Accepted: 5 April 2018
DOI: 10.1111/and.13041

ORIGINAL ARTICLE

WILEY ANDROLOGIA

Comparative evaluation of the effects of *Withania somnifera* with pentoxifylline on the sperm parameters in idiopathic male infertility: A triple-blind randomised clinical trial

R. Nasimi Doost Azgomi[1] | H. Nazemiyeh[2] | H. Sadeghi Bazargani[3] | S. M. B. Fazljou[1] | F. Nejatbakhsh[4] | A. Moini Jazani[1] | Y. Ahmadi AsrBadr[5] | A. Zomorrodi[6]

[1]Department of Iranian Traditional Medicine, School of Traditional Medicine, Tabriz University of Medical Sciences, Tabriz, Iran

[2]Research Center for Pharmaceutical Nanotechnology, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran

[3]Road Traffic Injury Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

[4]Department of Iranian Traditional Medicine, School of Traditional Medicine, Tehran University of Medical Sciences, Tehran, Iran

[5]Department of Urology, Sina Hospital, Tabriz University of Medical Science, Tabriz, Iran

[6]Department of Urology, Emam Reza Hospital, Tabriz University of Medical Sciences, Tabriz, Iran

Correspondence
Afshar Zomorrodi, Department of Urology, Emam Reza Hospital, Tabriz University of Medical Sciences, Tabriz, Iran.
Email: dr_zomorrodi@yahoo.com

Funding information
The present study was funded and supported by the Vice Chancellor for Research of Tabriz University of Medical Sciences. It was presented as a PhD thesis (Ramin Nasimi Doost Azgomi, No: 3) at the School of Traditional Medicine, Tabriz University of Medical Sciences.

Summary

In this triple-blind randomised clinical trial, we compared the effects of *Withania somnifera* and pentoxifylline on the sperm parameters in idiopathic male infertility. One hundred infertile male patients were randomly allocated into either *W. somnifera* or pentoxifylline groups. Patients in the herbal group received six capsules containing 5 g/daily of *W. somnifera* root, and subjects in the pentoxifylline group received six capsules containing 800 mg/daily of pentoxifylline and placebo for 90 days. Sperm parameters were analysed at the beginning and end of the study. *W. somnifera* increased mean sperm count (12.5%) and progressive motility (21.42%) and improved sperm morphology (25.56%) compared to the baseline (p = .04, p = .001 and p = .000 respectively). Moreover, pentoxifylline increased mean semen volume (16.46%), progressive motility (25.97%) and improved sperm morphology (13.28%) versus the baseline (p = .02, p = .003 and p = .01 respectively). Intergroup comparison showed no significant differences between the two groups regarding semen volume (p = .11), sperm count (p = .09), morphology (p = .12) and progressive motility (p = .77) after treatment. No major complication was reported in either of the two groups. *W. somnifera*, a traditional medicine remedy, improves sperms parameters in idiopathic male infertility without causing adverse effects. Therefore, this medication can be considered to be an alternative to pentoxifylline in this regard.

KEYWORDS
male infertility, pentoxifylline, sperm parameters, traditional medicine, *Withania somnifera*

1 | INTRODUCTION

Infertility is described as an individual's inability to conceive after 1 year or more of unprotected intercourse. It is a common disorder affecting 50–80 million partners worldwide (Cooper et al., 2010; Lindsay & Vitrikas, 2015). The male factor is responsible for 20%–50% of all cases of infertility (Sharlip et al., 2002). The pathogenesis of male factor infertility is undetermined in up to 25% of patients and remains idiopathic (Tahvilzadeh, Hajimahmoodi, Toliyat, Karimi, & Rahimi, 2016). However, sperm parameter abnormality can be found in 30%–40% of cases (Godmann, Lambrot, & Kimmins, 2009).

Due to the unknown and heterogeneous nature of male factor infertility, its treatment remains a major therapeutic dilemma and is difficult, costly and unachievable in a majority of the cases (Ahmad

et al., 2010; Gupta et al., 2013). Assisted reproductive techniques (ART) and medications such as hormone therapy and anti-oxidant compounds are two of the main therapeutic strategies developed for male factor infertility (Tahvilzadeh et al., 2016). However, ART is an invasive and costly procedure, and its success rate is low (Bioos et al., 2015). Thus, pharmacological treatment is being preferred by both patients and clinicians (Safarinejad, 2011).

Pentoxifylline is a phosphodiesterase inhibitor (PDEi) that is used in male infertility treatment. It has been shown that pentoxifylline improves sperm motility, fertilisation capacity and acrosome reaction and protects spermatozoa against oxidative stress (Kinutani, 1999; Nabi, Khalili, Fesahat, Talebi, & Ghasemi-Esmailabad, 2017). Nevertheless, its use has been reported to cause side effects including chest pain and gastrointestinal complaints, such as nausea, vomiting, bloating and dyspepsia (Ward & Clissold, 1987). On the other hand, according to a recent meta-analysis, the current medications for male factor infertility do not exert significant effects on sperm parameters, and their efficacy has not yet been proven (Ahmad et al., 2010; Speroff & Fritz, 2005). Therefore, there is an urgent need to find and implement alternative strategies for this purpose (Ahmad et al., 2010). Among these, complementary and alternative medicine (CAM), especially herbal medicine, because of its low rate of adverse effects, high efficacy and social acceptance, is receiving unprecedented attention (Ahmad et al., 2010; Nazari, Naseri, Mokri, Davati, & Kamalinejad, 2013). Sperm parameter abnormalities, anatomical abnormalities of the male reproductive system, decreased libido and erectile dysfunction have all been addressed in Persian Medicine (PM), due to their impacts on sexual function (Tahvilzadeh et al., 2016). PM researchers have proposed specific treatment strategies for these disorders. Among these strategies, the use of medicinal plants is of interest because of their positive effects on organs such as the heart, brain and testes and their favourable side effect profile (Nejatbakhsh, Shirbeigi, Rahimi, & Abolhassani, 2016).

Withania somnifera (WS), also referred to as ashwagandha, winter cherry, horse smell, Indian ginseng and Kaknaje Hindi, is a member of Solanaceae family. This medicinal herb has been used in Iranian and Indian traditional medicine as an aphrodisiac and a therapeutic agent for male factor infertility (Bakhtiar & Gruner, 1999; Gupta et al., 2013; Razi, 2005). The plant is found in dry and hot semiarid climate regions and is distributed in the southern Mediterranean, from northern Africa to India, the Canary Islands, Sri Lanka, Afghanistan, Jordan, Sudan, Palestine, Iran, Egypt and China. Different parts of this plant, especially its roots, have been used as medicinal remedies in various traditional medicines. Its main active ingredients are withanolides, such as withaferin-A, withanolide-D and withanone. In addition, WS contains sitoindosides, beta-sitosterol, iron and alanine, aspartate, fructose, lactate and glutamine, which affect fertility status. Evidence suggests that WS root possesses high amounts of withanolides, which have anti-oxidant, anti-apoptosis and anti-inflammatory properties and exert positive effects on the male reproductive system by improving semen quality, regulating sex hormones levels and inhibiting lipid peroxidation (Nasimi Doost Azgomi et al., 2018; Sengupta et al., 2018). Also, evidence shows that the regular use of WS root in infertile males improves sperm parameters, increases LH and testosterone and decreases FSH (Ambiye et al., 2013; Kaspate et al., 2015; Kumar et al., 2015). However, no randomised clinical trials have assessed its effects on the mentioned parameters or have compared them with the effects of standard medications for this purpose, such as pentoxifylline.

Therefore, this study aimed to compare the efficacies of WS and pentoxifylline on sperm parameters in idiopathic male infertility.

2 | MATERIALS AND METHODS

2.1 | Study design and setting

This was a triple-blind, randomised, two-arm clinical trial with a parallel design conducted between February 2015 and February 2016 at Alzahra Infertility Center, affiliated to Tabriz University of Medical Sciences (TUOMS), Iran to assess and compare the impacts of WS and pentoxifylline on sperm parameters in idiopathic male infertility.

2.2 | Population

The studied population consisted of married infertile male patients aged between 18 and 45 years. Infertility was defined as one's inability to conceive after 1 year or more of having unprotected sexual intercourse. Other criteria for inclusion were as follows: (i) an idiopathic abnormal spermogram defined as sperm count <20 million/ml Or <40 million/specimen, sperm percentage with normal morphology <30% or sperm percentage with normal motility <50% (World Health Organization criteria); (ii) nonsmoker and not addicted to opiates or alcohol; (iii) no urogenital infection or anatomical abnormality of this system, including varicocele; (iv) no prior history of surgery in the pelvic area; (v) no uncontrolled systemic illness, such as liver and gallbladder cancers, diabetes mellitus, renal failure, thyroid disorders, uncontrolled hypertension and cerebral haemorrhage; (vi) no prior history of trauma to the testes; (vii) no unilateral testicular atrophy; (viii) no previous history of chemotherapy or treatment with anticoagulants, corticosteroids, testosterone and anti-androgen medications during the 8 weeks before the study; and (ix) a healthy female factor.

Every patient who used less than 80% of the medications, experienced significant side effects, was a smoker/addict, or used any other medication was excluded from the study.

2.3 | Outcomes

The primary outcome of this study was the improvement of sperm parameters (i.e., sperm count, sperm motility, and sperm morphology, and semen volume) of infertile males after 90 days of treatment with the medications and their comparison between the study groups. The lack of major adverse effects in the studied groups was considered the secondary outcome of the study.

2.4 | Collection of sperm samples and analysis

After 72 hr of abstinence from intercourse/last dose of medication, semen samples were obtained from the subjects and collected in sterile plastic boxes at the beginning and after 90 days of study. These samples were stored at 37°C, and sperm analysis was performed by a professional according to the WHO guidelines (Organization, 2010) in <1 hr. Sperm parameters were sperm count, velocity (4 grades: A, B, C and D) and morphology, semen volume and the number of round cells.

2.5 | Collection of possible adverse effects of the medications

Potential adverse effects of the treatment were collected using a specific form designed for this purpose. In addition, the patients were examined by the specialist at periodic physical examinations, and side effects were measured on days 45 and 90. Patients were also free to call the specialist to report possible side effects.

2.6 | Methods

In this study, a urologist evaluated 167 patients; after a physical examination and necessary laboratory assessments, 100 patients were considered eligible for inclusion and were selected using convenience sampling. We applied fixed-size block randomisation to allocate the subjects into two equally sized treatment groups (n = 50 each). The patient randomisation sequence was generated using the Randlist version 11 software package.

The medications were placed in capsules that were consistent in shape, colour and size that were coded A or B. The capsules were prepared by Shafa Pajhohan Sabz Co. (East Azerbaijan region, Iran). Then, these capsules were placed into numbered opaque packets to ensure blinding. To avoid smell bias, the herbal medication and pentoxifylline capsules were stored in the same place. To reduce the risk of bias, the weight of the pentoxifylline capsules was equalised using lactose powder (the double-dummy technique). All researchers and subjects were blinded to the content of these capsules. The researchers and statisticians were also blinded to the capsule codes until data analysis. We explained the study methods to all the participants and obtained written informed consents.

2.7 | Protocol

In this study, 100 eligible subjects were randomly allocated to two groups (n = 50 each): (i) WS root group, and (ii) pentoxifylline group.

In the WS group, patients received a daily dose of six capsules designed in two different colours (containing 5 g of WS root) (Mahdi et al., 2011; Shukla et al., 2011) in three divided doses; in the pentoxifylline group, subjects received six capsules in two different colours (containing 800 mg of this drug and a placebo) (Mehni, Ketabchi, & Hosseini, 2014) three times a day over 90 days. Every meal of the patient must have included capsules from both colours.

Five cases from the pentoxifylline group and four cases from the WS group left the study for various reasons, such as work in other provinces; the study continued with 45 subjects in the pentoxifylline group and 46 subjects in the WS group. No other medication was used by the patients throughout the study.

2.8 | Treatments and plant samples

We purchased pentoxifylline granules from Farabi Pharmaceutical Company, Isfahan, Iran. We obtained WS roots from a local market in Tabriz, Iran, as well as the herbarium of the Faculty of Pharmacy of Tabriz University of Medical Sciences and confirmed their identity using specific examinations. We then washed, dried and powdered the roots and assessed their microbial content. We stored all medications at 2-5°C.

2.9 | Preparation of the extract

We performed the extraction and sample preparation according to the method proposed by Sangwan et al. (Chaurasiya et al., 2008) with some modifications. Briefly, we powdered approximately 4 g of WS root, which was then weighed and extracted with 20 ml of a water–methanol (3:1 v/v) mixture at room temperature (25°C). We placed the mixture in an ultrasound bath for 5 min to facilitate the extraction and repeated this process two more times. Then, we collected the filtrates and extracted them with petroleum ether (3 × 60 ml). We disposed of the petroleum ether fraction and extracted the methanol–water fraction with chloroform (3 × 60 ml). Afterwards, we dissolved the extract in methanol and passed it through a filter and adjusted its volume to 1 ml.

2.10 | HPLC profiling of the WS root extract

We conducted HPLC analysis using a Shimadzu-LC 8A system (Shimadzu Co., Japan). We injected 20 μl of extract (1.014 mg/ml in methanol) manually and eluted the sample through the column with a mobile phase linear gradient consisting of acetonitrile (B) and water (A) with a mobile phase program of 20:80–100:0 over 25 min; the flow rate was maintained at 1 ml/min. A diode array detector was used to record the UV spectra in the range of 190-400 nm at a wavelength of 217 nm (Figure 1a, b).

2.11 | Quantification of withaferin-A

The principle bioactive constituents of WS are withanolides. According to the published data, Withaferin-A is one of the main active withanolides in WS and has been used to standardise WS extracts in numerous studies (Patel, Rao, & Hingorani, 2016; Patel, Singh, & Patel, 2013; Vanden Berghe, Sabbe, Kaileh, Haegeman, & Heyninck, 2012). So we weighed an accurate quantity (1.10 mg) of withaferin-A (Fluka; BCBN9022 V; purity 99.0%) and dissolved it in methanol; the volume was then made up to 1 ml with methanol. The injection volume was 20 μl and, and the conditions for the analysis were the same as those outlined above (Figure 1a, b). We repeated

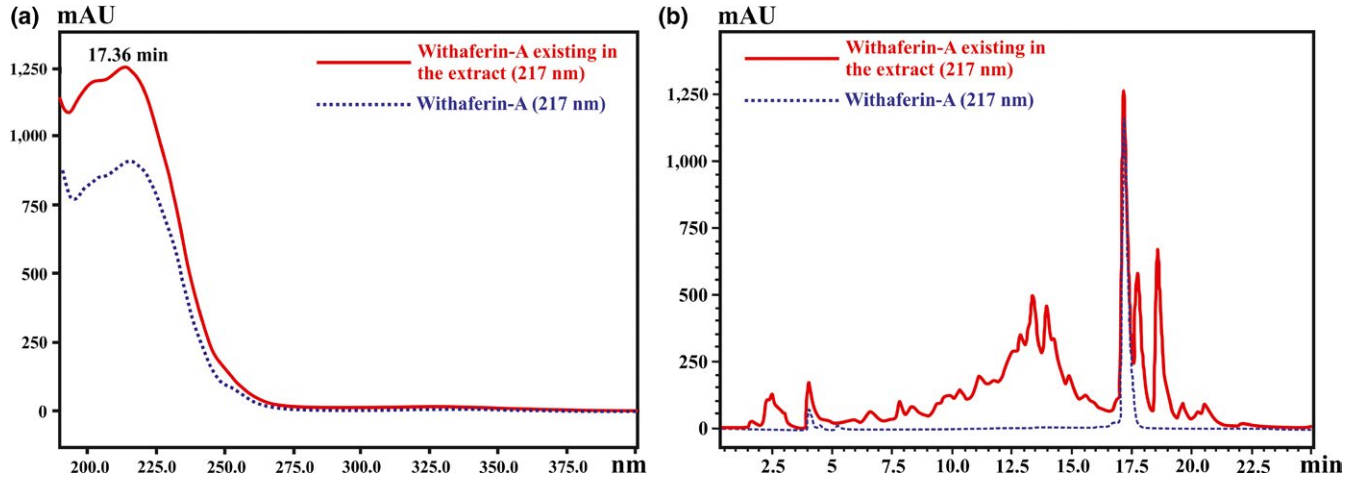

FIGURE 1 (a & b), UV On line and HPLC chromatogram of standard withaferin-A and withaferin-A existing in the extract

all experiments 3 times and used the mean AUC value to calculate the concentration.

2.12 | Ethics

This study was conducted in compliance with the requirements of the Declaration of Helsinki (General Assembly of the World Medical Association, 2014) and was approved by the Ethics Committee of Tabriz University of Medical Sciences, Iran (code: TBZMED.REC.1394.529). This clinical trial was also registered at Iranian Registry of Clinical Trials and received the registration number of IRCT2015092224135N1.

2.13 | Sample size calculation

The sample size for comparison of means was estimated using STATA statistical software package version 13. Absolute improvements of 8% ± 1.75% and 9% ± 1.5% in the motility parameter were used for herbal root and pentoxifylline respectively. Based on the following formula and with a power of 80% and a type one error of a maximum of 5%, 42 subjects were considered for each group. However, taking into account the dropouts, we decided to have 50 patients in each group.

$$n = \frac{2(Z1 - a/2 + Z1 - b)2.pq}{(p1 - p2)2}$$

2.14 | Statistical analyses

Intention-to-treat analysis was used as the basis for all statistical analyses in this study. Quantitative data are presented as means ± *SD*, while categorical data are presented as percentage and frequency. For comparison of quantitative data, independent *t*-test and Mann–Whitney *U* test were used. Also, for comparison of categorical data between the groups, chi-square and Fisher's exact tests were applied. To compare the mean values of semen parameters before and after the intervention, paired sample *t*-test was applied. The change in each semen parameter before and after intervention was compared for the WS and pentoxifylline groups using independent *t* test. The test assumptions such as normality for parametric tests were assessed using appropriate methods. In all comparisons, $p < .05$ was considered statistically significant. All statistical calculations were performed using STATA statistical software package version 13(StataCorp LP, College Station, TX, USA).

3 | RESULTS

3.1 | HPLC quantitation of withaferin-A

The obtained HPLC chromatogram of standard withaferin-A (Figure 1a, b) showed a retention time at 17.36 min under the experimental conditions. This analysis showed that the WS available on the market contains 4.02% ± 0.3% withaferin-A.

3.2 | General study characteristics

We evaluated a total of 167 infertile male patients, and 100 patients were considered eligible for this study. These patients were randomly assigned to the WS and pentoxifylline groups ($n = 50$ each). Five and four patients from the pentoxifylline and herbal groups refused to continue the study respectively. Thus, we resumed the study with 45 and 46 patients in each group respectively (Figure 2).

The mean age of the study population was, respectively, 32.48 ± 5.49 and 34.74 ± 5.59 years in the WS and pentoxifylline groups. The mean body mass indexes (BMIs) of the patients were 25.93 ± 3.85 and 26.18 ± 3.94 in the WS and pentoxifylline groups respectively. There was no significant difference in age or BMI between the groups. We found mean infertility periods of 4.54 ± 3.63 and 6.3 ± 4.98 years in the WS and pentoxifylline

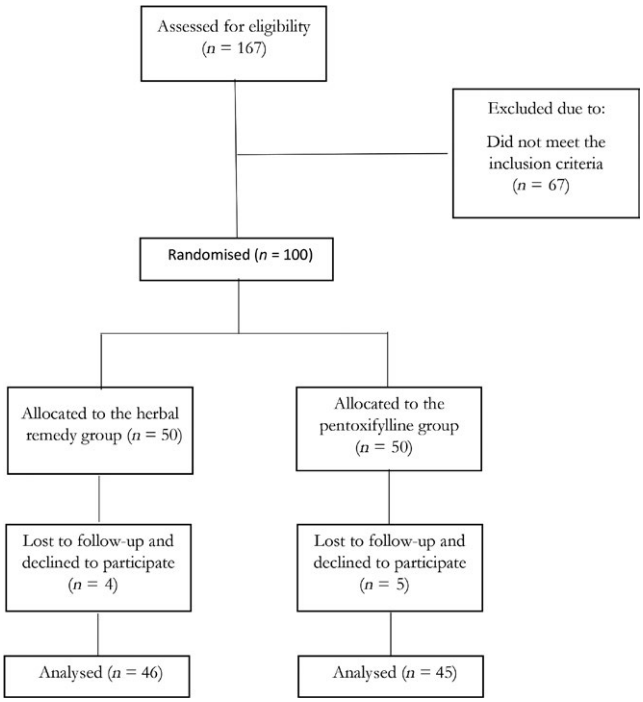

FIGURE 2 CONSORT statement flow diagram of the study

groups, respectively, and this difference was significant ($p = .046$). None of these variables were shown to affect the outcome of the study. Other demographic and paraclinical data of the patients are presented in Table 1.

3.3 | Primary study outcomes

Our findings showed no significant differences between the baseline sperm parameters of the patients in the two study groups (Table 2). We found that WS administration markedly increased mean sperm count (12.5%) and progressive motility (21.42%) and improved sperm morphology (25.56%) compared to the baseline values ($p = .04$, $p = .001$ and $p = .000$ respectively). However, WS had no significant effect on the mean semen volume or the number of round cells (Table 3).

We also showed that pentoxifylline treatment markedly increased the mean semen volume (16.46%) and progressive motility (25.97%) and improved sperm morphology (13.28%) in comparison with the baseline values ($p = .02$, $p = .003$ and $p = .01$ respectively). However, pentoxifylline had no significant impact on the mean sperm count (Table 3). Based on the intergroup comparisons, there was no significant differences between the groups regarding semen volume ($p = .11$), sperm count ($p = .09$), progressive motility ($p = .77$) or morphology ($p = .12$) after 90 days (Table 4). The sperm motility classes in the studied population are presented in Table 5.

Although conception rate was not a study outcome, we showed a higher pregnancy rate in the partners of the patients who received WS compared to those who received pentoxifylline (18% ($n = 9$) vs 12% ($n = 6$) respectively).

TABLE 1 Baseline demographic and paraclinical data of the included population

Variables	WS	PTX	*p* value
Age	32.48 (5.49)	34.74 (5.59)	.44
Infertility period	4.54 (3.63)	6.3 (4.98)	.046
Primary infertility (%)	84	76	NS
Age of marriage (year)	26.76 (4.47)	25.96 (4.00)	.34
First coitus (year)	28.04 (5.27)	27.9 (6.42)	.90
BMI (kg/m^2)	25.93 (3.85)	26.18 (3.94)	.75
Drug history	36 (72%)	38 (76%)	NS
TSH (μIU/ml)	2.25 (1.87)	2.22 (1.44)	.92
FSH (mIU/ml)	4.38 (2.89)	4.48 (2.88)	.86
LH (mIU/ml)	5.49 (2.25)	4.98 (2.37)	.27
WBC × 10^9 cells/L	6172 (1239.80)	6234.49 (1472.91)	.82
Hb (gr/dl)	15.65 (1.03)	15.54 (1.20)	.63
HCT (%)	45.18 (2.775)	44.65 (3.23)	.39
FBS (mg/dl)	88.97 (7.12)	88.8 (9.81)	.92
AST (IU/L)	24.18 (11.18)	24.24 (9.84)	.97
ALT (IU/L)	26.64 (9.71)	30.87 (19.32)	.20
ALP (IU/L)	184.70 (44.32)	189.34 (53.19)	.65
TES (ng/ml)	5.13 (2.27)	4.69 (1.68)	.27
PRL (mIU/ml)	194.36 (134)	183.65 (136.1)	.69

ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; FBS, fasting blood sugar; FSH, follicular stimulating factor; Hb, haemoglobin; HCT, haematocrit; LH, luteinising hormone; NS, nonsignificant; PRL, prolactin; PTX, pentoxifylline; TES, testosterone; TSH, thyroid stimulating hormone; WBC, white blood cell; WS, *Withania somnifera*.

TABLE 2 Sperm parameters before intervention in the studied population. Intergroup comparison showed no significant difference in the baseline sperm parameters between two groups

Variables	WS	PTX	*p* value
Semen volume (ml)	3.45 (1.54)	3.28 (1.38)	.56
Sperm count (×10^6)	55.82 (28.45)	56.68 (28.92)	.88
Motility progressive (A+B) (%)	18.9 (9.32)	17.71 (8.63)	.51
Total motility sperm count (×10^6)	32.36 (22.44)	31.98 (21.70)	.93
Normal morphology (%)	15.1 (6.18)	14.68 (6.34)	.73
Round cells (10^6/ml)	5.66 (4.89)	6.94 (4.57)	.18

A, Motility class A; B, Motility class B; PTX, pentoxifylline; WS, *Withania somnifera*.

3.4 | Secondary study outcome

The patients and clinicians reported no major adverse effects or withdrawal signs either in the WS or pentoxifylline groups. However,

TABLE 3 Intragroup comparison of sperm parameters before and after intervention in the studied groups

Variable	WS before intervention	WS after intervention	*p* value	PTX before intervention	PTX after intervention	*p* value
Semen volume (ml)	3.45 (1.54)	3.48 (1.71)	.84	3.28 (1.38)	3.82 (1.75)	.02
Sperm count ($\times 10^6$)	55.82 (28.45)	62.65 (33.66)	.04	56.68 (28.92)	55.68 (27.52)	.75
Progressive motility (A+B) (%)	18.9 (9.32)	22.95 (11.05)	.001	17.71 (8.63)	22.31 (11.58)	.003
Total motility sperm count ($\times 10^6$)	32.36 (22.44)	41.60 (26.89)	.003	31.98 (21.70)	35.47 (23.44)	.21
Normal morphology (%)	15.1 (6.18)	18.96 (6.67)	.000	14.68 (6.34)	16.63 (6.28)	.01
Round cells (10^6/ml)	5.66 (4.89)	5.08 (4.50)	.51	6.94 (4.57)	4.86 (5.12)	.01

A, Motility class A; B, Motility class B; PTX, pentoxifylline; WS, *Withania somnifera*.

TABLE 4 Sperm parameters change before and after intervention in the studied groups

Variables	Parameters change before and after intervention		*p* value
	WS	PTX	
Volume (ml)	0.03	0.54	.11
Sperm count ($\times 10^6$)	6.83	−0.99	.09
Progressive motility (A + B) (%)	4.05	4.59	.77
Total motility sperm count ($\times 10^6$)	9.23	3.49	.16
Normal morphology (%)	3.86	1.95	.12
Round cells (10^6/ml)	−0.58	−2.08	.2

A, Motility class A; B, Motility class B; PTX, pentoxifylline; WS, *Withania somnifera*.

three (6.66%) patients experienced nausea and epigastric pain with pentoxifylline use. These symptoms occurred only in one (2.17%) patient in the WS group.

4 | DISCUSSION

Male factor infertility has become a significant healthcare problem, and studies show that its prevalence is increasing (Ayaz, Kothandaraman, Henkel, & Sikka, 2018). Both genetic and environmental factors contribute to this issue; however, in 40%–50% of cases, the causative agent remains unclear (Ambiye et al., 2013; Skakkebaek et al., 2016). Evidence suggests that the quality and quantity of spermatogenesis are among the most critical factors in the pathogenesis of male infertility (Ambiye et al., 2013). Increased sperm motility and count, besides improved sperm morphology, have been shown to bear a good prognosis in this regard (Hassanzadeh, Emdady, & Maden, 2010).

To the best of our knowledge, this is the first study comparing the effects of WS, a herbal remedy and pentoxifylline on sperm parameters in idiopathic male infertility. Nonetheless, the effects of these medications alone on sperm parameters have been previously investigated in infertile males.

In the present study, WS root administration for 90 days improved sperm parameters in infertile men. The descriptive results showed greater improvements in sperm count, total motile sperm

TABLE 5 Change in the different classes of sperm motility before and after intervention in the studied groups

Classes of sperm motility	WS before intervention	WS after intervention	*p* value	PTX before intervention	PTX after intervention	*p* value	Change before and after intervention		*p* value
							WS	PTX	
Motility class A (%)	1.7	2.1	.77	1.32	1.8	.95	0.4	0.48	.93
Motility class B (%)	17.3 (8.09)	20.70 (8.80)	.003	16.29 (7.34)	19.61 (9.41)	.01	3.40	3.32	.95
Motility class C (%)	34.4 (12.52)	38.08 (10.38)	.09	35 (12.20)	34.87 (10.03)	.94	3.68	−0.12	.16
Progressive motility (A + B) (%)	18.9 (9.32)	22.95 (11.05)	.001	17.71 (8.63)	22.31 (11.58)	.003	4.05	4.59	.77
Immotile (%)	46.7 (18.22)	38.97 (17.62)	.007	47.28 (17.85)	43.65 (18.68)	.13	−7.72	−3.62	.26

PTX, pentoxifylline; WS, *Withania somnifera*.

count and number of spermatozoa with normal morphology with the regular use of WS roots, compared to pentoxifylline. However, these differences were not significant. Consistent with these findings, various studies have shown that various parts of the WS plant, especially its root, can improve sperm count and motility in infertile males. The exact mechanisms underlying these effects are unclear; however, several studies have shown that anti-oxidative properties, sex hormone regulation, improvements in detoxification and GABA-mimetic features of WS phytochemical components may exert the greatest effects on the male reproductive system.

In a clinical trial by Ambie et al., it was shown that WS administration in oligospermic infertile males increases semen volume by 53%, sperm count by 167% and sperm motility by 57% (Ambiye et al., 2013). WS also has protective impacts on Leydig cells and spermatogenesis, increases gonad weight and regulates sexual hormone levels (Abdel-Magied, Abdel-Rahman, & Harraz, 2001; Al-Qarawi et al., 2000; Kaspate et al., 2015; Kiasalari, Khalili, & Aghaei, 2009; Kumar et al., 2015; Patil, Vora, & Pillai, 2012;. Indeed, it has been shown that WS compensates for decreased or increases in LH and FSH levels in the diabetic rat (Nasimi Doost Azgomi et al., 2018). Accordingly, WS has been widely used for the treatment of impotence and to increase libido and fertility (Ambiye et al., 2013; Kumar et al., 2015).

WS treatment has been shown to increase the levels of, zinc, copper, gold and iron ions in seminal plasma. These ions are essential cofactors for enzymes in the seminal fluid and thus increase semen quality (Shukla et al., 2011).

A previous study found that oxidative stress and increased reactive oxygen species production can disrupt the hypothalamus–hypophysis–gonad axis and decrease spermatogenesis (Kumar et al., 2015). Extensive evidence shows that anti-oxidant, anti-inflammatory and anti-apoptotic properties of WS may be responsible for these effects (Ambiye et al., 2013). WS contains essential cofactors for anti-oxidant enzymes and nutrients that reduce oxidative stress and subsequent apoptosis and sperm death. WS root also enhances alanine transaminase activity, which leads to an increase in the semen alanine content. Alanine then protects spermatozoa against oxidative stress-induced damage and increases sperm count and motility (Gupta et al., 2013; Shukla et al., 2011). Steroidal lactones and withanolide contents of WS including sitoindosides VII-X and withaferin-A appear to be responsible for the therapeutic effects of this medicinal herb (Nasimi Doost Azgomi et al., 2018).

Further, WS root possesses gamma-aminobutyric acid (GABA)-mimetic effects and stimulates gonadotropin-releasing hormone-secreting (GnRH)-producing neurons, increases Leydig cell testosterone production and improves spermatogenesis (Bhattarai, Ah Park, & Han, 2010).

However, we also found that the chronic and regular use of pentoxifylline improves sperm parameters in infertile male patients. Similarly, Cetintas et al. showed that pentoxifylline improved sperm parameters in asthenozoospermic and oligo-asthenozoospermic males (Çetintaş et al., 2007). Pentoxifylline is rapidly and extensively absorbed through the alimentary tract upon oral administration. It reaches its peak plasma concentration 0.29 and 0.41 hr after administration (Smith et al., 1986). Further studies have revealed that pentoxifylline inhibits cAMP PDE activity and increases ATP levels inside cells. This may improve sperm motility, the acrosome reaction and sperm penetration to the zona pellucida of the oocyte (Çetintaş et al., 2007; Kinutani, 1999). Pentoxifylline also improves epididymis microcirculation and thus sperm motility (Moein, Khalili, & Davoudi, 2005). It has been found that pentoxifylline possesses anti-oxidant effects and that through this mechanism, it exerts positive effects on sperm parameters (Shen, Chiang, Yang, Hong, & Chen, 1991).

In this study, the two treatments did not appear to be significantly different regarding the improvement of sperm parameters in infertile males. However, considering the high social acceptance of herbal remedies, its low cost in our region and the positive effects of WS on the studied sperm parameters make WS useful in medical decisions; thus, WS represents a potential alternative for the treatment of idiopathic male infertility.

Our study had several limitations. First, we could not follow up with the patients for longer periods. Second, in this study, we used capsules to deliver WS to the patients; this method is essentially different from the way in which traditional Persian practitioners administer medication to their patients. This difference might also have affected the outcome of this study.

In conclusion, our results showed that both WS and pentoxifylline meaningfully improve sperm parameters in idiopathic male infertility. However, the impacts of the two medications were not significantly different. Due to high social acceptance of WS, this medication may be a low-cost alternative for the treatment of idiopathic male infertility. Future larger-scale studies with a longer follow-up could be beneficial for elucidating further details of the efficacy and effectiveness of *W. somnifera* in idiopathic male infertility.

ACKNOWLEDGEMENTS

We are grateful to all our patients for their patience and their contribution to this study.

CONFLICT INTERESTS

The authors declare that they have no conflict of interests.

ORCID

R. Nasimi Doost Azgomi http://orcid.org/0000-0002-3836-3240

REFERENCES

Abdel-Magied, E., Abdel-Rahman, H., & Harraz, F. (2001). The effect of aqueous extracts of *Cynomorium coccineum* and *Withania somnifera* on testicular development in immature Wistar rats. *Journal of Ethnopharmacology*, *75*(1), 1–4. https://doi.org/10.1016/S0378-8741(00)00348-2

Ahmad, M. K., Mahdi, A. A., Shukla, K. K., Islam, N., Rajender, S., Madhukar, D., … Ahmad, S., (2010). *Withania somnifera* improves semen quality by regulating reproductive hormone levels and oxidative stress in seminal plasma of infertile males. *Fertility and Sterility*, *94*(3), 989–996. https://doi.org/10.1016/j.fertnstert.2009.04.046

Al-Qarawi, A., Abdel-Rahman, H., El-Badry, A., Harraz, F., Razig, N., & Abdel-Magied, E. (2000). The effect of Extracts of *Cynomorium coccineum* and *Withania somnifera* on Gonadotrophins and ovarian follicles of immature Wistar rats. *Phytotherapy Research*, *14*, 288–290. https://doi.org/10.1002/(ISSN)1099-1573

Ambiye, V. R., Langade, D., Dongre, S., Aptikar, P., Kulkarni, M., & Dongre, A. (2013). Clinical evaluation of the spermatogenic activity of the root extract of Ashwagandha (*Withania somnifera*) in oligospermic males: A pilot study. *Evidence-Based Complementary and Alternative Medicine: eCAM*, *2013*, 1–6.

Ayaz, A., Kothandaraman, N., Henkel, R., & Sikka, S. C. (2018). Impact of environmental factors on the genomics and proteomics landscapes of male infertility. In S. C. Sikka, W. J. G. Hellstrom (Eds.), *Bioenvironmental issues affecting men's reproductive and sexual health*(pp. 335–353). Elsevier. https://doi.org/10.1016/B978-0-12-801299-4.00021-9

Bakhtiar, L., & Gruner, O. C. (1999). *The canon of medicine (al-Qānūn Fī'l-ṭibb) (Vol. 1)*. India: Abjad Book Designers & Builders.

Bhattarai, J. P., Ah Park, S., & Han, S. K. (2010). The methanolic extract of *Withania somnifera* ACTS on GABAA receptors in gonadotropin releasing hormone (GnRH) neurons in mice. *Phytotherapy Research*, *24*(8), 1147–1150.

Bioos, S., Nazem, E., Keshavarz, M., Siahpoosh, M., Sohrabvand, F., Sohanaki, H., & Nejatbakhsh, F. (2015). A traditional Iranian medicine (Majoon-e Loboob) for idiopathic male infertility: A case series. *Traditional and Integrative Medicine*, *1*(1), 47–51.

Çetintaş, S., Esinler, İ., Durak, T., Öktem, M., Kuşçu, E., & Zeyneloğlu, H. (2007). The affect of Pentoxifylline on sperm parameters in Normozoospermic, Asthenozoospermic and Oligo-Asthenozoospermic Males. *Gynecology Obstetrics & Reproductive Medicine*, *13*(1), 38-42.

Chaurasiya, N. D., Uniyal, G. C., Lal, P., Misra, L., Sangwan, N. S., Tuli, R., & Sangwan, R. S. (2008). Analysis of withanolides in root and leaf of *Withania somnifera* by HPLC with photodiode array and evaporative light scattering detection. *Phytochemical Analysis*, *19*(2), 148–154. https://doi.org/10.1002/(ISSN)1099-1565

Cooper, T. G., Noonan, E., Von Eckardstein, S., Auger, J., Baker, H., Behre, H. M., … Mbizvo, M. T., (2010). World Health Organization reference values for human semen characteristics. *Human Reproduction Update*, *16*(3), 231–245. https://doi.org/10.1093/humupd/dmp048

General Assembly of the World Medical Association. (2014). World Medical Association Declaration of Helsinki: Ethical principles for medical research involving human subjects. *Journal of the American College of Dentists*, *81*(3), 14.

Godmann, M., Lambrot, R., & Kimmins, S. (2009). The dynamic epigenetic program in male germ cells: Its role in spermatogenesis, testis cancer, and its response to the environment. *Microscopy Research and Technique*, *72*(8), 603–619. https://doi.org/10.1002/jemt.20715

Gupta, A., Mahdi, A. A., Shukla, K. K., Ahmad, M. K., Bansal, N., Sankhwar, P., & Sankhwar, S. N. (2013). Efficacy of *Withania somnifera* on seminal plasma metabolites of infertile males: A proton NMR study at 800 MHz. *Journal of Ethnopharmacology*, *149*(1), 208–214. https://doi.org/10.1016/j.jep.2013.06.024

Hassanzadeh, K., Emdady, V., & Maden, S. (2010). Effect of oral Pentoxifylline on semen parameters in Oligoastheno. *Journal of Ardabil University Of Medical Sciences*, *10*(3), 217–222.

Kaspate, D., Ziyaurrahman, A., Saldanha, T., More, P., Toraskar, S., Darak, K., … Narkhede, S., (2015). To study an aphrodisiac activity of hydroalcoholic extract of *Withania somnifera* dried roots in female Wistar rats. *International Journal of Pharmaceutical Sciences and Research*, *6*(7), 2820.

Kiasalari, Z., Khalili, M., & Aghaei, M. (2009). Effect of *Withania somnifera* on levels of sex hormones in the diabetic male rats. *International Journal of Reproductive Biomedicine (Yazd)*, *7*(4), 163–168.

Kinutani, M. (1999). Effects of pentoxifylline on sperm motion characteristics in normozoospermic men defined by a computer-aided sperm analysis. *Hiroshima Journal of Medical Sciences*, *48*(4), 117–122.

Kumar, A., Kumar, R., Rahman, M. S., Iqubal, M. A., Anand, G., Niraj, P. K., & Ali, M. (2015). Phytoremedial effect of *Withania somnifera* against arsenic-induced testicular toxicity in Charles Foster rats. *Avicenna Journal of Phytomedicine*, *5*(4), 355.

Lindsay, T. J., & Vitrikas, K. R. (2015). Evaluation and treatment of infertility. *American Family Physician*, *91*(5), 308-314.

Mahdi, A. A., Shukla, K. K., Ahmad, M. K., Rajender, S., Shankhwar, S. N., Singh, V., & Dalela, D. (2011). *Withania somnifera* improves semen quality in stress-related male fertility. *Evidence-Based Complementary and Alternative Medicine*, *2011*, 66.

Mehni, N. M., Ketabchi, A. A., & Hosseini, E. (2014). Combination effect of Pentoxifylline and L-carnitine on idiopathic oligoasthenoteratozoospermia. *Iranian Journal of Reproductive Medicine*, *12*(12), 817.

Moein, M. R., Khalili, M. A., & Davoudi, A. (2005). The effect of oral administration of Pentoxifylline on sperm motility of asthenozoospermic ejaculates from men with or without testicular varicoceles. *International Journal of Reproductive BioMedicine*, *3*(1), 25–29.

Nabi, A., Khalili, M. A., Fesahat, F., Talebi, A., & Ghasemi-Esmailabad, S. (2017). Pentoxifylline increase sperm motility in devitrified spermatozoa from asthenozoospermic patient without damage chromatin and DNA integrity. *Cryobiology*, *76*, 59–64. https://doi.org/10.1016/j.cryobiol.2017.04.008

Nasimi Doost Azgomi, R., Zomorrodi, A., Nazemyieh, H., Fazljou, S. M. B., Sadeghi Bazargani, H., Nejatbakhsh, F, … Ahmadi AsrBadr, Y. (2018). Effects of *Withania somnifera* on reproductive system: A systematic review of the available evidence. *BioMed Research International*, *2018*, 1–17.

Nazari, S. M., Naseri, M., Mokri, A., Davati, A., & Kamalinejad, M. (2013). Hab-o Shefa (an Iranian traditional medicine compound) in withdrawal syndrome and its effects in acute detoxification of opiates addict: A randomized, double blind, clinical trials. *Journal of Medicinal Plants Research*, *7*(22), 1628–1635.

Nejatbakhsh, F., Shirbeigi, L., Rahimi, R., & Abolhassani, H. (2016). Review of local herbal compounds found in the Iranian traditional medicine known to optimise male fertility. *Andrologia*, *48*(8), 850–859. https://doi.org/10.1111/and.12675

Organization, W. H. (2010). *WHO laboratory manual for the Examination and processing of human semen*. Switzerland: WHO press.

Patel, S. B., Rao, N. J., & Hingorani, L. L. (2016). Safety assessment of *Withania somnifera* extract standardized for Withaferin A: Acute and sub-acute toxicity study. *Journal of Ayurveda and Integrative Medicine*, *7*(1), 30–37. https://doi.org/10.1016/j.jaim.2015.08.001

Patel, K., Singh, R. B., & Patel, D. K. (2013). Pharmacological and analytical aspects of Withaferin A: A concise report of current scientific literature. *Asian Pacific Journal of Reproduction*, *2*(3), 238–243. https://doi.org/10.1016/S2305-0500(13)60154-2

Patil, R. B., Vora, S. R., & Pillai, M. M. (2012). Protective effect of spermatogenic activity of *Withania somnifera* (Ashwagandha) in galactose stressed mice. *Annals of Biological Research*, *3*(8), 4159–4165.

Razi, M. (2005). *Al-havi* (pp. 227). Tehran, Iran: Academy of Medical Sciences Islamic Republic of Iran.

Safarinejad, M. R. (2011). Effect of pentoxifylline on semen parameters, reproductive hormones, and seminal plasma antioxidant capacity in men with idiopathic infertility: A randomized double-blind placebo-controlled study. *International Urology and Nephrology*, *43*(2), 315–328. https://doi.org/10.1007/s11255-010-9826-4

Sengupta, P., Agarwal, A., Pogrebetskaya, M., Roychoudhury, S., Durairajanayagam, D., & Henkel, R. (2018). Role of *Withania somnifera* (Ashwagandha) in the management of male infertility. *Reproductive Biomedicine Online*, *36*(3), 311–326. https://doi.org/10.1016/j.rbmo.2017.11.007

Sharlip, I. D., Jarow, J. P., Belker, A. M., Lipshultz, L. I., Sigman, M., Thomas, A. J., … Damewood, M. D., (2002). Best practice policies for male infertility. *Fertility and Sterility*, *77*(5), 873–882. https://doi.org/10.1016/S0015-0282(02)03105-9

Shen, M., Chiang, P.-H., Yang, R., Hong, C., & Chen, S.-S. (1991). Pentoxifylline stimulates human sperm motility both in vitro and after oral therapy. *British Journal of Clinical Pharmacology*, *31*(6), 711–714. https://doi.org/10.1111/j.1365-2125.1991.tb05600.x

Shukla, K. K., Mahdi, A. A., Mishra, V., Rajender, S., Sankhwar, S. N., Patel, D., & Das, M. (2011). *Withania somnifera* improves semen quality by combating oxidative stress and cell death and improving essential metal concentrations. *Reproductive Biomedicine Online*, *22*(5), 421–427. https://doi.org/10.1016/j.rbmo.2011.01.010

Skakkebaek, N. E., Rajpert-De Meyts, E., Louis, G. M. B., Toppari, J., Andersson, A.-M., Eisenberg, M. L., … Sapra, K. J. (2016). Male reproductive disorders and fertility trends: Influences of environment and genetic susceptibility. *Physiological Reviews*, *96*(1), 55–97. https://doi.org/10.1152/physrev.00017.2015

Smith, R. V., Waller, E. S., Doluisio, J. T., Bauza, M. T., Puri, S. K., Ho, I., & Lassman, H. B. (1986). Pharmacokinetics of orally administered pentoxifylline in humans. *Journal of Pharmaceutical Sciences*, *75*(1), 47–52. https://doi.org/10.1002/jps.2600750111

Speroff, L., & Fritz, M. A. (2005). *Clinical gynecologic endocrinology and infertility*. Philadelphia, PA: lippincott Williams & wilkins.

Tahvilzadeh, M., Hajimahmoodi, M., Toliyat, T., Karimi, M., & Rahimi, R. (2016). An evidence-based approach to medicinal plants for the treatment of sperm abnormalities in traditional Persian medicine. *Andrologia*, *48*(8), 860–879. https://doi.org/10.1111/and.12676

Vanden Berghe, W., Sabbe, L., Kaileh, M., Haegeman, G., & Heyninck, K. (2012). Molecular insight in the multifunctional activities of Withaferin A. *Biochemical Pharmacology*, *84*(10), 1282–1291. https://doi.org/10.1016/j.bcp.2012.08.027

Ward, A., & Clissold, S. P. (1987). Pentoxifylline. *Drugs*, *34*(1), 50–97. https://doi.org/10.2165/00003495-198734010-00003

How to cite this article: Nasimi Doost Azgomi R, Nazemiyeh H, Sadeghi Bazargani H, et al. Comparative evaluation of the effects of *Withania somnifera* with pentoxifylline on the sperm parameters in idiopathic male infertility: A triple-blind randomised clinical trial. *Andrologia*. 2018;50:e13041. https://doi.org/10.1111/and.13041