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Original article

Comparing the Effect of *Nigella sativa* oil Soft Gel and Placebo on Oligomenorrhea, Amenorrhea and Laboratory Characteristics in Patients with Polycystic Ovarian Syndrome, a Randomized Clinical Trial

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Abstract

Background and objectives: Oligo-amenorrhea is one of the most common symptoms in poly cystic ovarian syndrome (PCOS) patients and *Nigella sativa* is a medicinal plant used in Iranian traditional medicine for the treatment of oligo-amenorrhea. The aim of this study was to evaluate the effect of *N. sativa* oil on oligo-amenorrhea in patients with PCOS. **Methods:** This study was a double-blinded placebo-controlled clinical trial conducted on 84 PCOS patients with oligo-amenorrhea. Patients were randomly assigned to intervention and placebo group. They used two soft gel capsules of *N. sativa* oil (500 mg, each capsule) or placebo at night for sixteen weeks. Four indices were used to assess menstruation; the interval between menstruations, duration of menstruation, the occurrence of menstruation and the severity of bleeding. The two groups were compared using analysis of covariance. **Result:** Fifty five patients completed the study (32 patients in *N. sativa* group and 23 patients in placebo group). The menstrual interval after the study in the intervention group (45 days, 95% CI) was significantly lower than the control group (86 days). The frequency of menstrual cycle in the intervention group (0.79) was significantly higher than the placebo group (0.48). No serious complication was reported in this clinical trial. **Conclusion:** findings suggest that *N. sativa* is an alternative treatment and could be useful for menstrual irregularities in women with PCOS. Further studies are recommended to find the exact mechanisms of *N. sativa* and its different derivatives.

Keywords: Iranian traditional medicine; *Nigella sativa*; Oligo-amenorrhea; PCOS

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Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine disorder that affects 4-10% of childbearing-age women [1]. PCOS is characterized by chronic hyperandrogenic anovulatory cycle causing abnormal hair distribution, acne, irregular menses, and infertility. Its prevalence is different based on various resources due to use of different criteria to diagnose this syndrome [2]. The first definition for PCOS was explained by the National Institutes of Health (NIH), 1990 which concluded that PCOS is a clinical disorder with clinical and/or biochemical hyperandrogenism which is related to a menstrual disorder. To make a right diagnosis, other conditions such as Cushing syndrome, congenital adrenal hyperplasia and

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hyperprolactinemia should be excluded [3]. It is believed that multiple factors like genetic, behavioral, and environmental factors paly role in PCOS etiology and the exact cause is not clear [1]. PCOS is associated with many metabolic and cardiovascular conditions like insulin resistance, obesity, type 2 diabetes mellitus, hypertension, dyslipidemia, inflammation, and subclinical cardiovascular disease [4]. PCOS patients also experience anxiety, depression, and reduced quality of life [5]. Irregular menses include oligomenorrhea, amenorrhea, and prolonged unpredictable menstruation are frequently observed in PCOS patients. Although 30% of women with PCOS have normal menses, about 85%-90% of women with oligomenorrhea have PCOS whereas 30%-40% of women with amenorrhea have PCOS [4].

The current treatments for PCOS are not totally effectiveon controlling symptoms and preventing complications [6]. The main step to regulate the amenorrhea and oligomenorrhea derived from PCOS is using the oral contraceptive pill (OCP) which can have different complications. OCP has many side effects like acne, weight gain, breast tenderness, dizziness, headache, change in sexual behavior and mood, increase in blood pressure and vomiting. OCP is contraindicated in women over 35 years old, smokers and those with hyperlipidemia. In women who reject oral contraceptives or contraceptives are contraindicated, progesterone is used periodically as alternative. Progestins have many side effects like increase in appetite and weight, tiredness, bloating, breast tenderness, fluid retention, headache, skin rash, decreased libido, mood changes like anxiety, irritability, and depression. Other treatment options for PCOS are oral hypoglycemic agents like metformin which not only improve insulin resistance but also regulate the menstrual cycles and induced ovulation. However, these hypoglycemic agents can cause gastrointestinal discomfort and hypoglycemic condition [6]. Despite extensive research on PCOS and its contributing factors, there are still many problems with standard treatment in many patients [4].

According to the new approach of World Health Organization (WHO) to complementary medicine, it is logical to conduct researches in the PCOS field [7]. Iranian traditional medicine (ITM) is one of the holistic and active complementary systems of medicine in the world and has a comprehensive approach to diseases. The method of treatment in ITM is taking natural medicine along with modifying the lifestyle [8,9]. *Nigella sativa* with a rich history in medicine is one of the herbal medications which has been used in treating oligo-amenorrhea [10,11]. *Nigella sativa* is from Ranunculacea family and is native to southern Europe, northern Africa, and Asia [11,12]. Iranian physicians have used *N. sativa* for treating headache, nasal congestion, asthma, allergy, strengthening the immune system, toothache, intestinal worms, hypertension, gastrointestinal problems, rheumatoid arthritis, induced menstruation and increase lactation [12-14]. Many researches have been conducted on *N. sativa*. It has been reported in many scientific papers that *N. sativa* has immunomodulatory [14], anti-inflammatory [11,12], anti-oxidant [15], anti-asthmatic [16], antimicrobial [17], and antitumor [18] properties. It also protects liver, kidney [12,13], nervous system [19], and cardiovascular system [20], and improves digestive system [21], lipid profile [22], blood pressure [23] and blood sugar [24]. Hypoglycemic and hypolipidemic effects are metabolic effects of *N. sativa* and its components that have been identified in numerous studies [25]; therefore, *N. sativa* can be effective in treating metabolic aspects of PCOS. In the study of Latif et al., the effect of *N. sativa* as a supplement to improve the metabolic profile in women perimenopause was investigated [26]. Also, another study has been conducted on the positive effect of *N. sativa* on dysmenorrhea and mastalgia in Iran [27].The Naeimi et al. pilot study about the effect of *N. sativa* on oligomenorrhea, amenorrhea and the other parameters of PCOS showed significant improvement on duration of menstruation, interval between menstruation and level of metabolic factors in serum after 4 months [29]. From ITM and modern medicine points of view there are no life-threatening reports about side effects of *N. sativa* [14,29]. Therefore, this study aimed to examine the effectiveness of *N. sativa* on oligomenorrhea and amenorrhea in PCOS patients.

Materials and Methods

Ethical considerations

This was a randomized double-blinded placebo-controlled study, among PCOS patients with oligo-amenorrhea during December 2015 to July

2017. This research was carried out with the approval of the Ethics Committee of the Shahid Beheshti University of Medical Sciences, Iran, with the code of ethics IR.SBMU.REC.1394.157 and registered with the code of IRCT2016041827455N1 in Iranian Registry of Clinical Trials website at the Ministry of Health.

Plant material

N. sativa capsules and placebo were prepared in Barij Essence Pharmaceutical Company, Kashan, Iran. The characteristic of the oil was as following: density: 0.921 g/cm^3, refractive index: 1.4700, acid value: 11 mg KOH/g oil, iodine value: 121 g I_2/100 g, saponification value: 188 mg KOH/g oil, oleic acid: 23%, linoleic acid: 62%. Each package contained either 60 capsules of (500 mg) *N. sativa* oil or 60 capsules of sunflower oil (placebo). The patients consumed two capsules of *N. sativa* oil (1000 mg) or placebo (sunflower oil) at night. All candidates were advised to follow similar recommended dietary and exercise plan.

Study design

The purpose of the research was explained for qualified individuals and written consent was obtained Based on Naeimi et al. the sample size in each group was considered 42 patients [29].

The criteria for entering the study included the diagnosis of PCOS based on NIH criteria by a gynecologist, age between 18 to 38 years old with irregular menses, person's willingness to enter the study, informed consent and not taking hormonal medications and metformin in the last sixteen weeks. Exit criteria included using lowering blood pressure and blood glucose medications, insulin sensitizing drugs, aspirin and other anticoagulants, oral contraceptives and antiprostaglandins. Other exclusion criteria were taking using herbal remedies, having a history of uncontrolled hypertension, stroke, heart attack, myocardial infarction, and other cardiovascular diseases, liver, kidney, thyroid disorders, type 1 and type 2 Diabetes melitus, cancer, pregnancy, lactating and smoking.Patients who used hormonal drugs during the study, needed other therapeutic interventions and surgery, and who decided to leave the study with a personal request and patients with possible side effects during treatment, were excluded from the study.

Eligible patients were selected randomly using stratified random block method with block sizes of 4 and 1:1 allocation ratio. Two strata were considered according to body mass index (BMI), below 30 and above 30, and randomization was performed in each of these two strata separately. Patients and physicians did not know the pearls (capsule) content in the containers.

The patients with PCOS who had completed initial diagnostic tests and were diagnosed by a gynecologist based on NIH criteria were included in the study. After explaining the method of study and taking informed consent the researcher filled the personal information, medical history forms, and menstrual and Freeman-Galway (scores of hirsutisms) questionnaires.

Next, the researcher performed primary physical exam, took blood pressure and measured weight and BMI, the waist and hip circumference.

At the beginning of the study, blood sample was taken after 12 h fasting in the follicular phase (third day) in the presence of menstrual cycles or otherwise induced menstruation by progesterone and then blood sample was taken on the third day of menstruation. The first series of blood laboratory indices for all candidates included: LH, total testosterone, FBS, GTT and fasting insulin. All blood analysis was done in one laboratory by giving referral forms. The hormonal blood tests were assessed by electrochemiluminescence method with Cobase and Elecsy analyzer 2010 (Germany). The other blood tests were assessed by enzymatic method (Pars Azemoon company's kits, Iran). All physical and blood tests were repeated at the end of the study with the maximum duration sixteen weeks. Patients were visited at the end of each cycle and received the next month medication.

Four indices were used to evaluate menstrual characteristics; 1) the interval between menstruations that was measured in days from the first day of menstruation to the first day of next menstruation, 2) duration of menstruation (number of bleeding days), 3) the occurrence of menstruation or the frequency of menstrual cyclicity during the intervention that was calculated as the ratio of cycle per month and 4) severity of bleeding by using Higham chart to measure the severity of menstruation. In case of menstruation, the pictorial blood assessment chart (Higham chart) was filled by patients and given to the research coordinator [30]. Any possible side effects of *N. sativa* were recorded in the form of drug complications. A contact

number of research coordinator was available to answer candidates' questions.

Statistical analysis

Data were analyzed using descriptive statistics including mean and standard deviation for continues and frequency (percent) for categorical variables. The normal distribution of quantitative variables was assessed by Kolmogrov-Smirnov test and the significance level in all tests was considered as $p < 0.05$. The two groups were compared using independent T-test or equivalent nonparametric Mann-Whitney test for continuous variables. The categorical variables were compared using Chi square test. To adjust for potential confounders and baseline covariates, analysis of covariance was used and marginal means with 95% confidence interval were estimated. All analysis was performed in SPSS version 19.

Results and Discussion

Among 84 participants recruited, 55 participants (32 patients in *N. sativa* group and 23 patients in placebo group) completed the study. Figure 1 shows the flowchart of study population. The mean age of subjects was 24 years (±5.7) with a minimum of 18 years and a maximum of 38 years. The average time of having PCOS was 76 months (±64) and the mean age of menarche was 13 years (±1.8). Table 1 shows the distribution of baseline characteristics. There was no significant difference in terms of demographic and medical history except for the history of menstruation (cause of referral). The reason for amenorrhea in the intervention group (44%) was significantly higher than the placebo group (17%).

Table 2 shows menstrual, anthropometric, and laboratory characteristics before the study in intervention and placebo groups. Anthropometrics, biochemical and hormonal tests did not show any significant differences between the two groups before the study. The intervention group showed significantly longer menstruation interval than the placebo group (p-value=0.03). All other menstrual and laboratory indices were not significantly different in the two groups.

Table 3 shows comparison of menstrual and laboratorial indexes between the two groups after-intervention using covariance analysis. There were no significant differences between the two groups in the duration of menstruation, anthropometric and laboratorial indexes. The menstrual interval after the study in the intervention group (45 days, 95% CI: 34-56) was significantly lower than the placebo group (86 days, 95%CI: 69-104). Figure 2 shows the comparison of menstrual interval index between the two groups before and after the intervention. In the intervention group, the menstrual interval significantly decreased from 96 to 45 days (p-value=0.001) and the placebo group showed significantly increased menstrual interval from 66 to 86 days (p-value=0.004). The frequency of menstrual cycle in the intervention group sixteen weeks after the intervention (0.79, 95%CI: 0.71-0.87) were significantly higher than the placebo group (0.48, 95%CI: 0.35-0.61).

Table 1. Baseline characteristics of study population in intervention and placebo groups

Characteristics	Intervention group N=32	Placebo group N=23	P-value
Age (year) Mean age (SD)	24 (5.9)	24 (5.3)	0.62
Reason for referral (%)			
Amenorrhea	14 (44)	4 (17)	0.001
Oligomenorrhea	18 (56)	19 (83)	
Mean Menarche age (year) Mean (SD)	13 (2)	13 (1.5)	0.44
Birthplace (%)			
urban	30 (94)	22 (96)	0.67
rustic	2 (6)	1 (4)	
Marital status (%)			
Single	18 (56)	10 (43)	0.43
Married	14 (44)	13 (57)	
Education (%)			
Diploma and below	18 (56)	13 (57)	0.43
Higher than diploma	14 (44)	10 (43)	
PCO History(%) *			
No	17 (53)	12 (52)	0.56
Yes	15 (47)	11 (48)	
PCOS duration (months) Mean (SD)	75 (46)	81 (75)	0.39
Family history of DM (%)			
No	15 (47)	12 (52)	0.29
Yes	17 (53)	9 (48)	
Family history of high blood pressure (%)			0.93
No	21 (66)	16 (70)	
Yes	11 (34)	7 (30)	
Family history of Hyperlipidemia (%)			0.68
No	17 (53)	14 (61)	
Yes	15 (47)	9 (39)	
Spotting (%)			
No	21 (66)	16 (69.5)	0.93
Yes	11 (44)	7 (30.5)	
Dysmenorrhea (%)			
No	22 (69)	9 (39)	0.43
Yes	10 (31)	14 (61)	
Number of children (%)			
None	25 (78)	19 (83)	0.40
1 and more	7 (22)	4 (17)	
Treatment history (%)			
No	4 (12.5)	4 (17)	0.76
Yes	28 (87.5)	19 (83)	

*PCO family history

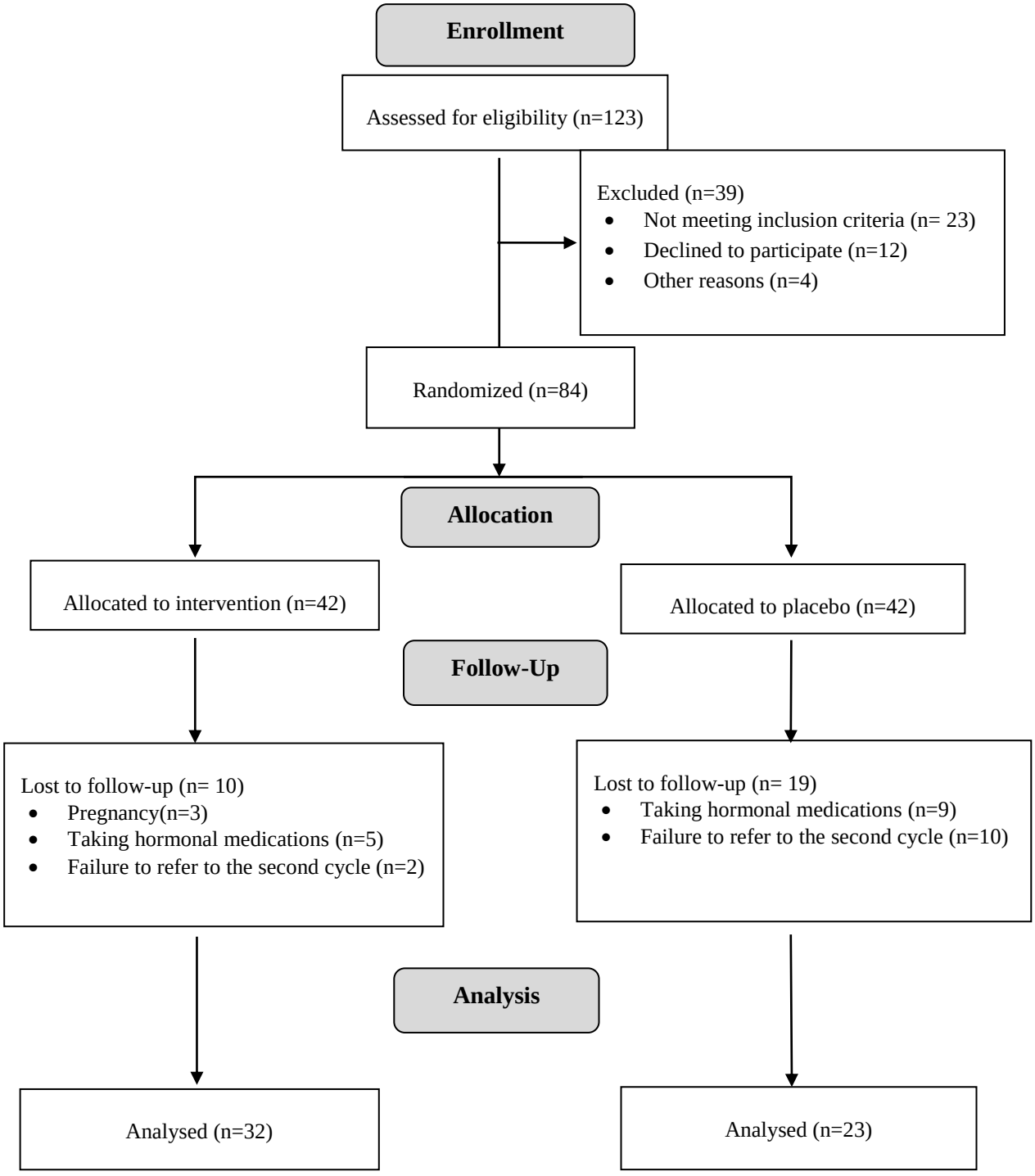

Figure1. Flowchart of study according to CONSORT

Table 2. Menstrual, anthropometric and laboratory characteristics of study population in the two groups before the intervention

	Intervention group Mean (SD)	Placebo group Mean (SD)	P-value*
Duration of menstruation	6.3 (1.9)	6.5 (1.8)	0.68
Interval between two menstruations	96 (56)	66 (38)	0.03
Frequency of menstruation in a month	0.41 (0.24)	0.50 (0.17)	0.33
Menstruation severity 0 1 2	 9 (28) 19 (60) 4 (12)	 11 (48) 11 (48) 1 (4)	0.25
Body mass index	27 (5)	26 (5)	0.29
Waist-hip ratio	0.8 (0.07)	0.8 (0.06)	0.05
Blood pressure (systolic)	106 (8.6)	104 (8.9)	0.31
Blood pressure (diastolic)	70.2 (7.9)	70 (8.1)	0.21
Hirsutism	5.8 (3.2)	5.8 (3.3)	0.99
LH Level	10.2 (8.5)	7.7 (4.2)	0.17
Total testosterone	18 (26)	5 (11)	0.02
One-hour GTT	118 (36)	109 (27)	0.25
Two-hour GTT	104 (35)	100 (15)	0.59
FBS	90 (11)	87 (5.4)	0.17
Insulin	13 (10)	9.6 (6)	0.15
HOMA	5.00 (0.64)	4.83 (0.3)	0.15

*based on Mann-Whitney test

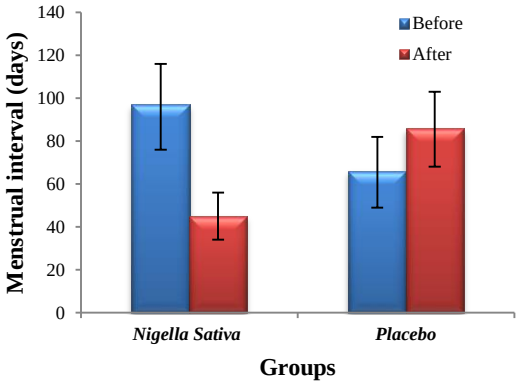

Figure 2. Menstruation interval before and after the study in the intervention and placebo group (mean values with 95% confidence interval)

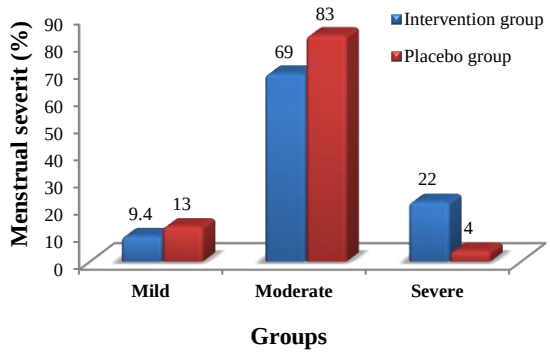

Figure 3. Menstrual severity after sixteen weeks in the intervention and placebo groups

Table 3. Comparison of menstrual and laboratory indices in the intervention and placebo groups*

Index	Intervention group**	Placebo group**	F value	P-value
Menstruation Duration	7.02 (6.59-7.44)	6.25 (5.78-7.26)	1.36	0.61
Menstruations Intervals	45 (34-56)	86 (69-104)	18.28	0.001
Menstruation frequency (per month)	0.79 (0.71-0.87)	0.48 (0.35-0.61)	18.62	0.001
Testosterone level	15.6 (12.3-18.8)	12.9 (7.4-18.3)	0.69	0.41
LH level	9.1 (7.6-10.6)	7.5 (6.7-8.4)	3.46	0.07
One-hour GTT	112 (104-121)	110 (96-125)	0.05	0.82
Two-hour GTT	109 (97-120)	96 (75-116)	1.19	0.28
FBS	87 (85-89)	85 (81.3-88.9)	1.27	0.26
Fasting insulin	10 (8.8-11.9)	11 (8.6-13.8)	0.35	0.56
HOMA	4.8 (4.7-4.9)	4.7 (4.5-4.9)	0.78	0.54
Hirsutism	5.4 (5.2-5.6)	5.3 (4.9-5.7)	0.13	0.72
Body mass index	26 (26-27)	26 (25-27)	0.02	0.89
Waist-hip Ratio	0.82 (0.81-0.83)	0.82 (0.81-0.83)	0.02	0.89
Systolic Blood Pressure	104 (103-105)	105 (102-107)	0.23	0.63
Diastolic Blood Pressure	70 (70.2-80.3)	70.1 (70-80.5)	0.03	0.87

*adjusted for baseline covariates and referral reason
**Mean values with a confidence interval of 95%

Regarding to menstrual severity, 22% of intervention group had severe menstruation compared to 4% in the placebo group. However, there was no statistically significant difference between the two groups in terms of menstrual severity (p-value=0.13). Figure 3 shows the menstrual severity between the two groups after the study.In the present study, the effect of soft gelatin capsules containing 500mg *N. sativa* oil was investigated on oligo-amenorrhea of PCOS patients. Taking *N. sativa*s gelatin capsules for maximum sixteen weeks led to decrease in menstrual cycle intervals and increase in the frequency of menstruation (cyclicity). Four menstrual related indices were used in this study which two showing a significant difference between intervention and placebo groups. The current research was the first systematic study about the effect of *N. sativa* on oligo-amenorrhea which was designed as a double-blinded study. Naeimi et al. had previously conducted a before-after study about the effect of *N. sativa* on PCOS patients with oligo-amenorrhea, with no placebo group, and the medicine was in the form of

powder and the dose as half of the present study [29]. Compared to this study, the severity of menstruation and insulin resistance parameters had improved in the Naeimi et al. study by consuming *N. sativa* powder-contained capsules [29].

Unfortunately, the definitive mechanism for the positive reaction of *N. sativa* to decrease menstrual interval and improve menstruation is not certain. It is not exactly known which component of *N. sativa* effects on uterus and ovary that leads to improve in menstruation; however, it seems that *N. sativa* has phytoestrogenic effects [26]. Phytoestrogens are weak estrogenic compounds that are found in many plants. The exact mechanism of phytoestrogens is not known and they react to the estrogen receptors both dependently and independently [31]. They show agonistic and antagonistic effects and it seems that *N. sativa* acts with both mechanisms [32]. Estrogenic activity of *N. sativa* can be attributed to its non-saturated fatty acid contents including linoleic acid and oleic acid. The estrogenic effects of these non-saturated fatty acids have been reported in many studies [33]. Several animal studies have been conducted to confirm this claim. For example, Parhizkar et al. compared the effect of methanol and hexane extracts of *N. sativa* and conjugated estrogen on the improvement of vaginal epithelial cells of menopause mice and found that methanol extract of *N. sativa* showed estrogenic effects. The extract demonstrated less estrogenic effects compared to crude powder of *N. sativa* and this effect was even weaker compared to conjugated estrogen [34]. In another study, the same group examined the estrogenic effect of *N. sativa*, which included uterotrophic assay and serum estradiol level on ovariectomized rats and compared them with conjugated estrogen control group and negative control group. The results showed increase in uterine weight and serum estradiol level in comparison to the negative control group, which indicated the estrogenic effect of *N. sativa* [32]. In addition, Intan et al. reported an increase in the blood estradiol of ovariectomized rabbits after 14 days of consuming *N. sativa* supplements [35].

Besides *N. sativa*, there are studies about the effects of other plants such as *Mentha longifolia, Sesamum indicume,* and *Matricaria chamomilla* on oligo-amenorrhea of PCOS patients in which phytoestrogenic effects were one of proposed mechanisms [36,37].

Based on PCOS pathophysiology, one of the potential mechanisms of *N. sativa* on oligo-amenorrhea is its anti-inflammatory and antioxidant properties [15]. Oxidative stress indicates an imbalance between reactive oxygen species and antioxidant capacity of biologic system of the body [40]. Recent studies have shown that regarding the significant role of oxidative stress in PCOS pathogenesis, using antioxidants has been effective in improving these symptoms [41]. Many researches have been done to evaluate the antioxidant mediators of *N. sativa* and these materials have been analyzed by enzymatic methods [42]. On the other hand, inflammatory mediators such as interleukin 6 and tumor necrosis factor-α cause anovulation and premature atherosclerosis by oxidative stress affect on the ovary and endothelial system in PCOS patients [43]. Indeed, inflammatory mediators are the initiating responses of immune system to obesity and insulin resistance in PCOS patients. The antioxidant property of *N. sativa* positively effects on ovarian function [44].

The effects of *N. sativa* on glucose metabolism and insulin resistance manifested by weight loss and lipid and glucose profile changes are other possible mechanisms which affect menstruation. This hypothesis has been supported by other researches [45]. The anti-inflammatory and antioxidant characteristics of *N. sativa* can lead to improvement of insulin secretion (which preserves β cells of pancreas) and decrease insulin resistance that in turn controls the blood sugar [46]. In a clinical trial study on human, the findings showed that taking *N. sativa* oil and powder (2-3g daily) for 12 weeks in patients with Diabetes mellitus type 2 led to improvement of glycemic index, insulin resistance, performance of pancreas's β cells and improvement of blood lipid profile [44,46]. The increase of insulin (in insulin resistance) causes synthesizing of adrenal and ovarian free androgens and decreases production of sex hormone binding globulin (SHBG) by liver. Consequently, the level of free androgen increases in the serum and prevents ovarian follicles to grow appropriately. Therefore, decrease in insulin level result in decrease of serum androgen, LH and LH/FSH ratio and increase of SHBG which improves the menstrual cycles with normal ovulation [47].

Contrary to the above mentioned studies, the effect of *N. sativa* on improving fasting blood sugar, insulin, GTT and HOMA index were not significant on intervention group compared with placebo group in this study. We suspect *N. sativa* oil might have lost certain components present in its structure in powder form, which reduced its effectiveness on insulin resistance and blood sugar. No serious complication was reported in this clinical trial, therefore there was no need to stop the treatment.

In conclusion, our findings suggested that *N. sativa* could be an alternative treatment compared to standard treatments and could be useful for menstrual irregularities in PCOS women. However, there is a need for more accurate studies to find the exact mechanism of *N. sativa* and its components. Studies with increased time (more than three menstrual cycles) might be needed.

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Author contributions

Dr. Mojgan Tansaz designed the study; Seyedeh Atieh Naeimi conducted the study and drafted the manuscript; Sojdeh Saber analyzed the data Homa Hajimehdipoor was the pharmaceutical consultant.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

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Abbreviations

BMI: Body Mass Index; CI: confidence interval; DM: diabetes mellitus; FBS: fasting blood sugar; FSH: follicular stimulating hormone; GGT: gamma glutamyl transferase; HOMA-IR: homeostatic model assessment of insulin resistance; ITM: Iranian traditional medicine; KOH: potassium hydroxide; LH: luteal hormone; NIH: national institutes of health; OCP: oral contraceptive; PCOS: polycystic ovarian syndrome; SHBG: sex hormone binding globulin; WHO: World Health Organization