Withania somnifera improves semen quality by regulating reproductive hormone levels and oxidative stress in seminal plasma of infertile males

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Objective: To investigate the impact of *Withania somnifera* roots on semen profile, oxidative biomarkers, and reproductive hormone levels of infertile men.
Design: Prospective study.
Setting: Departments of Biochemistry and Urology, Chhatrapati Shahuji Maharaj Medical University, Lucknow, India.
Patient(s): Seventy-five normal healthy fertile men (control subjects) and 75 men undergoing infertility screening.
Intervention(s): High-performance liquid chromatography assay procedure for quantization of vitamin A and E in seminal plasma. Biochemical parameters in seminal plasma were estimated by standard spectrophotometric procedures. Estimation of T, LH, FSH, and PRL in blood serum by RIA methods.
Main Outcome Measures(s): Before and after the treatment, seminal plasma biochemical parameters, antioxidant vitamins, and serum T, LH, FSH, and PRL levels were measured.
Result(s): *Withania somnifera* inhibited lipid peroxidation and protein carbonyl content and improved sperm count and motility. Treatment of infertile men recovered the seminal plasma levels of antioxidant enzymes and vitamins A, C, and E and corrected fructose. Moreover, treatment also significantly increased serum T and LH and reduced the levels of FSH and PRL.
Conclusion(s): The treatment with *W. somnifera* effectively reduced oxidative stress, as assessed by decreased levels of various oxidants and improved level of diverse antioxidants. Moreover, the levels of T, LH, FSH and PRL, good indicators of semen quality, were also reversed in infertile subjects after treatment with the herbal preparation. (Fertil Steril® 2010;94:989–96. ©2010 by American Society for Reproductive Medicine.)

Key Words: *Withania somnifera,* male infertility, alternative therapy, reproductive hormone, oxidative stress, antioxidant

Infertility may be defined as failure to conceive by a couple after 12 months of unprotected sexual intercourse (1). Infertility affects 15% of all couples, and approximately 50% of these have an abnormality detectable in the male partner as the cause of infertility. Specific and directed treatment for male infertility is not available owing to the unexplained and heterogeneous nature of the disorders (2). Under such circumstances, only assisted reproductive technologies are of some help. However, these treatments are expensive and inaccessible to all. The lack of available specific therapies for men with infertility demands the exploration of alternative therapies. Given the lack of knowledge about etiologic factors, a nondirected but general therapy may yield good results in a subcategory of patients. The rationale for the use of these therapies is based on the speculation that some forms of male infertility are caused by oxidative insult and hormonal imbalance, and the use of alternative therapies may improve male fertility potential and semen quality (3). The latter is also supported by our previous studies (4–6).

Aerobic metabolism of human sperm produces different reactive oxygen species (ROS), which are essential for sperm capacitation, acrosome reaction, and oocyte fusion (7). To counteract the toxic effects of ROS, seminal plasma and spermatozoa are well endowed with an array of antioxidant mechanisms. The antioxidant enzymes catalase, superoxide dismutase (SOD), glutathione peroxidase, and glutathione reductase have all been detected in seminal plasma (8). In addition, semen contains high concentration of thiol groups, ascorbic acid and uric acid, as well as less substantial

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amounts of glutathione and α-tocopherol (9). Spermatozoa themselves also possess high concentration of thiol groups, as well as smaller amounts of ascorbic acid, α-tocopherol, uric acid, and glutathione (10). However, uncontrolled and excessive production of ROS may result in seminal oxidative stress (11). The imbalance between ROS production and ROS degradation has been hypothesized as a cause of oxidative stress in semen, with peroxidative injury to the sperm membrane and a consequent impairment of the related functional properties, such as sperm motility and morphology. (12). Growing evidence suggests that such seminal oxidative stress is involved in many cases of idiopathic male factor infertility (11).

In the Ayurveda and Unani systems of medicine practiced in India, several plants and plant products have been documented to fight against stress, impotence, infertility, and the aging process (13). *Withania somnifera*, also known as Indian ginseng, has been described in folk medicine as an aphrodisiac and geriatric tonic. Different investigators have reported that *W. somnifera* possesses antiserotogenic, anticancer, and anabolic activity and is beneficial in the treatment of arthritis, geriatric problems, stress, and male sexual dysfunction. It also possesses adaptogenic, cardiotropic, cardioprotective, and anticoagulant properties (14). *W. somnifera* has been shown to inhibit lipid peroxidation in stress-induced animals (15). Earlier studies have shown that aqueous extract of this plant elicits changes in pituitary gonadotropins coupled with an enhancement in epididymal sperm pattern in adult male rats and folliculogenesis in immature female rats (16). *W. somnifera* induced testicular development and spermatogenesis in immature Wistar rats by directly affecting the seminiferous tubules (17). In view of the above considerations, the present study was undertaken to investigate the impact of *W. somnifera* on semen profile, oxidative biomarkers, and reproductive hormone levels of infertile men.

MATERIALS AND METHODS

Plant Materials

The roots of *W. somnifera* were procured from the Central Council for Research in Unani Medicine, New Delhi. The roots were dried under shade and made to fine powder using a laboratory grinder.

Study Design

The Institutional Review Board and Ethics Committee of Chhatrapati Shahuji Maharaj (CSM) Medical University, Lucknow, approved this study. The study was conducted between February 2007 and August 2008, and the study population included normal healthy and fertile men (n = 75) and infertile patients (n = 75), aged 25–40 years, recruited from the Outpatient Department of Urology, CSM Medical University. The control group comprised age-matched healthy men who had previously initiated at least one pregnancy and exhibited normal semen profile (sperm count $>20 \times 10^6$/mL, motility >40%, and >40% normal morphology). Normozoospermic infertile men (n = 25) had normal semen profile (defined as in the control group) and infertility of unknown etiology. Oligozoospermic infertile men (n = 25) had a sperm count of $<20 \times 10^6$/mL, motility >40%, and >40% normal morphology. Asthenozoospermic infertile men (n = 25) had a sperm count of $>20 \times 10^6$/mL, motility <40%, and >40% normal morphology. Before enrollment in the study, each subject's informed written consent was obtained in response to a fully written and verbal explanation of the nature of the study. The potential participants, each with infertility persisting longer than 1 year, were carefully examined. As an inclusion criterion, the infection of accessory glands was ruled out in all subjects. Moreover, subjects having diabetes, hypertension, arthritis, tuberculosis, or human immunodeficiency, those on drugs, and those having other conditions known to influence oxidative stress were excluded. Complete physical, biochemical, and semen examinations were performed as the screening tests. Additionally, medical histories of patients and their female partners were recorded. Infertile cases where a problem could be diagnosed in the female partner were excluded from the study. All subjects were instructed to continue normal diets without switching to dietary supplements during the course of treatment. To further ensure this, the details of the diet were taken from the patients at monthly intervals.

Treatment

Infertile men were prescribed *W. somnifera* root powder (5 g/day) orally for 3 months with milk. This dosing schedule was as reported earlier by Singh (18).

Sample Collection and Preparation

Semen samples were collected into sterile plastic containers by masturbation after 3–4 days of abstinence and allowed to liquefy for 30 minutes. Semen volume was recorded after liquefaction; an aliquot was taken to assess sperm motility and count. Semen profile was constructed with the procedures described by the World Health Organization (19). Liquefied semen samples were centrifuged at 1,200*g* for 20 minutes for separation of seminal plasma. The supernatant (seminal plasma) was centrifuged at 10,000*g* for 30 minutes to eliminate all possible contaminating cells. Seminal plasma was quickly frozen and stored at −20°C until the assessment of different biochemical parameters. Venous blood samples were drawn between 8 a.m. and 10 a.m. and centrifuged at 3,000*g* at 4°C for 10 minutes and serum was aspirated out for hormone assays.

Hormonal Assays

Serum T, LH, FSH, and PRL were measured by a double-antibody RIA method using Gamma Counter (Stratec Biomedical System, Birkenfeld, Germany) (20). Intra- and interassay coefficients of variation in T, LH, FSH, and PRL were 10.0%, 14.0%, 8.5%, and 12.5%, respectively. Receiver's operating