

## Ginsenoside Re Enriched Fraction (GS-F3K1) from Ginseng Berries Ameliorates Ethanol-Induced Erectile Dysfunction *via* Nitric Oxide-cGMP Pathway

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**Abstract** – Erectile dysfunction (ED) is a highly prevalent disorder that affects millions of men and considered to be an early symptom of atherosclerosis and a precursor of various systemic vascular disorders. The aim of the present study was to prepare ginsenoside Re enriched fraction (GS-F3K1, ginsenoside Re 10%, w/w) from ginseng berries flesh and to investigate the enhanced activities of GS-F3K1 on alcohol-induced ED. GS-F3K1 was prepared by the continuous liquid and solid separating centrifugation and circulatory ultrafiltration from ginseng berries flesh. GS-F3K1 was administered for 5 weeks in ethanol-induced ED rat by oral administration of 20% ethanol. To investigate the effects of GS-F3K1 on ED model, the levels of nitrite expression, cyclic guanosine monophosphate (cGMP) and erectile response of the penile corpus cavernosum of rat were measured. The erectile response of the corpus cavernosum was restored after GS-F3K1 administration, to a level similar to the normal group. The level of nitrite and cGMP expression in the corpus cavernosum of GS-F3K1-administered male rats was increased significantly compared to positive control group. GS-F3K1 from ginseng berries should effectively restore ethanol-induced ED in male rats and could be developed as a new functional food for the elderly men.

**Keywords** – GS-F3K1, Ginseng berry, Ethanol-induced erectile dysfunction, Nitrite, Cyclic guanosine monophosphate (cGMP)

### Introduction

The root of *Panax ginseng* (Korean ginseng) has been considered as one of the traditional and folk medicines which are widely used for many therapeutic purposes in the oriental countries such as Korea, China and Japan for thousands of years. A novel source plant of ginseng is *Panax ginseng* Meyer (Araliaceae), a herb with fleshy roots which grows abundantly in cool and shady forests extending from Korea and North eastern China to far eastern Siberia.<sup>1</sup> Ginseng has long been reported to have aphrodisiac properties tends to increasing stamina. In Asia, ginseng is routinely included in herbal formulations used for the treatment of sexual dysfunction. Ginsenosides

have been characterized as an therapeutic component of ginseng which are distributed in many parts of the ginseng plant, including the root, leaf and berry.<sup>1,2</sup> Particularly, it has recently been reported that ginseng berry showed a distinctive saponin profile different from that of root and nevertheless, it has not traditionally been used for therapy. Korean ginseng berries, a flesh part of the berries, was already found to contain as much as approximately 6% of ginsenoside Re, while Korean ginseng roots contains 3 to 4% of saponins, including different ginsenosides namely, Rb1, Rb2, Rc, Rd, Rg1 and Re.<sup>3</sup> In recent times, ginseng berries have been known to exhibit enormous significant beneficial effects on various problems such as obesity-induced insulin resistance, diabetes, abnormal immune responses and penile erection.<sup>4,5</sup> Furthermore, Zhang *et al.* have reported that ginsenoside Re increases as then ozoospermic human sperm motility by enhancing the induction of nitric oxide synthase as well as promotes

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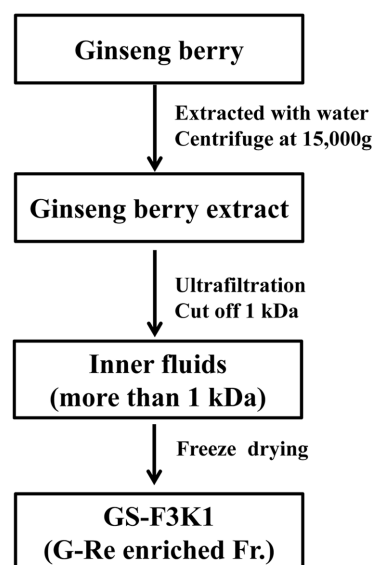
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human sperm capacitation through nitric oxide-dependent pathway.<sup>6,7</sup> In a parallel study, it was also found that ginsenoside Re-induced vasodilation which was mediated by releasing NO through a membrane sex steroid receptors, followed by relaxing vascular smooth muscle, acting on K channel.<sup>8</sup> The phytoestrogen ginsenoside Re activates potassium channels of vascular smooth muscle cells through PI3K/Akt and nitric oxide pathways.<sup>8</sup> This is the reason why research focused on the development of ginsenoside Re enriched fraction from ginseng berries flesh to induce the vasodilation and relaxation of penile corpus cavernosum. However, ginsenoside Re in the berries flesh of ginseng has been reported to be easily decomposed due to organic acid based hydrolysis by heating in the manufacturing process. In the previous study, we confirmed that ultrafiltration (UF) is the most efficient method to obtain biologically active compounds and to remove low molecular organic acid.<sup>9</sup> Erectile dysfunction (ED) is a general problem in middle-aged male worldwide and defined as the insufficient maintenance of penile erection in sexual intercourse.<sup>5,10</sup> ED induced by reduced nitric oxide (NO) bioavailability causes the decreased inflow of blood in the penile corpus cavernosum in chronic diseases such as diabetes, vascular problem and/or high alcohol consumption.<sup>5,11</sup> The sexual and erectile activities of ginseng extract including ginseng berry on ED model have been previously reported,<sup>5</sup> but very few are known to be effective on the next signaling pathway such as NO-cGMP pathway. Accordingly, we describe ginsenoside Re enriched fraction (GS-F3K1), which has been purified from ginseng berries flesh by ultrafiltration method, and the mechanisms of the action of GS-F3K1 on alcohol-induced erectile dysfunction.

## Experimental

**Material** – The ginseng berries were collected from cultivated *Panax ginseng* at Geumsan (Korea) on July 15, 2013. The Red Ginseng (*Panax ginseng* Meyer) used in this experiment were purchased from local market Geumsaninsam cooperative association (Geumsan, Korea). The specimen of ginseng berry (No.; GS201307) and root (No.; GS201105) were deposited in the International Ginseng and Herb Research Institute. The instruments used for ultrafiltration was Flex Stand benchtop pilot hollow fiber system (GE Healthcare, U.K.) with Hollow Fiber cartridge (Model; UFP-1-C-4M, molecular weight cut off (MWCO); 1 kDa, membrane area; 650 cm<sup>2</sup>, GE Healthcare, U.K.). Ginsenoside standards were purchased from Ambo Institute (Daejeon, Korea). The acetonitrile



**Fig. 1.** Schematic representation of the purification procedure of GS-F3K1 from ginseng berry. Ginsenoside Re enriched fraction (GS-F3K1) from ginseng berries flesh was prepared by a combination of continuous centrifugation and ultrafiltration to remove low molecular weight compounds.

and methanol were of HPLC grade (Fisher Scientific, USA). All other chemicals were of analytical grade.

**Preparation of ginseng berry flesh and red ginseng extracts** – Several kinds of ginseng berry flesh extracts were prepared to measure the change in ginsenoside composition based upon heating process. A flesh part of the ginseng berries were freeze dried to produce ginseng berry flesh powder (GBFP), which was extracted with distilled water for 1 hour at room temperature using microwave, followed by freeze drying to produce ginseng berry flesh extract (GBFE). Another water extract was evaporated and dried at 60 °C *in vacuo* to prepare ginseng berry flesh extract (EGBE), and also to prepare heated ginseng berry flesh extract (HGBE) by heating process at 90 °C for 3 hours. To prepare ginsenoside Re enriched fraction (GS-F3K1), ginseng berries flesh was mixed with the same amount of distilled water and then was centrifuged at 15,000 × g using the continuous liquid and solid separating centrifuges (HANILBIOMED Co., Ltd, Gwangju, Korea). The liquid part of flesh was concentrated to 50% (vol) by circulatory ultrafiltration system equipped with membrane filter (1 kDa). The inner fluids were freeze dried (GS-F3K1) and stored at –80 °C, and then immediately reconstituted before use (Fig. 1). The other liquid part of flesh after continuous centrifugation was freeze dried (GBE) for analysis of various ginsenosides. To produce RGE, a mixture (1 kg) of main body and tail root of red ginseng (4:6, w/w) were extracted with

1 liter of 70% ethanol under 70 °C for three times and concentrated *in vacuo*. The dried red ginseng extract (RGE) was found to contain ginsenoside-Rg1 7.4, Re 12.4, Rf 3.9, Rb1 32.6, Rc 22.7, Rb2 9.8, Rd 4.6 mg/g, respectively.

**Analysis of ginsenosides** – Ten mg of REG or 5 mg of GS-F3K1 powder was dissolved with 1 mL of methanol (MeOH) and filtered out by 0.45 µm membrane filter after extraction with ultrasonic waves for 30 min, then finally analyzed in HPLC. The HPLC system was Waters 1525 (Waters, MA, USA) with PDA detector (Waters, 2998). Waters Xbridge™ C18 column (250 mm × 4.6 mm, 5 µm, Waters, MA, USA) was also used. The detection wavelength, flow rate, injection volume, and column oven temperature were set at 203 nm, 1.0 mL/min, 20 µL, and 40 °C, respectively. A mixture of purified water (A) and acetonitrile (B) was used as the following gradient program: 0 min 18% B, 0 - 42 min 24% B, 42 - 46 min 29% B, 46 - 75 min 40% B, 75 - 100 min 65% B, 100 - 135 min 85% B, and 135 - 180 min 18% B.

**Animals** – Sixty-six male Sprague-Dawley rats (6 weeks old, specific pathogens free) were purchased from SAMTAKOBIO Korea (Osan, Korea). The experiments were performed in accordance with the principles and approval from Ethics Committee of the Wonkwang University (Approval No. WKU13-40). All animals, which were maintained in a temperature-controlled room (temperature 22 ± 2 °C, humidity 50 ± 5%) on a 12 h light/dark cycle, were acclimatized to the laboratory environment while housed in individual cages for 1 week before starting the experiment.

**Animal model for erectile dysfunction (ED)** – Rats were randomly divided into 6 groups: normal, control, low-dose (GS-F3K1, 0.1 g/kg), medium-dose (GS-F3K1 0.25 g/kg), high-dose (GS-F3K1, 0.5 g/kg) and positive control (RGE 0.5 g/kg). After a week of acclimation, the normal group was fed with standard chow without GS-F3K1 and water sterilized by ultraviolet radiation. All of the 30 rats, except for the rats comprising the normal group, were provided with water containing 20% ethanol, GS-F3K1, and RGE for 5 weeks. GS-F3K1 0.1, 0.25 and 0.5 g/kg, and RGE 0.5 g/kg alone were orally administered as low-, medium-, and high-dose groups, and positive control, respectively. We measured the average feeding rate for one week after the administration of GS-F3K1 but no significant differences were observed.

**Measurement of erectile responses** – Experimental animals were anesthetized with a 1:7 mixture of xylazine (23.3 mg/mL) and ketamine (57.68 mg/mL by intraperitoneal injection) (0.4 mL/250 g) and were maintained under

anesthesia with phenobarbital (Ruminal, Daihan Pharm Co., Ltd, Seoul, Korea) by intramuscular injection; this treatment had no effect on the blood pressure. Animals were sacrificed and erectile response was measured as reported previously.<sup>12</sup> Briefly, the ventro dorsally positioned carotid artery of the animals was exposed and connected to Polyethylene 50 (Dow Corning, Midland, MO, USA) filled with heparinized saline (100 IU/mL). The silicone tube was connected to channel of the pressure transducer (MLT1199BP Transducer, AD Instruments, Colorado Springs, CO, USA). The right major pelvic ganglion was exposed by a low abdominal midline incision, and the corpus cavernous nerve on either side of the prostate was isolated through a skin incision overlying the penis. A bipolar platinum electrode and Stimulator HC (ML155, AD Instruments, Dunedin, New Zealand) were used to directly stimulate the right cavernous nerve. Next, the right base of the penis was exposed, and the right corporal body was cannulated with a 22-gauge needle primed with 100 U/mL heparin saline and connected to channel 2 of the pressure transducer (MLT1199BP Transducer, AD Instruments, Dunedin, New Zealand). The electrostimulation was controlled by an amplifier (Podbridge, AD Instruments) connected to a data acquisition system (ML846 Powerlab 4/26, AD Instruments, Dunedin, New Zealand). The intracavernous pressure (ICP) was recorded and analyzed using a data analysis software (Powerlab Program, AD Instruments). The average maximum ICP was represented by maximum ICP/mean arterial pressure (MAP) × 100.

**Tissue extracts preparation** – After the erectile response test, blood samples and the corpus cavernosum tissue of penis were collected for further experiments. The blood and other connective tissues of the corpus cavernosum tissues were removed by washing with ice-cold saline. The isolated tissues were homogenized with 4 mL of 0.1 M potassium phosphate buffer (pH 7.4) per gram tissue (IKA T10 basic; IKA Laboratory Equipment, Staufen GmbH & Co., Germany) and supernatant was collected after centrifugation at 10,000 × g for 10 min at 4 °C.

**Measurement of the nitrite level** – To measure the nitrite level in the corpus cavernosum tissue, we used a nitric oxide detection kit (INTRON Biotechnology, Inc., Gyeonggi, Korea) based on colorimetry using the Griess reaction. The method was performed, and the results were analyzed according to manufacturer's protocol. Briefly, 50 µL of the sulfanilamide solution was added to 100 µL of the tissue-derived supernatant in a 96-well plate. The mixture was incubated at room temperature for 5 min. Naphthylendiamine (50 µL) was then added to each

well, and the plate was incubated for 5 min, and the sample absorbance was finally measured at 540 nm using UV spectrometry. The standard curve was determined by measuring the absorbance of 0.98 - 1,000  $\mu\text{M}$  nitrite standards, and the results were expressed as  $\mu\text{M}$  nitrite per gram of tissue.

**Measurement of the cGMP level** – To measure the cGMP level in the corpus cavernosum tissue, we used a cGMP kit (Enzo Life sciences, Inc., FL, USA). The method was performed, and the results were analyzed according to manufacturer's protocol. Briefly, 50  $\mu\text{L}$  of the conjugate and antibody solution was added to 100  $\mu\text{L}$  of the tissue-derived supernatant in a 96-well plate. The mixture was allowed for shaking incubation (500 rpm) at room temperature for 120 min. After washing out three times, pNpp substrate solution (200  $\mu\text{L}$ ) was added to each well, the plate was incubated for 60 min. Stock solution (50  $\mu\text{L}$ ) was added to each well, and the sample absorbance was measured at 504 nm by UV spectrometry.

**Statistical analysis** – All data were expressed as mean  $\pm$  SD and differences between groups were analyzed using independent *t* test. All analyses were performed using SPSS 21 (SPSS Inc., Chicago, USA). Each value was the mean of at least 3 separate experiments in each group, and data with different superscript letters are significantly different when *p* value is less than 0.05.

## Result

**Preparation of GS-F3K1 by ultrafiltration from ginseng berry extract** – To measure decomposition of ginsenoside Re by heat, three ginseng berry flesh extracts were prepared as follows; freeze dried ginseng berry extract (FGBE), evaporated ginseng berry extract at 60  $^{\circ}\text{C}$  (EGBE), and evaporated ginseng berry extract after heating at 90  $^{\circ}\text{C}$  for 3 hours (HGBE). As shown in Table 1, the content of ginsenoside Re was 50.1, 41.5, and 20.0 mg/g in FGBE, EGBE, and HGBE, respectively. The level of ginsenoside Re in HGBE was found to show lower values (60.1 and 51.8%) than those of FGBE and EGBE, indicating the possibility of decomposition by heating process such as concentration and sterilization.

Therefore, ginsenoside Re enriched fraction (GS-F3K1) from ginseng berries flesh was prepared by a combination of continuous centrifugation and ultrafiltration to remove low molecular organic acid and the ginsenoside Re content was analyzed. Extraction method by continuous centrifugation at 1,500  $\times$  g has found to increase 42.1% of ginsenoside Re content (GBE 86.4 mg/g vs FGBE 50.1 mg/g) to compare general extraction method (Table 1 and 2). GS-F3K1 was found to increase the content (109.0 mg/g) two times as much as that of FGBE (Table 1 and 2). The ginsenosides of GS-F3K1 used for this experiment was confirmed to be G-Rg1  $7.0 \pm 0.46$ , G-Re  $109.0 \pm 1.26$ , G-Rb1  $13.2 \pm 0.31$ , G-Rc  $10.5 \pm 0.54$ , G-Rb2  $12.4 \pm 0.18$ , and G-Rd  $12.1 \pm 1.02$  mg/g, respectively.

**Effect of GS-F3K1 on the erectile response** – The erectile response was assessed in all rats by measuring the maximum intra cavernous pressure (ICP) that was corrected by mean arterial pressure (MAP) in rats (Fig. 2). The ICP of the control group was significantly decreased compared with normal group ( $0.58 \pm 0.02$  and  $0.76 \pm 0.03$  mmHg, respectively). In contrast, when compared to the control group, ICP in both the GS-F3K1 and RGE groups were  $0.69 \pm 0.017$ ,  $0.63 \pm 0.71$ ,  $0.71 \pm 0.02$ , and  $0.66 \pm 0.02$  mmHg, respectively. Especially, treatment with 0.5 g/kg GS-F3K1 group exhibited markedly ICP compared with the control group, but no dose-dependent change was observed.

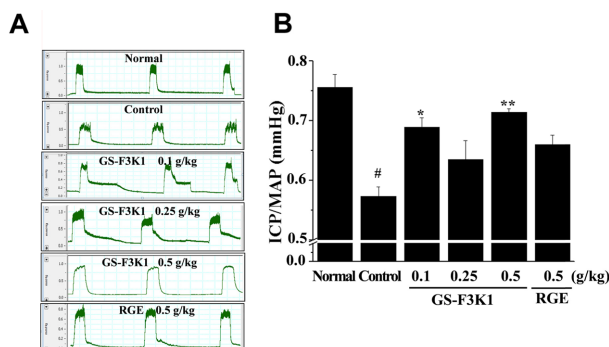
**Effect of GS-F3K1 on the nitrite** – In the corpus cavernosum, the nitrite level was decreased significantly in control group ( $11.37 \pm 0.12$  to  $9.93 \pm 0.08$   $\mu\text{M}$ ), and increased in dose-dependent manner at 0.1 g/kg, 0.25 g/kg, and 0.5 g/kg GS-F3K1 groups ( $10.39 \pm 0.10$ ,  $10.98 \pm 0.05$ , and  $11.67 \pm 0.13$   $\mu\text{M}$ , respectively) compared with the control group. In addition, although the nitrite level was higher in the 0.5 g/kg GS-F3K1 group compared with the 0.1 g/kg and 0.25 g/kg GS-F3K1 groups, but not increased compared to RGE group ( $11.57 \pm 0.14$   $\mu\text{M}$ ) (Fig. 3).

**Effect of GS-F3K1 on the cGMP concentration** – Finally, we investigated the effect of GS-F3K1 on cGMP level in ethanol-induced ED rats (Fig. 4). cGMP concentration was markedly decreased compared to normal

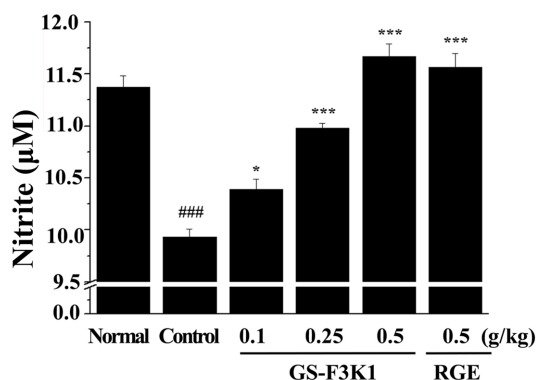
**Table 1.** Change in ginsenoside composition of ginseng berry extract by heat

Ginsenoside (mg/g)	Rg1	Re	Rf	Rb1	Rc	Rb2	Rd	S-Rg3	R-Rg3
FGBE <sup>a</sup>	4.7 $\pm$ 0.23	50.1 $\pm$ 2.51	1.4 $\pm$ 0.07	8.1 $\pm$ 0.41	3.3 $\pm$ 0.17	3.9 $\pm$ 0.20	4.0 $\pm$ 0.20	0.1 $\pm$ 0.00	0.0 $\pm$ 0.00
EGBE <sup>b</sup>	4.1 $\pm$ 0.20	41.5 $\pm$ 2.08	1.0 $\pm$ 0.05	6.5 $\pm$ 0.32	1.8 $\pm$ 0.09	2.3 $\pm$ 0.11	2.1 $\pm$ 0.10	0.2 $\pm$ 0.00	0.2 $\pm$ 0.00
HGBE <sup>c</sup>	1.7 $\pm$ 0.09	20.0 $\pm$ 1.00	1.1 $\pm$ 0.05	6.6 $\pm$ 0.33	1.1 $\pm$ 0.12	1.4 $\pm$ 0.32	1.5 $\pm$ 0.37	2.5 $\pm$ 0.12	3.0 $\pm$ 0.15

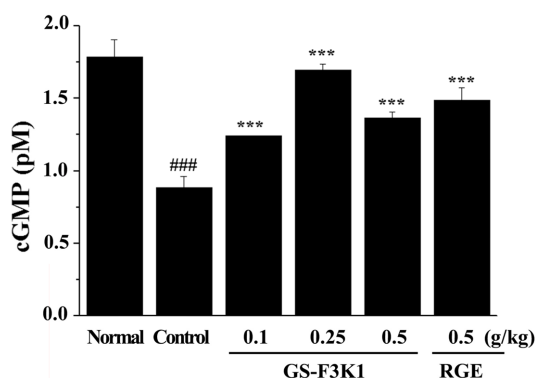
<sup>a</sup>FGBE; Freeze dried ginseng berry extract, <sup>b</sup>EGBE; evaporated ginseng berry extract, <sup>c</sup>HGBE; heated ginseng berry extract.



**Fig. 2.** Effects of GS-F3K1 and RGE on the erectile response to cavernous nerve stimulation in ethanol-induced ED rats. (A) Oscillations of the intracavernous pressure (ICP), and (B) Statistic analysis. Data are given as mean of value  $\pm$  SD ( $n=6$ ) from three independent experiments. # $p < 0.05$  when compared with the normal group. \* $p < 0.05$ , \*\* $p < 0.001$  when compared with the control group by independent  $t$  test.



**Fig. 3.** Effects of GS-F3K1 and RGE on nitrite production to cavernous nerve stimulation in ethanol-induced ED rats. Data are given as mean of value  $\pm$  SD ( $n=6$ ) from three independent experiments. ### $p < 0.001$  when compared with the normal group. \* $p < 0.05$ , \*\*\* $p < 0.001$  when compared with control group by independent  $t$  test.



**Fig. 4.** Effects of the GS-F3K1 and RGE on cGMP level to cavernous nerve stimulation in ethanol-induced ED rats. Data are given as mean of value  $\pm$  SD from three independent experiments. Level of significance was identified statistically using independent  $t$  test. ### $p < 0.001$  when compared with the normal group. \*\*\* $p < 0.001$  when compared with the control group by independent  $t$  test.

group in the corpus cavernosum of ethanol-induced ED rats ( $0.88 \pm 0.08$  and  $1.79 \pm 0.11$  pM, respectively). In contrast, levels of cGMP were significantly increased in all GS-F3K1 groups and RGE group ( $1.25 \pm 0.00$ ,  $1.69 \pm 0.04$ ,  $1.36 \pm 0.04$ , and  $1.49 \pm 0.08$  pM, respectively).

## Discussion

Korean ginseng has traditionally been reported to have aphrodisiac properties, for example, increasing the effectiveness of erection.<sup>13</sup> In Asia, ginseng is widely included in herbal formulations used for the treatment of sexual dysfunction. ED is a highly prevalent common disorder that affects millions of men in the world and considered to be an early symptoms of atherosclerosis and being noted as precursor of various systemic vascular disorders. *Panax ginseng* has been recently used as a tonic and restorative agent to maintain the physical vitality and shows a dose-dependent relaxing effect on the isolated rabbit corpus cavernosum strip.<sup>14</sup> Additionally, several kinds of clinical studies have shown that ginseng improves sexual function as follows. The effect of ginseng extract on psychogenic or mildly vasculogenic erectile dysfunction was investigated and compared to reference medication and placebo in a randomized trial including 90 patients (age range 26–70, mean age  $43.7 \pm 8.6$  years). For a period of 3 months, participants have received one of the following daily dosages: 1.8 g red ginseng extract, 25 mg trazodone, or placebo. Before and after the study period, symptoms were evaluated with a detailed questionnaire and serum testosterone sampling also noted. No change was observed regarding testosterone levels, frequency of intercourse, premature ejaculation or morning erections in either study group. However, patients under ginseng medication reported to have greater improvements in early detumescence, penile rigidity, libido, and satisfaction than participants in the other two groups.<sup>15</sup> A similar study was recently found to be conducted among 64 patients suffering from borderline organic and psychogenic erectile dysfunction over a period of 3 months, during which participants were treated with red ginseng powder (dosage not specified) alone or placebo. On subjective analysis, all parameters were improved under ginseng medication compared to placebo with a total improvement rate of about 76% in the ginseng group. Overall testosterone levels showed no significant changes but only 6 patients with initially reduced testosterone levels were normalized during the course of the trial.<sup>16</sup> Both of subjective and physiological parameters were successfully assessed in a randomized double-blind study among 26

men suffering from mild vasculogenic impotence. Before and after 3-months administration of either Red Ginseng or placebo, participants were evaluated using an available sexual function questionnaire, duplex sonography of the cavernous artery, and nocturnal penile tumescence monitoring. Interestingly, there is no statistically significant differences were found regarding physiological functions but scores on the subjective questionnaire for total improvement and improvement of sexual satisfaction were found to be higher among ginseng-treated patients.<sup>17</sup>

On the other hand, ginseng berry has received attention with more and more interest from researchers, since several studies for treating diabetes and obesity have shown that it would be normalized blood glucose level, improved sensitivity to insulin, lowered cholesterol levels, decreased weight by reducing appetite. However, only a very few report about sexual improving effect of ginseng berry are known despite of transmitting by word of mouth.

In 2012, Choi *et al.* have pointed out that oral administration of the standardized Korean ginseng berry extract (SKGB, 350 mg per tablet) improved all domains of sexual function in a multicenter, placebo-controlled, double-blind clinical study. One hundred nineteen men with erectile dysfunction were administered with 4 tablets of either SKGB or placebo, every day for 8 weeks. Efficacy estimated with International Index of Erectile Function (IIEF) showed the possibility of SKGB extract would be considered as an alternative medicine to improve sexual life in men with sexual dysfunction.<sup>18</sup> Penile erection is an important hemodynamic process that fully depends on complex interactions between peripheral factors and components of the central nervous system, resulting in smooth muscle relaxation and vasodilation in the corpus cavernosum.<sup>19</sup> In response to sexual stimulation, cavernous nerves and endothelial cells have released nitric oxide (NO), which promotes the production of cGMP via the cytosolic enzyme called as granulate cyclase. The resulting molecular cascade pathway has been found to decrease intracellular calcium levels, allowing relaxation of smooth muscle cells in the cavernosal bodies. Penile blood flow increases and sinusoidal spaces expand, preventing venous outflow of blood and resulting in erection, indicating it to be a hemodynamic event regulated by NO/cGMP pathway.<sup>20</sup> The NO is multifunctional substances and distributed in almost every tissues, and ubiquitous presence in higher organisms.<sup>21,22</sup> Basal level of NO contributes to maintain a vasodilatory tone through its interaction and subsequent activation of soluble guanylate cyclase, as the intracellular generation of cGMP in smooth muscle cells leads to vascular

relaxation.<sup>23,24</sup> Zhang *et al.* reported that ginsenoside Re induced nitric oxide synthase and increase nitric oxide.<sup>6,7</sup> Flesh of ginseng berries contain about 6% of ginsenoside Re. For the purpose of investigation of newly active fraction from ginseng berries flesh, we carried out to prepare ginsenoside Re enriched fraction (GS-F3K1, above ginsenoside Re 10%, w/w) by a combination of continuous centrifugation and ultrafiltration. GS-F3K1 was administered for 5 weeks to assess the improving effects on alcohol-induced erectile dysfunction (ED). In 2008, Ponizovbsky reported that men with alcohol dependence commonly suffer from alcohol-induced sexual dysfunction and have poor quality of life, suggesting alcohol dependence to be associated with erectile dysfunction.<sup>25</sup> All the experimental animals were divided into six groups; one normal group, one control group, three GS-F3K1-treated groups (0.1, 0.25, and 0.5 g/kg), and one positive control group (red ginseng extracts, RGE, 0.5 g/kg). We performed ethanol-induced ED model by oral administration of 20% ethanol instead of water every day for 5 weeks in order to investigate the effects of GS-F3K1 on the animal model, we measured levels of nitrite expression, cyclic guanosine monophosphate (cGMP) and erectile response of the penile corpus cavernosum of rat. The erectile response of the corpus cavernosum was successfully restored after GS-F3K1 administration, to a level similar to the normal group. The level of nitrite and cGMP expression in the corpus cavernosum of GS-F3K1-administered male rats was increased significantly compared to positive control group, indicating that GS-F3K1 should effectively restore ethanol-induced ED in male rats. We think that these results came from ginsenoside Re content. GS-F3K1 contains 10.9% of ginsenoside Re, while its content of RGE was confirmed to be 1.24%. From all of these above results, it might be concluded that ginsenoside Re enriched GS-F3K1 provide strong evidence for potent effects on ethanol-induced ED model. Furthermore, we demonstrate the improved effects of erectile response and increased nitric oxide and cGMP level by GS-F3K1 administration on ethanol-induced ED model, suggesting that GS-F3K1 would be responsible for an effective materials on ethanol-induced ED.

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