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RESEARCH ARTICLE

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Efficacy of black seed (*Nigella sativa* L.) on kidney stone dissolution: A randomized, double-blind, placebo-controlled, clinical trial

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Preclinical studies have shown beneficial effects of black seed (*Nigella sativa* L.) in the prevention and treatment of renal stones. Hence, we designed a study to evaluate the renal-stone-dissolving efficacy of black seed. Sixty patients with renal stones were randomly enrolled in two arms of a randomized, triple-blind, placebo-controlled, clinical trial. The patients were treated by black seed capsules (500 mg) or placebo two times per day for 10 weeks. Patients were assessed in terms of size of renal stones by using sonography before and after intervention. In the black seed group, 44.4% of patients excreted their stones completely, and the size of the stones remained unchanged and decreased in 3.7% and 51.8% of patients, respectively. In contrast, in the placebo group, 15.3% of the patients excreted their stones completely, 11.5% had reduction in stone size, 15.3% had increase in stone size, and 57.6% had no change in their stone size. The difference in the mean size of renal stones after the study was significant between the two groups ($p < 0.05$). *N. sativa* L., as compared with placebo, is demonstrated to have significant positive effects on disappearance or reduction of size of kidney stones.

KEYWORDS
black seed, herbal medicine, *Nigella sativa*, Persian medicine, urinary stones

1 | INTRODUCTION

Kidney stones are one of the most common urologic problems (Osterberg, 2018). In the United States, about 13% of men and 7% of women develop kidney stones during their lives. Prevalence of renal stones is increasing in developed countries. In 80% of renal stones, calcium salts are the main components, most of which are composed primarily of calcium oxalate or, less often, calcium phosphate (Singh et al., 2015).

Today, nonmedical therapies such as extracorporeal shock wave lithotripsy, percutaneous nephrolithotomy, open surgery, and medical expulsive therapy are often used for stone removal, which are often invasive and costly and require postoperative care (Raheem, Khandwala, Sur, Ghani, & Denstedt, 2017; Wood, Gorbachinsky, & Gutierrez, 2014). Hence, scientists in the field of complementary and alternative medicine (Ewertz, Qvortrup, & Eckhoff, 2015) have attempted to find potential new medical treatments of kidney stone removal. Phytotherapy is one of the most popular choices among complementary and alternative medicine modalities (Jabbari, Daneshfard, Emtiazy, Khiveh, & Hashempur, 2017).

Different medicinal plants have been proposed to reduce the size or excretion of urinary stones in some traditional or complementary medicine, but its effectiveness does not have enough clinical evidence (Faridi, Roozbeh, & Mohagheghzadeh, 2012). Generally, there are several concerns as to the efficacy of medicinal herbs for their applications in clinical practice (Grollman & Marcus, 2016; Izzo, Hoon-Kim, Radhakrishnan, & Williamson, 2016). Therefore, an evidence-based approach is indicated.

One of the traditional Persian medicine suggestions for treatment of urinary stones is black seed (*Nigella sativa* L.). It is a well-known medicinal herb from Hippocrates's (460–377 BC; Mosavat, Marzban, Bahrami, Parvizi, & Hajimonfarednejad, 2017) time and earlier. The most famous Persian physicians, such as Rhazes (865–925 AD; Hashempur, Hashempour, Mosavat, & Heydari, 2017), Haly Abbas (930–994 AD; Heyadri et al., 2015), Avicenna (980–1037 AD; Dalfardi, Heydari, Golzari, Nezhad, & Hashempur, 2014), and later Aghili Shirazi (18th century; Akbari, Nasiri, Heydari, Mosavat, & Iraji, 2017), discussed about different therapeutic potentials of black seed (*Siah daneh* in Persian). For example, in *Makhzan al-Advieh* (written by Aghili Shirazi in 1771) and in *Tuhfat ul-Momineen* (Mo'men tonekaboni, 17th century), the use of black seed, along with water and honey, was clearly indicated for the dissolution of kidney stones. Of course, in some of these traditional references, black seed alone is used, and in some others, black seed in combination with other herbs is listed as an effective drug on the treatment of kidney stones. Black seed is also referred as a diuretic and an antiurinary retention in these books (Aghili Shirazi, 2009; Tonkaboni, 2007). Black seed has been traditionally used for treatment of various diseases such as a gastro-tonic, hepato-tonic, diuretic, and emmenagogue agents (Kooti, Hasanzadeh-Noohi, Sharafi-Ahvazi, Asadi-Samani, & Ashtary-Larky, 2016). Moreover, several preclinical studies have shown beneficial effects of this herb in the prevention and treatment of renal diseases including renal stones and renal damages. For example, Ahmed and Abd El-Mottaleb have reported that *N. sativa* oil has renal protective effects against acetaminophen-induced renal injury in rats (Ahmed & El-Mottaleb, 2013). Also Hadjzadeh et al. have reported that the black seed oil inhibited collagen deposition and the severity of fibrosis in a bromobenzene-induced hepato-renal injury model (Hadjzadeh, Rad, Rajaei, Tehranipour, & Monavar, 2011; Hayatdavoudi, Rad, Rajaei, & Hadjzadeh, 2016). Previous studies on *N. sativa* L. demonstrated that aqueous-ethanolic extract of *N. sativa* L. significantly reduced the number and size of the kidney calcium oxalate deposits than did ethylene glycol group in male Wistar rats (Mousa-Al-Reza Hadjzadeh, Rad, & Maryam Tehranipour, 2011).

Regarding the traditional use of *N. sativa* L. in addition to their known beneficial effects in recent studies, we decided to carry out a randomized, controlled, clinical trial to evaluate the safety and renal-stone-dissolving efficacy of *N. sativa* L. in addition to its effects on some related urine and blood biochemical factors.

2 | MATERIALS AND METHODS

2.1 | Trial design

We designed a randomized, double-arm, double-blind, placebo-controlled, clinical trial. In this trial, we evaluated the safety and renal-stone-dissolving efficacy of *N. sativa* L. in addition to its effects on some related urine and blood biochemical factors. No changes occurred in the methods after trial commencement.

2.2 | Participants

Inclusion criteria for participants enrolled in this study were men and women aged 20 to 60 years with kidney stone disease that had been confirmed by sonography. They should have kidney stones larger than 5 mm. Signing the informed consent form was necessary for participants. The exclusion criteria were breastfeeding, pregnancy, any renal disorder that require new treatment protocols, and known allergy to black seed.

This study was carried out in the urology clinic of Emam Reza Hospital, affiliated to Mashhad University of Medical Sciences, Mashhad, Iran, from February 2016 to October 2016.

2.3 | Intervention

After diagnosis of renal stones by a urologist and confirmation of the diagnosis by a radiologist via sonographic assessment, eligible patients were divided into two groups. The participants were randomly assigned to receive either a 10-week dosing schedule of *N. sativa* L. capsules (500 mg two times per day) as the intervention group or placebo starch capsules (500 mg two times per day) as the control group.

The control and intervention groups consumed the capsules with honey syrup. To prepare honey syrup, a tablespoon of honey was diluted in 150 cm^3 of lukewarm water. Moreover, participants in both groups received written dietary commands including a proper diet for patients with kidney stones. Consumption of less than 70% of the drugs during the trial was considered as drug intolerance, and the patient was excluded from the trial.

2.4 | Preparation of drugs

The intact seeds of *N. sativa* L. were purchased from an herbal market in Mashhad (Iran), authenticated by a botanist (Voucher Number EZ-70), and kept at the Herbarium of the Faculty of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran. The intact black seeds were completely ground. The obtained powder was then passed through a 120 mesh sieve. Capsules weighing 500 mg were prepared by a nonautomatic filling capsule machine from the sieve powder. Starch powder was used to make placebo in the same size, weight, and shape as black seed capsules. It should be noted that the size, label, and shape of the placebo bottles were similar to the black seed bottles.

2.5 | Drug biochemical assay

2.5.1 | Gas chromatography–mass spectrometry analysis

The essential oil composition was determined using a Agilent (Model 7890A) gas chromatograph coupled to a Model 7000 triple quad mass spectrometer of the Agilent company. Separation was achieved on the DB-1 column (30 m × 0.25 mm × 0.25 μm). Helium was the carrier gas at a flow rate of 1.2 ml/min. The split ratio was 50:1. The injector and auxiliary temperatures were set at 250°C and 280°C, respectively. Initially, the oven temperature was set at 70°C and then raised by 3°C/min to a final temperature of 280°C (held for 4 min; Mallahi, Ramezanian, Saharkhiz, Javanmardi, & Iraji, 2018). The ionization energy was set at −70 eV. Identification of volatile constituents was made on the basis of their retention indices on column (Kovats index, 1965) and their mass spectra, which were compared with reference data (Khodadoust, Mohammadzadeh, Mohammadi, Irajie, & Ramezani, 2014).

2.5.2 | Free-radical-scavenging analysis

The free-radical-scavenging capacity of the black seed was tested by bleaching of the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). Briefly, the black seed was powdered and dissolved in EtOH, and five different concentrations were mixed with a methanolic solution of 100 μM of DPPH in triplicate. After 30 min of incubation at room temperature in the dark, the absorbance at 517 nm was recorded (Abolfazl, Khadijeh, Mojtaba, Seyed Hamdollah, & Aida, 2017). The concentrations were carefully chosen to produce a suitable dose–response curve. The percent inhibition of the radical was calculated on the basis of the absorbance of the mixture compared with the absorbance of DPPH solution alone. IC_{50} values were calculated by the software Curve-Expert (for Windows, Version 1.34). Values represent the mean of three to five experiments ± *SD*.

The Folin–Ciocalteu method was used to determine the amounts of total phenolic capacity. Briefly, 5 μl of the sample or standard (gallic acid) was mixed with 10 μl of Folin–Ciocalteu reagent (FCR) and diluted with 160 μl of distilled water. After 10 min of incubation, 30 μl of Na_2CO_3 (25% distilled water) was added, and the mixture was further incubated for 90 min at room temperature in the dark, and absorbance was measured at 725 nm using a UV–visible spectrophotometer. The concentration of the total phenolic was expressed as milligrams of gallic acid equivalents per gram of black seed.

2.5.3 | Primary outcome measure

The primary outcome measure in this trial was change in the size of renal stones assessed by using sonography. Sonographic evaluation was done with the device model Siemens 40 with the Acuson 15 L8 transducer by a radiologist who was not aware of the participant's medical case files before and 10 weeks after the intervention.

2.5.4 | Secondary outcome measures

Secondary outcome measures were changes in the levels of serum calcium and urine pH. Blood sampling was performed in the laboratory of the Emam Reza Hospital, affiliated to Mashhad University of Medical Sciences, Mashhad, Iran. Blood samples were centrifuged and stored at −20°C. Serum calcium was analyzed using Pars Azmoon kits (Pars Azmoon Co., Iran) by cresolphthalein complexion method. The participants also provided a spot midstream urine sample for evaluation of urine pH.

Any observed adverse event was also considered as the secondary outcome. No changes were made to trial outcomes after the trial commenced.

2.6 | Sample size

Regarding the expected difference of renal stone size as the primary outcome measure between the two groups of the study (obtained from previous study), and by taking into account two-sided significance level of 0.05 and power of 80%, the sample size was calculated from 30 patients in each group, with a total of 60 patients (Faridi et al., 2014).

2.7 | Safety assessment

In order to detect the potential possible patients' complaints, we followed up all the patients by physicians every 2 weeks. All participants were asked to report any drug side effects, especially abdominal pain, constipation, nausea, and vomiting.

2.8 | Randomization

Sixty eligible patients were randomized into two parallel groups. A statistician generated a randomized list by using NCSS (statistical software) with simple block randomization method. Then, the eligible patients were assigned to two groups by the secretary of the clinic according to the randomized list. All participants and investigators were blind to the allocation of the patients. Because placebo capsules were similar to black seed capsules in size, weight, shape, and green color, the physician, drug deliverer, and data analyst were blinded to the type of medicine.

2.9 | Ethical issues

The trial was in compliance with the Declaration of Helsinki (1989 revision) and was also reviewed, approved, and monitored by the ethics committee of Mashhad University of Medical Sciences (License Number IR.MUMS.REC.1394.659). The trial was registered with the Iranian Registry of Clinical Trials with the following code: IRCT20160619028530N. All the participants signed an informed consent form prior to enrollment in the study.

2.10 | Statistical methods

All the data were analyzed using Statistical Package for the Social Sciences (SPSS software Version 15). All data were described by mean ± standard deviation or number (percentage). The Kolmogorov–Smirnov and Shapiro–Wilk tests were used for investigating the normal distribution of the values. Chi-square, independent sample *t* test, and Mann–Whitney *U* tests were used for statistical comparison of baseline characteristics. The Wilcoxon signed rank test was used to determine the changes in outcomes between the two groups of the study. p values less than 0.05 were considered significant. Unfortunately, we did not have the information of the excluded participants after the study, so we had to analyze the data per protocol.

3 | RESULTS

3.1 | Chemical composition of the black seed essential oil

In order to verify the nature of the chemical components present in black seed essential oil, gas chromatography–mass spectrometry analysis was performed. The gas chromatography–mass spectrometry chromatogram of compounds is shown in Figure 1.

A number of monoquiterpenes, sesquiterpenes, and fatty acids were identified and listed in the Table 1 according to the peak numbers shown in the chromatogram with area percent.

Thirty-one components were characterized in the essential oil of black seed, representing 93.8% of the total oil components detected, which are listed in Table 1 with their percentage composition and Kovats index. Among them, *para*-cymene (29.5%), spathulenol (27.8%), and alpha-thujene (6.6%) were the major components. Normal alkane derivatives formed around 8.8% of the total Essential Oil (EO) and were present in trace amounts.

3.2 | Antioxidative activity and FCR assay

In vitro and in vivo studies demonstrate that oxalate induces lipid peroxidative injury through generation of reactive oxygen and nitrogen species, which in return result in nuclear, membrane, and cellular injuries (Thamilselvan, Khan, & Menon, 2003; Thamilselvan & Menon, 2005). Lots of studies provide evidence of correlation between antioxidant levels and kidney stones. It seems that antioxidant therapy reduced calcium oxalate precipitation and increased urinary oxalate excretion in patients with kidney stones (Davalos, Konno, Eshghi, & Choudhury, 2010; Grases et al., 2015; Selvam, 2002). Moreover, free radical scavengers like phenolic compounds provide protection against the deposition of stone (Ghalayini, Al-Ghazo, & Harfeil, 2011).

Free-radical-scavenging activity was defined as the amount of antioxidant necessary to decrease the initial DPPH radical concentration to 50% in 30 min (IC_{50}). Black seed exhibited good radical-scavenging activities with IC_{50} of 6.88 ± 0.92 mg/ml (quercetin as a positive control demonstrates IC_{50} = 3.01 ± 1.72 μM). Antioxidants react with reactive oxygen species such as hydroxyl, superoxide, and lipid peroxyl radical, which have a broad spectrum of chemical and biological activities. In this study, correlation between the amount of total phenolic compounds and antioxidants were observed. In the FCR assay, reduction reactions take place in the presence of sodium carbonate solution (pH 10). Total polyphenol content determined by FCR assay was 172.71 ± 4.01 mg gallic acid equivalent/g dry extract.

3.3 | Patients' enrollment

From February 2016 to October 2016, 79 volunteers were assessed for eligibility. Sixty patients who met the inclusion criteria and agreed to participate in the study were divided into two groups. Thirty patients were assigned to the black seed group and 30 to the placebo group. Three participants in the drug group and two in the placebo group were lost to follow-up. One person in the drug group was excluded from the study due to hydronephrosis, and one person was taken out of the placebo group due to pregnancy. Figure 2 is a flowchart of the groups' distribution, recruitment, intervention, follow-up, and analysis.

3.4 | Baseline clinical characteristics

The mean age of the participants in the trial was 41.78 (±6.04) and 42.73(±10.71) years in black seed and placebo groups, respectively. This did not show any significant difference between the two groups of the study (p = 0.690). Among the male participants, 45% were in the black seed group and 55% in the placebo group. Among the female participants, 55% were in the black seed group and 45% in the placebo group. None of the baseline clinical characteristics of the patients had significant difference(s) between the two groups of the trial (p > 0.05; Table 2).

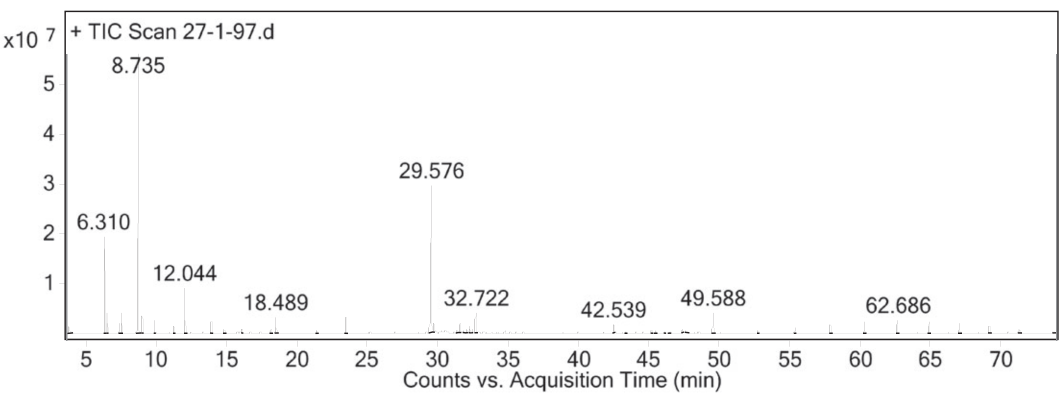

FIGURE 1 Gas chromatography chromatogram of black seed (*Nigella sativa* L.)

TABLE 1 Composition of the essential oil of Iranian black seed

Peak	Area	Compound	KI
1	6.593309	Thujene-α	924
2	1.365862	Pinene-α	932
3	0.793557	Sabinene	969
4	1.545814	Pinene-β	974
5	29.50027	Cymene-ρ	1,020
6	1.315712	Limonene	1,024
7	1.10921	Terpinene-γ	1,054
8	4.174288	Unknown	
9	1.212461	Terpinen-4-ol	1,177
10	0.486754	Cyclocitral-β	1,217
11	0.407104	Thymoquinone	1,248
12	0.495604	Thymol	1,289
13	1.920467	Carvacrol	1,298
14	0.356953	Longipinene-α	1,352
15	1.746416	Longipinene-β	1,400
16	27.8512	Spathulenol	1,577
17	0.944008	Caryophyllene oxide	1,583
18	1.368812	Unknown	
19	0.365803	Muurola-4,10(14)-dien-1-β-ol	1,631
20	0.634256	Aromadendrene epoxide-allo	1,641
21	2.572423	Caryophyllene-14-hydroxy-9-epi-*E*	1,668
22	0.997109	Hexadecanoic acid	1,960
23	2.781875	Sclareol	2,222
24	0.268452	Tricosane-*n*	2,300
25	0.610655	Tetracosane-*n*	2,400
26	1.041359	Pentacosane-*n*	2,500
27	1.295062	Hexacosane	2,600
28	1.495663	Heptacosane	2,700
29	1.333412	Octacosane	2,800
30	1.15346	Nonacosane	2,900
31	0.790607	Triacontane	3,000
32	0.581155	Untriacontane	3,100
33	0.274352	Dotriacontane	3,200

Abbreviation: KI, Kovat's Indices.

3.5 | Clinical response

Given the baseline size of kidney stones, stone disappearance rate and changes in stones size between the two groups of the study after the intervention are shown in Figure 3. The results suggest that the black seed is effective in the early stages of kidney stones when the stones are smaller, but once the stones increase in size, the efficacy is reduced.

Regardless of the baseline size of kidney stones, in the black seed group, 12 patients (44.4%) excreted their stones completely. In contrast, in the placebo group, four patients (15.3%) did so (p = 0.035). In fact, the likelihood of stone excretion in those who received the black seed was approximately three times that of the placebo group (relative risk = 2.88, confidence interval = 1.06–7.81). Moreover, the reduction of renal stone size was more significant after the study in the black seed group compared with the placebo group ($p < 0.05$; Table 2).

Regarding within-group changes, significant differences were observed in the mean of serum calcium (increase) and urine pH (decrease) in the black seed group after the intervention compared with baseline ($p < 0.05$). However, these differences were not seen in the placebo group ($p > 0.05$). Moreover, between-group analysis showed that there was a significant difference in the mean size of renal stones and urine pH level over the study period in the black seed group compared with placebo group ($p < 0.05$). The details of these changes are shown in Table 2.

3.6 | Safety assessment

One of the male patients in the black seed group presented with hydronephrosis and increase in blood pressure, so he was excluded from the study.

4 | DISCUSSION

Efficacy and safety of the *N. sativa* L. on dissolving kidney stones were evaluated in this study via a randomized, double-blind, placebo-controlled, clinical trial. *N. sativa* L., as compared with the placebo, is demonstrated to have significant positive effects on the disappearance or reduction in size of kidney stones.

Besides increasing attentiveness toward complementary and alternative medicine (Niazi, Hashempur, Taghizadeh, Heydari, & Shariat, 2017), there are several herbal remedies that reveal probable advantage for management of renal stones. Some of the commonly used herbs were *Ammi visnaga*, *Tribulus terrestris*, *Bergenia ligulata*, *Vediuppu chunnam*, *Dolichos biflorus*, and *Paronychia argentea* (Yadav et al., 2011).

The black seed, as a dry-and-warm-temperature herb, is used in traditional Persian medicine for the treatment of renal stones. A previous animal study showed that *n*-butanol fraction and *n*-butanol phase remnant of the *N. sativa* L. significantly reduced the number and size of kidney calcium oxalate deposits and had beneficial effect on calcium oxalate deposition in the rat kidney (Hadjzadeh et al., 2011). Another animal study showed that treatment of rats with ethanolic extract of *N. sativa* L. reduced the number of calcium oxalate deposits and could also lower the urine concentration of calcium oxalate (Khoei, Hadjzadeh, & Parizady, 2009). Zarei and colleagues investigated the effects of methanol extract and essential oil of *N. sativa* L. on kidney stones induced by ethylene glycol in rats. They concluded that alcoholic extract of *N. sativa* L. is more effective in the prevention of calcium oxalate crystal accumulations than in urinary tract and kidney stone treatment (Zarei & Rahmani, 2015). The antiurolithiasis effect of *N. sativa* L. in combination with other herbs such as *Butea monosperma* Lam. has been proven in previous in vitro and in vivo studies (Sikandari & Mathad, 2015). To the best of our knowledge, our study is the first human study that evaluated the safety and

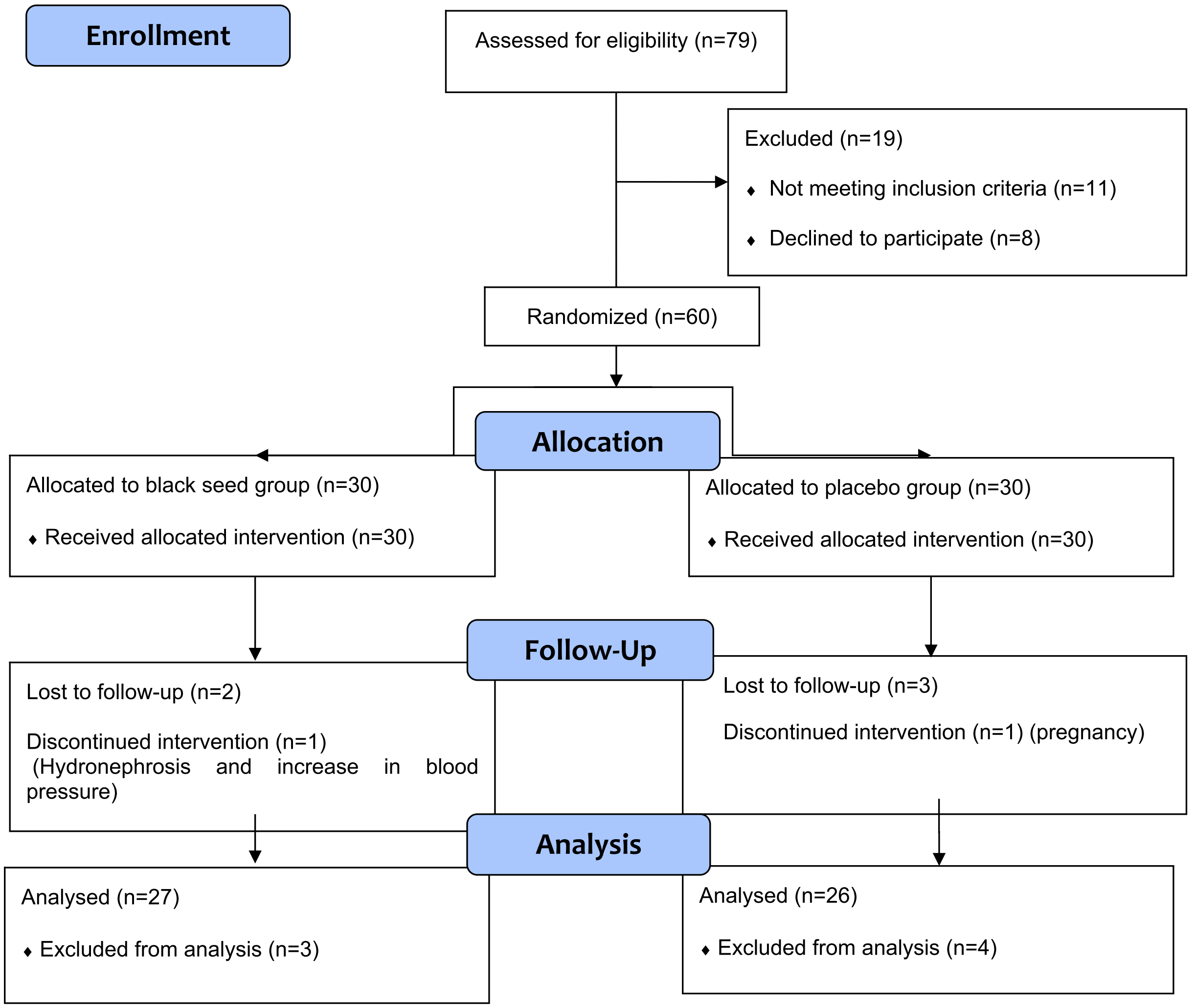

FIGURE 2 Flow diagram of the groups' allocation, enrolment, intervention, follow-up, and the analysis in both groups of the study [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 2 Laboratory indices and kidney stone size of the patients participating in the trial before and after the intervention in the two groups of the study

Variables		*M* ± *SD* Black seed	*M* ± *SD* Placebo	*p* value
Blood urea nitrogen (mmol/L)	Before	18.50 ± 2.94	18.36 ± 2.80	0.908
	After	18.12 ± 2.25	18.56 ± 2.54	0.775
	p value	0.073	0.395	
Serum creatinine (μmol/L)	Before	1.00 ± 0.09	0.93 ± 0.11	0.080
	After	1.01 ± 0.16	0.92 ± 0.09	0.099
	p value	0.177	0.644	
Urine pH	Before	5.19 ± 0.39	5.38 ± 0.49	0.055
	After	5.04 ± 0.19	5.38 ± 0.49	0.002
	p value	0.046	0.317	
Size of renal stone (mm)	Before	6.20 ± 1.65	6.41 ± 1.51	0.296
	After	2.66 ± 2.72	5.53 ± 2.91	0.000
	p value	0.000	0.098	
Serum calcium (mg/dl)	Before	9.08 ± 0.70	9.20 ± 0.52	0.324
	After	9.37 ± 0.69	9.28 ± 0.60	0.732
	p value	0.001	0.146	

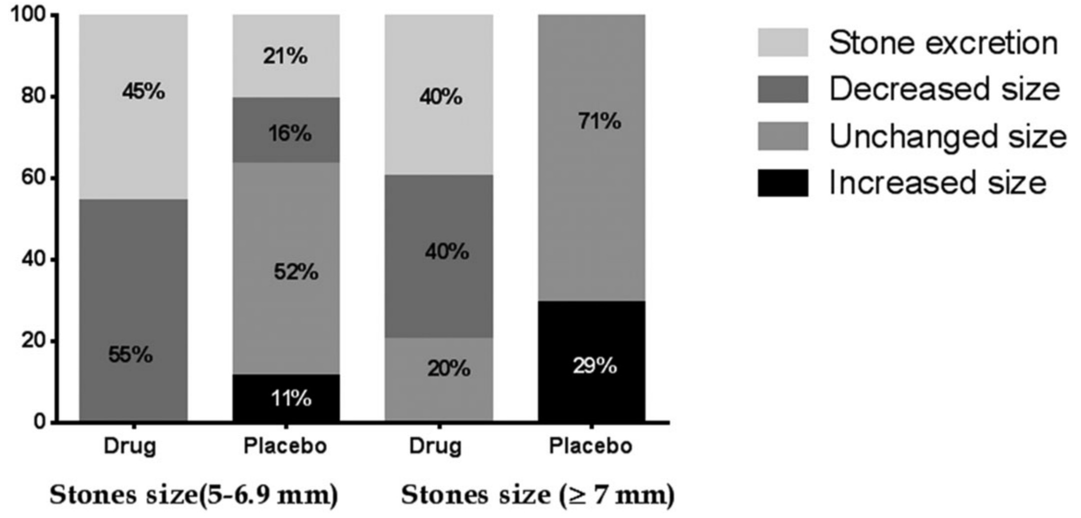

FIGURE 3 Disappearance rate and changes in the stones size between the two groups of the study after the intervention based on baseline stone size

renal-stone-dissolving efficacy of *N. sativa* L. in a clinical trial study. However, numerous human studies have examined the effectiveness of *N. sativa* L. in diseases such as hypertension, fatty liver, hyperlipidemia, diabetes, infertility, seizure, dyspepsia, and rheumathologic disorders (Gheita & Kenawy, 2012; Hosseini et al., in press; Kaatabi et al., 2015; Mohtashami et al., 2015). In most studies, the antidiabetic, anti-inflammatory, antioxidant, antibacterial, and anticarcinogenic effects of pepper have been attributed to thymoquinone, an active constituent of *N. sativa* L. (Hayatdavoudi et al., 2016). Moreover, previous studies showed that thymoquinone decreased the number and size of calcium oxalate deposits in the renal tubules of rats and has a preventive effect on calcium oxalate calculi formation in the kidneys of rats (Hadjzadeh, Mohammadian, Rahmani, & Rassouli, 2008). One of the assumptions that have been proposed for this effect is that renal stone depositions may damage the epithelial cells and lead to production of free radicals and superoxide anions and induction of heterogenic crystal nucleation. Alternatively, thymoquinone has an antioxidant effect, cleans free radicals and superoxide anions, and inhibits cyclooxygenase and 5-lipoxygenase pathways, so it inhibits the inflammatory process (Khan & Thamilselvan, 2000). Moreover, multiple in vivo and in vitro studies have showed that high levels of oxalate may have a toxic effect on renal epithelium through the intracellular oxidative stress process (Hosseinian, Ebrahimzadeh Bideskan, Shafei, Sadeghnia, & Soukhtanloo, 2018). Hence, black seed treatment may protect against the changes associated with oxidative stress and oxidative damage associated with lithogenesis (Bashir et al., 2010). As a result, it could be effective in the prevention and dissolving of renal stones. Also, thymoquinone has antimicrobial effects and could be effective in the prevention of stones with bacterial origin such as struvite stones (Khoei et al., 2009).

Although in our study one person was excluded from the study due to hydronephrosis and high blood pressure, previous studies have reported the safety of black seed in this dose. Many toxicological studies have been performed on black seeds, and no toxic effects were reported when black seed was given to mice via the stomach in an acute and chronic study. These studies suggest a wide margin of safety for therapeutic doses of black seed (Ahmad et al., 2013; Gilani, Jabeen, & Khan, 2004). Moreover, in similar clinical trials, black seed not only has not increased blood pressure but also has led to a decrease in blood pressure (Dehkordi & Kamkhah, 2008; Fallah Huseini et al., 2013; Qidwai, Hamza, Qureshi, & Gilani, 2009). Therefore, we cannot attribute the increased blood pressure of an individual in this study to black seed consumption easily.

However, our study had some limitations. Short time duration of the study was one of its main limitations. Because kidney stone formation and excretion are time dependent, by extending the study period, we could also evaluate the recurrence of renal stones. However, by prolongation of the study period, increase in patients' dropout rate could be predicted. Using the minimum acceptable sample size was another limitation of this study. The small sample size should be declared as another concern. However, this trial was a pilot study—we planned to evaluate our idea in a standard minimum sample size of patients with renal stones. Another important limitation was the lack of more accurate objective indices like computed tomography (Gonzaga do Nascimento-Neto et al., 2012) scan for the assessment of kidney stone size. However, due to the high cost, it was difficult to perform computed tomography scan. Considering the fact there was far greater success with the smaller stones, the future research should focus on longer term and reducing recurrence of kidney stones and larger sized clinical trial.

If we suppose that crushed powder of seeds of *N. sativa* L. used in this study would be the equivalent of adding the seeds in the diet (except that the capsule protects the powder from the saliva in the mouth), we could conclude the use of *N. sativa* L. seeds in the diet to prevent kidney stones and to remove early-forming stones, but to treat larger stones, a concentrated extract is required. The antioxidant and fatty acid components of the seeds have been extracted by others to demonstrate potent efficacy. So a future study should be the use of a concentrated extract of the seed powder in order to increase the potency and thus be able to be effective against larger kidney stones.

5 | CONCLUSION

This small preliminary study demonstrated that *N. sativa* L. in the diet, as compared with placebo, could help to prevent kidney stones and to remove early-forming stones. But to treat larger stones, a concentrated extract is required.

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CONFLICT OF INTEREST

None of the authors have a conflict of interest to declare.

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