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The Beneficial Effects of Herbal Therapy (Zayesh Booalidaroo + Safoof-e Hefz + Royal Jelly) on Male Infertility

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ABSTRACT

Background: Several studies have recently been conducted on herbal plants due to their growing medicinal usage. Since it is estimated that male partner causes 40% of infertility, we aimed to examine the effects of herbal combinations, including Zayesh Booalidaroo + Safoof-e Hefz + Royal jelly, in increasing male fertility in men with oligoasthenoteratozoospermia (OAT).

Materials and Methods: A total of 50 OAT men were chosen, confirmed, and diagnosed by a urologist. They were randomly divided into two groups: Herbal and routine therapy (Sperigen supplement). All the parameters in semen analysis, hormonal assay, sperm DNA fragmentation, protamine deficiency, and the concentration of seminal plasma antioxidants, including malondialdehyde (MDA), total antioxidant capacity (TAC), and superoxide dismutase (SOD), were assessed in both groups before and after the intervention.

Results: According to the obtained data, the herbal group showed improved total sperm and /mL count (P=0.0001 and P=0.007, respectively) along with increased progressive and total motility (P=0.02 and P=0.043, respectively), while routine therapy did not change any of sperm parameters significantly (P>0.05). Also, in hormonal assessment, there were no significant changes after interventions except in the herbal therapy group, where patients showed an elevated testosterone hormone level (P=0.00). Furthermore, the seminal concentration of all assessed antioxidant enzymes significantly increased in both groups (P≤0.05).

Conclusion: The antioxidant properties of herbal composition ingredients and their ability to improve the level of testosterone hormone are the main factors responsible for lessening the percentage of sperm DNA fragmentation and sperm parameters. So, this herbal therapy can be regarded as a practical option in improving semen quality.

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Introduction

Male infertility is defined as the inability of a man to conceive a fertile woman [1]. It accounts for 30% to 50% of human infertility [2]. Male infertility impacts approximately 7% of males worldwide [3]. Today's world has a high rate of male infertility due to several reasons, including aging, exposure to chemicals, and heredity. Semen quality can reflect the male fertility status, whereas deficiencies in the properties of the semen, such as sperm concentration, motility, and appearance, lead to male infertility [4]. Infertility in men can occur due to a multitude of factors, although there is no definite etiology in nearly 40% of infertile men [5]. Hormonal deficiencies, physical causes, sexually transmitted disorders, environment and lifestyle, and hereditary factors are among the most common causes of male infertility [6-9].

Because of significant psychological issues and a challenging and depressing life for parents, infertility has drawn the attention of the pharmaceutical and medical professions. A wide range of products, from in vitro fertilization procedures to fertility hormones and other medicines, are available for this problem [10]. The pharmaceutical industry investigates active drugs mostly driven from the plants used in traditional medicine. It is an important field of pharmacy and medicine, and there is a growing global public demand for this treatment [11]. The hypothesis that natural aphrodisiacs have less or no adverse effects with systemic effects as a source of nutrition and their accessibility, availability, and affordability may account for the interest in herbal medicine despite the availability of synthetic medications [2]. Also, people who experienced failure in past infertility treatments are looking for alternative herbal medicine to help them overcome their condition [12].

In this regard, the World Health Organization (WHO) has recommended that researchers investigate the appropriate use of medicinal plants as a source of novel medicines and has encouraged using these plants [13]. Therefore, scientists are trying to elucidate the impact of medicinal plants on male fertility due to the increased interest in these plants. According to the currently available research, several herbs boost sperm motility and count, while a number of them change the hormones that the testicles secrete [14]. Certain plants have phenolic chemicals and flavonoids that are strong antioxidants against reactive oxygen species (ROS). They enhance sperm quality and fertility parameters while shielding the sperm from free radicals [15].

Some aspects of male infertility, such as sexual inability, libido (sexual desire), erectile dysfunction, ejaculatory issues, and sperm abnormalities, including azoospermia and oligospermia, have been treated with different plants [16]. Medicinal herbs' vagotonic, anti-inflammatory, anti-edematous, and antioxidant qualities can enhance sperm parameters. They might also increase serum testosterone (T) levels as well as essential factors for the formation of sperm [17].

In men, the formation of ROS is required for optimal sexual activity; nevertheless, an increase in ROS can cause tissue injury and cell death due to increased sperm DNA fragmentation (SDF), which damages sperm and male infertility [18]. In other words, sperm DNA integrity is crucial for fertilization and embryonic development. Accordingly, multiple studies have found that in infertile men with high SDF levels (>30%), the fertilization rate is close to zero. The increased SDF level can be significantly associated with higher recurrent miscarriage and a lower success rate of assisted reproductive technologies [19, 20].

There are very few published clinical trials addressing the use of medicinal herbs to treat male infertility; instead, the majority of research on this subject has been done on laboratory animals [21]. Therefore, WHO has noted a lack of scientific research on the characteristics of herbal medicines despite their widespread use. So, it is crucial to assess how physiologically active herbal compounds affect male fertility and find natural substances with estrogenic and anti-estrogenic qualities [22].

To improve abnormal sperm parameters and the hormonal levels (luteinizing hormone [LH], follicle-stimulating hormone [FSH], and T of patients with oligoasthenoteratozoospermia (OAT), this study evaluated the effects of compound herbal medicine (Zayesh Booalidaroo® + Safof-e Hefz + Royal Jelly [RJ]). The positive impact of RJ on sperm count, maturation, motility, and plasma testosterone levels has been well documented previously [23-26]. Bee queens are given a natural food called RJ throughout their lives. RJ possesses many pharmacological actions, including antioxidant, anti-inflammatory, anticancer, antibacterial, and hypotensive; RJ is classified as a functional food (with a known safety profile). Indeed, RJ has been used extensively to treat several medical problems, including diabetes, cancer, and cardiovascular disorders, to mention a few. In addition, RJ improves the general health and fertility of queen bees, who may lay up to 3000 eggs per day and live up to five years as opposed to infertile workers who

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only live up to 45 days; it is hypothesized that RJ is a powerful promoter of healthy aging and fertility [27].

Concerning the compositions of herbal medication (which will be subsequently discussed in detail), it is possible that combining these substances will have a synergistic and significant effect on improving male infertility. In this study, we tried to compare the effects of the mentioned compound herbal medicine and routine therapy (Sperigen supplement) on increasing male fertility in men with OAT.

Participants and Methods

Study design and participants

This research was conducted as a phase 3 blinded, randomized clinical trial. In 2020, 50 males with OAT were enrolled in this study out of all the infertile men who were referred to the highly specialized Rooya Infertility Treatment Center, Academic Center for Education, Culture and Research (ACECR), Qom Branch, Iran. Before taking part in the trial, each participant provided their informed consent. Infertile couples without a history of reported pregnancy, a normal female spouse, and a male partner classified as having OAT according to the WHO (WHO, 2010) guidelines were enrolled in the study [28]. The exclusion criteria were a history of receiving corticosteroids, anticoagulants, testosterone, anti-androgens, or chemotherapy medicines within the two months before the study's commencement, infections in the genitalia, anatomical irregularities, chromosomal abnormalities, genital surgical history (varicocele), drug or alcohol abuse, ejaculatory issues patients, and systemic diseases (thyroid, liver, gallbladder, and cancer). The urologist's results of the semen analysis were used to choose each participant. Then, their demographic information was collected and randomly allocated into two herbal and routine therapy groups. In addition, patients in herbal therapy filled out the Hulbert index of sexual desire (HISD) form, which assesses the sexual desire of both genders, and the international index of erectile function (IIEF) form, and measured erectile function in cross-cultural settings detecting treatment-related changes in patients.

Because the participants were men with OAT who had been sent to an infertility clinic for treatment, it was not ethical to consider a placebo group, which means denying them treatment.

Randomization

Patients were randomly allocated to the herbal and routine groups. Urologists and individuals who assessed the outcomes were blinded to the assigned treatment.

Intervention and assessment

In the herbal group, eligible patients orally received combined herbal medication, including Zayesh Booalidaroo® + Safoof-e Hefz + RJ (produced in Booali Daroo Company, Iran) for 3 months (Table 1). Patients in the routine group consumed Sperigen supplements (Arvand Pharmed, Iran) for 3 months. Both before and after the interventions, assessing seminal parameters, SDF, chromatin maturity, total antioxidant capacity (TAC), lipid peroxidation, and hormonal parameters (LH, FSH, T, prolactin, and estradiol) were done.

Introduction of drugs and supplements (chemicals)

Combination of herbal therapy

Zayesh Booalidaroo® mixture contains pistachios, hazelnuts, chilgoza pine, coconut, almond, sesame, Fraxinus, *Lepidium satirum*, alfalfa, *Saccharum officinarum*, ginger, *Piper cubeba*, *Piper longum*, *Centaurea Behen*, frankincense, alyssum, and honey.

Saffof-e Hefz capsule contains frankincense, *Pistacia lentiscus*, cinnamon, *Echium vulgare*, lemon balm, and *pistacia alkekengi*. It is used to cleanse and strengthen the brain and increase instinctual heat.

The RJ capsule contains 12.5% protein, 11% simple sugars (monosaccharides), 6% fatty acids, and 3.5% 10-hydroxy-2-decanoic acid (10-HDA). It lacks the fat-soluble vitamins A, D, E, and K but does include trace minerals, antibacterial and antibiotic components, vitamin B5, vitamin B6, and trace levels of vitamin C.

Combination of routine therapy (Sperigen)

Sperigen contains vitamins A (5000 IU), C (100 mg), E (150 IU), D3 (600 mg), B1 (12 mg), B2 (5 mg), B6 (10 mg), and B12 (0.075 mg), folic acid (0.4 mg), selenium (0.1 mg), zinc (15 mg), pantothenic acid (10 mg), niacin (20 mg), biotin (0.15 mg), magnesium (60 mg), manganese (2 mg), chromium (0.05 mg), and glutathione (2.5 mg), iron (6 mg), copper (1 mg), L-carnitine (200 mg), coenzyme Q10 (15 mg), N-acetyl-L-cysteine (50 mg), lycopene (4 mg), L-arginine (10 mg), inositol (40 mg), and Siberian ginseng root extract (30 mg).

Semen analysis

Semen samples were collected after 2–7 days of sexual abstinence in sterile vials and were analyzed immediately after liquefaction. According to the 2010 WHO guidelines, semen parameters (volume, pH, sperm count, motility, and normal morphology) were determined. The morphological characteristics were assessed by Papanicolaou staining, and samples with less than 4% normal sperm morphology and 15% sperm count were considered OAT according to WHO criteria.

Hormonal analysis

After centrifugation of collected peripheral blood sample for 5 min at 3000 rpm (Hettich, EBA20, UK), serum samples were stored at -70 ˚C for future analysis. Using ELISA assay, the serum levels of FSH (mIU/mL, Cat.N.DE1288), LH (mIU/mL, Cat.N.DE1289), prolactin (PRL; ng/mL, Cat.N.DE1291), and total testosterone (TT; ng/mL, Cat.N.DE1559) in all samples were measured (Demeditec Diagnostics GmbH, Germany).

Assessment of DNA fragmentation

A sperm chromatin dispersion (SCD) test (the Halosperm kit, INDAS laboratories, Spain) was used to measure the fragmentation of DNA. For each sample, 500 spermatozoa were graded. The visualization technique employed was Diff-Quik staining. Spermatozoa with a large- or medium-sized halo were classed as normal spermatozoa; spermatozoa with a small halo without a halo, or those that were degraded, were defined as exhibiting fragmentation. These two patterns of halo surrounding the sperm head were classified. The DFI% (fragmented spermatozoa/total spermatozoa counted×100) was used to determine the fragmentation rate. According to the guidelines provided by the manufacturer, sperm DFI values above 30% were considered abnormal.

Assessment of protamine deficient sperms

Sperm chromatin condensation was assessed with chromomycin A3 (CMA3) staining, determining protamine deficiency. Chromomycin is a type of fluorochrome that exhibits competitive binding to the DNA's guanine-cytosine dinucleotide region, which binds with protamine. Theoretically, spermatozoa with elevated protamine concentrations will exhibit some chromomycin fluorescence. A bright green spermatozoon was considered protamine as protamine deficiency (CMA3 positive), and spermatozoa with dull yellow staining had normal amounts of protamine (CMA3 negative).

Assessment of lipid peroxidation

The Abnova ELISA Kit (Cat.N.KA3736, Abnova Corporation, Taiwan) was utilized to detect seminal malondialdehyde (MDA), which is a direct indicator of lipid peroxidation with a detection range of 0.125–2 mM (125–2000 mmol/L).

Assessment of seminal TAC

A colorimetric kit (Zell Bio GmbH, Wurttemberg, Germany) was employed to measure seminal TAC. The assay was based on the production of brown color with maximum absorbance at 490 nm, caused by antioxidants reducing copper (II) to copper (I). We matched the sample absorbance to a standard curve of known uric acid.

Determination of seminal superoxide dismutase (SOD) activity

SOD activity in seminal plasma was evaluated using a commercial kit (NO.A001-1, Nanjing Jiancheng Bioengineering Institute, Nanjing, China) following the instructions of the manufacturer. SOD activity was measured in units per milliliter (U/mL) of seminal plasma. It was based on creating red formazan dye, which was generated by the interaction of 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride with the superoxide radical produced by xanthine and xanthine oxidase.

Statistical analysis

SPSS software, version 16 (SPSS, Chicago, IL, USA) was used to analyze the data. The normal distribution of data was assessed by the Kolmogorov-Smirnov test. One-way ANOVA was also used to compare the mean values between the groups. Tukey's multiple comparison post hoc test compared the groups' differences. $P<0.05$ were considered statistically significant. The results are displayed as Mean±SD.

Results

All participants were homogenous in age, while the mean age of the herbal and routine groups was 34.7±4.17 and 33.6±3.8, respectively, which showed no significant difference ($P\leq0.05$). The following parameters were assessed in both groups, which received combined herbal therapy (Zayesh Booalidaroo + Safoof-e Hefz + RJ) and routine therapy (Spiregen oral supplement).

Table 1. Instruction on consuming herbal treatment

Drug-supplement	Dose	Recommended Daily Intake
Zayesh Booalidaroo®	8 g	16 g/d preferably with an empty stomach with warm milk
Saffuf-e Hefz	600 mg	1200 g/d after breakfast and dinner with water
RJ	600 mg	1200 g/d after breakfast and dinner with water

The results of semen analysis before and after herbal and routine therapies

The assessed sperm parameters are presented in Table 2. Herbal therapy significantly improved sperm total motility, progressive motility (a+b) per mL, and total sperm count. In routine therapy, there were no significant differences in sperm parameters before and after the treatment.

The results of DNA fragmentation and protamine deficiency tests in herbal and routine therapy groups

The mean percentage of DNA fragmentation in herbal and routine therapy groups was significantly decreased. At the same time, in the case of protamine deficiency, no significant change was observed after the interventions (Table 3).

The results of hormonal profile evaluation before and after herbal and routine therapies

In both herbal and routine therapy groups, the mean value of T hormone significantly increased after the intervention. In contrast, in none of the other assessed hormones, there was a statistically significant difference between before and after the interventions (Table 4).

The results of assessing antioxidant factors before and after herbal and routine therapies

In both herbal and routine therapy groups, the mean level of TAC and SOD significantly increased after the interventions, while there was a significant decrease in the level of MDA (Table 5).

The results of the IIEF questionnaire in the herbal therapy group

The mean percentage of sexual desire (7.40±1.08 vs 7.96±0.9) and erectile function (25.00±4.8 vs 27.00±2.8) significantly improved after herbal intervention (P=0.03 and P=0.004, respectively). There were no significant differences in orgasmic function (8.76±1.80 vs 9.00±1.63), intercourse satisfaction (11.48±2.85 vs 11.76±2.06), and overall satisfaction (8.96±1.27 vs 9.08±1.03) (P=0.45, P=0.62, and P=0.62, respectively).

The results of the HISD questionnaire in the herbal therapy group

The mean percentage of the Hulbert index of sexual desire (68.76±11.7 vs 69.28±13.2; P=0.85) was not significantly improved after treatment with the herbal therapy.

Table 2. Comparing semen parameters before and after the interventions

Sperm Parameters	Herbal Therapy (n=25)		P	Routine Therapy (n=25)		P
	Before	After		Before	After	
Volume (mL)	2.52±0.8	2.96±0.93	0.75	2.76±0.92	2.84±0.89	0.75
Total motility (%)	16.9±2.3	19±2.8	0.043*	23±2.9	23.40±3.01	0.73
Progressive motility (a + b) (%)	4.8±0.9	7.2±1.5	0.02*	8.20±1.8	9.60±1.8	0.14
Total sperm concentration ($\times 10^6$)	20.7±2.2	34±20	0.0001*	31.6±3.7	31.8±4.1	0.96
Sperm concentration ($\times 10^6$/mL)	8.28±8.6	12.22±1.7	0.007*	11.7±1.08	11.8±1.6	0.94
Normal morphology (%)	0.9±0.1	1±0.1	0.3	1.28±0.89	1.20±0.70	0.53

*Significant difference.

Table 3. Comparing sperm chromatin heath before and after the interventions

Sperm Chromatin Heath	Herbal Therapy (n=25)		P	Routine Therapy (n=25)		P
	Before	After		Before	After	
DFI	26.28±7.7	24.6±6.9	0.000*	22.80±6.344	20.08±5.93	0.000*
Protamine deficiency	32.04±10.1	31.6±8.7	0.49	33.32±6.1	32.6±5.07	0.12

Abbreviations: DFI: Sperm DNA fragmentation index; DFI: Sperm DNA fragmentation index.
*Significant difference.

Discussion

The statistical analysis of the present study showed that combined herbal therapy leads to improving sperm parameters and decreasing sperm DNA fragmentation, probably through increased T hormone and antioxidant activity, respectively. Previously, several clinical trials improved sperm parameters using some plants such as palm pollen [29, 30], ginseng [31, 32], sesame [33], *Nigella sativa* [34], ashwagandha [35], or *Alpinia officinarum* [36]. Also, the effects of some herbal compositions, including TOPALAF [37] and Speman [38], on male fertility through their antioxidant constituents have been reported. Some research revealed enhanced sperm function in low-fertility males following the use of RJ due to a variety of its biological functions, including increasing seminal fructose, ejaculate volume, sperm production, and motility [26]. In another study investigating the effect of RJ on sperm parameters and serum testosterone of infertile men. Although the sperm count in the patients before and after treatment did not differ significantly, there was a significant improvement in sperm motility, normal morphology, and serum T hormone level [39]. In the case of sperm parameters, according to Table 2, our results revealed improved count (total and /mL) and motility (total and progressive) ($P \leq 0.05$), while the percentage of normal morphology did not differ significantly ($P > 0.05$). However, none of the mentioned sperm parameters has shown significant improvement in the routine therapy group ($P > 0.05$) (Table 2). The association between serum levels of LH, FSH, T, inhibin B, and free T_4 and sperm characteristics was demonstrated in a study carried out by Meeker et al. in 2007 [40]. They found that LH and FSH were negatively associated to sperm count, motility, and morphology, while testosterone was favorably correlated with sperm motility, and inhibin B and free T_4 were positively correlated with sperm count. In the present study, given that only the herbal group's serum T level significantly increased after the intervention, the improvement in motility parameters and sperm count can be attributed to this hormone's rise. In fact, testosterone's direct impact on the Sertoli cells, tubular fluid, growth factors, and transferrin protein-all of which are crucial for the nourishment and proliferation of sexual cells change the sperm production [41]. However, neither the herbal nor the routine groups showed any significant changes in the measurement of the other investigated hormones (LH, FSH, estradiol, and TSH) (Table 4).

It has been established that sperm parameters cannot accurately reflect its quality, whereas sperm chromatin integrity, which is not routinely examined during conventional semen analysis, is negatively correlated with male fertility in both in vitro and in vivo studies [42]. Excessive ROS production [43, 44] and decreased semi-

Table 4. Comparing hormonal profile before and after the interventions

Hormonal Analysis	Herbal Therapy (n=25)		P	Routine Therapy (n=25)		P
	Before	After		Before	After	
FSH (mU/mL)	6.26±2.94	6.22±2.67	0.86	5.77±3.22	5.25±2.49	0.22
LH (mU/mL)	2.67±0.94	2.74±1.14	0.72	3.67±1.53	3.68±1.51	0.6
Estradiol (mU/mL)	58.57±6.91	58.38±6.41	0.92	33.63±5.51	35.38±6.32	0.52
Prolactin (mU/mL)	33.32±6.17	32.04±10.12	0.65	9.58±4.74	8.85±3.98	0.18
Testosterone (mU/mL)	4.48±2.52	4.9±2.4	0.00*	5.07±1.47	5.07±1.46	0.2

*Significant difference.

Table 5. Comparing TAC

Antioxidant Enzymes	Herbal Therapy (n=25)		P	Routine Therapy (n=25)		P
	Before	After		Before	After	
TAC	0.15±0.04	0.2±0.04	0.005*	0.09±0.01	0.16±0.02	0.015*
SOD	2.5±1.22	2.7±1.007	0.013*	3.34±1.8	3.8±1.9	0.000*
MDA	0.8±0.01	0.8±0.01	0.000*	0.8±0.01	0.7±0.01	0.000*

Abbreviations: TAC: Total antioxidant capacity; SOD: Superoxide dismutase; MDA: Malondialdehyde.
*Significant difference.

nal antioxidants [45] are two major causes of sperm DNA fragmentation. On the other hand, seminal plasma generated by accessory glands comprises an enormous antioxidant system, including enzymatic and non-enzymatic components for protecting spermatozoa against ROS damage. Since spermatozoa are highly susceptible to oxidative damage, these cells rely on the surrounding environment's strong antioxidant features [45]. Our study showed that routine and herbal therapies have protective effects against oxidative stress, proven by a significant decrease in SDF percentage after interventions ($P \leq 0.05$). In line with this result, a significant increase in SOD activity and TAC, as well as decreased MDA concentration, were observed in both groups ($P \leq 0.05$). These findings are consistent with previous studies demonstrating that defects in the seminal oxidative stress scavenging system play a role in the pathophysiology of sperm DNA damage, which impairs spermatogenesis and spermiogenesis [46-48].

The characteristics of the components in the herbal compound raise testosterone levels, which in turn improve sperm parameters and chromatin quality. For example, it has been demonstrated that treating busulfan-induced infertile mice with *Pistacia atlantica*, a species of Pistachio, contains phenolic compounds, including tannins, flavonoids, and phenolic acids, reduces oxidative stress and improves chromatin quality, sperm parameters, and testicular histopathology [49]. Also, another study found that pistachios increase testosterone levels, which in turn can increase libido and improve sexual performance in male rats [41]. Another herbal complex component employed in this study is Fraxinus, which contains chemicals like flavonoids, steroids, esters, phenols, lactams, and terpenoids and has been shown to improve cisplatin-induced testicular toxicity in male albino rats [50]. In addition, a study on young and old male rats revealed that a hazelnut-supplemented diet improves semen quality, plasma T level, and testicular antioxidant function [51]. In another study on the effects of hazelnut supplemented diet on doxorubicin-induced testicular and epididymal tissue damage of male rats, improvement of epididymis sperm quality, testicular and epididymal tissue injury, T level, epididymis oxidative stress index, and lipid peroxidation was observed [52]. The anti-oxidative components of ginger have been investigated in numerous in vitro studies, animal models, and clinical trials, showing significant reduction in induced lipid peroxidation, increased antioxidant enzyme levels, and improved sperm quantity and quality [53-56]. Furthermore, in a study, the potential therapeutic impact of balm lemon on the chromatin structure and sperm parameters in varicocele-induced rats was examined. It was found that the detrimental effects of varicocele on sperm parameters and chromatin structure were lessened by balm lemon [57]. The antioxidant properties of frankincense and cinnamon, in association with their enhancing effects on sperm parameters (count and motility) and T hormone levels, are reported in previous studies [58, 59]. Moreover, most of the other Zayesh and Safof-e Hefz ingredients, either as an ingredient of an herbal combination or as a single substance, have been researched or found to have the ability to increase male fertility [33, 60-62]. Finally, based on the IIEF questionnaire, sexual desire and erectile function were improved after herbal intervention, although the Hulbert index of sexual desire did not change significantly.

All of the findings mentioned above and justifications suggest that herbal intervention is a more effective way to treat male infertility than standard medical care, and it may be considered a different approach to improving the quality of semen.

Conclusion

The antioxidant properties of herbal composition ingredients and their ability to improve the level of testosterone hormone are the main factors responsible for lessening the percentage of sperm DNA fragmentation and sperm parameters. So, this herbal therapy can be regarded as a practical option in improving semen quality.

Ethical Considerations

Compliance with ethical guidelines

The Ethics Committee for Research Involving Human Subjects at Islamic Azad University, Qom Branch approved this prospective clinical trial (Code: IR.IAU.QOM.REC.1399.021). Also the study was registered by Iranian Registry of Clinical Trials (IRCT) (Code: IRCT20200929048878N1).

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Authors contribution's

Conceptualization: Leila Naserpour and Hoda Fazaeli; Data acquisition: Seyed Abbas Seyed Ebrahimi, Seyedeh Saeideh Sahraei and Zahra Ebrahimi; Data analysis and interpretation: Naser Kalhor and Leila Naserpour; Drafting of the manuscript: Hoda Fazaeli, Leila Naserpour, and Mohamad Heydari; Review and editing: Hoda Fazaeli and Seyedeh Saeideh Sahraei.

Conflict of interest

The authors declared no conflict of interest.

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