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Panax ginseng Improves Senile Testicular Function in Rats

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We reported previously that the administration of Korean red ginseng water extract (KRG-WE) protected the guinea pig testis against damage induced by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (a potent endocrine disruptor). We also found that crude saponin from ginseng was the active ingredient responsible for this protection. Here, we examined the biological role of KRG-WE in an animal model of age-induced dysfunction of spermatogenesis. Twenty-four male Sprague-Dawley (six 2-month-old and eighteen 12-month-old) rats were used. The young and old control groups received only vehicle. The ginseng saponin (GS)- and KRG-WE-treated groups received GS (40 mg/kg body weight/day) and KRG-WE (200 mg/kg body weight/day), respectively, for 4 months. The number of cells, Sertoli cell index, Johnsen's score, and sex hormone levels decreased significantly with age. However, the administration of KRG-WE and GS markedly improved the number of germ cells, seminiferous tubular size, and Johnsen's score in the old rats. Ginseng produced a distinct testicular histological improvement in old rats. KRG-WE and GS elevated testosterone levels, while attenuating the aberrant increase in follicle stimulating hormone and luteinizing hormone levels. Sperm kinematics evaluated by a computer-assisted sperm analyzer demonstrated improvement in the percentage of motile sperm, progressive sperm motility, and curvilinear velocity associated with sperm quality, supporting the beneficial role of red ginseng in senile spermatogenesis. Overall, the total water extract had a more potent effect than the corresponding saponin fraction. In conclusion, Korean red ginseng rejuvenated age-induced testicular dysfunction. Additionally, the total water extract was more potent than the corresponding saponin fraction.

Keywords: Red ginseng, Crude saponin, Sperm quality, Spermatogenesis, Aging

INTRODUCTION

Increasing life expectancy has raised issues concerning the impact of aging on the male endocrine system and sexuality. While female fertility ends at the entrance to menopause around the age of 50 years, men generally do not experience a clear-cut end to their reproductive capacity. A reduction in sperm motility may be due to increased latency and decreased frequency of intercourse with aging. Age-associated histomorphological alterations of the testis include a reduction in the number of Leydig cells [1], thickening and protrusions of the basal membrane of the seminiferous tubules [2], small areas of disturbed spermatogenesis, and malformed spermatids [3]. Decreased numbers of Leydig cells could be related to lower serum testosterone levels, which were decreased by approximately 0.4% annually starting at the age of 50 years [4]. Additionally, decreased testicular

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perfusion [5], reduced Sertoli cell function [6], and increased testicular connective tissue deposition have been suggested to be age-related changes that might impair spermatogenesis and reduce feedback from the testes to the pituitary, resulting in elevated luteinizing hormone (LH) and follicle stimulating hormone (FSH) levels [7]. Consistent with this, sperm morphology analyses show a reduced percentage of normal spermatozoa in men older than 50 years of age.

Drugs used to treat sexual dysfunction include phosphodiesterase-5 inhibitors [8], dopamine agonists [9], synthetic prostaglandins [10], and α-adrenergic receptor antagonists [11]. However, these drugs focus primarily on erectile dysfunction and only temporarily improve that symptom. Additionally, they are accompanied by side effects, including headache, flushing, dyspepsia, nasal congestion, and impaired vision. Rare, but serious, adverse effects found through post-marketing surveillance include priapism, severe hypotension, myocardial infarction, ventricular arrhythmias, stroke, increased intraocular pressure, and sudden hearing loss [12]. Consequently, agents with fewer side effects that improve sexual function are desirable.

Korean red ginseng (KRG) has been taken orally to improve physical strength by people in East Asia for more than 2,000 years. Studies have shown that KRG helps prevent diabetes mellitus [13], atherosclerosis [14], erectile dysfunction [15], immune dysfunction [16], carcinogenesis [17], and physicochemical stress [18] among other disorders. Additionally, we reported that the administration of KRG water extract (KRG-WE) protects testicular function [19], and improves sperm survival rate and quality in guinea pigs exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin [20]. KRG is used for many reasons, including its reasonable price, ready availability, and safety. In light of its long history of use and the results of modern scientific research, Korean ginseng appears to alleviate testicular dysfunction, including erectile dysfunction and stress-induced sexual dysfunction. However, no attempt has been made to examine the effects of Korean ginseng on age-related testicular dysfunction, or to compare the efficacy of the whole extract and crude saponin, which is the main active ingredient of *Panax ginseng*. Thus, we examined the benefits of KRG in age-induced testicular dysfunction.

MATERIALS AND METHODS

Materials

Six-year-old KRG-WE was procured from a local KRG distributor. The crude ginseng saponin (GS) was prepared as reported previously [21]. Briefly, 1 kg of KRG-WE was diluted in distilled water to make a 10% solution and passed through a glass column (5 L) containing Diaion HP-20 resin (3.5 L; Mitsubishi Chemical, Tokyo, Japan). The resin was subsequently washed with four bed volumes of distilled water. The crude GS fraction was obtained by eluting the water-washed resin with absolute ethanol. The ethanol eluate was dried *in vacuo* to obtain a dark brown powder (350 g). The ginsenoside contents of the ginseng preparations were determined as described in 'European pharmacopoeia (supplement 5.1 to the 5th edition)' with slight modifications. Briefly, the ginsenosides were analyzed using reverse phase HPLC with an ultraviolet detector using the following gradient of acetonitrile (CH_3CN) and water: 0 min, 20% CH_3CN; 40 min, 35% CH_3CN; 52 min, 45% CH_3CN; 62 min, 70% CH_3CN; and 80 min, 100% CH_3CN. The standard reference ginsenosides Rb_1, Rb_2, Rc, Rd, Re, Rf, Rg_1, Rg_2(S), Rg_2(R), Rg_3(S), Rg_3(R), and Rh_1 were isolated by our laboratory. Identification and purity tests were carried out as described in 'Herbal medicinal products' [22].

Experimental animals

Eighteen 12-month-old (750±20 g) and six 2-month-old (280±10 g) male Sprague-Dawley rats were purchased from Hanil Experimental Animal Breeding (Yeumsung, Korea) and acclimatized to the facility for at least 1 week before the experiment. They were fed a standard pellet diet and water *ad libitum* and kept at a constant temperature (23±2°C) and relative humidity (55±10%) on a 12/12-h light/dark cycle.

The rats were maintained in the Regional Innovation Center Experimental Animal facility in accordance with the Institutional Animal Care and Use Committee guidelines of Konkuk University. The study was approved by the Animal Ethics Committee in accordance with the 14th article of the Korean Animal Protection Law.

The rats were divided into four groups of six rats each: the young (YC) and old control (OC) groups received vehicle only; the GS and KRG-WE groups received the crude ginseng saponin fraction and Korean red ginseng water extract for 4 months at daily doses of 40 mg/kg body weight and 200 mg/kg body weight, respectively. The GS and KRG-WE were mixed evenly with sterilized standard diet and administrated orally after pelletization. The appropriate contents of GS and KRG-WE were controlled by weighing the rats weekly and the daily dietary intake. At the end of the experiment, all food was removed for 24 h before sacrifice. The rats

were euthanized under general anesthesia with diethyl ether. Blood samples were collected from the abdominal vein for blood chemistry panels and sex hormone level analysis. The liver, kidney, spleen, epididymides, and testes were isolated and weighed after removing any adhering adipose tissue.

Blood chemistry panels

To measure biochemical parameters, blood was collected in a SST® gel and clot activator tube (Becton Dickinson, Franklin Lakes, NJ, USA). Serum was separated by centrifugation (1,500× *g*, 10 min, room temperature). An automated chemistry analyzer (Hitachi-747, Hitachi Medical, Tokyo, Japan) was used to measure serum levels of aspartate transaminase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), γ-glutamyl transpeptidase (γ-GTP), albumen (Alb), calcium (Ca), phosphorus (P), blood urea nitrogen (BUN), and uric acid (UA). Serum testosterone, LH, and FSH contents were analyzed using a radioimmunoassay kit (Diagnostic Product Corporation, LA, USA).

Measuring sperm motility

Sperm samples were extracted from the left caudal epididymis by cutting it with scissors; one drop of caudal fluid was immediately placed in a culture dish containing 5 mL of Hanks' balanced salt solution prewarmed to 37°C and supplemented with 10 mg/mL bovine serum albumin (fraction V). After incubation for 5 min at 37°C, an aliquot of the suspension was collected with a micropipette and diluted to contain 40±10 sperm under the defined microscopic field (×100 magnification); then, 10 μL of the suspension were added to a 2X-CEL slide (depth, 80 μm; thickness, 0.15 mm; Hamilton Thorne Research, Beverly, MA, USA) that had been prewarmed in a CO_2 incubator (Sanyo, Osaka, Japan) at 37°C. Sperm motility was recorded using a computer-aided sperm analyzer (Hamilton Thorne Research) with a ×4 objective lens and a charge-coupled device camera. At least 200 sperm in each sample were monitored for motility pattern analysis.

Analysis of spermatogenesis-related parameters

To analyze the stages of spermatogenesis, germinal cells were divided into three broad morphological categories according to the developmental process: spermatogonia, spermatocytes, and spermatids in the seminiferous epithelium. Testes were stained with periodic-acid Schiff and hematoxylin and examined under a light microscope for categorization according to the criteria proposed by Hankinson [23] and developed by Russell et al. [24]. The Sertoli cell index (SCI, the ratio of the total number of germinal cells to the total number of Sertoli cells) was calculated and the numbers of sperm at different stages of maturation in the seminiferous epithelium were analyzed, as recommended by Russell et al. [24].

Histomorphological study

The left testis was cut into small pieces (5 mm^3) and fixed in Bouin's solution (saturated solution of picric acid, 40% formaldehyde and glacial acetic acid) for histopathological study. Fixed testicular tissues were dehydrated and embedded in paraffin wax using an automatic tissue processor (Leica ASP300; Leica Microsystems, Wetzlar, Germany), sectioned to 4-6 μm thickness with a microtome (Leica RM2245, Leica Microsystems), stained with hematoxylin and eosin, and examined using light microscopy (Olympus CX31; Olympus, Tokyo, Japan). More than 100 horizontally sectioned seminiferous tubules per group were analyzed to determine the spermatogenesis-related histology. All seminiferous tubules in one histological section of the testicular specimen were evaluated and scored on a scale of 1 to 10 using Johnsen's scoring system [25]. Briefly, the scoring is as follows: 10) complete spermatogenesis with many spermatozoa, determined by head form, and an organized germinal epithelium of regular thickness, leaving an open lumen; 9) many spermatozoa present, but with a disorganized germinal epithelium and marked sloughing or obliteration of the lumen; 8) only a few spermatozoa present; 7) no spermatozoa, but many spermatids present; 6) no spermatozoa and only a few spermatids present; 5) no spermatozoa and no spermatids, but several or many spermatocytes present; 4) only a few spermatocytes (<5), but no spermatids or spermatozoa present; 3) spermatogonia were the only germ cells present; 2) no germ cells, but Sertoli cells were present; and 1) no cells in a tubular section.

Statistical analyses

Results are expressed as the mean±SD. Statistical analysis was performed using ANOVA followed by Duncan's *t*-test ($p<0.05$ and $p<0.01$). Analyses were performed using the SAS ver. 9.1 (SAS Inc., Cary, NC, USA).

RESULTS

Ginsenoside content of the ginseng preparations

The ginsenoside content of the KRG-WE determined

on a dry weight basis was as follows: Rb_1 10.44 mg/g, Rb_2 4.61 mg/g, Rc 5.19 mg/g, Rd 3.30 mg/g, Re 3.08 mg/g, Rf 2.63 mg/g, Rg_1 1.90 mg/g, Rg_2S 3.30 mg/g, Rg_2R 2.45 mg/g, Rg_3S 4.19 mg/g, Rg_3R 1.84 mg/g, and Rh_1 1.40 mg/g. The amount of each ginsenoside in the GS fraction was approximately 19 times higher than that in KRG-WE.

Effects on organ weight and blood chemistry panels

As shown in Table 1, the liver, kidney, and spleen were about twice as heavy in old rats versus young rats (p<0.01), but no significant change in testicular weight was observed. However, the epididymides of old rats were significantly lighter than those of young rats. Interestingly, the ginseng treatments increased the epididymis weights of old rats, particularly the KRG-WE treatment. The blood chemistry panels worsened with aging, but there were no significant changes in the Alb, Ca, P, or UA levels. Liver function marker enzymes (AST, ALT, ALP, and γ-GTP) and BUN (a kidney function marker) were significantly increased in old rats (p<0.05). Generally, treatment with ginseng, especially KRG-WE, improved the changes in organ weight and blood chemistry panels in old rats, particularly epididymides weight and the AST, ALT, ALP, γ-GTP, and BUN levels.

Effects on sperm movement parameters

Sperm motility ratios in young and old rats were 84.6±10.0% and 38.5±18.9%, respectively (p<0.05) (Table 2). The progressive sperm motility ratios were 35.4±1.8% and 12.0±3.6% in young and old rats, respectively (p<0.01). Overall, sperm quality decreased with increasing age, while the average path velocity, straight line velocity, and curvilinear velocity (VCL) of old rats were markedly lower than in young rats (p<0.05-0.01). However, the parameters associated with sperm motility were improved significantly by treatment with ginseng preparations (p<0.05-0.01). There was no significant difference in sperm motility-related parameters between the GS and KRG-WE groups. The linearity, straightness, and wobbling of swimming sperm were the same in both young and old rats.

Effects on spermatogenesis-related parameters in the testis

There was no significant difference in the percentage of seminiferous tubules with sperm when comparing young and old rats (Table 3). However, the sperm counts per tubule, Sertoli cell counts per tubule, SCI, and seminiferous tubule size were significantly lowered in old than young rats. There was no marked difference in these spermatogenesis-associated parameters between the GS and KRG-WE groups. Tubular cross-sections of young rat testis showed the typical arrangement of cells at different stages (Fig. 1). Spermatogonia and Sertoli

Table 1. Effects of ginseng saponin and Korean red ginseng water extract (KRG-WE) on organ weights and blood chemistry panels

Variable	Group			
	Young control goup	Old control group	Ginseng saponin	KRG-WE
Organ weight (g)				
Liver	6.2±1.7	16.9±3.1††	17.0±1.6	19.7±2.3
Kidney	1.8±0.2	3.5±0.3††	3.6±0.3	3.5±0.4
Spleen	0.4±0.2	0.9±0.1††	1.0±0.1	1.0±0.1
Epididymides	1.8±0.3	1.0±0.2††	1.2±0.2	1.5±0.2*
Testes	3.5±0.2	3.6±0.2	3.5±0.4	3.4±0.5
Liver and kidney-related blood chemistry				
Aspartate aminotransferase (U/L)	138.4±75.1	351.8±72.9†	242.9±47.2*	258.2±61.5*
Alanine aminotransferase (U/L)	89.5±7.1	179.9±37.7†	163.7±44.4	134.2±48.3
Alkaline phosphatase (U/L)	113.8±12.4	176.3±75.1†	207.4±60.8	145.6±26.4
γ- glutamyl transpeptidase (U/L)	2.2±4.1	7.8±4.5†	2.1±1.4*	1.8±1.1*
Albumin (g/dL)	4.2±0.4	4.1±0.2	3.7±0.4	3.5±0.3*
Calcium (mg/dL)	8.9±0.7	9.7±0.5	9.1±0.4	8.9±0.2*
Phosphorus (mg/dL)	5.1±0.5	5.0±0.6	5.5±0.7	5.2±0.9
Blood urea nitrogen (mg/dL)	10.8±2.4	16.9±0.8†	17.9±10.3	11.9±3.2*
Uric acid (mg/dL)	1.1±0.3	1.3±0.2	1.3±0.6	1.2±0.4

Data are expressed as the mean±SD.
†p<0.05, ††p<0.01, compared with the young control group; *p<0.05, **p<0.01, compared with the old control group.

Table 2. Effects of ginseng saponin and Korean red ginseng water extract (KRG-WE) on sperm movement parameters

Variable	Group			
	Young control goup	Old control group	Ginseng saponin	KRG-WE
Motility (%)	84.6±10.0	38.5±18.9†	69.3±4.9*	73.3±8.0*
Progressive (%)	35.4±1.8	12.0±3.6††	24.3±1.2**	24.8±2.3**
Average path velocity (um/s)	382.8±45.9	173.9±74.7†	229.1±69.4	242.2±81.8
Straight line velocity (um/s)	258.7±31.2	129.7±53.5†	156.2±57.9	154.8±65.8
Curvilinear velocity (um/s)	524.2±84.5	258.8±106.3††	389.4±93.8	406.4±88.7*
Linearity (%)[1)]	49.4±0.8	49.3±2.5	42.3±1.5	42.0±1.2
Straightness (%)[2)]	67.6±2.6	69.7±1.2	65.7±0.5	66.0±1.2
Wobble (%)[3)]	73.0±1.5	66.8±4.0	58.8±2.7	58.3±1.6

Data are expressed as the mean±SD.
[1)]VSL/VCL×100; [2)]VSL/VAP×100; [3)]VAP/VCL×100.
$^{\dagger}p<0.05$, $^{\dagger\dagger}p<0.01$, compared with the young control group; $^{*}p<0.05$, $^{**}p<0.01$, compared with the old control group.

Table 3. Effects of ginseng saponin and Korean red ginseng water extract (KRG-WE) on spermatogenesis and serum sex hormone levels

Variable	Group			
	Young control goup	Old control group	Ginseng saponin	KRG-WE
Spermatogenesis				
% tubules with sperm	83.2±6.3	82.5±9.7	87.4±10.3	93.5±15.5
Sperm counts/tubule	3,632±342	2,730±287†	3,070±216	3,570±266*
Sertoli cell counts/tubule	25.3±1.3	21.6±3.3†	20.8±2.7	22.8±3.7
Germ cell counts/tubule	505.4±96.4	437.8±96.3	489.5±71.4	544.7±62.5*
Sertoli cell index	32.0±3.3	20.3±2.0††	23.5±3.0	23.9±2.5
Seminiferous tubule size (μm)	294.6±23.2	246.7±47.4†	255.5±38.7	279.6±47.3*
Johnsen's score[1)]	9.5±0.7	8.9±1.1†	9.1±1.1	9.5±1.4*
Sex hormone level				
Testosterone (ng/ml)	5.1±0.6	5.6±1.3	7.3±1.7	8.5±2.1**
Follicle stimulating hormone (mIU/ml)	10.6±2.6	22.6±3.2†	19.5±3.9	15.5±4.7*
Luteinizing hormone (mIU/ml)	12.5±2.0	25.6±3.0††	21.3±3.6	17.3±2.7*

Data are expressed as the mean±SD.
[1)]Johnsen's score was defined as the degree (1–10) of male fertility calculated from testicular biopsy specimens.
$^{\dagger}p<0.05$, $^{\dagger\dagger}p<0.01$, compared with the young control group; $^{*}p<0.05$, $^{**}p<0.01$, compared with the old control group.

cells rested on the basement membrane, surrounded by a concentric myofibroblast layer similar to that of the human testis. In particular, the tubules were densely packed with sperm cells at various stages. In contrast, the tubules of old rats were loosely packed. The number of fully mature spermatozoa at the center of the tubules was low, demonstrating late maturation arrest, degeneration around the tubule, and a decrease in the number of cells lining the tubular membrane. Sperm number per tubule in the OC ($p<0.05$), GS, and KRG-WE groups accounted for 75.2% ($p<0.05$), 84.5%, and 98.3% ($p<0.05$), respectively, compared with the number in the YC group, demonstrating that KRG-WE increased spermatogenesis significantly in old rats. The germ cell counts per tubule also increased markedly with KRG-WE treatment ($p<0.05$). The marked decrease in seminiferous tubular size and Johnsen's score in OC ($p<0.05$) were significantly elevated in the KRG-WE group ($p<0.05$). Rats in the GS and KRG-WE groups had denser tubule packing than those in the OC group (Fig. 1). Additionally, degenerated Leydig cells were rejuvenated by treatment with GS and KRG-WE, and were uniformly scattered within the interstitium. The rejuvenating effect of KRG-WE on spermatogenesis in old rats was more potent than the effect of GS.

Effects on serum sex hormone levels

Serum testosterone was not lowered by aging, but the FSH and LH levels increased almost two-fold in old rats (Table 3). KRG-WE significantly reduced the abnormal

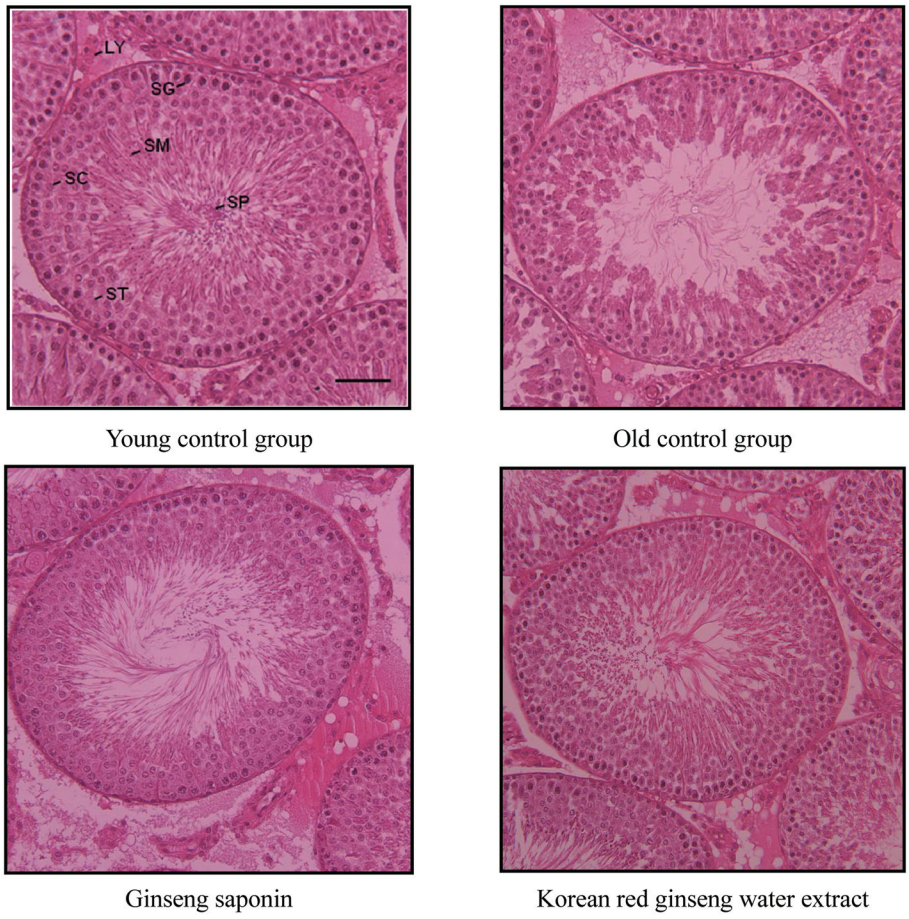

Fig. 1. Cross-sections of seminiferous tubules in the rat testis. All specimens were sectioned at 4 to 6-μm thick with a microtome, stained with hematoxylin and eosin, and examined using light microscopy (×200 magnification). Scale bar, 45 μm. LY, Leydig cell; ST, Sertoli cell; SG, spermatogonium; SC, spermatocyte; SM, spermatid; SP, spermatozoa.

production of FSH and LH in old rats ($p<0.05$). Interestingly, the testosterone level was increased significantly by treatment with the ginseng preparations, particularly by KRG-WE ($p<0.01$).

DISCUSSION

The age-related degenerative change that occur in the testis influences sperm quality, semen volume, sperm count, sperm motility, morphology and fertility. The changes are reflected by increases of FSH and LH in the serum, leading to a decline in fertility. These characteristics vary among individuals, and are strongly influenced by endogenous or exogenous stress and lifestyle. Age-related changes in male fertility are different from the female menopause, which is marked by the cessation of reproductive capacity connected with a sudden loss in endocrine function. To date, some drugs derived from natural products have been reported to improve sexual dysfunction in males. These drugs include yohimbine [26], krausianone [27], berberine [28], papaverine [29], and forskolin [30].

Panax ginseng is one of the oldest and best-known medicinal plants used to prevent sexual dysfunction. Ginseng has several pharmacological properties and potential therapeutic applications, and some studies suggest that the antioxidant and organ protective actions of ginseng are associated with enhanced nitric oxide (NO) synthesis in the endothelium of the lung, heart, kidney, and corpus cavernosum [31]. Enhanced NO synthesis causes vasodilatation and might be responsible for the aphrodisiac properties of ginseng. Although many researchers have recognized that *Panax ginseng* reverses erectile dysfunction [15,32], few attempts have been made to determine its effects on age-associated sexual function. Moreover, no reported trial has compared the efficacy of the whole KRG-WE and crude GS, which contains the main active ingredients of ginseng.

Here, we investigated the beneficial role of KRG on organ weight, blood chemistry panels, serum sex hor-

mone levels, histology, and parameters related to spermatogenesis in old rats. Testis weight was not changed by aging, but epididymis weight was reduced nearly 55% compared with the YC group (Table 1). KRG-WE increased the weight of the epididymides, but not the testes ($p<0.05$). As fully mature sperm cells are recruited from the seminiferous tubules to the epididymides, and wait there for ejaculation, the weight of the epididymides reflects the rate of sperm cell production. Our data indicate that ginseng improves the release of spermatozoa from spermatogonia in testicular gonocytes.KRG-WE significantly decreased AST, γ-GTP, and BUN levels. These results indicate that ginseng plays a beneficial role in liver and kidney function (Table 1). In traditional Asian medicine, kidney function is believed to be closely related to sexuality. No references demonstrating a direct relationship between organs other than the kidney are available. However, it can be deduced that decreases in the function of the liver and other organs may play a negative role in general physical condition, including sexual function.

The age-related declines in sperm motility were significantly improved by the GS and KRG-WE treatments ($p<0.05$) (Table 2), to levels equivalent to about 80% of the YC. Progressiveness and VCL were increased markedly in the KRG-WE group compared with the OC group ($p<0.05$). Thus, it seems reasonable to conclude that KRG counteracts the decline of spermatogenesis caused by aging, resulting in increased total distance of sperm movement in a unit period and the production of sufficient spermatozoa to cope with a competitive mating system. Moreover, KRG-WE was more effective than crude saponin in improving various parameters related to spermatogenesis, such as the Johnsen's score, seminiferous tubular size, percentage of tubules containing sperm, sperm count, and germ cell count. KRG-WE significantly improved the FSH and LH levels ($p<0.05$) (Table 3) and increased testosterone levels in old rats ($p<0.01$), consistent with an earlier report [33]. The age-related decline of Leydig cell steroidogenesis has potential implications for the decline of male fertility, and high testicular testosterone concentrations are required to maintain spermatogenesis [34]. Our results indicate that KRG-WE improves the reduced feedback from the testes to the pituitary gland with aging, resulting in an increase in the amount of testosterone secreted from LH-stimulated Leydig cells. In the histological study, the degeneration of spermatogonia and Sertoli cells caused by aging was improved by treatment with KRG-WE (Fig. 1). Spermatogonia and Sertoli cells rested on the basement membrane, surrounded by a concentric myofibroblast layer. Additionally, degenerating Leydig cells were rejuvenated by treatment with KRG-WE and were scattered uniformly within the interstitium.

We postulated that the ginseng saponin fraction would be more potent than KRG-WE. Surprisingly, our results demonstrated that KRG-WE was more effective than GS, which is thought to be the active fraction of KRG-WE. Ginsenosides may be the main active compounds in ginseng; nevertheless, the total extract was more potent than the saponin fraction alone. This suggests that the saponin and non-saponin fractions act in harmony to achieve their physiological effects. These results provide evidence that KRG plays a beneficial role in sexual dysfunction induced by aging. Our animal experiment results suggest that men with oligospermia or asthenospermia could benefit from taking KRG. However, this evidence is anecdotal, and a placebo-controlled clinical experiment should assess this hypothesis. We also demonstrated that KRG improved liver and kidney function, sperm motility, testosterone levels, and spermatogenesis in old rats. From these results, together with our previous reports [19-21], we conclude that KRG effectively overcomes the testicular dysfunction of aging. These physiochemical results lead us to propose additional biochemical studies and further development of ginseng for sexual dysfunction, especially to promote spermatogenesis in men.

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REFERENCES

1. Neaves WB, Johnson L, Porter JC, Parker CR Jr, Petty CS. Leydig cell numbers, daily sperm production, and serum gonadotropin levels in aging men. J Clin Endocrinol Metab 1984;59:756-763.
2. Johnson L, Petty CS, Neaves WB. Influence of age on sperm production and testicular weights in men. J Reprod Fertil 1984;70:211-218.
3. Holstein AF. Spermatogenesis in the aged--a borderland between normal and pathologic anatomy. Urologe A 1986;25:130-137.
4. Gray A, Feldman HA, McKinlay JB, Longcope C. Age, disease, and changing sex hormone levels in middle-aged

men: results of the Massachusetts Male Aging Study. J Clin Endocrinol Metab 1991;73:1016-1025.
5. Noguchi J, Yoshida M, Ikadai H, Imamichi T, Watanabe G, Taya K. Age-related changes in blood concentrations of FSH, LH and testosterone and testicular morphology in a new rat sterile mutant with hereditary aspermia. J Reprod Fertil 1993;97:433-439.
6. Tenover JS, McLachlan RI, Dahl KD, Burger HG, de Kretser DM, Bremner WJ. Decreased serum inhibin levels in normal elderly men: evidence for a decline in Sertoli cell function with aging. J Clin Endocrinol Metab 1988;67:455-459.
7. Plas E, Berger P, Hermann M, Pflüger H. Effects of aging on male fertility? Exp Gerontol 2000;35:543-551.
8. Corona G, Razzoli E, Forti G, Maggi M. The use of phosphodiesterase 5 inhibitors with concomitant medications. J Endocrinol Invest 2008;31:799-808.
9. Sivalingam S, Hashim H, Schwaibold H. An overview of the diagnosis and treatment of erectile dysfunction. Drugs 2006;66:2339-2355.
10. Costabile RA, Mammen T, Hwang K. An overview and expert opinion on the use of alprostadil in the treatment of sexual dysfunction. Expert Opin Pharmacother 2008;9:1421-1429.
11. van Dijk MM, de la Rosette JJ, Michel MC. Effects of alpha(1)-adrenoceptor antagonists on male sexual function. Drugs 2006;66:287-301.
12. Becker AJ, Stief CG, Schultheiss D, Truss MC, Jonas U. Pharmacological therapy of erectile dysfunction. Urologe A 1998;37:503-508.
13. Yuan HD, Shin EJ, Chung SH. Anti-diabetic effect and mechanism of Korean red ginseng in C57BL/KsJ db/db mice. J Ginseng Res 2008;32:187-193.
14. Qureshi AA, Abuirmeileh N, Din ZZ, Ahmad Y, Burger WC, Elson CE. Suppression of cholesterogenesis and reduction of LDL cholesterol by dietary ginseng and its fractions in chicken liver. Atherosclerosis 1983;48:81-94.
15. Choi HK, Choi YD, Adaikan PG, Jiang Y. Effectiveness of Korea red ginseng in erectile dysfunction-multi-national approach. J Ginseng Res 1999;23:247-256.
16. Sung H, Kang SM, Lee MS, Kim TG, Cho YK. Korean red ginseng slows depletion of CD4 T cells in human immunodeficiency virus type 1-infected patients. Clin Diagn Lab Immunol 2005;12:497-501.
17. Yun TK, Yun YS, Han IW. Anticarcinogenic effect of long-term oral administration of red ginseng on newborn mice exposed to various chemical carcinogens. Cancer Detect Prev 1983;6:515-525.
18. Kim HS, Jung MW, Jang CG, Park WK, Oh KW. Effects of ginseng total saponin on stress-induced analgesia. Korean J Ginseng Sci 1993;17:22-28.
19. Kim W, Hwang S, Lee H, Song H, Kim S. *Panax ginseng* protects the testis against 2,3,7, 8-tetrachlorodibenzo-p-dioxin induced testicular damage in guinea pigs. BJU Int 1999;83:842-849.
20. Hwang SY, Kim WJ, Wee JJ, Choi JS, Kim SK. *Panax ginseng* improves survival and sperm quality in guinea pigs exposed to 2,3,7,8-tetrachlorodibenzo- p-dioxin. BJU Int 2004;94:663-668.
21. Kim SK, Kwak YS, Kim SW, Hwang SY, Ko YS, Yoo CM. Improved method for the preparation of crude ginseng saponin. J Ginseng Res 1998;22:155-160.
22. Gaedcke F, Steinhoff B, Blasius H. Herbal medicinal products: scientific and regulatory basis for development, quality assurance and marketing authorisation. London: CRC Press, 2003.
23. Hankinson O. Role of coactivators in transcriptional activation by the aryl hydrocarbon receptor. Arch Biochem Biophys 2005;433:379-386.
24. Russell LD, Ettlin RA, Hikim AP, Clegg ED. The classification and timing of spermatogenesis. In:. Russell LD, Ettlin RA, Hikim AP, Clegg ED (Eds.). Histological and histopathological evaluation of the testis. Clearwater: Cache River Press, 1990.
25. Johnsen SG. Testicular biopsy score count--a method for registration of spermatogenesis in human testes: normal values and results in 335 hypogonadal males. Hormones 1970;1:2-25.
26. Ernst E, Pittler MH. Yohimbine for erectile dysfunction: a systematic review and meta-analysis of randomized clinical trials. J Urol 1998;159:433-436.
27. Drewes SE, Horn MM, Munro OQ, Dhlamini JT, Meyer JJ, Rakuambo NC. Pyrano-isoflavones with erectile-dysfunction activity from Eriosema kraussianum. Phytochemistry 2002;59:739-747.
28. Chiou WF, Chen J, Chen CF. Relaxation of corpus cavernosum and raised intracavernous pressure by berberine in rabbit. Br J Pharmacol 1998;125:1677-1684.
29. Zaher TF. Papaverine plus prostaglandin E1 versus prostaglandin E1 alone for intracorporeal injection therapy. Int Urol Nephrol 1998;30:193-196.
30. Mulhall JP, Daller M, Traish AM, Gupta S, Park K, Salimpour P, Payton TR, Krane RJ, Goldstein I. Intracavernosal forskolin: role in management of vasculogenic impotence resistant to standard 3-agent pharmacotherapy. J Urol 1997; 158:1752-1758.
31. Chen X, Lee TJ. Ginsenosides-induced nitric oxide-mediated relaxation of the rabbit corpus cavernosum. Br J Pharmacol 1995;115:15-18.
32. Choi HK, Seong DH, Rha KH. Clinical efficacy of Korean red ginseng for erectile dysfunction. Int J Impot Res

1995;7:181-186.
33. Fahim MS, Fahim Z, Harman JM, Clevenger TE, Mullins W, Hafez ES. Effect of *Panax ginseng* on testosterone level and prostate in male rats. Arch Androl 1982;8:261-263.
34. Ford WC, North K, Taylor H, Farrow A, Hull MG, Golding J. Increasing paternal age is associated with delayed conception in a large population of fertile couples: evidence for declining fecundity in older men. The ALSPAC Study Team (Avon Longitudinal Study of Pregnancy and Childhood). Hum Reprod 2000;15:1703-1708.