A Chinese Herbal Formula, Wuzi Yanzong Pill (五子衍宗丸), Improves Spermatogenesis by Modulating the Secretory Function of Sertoli Cells

ABSTRACT  Objective: To evaluate the effects of the Chinese herbal formula Wuzi Yanzong Pill (五子衍宗丸, WYP) on the spermatogenesis and specific secretory functions of Sertoli cells in rat model and to investigate the underlying mechanism. Methods: Five groups of male Sprague-Dawley rats including the control group, the model group, the low-dose WYP group, the medium-dose WYP group and the high-dose WYP group (5 in each group) were treated daily with vehicle, multiglycosides of Tripterygium wilfordii Hook f (GTW) either alone (20 mg/kg) or followed by WYP (0.5, 1.0, or 2.0 g/kg daily), respectively for 30 days. Serum levels of follicle-stimulating hormone (FSH), inhibin B (INHB) and testosterone (T) were evaluated using enzyme-linked immunosorbent assay. Androgen-binding protein (ABP) gene expression and transferrin (TF) protein expression in testis tissue specimens of all rats were determined using real-time reverse transcriptase polymerase chain reaction and Western blotting analysis, respectively. Histopathological alterations in the testis were determined using Johnsen's score. Results: The toxicity of GTW towards Sertoli cell secretory functions and spermatogenesis was accompanied by increased serum FSH concentrations and decreased INHB and T concentrations. Upregulated ABP mRNA levels, and decreased TF protein expression and Johnsen's scores were detected in the model group compared with the control group (P<0.05 or P<0.01). Oral high-dose WYP administrations to GTW-treated rats effectively alleviated all of the GTW-induced changes in specific secretory functions of Sertoli cells (ABP, INHB and TF). Furthermore, serum T level and Johnsen's score of the testis increased greatly compared with the model group (P<0.01). Conclusion: WYP has the ability to improve the spermatogenesis, possibly through modulating the secretory protein expression of Sertoli cells.

KEYWORDS  Wuzi Yanzong Pill, spermatogenesis, sertoli cell, secretory function, Chinese medicine

About 15% of couples do not achieve pregnancy after 1 year of regular, unprotected intercourse. A male factor is the only cause of infertility in 30% to 40% of couples. From the perspective of Chinese medicine, the etiology of male infertility is Kidney (Shen) essence insufficiency. Therefore, the principal therapy is supplementing Kidney essence. Wuzi Yanzong Pill (五子衍宗丸, WYP) has been used in Chinese medicine formula for over 463 years. It is widely used as a classical prescription for male infertility with insufficiency of Kidney essence and has been observed to improve spermatogenesis. To date, it has been found that WYP can enhance the content of Ca²⁺ in the sperm cytoplasm and mitochondria. However, it is unclear whether WYP has a direct effect on Sertoli cell secretions.

Sertoli cells are regarded as the "nurse cells" of the germ cells and play an important role in spermatogenesis. It has been shown that Sertoli cells provide structural and nutritional support to developing germ cells. Moreover, some specific secretions synthesized by Sertoli cells regulate or respond to pituitary hormone release and further influence spermatogenesis.

In this study, we investigated the effect of WYP on the secretory function of Sertoli cells through the observation of inhibin B (INHB), androgen-binding protein (ABP) and transferrin (TF) expression. In addition, the serum levels of follicle-stimulating hormone (FSH), testosterone (T) and the pathology of testicular abnormalities induced by multiglycosides of...
**METHODS**

**Experimental Animals**

Eight-week-old male Sprague-Dawley rats (SPF grade) with body weights of 300–350 g were obtained from Vital River Experimental Animal Center, Beijing, China [Certificate No. SCXK(Beijing)2012-0001].

**Plant Materials**

The WYP (composed of *Lycii Fructus, Cuscutae Semen, Rubi Fructus, Schisandrae Fructus, and Plantaginis Semen*), lot No. 2035053, was produced by Tongrentang Technologies Co., Ltd. (Beijing, China). GTW, 10 mg per tablet, was obtained from Huangshi, Feiyun Pharmaceutical Co., Ltd. (Hubei, China). In the experiments, the designated concentrations of the WYP and the GTW tablet were freshly prepared in 0.5% carboxymethyl cellulose (CMC) to freshly prepare the designated concentrations.

**Chemicals and Reagents**

Other materials included RNA extraction kit (Takara, China), M-MLV reverse transcriptase (RT, Tiangen, China), SYBR Green I real-time polymerase chain reaction (PCR) kit (Bioer, China), bicinehoninic acid (BCA) protein assay kit (Cwbiotech, China), polyvinylidene difluoride (PVDF) membranes (Millipore, MA, USA), Trizol reagent (Cwbiotech, China), protease inhibitor cocktail (Roche, IN, USA), enhanced chemiluminescence (ECL, Millipore, MA, USA), goat anti-rabbit IgG-HRP (Jackson, PA, USA), goat anti-mouse IgG-HRP (Jackson, PA, USA), rat FSH, INHB, T kits (CUSABIO, China).

**Establishment of Animal Model and Drug Administration**

According to the random number table, twenty-five rats were divided into five groups, i.e., control group, model group, low-dose WYP group, medium-dose WYP group and high-dose WYP group, with 5 in each group. The control group received 0.5% CMC (10 mL/kg) twice daily. The model group received GTW (20 mg/kg dose that induced testicular toxicity in rats) in the morning and 0.5% CMC (10 mL/kg) in the evening. Rats of the WYP groups received GTW (20 mg/kg) in the morning and WYP in the evening. The WYP was given at three dose levels: 0.5, 1.0, and 2.0 g/kg body weight (equivalent to 2.5, 5.0 and 10.0 times the human adult dose, respectively). All treatments were administered orally by gavage for 30 days. On the 31st day, the rats were anesthetized by intraperitoneal injection with chloral hydrate (350 mg/kg), and the serum and left testis were extracted for further analysis, including gene and protein examination in all rats. The experiments were approved by the China-Japan Friendship Hospital Institutional Animal Care and Use Committee.

**Determination of Serum Concentrations of FSH, INHB and T Using Enzyme-Linked Immunosorbent Assay**

Concentrations of FSH, INHB and T in serum samples were detected by enzyme-linked immunosorbent assay (ELISA) in accordance with the directions of the manufacturer. All absorbance were measured with an ELISA plate reader at a wavelength of 450 nm.

**Determination of ABP mRNA Expression in Testis Using Real-Time RT-PCR**

Total RNA was isolated from the testis using the Trizol reagent according to the manufacturer’s instructions. The integrity of the RNA was measured by 1% agarose and electrophoresis (90 V, 20 min; Liuyi Instrument Factory, Beijing, China). Reverse transcription was performed using an oligo (dt)12–18 primer and M-MLV RT according to the manufacturer’s instructions by incubation for 120 min at 42 °C, followed by 15 min at 95 °C. SYBR Green I PCR master mix reagent kits were used according to the manufacturer’s instructions for the quantification of gene expression. The primer is designed as follows: 159 bp, sense: 5'-GTGCAGTTTGACTGCACTGG-3', antisense: 5'-CTTAATGGAGCGAAAGCGCC-3'. The cycling conditions were as follows: 95 °C for 120 s followed by 45 cycles of 95 °C for 20 s, 59 °C for 25 s, and 72 °C for 30 s. After PCR, a melting curve analysis was performed to demonstrate the PCR product specificity, which was displayed as a single peak.

**Determination of TF Protein Expression in Testis Using Western Blotting Analysis**

Protein was extracted from testis and frozen at −80 °C by homogenizing the sample in radio immunoprecipitation assay (RIPA) buffer supplemented with protease inhibitor cocktail. The cellular debris was added to 50 μL RIPA and incubated on ice for 20 min, then protein was centrifuged at 13,000 r/min, 4 °C for 20 min. The clarified supernatant was stored at −80 °C. The protein content was determined using the BCA