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Original Article

Comparative Effect of Nigella Sativa+ Black Pepper and Letrozole + Tamoxifen on Female Infertility in Women with Polycystic Ovarian Syndrome: A Randomized Clinical Trial

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ABSTRACT

Background: Polycystic ovary syndrome (PCOS), one of the most common reasons for infertility in terms of anovulation, influences 4–7% of reproductive women.
Objectives: This study aimed to compare the impact of Nigella sativa + black pepper with Letrozole + Tamoxifen as a treatment for infertile women with PCOS.
Methods: 90 PCOS infertile women aged 18 and 42 who were sent to an infertility clinic took part in this randomized, double-blind and clinical experiment. Patients were split into intervention and control groups at random. Letrozole and Tamoxifen were utilized in the control group. In contrast, Nigella sativa and black pepper were employed in the intervention group (from the third to the seventh day of the menstrual cycle). Throughout therapy, transvaginal ultrasound measures such as ovarian follicular size, number, and endometrial thickness were monitored, and the medication regimen was maintained according to these characteristics.
Results: In the intervention and control groups, the pregnancy rate was 22 (48.8%) and 5(11.1%), respectively (p=0.021). On day 12 of the menstrual cycle, there was a significant difference between the two groups endometrial thickness, dominant follicle size, and the number of follicles ($p<0.05$). The incidence of Ovarian Hyperstimulation Syndrome (OHSS) did not significantly vary between the two groups ($p>0.05$).
Conclusion: Increased pregnancy rates are finally brought on by the favorable effects of Nigella sativa and black pepper on the size of dominant follicle and endometrial thickness. Consequently, despite the necessity for standardization by the applied research laboratory of medicinal plants and further clinical trial research, we advise using this low-cost, low-side-effect regimen to treat infertile PCOS patients.

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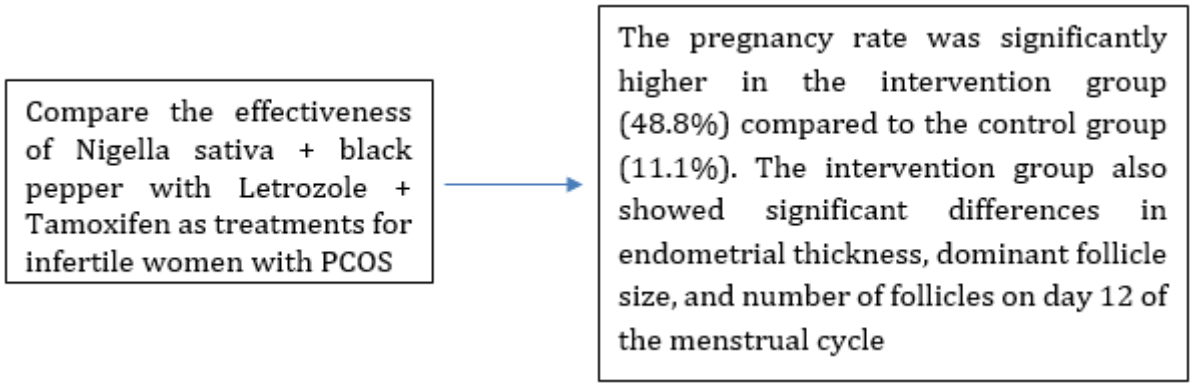
GRAPHICAL ABSTRACT



Introduction

Four to seven percent of women have polycystic ovary syndrome (PCOS), one of the most prevalent causes of infertility brought on by anovulation [1]. Anovulation and androgen excess the syndrome's defining diagnostic criteria [2]. At least two of the following criteria-oligo as well as anovulation, clinical and biochemical symptoms of hyperandrogenism, and polycystic ovaries during the ultrasound-are required for diagnosing PCOS, according to a consensus meeting conducted in Rotterdam [3]. While the exact cause of PCOS is still unknown, some investigations have shown that it may be an X-linked dominant condition [4]. s women with PCOS have metabolic abnormalities, including those with PCOS, which include insulin resistance, hyperinsulinemia, and dyslipidemia [5, 6]. Managing women with PCOS depends on the symptoms. These could be ovulatory dysfunction-related infertility, menstrual disorders, or androgen-related symptoms.

Third-generation selective, non-steroidal aromatase inhibitor letrozole has effectively induced ovulation in PCOS patients [7]. In the letrozole group for ovarian stimulation, Mitwally *et al.* observed favorable pregnancy outcomes and a decreased risk of multiple gestations [8]. Letrozole has no long-lasting anti-estrogenic effect since it does not reduce estrogen receptors (ER) in target tissues. The endometrium and cervical mucosa may not be negatively affected by it, which generally leads to mono-ovulation [9]. In recent years, Tamoxifen (Tx), another antiestrogenic substance with a structure quite similar to that of clomiphene citrate, has been investigated as a fertility aid. Ovulation rates have been reported as 50-90% and pregnancy rates as 30-50% [10] despite the lack lack of research on TTamoxifen for ovulation induction. Due to its antioxidant action, piperine, the most prevalent alkaloid in pepper, has anticancer, antitumor, and liver-protective properties [11]. Traditional Iranian medicine has employed Nigella Sativa (Cyah-daneh in Persian) seeds as a natural treatment for female menstruation, galactagogues, carminatives, laxatives, etc., and anti-parasitic properties [12].

Although Nigella sativa and black pepper are known to have several health benefits [13, 14], the specific mechanism of action by which they may improve fertility outcomes in women with PCOS is not well understood. Therefore, this study investigates the underlying mechanism of action of this herbal intervention and compares it with the mechanism of action of Letrozole and Tamoxifen.

Materials and Methods

Study design

From September 2017 to February 2018, 90 infertile PCOS women sent to an infertility clinic associated with Jahrom University of Medical Science in Iran underwent this double-blinded clinical trial investigation.

According to El-Gharib *et al.* study [15] the sample size was determined to be 90 participants, with a consideration of a 0.6 effect size, a type 1 error of 5%, and a power of 80%,

resulting in 45 individuals per group. Also, we performed power analysis using the G*Power program by kind of A priori method [16] based on data from the initial study [15], and actual power (1-β err prob) was calculated as 0.8036969.

Participants

According to the Rotterdam criteria, the inclusion criteria for infertile PCOS women included the presence of two symptoms: (i) oligomenorrhea or amenorrhea, and (ii) clinical (hirsutism) or biochemical signs of hyperandrogenism and adherence to the treatment regimen as well as the absence of surgery, internal disease, endocrine disorders, virilizing tumors, renal or liver disease, and any dietary or drug regimen for fertility. research did not include individuals unable to continue taking their medications due to severe gastrointestinal problems, such as an allergic reaction. Semen analysis, pelvic ultrasound, hysterosalpingography, and several hormonal assays (TSH, T3, T4, FSH, LH, PROLACTIN, and DHEA-S) were all used to evaluate the factors impacting fertility. Participants with aberrant lab results were eliminated from the research. Through random allocation, eligible patients were split into two groups.

Randomization and blindness

This double-blind, randomized clinical experiment was conducted on 90 infertile 18-42-year-old women with PCOS. Among the patients sent to the infertility clinic, 45 infertile women with PCOS were chosen for each group (Figure 1). luckRandom allocation was performed using Random Allocation Software as an arbitrary division with the nine-way lucky block approach. Using a lottery, the letters A and B will represent one of the two intervention and control groups. The following stage included placing each of the nine codes in a sealed envelope that could not be opened. Upon the patient's visit, one of the envelopes was chosen at random, and the patients were randomized to one of the research arms based on the sequence of letters on the envelope. This research will be done neither the participant nor the researcher will be aware of the method used to allocate individuals to treatment groups.

Hormonal assessment before the intervention

'Patients' hormonal tests (FSH-LH-TSH-DHEA-S and prolactin) were done on the third day of menstruation before the intervention.

Intervention

Nigella Sativa and black pepper samples were collected from the central herbal market of Jahrom City and authenticated by pharmacognosy specialists at Jahrom University of Medical Sciences Faculty of Pharmacy. We determined the dosage of the herbal regimen using the Physician's Desk Reference (PDR) [17]. Herbal pharmacologist at Jahrom University of Medical Sciences, Iran, has found herbarium Nigella sativa code number A4017009P and black pepper code number PM 1310-Peper nigrum L. The dosage administered to the control group was determined based on prior research [18]. Intervention group: From the third to the seventh day of the menstrual cycle for three months, take five capsules of NigellaSativa (each capsule contains 550 mg of pure powder) and five capsules of black pepper (each capsule contains 550 mg of pure powder).Control Group: 10 mg Tamoxifen (two tablets per day, SHAFA Company) and 2.5 mg Letrozole (two tablets per day, Alborz Company) from the 3rd to 7th day of the menstrual cycle.

Data collection

Patients were given a demographic questionnaire that included questions about their age, height, weight, underlying conditions, gestational age, and other details. Transvaginal ultrasound parameters including ovarian follicular size, number, and endometrial thickness were assessed on the third, seventh, and twelfth days of the menstrual cycle. Depending on the size of follicles, the next step is established.

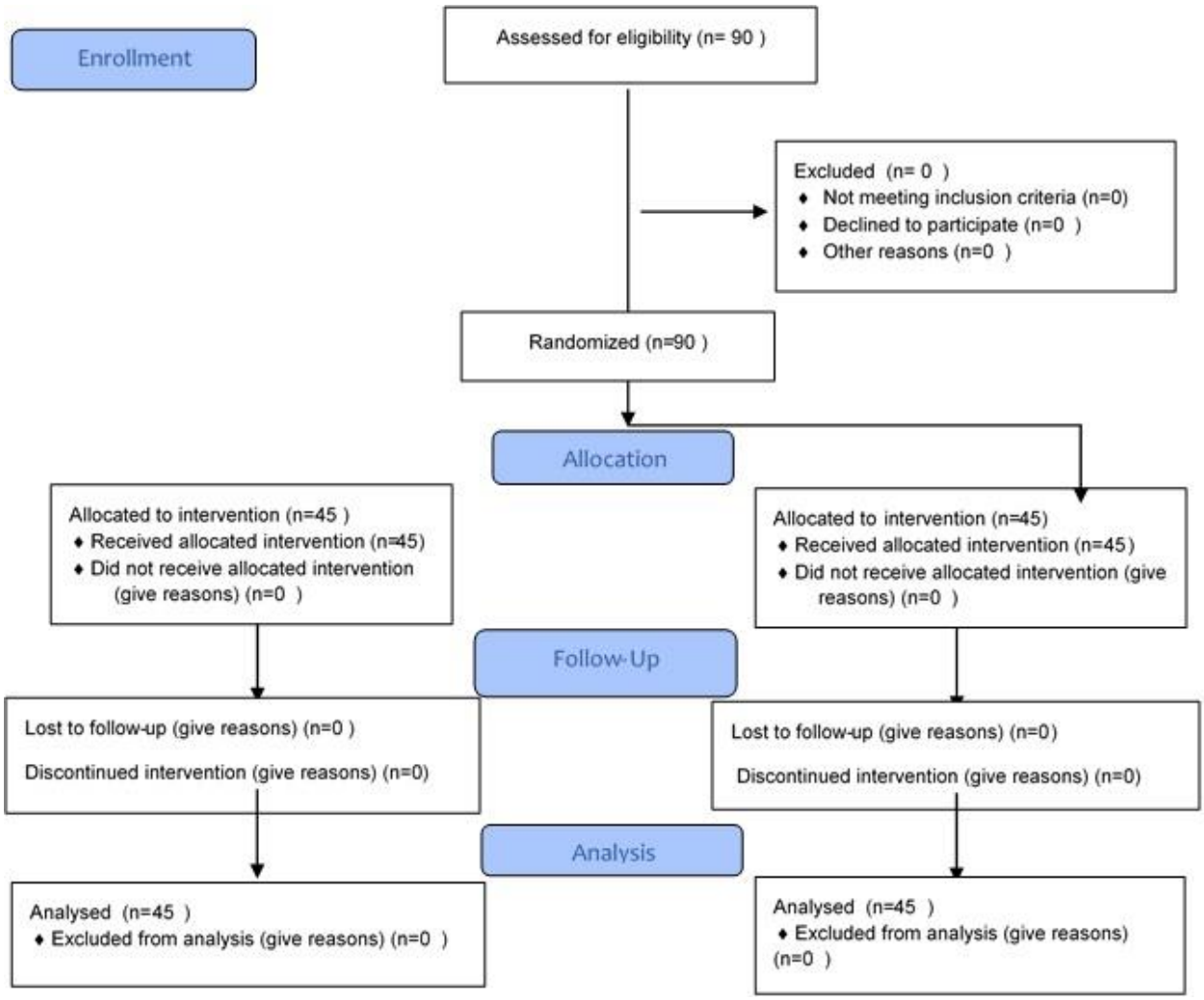


Figure 1: Consort diagram of study participants

If the follicles were 25 mm or larger, the medication was discontinued, and if they were less than 18 mm, the drug was maintained for four days. The trigger medicine is administered when the follicles are 18 to 25 mm in diameter (5,000 to 10,000 HCG units), and the dosage varies according to the quantity and size of the follicles. The dosage of HCG is reduced if there are more than 25 mm in diameter and roughly 10 mm in the number of follicles. During the thirteenth, fifteenth, and seventeenth days of their menstrual cycle, patients were instructed to engage in sexual activity. A β.HCG test was run if the lost period was present. The treatment went on for three menstrual cycles if pregnancy did not happen.

In this study, the primary endpoints included follicle size, follicle count, and endometrial thickness, measured by ultrasound on days 7 and 12 of the menstrual cycle. The secondary endpoint was ovarian hyperstimulation syndrome, which was evaluated by ultrasound during the treatment period.

Statistical analysis

Versions of GraphPad Prism 8 and SPSS software (version 21) were used for data analysis. The Kolmogorov-Smirnov test was initially employed to determine if the data were normal. Results were presented as mean, standard deviation, frequency, and percentage in the descriptive statistics section. Chi-square, Fisher's exact test, and independent-sample t-test were used in the analytical statistics part. In this investigation, significant values were defined as $p < 0.05$.

Results and Discussion

Population characteristics

The average age of the patients in the intervention and control groups was 25.7±3.41 and 27.67±7.04, respectively (p=624). Moreover, no significant difference was found between the two groups in terms of weight, height, BMI, and hormonal characteristics in serum levels like FSH, LH, DEHA-S, Prolactin, and TSH (p>0.05) (Table 1 and Figure 2).

Comparison of therapeutic effects

According to the findings, there was no discernible difference in the two groups' hormone levels at the research's opening and conclusion (P> 0.05). Except for serum LH, which was considerably lower in the Nigella sativa+ black pepper group (P = 0.001) than in the Tamoxifen + Letrozole group (P> 0.05), there was no discernible difference in the blood levels of these hormones in either group after the intervention (P> 0.05) (Figure 2).

Table 1: The comparison of two groups in terms of age, weight, height, and BMI under study before the intervention

Variable	Groups		P-value*
	Intervention group (Nigella sativa + black pepper) (N= 45)	Control Group (Letrozol+Tamoxifen) (N= 45)	
Age (yr), mean±SD	25.7±3.41	27.67±7.04	0.624
Weight (cm), mean±SD	67.4±16.49	69.67±13.38	0.648
Height (cm), mean±SD	150.93±21.77	161.42±5.58	0.312
BMI (kg/m^2), mean±SD	31.55±13.63	26.57±3.67	0.614

*BMI: Body mass index, SD: standard deviation,*independent t-test, and significant level<0.05.*

As indicated in Table 2, follicular size, number, and endometrial thickness were assessed in each group on day 3 of the menstrual cycle. Endometrial thickness and follicular number in both groups were not statistically significant at baseline (p>0.05), but follicular size in both groups was statistically significant (p=0.009) (Table 2). Each group's ovarian and endometrial parameters were assessed on days 7 and 12 of the menstrual cycle. The endometrium thickness, the size of follicles, and their numbers were significantly different in the intervention group (p<0.05) (Table 2). The pregnancy rate was 48.8% in the intervention group and 11.1% in the control group (p=0.021). Incidence of Ovarian hyperstimulation syndrome (OHSS) was nil in the intervention group and 2.22 percent in the control group (p = 0.10). One patient in the intervention group (2.2%) had an abortion, whereas three patients in the control group (6.6%) do (p=0.306) (Table 3).

This clinical trial aims to compare Letrozole + Tamoxifen with the Nigella sativa + black pepper regime to treat infertility in women with polycystic ovary syndrome. Letrozole and Tamoxifen are recommended as an alternative for ovulation induction in infertile patients with polycystic ovary syndrome [9].

Tamoxifen may improve ovulation and pregnancy in clomiphene-resistant individuals because of estrogen's actions on the endometrium and cervical mucosa, producing a favorable environment for follicle formation. The results of this study indicate that the group receiving a combination of Nigella sativa and black pepper had a significantly higher pregnancy rate (48.8%) compared to the letrozole + tamoxifen group (11.1%) (p=0.021). Furthermore, on day 12 of the menstrual cycle, the two groups exhibited significant differences in dominant follicle size, number of follicles, and endometrial thickness (p <0.05). However, there was no significant difference in the incidence of Ovarian hyperstimulation syndrome (OHSS) between the two groups (p>.05). In addition, some studies have shown that endometrial thickness in the letrozole group is significantly greater than that in the clomiphene group. Relevant references have been provided for further reading [19, 20].

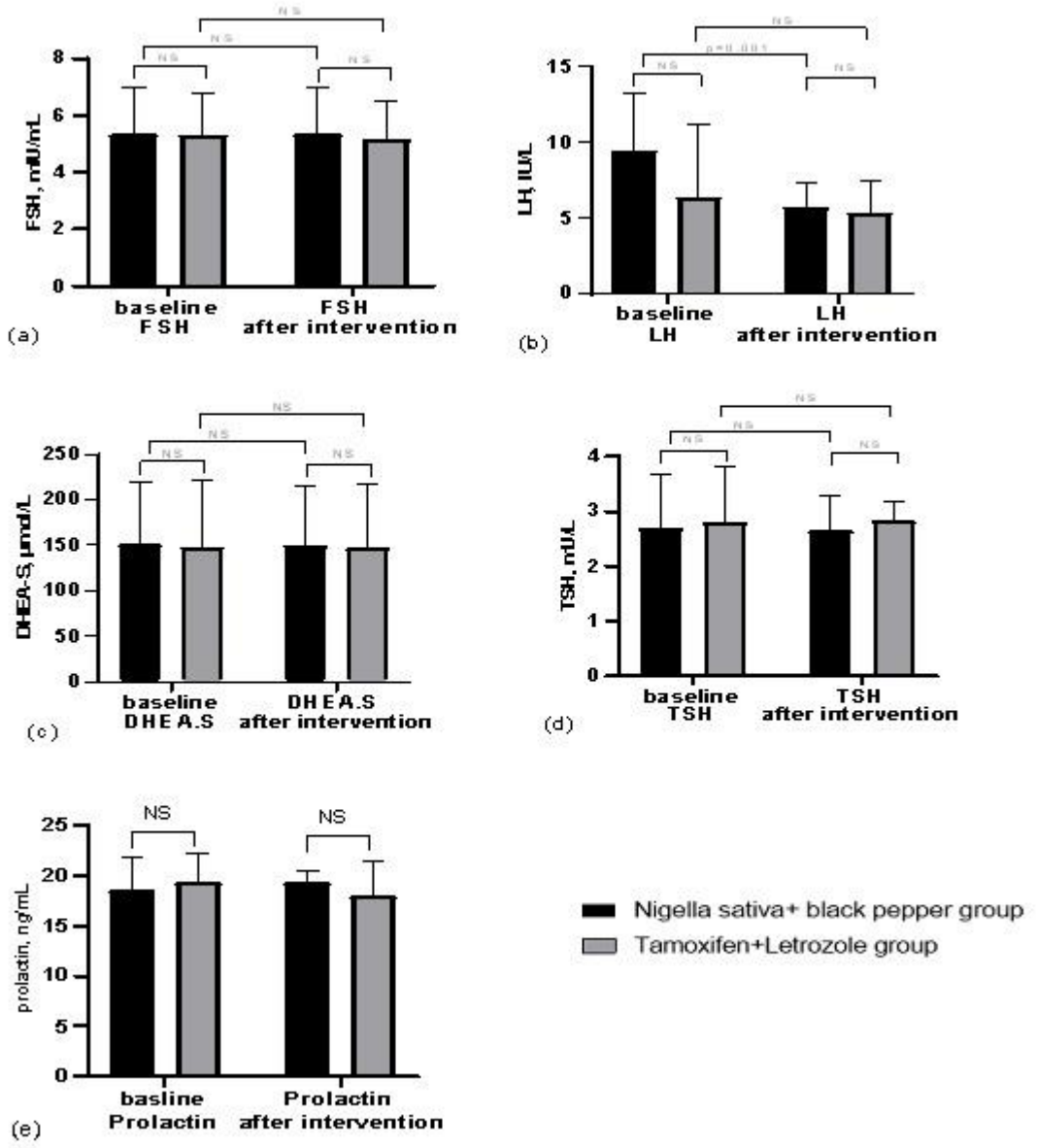


Figure 2: Comparison of hormonal tests in two Treatment Groups. Baseline and post-intervention levels of FSH (a), LH (b), DEHA-S (c), TSH (d), and prolactin (e) are presented for the Nigella Sativa + black pepper and letrozole + tamoxifen groups. NS denotes non-significance

Table 2: Mean and standard devotion of follicular size, number, and endometrial thickness in each group based on days of the menstrual cycle

Variable	Day 3 Menstrual cycle			Day 7 Menstrual cycle			Day 12 Menstrual cycle		
	Intervention group	Control Group	P-value*	Intervention group	Control Group	P-value*	Intervention group	Control Group	P-value*
Endometrial thickness (mm)	3.11±0.59	3.38±0.73	p=0.057	6.89±0.96	4.84±0.92	<0.001	8.63 ± 1.49	6.16 ± 1 .08	<0.001
Follicular size (mm)	4.91±0.41	4.60±.65	p=0.009	10.08±4.02	8.26±1.24	<0.005	13.29±5.48	8.33 ± 2.99	<0.001
Number of follicles	1.04±0.20	1.00 ±. 00	p=0.16	1.04 ±0.20	10.06±0.25	<0.001	1.13±0.40	10.01±0.002	<0.001

*Intervention group: Nigella sativa + black pepper, control group: Letrozol+Tamoxifen,*independent t test, and significant level<0.05.*

Table 3: The comparison of Nigella sativa + black pepper and letrozole + tamoxifen groups in terms of fertility rate in two groups under study

Variables	Category	Groups		P-value
		Intervention group (Nigella sativa + black pepper) N (%)	Control group (Letrozol+Tamoxifen) N (%)	
Pregnancy [a]	Positive	22(48.88)	5(11.11)	0.021*
	Negative	23(51.12)	40(88.89)	
OHSS [b]	Yes	0(0)	1(2.22)	0.103**
	No	45(100)	44(97.78)	
Abortion [c]	Yes	1(2.22)	3(6.66)	0.306**
	No	44(97.78)	42(93.34)	

*βHCG: Beta-Human Chorionic Gonadotropin, OHSS: Ovarian hyperstimulation syndrome, * Chi-square test, and** Fisher's exact test, significance level<0.05.*

a: Pregnancy was determined by BHCG after not menstruating.

b: OHSS was determined by sonography after the end of each treatment regimen.

c: Abortion was determined by measuring βHCG titer immediately after one day of menstruation and titration again 48 to 72 hours later. If the titer dropped, it was considered an abortion .Also, ultrasound in the 6th week of pregnancy.

Akbari *et al.*, in intrauterine insemination cycles, letrozole, and clomiphene citrate coupled with gonadotropins were compared. In their research, endometrial thickness was considerably greater in the letrozole group, whereas clinical pregnancy rates were similar between the two groups. They discovered that Letrozole is an effective and cost-efficient substitute for Clomiphene Citrate-Intrauterine Insemination (CC-IUI) cycles [20].

The proper function of ovary is essential for maintaining fertility and overall health. There is limited evidence of the effects of oxidative stress on the health of early follicles, growth, and ovulation, as well as primary oocytes and embryos with cellular and molecular targets [21]. Research points to the antioxidant and anti-inflammatory capabilities of both black oil and the active constituent components, particularly thymoquinone (TQ). It inhibits inflammatory mediators, including prostaglandins and leukotrienes, to provide anti-inflammatory effects.

The histological alterations brought on by methotrexate usage are significantly reduced by thymoquinone, which is present in the Nigella sativa extract [22]. Nigella sativa's currant extract suppresses the breakdown of beta cells in the Langerhans Islands owing to toxins like streptozotocin and alloxan and lowers the formation of free oxygen radicals. When Nigella sativa is prescribed, the streptozotocin-induced animal model of diabetes mellitus sees an increase in beta cell regeneration and proliferation [23]. In rats with diabetes caused by STZ, Nigella sativa exerts (NS) a protective action against β-cell damage. In diabetic rats, NS therapy stimulates pancreatic β-cell regeneration, which raises low levels of blood insulin and subsequently lowers high levels of serum glucose [24]. According to Sarvesh Kumar *et al.* research, taking pepper extracts or their chemical components as dietary supplements may help fend against various illnesses, including asthma, COPD, cancer, and cardiovascular disease [25]. It has long been known that insulin resistance is linked to PCOS and is caused by ovarian hyperandrogenism.

Many studies have shown that PCOS-positive women have a higher risk of acquiring type 2 diabetes [26-28]. Prospective clinical investigations have shown that type 2 diabetes and impaired glucose tolerance is more common in PCOS-affected women (31-35% and 7.5–10.0%, respectively) [25]. Moreover, it has been shown that women with PCOS have a 5- to 10-fold greater risk of developing type 2 diabetes from reduced glucose tolerance [29]. The development of this illness is significantly influenced by these women's obesity and elevated insulin resistance. Nami Kim *et al.*

investigated piperine's role in skeletal muscle when used as a treatment for several metabolic disorders to characterize its metabolic effect thoroughly. They cconcluded that piperine regulates metabolic processes by activating the AMPK signaling pathway in a way that makes it a strong candidate for clinical metabolic therapy [16]. According to studies on the treatment of infertile women, black pepper, and Nigella sativa may help decrease insulin resistance, obesity, and the LH to FSH ratio, which may lead to an increase in ovulation and fertility [14, 30]. Darand *et al*. showed in a review study that the treatment with N. sativa has improved the sperm parameters, semen fluid in men and Leydig cell count, graph follicle count, corpus luteum, and level of sex hormones such as testosterone and progesterone in women ($p<0.05$) [31]. However, to our knowledge, there have been no previous studies on the combined effects of Nigella sativa and black pepper on female fertility. Therefore, this study provides novel and promising results for the potential use of this combination in fertility treatments.

Strengths and limitations

The study's strength was the comparison of this herbal combination with a licensed medication for the infertility treatment, which showed rather excellent effectiveness in an appropriate patient group. The study's short-term follow-up and limited sample size were two weaknesses. In addition, the limited number of similar studies on the effects of nigella sativa and black pepper in the infertility treatment was a limitation of this study, which made it difficult to compare the findings of this study with those of other studies.

Conclusion

In the end, we concluded that Nigella sativa + black pepper consumption affects the thickness of the endometrium and increases the size of dominant follicle, ultimately increasing pregnancy rates in infertility women with PCOS. However, more clinical studies with larger sample sizes are still required. Chemical medications may eventually replace herbal regimens due to their minimal side effects, accessibility, and high level of acceptability, albeit the Applied Research Laboratory of Medicinal Plants should initially standardize them.

Disclosure Statement

No potential conflict of interest was reported by the authors.

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Authors' Contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

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