

The Effects of SS-cream and Its Individual Components on Rabbit Corpus Cavernosal Muscles

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SS-cream (Severance Secret cream) is made up of extracts from 9 natural products for treating premature ejaculation (PE). SS-cream has been proved to be effective in the treatment of PE in pilot clinical studies. It has also been found to have a potentiating effect of their erectile capacity in some patients. Therefore, we investigated the pharmacological actions of SS-cream and the extracts of its individual components in rabbit corpus cavernosal smooth muscle to realize the effect of SS-cream on penile erection.

Extracts of Bufonis Venenum induced a dose-related contraction of rabbit corpus cavernosal muscle, which was significantly inhibited by phentolamine. Extracts of Caryophylli Flos induced a dose-related relaxation in the muscle strips precontracted with phenylephrine ($5 \times 10^{-6}M$; PHE). Caryophylli Flos caused a dose-dependent inhibition of the PHE induced contraction and also inhibited the contractility of Bufonis Venenum. Other extracts, when used individually or in a mixture, induced a dose-related relaxation in the precontracted muscle strips with PHE. SS-cream began to exert a relaxing effect at the concentration of 0.05 mg/ml in the muscle strips precontracted muscle strips with PHE ($5 \times 10^{-6}M$); causing dose-dependent relaxation with a maximal effect at 0.2 mg/ml. The relaxation effect of SS-cream was partially inhibited by endothelial disruption and by pretreatment with methylene blue, pyrogallol, atropine, and indomethacin, although they were not statistically significant.

The results show that SS-cream has a relaxing effect on cavernosal smooth muscle. And it is partly related with enhancing the NO/cyclic GMP pathway although the relaxation mechanism in detail remains to be elucidated. Therefore, SS-cream may be effective for future treatment of mild erectile dysfunction, in addition to its role for premature ejaculation.

Key Words: Premature ejaculation, cream, cavernosum, rabbit, erection

Premature ejaculation (PE) is uncontrolled ejaculation, which classically represents ejaculation that either precedes vaginal entry or occurs immediately after vaginal entry, and it

is also the most common type of ejaculatory disorders (Lee, 1988; Kaplan, 1989; Seong *et al.* 1994). In general, causes of PE have been thought to be psychologic (Lee, 1988; Kaplan, 1989). However, our recent studies of penile biothesiometry and somatosensory evoked potential (SEP) in patients with primary premature ejaculation have shown that patients with PE have penile hypersensitivity and/or hyperexcitability (Xin *et al.* 1995a, b; Xin *et al.* 1996a), which is thought to be an organic implication of PE.

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SS-cream is made up with extracts of natural products including Ginseng Radix alba, Angelicae Gigantic Radix, Cistanchis Herba, Zanthoxylli Fructus, Torilidis Semen, Asiasari Radix, Caryophylli Flos, Cinnamoni Cortex, and Bufonis Venenum (Choi *et al.* 1993; Xin *et al.* 1994). SS-cream was formulated according to the specific composition of these ingredients and believed to have the local desensitizing effect and the enhancing capability on local blood flow. We have evaluated penile biothesiometry and SEP in PE patients treated with SS-cream and these studies showed that SS-cream increased penile sensory perception threshold, prolonged the latencies of SEP, and decreased amplitudes of SEP (Xin *et al.* 1995a, b). These results suggest that SS-cream has a local desensitizing effect on penile hypersensitivity and/or hyperexcitability in patients with PE.

SS-cream has been effective in the treatment of PE. It has also been found to have a potentiating effect on erectile capacity in some patients (Choi *et al.* 1993; Xin *et al.* 1994). Therefore, in order to realize the effects of SS-cream on penile erection, we elucidated the effects of SS-cream and each individual active ingredients on cavernosal smooth muscles.

MATERIALS AND METHODS

Materials

We used 80 post-pubertal male New Zealand White rabbit (weighing 2.5 to 3.0 kg.).

Methods

Animal preparation: The animal were sacrificed by exsanguination following anesthesia by intravenous injection of pentobarbital sodium (30~50 mg/kg). The penis was dissected and harvested en bloc and immediately stored in Tyrode solution with continuous oxygen flow. Using dissecting microscope, cavernosal tissues were trimmed and 2×2×6 mm sized strips were made.

Preparation of tissue in organ chambers: Strips of rabbit corpus cavernosum tissue were submerged in 10 ml organ chamber con-

taining Tyrode buffer solution. The strips were suspended with silk ties to a force transducer on one end and fixed to a metallic support on the opposite end. The solution was gassed with 95% air and 5% CO₂. The pH of the solution was 7.4 and the temperature was maintained at 37C. Isometric tension was measured with a force transducer and monitored with a physiography (Biopac systems, Santa Barbara, California, USA). The corpus cavernosum tissue was stretched incrementally for a period of 2 hours and the optimal resting isometric tension for contraction was determined. After every three stretches (0.5 gm. tension/stretch), the tissue was contracted with phenylephrine (5×10^{-6} M, PHE). When the amplitude of the contraction was within 10% of the previous contraction, that tension was considered optimal for isometric contraction.

Experiment: At the basal state of muscle strips, tension changes were observed with solution of SS-cream and individual ingredients of SS-cream. Solution were prepared from extracts of 9 natural products and treated from 0.01 mg/ml in cumulative increments. Relaxations were studied in muscle strips precontracted with PHE (5×10^{-6} M). After muscle strips precontracted with PHE (5×10^{-6} M) were stabilized, solutions were treated with increasing concentration from 0.01 mg/ml. Relaxing activities of SS-cream were observed in the deendothelialization tissue and preparation with the treatment of methylene blue (10^{-4} M), pyrogallol (10^{-4} M), atropine (5×10^{-6} M), and indomethacin (10^{-4} M). Disruption of the endothelium was achieved by rubbing cavernosal tissue strips between the thumb and index finger for about 20 sec. After rinsing with chilled Tyrode solution, tissue strips were gently rolled across a dry paper towel to generate shear forces across the endothelial surfaces of the lacunar spaces. Removal of the endothelium was confirmed by the absence of the relaxation response of the strip to acetylcholine (10^{-5} M) or when relaxed within a 10% range in the control state. Methylene blue, atropine, and indomethacin were added to the muscle strips precontracted with PHE (5×10^{-6} M) and incubated for 20 minutes prior to the addition

of SS-cream. Pyrogallol was added to the muscle strips precontracted with PHE ($5 \times 10^{-6}M$) for 5 minutes before the addition of SS-cream.

Solutions & Drugs

Phenylephrine hydrochloride, acetylcholine chloride, atropine sulfate, and phentolamine hydrochloride were obtained from Sigma Chemical Company (St. Louis, MO, USA).

Compositions of Tyrode solution were Na^+ 153.6, K^+ 5.3, Ca^{++} 3.0, Mg^{++} 1.2, Cl^- 157.2, $H_2PO_4^-$ 0.6, SO_4^- 1.2, HCO_3^- 7.1, and glucose 11.4 (all units in mEq/L).

Data and statistical analysis

All relaxant responses were expressed as a percentage of maximal relaxation which was calculated from the perpendicular vertical distance between PHE-induced maximal contraction point and the largest downward deflection in the tracing at any given experiment. Inhibitory actions on contractile responses were also expressed as a percentage of the contraction in the control state. The statistical analysis was performed using student's t test for paired observation and Mann-Whitney U test for unpaired observation. $P < 0.05$ was taken as being of statistical significance.

RESULTS

Effect of SS-cream and its individual ingredients at the basal state of muscle strip

SS-cream did not induce any contraction or relaxation of muscle strips in their basal state. Among the active ingredients comprising SS-cream, Bufonis Venenum extract induced contraction of the muscle strip in a dose dependent manner ($p < 0.001$) (Fig. 1). The magnitude of contraction was similar to that induced by PHE. Other components of SS-cream did not influence the basal tension of the muscle strip.

Effects of SS-cream and its individual ingredients on the submaximally precontracted muscle strip with PHE

On the precontracted muscle strip with

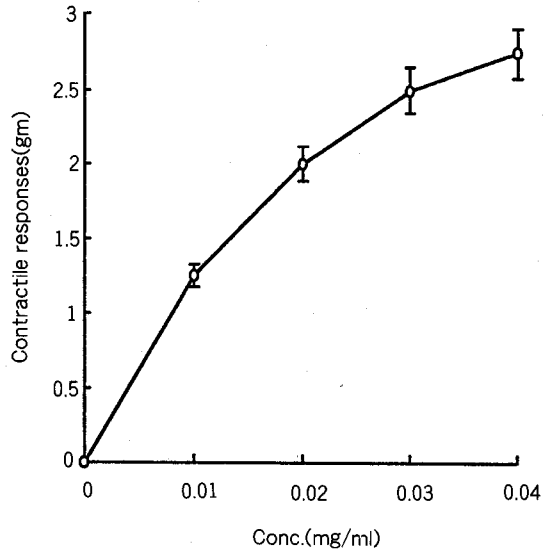


Fig. 1. Effect of Bufonic Venenum(BV) on the basal tension of rabbit corpus cavernosum muscle. It showed dose dependent contractile responses ($p < 0.001$, $n = 5$). Values were means \pm SD and were expressed as percent of the initial contraction.

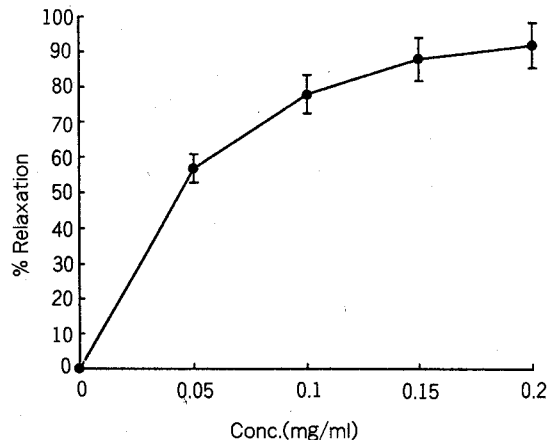


Fig. 2. Relaxation effect of SS-cream on the phenylephrine($5 \times 10^{-6}M$) induced contraction in the rabbit corpus cavernosum strips. SS-cream have a dose dependent relaxation effect on the isolated rabbit corpus cavernosum muscles ($p < 0.001$, $n = 5$). Values were means \pm SD and were expressed as percent of the relaxation.

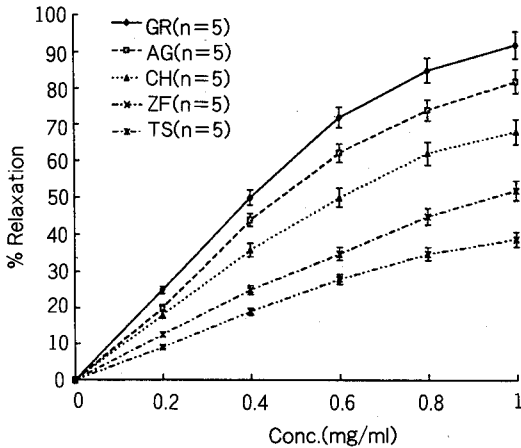


Fig. 3. Each relaxation effect of extracts of Ginseng Radix (GR), Angelicae Gigantis (AG), Cistanchis Herba (CH), Zanthoxyli Fructus (ZF) and Torilidis Semen (TS) on the $5 \times 10^{-6} M$ of phenylephrine induced contraction in the corpus cavernosal strips. A dose dependent relaxation effects were shown with each ingredients ($p < 0.001$). Values were means \pm SD and were expressed as percent of the relaxation.

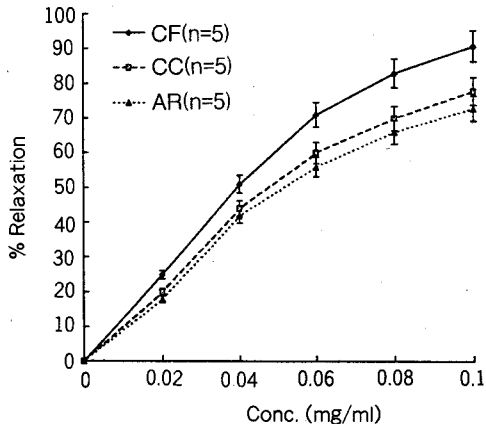


Fig. 4. Each relaxation effects of extracts of Caryophylli Flos (CF), Cinnamoni Cortex (CC) and Asiasari Radix (AR) on the $5 \times 10^{-6} M$ of phenylephrine induced contraction in the rabbit corpus cavernosal strips. A dose dependent relaxation effects were shown with each ingredients ($p < 0.001$). Values were means \pm SD and were expressed as percent of the relaxation.

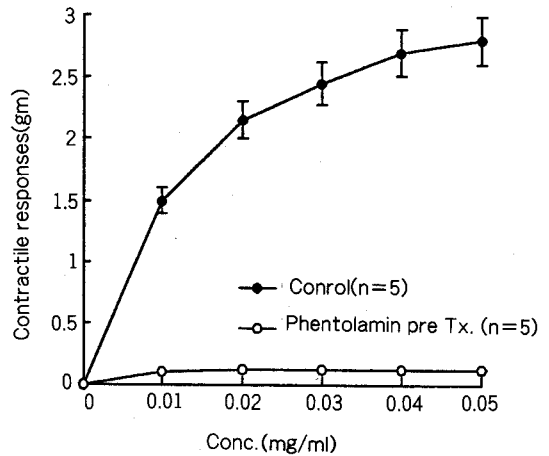


Fig. 5. Effect of phentolamine on the contractile responses of *Bufo venenum* (BV). The contractile response of the isolated cavernosal muscle strips to extract of *Bufo venenum* were inhibited by phentolamine ($p < 0.001$). Values were means \pm SD and were expressed as tension (gm) of the contraction.

PHE ($5 \times 10^{-6} M$), SS-cream began to exert a relaxing effect at the concentration of 0.05 mg/ml and reach the 90% relaxation effect at the concentration of 0.2 mg/ml; causing a dose-dependent relaxation ($p < 0.001$) (Fig. 2).

Among the individual ingredients comprising SS-cream, *Bufo venenum* extract induced contraction on the PHE-induced precontracted muscle strip in a dose-dependent manner. The extract of *Caryophylli Flos* induced a dose-related relaxation in the muscle strips precontracted with PHE ($5 \times 10^{-6} M$). Other extracts of the SS-cream, when used individually or in mixture, induced a dose-related relaxation in the muscle strips precontracted with PHE ($p < 0.001$) (Fig. 3, 4).

Effects of various treatment on the contractile activity of individual ingredients of SS-cream

Extract of *Bufo venenum* induced a dose-related contraction of the muscle strip, which was significantly inhibited by phentolamine ($p < 0.001$) (Fig. 5). The contractility of *Bufo venenum* was also inhibited by *Caryophylli Flos* ($p < 0.001$) (Fig. 6).

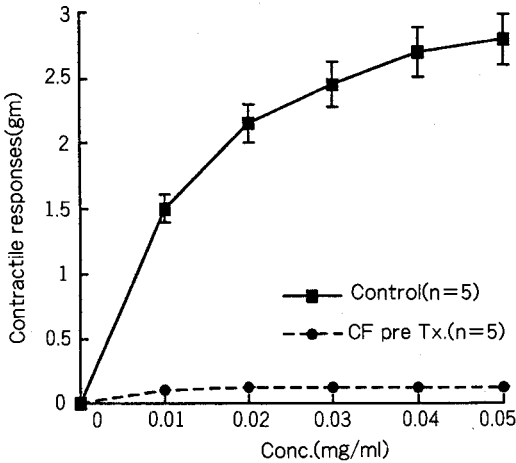


Fig. 6. Effect of extract of *Carynhamoni Flos*(CF) on the *Bufo venenum*(BV) induced contraction in the cavernosal muscle strip. *Carynhamoni Flos* inhibits the contractile response of the isolated rabbit cavernosal muscle strips to *Bufo venenum* ($p < 0.001$). Values were means \pm SD and were expressed as tension (gm) of the contraction.

Effects of various treatment on the relaxing activity of SS-cream

SS-cream had a dose-dependent relaxation in the muscle strips precontracted with PHE. Deendothelialization partially blocked but did not completely abolished the SS-cream induced relaxation of cavernosal muscle. Furthermore (in addition), pretreatment with a guanylate cyclase inhibitor, methylene blue (10^{-4} M) or a NO scavenger, pyrogallol (10^{-4} M), inhibited the relaxation of the muscle strips partially. The relaxing action of SS-cream was partially inhibited by atropine but was not affected by indomethacin ($p > 0.05$) (Fig. 7).

DISCUSSION

SS-cream is made up of extracts of 9 major ingredients including Ginseng Radix Alfa. Generally, several active components are present in each natural product. Each components

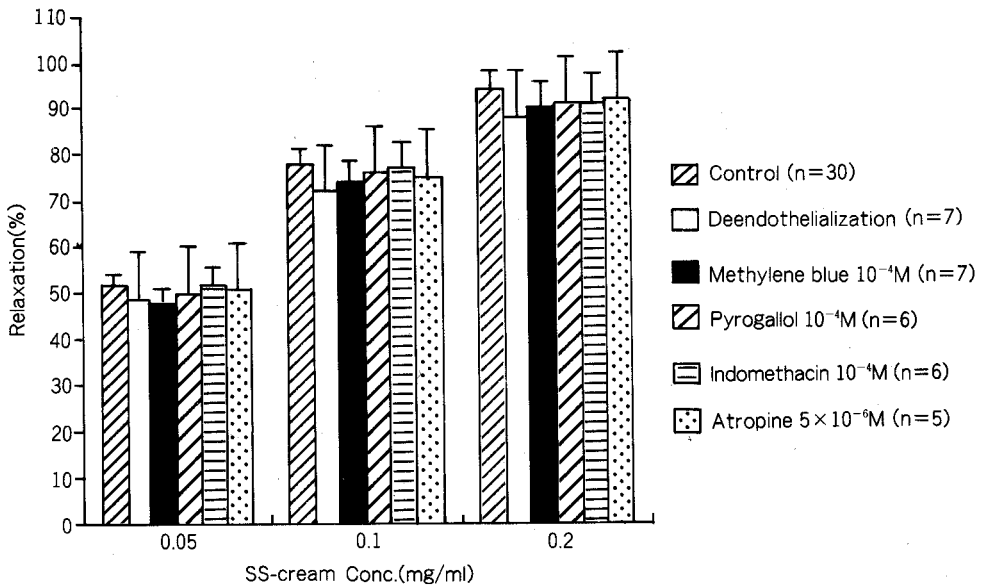


Fig. 7. Effect of various treatments on the SS-cream induced relaxation in the cavernous muscle strip. The relaxation effects were partially inhibited by deendothelialization, pretreatment of methylene blue, pyrogallol, indomethacin and atropine, but not statistically significant ($p > 0.05$). Values were means \pm SD and were expressed as percent of the relaxation.

has its own specific structures and exert various actions. Among the ingredients found in SS-cream, bufalin in *Bufo venenosus*, euganol in *Caryophylli Flos*, methyleuganol in *Asiasari Radix*, and Sanshool in *Zanthoxyli Fructus* have a local desensitizing effect (Yoshida *et al.* 1976; Han, 1988). When the effect of SS-cream was evaluated on the corneal reflex, it inhibited the reflex in a dose-dependent manner (Xin *et al.* 1996b). The topical application SS-cream on the glans penis also significantly prolonged the latency of penile SEP (Xin *et al.* 1995a). These local effects are believed to be responsible for the inhibition of penile hypersensitivity, often suspected as the main organic causes of premature ejaculation.

Male sexual responses included sexual desire, penile erection, and ejaculation. Premature ejaculation is a disorder of the ejaculatory function, in addition, 20~40% of those have mild form of erectile dysfunction (Seong *et al.* 1994; Song *et al.* 1994). Therefore, desirable management of premature ejaculation requires not only to desensitize the penile hypersensitivity and restore the normal ejaculatory reflex but also enhance the penile erection.

Penile erection primary controlled by contraction and relaxation of penile smooth muscle (Lue and Tanagho, 1987). Therefore, corporal smooth muscle relaxation plays a principle role in erection, which is largely mediated by a nonadrenergic, noncholinergic (NANC) mechanism, however, endothelium-dependent cholinergic neurotransmission (EDRF) may also mediate penile erection (Speeding *et al.* 1986; Kimoto *et al.* 1990). Recent studies have shown that nitric oxide (NO) is the major neuronal mediator of erection.

NO was first described in 1979 as a potent relaxant of peripheral vascular smooth muscle, with an action mediated by cyclic GMP. Acetylcholine was postulated to stimulate the formation of EDRF, which was subsequently identified as being either NO or a chemically unstable nitroso precursor. NO is synthesized from endogenous L-arginine by the nitric oxide synthase system, located in the vascular endothelium.

Our data demonstrate SS-cream relaxed isolated rabbit corporal smooth muscle strips. It

was also found that the removal endothelium of muscle strips inhibited relaxation by SS-cream. This result indicates that the vasorelaxant effect of SS-cream is mediated by the release or augmentation of the spontaneous release of EDRF. The present study also shows that both guanylate cyclase inhibitor, methylene blue, NO scavenger, and pyrogallol can inhibit the relaxation effect of SS-cream on the muscle strips. This indicates that the relaxing action of SS-cream in the isolated rabbit corporal muscle is mediated by NO/cGMP pathway. However, this inhibited relaxant response of muscle strips to SS-cream by removal of the endothelium or the addition of methylene blue and pyrogallol occurred partially, but not completely. This indicates that the relaxing action of SS-cream includes not only an endothelium mediated mechanism but others also.

With better understanding of the physiologic mechanisms on penile erection, the smooth muscle relaxants such as papaverine, phentolamine, and prostaglandin E1 have been widely studied and shown to be effective in the treatment of erectile dysfunction (Padma-Nathan *et al.* 1987; Goldstein *et al.* 1988; Lee *et al.* 1989; Rapoport *et al.* 1990).

In our experiment, epinephrine found in *Bufo venenosus* induced the contraction of smooth muscle, but other components such as ginsenoside Rb in *Ginseng Radix Alba*, decursin in *Angelicae gigantica Radix*, cinnamic aldehyde in *Cinnamomi Cortex*, euganol in *Caryophylli Flos*, methyleuganol in *Asiasari Radix*, and alkaloids in *Cistanchis Herba* induced a dose-dependent relaxation of cavernosal tissues and acted directly antagonistic to the effect of *Bufo venenosus*. Also in our study, pretreatment by phentolamine and *Caryophylli Flos* inhibited the contraction induced by *Bufo venenosus*. This is probably caused by euganol in *Caryophylli Flos*, acting as an alpha-adrenergic blocker. All these actions synergistically or additively induces powerful relaxation of cavernosal smooth muscles.

In our previous studies, the earlier compositions of SS-cream induced early contraction followed by late relaxation (Choi *et al.* 1995).

This phenomenon is closely related to the amount of Bufonis Venenum and Caryophylli Flos in the mixture. Thus not only the herbs but also the exact composition of these agents are also important in achieving optimal desired therapeutic benefit.

Conclusively, SS-cream had a dose-related relaxing effect on rabbit corpus cavernosal smooth muscles and other ingredients except Bufonis Venenum had a dose-related relaxing effect, but the exact relaxation mechanisms in the isolated corporal muscle were not clearly elucidated due to the compound nature of the drug.

Further studies are required at this point to clarify the individual mechanism of each component of SS-cream and purify and select the proper components for achieving better relaxation of corporal smooth muscle.

With these results we concluded SS-cream had a dose-related relaxing effect on rabbit corpus cavernosal smooth muscles, though the mechanisms of SS-cream in the isolated rabbit corporal smooth muscle were not clearly elucidated due to compound nature of the drug. Therefore, SS-cream is not only effective in the treatment of PE but also in the treatment of PE with mild erectile dysfunction.

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