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The effect of *Nigella sativa* on infertility in men and women: a systematic review

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Summary. *Objective:* Black seed (*Nigella sativa* L.) is a medicinal plant belonging to botanical family of Ranunculaceae. Given the effective role of N. sativa in treating different diseases, the present systematic review aimed to evaluate the effects of N. sativa on the reproductive system. *Materials and Methods:* The current systematic review was carried out on randomized controlled trials (RCTs) regarding human, animal and in vivo/in vitro studies published between 2014 and 2017. The related articles were collected by searching the databases of Medline, PubMed, Scopus, Science direct, and Google scholar only in English using the search keywords of *Nigella sativa*, Black Seed, Thymoquinone (TQ), Infertility, Reproductive System and Reproductive Parameters. Review articles, case reports, abstract in symposium and congress were excluded. *Results:* Finally, 24 articles were selected for analyses. The results showed 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 ($p<0.05$). *Conclusion:* According to the findings, *N. sativa* can improve the reproductive parameters and sex hormones in both genders.

Key words: *Nigella sativa*, black seed, thymoquinone, infertility, reproductive system, reproductive parameters

Introduction

World Health Organization refers to infertility as the inability of a sexually active, non-contracepting couple to achieve pregnancy during a year (1, 2). Globally, one of the most underappreciated reproductive problems is the infertility accounting for about 12-15% of couples. The prevalence rate of infertility in Iran in 2013 reportedly was 20.2%, meaning about three million infertile couples (2, 3). According to the results, the infertility rate is higher in the males than in the females, as the male infertility factors contribute to one half of the infertility cases (4). The infertility can potentially affect the life of couples, healthcare system and society, incurring huge financial burden, psychological consequences and physical problems (5, 6). There are many reasons responsible for the infertility, such as congenital disorders, hormonal abnormalities, genetic factors, and nutritional problems.

Among which, the leading causes can be attributed to unhealthy male anatomy, liver failure and cirrhosis, drug-related complications, lifestyle and environmental factors alone or in combination with other causes (4). The quality assessment of semen to improve the fertility is important because about 60% of infertilities occur due to impairments in semen and spermatozoa. Moreover, it should be noted that the female infertility is developed following the ovulation

deficiencies, endometriosis, low-quality eggs, polycystic ovarian syndrome (PCOS), and fallopian tube obstruction (7, 8). The treatment of infertility can be promising reportedly by consuming anti-oxidants and herbal medications as well as appropriate nutritional suppliments. One of the effective herbals in this regard is black seed (*Nigella sativa* L.) that has been important traditional therapy for infertility in Iran, which belongs to the family Rannculaceae. The plant has light or dark blue and/or white flowers containing white milky seeds that become black while exposing to air (9-11).It is one of the common and popular in medical history and religious, which has been widely used to heal diseases, This medicinal herbs have had extensive therapeutic purposes in the Middle East and Far East, including asthma, headache, diarrhea, infections, obesity, backache, hypertension, gastrointestinal problems, abscess, nasal congestion, and rheumatism. Previous studies reported anti-oxidant, anti-inflammatory, immune system booster, anti-histamine and diuretic characteristics (12-28).

Furthermore, researches regarding the active effects of black seed on reproductive functions and infertility treatment demonstrated significant improvements in respective factors, including sperms, semen, leydig cells count, follicular development, corpus luteum and gonadotropic hormones like testosterone and progesterone. *Nigella sativa* as an effective herbal for treating different disorders has shown no serious complications. Nevertheless, limited reviews are available on the study of the black seed effects to improve the reproductive parameters among males and females. Accordingly, the current systematic review was designed to evaluate is the effects of black seeds on preventing and treating the infertility.

Materials and Methods

The current systematic review was carried out on randomized controlled trials, including human, animal and in vivo/in vitro studies, published between 2014 and 2017. Because the last article in this subject (but only on men) was published in 2014, so we tried to look for more recently studies after 2014. The articles were collected by searching the databases of Medline, PubMed, Scopus, Science direct, and Google scholar. The searches were limited to studies in English including relevant keywords or phrases such as *Nigella sativa*، Black Seed, Thymoquinone, Infertility, Reproductive System and Reproductive Parameters.

The study design was restricted to RCTs; and review articles, case reports, abstract in symposium and congresses were excluded. All articles meeting the inclusion criteria were included in the study. Furthermore, the reference lists of potentially selected articles were checked to find additional related references. In the next step, two reviewers who were subject matter experts analyzed the titles and abstracts of initially selected articles to confirm the final articles for inclusion in the study. Duplication was also checked and unrelated articles (in content) were excluded (Figure1).

For each study, the following data were extracted: demographic characteristics of participants (age and gender), sample size, study design, method of dietary assessment, type of dietary black seed, dosage of black seed, and duration of consumption.

Characteristics of the evaluated studies have been summarised in Table 1.

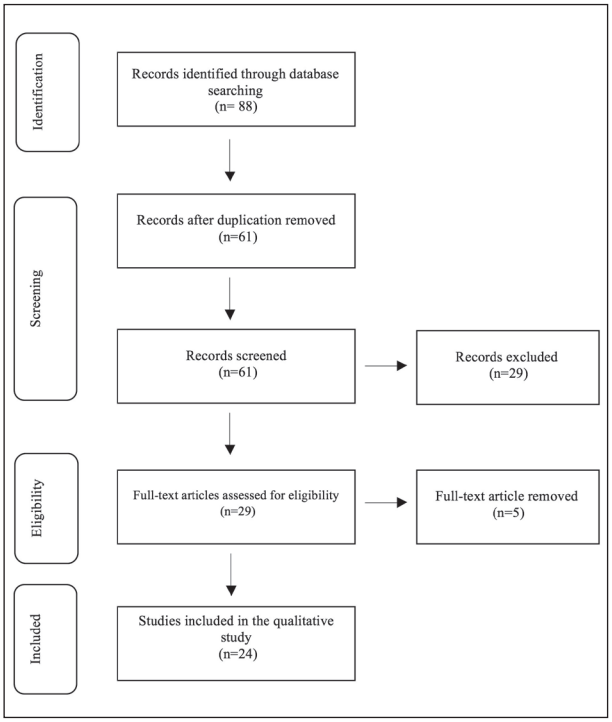

Figure 1: Trend of screening and choosing articles

Table 1. Characteristics of the eligible studies

Author/Year	Study Sample	Intervention	Dose/duration	Result
Human studies				
Kolahdooz, et al. (2014)[29]	78 infertile men	NSO [1]	2.5 mL oil (twice daily for 2 months)	↑ Sperm count, ↑sperm motility ↑ Normal Sperm morphology ↑ Semen volume ↑ Semen pH ↑ Round cells
Latiff LA, et al. (2014)[30]	69 perimenopausal women	pure powdered *N. sativa*	1600mg/day for 12 weeks	↓ of prevalence and severity of menopausal symptoms ↓ LDL and blood glucose
Animal studies				
Koutabadi L, et al. (2015)[32]	20 newly weaned rats	TQ [2]	15 mg/kg	↑ progesterone *no significant effect on FSH, LH and estrogen*
Arif M, et al. (2016)[31]	polycystic ovary (PCO) rats	TQ	(1 mM/ml)	NF-kB nuclear translocation, COX2, and ROS expression were repressed restoration of normal physio-molecular behavior of ovary normalization of key ovarian factors ↑ ovulation rate ↑ normal ovarian phenotype
Alyoussef A, et al.(2016)[35]	50 adult male rats	TQ	25 or 50 mg/kg daily by oral-gavage for twelve weeks	TQ ameliorated testicular tissue inflammation and restored the normal balance of sex hormones induced by sodium nitrite
Tüfek, N. H, et al. (2015)[37]	NA[3]	Intraperitoneal TQ	NA	↑ The number of healthy sperm ↓ sperm anomalies ↑ The testis weight and Volume ↑ mean volume of th seminiferous tubules ↑ the total number of spermatogonia, primary spermatocyte, and round spermatids ↓ CAT activity ↓ Apoptotic activity in the spermatogonia
Mabrouk, A,et al. (2014)[52]	NA	TQ	5 mg/kg body weight/day, per orally for 5 weeks	↑ plasma testosterone level ↑ epididymal sperm count
Mabrouk, A, et al. (2015)[53]	NA	TQ	5 mg/kg body weight/day, per orally for 5 weeks	↑ the antioxidant enzymes activities such as superoxide dismutase, glutathione peroxidase and catalase ↓ the level of malondialdehyde.
Mousa, A. M, et al. (2015)[54]	40 adult male rats	TQ	100 mg/kg through a gastric tube for 6 weeks	improvement in the histological, morphometric, and biochemical changes*
Rahman, S. A, et al. (2014)[55]	NA	TQ	10 mg/kg on alternate days	protection of reproductive tissues and may have a role in the recovery of spermatogenic cells and the epithelium.
Sayed, M. M, et al. (2014)[56]	40 male rats	TQ	NA	↑values of serum LPO GSH,SOD, CAT, testosteron, ↑sperm motility, sperm abnormalities normalization sperm density and histological appearance

Continued...

Author/Year	Study Sample	Intervention	Dose/duration	Result
Human studies				
Jasim, W. K, et al. (2016)[57]	12 females rats	NSO	(1ml /kg BW/ day)	↑LH, Estrogen ↑Gonadotropic Releasing Hormone (GnRH)
Ping et al,(2014)[58]	Sprague-Dawley male rats	NSO	6μL/100 g body weight	↑Sperm motility ↑Normal sperm and live ↓Lumen diameter Spermatogonia thinner ↑Spermatid-sperm width
Mosbah, R, et al. (2016)[17]	32 adult male Wistar rats	NSO	(1 ml/kg/day) for 4 weeks	↑testosterone, semen characteristics, GSH, and antioxidant enzymes ↓ the levels of free radicals.
Hassanin, K. M, et al. (2014)[59]	30 male adult albino rats	NSO	(2 ml/kg body weight) for 4 weeks	ameliorating the cadmium-induced elevation of the serum levels of testosterone, LH and FSH ↓levels of TNF-α and IL-1β
Hussein, O. A.-T, et al. (2014)[60]	40 adult male rats	NSO	1 ml/kg orally for 1.5 months	amelioration of the testicular regression ↓ the $AlCl_3$-induced spermatogenic damage and generation of free radicals
Mohamed, A. F, et al. (2015)[61]	40 adult male rats	NSO	5 ml/kg/day for 45days	↑in the epithelial height of seminiferous tubules ↑attenuated the testicular injury
Umar, Z, et al. (2017)[62]	20 adult male rabbits	NSO	5ml/kg body weight/day on daily orally for 60 days	↑ weight, length,circumference and volume of testis ↑testosterone concentration ↑percentages area of interstitial cells in relation to seminiferous tubules, the thickness of germinal layer, diameter, area, diameter of lumen, spermatogenic cell layer and number of spermatogonia of seminiferous tubules of testes Stimulation testicular function in adult rabbits.
Parhizkar, S, et al. (2016)[33]	40 rats	**in the first experiment** N.sativa **in the second experiment** methanol, hexane and SFE extracts of *N. sativa* **in the third experiment** linoleic acid gamma linolenic acid thymoquinone	300, 600 and 1200 mg/kg 300 mg/kg 50 mg/kg 10mg/kg 15mg/kg	low dose *N. sativa*, methanol extract and linoleic acid had prominent estrogenic like effects
Assi MA, et al. (2016)[34]	20 Sprague-Dawley rats	N.Sativa	200 mg/kg/ daily for 1 month	↑ The sperm concentration, general, and individual motilities ↑ The percentage sperm viability ↑ testosterone (TS) hormone concentration ↑ concentration of LH ↑ EST concentration
Mohajeri, D, et al. (2015)[63]	40 male rats	N.Sativa	10 % and 20 % in diet	↑ blood testosterone level and decreased testis ↓malondialdehyde level ↑ antioxidant enzymes activities ↑ spermatogenesis
Aithal M, et al. (2016)[36]	36 rats	N. sativa seed powder Thymoquinone	(300mg/Kg BW) (4mg/kg BW)	↑ the Testosterone levels

Summary of the studies and potential mechanisms

We carried out this systematic review on 24 RCTs. The articles with the same method were categoryzed into the same groups. We used the most relevant studies to our inclusion criteria in the results and discussion section.

Human studies in MEN:

Of 24 selected articles, one study evaluated effects of *N. sativa* (NS) in men.

Kolahdooz et al. indicated that 2.5 ml/day black seed oil, improved the count, mobility, and the morphology of sperms, semen volume, pH and the stellar cells after two months. In addition, this study showed that 5 ml daily intake of black seed oil improves semen quality including sperm count, morphology and motility and semen volume, pH and round cells in the infertile men having abnormal semen parameters (29). It should e noted that only one study was found about the effect of NS on the female fertility:

In a study of Latiff LA et al., 12-week administration of 1600mg/day pure powdered *N. sativa* in perimenopausal women decreased the prevalence and severity of menopausal symptoms, as well as LDL and blood glucose (30).

Animal studies in RATS:

According to a study by Arif et al., the administration of 2mg/kg/d TQ in treating the PCOS resulted in the suppression of NF-κB nuclear translocation, COX2, and ROS. Additionally, the treatment of the PCO rat model with TQ showed significant restoration of normal physio-molecular behavior of ovary, such as reduced cysts formation, increased ovulation rate, and normalization of key ovarian factors like TNF-α, COX2, matrix metalloproteinases and gelatinases activity during follicular maturation (31).

There are numerous articles on evaluating the effect of *N. sativa* on infertility in male rats; also there are more limited studies on females rats than male rats.

In a study conducted by Koutabadi et al., the treatment with 15 mg/kg of TQ for 30 days increases the progesterone level in the female rats, hereby preventing the fetus loss probably. It could not significantly affect luteinizing hormone (LH), follicle-stimulating hormone (FSH) and estrogen levels (32).

Parhizkar et al. conducted a series of experiments to investigate the effects of *N. sativa* (with the dosages of 300, 600 and 1200 mg/kg in the first experiment), (300mg/kg of methanol, hexane and SFE extracts of *N. sativa* in the second experiment) and (50 mg/kg of linoleic acid, 10mg/kg of gamma linolenic acid, and 15mg/kg of thymoquinone in the third experiment) on selected menopausal parameters of ovariectomized (OVX) rats for 21 days. The findings indicated that low-dose *N. sativa*, methanol extract and linoleic acid had prominent estrogenic like effects, which were significantly different from those of control group ($p<0.05$) in different experiments (33).

Assi et al. demonstrated that the administration of 200 mg/kg/daily of NS per overall survival increased sperm count, general and individual motilities, percentage of sperm viability and testosterone (TS) hormone concentration for a month. However, the abnormality was found to be lower in these rats compared to the negative control ($p<0.05$) (34).

According to Alyoussef et al., 25 or 50 mg/kg of daily oral-gavage TQ ameliorated the testicular tissue by alleviating inflammation and apoptosis, as well as restoring the normal balance of sex hormones. Although, the two doses of TQ had protective effects against the testicular damage, 50 mg/kg of TQ made better results, especially in the restoration of normal balance of sex hormones (35).

Aithal et al. concluded that the oral administration of (300mg/Kg BW) seed powdered from N. sativa and (4mg/kg BW) TQ in two groups of diabetic rats increased the count of Leydig cells in testis and testosterone levels (36).

In a research of Fouad et al., the rats treated with TQ showed higher serum testosterone level, and testicular GSH and SOD activity, and significantly lower levels of MDA compared to the control group ($P < 0.05$) (15).

In a study of Tufek et al., the administration of TQ increased the mean volumes of testis and seminiferous tubules, the count of spermatogenic cells and Leydig cells. Also, the count of healthy sperm was increased and the anomalies were significantly reduced using TQ (37).

Based on the results obtained from the reports of Mosbah et al., the orally administration of 1 ml/kg/day *N. sativa* oil (NSO) alone increased testosterone,

semen characteristics, GSH, and antioxidant enzymes and decreased the levels of free radicals. In addition, the administration of NSO caused a significant increase in relative weights of all reproductive organs, spermatid count and SCs, DSP, and motility and a decrease in dead and abnormal sperms. The NSO alone increased the levels of testosterone, LH, FSH, 17-KSR and17-HSD (17).

Discussion

Our systematic review was on the RCTs regarding the effect of *N. sativa* on the infertility among males and females. We decided to design this systematic review on both genders because the systematic review in 2014 studied *N. sativa* impact only on the male infertility (1). As complimentary study, the present work reviewed the effects of *N. sativa* on the inflammatory indexes of reproductive system among both genders regarding more articles on the effects of this medicinal plant on the testes and uterus and ovary, including 24 final articles. It should be noted that the results section covers the most relevant articles. Accordingly, the active impact of *N. sativa* was observed in the majority of studies on improving the male and female infertilities; and no study reported negative or neutral effects for this herb in treating the infertility. Moreover, the effects of *N. sativa* extract, oil, powder, and TQ with different doses and durations were found among the studies and *N. sativa* oil had the most effect on improving the infertility.

The mechanisms of action of *N. sativa* on improving the infertility have been reported different in studies.

The oxidative oxidants and antioxidants are important in the reproductive system of the female animals. The ROSs are key transitional factor to regulate the ovulation, oocyte maturation, corpus luteum formation, uterine activity, fetal cycle, embryo implantation and fetal development and the placenta function via multifarious signaling and transition pathways. The imbalance between the ROS production and antioxidants can trigger the infertility or the recurrent miscarriages, endometriosis, PCOS and other pregnancy-related abnormalities (37-39). *N. sativa* has been shown to increase the levels of progesterone in female rats subjected to oxidative and environmental stresses (32).

The uterus weight was elevated due to the administration of *N. sativa* that acts as a supplement to imitate estrogen activities in uterus. The linoleic acid as active ingredient of N. sativa has estrogenic effects, which increases gradually the blood levels estrogen, and increases vaginal epithelial cells (33). Anti-PMS Properties have been proven for the essential fatty acids (linoleic, linoleic and gamma linoleic acids). The levels of linoleic acid, reported as the main dietary source of omega-6 fatty acids in the body, have been shown to be more than normal in all females. However, deficient was observed in the levels of its anti-inflammatory metabolites, including gamma-linolenic acid, among all women as it can be said that inflammation is important role to develop the PMS (40). In addition, N. sativa showed less abnormal vaginal bleeding or spotting episodes and no breast tenderness (41).

The useful effects of *N. sativa* on the male infertility have been reportedbecause some positive outcomes. For example, protective effects of *N. sativa* have been expressed for oxidative status, superoxide anion scavenger, direct cytoprotective effects and indirect antioxidant and androgen activities, hereby protecting sperm and semen fluid against a testicular toxin (41). The TQ can prevent mitochondrial degeneration and improve the spermatogenesis in rats due to its antioxidant characteristics (42).

The steroidogenesis and spermatogenesis can be improved by antioxidants, including N. sativa that can neutralize the free radicals in the semen fluid or enhance the sperm factors. Among which, some compound accompany the antioxidants to remove ROS, including Flavonoids, anthocyanin, carotene, isothiocyanate and carotenoids that are found also the N. sativa (43). *N. sativa* oil due to the presence of UFAs, including linoleic acid (about 60%) and oleic acid (about 20%), are able to boost the sperm parameters like mobility and count, as well as to remove the abnormal sperms (44). The levels of testosterone and FSH hormones in testicular tissues are increased due to phenolic and alkaloid compounds (45, 46).

The testes and epididymis can be enlarged because of zinc, copper, magnesium and vitamins found in *N. sativa*. It also enhances the activity of several metabolic

enzymes, secretions of steroid hormones and serum protein enzymes (47). The fat-soluble TQ as a main effective substance of N. sativa can affect the activity of enzymes associated with oxidative phosphorylation (48). The NS oil has been found to be the best form of *N. sativa* in many studies to heal the infertility, and also NS aqueous extract and TQ in other investigations suppress the COX2 expression, lipid peroxidation, elevate the SOD levels in diabetic rats, and reinforce the testosterone levels and testes tissue (49).

A study reported that an increase in the levels of LH enhanced the levels of leydig cell-secreted testosterone hormone (50), which is positively effective in growth, development, function and weight of testes and epididymis as well as prostate and reproductive glands in men (47).

The bitter nature of *N. sativa* extract taste has been known to decrease food intakes compared to control group, resulting in weight loss (51). It also can significantly elevate the sperm count, mobility, normal morphology, and bioavailability and semen volume.

Despite existing many reports on the significant effects of *N. sativa* on improving both male and female infertility, it is suggested to conduct further RCTs on the positive effects of *N. sativa* on infertility with determined dosage, duration of treatment and consumption form to recommend it as an independent therapeutic medication in the management of the infertility.

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Transparency declaration

MD Conceptualized and designed the systematic review, wrote the manuscript and interpreted the findings. MH assisted in revising and English editing the manuscript. PM corrected the possible errors and did the final review. All authors critically reviewed the manuscript and approved the final version submitted for publication. None of the authors has conflict of interest to declare. Also, Parvin Mirmiran (lead author) affirms honest, accurate, and transparent of this manuscript, as well as assures that no important aspects of the study have been omitted and that any discrepancies from the study as planned.

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