Effect of Withania somnifera on Seminal Plasma Metabolites of Infertile Males: A Proton NMR Study

ashish gupta[1], Abbas Ali Mahdi[2], Mohammad K Ahmad[2], Navneeta Bansal[2], Pushplata Sankhwar[3], and Satya Narain Sankhwar[4]

[1]Centre of Biomedical Magnetic Resonance, SGPGIMS Campus, Lucknow, UP, India, [2]Department of Biochemistry, King George's Medical University, Lucknow, UP, India, [3]Department of Obstetrics & Gynecology, King George's Medical University, Lucknow, UP, India, [4]Department of Urology, King George's Medical University, Lucknow, UP, India

SYNOPSIS: Efficacy of *Withania sonmifera* (*W. somnifera*) were evaluated on metabolic profile of seminal plasma of infertile patients (n=180). Patients were administered (5g/day) *W. somnifera* root powder for three months. Lactate, alanine, choline, citrate, glycerophosphocholine (GPC), glutamine, tyrosine, histidine, phenylalanine, and uridine were measured in seminal plasma using ^{1}H NMR spectroscopy. *W. somnifera* therapy repairs the disturbed concentration of lactate, alanine, citrate, GPC, histidine and phenylalanine in seminal plasma and recovers the quality of semen of post-treated infertile men with compared to pre-treated. Results suggest that *W. somnifera* may be used as an empirical therapy for infertility treatment and clinical management.

INTRODUCTION: Infertility is demarcated as failure to conceive by a couple after 12 months of unprotected regular sexual intercourse and it is a global health issue estimated to affect 10-15% of all couples worldwide (8) and in nearly 50% of the cases causes male infertility. Male infertility is often inexplicable etiology and the most difficult form of infertility to treat (25). Traditional Indian Systems of Medicine (Ayurveda and Unani) use roots of *Withania somnifera* (*W. somnifera*) for impotence, infertility treatment, fight against stress and aging process. The aim of the present work is to explore the scientific basis to validate the pre- and post-treatment efficacy of *W. somnifera* on seminal plasma of infertile men, which remains unexplored to date.

MATERIALS AND METHODS: One hundred eighty infertile patients comprised normozoospermic (NZ, n=60), oligozoospermic (OZ, n=60) and asthenozoospermic (AZ, n=60), aged 22 to 45 years, were recruited randomly from the list of infertility clinic, K. G. Medical University, Lucknow. The study also comprised age-matched normal healthy fertile men (n=50) as control group (CZ). After thorough analysis of the semen samples, infertile men were prescribed root powder of *W. somnifera* (5 g/day) orally in a single dose for 3 months with milk. Semen and blood samples were collected before and after 3 months of treatment with *W. somnifera*. Semen samples were collected and prepared according to World Health Organization protocol to perform various studies. Blood serum samples were also prepared for the measurement of LH, FSH, T, and PRL hormone. 500 µl of each semen sample was taken in 5mm NMR tubes. For field-frequency-lock and quantitative measurements of metabolites, a sealed reusable capillary containing 25µL of 0.375% sodium salt of tri-methylsilylpropionic acid (TSP) in deuterium oxide was inserted into the NMR tube. For all samples, one-dimensional ^{1}H NMR experiments were performed on a Bruker Avance III 800 MHz spectrometer at 22^{o}C using one-pulse sequence with suppression of the water resonance by pre-saturation. The acquisition parameters were spectral width, 16500Hz; time domain points, 64K; relaxation delay, 10sec; pulse angle, 90^o; number of scans, 64; spectrum size, 64K. The quantification of metabolites was obtained using the integral area of respective metabolite marker signal with reference to the integral area of TSP.

RESULT: The concentrations of alanine, citrate, GPC, and histidine were significantly reduced concomitantly phenylalanine was significantly increased in the pre-treated NZ infertile patients when compared with CZ (Fig. 1). After 3 months of *W. somnifera* treatment, the pool size of alanine, citrate, GPC and histidine were significantly increased and phenylalanine was decreased in post treated NZ patients with compared to pre-treatment. The decreased concentration of lactate, alanine, citrate, GPC, histidine and the concurrently increased concentration of phenylalanine were perceived in the pre-treated OZ patients when compared to CZ (Fig. 1). In contrast to pre-treatment, the concentrations of lactate, alanine, citrate, GPC and histidine were significantly increased and phenylalanine was decreased over 3 months of *W. somnifera*-treated OZ patients. Lactate, alanine, citrate, GPC, and histidine were diminished in pre-treated AZ when matched to CZ. When compared to pre-treated AZ, the amount of lactate, alanine, citrate, GPC, and histidine were significantly increased after three months of *W. somnifera* treated AZ patients. Harmonic balance of sperm concentration, motility, lipid peroxide, and enzymatic activities of ALT, AST, LDH and IDH of seminal plasma were corrected after three months of *W. somnifera* treatment in NZ, OZ, and AZ. Serum hormone levels of LH, FSH, T and PRL were also improved after three months of *W. somnifera* treatment in NZ, OZ, and AZ.

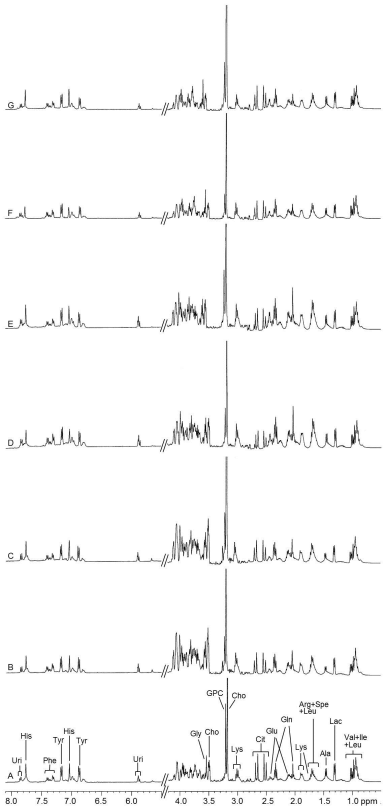

Fig.1: Typical ^{1}H NMR spectra of human seminal plasma from (A) CZ, (B) NZ (pre-treatment), (C) NZ (post-treatment), (D) OZ (pre-treatment), (E) OZ (post-treatment), (F) AZ (pre-treatment), (G) AZ (post-treatment) Key: Val, valine; Ile, isoleucine; Leu, leucine; Lac, lactate; Lys, lysine; Ala, alanine; Arg, arginine; Spe, spermine; Glu, glutamate; Gln, glutamine; Cit, citrate; Cho, choline; GPC, Glycerophosphocholine; Gly, glycine; Tyr, tyrosine; Uri, uridine; His, histidine; Phe, phenylalanine.

DISCUSSION: The decreased LDH activity observation supports the reduction in lactate and reduced sperm motility in pre-treated infertile patients. Roots of *W. somnifera* treatment increased lactate, LDH activity and sperm motility after three months of treatment of infertile patients. The diminished ALT activity observation vindicates the lowering of alanine concentration and advocates an altered oxidative process or increased reactive oxygen species production, LPO, in pre-treated infertile patients. Root of *W. somnifera* is rich source of alanine which facilitates to increase the alanine concentration, ALT activity, and improve LPO in post-treated infertile patients. The IDH activity and citrate content are significantly reduced in pre-treated infertile patients. After 3 months of *W. somnifera* treatment, increased level of testosterone, citrate content and IDH activity were observed. The observation reveals that the GPC content is significantly reduced in pre-treated infertile patients. *W. somnifera* contains immense amount of essential and non-essential fatty acids and sterols. These fatty acids play important roles in the synthesis of GPC, lipoproteins and facilitates in retaining the structural and functional integrity of the spermatozoa which leads to improved outcome exhibited in NMR spectroscopy, enzymatic activity and clinical variables results in post-treated infertile patients. At the cost of histidine levels the lipid peroxidation process increases, in pre-treated infertile patients. *W. somnifera* therapy enhances histidine production in seminal plasma and concomitantly falls in lipid peroxidation level. Marginally elevated level of phenylalanine was detected in pre-treated infertile patients. *W. somnifera* contains substantial amount of tyrosine hydroxylase and tyrosine content, which increases this enzyme in the human system over long-term therapy with *W. somnifera* and reduces the phenylalanine content in infertile patients.

References: Am. J. Mat. Chil. Nurs. 2010; 35 (3): 174. (2) Hum. Repro. 1998; 13 (Suppl. 1): 33-44.