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# A proton NMR study of the effect of *Mucuna pruriens* on seminal plasma metabolites of infertile males

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ABSTRACT

The objective of this study was to employ proton nuclear magnetic resonance ($^1$H NMR) spectroscopy to evaluate the impact of *Mucuna pruriens* seeds on the metabolic profile of seminal plasma of infertile patients. A total of 180 infertile patients were administered *M. pruriens* seed powder for a period of three months. Age-matched healthy men comprised the control ($n = 50$) group in the study. Lactate, alanine, choline, citrate, glycerophosphocholine (GPC), glutamine, tyrosine, histidine, phenylalanine, and uridine were measured in seminal plasma by $^1$H NMR spectroscopy. To evaluate the degree of infertility and extent of hormonal imbalance induced by this milieu, separate sperm concentration, motility, lipid peroxide in seminal plasma and LH, FSH, T, and PRL hormone concentration in serum were measured using standard laboratory methods and RIA, respectively, in the same subjects. *M. pruriens* therapy rectifies the perturbed alanine, citrate, GPC, histidine and phenylalanine content in seminal plasma and improves the semen quality of post-treated infertile men with compared to pre-treated. Concomitantly, clinical variables in seminal plasma and blood serum were also improved over post therapy in infertile men. On the basis of these observations, it may be proposed that *M. pruriens* seed powder not only reactivates the enzymatic activity of metabolic pathways and energy metabolism but also rejuvenates the harmonic balance of male reproductive hormones in infertile men. These findings open more opportunities for infertility treatment and management by improving semen quality.

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## 1. Introduction

Infertility, defined as failure to conceive by a couple after 12 months of unprotected sexual intercourse, is a global health issue affecting 15% of all couples worldwide [1]. Although rarely socially acknowledged, male infertility contributes to nearly 50% of all cases worldwide and is often the most difficult form of infertility to treat [2]. The high rates of infertility in the developing world have a variety of causative factors including environmental, physiologic, endocrine, and genetic mutations, but in 10 to 20% of cases, no definitive cause can be identified, and the infertility remains unexplained [3]. Specific and directed treatment for male infertility is not available due to the unexplained and heterogeneous nature of the causative disorders. It can be cured by both medical and surgical treatment modalities, but given its etiologic heterogeneity successful treatment of male infertility is quite cumbersome, expensive and inaccessible to many. The lack of easily available specific therapies for male infertility demands the exploration of alternative remedies. Thus, many patients are attracted to the natural herbal remedies, and these have becoming popular alternative therapies to improve fertility because these herbal remedies are easy to obtain, and are often more affordable compared to prescription medications, despite the availability of effective conventional medical treatments.

Traditional Indian Systems of Medicine (Ayurveda and Unani) use seeds of *Mucuna pruriens* for improving fertility. This plant is seen as an aphrodisiac. The seeds of *M. pruriens* contain a variety of alkaloids, triterpenes, sterols, lecithin, vernolic acid, gallic acid, and various proteins including L-3,4 dihydroxyphenyl alanine (L-DOPA), and a large number of other bioactive substances such as tryptamine, alkylamines, flavonoids and metals such as magnesium, copper, zinc, manganese, and iron [4–7]. The seed and bark of this plant improve semen quality and decrease spermatorrhea. It is reported in Ayurveda that the plant acts not only as a restorative and invigorating tonic but also as a prophylactic against oligospermia, and that it increases sperm count [7]. It is often used

*Abbreviations:* TSP, tri-methylsilylpropionic acid; CZ, control group; NZ, normozoospermic; OZ, oligozoospermic; AZ, asthenozoospermic.

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for treatment of psychogenic impotence and inexplicable infertility [6]. These perceptions are supported by a recently reported biochemical study [7].

Further scientific validation using nuclear magnetic resonance (NMR) spectroscopy is required to evaluate the pre- and post-treatment efficacy of *M. pruriens* on seminal plasma of infertile men, which remains unexplored to date. This technique fulfils the requisite of sensitivity and specifies to detect subtle changes at clinically relevant concentration of endogenous metabolites [8]. NMR spectroscopy is a newly emerging field in biomedical analysis and is one of the major techniques used in dynamic metabolic studies for various complex disease panoramas by means of a variety of biological fluids [9–11]. Therefore, the present study was designed to address this issue and to lend support to the existing data pertaining to the beneficial effects of *M. pruriens* in the treatment and improvement of clinical management of male infertility.

## 2. Materials and methods

### 2.1. Plant material

Seeds of *M. pruriens* were purchased from an authorized dealer of Ayurvadic and Unani medicine in Lucknow, India. The seeds were identified and authenticated by the National Botanical Research Institute (Lucknow), and were dried under shade, and ground to a fine powder with a laboratory grinder. The seeds contain L-DOPA, ascorbic acid, proteins, lipids, alkaloids and other bioactive compounds, and some metals [7].

### 2.2. Patient selection and treatment

The study protocol was approved by the institutional review board and ethics committee of the Chhatrapati Sahuji Maharaj (C.S.M.) Medical University, Lucknow, India. Before participants were enrolled in the study, written informed consent was obtained from each subject in response to a full written and verbal explanation of the nature of the study. Potential participants with infertility persisting longer than one year were clinically examined before the study was conducted. Complete physical, biochemical, and semen examinations were performed as the screening test. Medical histories of patients and their female partners were recorded. Exclusion criteria included: infections of accessory glands, smoking, stress, diabetes, hypertension, arthritis, malignancies, tuberculosis, HIV, urinary tract infections, endocrine disorders, drug abuse, and other conditions known to influence sperm physiology and male fertility.

One hundred eighty men, aged 22–45 years, were selected from the couples attending the Infertility Clinic of the Department of Obstetrics and Gynecology and the Outpatient Department of Urology, C.S.M. Medical University. The study also included normal healthy fertile men ($n = 50$) as control group (CZ). The healthy fertile men (CZ) had previously initiated at least one pregnancy and exhibited a normal semen profile (>$20 \times 10^6$ spermatozoa/ml, >40% motility, and >40% normal morphology). The patient group consisted of three subgroups. The first subgroup included normozoospermic (NZ) infertile males ($n = 60$) who had a normal semen profile (>$20 \times 10^6$ spermatozoa/ml, >40% motility, and >40% normal morphology) and fertile female partners who had undergone extensive infertility evaluation without showing any detectable gynecological abnormalities. The second subgroup included oligozoospermic (OZ) males ($n = 60$) who had a sperm concentration <$20 \times 10^6$/ml, >40% motility, and >40% normal morphology. The third subgroup included asthenozoospermic (AZ) males ($n = 60$) who had a sperm concentration >$20 \times 10^6$/ml, <40% motility, and >40% normal morphology. After examination of the semen samples, infertile men were prescribed *M. pruriens* seed powder (5 g/day) orally in a single dose with milk for 3 months. This dosing schedule was adapted from the literature [7]. Semen and blood samples were collected before and after 3 months of treatment with *M. pruriens*.

A semen sample profile was constructed according to the procedures described by the World Health Organization (WHO) [8]. Semen samples were collected by masturbation after 3–4 days of abstinence and divided into two parts. In one part, the semen was liquefied, and an aliquot was taken to assess sperm concentration and motility. In the second part, after liquefaction the semen sample (~1.0–1.5 ml) was centrifuged at $1200 \times g$ for 20 min for separation of seminal plasma. The supernatant (seminal plasma) was centrifuged at $10{,}000 \times g$ for 30 min to remove all possible contaminating cells and rapidly frozen in liquid nitrogen.

Venous blood samples were withdrawn between 8 a.m. and 10 a.m. which were clotted for 1 h at room temperature. Serum was separated after centrifugation at $3000 \times g$ at 4 °C for 10 min. and hormone levels were determined. All chemicals and RIA kits for luteinizing hormone (LH), follicle stimulating hormone (FSH), testosterone (T), and prolactin (PRL) were of analytic grade and were purchased from sigma chemicals (Sigma–Aldrich corporation, Bangalore, India). Serum LH, FSH, T, and PRL were measured by a double-antibody RIA method using Gamma Counter (Stratec Biomedical Systems Ag, Gewerbestr. 37, Birkenfeld, Germany), as explained previously [7].

### 2.3. Nuclear magnetic resonance experiments

The frozen seminal plasma samples were thawed and 500 μL of each sample was taken separately in 5 mm NMR tubes. For field-frequency-lock and quantitative measurements of metabolites, a sealed reusable capillary containing 25 μL of 0.375% sodium salt of tri-methylsilylpropionic acid (TSP) in deuterium oxide was inserted into the tube, and the spectra were obtained. For all samples, one-dimensional $^1$H NMR experiments were performed on a Bruker Avance 400 MHz spectrometer (Bruker BioSpin, Fällanden, Switzerland) at 22 °C using one-pulse sequence with suppression of the water resonance by pre-saturation. The acquisition parameters were spectral width, 8000 Hz; time domain points, 32 K; relaxation delay, 20 s; pulse angle, 90°; number of scans, 128; spectrum size, 32 K. The spectra were processed with line broadening: 0.3 Hz. Metabolites were quantified with an in-house custom program based on the integration area of identified metabolites relative to the area of the reference signal from TSP [8].

### 2.4. Statistical analysis

The four independent groups – CZ, pretreated NZ, pretreated OZ, and pretreated AZ – were compared by ANOVA (one-way analysis of variance) followed by Dunnett test. A paired $t$ test was used to analyze the significance of difference between pre- and post-treatment of infertile groups. Relative associations among NMR-derived metabolites and clinical variables were determined by Pearson's correlation coefficient. A $p$ value of <0.05 was considered statistically significant. All statistical tests were carried out with the Graph Pad INSTAT 3.0 commercial software. The results obtained for the NMR-based quantified metabolites (mg/dl) are expressed as mean ± S.D. (Table 1). The seminal plasma-based clinical laboratory variables comprising the sperm concentration (million/ml), motility (in percentage) and lipid peroxide (nmol MDA/ml) content information (Table 2) are also given. Serum hormone profile containing LH (mIU/ml), FSH (mIU/ml), T (ng/ml) and PRL (ng/ml) are expressed as mean ± S.D. (Table 3). Pearson correlation coefficients of NMR-derived and clinical variables between pre- and post-treated infertile patients are given in Table 4.

**Table 1**
Absolute concentrations (mg/dl) of NMR-derived metabolites quantified in seminal plasma of control, pre and post treated infertile patient groups, using homemade computer program with respect to a known concentration of tri-methylsilylpropionic acid. All results are shown as mean ± S.D. GPC, glycerophosphocholine.

| Metabolite name | Control ($n = 50$) | Normozoospermic ($n = 60$) | | Oligozoospermic ($n = 60$) | | Asthenozoospermic ($n = 60$) | |
|---|---|---|---|---|---|---|---|
| | | Pretreatment | Posttreatment | Pretreatment | Posttreatment | Pretreatment | Posttreatment |
| Lactate | 114 ± 37 | 119 ± 67 | 120 ± 55 | 107 ± 37 | 116 ± 31 | 90 ± 24 | 102 ± 12 |
| Alanine | 72 ± 45 | 49 ± 27[a] | 61 ± 25[c] | 65 ± 25 | 72 ± 22 | 45 ± 23[a] | 70 ± 15[c] |
| Glutamine | 42 ± 12 | 39 ± 11 | 38 ± 12 | 44 ± 15 | 40 ± 15 | 41 ± 12 | 44 ± 15 |
| Citrate | 480 ± 74 | 361 ± 84[b] | 428 ± 133[c] | 315 ± 20[b] | 365 ± 85[c] | 272 ± 70[b] | 339 ± 56[c] |
| Choline | 207 ± 64 | 184 ± 78 | 197 ± 67 | 199 ± 59 | 203 ± 55 | 171 ± 75 | 191 ± 58 |
| GPC | 217 ± 77 | 107 ± 50[b] | 149 ± 32[d] | 115 ± 40[b] | 134 ± 30[c] | 102 ± 12[b] | 120 ± 10[c] |
| Tyrosine | 134 ± 48 | 145 ± 68 | 136 ± 33 | 153 ± 55 | 139 ± 22 | 156 ± 43 | 143 ± 27 |
| Histidine | 110 ± 73 | 85 ± 40 | 100 ± 29[c] | 84 ± 32 | 97 ± 26[c] | 78 ± 21[a] | 93 ± 22[c] |
| Phenylalanine | 28 ± 14 | 40 ± 8[b] | 35 ± 5[d] | 37 ± 12[b] | 32 ± 7[c] | 33 ± 10 | 31 ± 6 |
| Uridine | 34 ± 16 | 35 ± 20 | 38 ± 14 | 41 ± 20 | 39 ± 11 | 44 ± 21 | 39 ± 10 |

[a] $p < 0.05$ as compared to the control.
[b] $p < 0.01$ as compared to the control.
[c] $p < 0.05$ as compared to the pretreatment.
[d] $p < 0.01$ as compared to the pretreatment.

**Table 2**
The seminal plasma clinical variables; sperm concentration (million/ml), sperm motility (%), and lipid peroxide (LPO) (nmolMDA/ml) as observed in control, pre and post treated infertile patient groups. All results are shown as mean ± S.D.

| Clinical parameters | Control ($n = 50$) | Normozoospermic ($n = 60$) | | Oligozoospermic ($n = 60$) | | Asthenozoospermic ($n = 60$) | |
|---|---|---|---|---|---|---|---|
| | | Pretreatment | Posttreatment | Pretreatment | Posttreatment | Pretreatment | Posttreatment |
| Sperm concentration | 76 ± 16 | 63 ± 30 | 75 ± 20[b] | 33 ± 25[a] | 55 ± 17[c] | 52 ± 24 | 67 ± 12[b] |
| Motility | 73 ± 9 | 70 ± 11 | 75 ± 8[b] | 45 ± 12[a] | 58 ± 8[c] | 22 ± 6[a] | 29 ± 6[a] |
| LPO | 1.7 ± 0.2 | 2.6 ± 0.3[a] | 2.2 ± 0.2[c] | 2.3 ± 0.4[a] | 2.0 ± 0.2[c] | 2.5 ± 0.2[a] | 2.2 ± 0.1[c] |

[a] $p < 0.01$, as compared to the control.
[b] $p < 0.05$ as compared to the pretreatment.
[c] $p < 0.01$ as compared to the pretreatment.

**Table 3**
The blood serum clinical variables; LH (mIU/ml), FSH (mIU/ml), T (ng/ml) and PRL (ng/ml) as observed in control, pre and post treated infertile patient groups. All results are shown as mean ± S.D.

| Hormonal parameters | Control ($n = 50$) | Normozoospermic ($n = 60$) | | Oligozoospermic ($n = 60$) | | Asthenozoospermic ($n = 60$) | |
|---|---|---|---|---|---|---|---|
| | | Pretreatment | Posttreatment | Pretreatment | Posttreatment | Pretreatment | Posttreatment |
| LH | 7.9 ± 1.1 | 6.3 ± 0.4[a] | 7.9 ± 0.5[b] | 4.1 ± 0.5[a] | 6.4 ± 0.6[b] | 4.0 ± 0.5[a] | 5.6 ± 0.5[b] |
| FSH | 5.8 ± 0.6 | 6.7 ± 0.6[a] | 5.7 ± 0.6[b] | 8.2 ± 0.7[a] | 6.1 ± 0.7[b] | 6.8 ± 0.7[a] | 6.0 ± 0.9[a] |
| T | 6.9 ± 0.7 | 5.9 ± 0.8[a] | 6.9 ± 0.6[b] | 3.7 ± 0.4[a] | 5.3 ± 0.4[b] | 4.4 ± 0.9[a] | 5.7 ± 0.6[a] |
| PRL | 6.8 ± 0.9 | 6.9 ± 0.6 | 6.0 ± 0.6[b] | 10.9 ± 1.2[a] | 7.4 ± 0.8[b] | 7.6 ± 0.8[a] | 6.8 ± 0.5[b] |

[a] $p < 0.01$ as compared to the control.
[b] $p < 0.01$ as compared to the pretreatment.

**Table 4**
Pearson correlation coefficients of NMR-derived and clinical variables between pre and post treated infertile patients.

| Variables | Normozoospermic | Oligozoospermic | Asthenozoospermic |
|---|---|---|---|
| Lactate | 0.99[b] | 0.94[b] | 0.97[b] |
| Alanine | 0.87[b] | 0.91[b] | 0.76[b] |
| Glutamine | 0.11[c] | −0.12[c] | −0.13[c] |
| Citrate | 0.94[b] | 0.90[b] | 0.72[b] |
| Choline | 0.95[b] | 0.93[b] | 0.96[b] |
| GPC | 0.33[a] | 0.54[b] | 0.81[b] |
| Tyrosine | 0.12[c] | −0.15[c] | −0.17[c] |
| Histidine | 0.97[b] | 0.96[b] | 0.93[b] |
| Phenylalanine | 0.47[b] | 0.54[b] | 0.62[b] |
| Uridine | 0.93[b] | 0.90[b] | 0.88[b] |
| Sperm concentration | 0.91[b] | 0.76[b] | 0.87[b] |
| Motility | 0.85[b] | 0.87[b] | 0.79[b] |
| LPO | 0.48[b] | 0.83[b] | 0.36[c] |
| LH | 0.002[c] | 0.43[a] | 0.61[b] |
| FSH | 0.27[c] | 0.25[c] | 0.70[b] |
| T | 0.54[b] | −0.07[c] | 0.19[c] |
| PRL | 0.84[b] | 0.13[c] | 0.46[a] |

[a] Significant ($p < 0.05$).
[b] Highly significant ($p < 0.01$).
[c] Not significant ($p > 0.05$).

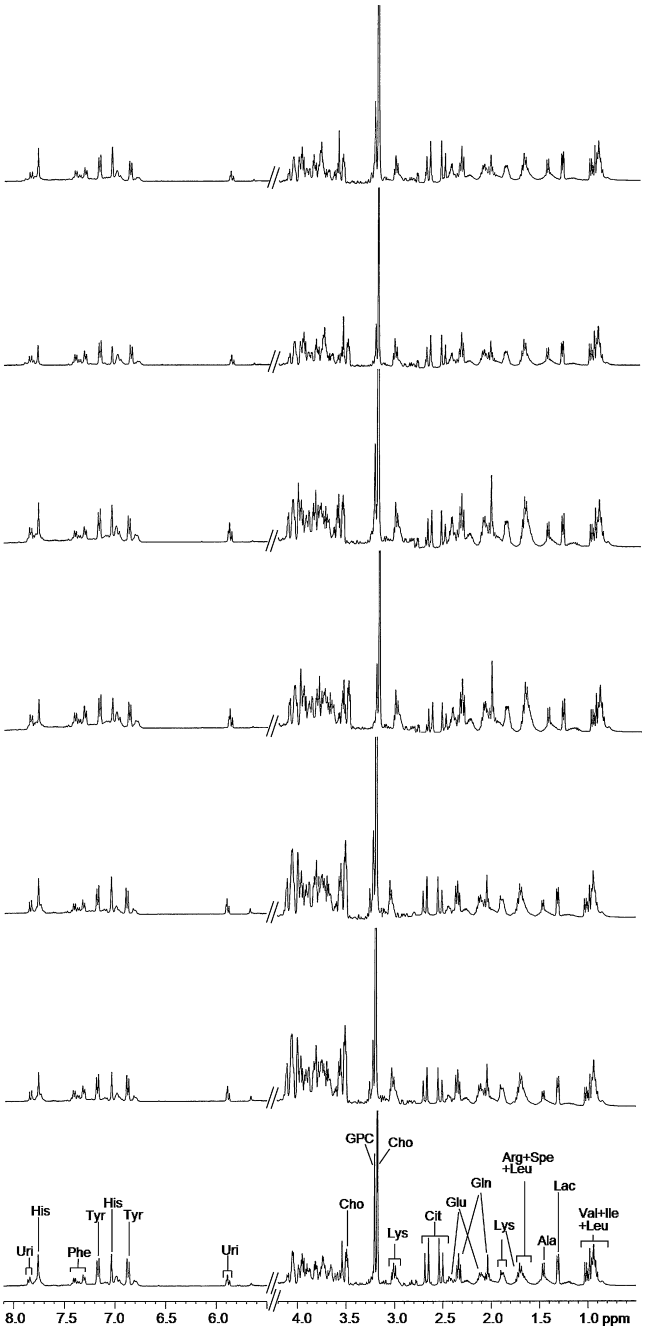


**Fig. 1.** Typical $^1$H NMR spectra of human seminal plasma from different groups viz: (A) control subjects, (B) normozoospermic (pre-treatment), (C) normozoospermic (post-treatment) (D) oligozoospermic (pre-treatment), (E) oligozoospermic (post-treatment), (F) asthenozoospermic (pre-treatment) and (G) asthenozoospermic (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.

## 3. Results and discussion

Fig. 1 shows the typical $^1$H NMR spectra of a complete metabolic profile and chemical shift assignments of different resonances in seminal plasma from the control along with pre and post treatment of three infertile groups. Resonance signals of various metabolites in seminal plasma were assigned by using known chemical shift and coupling constant parameters [8,12]. Several metabolite resonances emerged in the complex pattern. Valine, leucine, isoleucine, arginine, glutamate, and lysine were not quantified because of overlapping of resonance assignments. Ten metabolites (lactate, alanine, glutamine, citrate, choline, GPC, tyrosine, histidine, phenylalanine, and uridine) were unequivocally and ubiquitously present in all samples, and their quantities were estimated from their respective resonances as previously described [8] and subjected to statistical analysis. The chemical shift and multiplicity of these ten metabolites are presented in Table 5.

**Table 5**
Chemical shift, multiplicity and resonance assignments of the selected and measured metabolites in seminal plasma of all samples.

| Metabolites | Chemical shift ($\delta$) | Multiplicity | Assignment |
|---|---|---|---|
| Lactate | 1.32 | *d* | $CH_3$ |
| Alanine | 1.47 | *d* | $CH_3$ |
| Glutamine | 2.36 | *m* | $\beta$-$CH_2$ |
| Citrate | 2.50 | *d* | $CH_2$ |
| Choline | 3.20 | *s* | $N^+(CH_3)_3$ |
| GPC | 3.23 | *s* | $N^+(CH_3)_3$ |
| Tyrosine | 6.89 | *d* | H3, H5 |
| Histidine | 7.05 | *s* | H4 |
| Phenylalanine | 7.28–7.43 | *m* | H2, H3, H4, H5, H6 |
| Uridine | 7.84 | *d* | Cyclic H6 |

*s*, singlet; *d*, doublet; *m*, multiplet.

Lactate, choline, glutamine, tyrosine, and uridine did not demonstrate any differences among the univariate analyses, which is consistent with a previous report [8]. Although among these, some metabolites were playing a major role as a cumulative factor for differentiation and segregation of the different groups of infertility through the discriminant function analysis (DFA) because DFA reveals the profound descriptor with providing sensitivity, specificity, and predictive probability. Table 1 reveals that the NMR-derived metabolites concentrations of alanine, citrate, GPC were significantly decreased and phenylalanine was significantly increased in the pre-treated NZ infertile patients when it compared to the CZ (controls). In comparison to pre-treatment, the concentration of alanine, citrate, GPC and histidine were significantly increased and phenylalanine was decreased over 3 months of *M. pruriens*-treated NZ patients. The decreased amount of citrate and of GPC and the concomitantly increased amount of phenylalanine were observed as signature biomarkers to make the difference in the pre-treated OZ patients and CZ. The post-treatment of OZ with *M. pruriens* for 3 months demonstrated an increased amount of citrate, GPC, and histidine along with a decreased amount of phenylalanine compared to pre-treated OZ patients. The pool size of alanine, citrate, GPC, and histidine were decreased in pre-treated AZ compared to CZ. Compared to pre-treated AZ, the pool size of alanine, citrate, GPC, and histidine were statistically increased after three months' *M. pruriens* treatment of AZ patients. Fig. 2 shows the expanded view of these important metabolite resonances.

Similarly, the seminal plasma clinical variables and serum hormone level statistics of control compared with pre-treated patients, and post-treated patients compared with respective pre-treated patients are described in Tables 2 and 3, respectively.

Table 4 shows the Pearson's correlations between pre- and post-treatment of NMR-derived and clinical variables in infertile patients. In NZ patients all NMR and clinical variables were positive and significant; however, glutamine, tyrosine, LH, and FSH showed opposite association. Pearson's correlation of all NMR and clinical variables were positive and significant, except glutamine, tyrosine, FSH, T, and PRL in pre- and post-treated OZ patients. Besides glutamine, tyrosine, LPO, and T, all other metabolites and clinical variables were positive and significant in pre- and after three months-treated AZ patients with *M. pruriens*.

Given the lack of knowledge about etiological factors, a non-directed general therapy may yield good results in a subcategory

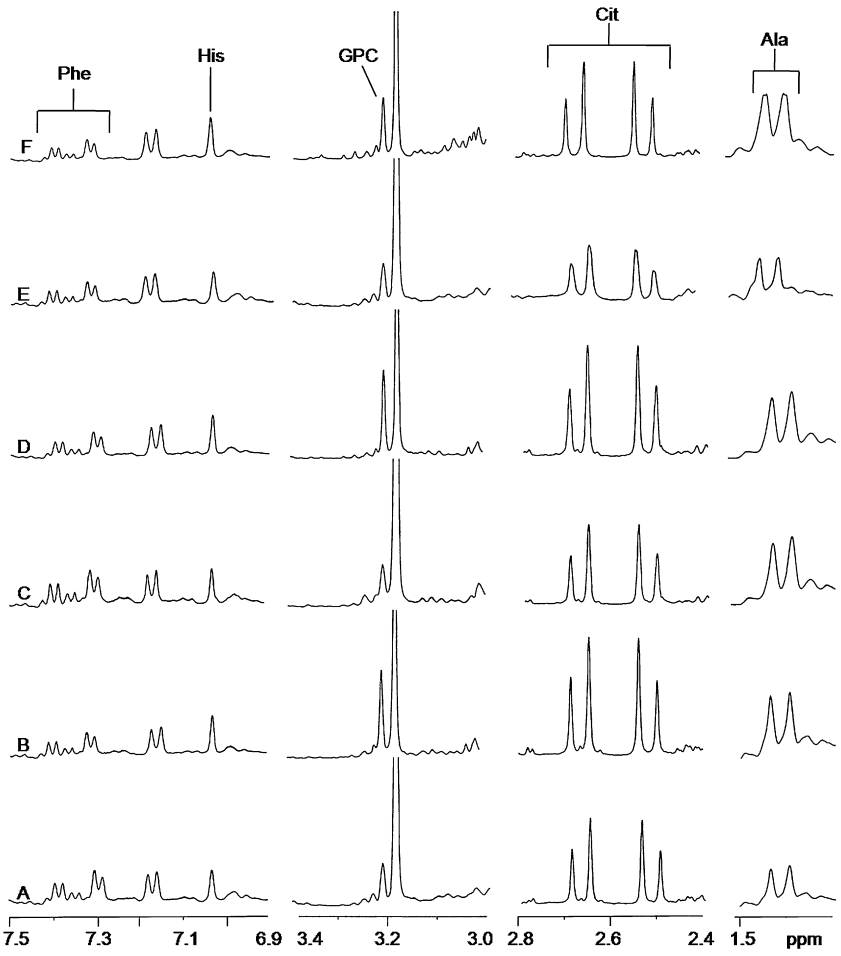


**Fig. 2.** $^{1}$H NMR spectra of selected metabolites of human seminal plasma from different patient groups viz: (A) pretreated normozoospermic (B) post-treated normozoospermic, (C) pre-treated oligozoospermic, (D) post-treated oligozoospermic, (E) pre-treated asthenozoospermic and (F) post-treated asthenozoospermic. *Key*: Ala, alanine; Cit, citrate; GPC, glycerophosphocholine His, histidine; Phe, phenylalanine. The difference in integration between (A) vs. (B) in Ala; 1.2894, Cit; 1.2771, GPC; 1.4435, His; 0.21677, Phe; −0.35594, (C) vs. (D) in Ala; 0.2807, Cit; 1.1984, GPC; 1.2435, His; 0.20187, Phe; −0.3992, (E) vs. (F) in Ala; 1.0241, Cit; 0.9728, GPC; 0.9005, His; 0.24692, Phe; −0.0393. Here difference in integration with (−) sign is indicating reduction in metabolite resonance.

of infertile patients. The rationale for the use of these therapies is based on the speculation that some forms of male infertility are caused by perturbation in the orchestra of central nervous system leading to imbalance in the metabolic and hormonal pathways, and that the use of alternative herbal remedies may improve male fertility potential and semen quality by means of rectifying these irregular cellular metabolic systems. A variety of complex disease mechanisms have been documented [8,12] with their dynamic metabolic studies using leading branch of NMR spectroscopy for biomedical analysis. It has been applied in non-selective detection of a number of molecular markers of infertility and sub-fertility [8] because NMR spectroscopy has advantages over other techniques such as biochemical and mass spectroscopy, e.g. it requires only minimal sample preparation, is non-destructive and inherently quantitative. These rewarding traits prompted investigators to study the effect of *M. pruriens* treatment on fertility with non-selective manner.

Alanine, citrate, GPC, histidine and phenylalanine were observed as important metabolites for NZ patients. Citrate, GPC, histidine and phenylalanine were observed as important metabolites for OZ patients. Similarly, alanine, citrate, GPC, and histidine were observed as important metabolites for AZ patients. The present study demonstrates that oral administration of *M. pruriens* to infertile patient for three months results in significant improvement of seminal plasma metabolic profile parallel to clinical variables.

Alanine is the product of alanine aminotransferase. The activity of this enzyme was shown to be significantly decreased in seminal plasma of infertile patients [13], a finding that supports the outcome of the present study in pre-treated infertile patients. The decreased endogenous metabolite, alanine, suggests an altered oxidative process via the pyruvate or the acetyl-CoA pathway. Probably *M. pruriens* treatment may have indirect or direct effects on alanine aminotransferase activity via an oxidative process and may enhance the production of alanine along with energy metabolism, which we observed in the seminal plasma of post-treated infertile patients.

Decreased amount of citrate was observed in pre-treated infertile patients possibly due to the perturbation in prostate function, either the consequence of an inflammatory milieu of prostate or an autoimmune response causing a diminished prostate function and citrate content [14]. Other reports says that testosterone has specific effects on the biosynthesis of mitochondrial aspartate aminotransferase (mAAT) and consequently regulates prostate citrate production via a stimulatory effect on mAAT, causing enhanced synthesis of mitochondrial citrate from aspartate in prostate epithelial cells [15]. This report supports the outcome of the present study, where we observed decreased amount of testosterone and citrate in blood and seminal plasma samples, respectively, in pre-treated infertile patients. After 3 months of *M. pruriens* treatment, an elevated level of testosterone and citrate were observed. The seeds of *M. pruriens* contain a substantial amount of L-DOPA

and other relevant metabolites such as epinephrine and norepinephrine. The action of L-DOPA over the sex hormone and lipid peroxide homeostasis has been explained in detail [7,16,17]. Briefly, L-DOPA and catecholamine activate the β-adrenergic system by increasing the cyclic adenosine monophosphate (cAMP) content, which in turn regulates the carbohydrate metabolism and lipolysis of fat. The effect of *M. pruriens* on key metabolic enzymes involved in carbohydrate metabolism has also been reported [18]. The cAMP level is significantly reduced in infertile patients when compared to healthy fertile men [19]. Moreover, after treatment with clomiphene citrate, not only elevated level of cAMP but also increased sperm motility were observed in the seminal fluid of infertile patients [20,21]. The above mentioned facts and information gleaned from the literature [7,16,17,22–25] support the finding that *M. pruriens* therapy increases dopamine levels which in turn rectifies the balance of hormones, enzymatic activity of TCA cycle, and energy metabolism in infertile patients, leading to improve citrate production via prostate cellular system, as observed in seminal plasma of post-treated infertile patients in this study.

NMR spectroscopy determined the decrement of GPC content in pre-treated seminal plasma samples of infertile patients, which is agreed to the literature [8]. The observation accounted that phosphatidylcholine of blood lipoprotein could not generate GPC, possibly because of the consequences of the abnormal milieu of the vas deferens and epididymis [26] causing a diminished GPC content in seminal plasma. *M. pruriens* contains immense amounts of total lipids (β-sitosterol, lecithin) [7]. The irrefutable information from the literature [27] suggests that these lipids play key roles not only in the synthesis of GPC and lipoproteins but also in maintaining the structural and functional integrity of the spermatozoa. In light of the information from the literature, and our results suggest that *M. pruriens* therapy increases important lipids, which in turn repair the harmonic balance of GPC, lipoproteins, essential lipids, morphology of spermatozoa, and energy metabolism of lipids in infertile patients leading to increase GPC excretion via vas deferens and epididymal epithelial cells, as observed in seminal plasma by NMR spectroscopy and clinical variables in serum samples of post-treated infertile patients in the present study.

The lowered pool size of histidine was observed in all pre-treated infertile patients when comparing to controls. Except AZ patients, it was not statistically significant in NZ and OZ patients, when compared to CZ. Fibrotic thickening of the testicular peritubular region is a common observation in infertile patients and parallels the increased numbers of activated testicular mast cells in these regions. The mast cells, via their major secretory product histamine, are crucially and causally involved in these events and closely associated with male infertility [28]. In mammals, the only source of histamine is histidine, and it is synthesized through histidine decarboxylase (HDC). HDC is localized in the acrosomes of spermatids and spermatozoa. A substantial amount of histamine and HDC activity has been detected in the testis, epididymis, and spermatozoa [29]. $W/W^V$ mice, known to lack most of their germ cells in the seminiferous tubules, were found to lack HDC protein expression as well as HDC activity in the testis [29]. These reports support the decreased amount of histidine in seminal plasma of infertile patients in our study. Reports suggest that the role of histidine is to prevent the increase in concentration of lipid hydroperoxides and the loss of polyunsaturated fatty acid content in sperm plasma phospholipids [30]. Conversely, loss of histidine levels lead to increases in the lipid peroxidation process, as observed in our study in pre-treated infertile patients when compared to the NMR-derived histidine level and the LPO of clinical variables. Three months of *M. pruriens* treatment augments histidine production in seminal plasma and concomitantly reduction in lipid peroxidation, as observed in post-treated infertile patients.

A substantial amount of phenylalanine was observed in pre-treated NZ and OZ infertile patients, this agreeing with a previous report [8]. The digestion of phenylalanine follows one of the two pathways. First, conversion into tyrosine by phenylalanine hydroxylase and second, binding to a large neutral amino acid transporter (NAAT) to be carried over the blood–brain barrier [31]. Phenylalanine and tyrosine, and various other metabolites (such as tryptophane, methionine, and the branched-chain amino acids) compete with each other at the binding site of NAAT, because this is the only way in which they can cross the blood–brain barrier [31]. Consequently, with very high competition for NAAT transporter among them leads to a large quantity of one amino acid in the blood stream occupying most of this transporter. This results in a phenylalanine overload in the surrounding areas, greatly limiting the amount of important amino acids entering the brain [31]. Probably, phenylalanine having high specificity for NAAT, as compare to tyrosine, a compromised dopamine production will result because phenylalanine binds more frequently and freely than tyrosine owing to its higher concentration, and thus lead to lower concentration of dopamine in the brain [31], supporting the lower concentration of dopamine in pre-treated infertile patients [16]. The lower concentration of dopamine conversely contributes to an increase in the tyrosine level in the brain, as this is a very important amino acid acting as a precursor of DOPA via tyrosine hydroxylase, and the DOPA converts into dopamine through the decarboxylation process. The report says that lower tyrosine hydroxylase activity as well as content leads to increased phenylalanine in the blood [31]. *M. pruriens* seeds contain the enzyme tyrosine hydroxylase [4,5], which increases this enzyme in the human system over long-term therapy with *M. pruriens* and reduces the phenylalanine content in infertile patients. The other possible mechanism explains that the decreased cAMP activity causing lowered complexity of the tight junctions of endothelial cells of blood–brain barrier results compromised NAAT transporter activity consequently increase in phenylalanine content [31]. Long-term of *M. pruriens* treatment possibly augments phenylalanine catabolism with the respective enzyme and increases the cAMP activity from adenosine triphosphate, as explained above, in infertile patients. Therefore, lowered phenylalanine content was observed in seminal plasma of post-treated NZ and OZ infertile patients.

## 4. Conclusions

In essence, it may be stated that alterations of endogenous metabolites commonly precede the onset of infertility, and *M. pruriens* therapy rectifies the harmonic balance of these endogenous metabolites through the orchestra of the central nervous system of the brain. *M. pruriens* therapy is capable of restoring sexual interest and power by acting as a restorative invigorating tonic and aphrodisiac. This study demonstrated the positive impact of *M. pruriens* on the harmonic balance of hormones, carbohydrate metabolism, lipolysis of fat, enzymatic activity of TCA cycle, and energy metabolism and repair alanine, citrate, GPC, histidine, and phenylalanine metabolites contents in seminal plasma. In brief, *M. pruriens* is able to resolve the complex disease panorama of infertility by its action on the various hormonal and metabolic pathways. *M. pruriens* may be used as a best surrogate drug for the improvement and therapeutic management of infertility.

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