From the past centuries in Iranian traditional medicine, different parts of walnut such as leaves, bark, and fruits were used to treat several diseases including high blood pressure, diabetes mellitus, diarrhea, parasite infections, and dyslipidemia. The different parts of walnut tree have different bioactive compounds, therefore it can have various efficacies in treatment of diseases.[16,17] In recent two decades, researchers have focused on lipid-lowering effects of walnut. For instance one clinical trial study revealed walnut oil can improve the lipid profile of diabetic patients with dyslipidemia.[18] Another study showed that 6-week dietary walnut consumption improved lipid profile in old men along with excercise.[19] Moreover, Hosseini S *et al.*[20] reported *J.regia* L. leaf extract decreased TG and TC in type II diabetic patients. An additional randomized clinical trial showed that walnut-rich diet reduced CVD adverse events, including stroke, myocardial infarction and death in 7747 Spain participants who did not suffer from CVD, but they had diabetes mellitus or CVD risk factors.[21] Based on this trial, European Society of Cardiology (ESC)/European Atherosclerosis Society (EAS) dyslipidemia guideline recommends using walnut to prevent CVD.[22]

The internal septum is one of the most important parts of the walnut. One animal study revealed that the internal septum extract (ISE) of walnut could reduce TC, LDL-C, and TG in alloxan-induced diabetic rats.[23] The phytochemical analysis showed that the internal septum of *J. regia* L. is a rich source of the phenolic, flavonoid, cyanidin, and tocopherol compounds.[24] These bioactive compounds have antioxidant and anti-inflammatory effects and prevent cholesterol oxidation.[25] In addition, phenolic compounds in terms of their inhibitory effects on inflammatory cytokines like interleukin 6 can reduce Lp(a) gene expression.[26] Therefore, the internal septum of walnut may be used alone or in combination with other lipid-lowering drugs to prevent primary and secondary CVDs. The present study was designed to evaluate the effect of *J. regia* L. ISE on lipid profile of type 2 diabetic patients with dyslipidemia.

Materials and Methods

Preparation of the plant material formulations

Chemicals and reagents

Gallic acid, Folin-Ciocalteau (FC) reagent, and sodium bicarbonate were purchased from Sigma-Aldrich chemicals Co., (USA). Ethylic alcohol 99.8% was purchased from kimia alcohol Co., (IRAN). All other chemicals were of analytical grade.

Plant material

J. regia L. dried fruit was collected from Tuyserkan area, Hamadan, Iran. After identification by a botanist, it was deposited (Voucher No. PMP-2665) at the Herbarium Center in the School of Pharmacy, Tehran university of medical sciences (TUMS), Tehran, Iran.

Extraction procedure

One hundred kilograms of the internal septum was separated from the fruit of the walnut and ground to a semi fine powder. Afterward, the sample was extracted by the percolation method with 70% ethanol for 48 hours for three times.[27] The extract was filtered and evaporated by a rotary evaporator and was dried by a freeze dryer to produce the dried powder.

Determination of total phenolic content (TPC)

TPC was estimated using FC method.[28] The extract (0.1 ml, 2.5 mg/ml) was added to 7.9 mL distilled water and 0.5 ml FC reagent. Then, the solution was mixed for 3 minutes, and sodium bicarbonate (1.5 ml, 200 mg/ml) was added. The sample was incubated for 3 hours at room temperature in darkness, and the absorbance was measured by a UV mini 1240 UV-Visible spectrophotometer (Shimadzu, Japan) at 765 nm. The result was expressed as milligram gallic acid equivalent/gram of dry extract (mg GAE/g DE).

Determination of total flavonoid content (TFC)

The aluminum chloride ($AlCl_3$) colorimetric method was used to determine TFC.[29] The *J.regia* L. ISE was prepared at a concentration of 100 mcg/ml. Then, 1 ml of sample solution was added to 4 ml distilled water and then mixed with 0.3 ml of 5% NaNO2 in a 10 ml volumetric flask. After 5 min, 0.3 ml of 10% AlCl3 was added to the solution. Later, 2 mL of 1 Molar NaOH was added to the flask and made up to 10 ml with distilled water. After ten minutes at room temperature in the dark, the sample's absorbance at 510 nm was evaluated using a UV mini 1240 UV-Visible spectrophotometer (Shimadzu, Japan). As standards, quercetin solutions ranging from 0 to 100 mcg/ml were utilized. The result was represented in terms of milligram quercetin equivalent/gram of dry extract (mg QE/g DE).

Formulations plant material and placebo

The dried extract powder was passed through sieve No. 40 mesh. To improve the flow, 0.5% magnesium stearate was added, and finally, 500 mg of the extract powder was weighed in each gelatin capsules 0 size. 500 mg of microcrystalline cellulose was used in each gelatin capsule 0 size with the same color to prepare a placebo.

Study design

This study was a double-blind placebo-controlled randomized clinical trial. The participants were selected from among those referred to the endocrinology clinic in Imam Khomeini Complex Hospital and Clinic No. 2 Diabetes and Metabolic Diseases, affiliated with TUMS, Tehran, Iran. The study inclusion criteria was type 2 diabetic adult patients (age of 25 to 70 years) with hemoglobin A1c (HbA1c) 6.5% to 8% who have only received metformin 500-2000 mg/day as antidiabetic agents. Patients should also have uncontrolled dyslipidemia (serum LDL-C more than goal based on 2019 ESC/EAS dyslipidemia guideline) despite receiving the maximum or maximum tolerable dose of statins. The therapeutic goals of LDL-C blood levels vary between 55 and 100 mg/dl based on patients' diseases and other conditions.[22]

The exclusion criteria was patients who received injectable antidiabetic drugs, or any herbal medicines, patients with history of allergy to walnut, , immune system defects, pregnancy, lactation, renal failure (estimated glomerular filtration rate < 30 mL/min/1.73m^2), chronic liver disease (aspartate transaminase (AST) or alanine transaminase (ALT) > 3 times more than the upper limit of normal),[22] uncontrolled thyroid disease (thyroid-stimulating hormone (TSH) level > 4.5 mIU/L or < 0.4 mIU/L), concomitant use of any medication containing estrogen or progesterone, systemic glucocorticoids, cyclosporine, tacrolimus, everolimus, sirolimus, antipsychotic drugs, psychotropic substances or alcohol, neurological or psychiatric disorders that affect the acceptance of medication and the correct implementation of the study protocol.

Randomization and study process

At first, 730 diabetic patients with dyslipidemia were assessed for eligibility according to inclusion and exclusion criteria, and finally 86 patients were enrolled in the trial under the supervision of an endocrinologist. Participants were randomly allocated to two equal groups (n=43 in each group) using the permuted block randomization technique by a clinical pharmacist. For this purpose, 43 blocks of 2 patients (block size; 2) with different sequences but in equal proportions were formed from two groups (A and B) and were numbered from 1 to 43. Using the RANDBETWEEN function in Excel, the blocks corresponding to these numbers were placed in a row, and a random chain was formed from groups A and B. Group A was assigned as the intervention group and Group B as the concurrent comparison group. In order to concealment of the random allocation, 86 aluminum envelopes were prepared (to make the contents of the envelopes unclear). Then the letters A and B were recorded on the cards and placed in the envelopes in random order. Patients were consecutively went to the department secretary (who was completely unaware of the contents of the envelopes) and they received one envelope. Finally, the patients opened the envelopes in order and their assigned group was revealed.

The study was a double blind controlled trial. Patients and caregivers and also outcome assessors were blinded while only a pharmacist (responsible for the production line) in the pharmaceutical company was not blinded to content of capsules. In the next step, each group received capsules containing 500 mg of *J. regia* L. ISE or placebo three times a day for 12 weeks.

The serum level of the TC, TG, LDL-C, HDL-C, Lp(a), AST, ALT, alkaline phosphatase (ALP), total bilirubin, direct bilirubin, HbA1c, SCr and urea were measured in the baseline and end of the 12 weeks in both groups. Besides, weight, waist, waist to hip ratio (WHR), systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured at the beginning and after the 12 weeks intervention. All paraclinical tests were performed in the same laboratory center. Blood samples were obtained after participants fasted for at least 8 hours. Lipid profile, LFT, SCr and urea were assessed by enzymatic methods. [30]

Weight was measured through an electronic floor scale while participants were fasting, with no shoes and with light-weight clothing. To measure waist and hip circumference, patients stood with feet close together and arms at the side by using a flexible measuring tape. Waist circumference was measured when the tape placed snugly around the middle, just above their hipbones. The hip circumference was measured from the widest part of the buttocks. WHR was estimated by dividing waist circumference by hip circumference.

BP was evaluated by using a calibrated digital BP device with cuff sizes based on measured arm circumference. Two BP readings were made separated by the 15 minutes rest period, after the patients sat at least for 5 minute. The average e of the two readings was used to determine BP. [31]

All participants were advised not to change their previous medications, diet (particularly walnut consumption), and physical activity during the study period. The compliance of the patients was checked at the 6 and 12 weeks of intervention, and only who took more than 80% of all study capsules have remained in the study. During the study, patients were followed up for side effects by telephone or WhatsApp messenger for 12 weeks, and in case of possible complications, Naranjo scale was evaluated and recorded.[32]

Study outcomes

The primary outcome of the study was measuring LDL-C at baseline and 12 weeks after the intervention. Lp(a), liver function test (LFT), serum creatinine (SCr), and urea were evaluated as secondary outcomes at baseline and on week 12.

Statistical analysis

The minimum sample size was determined to be 30 patients in each group, considering the significance level of 0.05 with a statistical power of 95% for detecting at least a mean difference of 30 mg/dl in LDL-C between two groups.[18] To perform the parametric tests, the log transform was applied on the non-normal response variables. The paired sample t-test and independent sample t-test were performed to compare the differences within and among the groups, respectively. Descriptive data of demographic characteristics and paraclinical parameters were reported as mean (standard deviation [SD]). A *p*-value less than 0.05 was considered statistically significant. Statistical analyses were performed by Stata version 14.

Results

The mean (SD) of TPC and TFC were measured 74.57 (5.20) mg GAE/g DE and 14.11 (2.73) mg QE/g DE, respectively. During the trial, 26 patients lost follow-up in terms of coronavirus disease 2019 (COVID-19) or changes in lipid-lowering drugs, and the study continued with remaining 60 (n=30 in each group) patients (Figure 1). The mean (SD) age (year) of participants was 48.70 (20.35) in *J. regia* L. extract group and 45.20 (18.75) in the placebo group (p=0.41). The sex distribution was homogeneous (p=0.59) between the *J. regia* L. and placebo groups (19 vs. 17 male and 11 vs. 13 female, respectively).

The mean (SD) of HbA1c at baseline was 7.57 (1.01) and 7.47 (0.92) for *J. regia* L. and placebo-treated groups, respectively, and there was no significant difference between two groups (p =0.11). After 12 weeks, no significant (p =0.65) difference was observed in HbA1c among the groups. The mean (SD) dose of metformin was calculated 1616 (386.16) and 1466 (571.64) mg/day for *J. regia* L. and placebo treated groups, respectively, (p=0.09).

All participants were used atorvastatin (n=51) or rosuvastatin (n=9). The mean (SD) dose of atorvastatin was 24.16 (11.00) in *J. regia* L. group and 24.80 (11.22) mg/day in placebo group, (p =0.65) while, rosuvastatin dose was measured 21.00 (13.10) and 19.87 (13.43) in *J. regia* L. and placebo treated groups, respectively, (p =0.70).

Table 1 exhibited demographic characteristics and paraclinical parameters of patients in *J. regia* L. and placebo treated groups before and after intervention. The baseline demographic characteristics and paraclinical parameters of participants were homogeneous among the groups (p>0.05). As shown, there were no significant (p >0.05)

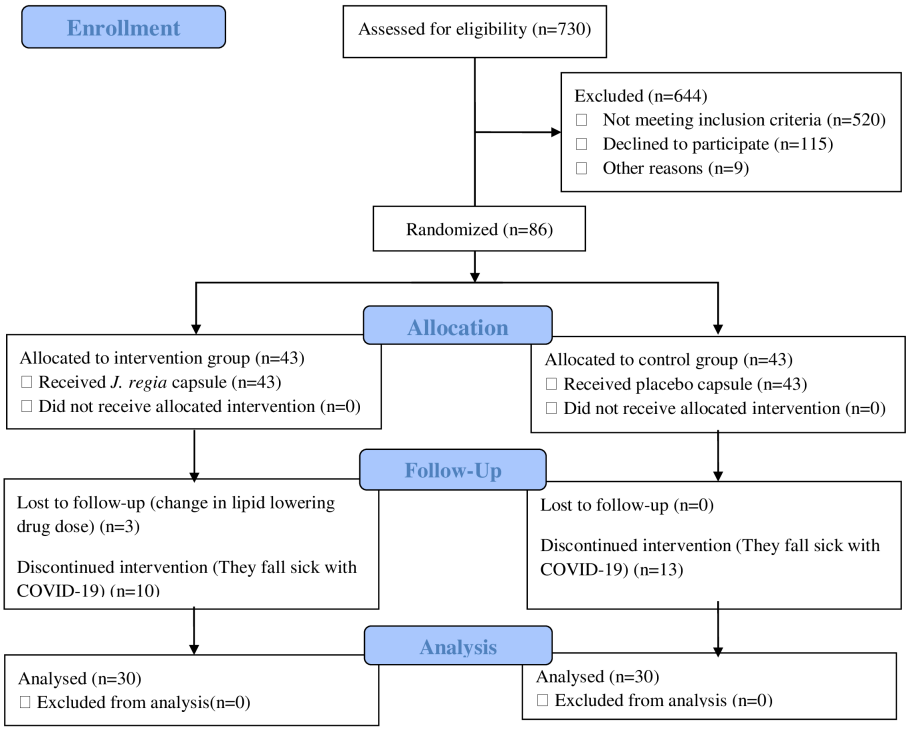

Figure 1. The CONSORT diagram of study.

Table 1. Demographic characteristics and paraclinical parameters of patients in *J. regia* L. ISE and placebo treated groups before and after intervention (N=60).

Parameters	Placebo group		*p*-value*	*J. regia* L. group		*p*-value*	*p*-value#
	Week 0§	Week 12		Week0§	Week 12		
Weight (kg)	68(12)	68(14)	0.22	73(22)	73(22)	0.91	0.12
WC (cm)	120(26)	120(25)	0.73	124(32)	126(33)	0.83	0.18
WHR	1.13(0.28)	1.14(0.26)	0.70	1.16(0.31)	1.17(0.31)	0.79	0.28
SBP (mmHg)	116(20)	119(21)	0.37	119(21)	121(25)	0.43	0.21
DBP (mmHg)	76(10)	75(9)	0.40	80(14)	77(11)	0.31	0.19
TC (mg/dl)	145(40)	146(32)	0.60	126(26)	131(27)	0.21	0.42
LDL-C (mg/dl)	84(30)	84(25)	0.27	72(17)	74(18)	0.15	0.44
HDL-C (mg/dl)	44(11)	44(11)	0.78	42(7)	43(7)	0.67	0.99
TG (mg/dl)	164(77)	154(83)	0.09	134(54)	133(63)	0.82	0.32
Lp(a) (mg/dl)	27(36)	29(36)	0.18	24(31)	26(39)	0.41	0.55
ALT (U/L)	25(9)	25(9)	0.61	25(15)	25(13)	0.90	0.73
AST (U/L)	23(9)	23(8)	0.91	24(15)	26(15)	0.26	0.48
ALP (U/L)	179(46)	197(51)	0.11	182(39)	194(55)	0.27	0.54
Total bilirubin (mg/dl)	0.66(0.33)	0.69(0.37)	0.56	0.70(0.36)	0.65(0.26)	0.65	0.56
Direct bilirubin (mg/dl)	0.24(0.13)	0.29(0.22)	0.60	0.24(0.10)	0.25(0.12)	0.90	0.71
SCr (mg/dl)	0.87(0.17)	0.89(0.13)	0.56	0.77(0.20)	0.76(0.15)	0.92	0.34
Urea (mg/dl)	30(8)	29(7)	0.15	26(6)	26(7)	0.93	0.86

Descriptive data expressed as mean (SD). *P*-value <0.05 was considered as significant. §there were no significant (p>0.05) differences in baseline data between two groups. *p-values obtained from paired t-test. #p-values obtained from independent sample t-test for comparing difference between two groups at week 12. ISE: internal septum extract, SD: standard deviation, WC: waist circumference, WHR: waist to hip ratio, SBP: systolic blood pressure, DBP: diastolic blood pressure, TC: total cholesterol, LDL-C: low density lipoprotein cholesterol, HDL-C: high density lipoprotein cholesterol, TG: triglyceride, Lp(a): lipoprotein(a), ALT: alanine transaminase, AST: aspartate transaminase, ALP: alkaline phosphatase, SCr: serum creatinine.

differences in the weight, waist circumference, waist-to-hip ratio, SBP, DBP, TC, LDL-C, HDL-C, TG, Lp(a), LFT, SCr, and urea of patients within the groups. Moreover, no significant (p >0.05) differences were observed in demographics features and paraclinical parameters between two groups at the end of study.

Only 9 (15%) patients had a baseline Lp(a) more than the upper limit of normal (50 mg/dl).[33] The mean (SD) of Lp(a) of these 9 patients was 115 (31) and 120 (38) mg/dl in *J. regia* L. and placebo-treated groups, respectively, (p =0.80). No adverse effect was reported in study patients during the follow-up period.

Discussion

This study aimed to evaluate the effect of the *Juglans regia* L. ISE on LDL-C as a primary outcome and Lp(a), LFT, SCr and urea as secondary outcomes. According to our results, *J. regia* L. ISE had no significant effect on blood LDL-C, TC, HDL-C, TG and Lp(a). In addition, no adverse effect on liver and kidney functions were observed.

Despite of our results, some other clinical trials have shown the different parts of *J. regia* L. such as oil, leaves and fruit can improve lipid profile in dyslipidemic patients.[18-20] one randomized, placebo-controlled clinical trial has indicated that Persian walnut oil (15 ml/day) significantly decreases TC (p <0.001), LDL-C (p <0.001) and TG (p =0.021) compared to placebo group in 100 type 2 diabetic patients after 90 days.[18] In another study, 61 diabetic patients received 100 mg *J. regia* L. leaf extract or placebo twice a day. After 3 months, TC and TG significantly (p <0.05) decreased in *J. regia* L. leaf extract group. Although no significant (p >0.05) differences were observed in LDL-C and HDL-C levels between two groups.[20] One animal study, as well, reports that the hydroalcoholic ISE of *J. regia* L. has decreased TG (p <0.05), TC (p <0.001), and LDL-C (p <0.001) in alloxan-induced diabetic rats.[23]

In general, the duration of clinical trials in order to see the lipid-lowering effect of agents were 4 to 12 weeks[34,35] and the duration of our study was considered 12 weeks which seems to be sufficient. However, it is possible that by increasing study duration the *J. regia* L. ISE would show lipid-lowering effect. The insignificant effect of *J. regia* L. ISE on lipid profile can also be due to insufficient absorption or dissolution time of the extract capsules in the gastrointestinal tract. The dose of *J. regia* L. ISE was calculated 1500 mg/day via the conversion of animal dose to human dose.[36] yet, lipid profiles may be improved by increasing the dose of the extract.[37]

Our study was conducted during the COVID-19 pandemic. COVID-19 can cause metabolic disorders by

affecting different endocrine glands or organs such as the pituitary gland, thyroid, adrenal gland, pancreas, and fat.[38] Although we had excluded participants with COVID-19, some patients might have had an asymptomatic infection, so their lipid profiles may not be reliable at the end of 12 weeks. Besides, it is possible that the participants had changed their previous physical activity or lifestyle during the study period due to limitations of COVID-19 pandemic which can also be a reason that the lipid profile showed no change.

In our trial, participants did not report any side effects. Besides, no significant differences were observed in LFT, SCr, and urea before and after intervention in terms of using the *J. regia* L. ISE, while most lipid- lowering agent, such as statin, niacin and fibrates cause hepatotoxicity.[39] In Hosseini *et al.*[20] study , *J. regia* L. leaf extract caused mild diarrhea in 11(34.4%) diabetic patients at the beginning of study but In line with our study, no significant (p >0.05) changes were found in liver and kidney function tests. Hence, regarding the lack of any serious side effects during our study, this could be an advantage over other drugs.

One study in 2017 assessed the effect of a walnut (42 g/day) enriched diet on CVD risk factors in 100 overweight participants and has reported that it had improved BP, weight, and waist circumference (p<0.001) after 3 months and TC and LDL-C (p<0.05) at 6 months.[40] Whereas, our study showed no significant differences in systolic and diastolic BP, weight, waist circumference, waist-to-hip ratio and lipid profile by *J. regia* L. extract in comparison with placebo after 12 weeks. These different may be in terms of different amounts of bioactive compounds in the internal septum and fruit.[24] Moreover, different species of walnut have different amounts of compounds, and in the former study the walnut species was not determined.[41]

In AIM-HIGH trial, a high dose of niacin (1500-2000 mg/day) decreased Lp(a) by 21% from the baseline after one year. The baseline Lp(a) means of 1427 patients was measured 38.2 mg/dl, and response rate was increased in the patients with higher Lp(a).[42] In the present trial, the baseline Lp(a) mean of patients in *J. regia* L. group (24 mg/dl) was lower in comparison with AIM-HIGH trial. In addition, only 15% participants had a baseline Lp(a) greater than 50 mg/dl in both groups. Data analysis of these 15% participants exhibited no significant difference in Lp(a) between *J. regia* L. and placebo treated groups at the end of study. However, the sample size is small and it is recommended to evaluate Lp(a) in a greater population. Moreover, the duration of AIM-HIGH trial was one year while our study duration was 12 weeks.

Type 2 diabetes and insulin resistance can cause lipid abnormality including increased TG and LDL-C and decreased HDL-C.[43] In our study, no significant differences were observed in HbA1c of patients at the start and after 12 weeks of study between two groups. Therefore, diabetes mellitus has not acted as a confounding factor.

Although, statins can modestly increase the risk of diabetes, the 2022 American Diabetes Association (ADA) guideline recommends use of statins in diabetic patients to prevent primary and secondary CVDs.[44] In spite of statins, the *J.regia* L. ISE had no adverse effect on HbA1c in our study.

At the beginning of this study, patient's medication was evaluated because some drugs have an effect on blood lipids. All patients used atorvastatin or rosuvastatin, but there were no significant differences in the dose of statins among the groups. Metformin can improve lipid profile through changing the production of chylomicrons and synthesis of TG and fatty acid in terms of decreased intestinal lipoprotein synthesis.[45] There was also no significant difference in metformin received dose between the two groups in the present trial. Therefore, the cofounding effects of these drugs were ruled out.

In Iran, people sporadically use the *J.regia* L. ISE to improve lipid profile. According to our findings, we do not recommend the use of the *J.regia* L. ISE unless a larger studies confirm its lipid-lowering effect. We suggest higher doses of *J.regia* L. ISE and using for longer duration in future studies. In addition, the pharmacokinetic behavior of the extract, especially its absorption should be investigated. Moreover, studies can be performed in the patients with higher blood LDL-C and Lp(a).

This study did have some limitations. The first issue was insufficient sample size due to COVID-19 pandemic. Another limitation was the lack of pharmacokinetic characteristics of the *J.regia* L. ISE.

Conclusion

In conclusion, our findings showed that *J.regia* L. ISE had no significant effect on lipid profile compared to placebo. However, it needs further studies after considering the limitations of the present study to make better decisions. Besides, patients did not report any adverse events within the follow-up period.

Ethical Issues

This study was approved by ethics committee of Tehran University of Medical Sciences (TUMS) and received ethical code number IR.TUMS.TIPS.REC.1399.121. Then, it was registered in the Iranian registry of clinical trials (IRCT ID: IRCT20201227049850N1). All patients completed the written informed consent form for trial participation and publication of study finding.

Data Sharing

It would be done per request from the corresponding author via email address.

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Author Contribution

Study concept and design: SN, AZ, NSH, MH, FA, MKH and MM. Acquisition of data: SN, FA, ME and MM. Analysis and interpretation of data: MM and SN. Drafting of the manuscript: MM and SN. Critical revision of the manuscript for important intellectual content: MM and SN. Statistical analysis: MM. Administrative, technical, and material support: SN and MM. Study supervision: SN, AZ, NSH, MH and ME.

Conflict of Interest

The authors report no conflicts of interest.

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