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ARTICLE

Withania somnifera improves semen quality by combating oxidative stress and cell death and improving essential metal concentrations

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Professor Abbas Ali Mahdi has made pioneering contributions in the emerging area of free radical biology and antioxidants. He has carried out in-depth studies related to perturbation of antioxidant levels consequent to oxidative stress in seminal plasma of infertile men. His group has demonstrated significant improvement in sperm count and motility following inhibition of lipid peroxidation in seminal plasma of infertile men by *Mucuna pruriens*. Recently, Dr. Mahdi has explored the role of *Withania somnifera* in improving stress-related infertility.

Abstract This study investigated the effect of a 3-month treatment with *Withania somnifera* on apoptosis and intracellular reactive oxygen species (ROS) concentration of spermatozoa and the metal ions copper, zinc, iron and gold in seminal plasma from infertile men (normozoospermic, $n = 25$; oligozoospermic, $n = 25$; and asthenozoospermic, $n = 25$). The apoptotic and necrotic cell distribution were analysed by annexin-V binding and propidium iodide uptake using flow cytometry. ROS generation was measured by fluorescence intensity and metal ions were analysed by atomic absorption spectrophotometry. The results demonstrated that, prior to treatment, sperm apoptosis and intracellular ROS concentrations were significantly higher in all groups of infertile men compared with controls ($P < 0.01$ to $P < 0.001$). Similarly, the concentrations of the essential metal ions Cu^{2+}, Zn^{2+}, Fe^{2+} and Au^{2+} in seminal plasma were lower. Treatment with *W. somnifera* significantly reduced apoptosis in normozoospermic and oligozoospermic men and ROS concentrations in oligozoospermic and asthenozoospermic men (all $P < 0.05$). Treatment also significantly improved metal ion concentrations in infertile men ($P < 0.01$). It is concluded that *W. somnifera* improves semen quality by reducing oxidative stress and cell death, as well as improving essential metal ion concentrations.

KEYWORDS: apoptosis, male infertility, metals, ROS, *Withania somnifera*

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Introduction

Male infertility is globally increasing and accounts for approximately 50% of infertile couples (Nallella et al., 2006). There are a number of factors responsible for male infertility that include excessive formation of reactive oxygen species (ROS) (Guzick et al., 2001). Moreover, numerous reports indicate that apoptosis occurs during spermatogenesis in humans (Moskovtsev et al., 2009). The process of suicidal cell death therefore, plays an important role in maintaining a healthy stream of spermatozoa by eliminating the immature or defective sperms. Alterations in spermatogenesis may increase the number of defective cells in the spermatozoa lineage, resulting in the elevation of apoptosis as an effect (Sakamoto et al., 2008).

ROS in the testis is generated by Sertoli cells, the controlled amount of which is necessary for spermiogenesis, capacitation and acrosome reaction (Baumber et al., 2003; Hipler et al., 2001). The testicular environment is metabolically very active, thereby resulting in high production of ROS (Saleh and Agarwal, 2002). Though ROS is required for sperm function, excessive production may result in oxidative insult (Shamsi et al., 2009; Shukla et al., 2008). Excessive ROS production causes lipid peroxidation of sperm plasma membranes, resulting in alteration of sperm function and fertilizing capacity (Bonde et al., 1998). ROS is also known to affect the sperm genome, causing high frequencies of single- and double-strand DNA breaks (Szarrowki and Nathan, 1991). Both superoxide (O_2^-) and the hydroxyl radical (OH′) are known to cause chromosome deletions, dicentrics and sister chromatid exchanges (Leduc et al., 2008). There is a strong association between the presence of nuclear DNA damage in the mature spermatozoa of men and poor semen parameters (Fariello et al., 2009; Venkatesh et al., 2009). The antioxidant system of the body maintains a fine balance between ROS generation and elimination, which is required for normal fertility.

Zinc, as a component of enzyme complexes, has a fundamental role in the antibacterial activity of seminal plasma (Chia et al., 1992). Apart from the maintenance of immunocompetence, an important function of zinc, in conjunction with copper, is to protect the body against free radicals through superoxide dismutase, a cuprozinc enzyme which accelerates the dismutation of free radicals (Smith et al., 1983). This enzyme plays a major role in protecting human spermatozoa against peroxidative damage of cellular enzymes and structures. Another important metal ion, iron, is bound to transferrin. Iron is required for haemoglobin, myoglobin, cyclo-oxygenases, cytochromes, hydroxylases, oxidase enzymes, ribonucleotide reductase, aconitase, succinate dehydrogenase, catalase and many others (Ahmad et al., 2008). Administration of gold chloride to immature Wister rats improved steroidogenesis and gametogenesis, indicating an important role of gold in these processes (Biswas et al., 2004). Another study prescribed a gold-containing Ayurvedic drug to human males, reporting improvement in sperm motility and prostatic activity without any toxic effects (Sharma et al., 2001). From the cited studies it is evident that metals play an important role in enzyme activity related to ROS scavenging.

It has been recently shown that *Withania somnifera* improves endurance against stress, thereby helping in management of male sexual disorders like psychogenic impotence and unexplained infertility (Shukla et al., 2009a, 2010; Ahmad et al., 2010). *W. somnifera*, also known as Indian ginseng, has been described in folk medicine as an aphrodisiac and geriatric tonic and it induced testicular development and spermatogenesis in immature Wistar rats by directly affecting the somniferous tubules (Abdel-Magied et al., 2000). *W. somnifera* exerts its pro-fertility activity by regulating reproductive hormone concentrations and oxidative stress; however, it is believed that the adaptogenic nature of this herb may not be just due to one factor. Therefore, the present study was undertaken in search of other mechanisms underlying its male pro-fertility activity with the following objectives: (i) to compare concentrations of ROS in infertile versus fertile individuals; (ii) to compare apoptosis in infertile versus fertile men; (iii) to compare concentrations of important metal ions in infertile versus fertile men; and (iv) to evaluate the effect of *W. somnifera* administration on apoptosis and the concentrations of ROS and metal ions in infertile individuals.

Materials and methods

Study group

The study was approved by the Institutional Review Board of the CSM Medical University, Lucknow, India. Semen samples were obtained by masturbation into a wide-mouthed sterile specimen jar after 3 days of sexual abstinence (World Health Organization, 1999).

The cases were selected from the couples attending the Out Patient Department of Urology, CSM Medical University, Lucknow. The inclusion criteria included infertility persisting longer than a year with no obvious fertility problem in the partner. The sample size was calculated assuming a confidence interval of 8 and unknown population size using the online calculator at http://www.surveysystem.com/sscalc.htm. Written informed consent from each subject was obtained in response to a fully written and verbal explanation of the nature of the study. The potential participants were clinically examined before being included in the study. Complete medical history of subjects and their female partners was also recorded. Subjects having varicocele, diabetes, hypertension, arthritis, malignancy, tuberculosis, human immunodeficiency virus positive, infections, other endocrinal disorders and those on drugs and conditions known to influence oxidative stress and serum cortisol concentration were excluded. The cases having leukocytospermia as per the definition of WHO (1999) were excluded from the study.

The sample collection following the above inclusion and exclusion criteria was carried on untill 25 in each patient group was reached. Out of a total of 75 men recruited for the study, 25 were normozoospermic (defined as the control group), oligozoospermic ($<20 \times 10^6$ spermatozoa/ml, >50% motility and >30% normal morphology) and asthenozoospermic ($>20 \times 10^6$ spermatozoa/ml, <50% motility and >40% normal morphology), with details provided in an earlier study on the same cohort (Ahmad et al., 2010). The