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Intervention Effects of Jinkuishenqiwan Pills on the Kidney-yang Deficiency in Rats

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Abstract

With the aim of investigating the ability and possible mechanism of Chinese medicine Jinkuishenqiwan pills to inhibit the spermatgoenic arrest infertile, 100 mature male rats were randomly divided into five groups: normal control group, model group, test group treated with high dosage of Jinkuishenqiwan pills, test group treated with middle dosage of Jinkuishenqiwan pills, and test group treated with low dosage of Jinkuishenqiwan pills. Except the normal group, the other four groups were made into model of kidney-yang deficiency by injecting hydrocortisone. Simultaneously, the treatment of Jinkuishenqiwan pills was also irrigated into the stomachs of the three treated groups. After 30 days，the concentration of testosterone, the sperm density and the motility rate of sperm were examined, the microstructure of testicle was observed by optical microscope. Results showed that Jinkuishenqiwan pills can inhibit the spermatgoenic arrest infertile, there exists statistical difference between test group and the model group (P<0.05). In conclusion: Chinese medicine Jinkuishenqiwan pills may have a certain therapeutic effects on the rats with kidney-yang deficiency.

Keywords: Jinkuishenqiwan; Model of Kidney-Yang deficiency; Spermatogenic arrest; Testosterone

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1. Introduction

Chinese medicine is the general name of traditional drugs in China, which comes from natural plants, animals and minerals, includes three kinds of formats such as Chinese herbs, drink-slips and nostrum [1]. Traditional Chinese medicines are composed of complicated compositions, which decide the pharmaceutical complexity of traditional Chinese medicines. Traditional Chinese medicine exhibit obvious therapeutic effects on some chronic diseases, however, Chinese medicine's therapeutic mechanism is still not clarified well. How to clarify the mechanism of traditional Chinese medicine is a great technological challenge. Nanotechnology brings a new chance to solve current problems in development of traditional Chinese medicine [2,3]. Nanoscale Chinese medicine has become a new topic in recent years. Nanoscale traditional Chinese medicine is defined as the effective components with less than 100nm in diameter, effective locations, primary drugs and compound pharmaceutics that are fabricated by nanotechnology. When the traditional Chinese medicine components reach less than 100 nm in diameter, these components are easily separated by using chemical means, qualitatively analyzed by physical tools, and quantitatively analyzed by physical chemical methods, which is helpful to clarify the therapeutic mechanism of traditional Chinese medicine [4,5].

Jinkuishenqiwan pills, also called as kidney-QI pills, composed of nanoscale pills, were originally mentioned in Medical Treasures of the Golden Chamber written by Zhang Zhongjing [6]. It can cure illness in

the metabolism of urine by recovering the kidney controlling water metabolism. However, kidney controlling reproduction is often ignored in current medical therapeutic area. This experiment was designed with the aim of investigating intervention effects of Jinkuishenqiwan pills on rats with the kidney-yang deficiency.

2. Materials and Methods

2.1. Materials

2.1.1 Animals

100 mature male rats, aged 2.5~3 months, weighed 200~220g, were provided from Animal Experiment Center of Fourth Military Medical University. Under 20±3.0℃, the rats were raised in the circulation of light and darkness. With free ingestion and drinking, the rats were raised for a week.

2.1.2 Medicine and agents

Jinkuishenqiwan pills, with National Approval Number Z11020147, were produced by Beijing Tongrentang Co., LTD). Testosterone ELISA kit was purchased from Beijing Zhongshan Biomedical Engineering Co., LTD.

2.1.3 Equipment

Blood cell counting chamber was purchased from Zhejiang Yuhuan Hardware and Optical Instrument Company. Sartorius *Electronic Balance* BP-61 was from Germany. Olympus biological microscope was from Japan Olympus Company. Inverted microscope XDS-1 was from Chongqing Optical Instrument company. High-capacity refrigerated centrifuge LDR-4.SC was from Beijing Clinical Centrifuge Company.

2.2 Methods

2.2.1 Grouping of experimental rats

After rats were raised for one week, 20 rats were randomly selected from the 100 rats as normal group, the rest 80 rats were divided into 4 experimental groups: model group, test group treated with high dosage of Jinkuishenqiwan pills，test group treated with middle dosage of Jinkuishenqiwan pills, and test group treated with low dosage of Jinkuishenqiwan pills.

2.2.2 Establishment of kidney-yang deficiency model

Except the normal group, each rat in other 4 groups was injected hydrocortisone, 10mg a day, for continously 12 days, thus establishing kidney-yang deficiency model. Successful model can be judged according to objective manifestation [7]: dreading cold weather, preferring warmth, four limbs feeling low temperature, scrunching, depressing, showing emaciation, and with dim hairs.

2.2.3 Medication

Jinkuishenqiwan was made into 3 liquids: high density, middle density, and low density. The rats were drenched once a day, for 30 days. All the rats were weighed once a week so as to adjust the dosage of medicine. Each day, 1mL/100g distilled water was for rats in both the normal group and the model group; 1mL/100g Jinkuishenqiwan liquid was for the 3 treated groups (2.50g crude drug /kg for high dosage,1.25g crude drug /kg for middle dosage, 0.625g crude drug /kg for the low dosage).

2.2.4 Observation index and testing methods

(1) Physical condition observation: The rats' physical state and hairs was recorded; the rats were weighed once a week.

(2) Test of motility rate and density: The methods from "Modern Pharmocology Experimental Methods" eidted by Zhang Juntian was also adopted in this experiment. After killing a rat, the right epididymis was immediately taken. Then a small mouth was cut from deferent duct. A small quantity of sperma was put on a glass slide. Diluted by physiological saline, spermatic activity could be observed under microscope. Thus, the motility rate could be calculated. The test of sperma density was followed by Ni's methods. After 30 days' raise, the rats in all the 4 treated groups were killed. The left epididymis was immediately taken. The tails of the left epididymis were cut several times, then were put into 2 mL physiological saline. Under 37 ℃for 10-15 minutes; the physiological saline was 20 times diluted. One drop was put on the blood cell counting chamber. The number of sperma was counted under high power lens, with the unit of 10^6/ml [8].

2.2.5 Test of sex gland and accessory sex gland module

With organ module=organ weight/rat weight×100%, each group were weighed beore being killed. After killing, the humid weight of didymus, epididymis, and glandula seminalis was conducted.

2.2.6 Test of serum testosterone level

10 mL blood was taken from the arteria cruralis of rats, and was placed under room temperature for 30 minutes. Serum was made by centrifugalization for 20 minutes. The serum testosterone level was measured by enzyme immunoassay.

2.2.7 Observation of testicle microstructure

Paraffin imbedding was made on 1-4mm testicle of rats, sliced with 3 μm thick coronal plane. In dry air, defatted by xylene, gradiently dewatered by alcohol, dyed by HE, the coronal plane was observed under 40× microscope.

Table 1. Comparison of the sperma density, the motility rate, and serum testosterone concentration ($\overline{X} \pm S$, n=20)

Groups	the sperm density（$\times 10^9$/L）	the motility rate（%）	serum testosterone concentration (μg/L)
normal group	71.2±9.6[a]	89.3±7.6[a]	3.4±0.7[a]
model group	15. 3 ±8.7	8.2±6.1	2.0±0.5
Treated group with high dosage	69.3±7. 5[a]	81. 4 ±5.3[a]	3.1±0.6[a]
Treated group with middle dosage	63. 7 ±9.6[a]	78. 4 ±2.4[a]	3.0±0.1[a]
Treated group with low dosage	61. 3 ±6.8[a]	74. 4 ±8.0[a]	2.7±0.4

[a]P<0.05 vs model group.

2.3 Statistical analysis

Experimental data was expressed by $\overline{X} \pm S$. Analysis of variance was used for the comparison of groups. Statistics software SPSS 11.0 involved. P<0.05 showed statistical significance.

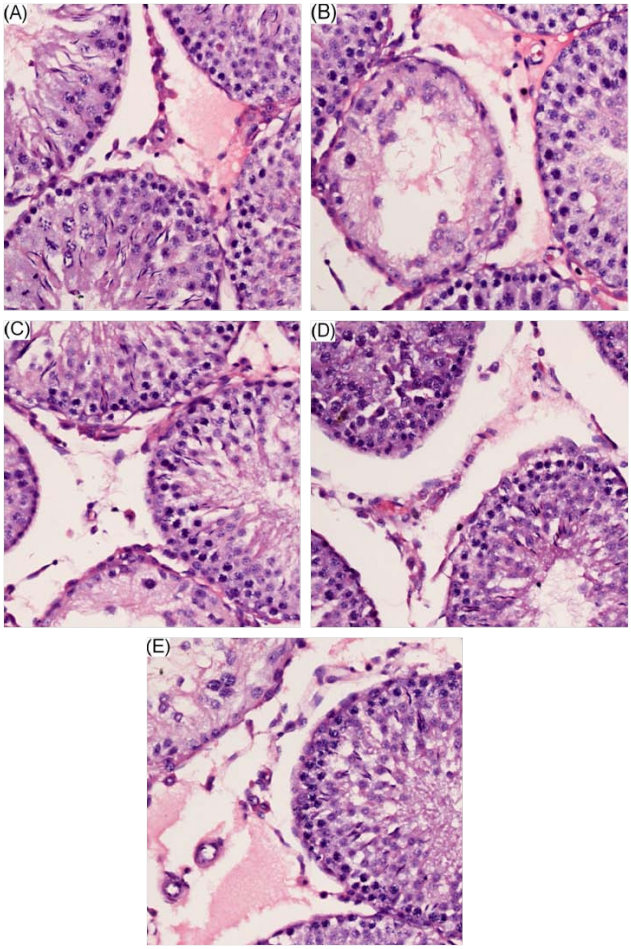

Figure 1. Rats'testicle microstructure under microscopy

A: HE staining in normal group (40×), B: HE staining in model group (40×), C: HEstaining in treated group with low dosage (40×), D: HE staining in treated group with middle dosage (40×), E: HE staining in treated group with high dosage (40×).

3. Results

3.1 Effects of Jinkuishenqiwan pills on rat sperm

Both the motility rate and the density of sperm of rats in model group decreased. Jinkuishenqiwan could, to different extents, improve the motility rate and the density. There was no statistical difference among the 3 test groups (P>0.05), which performed better than the model group. Obvious effect was especially showed in the 2 test groups treated with high dosage, and middle dosage (Table 1). After 30 days of experiment treatment, there existed statistical difference of the motility rate and the density of sperm of rats between the model group and the normal group (P<0.05). After 30 days of experiment treatment, there existed statistical significance of the motility rate and the density of sperm of rats between the model group and the treated group (P<0.05, Table 1).

3.2 Effects of Jinkuishenqiwan pills on rat serum testosterone level

The serum testosterone level of rats in model group decreased. Jinkuishenqiwan pills could, to different extents, increase the serum testosterone level (Table 1). There was no statistical significance between the test group treated with low dosage and the model group. There existed statistical difference between the serum testosterone level of the model group and the two test groups treated with high and middle dosage (P<0.05). There also existed statistical difference between the model group and the normal group (P<0.05, Table 1).

3.3 Effects of Jinkuishenqiwan pills on testicle structure of rats

In the normal group, the rats' seminiferous tubule had a complicated structure, with seminiferous epithelium lining up in order. Androgone, primary spermatocyte, spermatid, and sperma could be observed. Leydig inside interstitial tissue had a big volume, with grouping spread, abundant cytoplasm, and integrated basal membrane, and was surrounded by fusiform myoid cells (Fig. 1A). In the model group, the number of seminiferous tubule decreased dramatically, with caliber shrinking, cell layer decreasing; the array of seminiferous epithelium was in irregular order. Some lumens had no spermatid and sperm. The volume and number of Leydig inside interstitial tissue decreased. Interstitial tissues among spermiducts proliferated, with decreasing number of interstitial cells, showing atrophia testiculi (Fig 1B). In the three test groups, testicle structure exhibited obvious recovery improvement. Cell layers of seminiferous tubule increased, with increased num-

ber of spermatid and sperm; the number of interstitial cell recovered; acidophilia improved. Especially, better recovery results showed in the test groups treated with high dosage and middle dosage (Figs. 1 C-E).

4. Discussion

The Chinese medicine Jinkuishenqiwan pills is considered to be able to affect not only yin-yang, Qi and blood, chills and fever, reinforcing and reducing, but also affect the function of organs as heart, liver and spleen. Aconite root is benefit for fillingYin, and dried root of adhesive rehmannia is helpful to supply Yang; ramulus cinnamomi for supplied Qi, and cortex moutan for supplied blood; aconite root for supplied heat, and cortex moutan for supplied frigidity. Rehmannia is benefit for kidney-yin. Batatatis is benefit to spleenic yin. Alisma rhizoma cleans kidney, but not hurt kidney-Qi. Cortex moutan cleans liver, alisma rhizoma relieves deficient fire. Hoelen invigorates the spleen and alleviates water retention [1,7,8]. Combined together, the function of kidney, liver, and spleen could be improved. According to the experiment by Zhang Baili, Jinkuishenqiwan could increase the number of sperm, and motile sperm percentage, promoting the growth of genitical gland [9]. Jinkuishenqiwan could increase serum testosterone level. It was discovered by He Qinghu that Jinkuishenqiwan could improve the quality of sperm, and effectively cure male infertility of insufficiency of kidney-YANG [10]. This experiment showed that Jinkuishenqiwan pills could promote serum testosterone, especially in the 2 test groups treated with high dosage and middle dosage. The experiment also shows that Jinkuishenqiwan pills could increase the number of sperm, thus, being effective in treating hypospermia.

Although Chinese medicine Jinkuishenqiwan pill has been proved to have therapeutic effects on some diseases such as kidney-Yang deficiency, etc., its mechanism is not completely accepted by clinical doctors and pharmaceutical scientists. We think that Chinese medicine Jinkuishenqiwan pills may be composed of nanoscale components, whose therapeutic efficacy may be closely associated with its chemical components. Further work will focus on investigating its chemical components and this component function.

In conclusion, Chinese medicine Jinkuishenqiwan pills could treat the syndrome of deficiency of kidney-YANG, which help to recover reproduction function. The experiment provides evidence for therapeutic effects of the traditional medicine Jinkuishenqiwan pills. The theory of kidney controlling reproduction is also further verified.

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