

토끼의 음경해면체평활근에 대한 인진쑥 성분인 scoparone의 효과

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The Effect of Scoparone from *Artemisia Capillaris* on the Smooth Muscle of Rabbit Penile Corpus Cavernosum

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Purpose: 본 연구는 토끼의 음경해면체 조직에 대한 인진쑥의 에탄올 추출물과 그 성분 중의 하나인 scoparone의 효능을 평가하고자 하였다.

Methods: 체중이 2.5-3.0 Kg의 New Zealand백색 가토를 사용하여 음경 전체를 골반골로부터 분리하여 요도를 제거한 후 백막을 보유한 음경해면체를 2 mL organ chamber 관류모형에 연결하고, 10^{-5} M의 phenylephrine을 관류하여 음경해면체를 수축시킨 후 0.1, 0.5, 1, 2 mg/mL의 에탄올 추출물 또는 10^{-7} , 10^{-6} , 10^{-5} , 10^{-4} M의 scoparone을 녹인 용액으로 관류시켜 음경의 장력 변화를 측정하였다. 기존의 발기부전 치료제로 사용되는 실테나필에 대한 인진쑥과의 상호작용을 검토하기 위하여 10^{-6} M의 scoparone과 10^{-8} M의 실테나필을 병용 사용 시 음경해면체 조직에 대한 효과도 함께 검토하였다.

Results: 인진쑥의 에탄올 추출물은 phenylephrine으로 전처리하여 수축시킨 음경해면체 평활근을 농도 의존적으로 효과적으로 이완시켰으며 scoparone도 음경해면체 조직에 대한 이완작용 또한 농도 의존적으로 강한 효과를 나타내었다. 실테나필로 전처리한 음경해면체 조직에 대한 scoparone의 효과는 scoparone만을 단독으로 관류시킨 조직에서의 효과보다 강하게 나타났으며 실테나필과 scoparone의 병용사용은 실테나필의 효능을 2배 이상 증가시켰다.

Conclusions: Scoparone은 실테나필의 음경해면체평활근 작용을 증가시킴으로써 실테나필에 완전히 반응하지 않는 환자들의 발기부전 치료효과 향상에 유용할 것으로 전망된다.

□ Key words - *Artemisia capillaris*, scoparone, penile corpus cavernosum, sildenafil citrate

Erectile dysfunction (ED), the inability to achieve and maintain an erection sufficient to permit satisfactory sexual intercourse, is a common and important medical problem.¹⁻³⁾ According to the Massachusetts Male Aging Study, the prevalence of erectile dysfunction between the ages of 40 and 70 years was 52%. Although subject age was the most strongly associated with ED, it was also correlated with heart disease, hypertension, diabetes, associated medications, and indexes of anger and depression. Men who are under treatment for heart disease and hyper-

tension are also at a significantly increased risk of severe ED.⁽⁴⁻⁶⁾ Failure to achieve penile erection may be due to impaired relaxation of the smooth muscle of the corpus cavernosum, which is mediated through nitric oxide (NO) via cyclic guanosine monophosphate (cGMP)-mediated intracellular signaling.⁷⁾

Artemisia capillaris Thunb. (Compositae) has been used as food materials and herbal medicine sources to liver cirrhosis, liver cancer, jaundice, and cholecystitis in Asian countries, such as Korea, China, and Japan.⁸⁻¹³⁾ Scoparone (6,7-dimethoxy coumarin), a major component from *A. capillaris*, has been used as antipyretic, anti-inflammation, diuretic, and choleric for the treatment of hepatitis and bilious disorder.^(14,15) Many studies also have demonstrated the free radical scavenging, immuno-

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suppressive and vasodilator activities.¹⁶⁻¹⁹⁾

In the present study, we evaluated the relaxant effect of ethanol extract from *A. capillaris* and scoparone on the smooth muscle of rabbit penile corpus cavernosum (PCC) to examine further the action of scoparone on the erectile dysfunction. We also examined the synergistic effect of scoparone on sildenafil citrate pre-incubated PCC tissue.

MATERIALS AND METHODS

Plant Extraction

A. capillaris were collected on September 2010 from Jinan, Korea and identified by Dr. C.Y. Kim. The voucher specimen (accession number AC-1) has been deposited at the Natural Products Research Center of KIST Gangneung Institute (Gangneung, Korea). The shade-dried *A. capillaris* (600 g) were pulverized and extracted 3 times with 2400 mL of ethanol for 3 h using an ultrasonic bath (model 8510 DHT; Branson, CT, USA). After filtration, the extracts were evaporated *in vacuo* and lyophilized to yield the total extract (23.5 g). The sample was stored at 4°C until use.

Chemicals and Reagents

L-phenylephrine (Phe) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Sildenafil citrate was donated from Dong A Pharmaceutical Company (Seoul, Korea). Scoparone (Figure 1), a major component from *A. capillaris*, was also purchased from Sigma-Aldrich. All other chemicals were purchased from standard suppliers. Sildenafil was dissolved in distilled water. The extractions were dissolved in HEPES buffer and subsequently diluted in the buffer to the final concentration

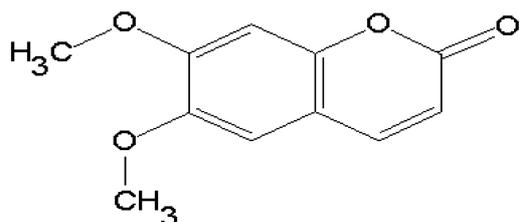


Fig. 1. The structure of scoparone from *A. capillaris*.

(0.1, 0.5, 1, or 2 mg/mL). Scoparone was dissolved in ethanol, and subsequently diluted in the buffer to the final concentration (10^{-7} , 10^{-6} , 10^{-5} , and 10^{-4} M).

Tissue Preparation

This research was conducted in accordance with the internationally accepted principles for laboratory animal use and care as found in the European Community guidelines (EEC Directive of 1986; 86/609/EEC) or the guidelines (NIH publication #85-23, revised in 1985). Strips of rabbit PCC smooth muscle (1.5×1.5×7 mm) were prepared from healthy control male New Zealand White rabbits weighing 2.5-3.0 kg. The rabbits were anesthetized with ketamine (50 mg/kg intravenously) plus rumpun (25 mg/kg), and exsanguinated. The penis was excised rapidly and the PCC smooth muscle was then carefully dissected free from the surrounding tunica albuginea. During the preparation, each step was undertaken cautiously to prevent damage of functional endothelium or overstretching of the tissue.

Measurement of Tension on PCC

The strip of PCC smooth muscle was vertically placed in a 2 mL organ chamber with one end connected with a cotton thread to the prong of a force transducer (FT03, Grass Telefactor; West Warwick, RI, USA), and the other end was secured with a cotton thread to a holder for isometric tension measurement. The organ chamber containing a HEPES buffer (36°C) was constantly aerated with 100% O₂. The HEPES buffer contained the following (mM): NaCl, 118; KCl, 4.7; CaCl₂, 2.5; MgCl₂, 1.2; NaHCO₃, 25; glucose, 10.0; and HEPES, 10 (with NaOH [pH 7.4.]) After mounting, the strip was equilibrated for 60 mins with several adjustments of length until a baseline force stabilized at 1 g, and the oxygenated buffer was replaced every 15 min. After stabilization, 10^{-5} M Phe was added to adjust the maximal contractile tension, and then the samples were added to the organ chamber with the desired final concentration. The relaxation effect of extraction was studied by cumulative addition at concentrations from 0.1-2 mg/mL at the plateau of the Phe-

induced concentration. Scoparone from *A. capillaris* were tested at final concentrations of 10^{-7} , 10^{-6} , 10^{-5} , and 10^{-4} M. The change in isometric force was measured and recorded using the PowerLab data 400 acquisition system (Software Chart, version 5.2; AD Instruments, Castle Hill, Australia).

Interaction between *A. capillaris* and sildenafil citrate

The PCC tissue was pre-incubated with sildenafil (10^{-8} M) for 30 mins, then scoparone (10^{-6} M), including sildenafil citrate, was added to the organ chamber after Phe-induced contraction. The penile tissue pre-incubated with scoparone was also performed with sildenafil citrate after Phe-induced contraction.

Statistical Evaluation

The submaximal penile contractile responses induced by Phe were considered to be the 100% values, and all subsequent responses to *A. capillaris* ethanol extract were expressed as a percentage of this value. The results were expressed as the mean \pm SD, and *n* represents the number of tissues in each group. The statistical significance of differences was calculated by one-way analysis of variance (ANOVA), followed by Bonferroni's multiple comparison test. A probability value < 0.05 was considered significant.

RESULTS

Effect of *A. capillaris* Ethanol Extract on PCC

Experiments were performed to investigate the cumulative dose-dependent relaxation responses to ethanol extract from *A. capillaris* in the pre-contracted PCC with Phe. Figure 2 shows that *A. capillaris* ethanol extract exerted a significant and concentration-dependent relaxing effect. The relaxations induced by 0.1, 0.5, 1, and 2 mg/mL of ethanol extracts were 6.26 ± 2.46 , 21.24 ± 3.53 , 42.33 ± 4.86 , and $68.50\pm 3.47\%$, respectively.

Evaluation of Cumulative Dose of Scoparone

Experiments were performed to investigate the cumu-

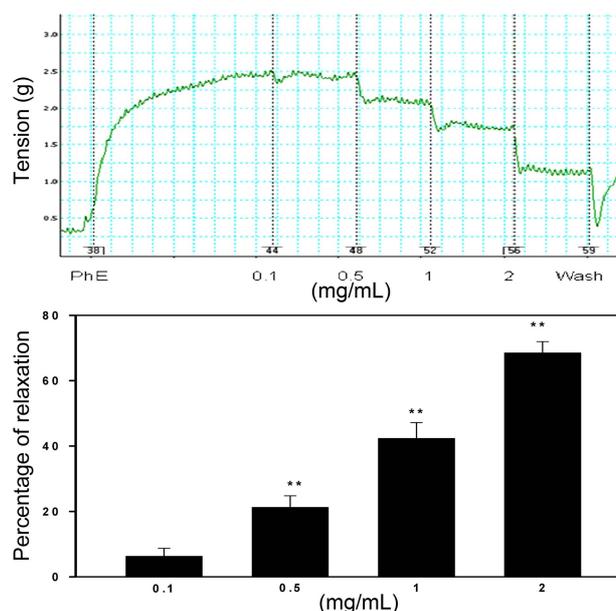


Fig. 2. Percentage of relaxation induced by the ethanol extract of *A. capillaris* ($n = 4$). The pre-contracted penile corpus cavernosum tissue with 10^{-5} M Phe was treated at 4 concentrations of the ethanol extracts. The submaximal penile contractile responses induced by Phe were taken as the 100% values, and all subsequent responses to the *A. capillaris* ethanol extracts were expressed as a percentage of this value. Each point represents the mean \pm SD of the percentages. ** $P < 0.01$ vs 0.1 mg/mL.

lative relaxation responses to scoparone in the pre-contracted PCC with Phe. As shown in Figure 3, scoparone relaxed dose-dependently the PCC with a maximum value of $92.82\pm 2.14\%$ at a concentration of 10^{-4} M.

Effect of Scoparone in the PCC Incubated with Sildenafil Citrate

The relaxation induced by a single dose of sildenafil citrate (10^{-8} M) on Phe pre-contracted tissue was $17.22\pm 1.26\%$ and the single use of scoparone (10^{-6} M)-induced relaxation on Phe pre-contracted PCC tissue was $24.42\pm 3.38\%$. The relaxation of scoparone was $49.85\pm 4.37\%$ on Phe pre-contracted PCC tissue, which had been pre-incubated on sildenafil citrate and the relaxation of sildenafil was $30.86\pm 6.97\%$ on Phe pre-contracted PCC tissue, which had been pre-incubated on scoparone. The active component, scoparone, efficiently enhanced sildenafil citrate-induced relaxation more than two-folds, as shown in

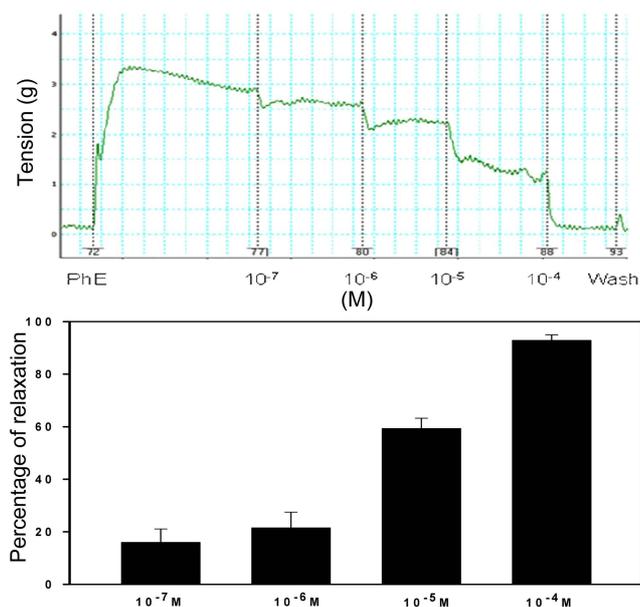


Fig. 3. Percentage of relaxation induced by scoparone of *A. capillaris* ($n = 4$). The pre-contracted penile corpus cavernosum (PCC) tissue with 10^{-5} M Phe was treated at four concentrations of scoparone. The submaximal penile contractile responses induced by Phe were taken as the 100% values, and all subsequent responses to scoparone were expressed as a percentage of this value. Each point represents the mean \pm SD of the percentages.

Figure 4.

DISCUSSION

The present study showed that *A. capillaris* ethanol extract had a significant relaxation effect in a concentration-dependent manner on the smooth muscle of rabbit PCC at a range of 0.1, 0.5, 1, and 2 mg/mL. The relaxation on Phe pre-contracted tissue induced by scoparone, which is the major component from *A. capillaris*, was in a cumulatively dose-dependent manner with a range of 10^{-7} to 10^{-4} M.

Although many medicines are now available for treating ED, finding a new medicine or an alternative medicine to treat ED and understanding its mechanism of action is still significant study field. Current pharmacologic treatment for ED includes the oral, intracavernosal, and intraurethral administration routes of erectogenic medicines. Oral treatment is the most effective therapy for ED and has the

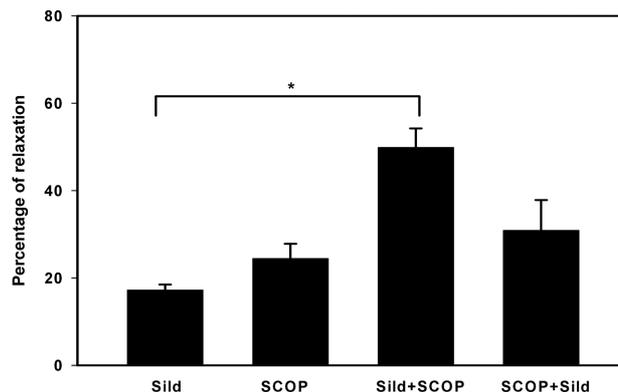


Fig. 4. Percentage of relaxation induced by combination with scoparone (10^{-6} M) and sildenafil (10^{-8} M; $n = 4$). Each point represents the mean \pm SD of percentages of maximal relaxation of the preceding submaximal contractile responses. SCOP means scoparone. Sild+SCOP means administration of Scoparone on the corpus cavernosum smooth muscle tissue contracted by Phe (10^{-5} M) which was pre-incubated with sildenafil. SCOP+Sild means administration of sildenafil on the corpus cavernosum smooth muscle tissue contracted by Phe (10^{-5} M) which was pre-incubated with scoparone. * $P < 0.05$ vs. control.

highest patients' preference. Sildenafil, an oral phosphodiesterase type 5 inhibitor, has been used commonly for the treatment of the patients with ED in Korea²⁰ and proven to be a valuable therapy in the management of ED, but there are limitations. Our results showed that scoparone efficiently increased sildenafil citrate-induced relaxation. The relaxation effect of scoparone on sildenafil citrate pre-incubated PCC tissue was higher than the relaxation on the scoparone pre-incubated tissue. Furthermore, the active component, scoparone significantly enhanced the sildenafil citrate-induced relaxation more than two-folds.

The primary intracellular activation pathway for cavernous smooth muscle relaxation appears to be mediated through the NO-cGMP signal pathway, and the cAMP system apparently functions as a secondary pathway.²¹⁻²³ The mechanism of action by which scoparone induces the relaxation on PCC, is still not known. Therefore, it seems worthy to continue the research about the clarification of mechanism and the development of new medicines from natural products with a clinical application in ED.

In conclusion, the scoparone from *A. capillaris* signif-

icantly had relaxation effect on the Phe pre-contracted PCC smooth muscle and exerted an increasing effect on the sildenafil citrate-induced relaxation. This study may provide the possibility that scoparone of *A. capillaris* may be a new medicine or supplement to treat the patients with erectile dysfunction.

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