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Original Article

Effects of *Morindae Officinalis Radix* on the Spermatogenesis and Antioxidant Activities in the SD Rat

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Objectives : This study was conducted to investigate the effects of *Morindae officinalis Radix* (巴戟) on the spermatogenesis and antioxidant activities in the Sprague Dawley (SD) rat.

Materials and Methods : We choose the 2-month-old SD rats, and administered the extract powder of *Morindae officinalis Radix* once in a day for 28 days. The control rats were administered normal water in the same way and duration. We observed changes of body weight, surgically isolated testis, epididymis, vascular gland and prostate gland before and after administration of *Morindae officinalis Radix* extracts in SD Rats. Also we compared the testicular tissue, especially seminiferous tubules between the control and treated groups by histochemical methods. In addition, we examined the total, normal, morphologic and motile sperm in the cauda epididymis, and the activity of catalase and peroxidase in the isolated testis tissue.

Results : There was no significant difference between control and treatment groups in the body weight, testis, vascular and prostate gland, but the weight of epididymis showed significant difference in the control group. The concentration of total sperm, the motility and normality of spermatozoa was significantly different when compared with the control group, respectively. In the histological examination of testicular tissues, the tendency of increasement of angiogenesis between seminiferous tubules was observed. And the concentration of spermatogonia, primary and secondary spermatocyte and sperm were higher than that of control testicular tissues. Finally, the activity of catalase and peroxidase related inhibitory molecules of oxidation were slightly increased in the treatment group than those of control group.

Conclusions : This study shows that *Morindae officinalis Radix* has the beneficial effect on the concentration, morphology and motility of sperm, the important factor in male fertility. We can suggest that *Morindae officinalis Radix* has an effect on the spermatogenesis in the SD rat.

Key Words: *Morindae officinalis radix*, SD rat, Spermatogenesis, Antioxidants, Infertility

Introduction

The definition of infertility is the inability to conceive after 1 year of frequent intercourse without contraception[1]. A male factor is responsible in about 50% of infertile couples but it is occasionally ignored in diagnosis and treatment because of developing of assisted reproductive technique as in vitro fertilization, micromanipulation[2]. However these remedies are too expensive and inevitable of accompanied treatment in normal female, so the treatment of male factor is getting important[3].

Male infertility is related with the disorder of spermatogenesis (90%), defect of sperm transportation, impotence, hypogonadism, dysspermia and so on[1].

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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[4]. The causes of male sterility were presented to be Sinhue mostly and Sinyanghue particularly[5]. We made it a rule to tonify the kidneys to strengthen spontaneous emission (補腎益精)[6].

Morindae officinalis Radix (巴戟) is the representative medicine of reinforcing the kidneys Yang and has the effects of curing impotence, curing female infertility from cold uterus, curing dysmenorrhea[7]. Also *Morindae officinalis Radix* was reported to have effects that inhibit the bronchi muscle contraction, increase uterine muscle contraction[8], improve osteoporosis[9] and improve ovulation, fertilization and in vitro fertilization[10]. However there is no experiment about spermatogenesis and the effect to antioxidant enzyme yet.

This study was conducted to investigate the effects of *Morindae officinalis Radix* on the reproduction and in vitro developmental competence in 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 *Morindae officinalis Radix*, the roots of Morindae officinalis How (Rubiaceae) bought in Kyung Hee Univ. Oriental medical center were used as test medicine material.

2) Test animals

Ten male Sprague Dawley rats, 8-week old and weighing 292±3.79g, 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

400g of *Morindae officinalis Radix* were extracted with 1 *l* boiled water for 3 days. Then, the extract filtrated and was evaporated under reduced pressure. And the extract was freeze-dried for 24 hours to obtain 4.15g.

2) Grouping and *Morindae officinalis Radix* Administration

10 rats were divided at random into 2 groups of 5 animals each. The experimental groups were garaged *Morindae officinalis Radix* at a dose of 1 mg in 1 ml water/kg/day for 28 days. The controls were given a similar amount of normal saline.

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 on the administration of the medicine, testis and epididymis was extracted from the killed treated mice. Under optic microscope (Nikon, Japan) the epididymis was divided from 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.

Tabel 1. Effect of *Morindae officinalis Radix* on the Body Weight and Weight of Testis, Epididymis, Vascular Gland and Prostate Gland in SD Rat

Groups	Initial body weight (g)	Final body weight (g)	Testicular weight (g)	Epididymis weight (g)	Vascular gland weight (g)	Prostate gland weight (g)
Control group (n=5)	292.00±4.69	354.80±9.09	1.52±0.05	0.19±0.01	0.46±0.15	0.21±0.05
MO group (n=5)	292.40±3.21	332.00±54.5	1.58±0.15	0.26±0.03*	0.35±0.11	0.22±0.05

*; $p<0.01$

Control group: Rats administered normal saline

MO group: Rats administered *Morindae officinalis Radix*

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) and hematocylin-eosin-staining.

7) Testicular peroxidase and catalase activity

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 ELISA reader (Tecan, USA) and every sample were 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 testis was 1.56±0.15g in *Morindae officinalis Radix* group and it was higher than 1.52±0.05g of controls but had no statistical significance. The weight of epididymis was 0.26±0.03g in *Morindae officinalis Radix* group and it was signicantly higher than 0.19±0.01g of controls ($p<0.01$). The weight of vascular gland was 0.35± 0.11g in *Morindae officinalis Radix* group and it was lower than 0.46±0.15g of controls but had no statistical significance. The weight of prostate gland was 0.22± 0.05g in *Morindae officinalis Radix* group and 0.21± 0.05g in control group and there is no significant difference. (Table Ⅰ).

The increase of vascular distribution besides the seminiferous tubule was observed in *Morindae officinalis Radix* 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 *Morindae officinalis Radix* group compared with the control (Fig.1, 2).

The concentration of the epididymal sperm was $100.00\pm13.62\times10^6/ml$ and it is significantly higher than $57.60\pm4.51\times10^6/ml$ in the control group

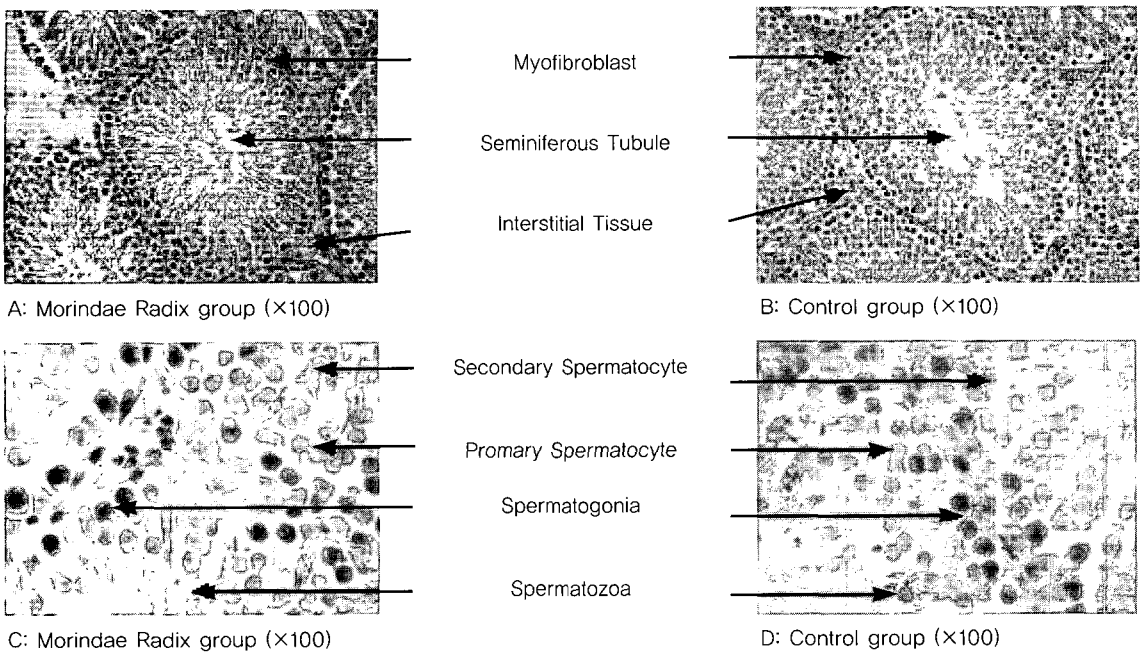

Fig. 1. Effect of Morindae officinalis Radix on the spermatogenesis in SD rats

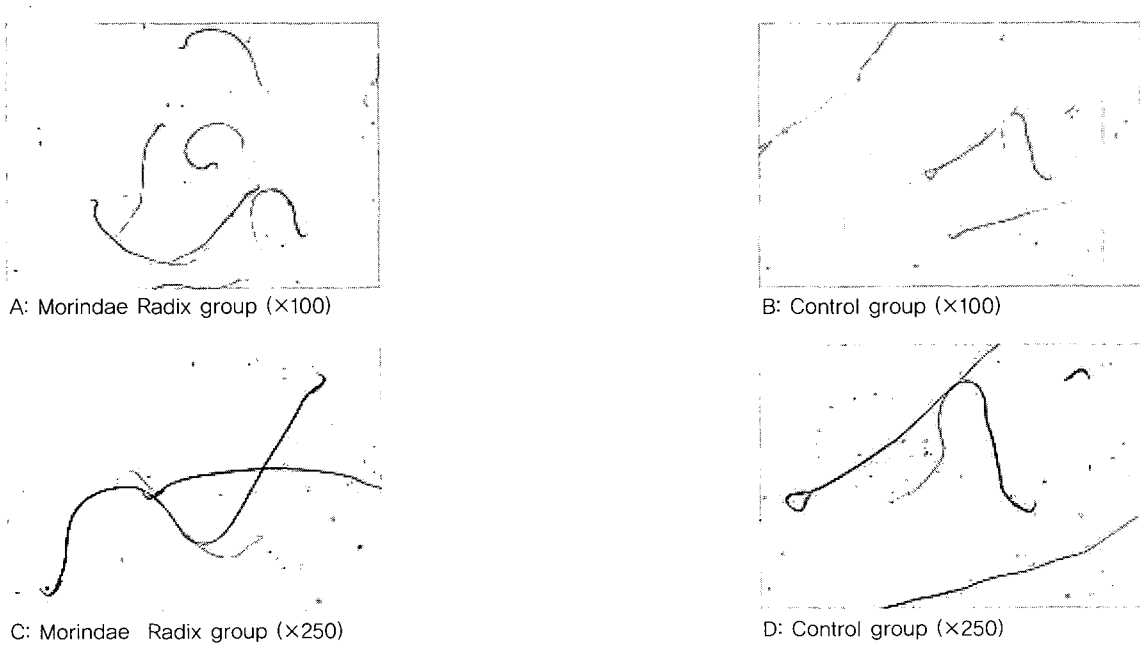

Fig. 2. Effect of *Morindae officinalis Radix* on the sperm count and morphology in SD rats

Tabel 2. Effect of *Morindae officinalis Radix* on the Sperm Concentration, Morphology and Motility in SD Rat

Groups	Sperm concentration (x 10^6 cell/ml)	Normal sperm (x 10^6 cell/ml)	Motile sperm (x 10^6 cell/ml)
Control group (n=5)	57.60±4.51	37.80±3.56(65.62%)	36.80±6.83(63.89%)
MO group (n=5)	100.00±13.62*	87.00±12.87(87.00%)*	85.60±11.37(85.60%)*

*; $p<0.01$

Control group: Rats administered normal saline

MO group: Rats administered *Morindae officinalis Radix*

Tabel 3. Effect of *Morindae officinalis Radix* on Catalase and Peroxidase Activity in SD Rat Testis

Groups	Testicular catalase activity (nmol/min/ml)	Testicular peroxidase activity (nmol/min/ml)
Control group (n=5)	0.38±0.06	14.00±0.707
MO group (n=5)	0.41±0.02	16.20±2.17

Control group: Rats administered normal saline

MO group: Rats administered *Morindae officinalis Radix*

($p<0.01$). The number of normal morphologic sperm was $87.00\pm12.87\times10^6/ml$ in *Morindae officinalis Radix* group and it was significantly higher than $37.80\pm3.56\times10^6/ml$ of control group ($p<0.01$). The number of motile sperm was $85.60\pm11.37\times10^6/ml$ in *Morindae officinalis Radix* group and it was significantly higher than $36.80\pm6.83\times10^6/ml$ of control group ($p<0.01$). (Table Ⅱ)

Testicular catalase activity was 0.41± 0.02 nmol/min/ml in *Morindae officinalis Radix* and 0.38± 0.06 nmol/min/ml in control group and there is no statistically significant difference. Testicular peroxidase activity was 16.20±2.17 nmol/min/ml in *Morindae officinalis Radix* group and 14.00±0.707 nmol/min/ml in control group, and it showed no significant difference between groups (Table Ⅲ).

Discussion

Approximately 15% of couple are sterile and the male factor is responsible in about 50% and 5-10% of married men diagnosed as a male infertility[1].

Recently male infertility has increased[11] and the count, motility of sperm and the ratio of normal sperm has diminished regardless of age[12]. As the reason, it is suggested the increase of genital diseases - testis cancer, cryptorchism - caused by drug, environmental pollution, stress, oxidative stress and hormonal disorders[13].

The causes of male infertility are disorders of spermatogeness, obstruction the route of sperm transport, dysspermia, intercourse disorder, endocrine disorder. In the clinical research, it is related with varicosity, ductal obstruction, sex chromosome defect, infectious disease, trauma and so on[14].

To diagnosis of male sterile, the history taking is very

important and many tests as hormonal study, chromosome test, X-ray, spermatozoal function test are used. Semen analysis has been used as a basic, essential and important tool to evaluate the male infertility[15].

Male infertility is treated by internal medicine, operation and assisted reproductive technology. With the development of micromanipulation, it shows high therapeutic index to external disease, but the internal medicine as antiestrogen, antibiotics, steroids and α-adrenergic agonist, are not distinguished effective[14].

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[4].

From the past, many books have dealt with male infertility. <Sub Chun Sa's Obstetrics & Gynecology> said it is caused from male factors in many cases and administered many prescription including *Morindae officinalis Radix*[15].

Morindae officinalis Radix (巴戟) has been used to deficiency of kidney Yang (腎陽虛) and applied to many symptoms of cold-damp[7].

Yang[16] reported Shao-Fu-Zhu-Yu-Tang activate acrosin and increase sperm concentration and motility and Kim[17] pointed the effect of Panax Ginseng water extract on increase human sperm count without virulence. Han[18] reported the effect of Cuscutae Semen on increase sperm count and motility. Siterman[19] reported the acupuncture treatment affect sperm density in males with very low sperm count.

Therefore, this experiment was conducted for the purpose of examining the effects of *Morindae officinalis Radix* on virility in male rat.

There was no signigicant effectiveness in the weight of testis, prostate and vascular gland. But the significant increase of weight of epididymis was observed.

The volume of testis is an index of testicular function and the decline of testicular volume means dysplasia or degeneration of seminiferous tubule[20].

Epididymis has function of transport, maturation, maintenance and absorption of sperm. Sperm get the fertility while it pass through epididymis and the relationship between the weight and function of epididymis[21].

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 *Morindae officinalis Radix* group compared with control group.

In the semen analysis, sperm concentration, motility and morphology were significantly increased in *Morindae officinalis Radix* group compared with control group.

Dahlberg B[22] 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. Park[14] made it clear that relationship between spermatozoal count and motility. Kruger[23] reported the morphology of sperm influence the fertility.

Considering above reports, *Morindae officinalis Radix* has an effect on male infertility by improving sperm concentration, motility, morphology.

To study the mechanism of *Morindae officinalis Radix* on spermatogenesis, we measured testicular catalase activity and peroxidase activity. The activity of catalase and peroxidase increased in *Morindae officinalis Radix* group, but there was no significant difference between groups.

Catalase remove O_2 made in leucocyte and protect sperm from genital inflammation. Peroxidase influence the improvement of spermatozoal mobility by suppression lipid hyperoxigenation[24].

Recently it is focused on the relevance between ROS (Relative Oxygen Spicies) and sterility. The membrane of sperm contain unsaturated fatty acid a lot and more frail to ROS. Therefore high level of ROS act as a factor

of sterility by diminishing spermatozoal mobility and fertility[25-27].

The existence of catalase and peroxidase can protect sperm against ROS unless there are too many leucocytes[24].

I think more research should be progressed into the mechanism of *Morindae officinalis Radix* on the spermatogenesis through other antioxidant enzyme, SOD (superoxide dismutase), or acrosin activity.

In these days the assisted reproductive technique is used widely but it's not the fundamental remedy. Hereafter it will be necessary to develope oriental drugs which strengthen kidney Yang for treatment of male infertility.

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