Effect of SA1, a Herbal Formulation, on Sexual Behavior and Penile Erection

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SA1 is a mixture of 9 Oriental herbs (Korean red ginseng, fermented soybean, Tribulus terrestris, Fructus Rubi, Fructus Lycii, Semen Cuscutae, Dioscorea Rhizome, Fructus Corni and Fructus Crataegi) that are widely used as energizers and vitalizers in the indigenous system of medicine and have been alleged to improve the sexual functions in men. This study evaluated SA1 using both in vitro and in vivo experiments on laboratory animals in order to determine its effect on the sexual behavior and penile erection. The male rats used to examine the copulatory behavior were administered either the vehicle or SA1 (30, 100, 300, 600 mg/kg) orally for 2 weeks. The intracavernous pressure and systemic blood pressure were recorded in anesthetized rats. The responses to acetylcholine and SA1 of rabbit corpus cavernosum strips were also examined. There was an overall increase in the copulatory behavior parameters in the SA1-treated rats, which was reflected by a decrease in the mount and intromission latencies and an increase in the ejaculation latency and mount frequency. SA1 significantly increased the ratio of the intracavernous pressure to mean arterial pressure. In vitro, SA1 significantly enhanced the relaxation responses to acetylcholine. These results suggest that SA1 improves the sexual activity and erectile function.

Key words SA1; copulatory behavior; erectile function; intracavernous pressure; cavernosal strip

The sexual dysfunction is a common disease, and with an increasing incidence as a result of the longer lifespan, the increasing prevalence of degenerative diseases as well as the increase in injuries and stress associated with industrialized lifestyles. Both medical and surgical treatment modalities are available for treating sexual dysfunction. However, despite the increasing availability of effective conventional medical treatments, plant-derived and herbal remedies continue to be a popular alternative for men and women seeking to improve their sex life.1) Nevertheless, the efficacy of most of these herbal agents in treating sexual dysfunction remains unclear.

SA1, which was introduced by Chungbuk Oriental Medicine Center, is a mixture consisting of 9 Oriental herbs that is used to treat sexual health and problems (Table 1). Panax ginseng C. A. Meyer has been used as a tonic and restorative to maintain the physical vitality, and shows a dose-related relaxing effect on the isolated rabbit corporal smooth muscle strip.2) Tribulus terrestris exhibits aphrodisiac activity due to its androgen increasing property.3) Rubus coreanus Miqel increases the levels of testosterone in the blood4) and Lycium chinense Miller improves the quality of the sperm.5) Cuscuta chinensis Lamark invigorates the reproductive system and reproductive endocrine function in male rats6) and Cornus officinalis Sieb et Zucc increases the levels of RNA in the interstitial cells of the testicle.7) Crataegus pinnatifida Bge. var. major N. E. Br. inhibits the expression of a vasoconstrictive mediator,8) and fermented soybean enhances the level of thrombolysis near the vessel wall.9) The antioxidant and anti-aging properties of the Dioscorea batatas Decne have been suggested.10) Therefore, SA1 might combine the therapeutic effects from each herb on sexual dysfunction.

Accordingly, this study evaluated the sexual and erectile activities of SA1 in vivo and in vitro.

Table 1. Composition of SA1

<table>
<thead>
<tr>
<th>Herb</th>
<th>Percentage</th>
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<tbody>
<tr>
<td>Powdered extract of Panax ginseng C. A. Meyer root</td>
<td>20</td>
</tr>
<tr>
<td>Powdered extract of fermented soybean</td>
<td>12</td>
</tr>
<tr>
<td>Powdered extract of Tribulus terrestris L.</td>
<td>8</td>
</tr>
<tr>
<td>Powdered fruit of Rubus coreanus Miqel</td>
<td>10</td>
</tr>
<tr>
<td>Powdered fruit of Lycium chinense Miller</td>
<td>10</td>
</tr>
<tr>
<td>Powdered seed of Cuscuta chinensis Lamark</td>
<td>10</td>
</tr>
<tr>
<td>Powdered rhizome of Dioscorea batatas Decne</td>
<td>10</td>
</tr>
<tr>
<td>Powdered fruit of Cornus officinalis Sieb et Zucc</td>
<td>10</td>
</tr>
<tr>
<td>Powdered fruit of Crataegus pinnatifida Bge. var. major N. E. Br.</td>
<td>10</td>
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</table>

SA1 was prepared by blending the above medicinal plants at the percentage indicated in the Table.

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Materials and Methods

Animals Adult male and female Sprague-Dawley rats (200—250 g, 6—7 weeks old) as well as the male New Zealand White rabbits (2.5—3.5 kg, 10—14 weeks old) were obtained from the Jeil Animal Breeding Company of Korea. The animals were acclimated to the laboratory conditions at Sungkyunkwan University for at least one week. During this period, food (Samyang Co., Korea) and tap water were supplied ad libitum. The animals were kept in a temperature- and humidity-controlled room (25 ± 1°C and 55 ± 5%, respectively) with a light–dark cycle of 12 h. The Sung.
kyungkwan University Animal Care and Use Committee approved all the protocols.

**Preparation of SA1** The SA1 was prepared at the Chungbuk Oriental Medicine Center, Jecheon, Korea. The powdered Oriental herbs were purchased from the Gyeongdong Herbal Market in Korea. Dr. D.H. Shin, who is a co-author, authenticated the dried herbs. Voucher specimens were deposited in the herbarium of the Chungbuk Oriental Medicine Center, Jecheon, Korea. SA1 was prepared by blending the powdered herbs at the percentage indicated in Table 1.

**Administration of SA1** In the copulatory behavior study, SA1 was suspended in 0.5% methylcellulose and administered orally at four different doses of 30, 100, 300 and 600 mg/kg of body weight once per day for 2 weeks. In the *in vivo* erectile experiment, 300 mg/kg SA1 was chosen based on the copulatory tests. In the *in vitro* erectile experiment, SA1 was dissolved in 50% dimethyl sulfoxide (0.05% final concentration) and added at doses of 5, 10, 20 and 40 μg/ml.

**Copulatory Behavior** The copulatory tests were carried out according to the method reported previously. The male rats were divided into five groups. The animals in the first group were used as the controls. The rats in the four test groups were treated with SA1 at the above-mentioned doses. The female rats were ovariec-tomized and only those exhibiting good sexual receptivity were used as the mating stimulus. The female rats were brought into estrus with single subcutaneous dose of 0.1 mg estradiol benzoate and 1.0 mg progesterone, 72 and 6 h before the tests, respectively. All the tests were initiated approximately 4 h after light offset in a 12:12 light–dark cycle. The male rats were first placed in the observation arena for an adaptation period of 3—5 min, and a sexually receptive female rat was then introduced. The following parameters of copulatory behavior were recorded: i) the mount latency (ML), the time from introducing the female to the first mount; ii) the intromission latency (IL), the time from introducing the female to the first intromission; iii) the ejaculation latency (EL), the time from the first intromission to the terminal ejaculation; iv) the mount frequency (MF), the number of mounts without intromission within a series; v) the intromission frequency (IF), the number of intromissions in a series; vi) the ejaculation frequency (EF), the number of ejaculations in a series; and vii) the post-ejaculating interval (PEI), the time from ejaculation to the initiation of a new series, as indicated by the next intromission. The tests were terminated when one of the four conditions were fulfilled: i) when there was no intromission within 30 min from the beginning of the test; ii) when there was no ejaculation within 20 min of the first intromission; iii) when there was no intromission within 15 min after ejaculation, or iv) when there was the first intromission following ejaculation. All the copulatory events were recorded automatically using a computerized video camera recorder (BW-2302, Samsung Techwin Co. Ltd., Seoul, Korea).

**Intracavernous Pressure/Mean Arterial Pressure Ratio** The rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (40 mg/kg), and the bladder and prostate were then exposed by a tranperitoneal midline incision. The pelvic trunk located at the posterolateral wall of prostate was identified, and the cavernous nerve was isolated. A platinum electrode was placed on the cavernous nerve, connected to an electric stimulator (Harvard Apparatus, Inc., MA, U.S.A.) and electrically stimulated at 5 Hz and 5 V. The corpus cavernosum was isolated after incising the penile skin. The intracavernous pressure (ICP) was measured by placing a 26 gauge needle into the corpus cavernosum, and the mean arterial pressure (MAP) was monitored by placing a 22 gauge needle into the right carotid artery. The needle was connected via a PE-tube to a blood pressure transducer (PT300, Grass Instrument Co., RI, U.S.A.). Heparinized saline (50 U/ml) was used to prevent clotting of the pressure line and catheters. Data acquisition was achieved using a physiograph (PowerLab/400, ADInstruments Pty. Ltd., Co., U.S.A.).

**Organ Bath Experiments** The corpus cavernous strips were prepared from the penises of rabbits and mounted in tissue baths containing 10 ml Krebs bicarbonate solution at 37 °C equilibrated with 95% O2 and 5% CO2. The strips were suspended with silk ties to a force-displacement transducer (FT03, Grass Instrument Co., RI, U.S.A.) and isometric tension was recorded on the physiograph. After 60 min equilibration, the strips of corpus cavernosum were loaded with a resting tension of 2 g. The tissue was contracted using phenylephrine (5 × 10−6 M) in order to determine the optimal resting tension for contraction. The tension was considered to be optimal for an isometric contraction when the amplitude of the contraction was within 10% of three separate rounds of contractions. The relaxations were examined in the muscle strips precontracted with phenylephrine (5 × 10−6 M). After the strips had been stabilized, acetylcholine (ACh) at increasing concentrations from 10−8 M was added to achieve relaxation. SA1 was added in increasing concentrations from 5 μg/ml. In some experiments, SA1 was administered to determine if there was any enhancing effect on relaxation due to the long-term administration other than the proven direct relaxation effect. The values are expressed as a percentage of relaxation referenced to the contraction by phenylephrine.

**Statistical Analysis** All the data is expressed as a means±S.E.M. Experimental groups were compared using one-way analysis of variance with post hoc Bonferroni’s test or unpaired Student’s *t* test. *p* < 0.05 was considered statistically significant.

**RESULTS**

**Sexual Behavior** Table 2 shows the copulatory behavior with the females after treating the male rats with SA1. There were no significant changes in copulatory behavior compared with the control after a single administration (30, 100, 300, 600 mg/kg) of oral SA1 (data not shown). Two weeks administering the SA1 at doses of 100, 300 and 600 mg/kg, there were significantly lower mount and intromission latencies and increased ejaculation latency and mount frequency. The administration of SA1 at 300 mg/kg significantly increased the intromission frequency, whereas it decreased the post-ejaculating interval. No significant change in ejaculation frequency was observed in the SA1-treated animals as compared with the control rats.

**Intracavernous Pressure/Mean Arterial Pressure Ratio** Under normal conditions the MAP in the controls and SA1 animals were 114.7 ± 6.3 and 121.6 ± 8.1 mmHg, respectively. There was no difference in the MAP in the controls and SA1 groups under the same stimulation of the cavernous nerve.
The strips with 2 weeks administration of SA1 also showed dose-dependent relaxation by ACh (ACh $10^{-8}$ M, 2.0 $\pm$ 0.54%; $10^{-7}$ M, 18.24 $\pm$ 3.98%; $10^{-6}$ M, 51.83 $\pm$ 6.78%; $10^{-5}$ M, 74.10 $\pm$ 7.58%; $10^{-4}$ M, 79.41 $\pm$ 8.03%). The administration of SA1 produced more pronounced relaxation than the control (Fig. 3).

**DISCUSSION**

A variety of plants have been used in traditional medicine as sexual stimulants in many countries. However, the scientific rationale for their use is unclear.

In this study, the rats showed an improvement in their sexual behavior pattern after 2 weeks administration of SA1 at different doses. The copulatory behavior of the sexually normal adult male rats is characterized by a series of alternate mounts and intromissions, which were well defined in number of each animal, and culminated in ejaculation. However, inexperienced laboratory rats take some time to establish their copulatory pattern. Usually, tests are necessary once or twice a week to determine each rat’s individual profile, as represented by the number of parameters that remain virtually unchanged for some time. As a result of this stability, the effects of drugs that potentially inhibit or stimulate the male sexual behavior can be examined. Sexually active rats were selected for the copulatory tests. In this study, a dose level of 100, 300 and 600 mg/kg SA1 caused a significant decrease in the mount and intromission latencies and an increase in the ejaculation latency and mount frequency. The intromission frequency was increased at 300 mg/kg SA1. This suggests

### Table 2. Effect of SA1 on Sexual Behavior in Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>ML (s)</th>
<th>IL (s)</th>
<th>EL (s)</th>
<th>MF</th>
<th>IF</th>
<th>EF</th>
<th>PEI (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>173.0 $\pm$ 39.2</td>
<td>519.3 $\pm$ 70.9</td>
<td>168.4 $\pm$ 65.2</td>
<td>10.7 $\pm$ 1.4</td>
<td>6.9 $\pm$ 2.4</td>
<td>1.6 $\pm$ 0.2</td>
<td>538.8 $\pm$ 38.1</td>
</tr>
<tr>
<td>SA1</td>
<td>30</td>
<td>34.4 $\pm$ 8.0**</td>
<td>259.7 $\pm$ 110.4</td>
<td>299.2 $\pm$ 44.0</td>
<td>19.4 $\pm$ 2.4*</td>
<td>14.5 $\pm$ 2.5</td>
<td>2.2 $\pm$ 0.3</td>
<td>364.2 $\pm$ 56.8</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>32.2 $\pm$ 9.0**</td>
<td>244.0 $\pm$ 86.5**</td>
<td>440.1 $\pm$ 48.5**</td>
<td>18.2 $\pm$ 1.8**</td>
<td>12.0 $\pm$ 2.6</td>
<td>2.4 $\pm$ 0.3</td>
<td>280.3 $\pm$ 46.0**</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>12.5 $\pm$ 1.3**</td>
<td>132.3 $\pm$ 56.8**</td>
<td>407.3 $\pm$ 42.0**</td>
<td>22.0 $\pm$ 8.8**</td>
<td>16.3 $\pm$ 3.4*</td>
<td>2.4 $\pm$ 0.2</td>
<td>320.9 $\pm$ 33.1*</td>
</tr>
<tr>
<td></td>
<td>600</td>
<td>39.4 $\pm$ 9.4*</td>
<td>214.7 $\pm$ 48.3**</td>
<td>452.9 $\pm$ 61.3**</td>
<td>20.5 $\pm$ 8.7**</td>
<td>8.3 $\pm$ 2.0</td>
<td>2.0 $\pm$ 0.2</td>
<td>533.9 $\pm$ 68.3</td>
</tr>
</tbody>
</table>

Values are means $\pm$ S.E.M. from 10—12 rats per group. *, ** Significantly different (p<0.05, p<0.01) from the control group. ML, mounting latency; IL, intromission latency; EL, ejaculation latency; MF, mounting frequency; IF, intromission frequency; EF, ejaculation frequency; PEI, post-ejaculating latency.

Fig. 1. Effect of SA1 on the ICP/MAP Ratio in the Response to Electrical Stimulation of a Rat

The values are the means $\pm$ S.E.M. for 10—12 rats per group. ** Significantly different (p<0.01) from the control group. ICP, intracavernous pressure; MAP, mean arterial pressure.

Fig. 2. Effect of SA1 on Acetylcholine-Induced Relaxation of the Isolated Rabbit Corpus Cavernous Strips Precontracted with Phenylephrine

The strips were precontracted with phenylephrine (5$\times$10$^{-6}$ M) and the cumulative response curves were constructed for acetylcholine ($10^{-6}$—$10^{-4}$ M). The values are represented as the means $\pm$ S.E.M. and are expressed as the percentage of relaxation.

* * * Significantly different (p<0.05, p<0.01) from control group. n=6 in each group.

(106.6 $\pm$ 7.7 and 112.0 $\pm$ 10.3 mmHg, respectively). In the control group, the ICP/MAP ratio was 21.9 $\pm$ 3.7% after cavernous nerve stimulation. Treatment with 300 mg/kg SA1 markedly increased the ICP/MAP ratio compared with the control group (Fig. 1).

**Organ Bath Experiments** In the present active muscle tone induced by phenylephrine, the corpus cavernosum immediately relaxed after applying ACh in a concentration-dependent manner ($10^{-8}$ M, 1.84 $\pm$ 0.47%; $10^{-7}$ M, 11.90 $\pm$ 3.40%; $10^{-6}$ M, 47.68 $\pm$ 8.29%; $10^{-5}$ M, 66.73 $\pm$ 8.00%; $10^{-4}$ M, 67.90 $\pm$ 4.42%). SA1 at 5 and 10 $\mu$g/ml had no effect on this relaxation response, but SA1 at 20 and 40 $\mu$g/ml caused significant relaxation in the ACh-mediated relaxation (Fig. 2). The strips with 2 weeks administration of SA1 also showed dose-dependent relaxation by ACh (ACh $10^{-8}$ M, 2.0 $\pm$ 0.54%; $10^{-7}$ M, 18.24 $\pm$ 3.98%; $10^{-6}$ M, 51.83 $\pm$ 6.78%; $10^{-5}$ M, 74.10 $\pm$ 7.58%; $10^{-4}$ M, 79.41 $\pm$ 8.03%). The administration of SA1 produced more pronounced relaxation than the control (Fig. 3).
that SA1 improves the sexual behavior.

A penile erection requires a well-coordinated system of vascular, endocrine, and neural networks supplying the male sexual organ. In the flaccid status, the cavernous tissues are contracted by a dominant sympathetic control to the arterioles and the cavernous smooth muscles.\(^\text{15}\) After receiving sexual stimulation, the parasympathetic nerve activity dominates and influences the local factors and there is an increasing blood flow through the cavernous artery. During an erection, which is mainly via the parasympathetic effect, the cavernous tissues are relaxed and the influx of a large amount of blood induces a rapid increase in intracavernous pressure.\(^\text{18}\) Electrical stimulation of the cavernous nerve produced a transient decrease in the mean arterial pressure and a sustained increase in the intracavernous pressure, which persisted beyond the period of stimulation. The ICP/MAP ratio provides an index of erectile activity, which controls the changes in the mean arterial pressure.\(^\text{19}\) The effect of SA1 was tested in an established \textit{in vivo} rat model, in which an erection was induced by stimulating the cavernous nerve.\(^\text{20}\) Electrical field stimulation was delivered unilaterally at a constant frequency of 5, 10, or 15 Hz for 30 s, at a pulse duration of 5 ms and 5 V. Low frequency stimulation (5 Hz) of the cavernous nerve after 2 weeks administration of SA1, resulted in a significantly higher mean intracavernous pressure. However, SA1 had no effect on the intracavernous pressure at the higher frequencies.

The relaxation of the cavernous smooth muscle requires the action of nitric oxide (NO).\(^\text{21}\) NO is a physiologic signal essential to penile erection, and disorders that reduce NO synthesis or release in the erectile tissue are commonly associated with erectile dysfunction. While both constitutively expressed neuronal NO synthase (nNOS) and endothelial NO synthase (eNOS) isoforms mediate penile erection, nNOS initiates cavernous tissue relaxation, while activated eNOS facilitates attainment and maintenance of full erection.\(^\text{22}\) ACh facilitating the production and release of NO require the endothelium.\(^\text{23}\) The released NO activates guanylate cyclase in the cytoplasm of the smooth muscle cells, producing cyclic guanosine monophosphate (cGMP).\(^\text{24}\) This product then stimulates a cGMP-dependent protein kinase, protein kinase G, which critically induces the cavernous tissue relaxation response.\(^\text{25}\) Electrical stimulation of the cavernous nerve in rodent animal models results in rapid increases in eNOS. The phosphatidylinositol 3-kinase/Akt-induced eNOS phosphorylation mechanism operates under blood flow stimuli in the penis to sustain physiologic penile erection.\(^\text{26}\) The results showed that SA1 administered directly to the corpus cavernous tissue enhances the ACh-mediated relaxation of the corpus cavernous smooth muscles \textit{in vitro}. It is believed that SA1 has relaxing effects on the vascular smooth muscle and this relaxation is associated with the NO released from the vascular endothelium. These results showed that administering SA1 for 2 weeks potentiates the cavernous relaxations provoked by ACh, which might be indirect evidence of the effect of SA1 on NO.

In conclusion, SA1 improves the sexual behavior and potentiates the erectile response in normal animals. However, the precise mechanisms involved in the aphrodisiac and erectile effects of SA1 require further investigation.

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**REFERENCES**