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Experimental Research on Therapeutic Efficacy of Traditional Chinese Medicine Shengjing Capsule Extracts in Treating Spermatogenesis Impairment Induced by Oxidative Stress

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Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Background: To investigate antioxidant effects of traditional Chinese Shengjing capsule extracts (sperm-producing capsule, with functions of tonifying kidney and invigorating kidney essence) on testes, epididymides, and sperms of rats.





Material/Methods: We randomly divided 50 rats into 5 groups. G1: normal control group (treated with saline); G2: cadmium chloride group; G3: cadmium chloride+ low doses of drugs; G4: cadmium chloride + medium doses of drugs; and G5: cadmium chloride + high doses of drugs (equivalent dose: 0.45 g/kg). In addition to the normal control group, the other 4 groups started receiving intraperitoneal injection of cadmium chloride (1 mg/kg, i.p.). Testicular glutathione peroxidase (GSH-PX), superoxide dismutase (SOD), and malondialdehyde aldehyde (MDA) were measured by ELISA; epididymis histopathological examination was performed; testis serum testosterone (T) was measured; specimens of the epididymal semen were analyzed for sperm concentration, morphology, vitality, and DNA fragmentation rate.

Results: Sperm count and activity of rats in the model control group decreased significantly; their MDA concentration of testicular and epididymal homogenates increased greatly; while the vitality of SOD and GSH-Px dropped sharply. All indexes mentioned above were significantly different from those of the blank control group ($P < 0.05$); the sperm count and activity of rats treated with Shengjing capsule (sperm-producing capsule) decreased, but were still significantly higher than those of the model group ($P < 0.05$). MDA level of rats treated with Shengjing capsule were significantly lower than that of the model group ($P < 0.05$), while their SOD and GSH-Px activity were significantly higher than the model group ($P < 0.05$). The normal morphology rate and DNA integrity rate of groups treated with Shengjing capsule were significantly higher than those of the model group ($P < 0.05$).

Conclusions: Shengjing can enhance the activity of antioxidant enzymes and inhibit oxidative stress. It can also repair testicular and epididymal pathological damages, protect spermatogenesis, increase sperm count and activity, and improve normal morphology rate of sperm.

MeSH Keywords: **Asthenozoospermia • Medicine, Chinese Traditional • Reactive Oxygen Species • Testis**

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Background

In biological systems, free radicals and reactive oxygen species (ROS) are two key factors in maintaining normal physiological conditions, while free radicals, of an exceedingly high or low amount, will cause damages of different degrees on biological macromolecules. Reactive oxygen species (ROS) play a crucial role in sperm movement, capacitation and acrosome reaction. Therefore, a certain amount of ROS can sustain sperm motility, while excessive ROS will damage sperm membrane, inhibit sperm motility and decrease sperm vitality, which all have a bad influence on egg-sperm binding; the excessive ROS can also damage sperm DNA and the defective DNA might be passed to the fetus by sperm alteration. Researches have already proved that damages on sperm DNA are closely related to active oxygen [1]. Thus, currently, the antioxidant drugs have been attached great importance in treating male infertility both at home and abroad [2,3]. In this study, rats model of oligoasthenospermia prepared by cadmium poisoning were used to detect antioxidation effects of traditional Chinese Shengjing capsule (with functions of tonifying kidney and invigorating kidney essence) on rats' testes, epididymides and sperms and its protection against testicular pathological damages induced by oxidative stress. The mechanism of traditional Chinese medicine in treating male infertility has also been explored in this study.

Material and Methods

Laboratory animal

SPF Wistar rats, purchased from Experimental Animal Center of Southern Medical University; Laboratory Animal License Number: SCXK (Yue) 2011-0015. The rats were put into experiment after a week of conventional breeding. Animal experiment location: 1st Affiliated Hospital of Guangzhou University of Traditional Chinese Medicine; Certificate Number of Environment and Facilities: SYXK (Yue) 2013-0092; temperature: 20–25°C; humidity: 40–70%.

Tested drug

Shengjing capsule extracts, provided by Liao Yuan He Tang Pharmaceutical Co., Ltd.; clinical dosage: 0.4 g per capsule, 4 capsules per time, 3 times per day; in order to ensure the experimental quality, the experimental Shengjing capsule were extracted by ultrasonic extraction method according to certain formula.

Main instruments and reagents

Tu-1810PC UV / Vis spectrophotometer (Beijing Puxi General Instrument Co., Ltd.); XW-80A vortex mixer (Kylin-bell

Manufacturing Co. Ltd, Haimen, Jiangsu); MK3 microplate reader (Guangdong Stanley company); BX50 fluorescence microscope (Olympus Corporation of Japan); AE200 electronic analytical balance (Mettler). Formaldehyde, produced by Guangzhou Mid-South Chemical Reagent Co., Ltd. (Batch No: 131202); Chloral hydrate, produced by Sinopharm Chemical Reagent Co. Ltd (Batch No: 20080327); MDA, produced by Nanjing Jiancheng Bioengineering Institute (Batch No: 20130425); Glutathione peroxide enzyme, produced by Nanjing Jiancheng Bioengineering Institute (Batch No: 20130424); Superoxide dismutase, produced by Nanjing Jiancheng Bioengineering Institute (Batch No: 20130424); Rat T ELISA Kit From USA components assembled in China (Expiration 10/2013).

Experimental methods

methods of randomized control, model control and normal control were adopted in this study. 50 rats were randomly divided into five groups and each group included 10 rats. G1: normal control group (treated with saline); G2: cadmium chloride group; G3: cadmium chloride + low doses of drugs; G4: cadmium chloride + medium doses of drugs; G5: cadmium chloride + high doses of drugs (equivalent dose for rats: 0.45 g/kg). Once a rat died, another rat would be included into the group. The high, medium and low doses, which were 1.80 g/kg, 0.90 g/kg and 0.45 g/kg respectively, were 4, 2 and 1 times the equivalent dose, 0.45 g/kg. In addition to the normal control group, the other four groups started receiving intraperitoneal injection of cadmium chloride (1 mg/kg, i.p.) on the first day of the experiment and after 24 h of the injection, each group was administered the Shengjing capsule intragastrically. All rats took the same volume of solvent. The intragastric administration was given for 56d in a row. After that, the rats were anesthetized to obtain specimens for tests of corresponding indexes. The rats' testes, epididymides and seminal vesicles were weighted to get their organ indexes; their testicular glutathione peroxidase (GSH-PX), superoxide dismutase (SOD) and malondialdehyde aldehyde (MDA) were measured by ELISA; epididymis histopathological examination was taken; testis serum testosterone (T) was measured; specimen of rats' epididymal semens were taken to analyze sperm concentration, morphology, viability and to calculate DNA fragment rate.

To determine the sperm morphology, the cauda epididymis of all animals was separated and transferred into a Petri dish containing 1.5 mL of 0.9% normal saline, and minced. After squeezing the slices, spermatozoa were vented to the surrounding fluid. A drop of Petri dish solution was transferred onto Neubauer hemocytometer lam (HBG Company, Giessen, Germany) (Tiefe Depth Profondeur 0.100 mm; area: 0.0025 mm²). Then, the exact sperm morphology was manually assayed in white blood cell chambers by light microscopy (Olympus Light Microscope, Tokyo, Japan).

Table 1. Influences of Shengjing capsule on GSH-PX, SOD, and MDA levels of model rats' testes ($\bar{x}\pm S$, n=10).

Group	Dose (g/kg)	GSH-PX (U/ mgprot)	MDA (nmol/mgprot)	SOD (U/mgprot)
Normal control group	/	33.4±8.2*	29.4±4.4*	17.3±4.3
Model control group	/	23.6±9.7	35.6±5.7	12.9±2.4
Low dose group	0.45	31.3±9.7	34.0±8.4	15.0±2.4
Medium dose group	0.90	34.2±6.2**	29.6±5.5*	15.9±2.5*
High dose group	1.80	32.5±19.7*	27.6±6.0**	17.3±4.3*

Compared with the model control group: * P<0.05; ** P<0.01.

Table 2. Impacts of Shengjing capsule on T serum level of rats model ($\bar{x}\pm S$, n=10).

Group	Dose (g/kg)	T (nmol/L)
Normal control group	/	4.42±1.09*
Model control group	/	3.24±0.83
Low dose group	0.45	3.94±1.14
Medium dose group	0.90	4.18±0.99*
High dose group	1.80	4.30±0.71*

Compared with those of the model control group: * P<0.05.

Sperm viability was assessed by the staining of eosin 1% (Merck Chemical Co., Darmstadt, Germany) used for the evaluation of live (unstained) and dead (red stained) sperm. After leaving this stain on each blood cell chamber of the Neubauer hemocytometer for 30 seconds, we manually counted the total number of live spermatozoa within two minutes and expressed it as the sperm viability percentage.

Statistical analysis

Statistical analyses were performed by SPSS statistical analysis software; all data were shown in ($\bar{x}\pm S$). P <0.05 means that the difference is of statistical significance; P <0.01 means it is of significant statistical significance.

Results

Influences of Shengjing capsule on GSH-PX, SOD and MDA levels of model rats' testes

Significant differences could be found in testicular GSH-PX, SOD and MDA levels between the normal group and the model group, which meant that the experimental model was established successfully. After drug intervention, GSH-PX and SOD levels of medium-dose group and high-dose group increased significantly, while the MDA levels of the two groups decreased sharply (Table 1).

Influences of Shengjing capsule on rats model's T serum

T serum level of rats in the model group was significantly lowered and there was a significant difference between it and that of the normal control group, which meant that the rat model was successfully established. The T serum levels of rats in medium and high doses group increased significantly after drug intervention (Table 2).

Influences of Shengjing capsule on indexes of model rats' testes, epididymides and seminal vesicles

The testis indexes, epididymis indexes and seminal vesicle indexes of model group lowered more significantly than those of normal control group. The testis indexes and epididymis indexes of medium dose and high dose group increased more than those of the model group; the seminal vesicle index of high dose group increased; organ indexes of the four groups increased more significantly than those of the model group (Table 3).

Influences of Shengjing capsule on sperm motility of rats model

Compared with normal control group, sperm motility of model group decreased more significantly. The sperm motility a+b of high dose, medium dose and low dose group increased remarkably, while the sperm motility d of them reduced significantly.

Table 3. Influences of Shengjing capsule on testis index, epididymis index and seminal vesicle index ($\bar{x}\pm S$, n=10, g/100 g).

Group	Dose (g/kg)	Testis index	Epididymis index	Seminal vesicle index
Normal control group	/	0.424±0.014**	0.174±0.025**	0.468±0.053**
Model control group	/	0.372±0.020	0.132±0.031	0.365±0.039
Low dose group	0.45	0.414±0.040*	0.155±0.024	0.405±0.044
Medium dose group	0.90	0.416±0.050*	0.164±0.032*	0.405±0.050
High dose group	1.80	0.423±0.025**	0.171±0.020**	0.406±0.034*

Compared with model control group: * P<0.05; ** P<0.01.

Table 4. Influences of Shengjing capsule on sperm motility of rats model.

Group	Dose (g/kg)	Mobility a+b	Mobility c	Mobility d
Normal control group	/	43.8±9.3**	6.17±4.67	50.0±6.4**
Model control group	/	12.2±3.5	4.46±1.97	83.3±4.8
Low dose group	0.45	36.9±6.7**	6.61±1.78*	56.5±5.6**
Medium dose group	0.90	48.8±3.2**	7.85±2.76**	43.3±3.9**
High dose group	1.80	40.5±10.4**	5.44±3.96	54.0±10.2**

Compared with the model control group: * P<0.05; ** P<0.01.

Table 5. Influences of Shengjing capsule on model rats' sperm morphology.

Group	Dose (g/kg)	Abnormal sperm morphology rate	Normal sperm morphology rate
Normal control group	/	2.39±0.87**	97.6±0.9**
Model control group	/	8.67±4.55	91.3±4.6
Low dose group	0.45	6.42±2.72	93.6±2.7
Medium dose group	0.90	3.83±1.17**	96.2±1.2**
High dose group	1.80	4.78±4.19	95.2±4.2

Compared with the model control group: ** P<0.01.

The sperm motility c in medium dose group and low dose group increased prominently (Table 4).

Influences of Shengjing capsule on model rats' sperm morphology

Abnormal sperm morphology rate of the medium dose group decreased abruptly, while its normal sperm morphology rate increased significantly; the abnormal sperm morphology rate of the high dose group showed a downward trend, while there was an increasing trend of its normal morphology rate, but both of them were of no statistical significance (Table 5).

Influences of Shengjing capsule on model rats' DNA fragments

Normal DNA fragment rate of high dose, medium dose and low dose group were significantly higher than before, while their abnormal rate decreased abruptly (Table 6).

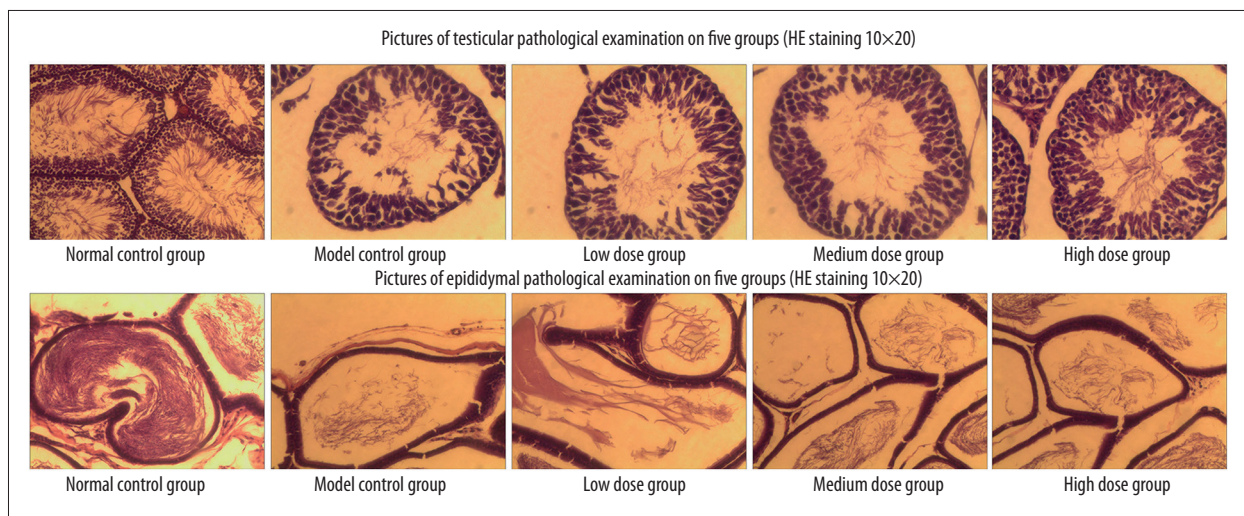
Histopathological observation on reproductive toxicity of Shengjing capsule

Compared with the normal control group, spermatogenesis of model control group was reduced more. It was manifested by atrophy and sparse arrangement of contorted seminiferous tubules, reduced spermatogenic cells, increasingly thinning seminiferous epithelium, less intracavitary mature sperms

Table 6. Influences of Shengjing capsule on model rats' DNA fragments.

Group	Dose (g/kg)	Normal DNA fragment rate	Abnormal DNA fragment rate
Normal control group	/	97.0±1.6**	3.05±1.57**
Model control group	/	92.9±2.5	7.13±2.54
Low dose group	0.45	96.6±1.6**	3.44±1.61**
Medium dose group	0.90	96.2±1.7**	3.85±1.67**
High dose group	1.80	96.8±1.8**	3.20±1.78**

Compared with model control group: ** P<0.01.

**Figure 1.** Histopathological observation on reproductive toxicity of Shengjing capsule.

and seminal vesicle secretions, and atrophic papillary epithelial. The incidence of spermatogenesis hypofunction gradually decreased as the dosage of Shengjing capsule increased, which meant that the therapeutic efficacy and dosage were closely interrelated. (Figure 1) This study has proved that Shengjing capsule had certain therapeutic effects on spermatogenesis hypofunction.

Discussion

ROS is a highly active oxide containing free radicals, such as H_2O_2 , O_2^- , OH^- , and NO . It can alter cell functions and endanger cell survival. Normally, ROS in testis and epididymis must be constantly inactivated to keep it in control in order to maintain sperms' normal physiological functioning. The antioxidant system of reproductive system includes superoxide dismutase (SOD), a variety of peroxidases, such as glutathione peroxidase (GPX), catalase (CAT) and other heme protein peroxidases. SOD, which can eliminate O_2^- and protect sperm from ROS toxicity, is the body's most important antioxidant enzyme. It plays an important role in maintaining the balance of our antioxidant defense system. There is a positive correlation between

SOD levels and sperm count [4]. GSH-Px reacts directly with hydrogen peroxide, superoxide anion and hydroxyl through its sulfate groups. Thiol groups can react with alkoxy and hydrogen peroxide and have a strong scavenging ability to H_2O_2 produced by active oxygen species and active tissues, which can effectively protect the integrity of the structure and function of cell membrane. Once any member of these mutual protection systems was weakened or reduced, the whole enzyme protection system might crumble, leading to irreversible cell damages [5].

Sperms are particularly sensitive to damages caused by excessive ROS, because there are a large number of membrane polyunsaturated fatty acids (PUFA) and ROS scavenging enzymes of low concentration in sperms' plasmalemma. The intracellular antioxidant enzyme cannot protect the plasma membrane which covers the top and end of cell body. It forces sperms to rely on the protection provided by seminal plasma to support its own limited antioxidant system. Excessive production of ROS in reproductive tract can both damage the fluidity of sperm plasma membrane and the integrity of DNA in sperm nucleus, which can lead to spermatogenesis dysfunction and toxic effects on sperms, causing the lipid peroxidation of sperm membrane.

The lipid peroxidase and their degradation products, such as MDA, etc. may cause metabolic disorder, abnormal morphology and sperm dysfunctions, leading to male infertility [6–8]. It has been proved that excessive ROS can mediately damage sperm DNA and produce abnormal sperms, which will work vice versa [9]. Malondialdehyde (MDA) is the end product of membrane lipid peroxidation; it serves as a marker of degree of oxidative damage in a cell and reflects the conditions of free radical production. The MDA concentration of testicular and epididymal homogenates of the model group increased significantly, which suggested severe oxidative damages in rat testes and epididymides.

Shengjing capsule is one of the most effective drugs for male infertility. It is also the only traditional Chinese medicine approved by the FDA for male infertility with indications of aspermia, asthenoligospermia, and semen unliquefaction. It is composed of velvet antler (*Cornu cervi pantotrichum*), dodder (*Semen cuscuta*), and medlar (*Fructus lycii*) and works especially well for patients with asthenoligospermia. The SOD and GSH-Px vitality of testicular and epididymal homogenates in Shengjing capsule treatment groups was much better than that of the model group, while the MDA concentration of the former was significantly lower than the latter. Compared with the model group, rats in the Shengjing capsule groups possessed more complete seminiferous epithelium and more sperm of better vitality, which indicates that Shengjing capsule extracts can enhance antioxidant enzyme activity of the reproductive system, antagonize oxidative stress, repair damaged seminiferous epithelium, and protect spermatogenesis. It can also safeguard sperm membrane and improve sperm vitality. Shengjing capsule is composed of 19 ingredients, including: velvet (*Cornu cervi panto trichum*), *Cordyceps sinensis*, ginseng, polygonatum, dodder (*Semen cuscuta*), medlar (*Fructus lycii*), *Astragalus complanatus*, epimedium (*Herba epimedii*), eucommia bark (*Cortex eucommiae*), Cherokee rose fruit (*Fructus rosae laevigatae*), verbena (*Herba verbenae*), raspberry (*Fructus rubi*), curculigo (*Rhizome curculigins*), and Psoraline (*Fructus psoraleae*).

Based on the tian-gui theory in *Plain Questions: Treatise of Heavenly Truth from Remote Antiquity*, velvet and ginseng are used as sovereign drugs in this formula to replenish vigor, warm and nourish governor meridian, engender essence, and boost marrow. Dodder (*Semen cuscuta*), medlar (*Fructus lycii*), polygonatum, *Cordyceps sinensis*, and curculigo (*Rhizome curculigins*) are jointly prescribed as ministerial drugs to tonify the life-gate fire, improve sexual function, replenish yin essence, and warm seminal fluid. The essence and qi are invigorated by reinforcing yin from yang, boosting yang from yin, and yin-yang mutual complementing. Cherokee rose fruit (*Fructus rosae laevigatae*), raspberry (*Fructus rubi*), and *Astragalus complanatus* are used as adjuvant drugs to arrest spontaneous

seminal emission and reduce urination frequency. Verbena (*Herba verbenae*), *Sargentia loryvine* stem, ginkgo leaves, and raspberry (*Fructus rubi*) are used as conductant drugs to eliminate dampness, dispel toxins, promote blood circulation, and remove channel obstructions. The drugs of the 4 classifications mentioned above work together to tonify the kidneys, arrest spontaneous emission, nourish yin, and invigorate yang. Modern pharmacological studies have confirmed that *Lycium barbarum* polysaccharide (LBP) contained in medlar (*Fructus lycii*) can enhance the activity of SOD in rats [10] and inhibit testicular cell damages induced by H₂O₂ [11,12]. Yang Xin et al. [13] found that dodder water extracts could improve SOD activity of sperm suspension, decrease MDA content, and intervene in lipid peroxidation of ROS to sperm membrane by antioxidation. Polysaccharide can significantly reduce the activity of SOD in *in vitro* cultured rat testicular interstitial cells, reduce MDA content, and enhance vitality of GSH-Px [14]. Pilose antler polypeptides are active ingredients of velvet antler, with antioxidant and anti-aging effects [15]. Gonadal hormone and testosterone play a prominent role in spermatogenesis. Hence, it could be proved that the extracts applied in the present study could improve sperm parameters via enhancing serum levels of testosterone.

Conclusions

This study showed that Shengjing capsule extracts can increase sperm count, enhance sperm vitality, and improve sperm form of rats with oligoasthenospermia. It also works well in increasing rats' T serum, reducing sperm DNA fragmentation rate, and MDA content in testes and epididymides. It can enhance the activity of antioxidant enzymes, such as SOD and GSH-Px, safeguard the antioxidant enzyme system, and repair pathological damages of testis and epididymis to protect spermatogenesis, all of which suggest that this traditional Chinese medicine can antagonize damages induced by oxidative stress in the reproductive system and provide new ideas and approaches for antioxidative treatment of male infertility. Although there might be an overall treatment mechanism of compound Chinese medicine in improving endocrine hormones and blood circulation, regulating immune system, preventing infections and supplying micronutrients to be detected, this study has proved that Shengjing capsule extracts antagonize oxidative stress injuries of the reproductive system and demonstrated that traditional Chinese medicine has scientific merit and is effective in treating male infertility, especially in treating oligoasthenospermia.

Competing interests

The authors declare that they have no competing interests.

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