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Original Articles

Effects of *Panax Ginseng* on the Sperm Motility and Spermatogenesis in the SD Rat

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Objective : This study was conducted to investigate the effects of *Panax Ginseng* (人蔘) on the sperm motility and spermatogenesis in the male rat.

Methods : We used 8-week-old Sprague-Dawley rats, and administered the extract powder of *Panax Ginseng* to 5 rats (treated group) and normal saline (control group) once a day for 28 days. We isolated their testes surgically, then observed the change of the body weights before and after administration of *Panax Ginseng* extracts and normal saline. We observed the weight of the testes, epididymis, vascular gland, and prostate. Also, we examined the total, normal motile sperm concentration, and the concentration of testicular catalase and peroxidase.

Results : We found that the concentration of normal, motile sperm in the testes of the *Panax Ginseng* group showed a significant difference compared with the control group. The angiogenesis of the seminiferous tubule was increased and the increasement of the number of spermatogonia, primary and secondary spermatocyte was observed in the *Panax Ginseng* group through a microscope. The body weight, the weight of the testes, epididymis, prostate and the concentration of testicular catalase and peroxidase were higher in the *Panax Ginseng* group but showed no significant difference.

Conclusion : This study shows that *Panax Ginseng* may have an effect on the morphology and motility of sperm, the important factor in male fertility, and can promote the concentration of antioxidants, catalase and peroxidase, which is the important factor in spermatogenesis.

Key Words: *Panax Ginseng,* male rat, spermatogenesis, reproductive competence, antioxidants, infertility

Introduction

The change of lifestyle and environmental pollution have caused a decline in sperm concentration, that is, the percentage of motile sperm and the percentage of normal sperm, and it has increased the ratio of male infertility[1-4].

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A male factor is responsible in about 50% of infertile couples, and it is related to the disorder of spermatogenesis, a defect of sperm transportation, impotence, hypogonadism, dysspermia, and so on[5].

In oriental medicine, male infertility is defined as masculinity sterility(男性不育), and the pathology is divided into four categories, deficiency of Qi, deficiency of essence, prospermia and cold semen[6]. The causes of male sterility were presented to be Sinhue mostly and Sinyanghue particularly. Some Studies[7] have been conducted about strengthening spontaneous emission(補腎益精) as the treatment of male infertility,

but there is no previous report about deficiency of Qi.

Ginseng(人蔘) is the representative herb of strengthening Qi and has the effects of curing consumption and promoting saliva regeneration and tranquilization[8]. A *Ginseng* infusion solution has been reported to have the effects of anti-stress[9], anti-aging[10], antioxidant[11] and gonadal function improvement[12].

This study was conducted to investigate the effects of *Ginseng* on the reproduction and in vitro developmental competence in the male rat observing the change of genital organ weight, sperm concentration, motility, morphology and testicular catalase and peroxidase, the antioxidant.

Materials and Methods

1. Medicinal stuff & Test animals

1) Test medicine material

The Korean *Panax Ginseng*, bought in Kyung Hee Univ. Oriental Medical Center was used as test medicine material.

2) Test animals

Ten male Sprague Dawley rats, 8-weeks old and weighing 280±10g, were used for this experiment. The animals were kept in breeding rooms with the temperature of 24℃, alternate light and darkness of 12 hours, and provided with enough water and food.

2. Methods

1) Concoction of medicine

200g of Panax *Ginseng* were extracted with boiled water for 3 days. Then, the extract as filtrated and was evaporated under reduced pressure. And the extract was freeze-dried for 24 hours to obtain 4.2g.

2) Grouping and Panax *Ginseng* Administration

Ten rats were divided at random into 2 groups of 5 animals each. The experimental groups were gavaged Panax *Ginseng* at a dose of 1 mg in 1 ml water/kg/day for 28 days. The controls were given a similar amount of distilled water.

3) Measure the body weight and weight of genital organs

Body weights were checked twice, before and after experiment.

The testes, prostate, seminal vesicles and epididymis were dissected and weighed.

4) Histologic observation of testis

One testis from each animal was fixed in Bouin's fixative and embedded in paraffin wax. 5μm sections were cut from the middle portion of the testis and stained with hematoxylin-eosin. The stained slides were examined under a light microscope.

5) Extraction of epididymal sperm

After 4 days of the administration of the medicine, the testis and epididymis was extracted from the killed treated mice. Under optic microscope (Nikon, Japan) the epididymis was divided from the testis and was immersed in M16 media and bovine serum albumin (Sigma, USA). The spermal clot of pyral past was extracted and suspended in CO2 culture medium for 1 hour.

6) The changes in the count, the motility and the morphology of epididymal sperm from the tested mice

The count, the motility and the morphology of the epididymal sperm was measured by markler sperm counting chamber (Sofi,Israel), sperm analyzer (CASA,Germany)andhematocylin-eosin-staining.

7) Testicular peroxidase and catalase activity

Table 1. Effect of *Ginseng* on the Body Weight and Weight of Testis, Epididymis, Vascular and Prostate Gland in SD Rat

Groups	Initial body weight (g)	Final body weight (g)	Testicular weight (g)	Epididymis weight (g)	Prostate gl. weight (g)	Vascular gl. weight (g)
Control (n=5)	293.2±10.4	359.8±20.54	1.487±0.02	0.180±0.01	0.426±0.09	0.599±0.10
Sample (n=5)	292.0±13.0	359.2±20.86	1.557±0.10	0.192±0.08	0.432±0.19	0.586±0.19

Control : Group with normal saline
Sample : Group with *Ginseng* extract

Testicular tissue was homogenized in a cold buffer (50mM patassium phosphate containing EDTA, pH 7.0) with a tissue concentration of 100 mg/mL. The homogenate was centrifuged at 10,000g for 15 min.

Testicular peroxidase activity were measured by chemiluminescent hydrogen peroxide detection kit (AssayDesigne, Inc., USA) and chemiluminometer (Tecan, USA) for 5 seconds and every sample were measured twice.

Testicular catalase activity were measured by catalase assay kit (Cayman chemical, USA) and the ELISA reader (Tecan, USA) and every sample was measured twice.

8) Analysis of results & statistical analysis

The results were analyzed using the Mann-Whitney U test. Differences at $p<0.05$ were considered statistically significant.

Results

There was no significant difference in body weight of before and after experiment. The weight of the testis was 1.557±0.10g in the *Ginseng group* and 1.487 ± 0.02g in the controls. The weight of epididymis was 0.192±0.08g in the *Ginseng* group and 0.180±0.01g in the control group. The weight of the prostate gland was 0.432±0.19g in *Ginseng* group and 0.426±0.09g in the control group. The weight of the vascular gland was 0.586±0.19g in the *Ginseng* group and 0.599± 0.10g in the controls. There was no significant difference between the groups(Table 1).

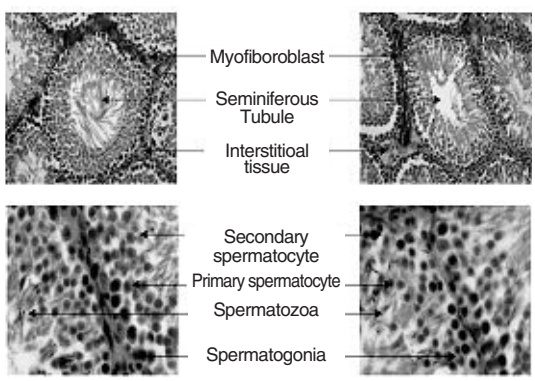

Fig. 1. Effect of *Ginseng* on the spermato -genesis in SD rat testis

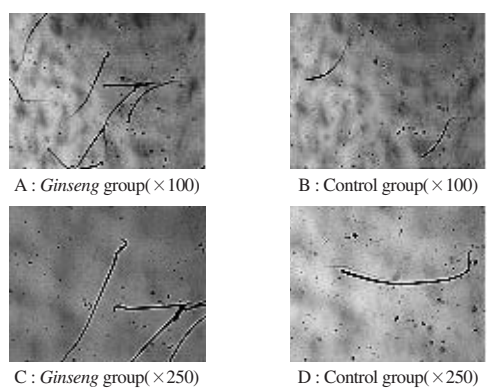

Fig. 2. Effect of *Ginseng* on the sperm count and morphology in SD rat

Table 2. Effect of *Ginseng* on the Sperm Concentration, Motility and Morphology in SD Rat

Groups	Sperm concentration (x 10^6 cell/*ml*)	Motile sperm (x 10^6 cell/*ml*)	Normal sperm (x 10^6 cell/*ml*)
Control (n=5)	59.6±6.07	29.4±12.4 (49.3%)	28.8±13.5 (48.7%)
Sample (n=5)	71.4±21.47	57.8±20.8 (81.0%)*	55.6±18.5 (77.9%)*

Control : Group with normal saline
Sample : Group with *Ginseng* extract
* ; $p<0.05$

Table 3. Effect of *Ginseng* on Catalase and Peroxidase Activity in SD Rat Testis

Groups	Testicular catalase activity (nmol/min/*ml*)	Testicular peroxidase activity (nmol/min/*ml*)
Control (n=5)	0.338±0.093	14.8±2.95
Sample (n=5)	0.426±0.045	15.4±3.36

Control : Group with normal saline
Sample : Group with *Ginseng* extract

The increase of vascular distribution besides the seminiferous tubule was observed in the *Ginseng* group compared with the control through a optical microscope. The number of spermatogonia, the primary and secondary spermatocyte on the basement membrane, and sperm on seminiferous tubule were increased in the *Ginseng* group compared with the control(Fig. 1, 2).

There is no significant difference in the sperm concentration between groups. The motility of the epididymal sperm was 57.8±20.8, 81.0% in *Ginseng* group and it was significantly higher than 29.4±12.4, 49.3% in control group ($p<0.05$). The normal ratio of sperm morphology was 55.6±18.5, 77.9% in the *Ginseng* group and it was significantly higher than 28.8 ±13.5, 48.7% of the control group ($p<0.05$) (Table 2).

Testicular catalase activity was 0.426±0.045 nmol/min/ml in the *Ginseng* group and it was higher than 0.338±0.093 nmol/min/ml in the control group but had no statistical significance. Testicular peroxidase activity was 15.4±3.36 nmol/min/ml in the *Ginseng* group and it was higher than 14.8±2.95 nmol/min/ml in the control group, but had no statistical significance (Table 3).

Discussion

If a male infertility factor is present, it is usually defined by abnormal semen analysis[13]. A difference in motility profile between sperm specimens from fertile men and sperm specimens from men in infertile units was found in an 8-hour in vitro test[14].

In this study, there was no signigicant effectiveness in the body weight, the weight of genital organs (testis, pididymis, vascular gland and prostate glad).

However the increase of vascular distribution and the number of spermatogonia, the primary and secondary spermatocyte on the basement membrane, and sperm on seminiferous tubule was observed in the *Ginseng* group compared with the control group. This fact indicates that *Ginseng* can promote spermatogenesis.

Also, it was effective to be treated with *Ginseng* on the increase of the motility and morphology of epididymal sperm but not effective on the change of the

concentration. Dahlberg B[14] pointed out the sperm motility is related to fertility and the motility of human sperm is recognized as playing the most important role in fertility. Robin[15] and Morgentaler[16] reported the morphology of sperm influence the fertility.

We supposed the machanism of *Ginseng* on improving sperm quality is antioxidant property but there is no significant increase in testicular catalase and peroxidase.

I think more research should be progressed into the mechanism of *Panax Ginseng* on the spermatogenesis.

We can suggest that *Panax Ginseng* has an effect on the improvement of reproductive competence in mail mice.

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