



Ningmitai capsule improves the semen quality of male infertile with chronic prostatitis by antioxidant and anti-inflammatory

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Academic Editor: Daishu Han, Chinese Academy of Medical Sciences & Peking Union Medical College, China

Received: May 16, 2023 **Accepted:** July 6, 2023 **Published:** December 14, 2023

Cite this article: Wu X, Ye Z, Li L, Zhang H, Huang X, Chai J, et al. Ningmitai capsule improves the semen quality of male infertile with chronic prostatitis by antioxidant and anti-inflammatory. *Explor Immunol.* 2023;3:590–7. <https://doi.org/10.37349/ei.2023.00123>

Abstract

Aim: To observe the effects of Ningmitai capsule on semen parameters of infertile patients with chronic prostatitis (CP) and explore the mechanisms.

Methods: A total of 43 patients diagnosed with CP were included in the study and administered Ningmitai capsules (4 capsules per dose) for a duration of 6 weeks. Subsequently, assessments were conducted on parameters including sperm concentration, forward progressive motility, total motility, oxidative and anti-oxidative indicators, as well as the concentration of interleukin-8 (IL-8) in seminal plasma before and after the treatment period.

Results: Compared to pre-treatment, forward progressive motility and total motility of the semen increased significantly ($37.15\% \pm 18.77\%$ vs. $45.44\% \pm 19.08\%$, $P < 0.05$ and $42.56\% \pm 21.22\%$ vs. $51.64\% \pm 19.48\%$, $P < 0.05$). And the expression of superoxide dismutase (SOD) and total antioxidant capacity (T-AOC) increased significantly after treatment as well ($11.36 \mu\text{mol/L} \pm 3.28 \mu\text{mol/L}$ vs. $12.79 \mu\text{mol/L} \pm 2.87 \mu\text{mol/L}$, $P < 0.05$ and $9.34 \text{ U/mL} \pm 3.22 \text{ U/mL}$ vs. $11.21 \text{ U/mL} \pm 4.87 \text{ U/mL}$, $P < 0.05$). In addition, the expression of malondialdehyde (MDA) and IL-8 decreased significantly after treatment ($41.06 \mu\text{mol/L} \pm 24.39 \mu\text{mol/L}$ vs. $32.17 \mu\text{mol/L} \pm 15.04 \mu\text{mol/L}$, $P < 0.05$ and $79.69 \text{ ng/L} \pm 26.24 \text{ ng/L}$ vs. $61.35 \text{ ng/L} \pm 23.41 \text{ ng/L}$, $P < 0.05$). No significant difference in sperm concentration and sperm DNA fragmentation index was observed after the treatment ($P > 0.05$).

Conclusions: Ningmitai capsule can enhance the antioxidant capacity and down-regulate the expression of cytokines in the semen, thereby improving the semen parameters of infertile patients with CP.



Keywords

Ningmitai, infertile patients, prostatitis

Introduction

Chronic prostatitis (CP) is a very common urogenital disease of male with prevalence ranging from 6.0% and 32.9% in China [1]. It is reported that approximately 40% of male infertilities suffer from CP, which has a certain influence on male fertility [2], and it has been speculated that inflammation in the prostate gland promotes an autoimmune response, which might be leading to deleterious effects on semen quality and function [3, 4]. There is also literature indicating that poor semen quality has been linked with the potential role of multiple cytokines, such as seminal interleukin-6 (IL-6), IL-8, IL-10, IL-12, and IL-18 and tumor necrosis factor- α (TNF- α) [4, 5]. Seminal reactive oxygen species (ROS) are known to be generated in response to infection or inflammation stimuli [6]. Increased level of ROS is associated with human infertility in several ways, which may lead to damage of cell membranes, intracellular proteins, organelles, and sperm DNA, with subsequent impairment of sperm motility and decreased seminal total antioxidant capacity (T-AOC) [6, 7]. Hence, the treatment of antioxidant and anti-inflammatory can be used to improve sperm quality of CP patients with male infertility, as a potential treatment.

A formulated Chinese medicine, Ningmitai capsule, has been used in CP patients for its antioxidant anti-inflammation function [8, 9]. Ningmitai capsule is composed of Touhualiao (Herba Polygoni Capitati), Baimaogen (Rhizoma Imperatae), Dafengteng (Radix Cocculi Trilobi), Sankezhen (Berberidis radix), Xianhecao (Herba Agrimoniae), and Lianqiao (Fructus Forsythiae suspensae). However, it is not clear whether Ningmitai capsules can improve the infertility caused by CP. In this study, a pilot trial was conducted to examine the impact of Ningmitai capsule on the semen parameters of infertile patients with CP and to explore potential underlying mechanisms.

Materials and methods

Study sample

From December 2014 to December 2015, a total of 43 male infertile patients were enrolled in this study, who were diagnosed with CP, in the Center of Reproductive Medicine, Tongji Medical College, Huazhong University of Science and Technology.

Diagnostic criteria

The diagnostic criteria for male infertility refers to men who have cohabitation and regular sex life without contraception for more than 1 year, and exclude the infertility factors caused by the female. Meanwhile, patients were diagnosed with CP as follows: (1) the main clinical symptoms: urinary tract stimulation syndrome, prostatic discharge, pain in the pelvic region, sexual function obstacles, and mental and neurological symptoms; (2) rectal examination: the surface of the prostate can be uneven, with uneven hardness or tenderness, the volume can be reduced, and there can be limited hard nodules and/or tenderness; (3) expressed prostate secretions (EPS) examination: decrease or disappearance of lecithin bodies in EPS, white cell count > 10/high power field (HPF).

Inclusion and exclusion criteria

Inclusion criteria

The study included patients who met the following criteria: (1) meeting the diagnostic criteria of CP infertility; (2) age from 20 to 40; (3) patients didn't take Chinese and Western medicine for treating CP in nearly 2 weeks.

Exclusion criteria

The study excluded patients who met the following criteria: (1) male infertility caused by non-CP including chromosomal abnormalities, endocrine diseases, and heredity; (2) patients suffering from acute urinary

system infections such as acute prostatitis, seminal vesiculitis, urethritis, and genital organic diseases; (3) patients suffering from primary cardiovascular, nervous system and other important organ diseases, and allergic; (4) patients with other medical history, such as diabetes mellitus and hypertension.

Research methods

Treatment method

The patients were treated with Ningmitai capsule (Guiyang Xintian Pharmaceutical Corporation, specifications: 0.38 g/capsule) orally, 4 capsules/time, 3 times a day, continuously for 6 weeks. During the treatment, it is forbidden to eat spicy food and other drugs for treatment of prostate disease.

Specimen collection

Semen samples were taken before and at the end of the treatment, and all subjects should be abstinent for 2 days to 7 days. Masturbation was adopted. Then semen was collected into a sterile container, and submitted within 5 min for semen analysis. Standard semen analysis performed according to World Health Organization criteria included total sperm count, normal sperm morphology, percent motility, and various sperm motility characteristics (velocity, linearity) using a computer-assisted semen analyzer (CASA, sperm analysis system WLJY-9000, WeiLi Medical, China). Sperm DNA fragmentation detection kit (BRED-002, Bred Life Science, China) was used to detect sperm chromatin dispersion (SCD), and the instructions were strictly followed. After acid denaturation and removal of nuclear proteins, by Reye's staining, sperm with fragments would not show a characteristic halo, while sperm without fragments would show a characteristic halo. The degree of DNA fragmentation was defined according to the presence and size of the halo. DNA-damaged sperm halos are small or absent, and DNA-intact sperm diffuse halos larger than 1/3 of the larger diameter of the head. Five hundred sperms were counted under high magnification and the proportion of sperm damaged by DNA was defined as the sperm DNA fragmentation index (DFI).

Biochemical assays

The seminal plasma was collected for biochemical parameter analysis. T-AOC was measured in accordance with the protocols of T-AOC assay kit (A015-1-1, Nanjing Jiancheng Bioengineering Institute, China). Lipid peroxidation was determined by monitoring the reaction of malondialdehyde (MDA) (A003-2-1, Nanjing Jiancheng Bioengineering Institute, China) with thiobarbituric acid as described in another study, and the MDA concentration was expressed as nmol/mg protein [10]. Superoxide dismutase (SOD) activities were also measured following the protocols of total SOD assay kit (A001-1-1, Nanjing Jiancheng Bioengineering Institute, China). One unit of SOD activity was defined as the amount of enzyme required to inhibit the oxidation reaction by 50% and was expressed as U/mg protein. Protein concentration was determined as a detection reagent for cut following the reduction of copper (Cu)²⁺ by protein in an alkaline environment by bicinchoninic acid (BCA) protein assay kit (catalog number: 23227, Thermo Scientific, USA). Measurements were performed on a microplate reader (Synergy™ HTX, BioSPX, USA), using a microwell plate protocol at 562 nm in accordance with the manufacturer's instructions. All biochemical parameters were measured in triplicate.

Cytokines and other immunologic factors in seminal plasma

Cytokine and chemokine determinations were performed on seminal plasma, using sandwich enzyme immunoassay for IL-8. Standard curves were developed for cytokine IL-8 according to the manufacturer's instructions. All test parameters were measured in triplicate.

Statistical analysis

Statistical analysis was performed using the Statistical Product and Service Solutions (SPSS) software (version 19.0) after checking and confirming. Mean ± standard deviation (SD) was employed for the measurement data. *t*-test was performed to compare the difference in each indicator before and after treatment. *P* < 0.05 was considered statistically significant.

Results

Baseline characteristics

A total of 57 patients were enrolled in the study, and 14 patients withdrew during the treatment (Figure 1). The average age was 30.6 years \pm 5.6 years. The average duration of CP was 3.1 years \pm 1.4 years in the patients. And the other clinical characteristics are shown in Table 1.

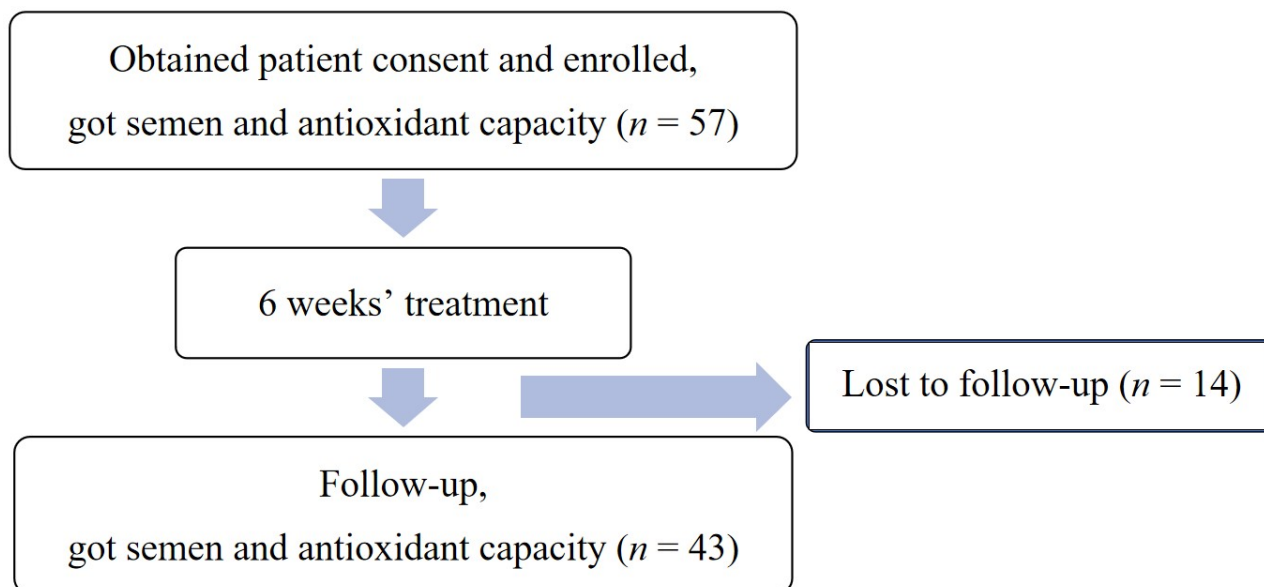


Figure 1. Patient screening flowchart

Table 1. Comparison of the semen parameters before and after treatment

Characteristics	Total (n = 43)
Age, years	30.6 \pm 5.6
Disease course, months	3.1 \pm 1.4
Body mass index	24.3 \pm 2.8
Storage symptoms	38 (88.4%)
Frequency	32 (74.4%)
Urgency	29 (67.4%)
Incontinence	25 (58.1%)
Nocturia	5 (11.6%)
Voiding symptoms	31 (72.1%)
Straining to void	24 (55.8%)
Hesitancy	20 (46.5%)
Slow stream	11 (25.6%)
Post micturition symptoms	29 (67.4%)
Feeling of incomplete emptying	25 (58.1%)
Terminal dribble	10 (23.3%)
Pains	37 (86.0%)

Ningmitai capsule improved the semen parameters of infertile patients with CP

Following 6 weeks' treatment with Ningmitai capsule, the forward progressive motility ($t = 2.031$, $P = 0.0454$) and the total motility ($t = 2.067$, $P = 0.0418$) of the semen increased significantly, but the sperm concentration and sperm DFI did not change compared to pre-treatment ($t = 0.3819$, $P = 0.7035$). These results indicated that Ningmitai capsule could improve the semen parameters of infertile patients with CP. The semen parameters of the patient are shown in Table 2.

Table 2. Comparison of the semen parameters before and after treatment

Group	n	Forward progressive motility (%)	Total motility (%)	Concentration (%)	Sperm DFI (%)
Pre-treatment	43	37.15 ± 18.77	42.56 ± 21.22	55.43 ± 66.96	20.5 ± 3.12
Post-treatment	43	45.44 ± 19.08*	51.64 ± 19.48*	60.73 ± 61.64	24.2 ± 2.71

* $P < 0.05$

Comparison of antioxidant capacity of semen pre- and post-treatment

To explore the mechanisms of the improvement of the semen parameters by Ningmitai capsule, the antioxidant ability of semen was evaluated, and the results showed that SOD ($t = 2.152$, $P = 0.0343$) and T-AOC ($t = 2.100$, $P = 0.0387$) increased significantly after six weeks' treatment, whereas MDA decreased significantly ($t = 2.034$, $P = 0.0451$) (Table 3).

Table 3. Comparison of the semen antioxidant capacity and expression of cytokines before and after treatment

Group	n	SOD (μmol/L)	T-AOC (U/mL)	MDA (μmol/L)	IL-8 (ng/L)
Pre-treatment	43	11.36 ± 3.28	9.34 ± 3.22	41.06 ± 24.39	79.69 ± 26.24
Post-treatment	43	12.79 ± 2.87*	11.21 ± 4.87*	32.17 ± 15.04*	61.35 ± 23.41*

* $P < 0.05$

Comparison of inflammation index of semen pre- and post-treatment

The levels of cytokines in the semen were assessed, revealing a significant decrease in IL-8 following treatment with Ningmitai capsules. Before the treatment of Ningmitai capsule, IL-8 was 79.69 ng/L, and after the treatment, the concentration was 61.35 ng/L ($t = 3.420$, $P = 0.0010$).

Discussion

Male infertility occurs in 40% of CP patients, and its pathogenesis is unclear. The possible reason is that the accumulation of ROS and cytokines leads to the decrease in sperm quality. A classical traditional Chinese medicine, Ningmitai capsule, with the function of antioxidant and anti-inflammatory, has been used in CP treatment. This study was conducted to appraise the function of Ningmitai capsule in male infertility of CP patients. The study aimed to examine whether the administration of Ningmitai capsules could enhance fertility in male patients with CP by improving sperm motility and semen quality.

Chinese medicine believes that CP belongs to the category of "semen turbidity" and "white turbidity". Hot and humid bets, blood stagnation and stasis, and deficiency of kidney often occur. Ningmitai capsule belongs to traditional Chinese medicine preparation. It is made up of Chinese herbs such as four seasons flowers, *Adenophora*, *Forsythia*, Rhizoma Imperatae, agrimony, three needles, lotus leaves, etc. It has the effects of heat-clearing, detoxifying, cooling and hemostasis, and diuresis-passing. It is effective in inhibiting bacteria, killing bacteria, diluting diuretics, and enhancing immunity [9]. The research of animal experiment found Ningmitai capsule can restore weight gain *in vivo* with bacterial prostatitis, eliminate local inflammation, inhibit glandular fibrous tissue proliferation, promote glandular secretion, and improve pathological vasodilation state [9]. Multiple meta-analysis results showed that Ningmitai treatment of CP could improve the score of the National Institute of Health's CP symptom index. The use is safe, the effect is better than that of Qianliekang, and the efficacy is equivalent to ofloxacin and Qianlieantong, and the combination of antibiotics can improve the curative effect [8].

Several studies have shown that Ningmitai capsule can effectively improve the semen quality of patients with CP infertility, increase semen antioxidant capacity, and inhibit the expression of inflammatory factors [9], which are consistent with the results of this study. The possible mechanisms are that Ningmitai capsule can inhibit the inflammation of genital tract, and relieve the obstruction of genital tract so that the stasis of prostatic fluid can be discharged. At the same time, it can relax the smooth muscles of the bladder and posterior urethra, reduce the pressure of the posterior urethra, prevent the reflux of urine and semen, and inhibit the production of anti-sperm antibodies. In addition, Ningmitai capsule can shorten the time of

semen liquefaction, reduce semen viscosity, and improve the content of trace elements and fructose in seminal plasma to improve sperm survival and vitality.

This study showed that Ningmitai capsule could reduce levels of oxidation and increase antioxidant capacity post-treatment. Chen et al. [8] illustrated that oxidative stress can induce peroxidation of membrane lipids and cause sperm damage. This study shows that after 6 weeks of treatment with Ningmitai capsules, the IL-8 chemokines were significantly reduced, indicating that Ningmitai capsules can inhibit local inflammatory responses by reducing the expression of inflammatory factors. IL-8 is a pro-inflammatory cytokine, which is generated by monocytes, macrophages, fibroblasts, endothelial cells, and multiple epithelial cells following inflammatory stimulation. The main feature is to help recruit granulocytes and monocytes into the inflammatory site and regulate the expression of leukocyte adhesion molecules, which is necessary for the above cells to leave the blood circulation and penetrate the tissue [11]. Pro-inflammatory cytokines can increase the expression of cyclooxygenase-2, inducible nitric oxide synthase, prostaglandins, and intercellular adhesion molecules. The increased expression product in turn causes damage to the prostate tissue and causes many complex symptoms of prostatitis through its respective mechanisms [12, 13]. Sperm lipid peroxidation rises concomitantly with the increase of IL-6 [14]. And Seshadri et al. [15] found that IL-6, IL-8, IL-10, IL-12, and TNF- α did not affect fertilization rates *in vitro* fertilization.

This study has initially found that Ningmitai capsules improve semen quality in male infertility patients with CP, which can further improve male fertility. However, due to the limited number of patients included in this study, further study will expand the study sample size, increase clinical pregnancy outcomes and other indicators, extend the follow-up time, and further explore its influence on male fertility and mechanism of action from the molecular level of cells. Further studies focused on other cytokines should be considered.

Abbreviations

CP: chronic prostatitis

DFI: DNA fragmentation index

IL-8: interleukin-8

MDA: malondialdehyde

ROS: reactive oxygen species

SOD: superoxide dismutase

T-AOC: total antioxidant capacity

Declarations

Author contributions

XW and ZY equally contributed to: Conceptualization, Investigation, Writing—original draft, Writing—review & editing. LL: Validation, Writing—review & editing, Supervision. HZ: Validation, Writing—review & editing. XH: Writing—review & editing, Supervision. JC: Conceptualization, Supervision. XZ: Validation, Supervision. All authors read and approved the submitted version.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Ethical approval

This study was approved by the Ethics Committee of Center of Reproductive Medicine of Tongji Medical College (201405). The study involving human research adheres to the ethical standards outlined in the Declaration of Helsinki.

Consent to participate

The informed consent to participate in the study was obtained from all participants.

Consent to publication

Not applicable.

Availability of data and materials

All relevant data is contained within the manuscript.

Funding

Not applicable.

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