

Full Length Research Paper

A new relaxant on human corpus cavernosum: *Ferulago syriaca* root extract

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***In vitro* effects of the extract of *Ferulago syriaca* used as an herbal medicine in Turkey on human corpus cavernosum were demonstrated. Corpus cavernosum tissue was collected from patients that penile prosthesis implantation had been applied to. Relaxing effect in organ bath was searched for by adding increasing dosages of the extract on tissue strips that were contracted with phenylephrine. It was repeated with incubating separately with N^G-nitro-L-arginine methyl ester (L-NAME) (NO-synthesis inhibitor) and oxadiazolo[4,3- α]quinoxalin-1one (ODQ) (selective guanilate cyclase inhibitor), respectively. In addition, the effects of the extract were also evaluated on the relaxing responses to Electrical field stimulation (EFS), acetylcholine, forskolin, and sodium nitroprusside. Extract caused relaxing on corpus cavernosum strips in a dose-dependent manner (relaxing with ratios of 17, 41, 61, and 87%, respectively). Relaxing of the tissue with extract could be inhibited with small dosages of L-NAME, and in all dosages of ODQ ($p < 0.01$). While incubating the tissue strips with the extract increased the relaxing responses to acetylcholin, it had no effect on the relaxing responses to EFS, sodium nitroprusside (SNP) and forskolin. *F. syriaca* root extract relaxes the human corpus cavernosum *in vitro* markedly. It ensures this relaxing, probably by activating NO- cyclic guanosine monophosphate (cGMP) pathway.**

Key words: Aphrodisiacs, bornyl acetate, penile erection, plant extracts, *in vitro*.

INTRODUCTION

Erectile dysfunction, the persistent inability to achieve or maintain an erection sufficient for satisfactory sexual performance, is estimated to affect up to 30 million men in the United States (NIH consensus, 1993). The disorder is age-associated, with estimated prevalence rates of 39% among men 40 years of age and 67% among those 70 years old. Penile erection is a physiological event involving relaxation of smooth muscle of the corpus cavernosum. This relaxation process results in an increased flow of blood into the trabecular spaces of the corpora cavernosa and could further cause the erection of penis (Lue, 1983; Andersson and Wagner, 1995).

Therapeutically, there are several drug types and agents that enhance through the NO-cGMP axis or cAMP, signal transduction, which may prove beneficial in treating erectile dysfunction (Jeremy et al., 1997; Tan et al., 2011). One such class of drugs is the phosphodiesterase (PDE) inhibitors that prevent the hydrolysis of cGMP and/or cAMP, thereby elevating levels of these cyclic nucleotides.

The inhibitors of cGMP-specific PDE type 5 are currently used as an oral therapy for the treatment of male erectile dysfunction (Moreland et al., 1998; Goldstein et al., 1998). On the other hand, several studies were attributed to the development of naturally occurring impotence-treating agents. Some authors found that several plant extracts produced relaxation in phenylephrine-precontracted corpus cavernosum obtained from experimental animals (Cehn et al., 2000;

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Chiou and Chen, 2002; Antunes et al., 2001). Furthermore, few studies confirm these relaxant effects of plant extracts using human corpus cavernosum in literature (Paick et al., 1996).

Turkey has a rich flora due to its location on the crossroad of three different phytogeographical regions: Euro-Siberian, Mediterranean and Irano-Turanien. More than 9,000 species grow naturally, and approximately 3,000 of them are endemic. In this study, we aimed to demonstrate effect of the lyophilized water extract of *F. syriaca* (*Umbelliferae*) roots growing naturally in Turkey on erectile tissue. It is known as "Çakşır" or "Çağşır" in Turkey and used traditionally as an aphrodisiac in South and Southeast Anatolia. In fact, many species that belong to *Ferula*, *Ferulago* and *Prangos* genera have been used for this purpose. They are used in rutting of sheep and goat, and also the water decoctions of the aerial parts and roots are administered orally as aphrodisiacs (Baytop, 1999).

Ferulago species are mostly well known for their aphrodisiac activities in Turkey like various plants in different countries (Ibrahim et al., 2010). In the course of our studies, we have found that water extract of *F. syriaca* roots produced relaxation in precontracted human corpus cavernosum. Thus, we attributed to investigate pharmacological profile of its relaxant effect by using isolated human corpus cavernosum tissue *in vitro*.

MATERIALS AND METHODS

Plant material

The plant material were collected from Hatay: Yayladağ Road, rocky slopes on the left side, altitude 480 m, 23/8/2001 (AEF 21613); 16/7/2002 (AEF 22458), identified by H. Duman. Voucher specimens are deposited at Herbarium of Ankara University, Faculty of Pharmacy.

Human corpus cavernosum tissue

The study was approved by our institution's review board for human studies. All tissue donors issued informed consent. Six patients (mean age 55 and range 44 to 65) on who were performed penile prosthesis implantations were included in our study. None of them had diabetes mellitus. In doppler analysis, arterial disease was observed in all patients. Penile corpus cavernosum tissues were obtained at the time of implantation of a penile prosthesis in patients. The tunica albuginea was incised with a penoscrotal, transverse scrotal or subcoronal skin incision to expose the underlying corporeal tissue. The erectile tissue was sharply dissected from the tunica albuginea to the central axis of the corpora. The corporeal tissue was removed and immediately placed in chilled physiological salt solution (PSS) and studied half an hour later. The cavernosal tissues were dissected into strip preparations measuring approximately 2 × 2 × 7 mm.

Chemicals

ACh, L-NAME, phenylephrine, KCl, forskolin, SNP and ODQ were obtained from Sigma Chemical Company (St. Louis, MO, USA).

Preparation of extract

For the extraction procedure, 50 g of root were grounded and macerated with 500 ml of distilled water for 4 h in 30 to 35°C. This material was filtered and lyophilized by using Labconco Freeze Dry System/Freezone® 4.5 (USA) device and 4.49 g extract was obtained. We calculated the lyophilized extract amount that equals to 1 g of plant material dissolved in 10 ml distilled water. It means that 10 ml solution contained 0.0898 g lyophilized extract. Distilled water was used to prepare the solution of extract due to the sensitivity of the organ bath components and tissue.

Organ bath experiments

Strip preparations were mounted approximately under 1 g resting tension in 20 ml organ baths for the measurement of isometric tension. The strips were tied to a wire connected to a force transducer with silk (Grass FT3, Quincy, Mass., USA) on one end, and fixed with silk ties to a metallic support on the other end. Organ baths contained PSS composed of (in mmol/l) NaCl 118.3, KCl 4.7, MgSO₄ 0.6, KH₂PO₄ 1.2, CaCl₂ 2.5, NaHCO₃ 25, calcium EDTA 0.026 and glucose 11.1. The solution was gassed with 95% air and 5% CO₂ during the study. The pH of the solution was 7.4 and the temperature was maintained at 37°C. One hour was allowed for equilibration. After thorough washout, relaxations response to extract in 1.8, 3.6, 5.4, and 9.0 mg doses were studied, respectively in strips of corpus cavernosum contracted with phenylephrine (10 μmol/l). After washing period, tissues were incubated with NO-synthase inhibitor, N^o-nitro-L-arginine methyl ester (L-NAME) (100 μM) and selective soluble guanylate cyclase inhibitor, 1*H*-1,2,4-oxadiazolo[4,3-*α*]quinoxalin-1-one (ODQ) (30 μM) for 30 min. Furthermore, effects of extract (3.6 mg) on EFS, acetylcholine, forskolin and sodium nitroprusside (SNP)-induced relaxation were also evaluated in human corpus cavernosum strips. A data acquisition system (MAY, Ankara, Turkey) connected to a personal computer simultaneously recorded contractile and relaxant responses to providing rapid data analysis.

Data analysis

The relaxation induced by each concentration of extract, ACh, forskolin and SNP was expressed as a percentage of active muscle tone induced by phenylephrine, running from 0 to 100% and used in the construction of the concentration-response curves. The results are expressed as mean ± S.E.M. Student's *t* test (one-tailed) was used for paired or unpaired observations where appropriate. A value of *p* < 0.05 was considered to be statistically significant.

RESULTS

F. syriaca plant extract caused a dose-dependent relaxation in phenylephrine-contracted human corpus cavernosal strips (Figure 1). The amplitude of contractile response to 120 mM KCl was not significantly different from the response to phenylephrine (data not shown), however; the relaxant activity of extract was greatly reduced in KCl contracted tissues (Figure 1). The degree of relaxation observed with extract (at 9 mg dose) was reduced from 86.7 ± 2.8 to 20 ± 3% (*p* < 0.01). 0.1 mM concentration of L-NAME reduced the extract-induced relaxation at 1.8 and 3.6 mg doses although L-NAME had no reduction in relaxant response of extract in high doses

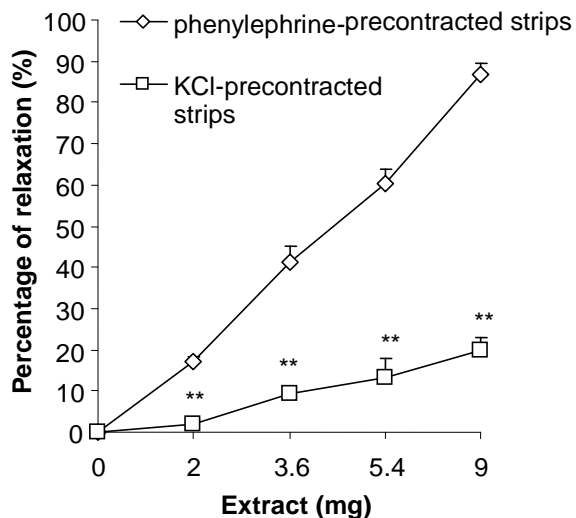


Figure 1. Relaxant effect of water extract of *F. Syriaca* roots in phenylephrine- and 120 mM KCl-precontracted human cavernosal strips, respectively. Data are shown as mean \pm S.E.M. for five preparations. ** $P < 0.01$ versus control.

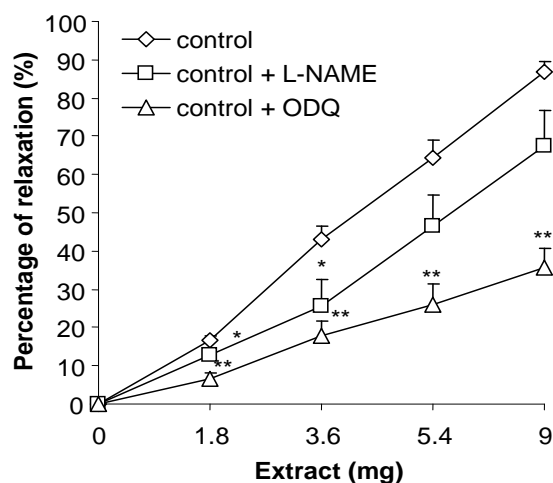


Figure 2: Effects N^G -nitro-L-arginine methyl ester (L-NAME) and 1H-[1,2,4]oxadiazolo [4,3- α] quinoxalin-11one (ODQ) on water extract of *F. syriaca* roots induced relaxation of the isolated human corpus cavernosum precontracted with phenylephrine. L-NAME or ODQ was added to human corporal preparation 20 min. Data are shown as mean \pm S.E.M for five preparations. * $P < 0.05$, ** $P < 0.01$ versus control.

(Figure 2). Pretreatment with ODQ (30 μ M) seriously suppressed the extract-induced relaxation (at 9 mg dose of extract, $86.7 \pm 2.8\%$ before, and $35.6 \pm 4.95\%$ after ODQ treatment, $p < 0.01$) (Figure 2).

As shown in Figure 3a, ACh (10^{-8} to 10^{-3} M) produced a dose dependent relaxation of the precontracted

cavernosal tissue. A higher concentration of extract (at 9 mg dose) augmented the relaxation of ACh as shown in dose response curve (Figure 3a). At 0.5 to 32 Hz frequency, electrical field stimulation (EFS) of human corpus cavernosum produced frequency-dependent relaxation (Figure 3b). Treatment of cavernosal strips with 9 mg extract did not affect the relaxation response curve to EFS (Figure 3b). In the presence of active muscle tone induced by phenylephrine, the corpus cavernosum relaxed upon application of forskolin (2 and 30 nM) and SNP (0.01 to 10 μ M). Figure 4 shows the effect of extract on the corporal relaxations induced by forskolin (Figure 4a) and SNP (Figure 4b), respectively. In the presence of extract, both agent evoked relaxations were not affected significantly.

DISCUSSION

In this study, we aimed to evaluate the relaxant effect of *F. syriaca* root's extract in human corpus cavernosum with *in vitro* studies. According to our results, the relaxant activities of *F. syriaca* root's extract significantly decreased in KCl precontracted strips. This study also showed that L-NAME and ODQ suppressed extract induced relaxation in human corpus cavernosum. The extract increased the relaxation response of strips with Ach incubation. On the other hand, *F. syriaca* root's extract did not affect the relaxation induced by EFS, forskolin and SNP.

Corporal smooth muscle relaxation plays a critical role in erection. Smooth muscle relaxation, which is mediated by nitric oxide (NO) during sexual stimulation, is synthesized in nerve terminals of parasympathetic nonadrenergic and noncholinergic nerves in the penis and also by the endothelial cells lining blood vessels and lacunar spaces of corpus cavernosum (Burnett et al., 1992; Trigo-Rocha et al., 1993; Burnett, 1995). As shown in Figure 1, corporal relaxation is induced by extract to a lesser extent under high KCl (120 mM) conditions. Potential sensitive calcium channels are activated by depolarization of the plasma membrane when the extracellular K^+ concentration is increased. The reduced response to extract in high K^+ medium suggest that extract does not act through a calcium channel antagonistic property to relax corpus cavernosum.

On the basis of these findings we conclude that extract relaxes the human cavernosal tissue. The findings that L-NAME reduced the relaxations elicited by extract indicate that it relaxed human corpus cavernosum tissues through the release of NO. Involvement of NO in the extract-induced human corpus cavernosum relaxations was further confirmed by the finding that ODQ, a potent and selective inhibitor of NO stimulated soluble guanylate cyclase, virtually inhibited relaxation. These results provide evidence that NO and cyclic guanosine monophosphate mediate the relaxant response elicited

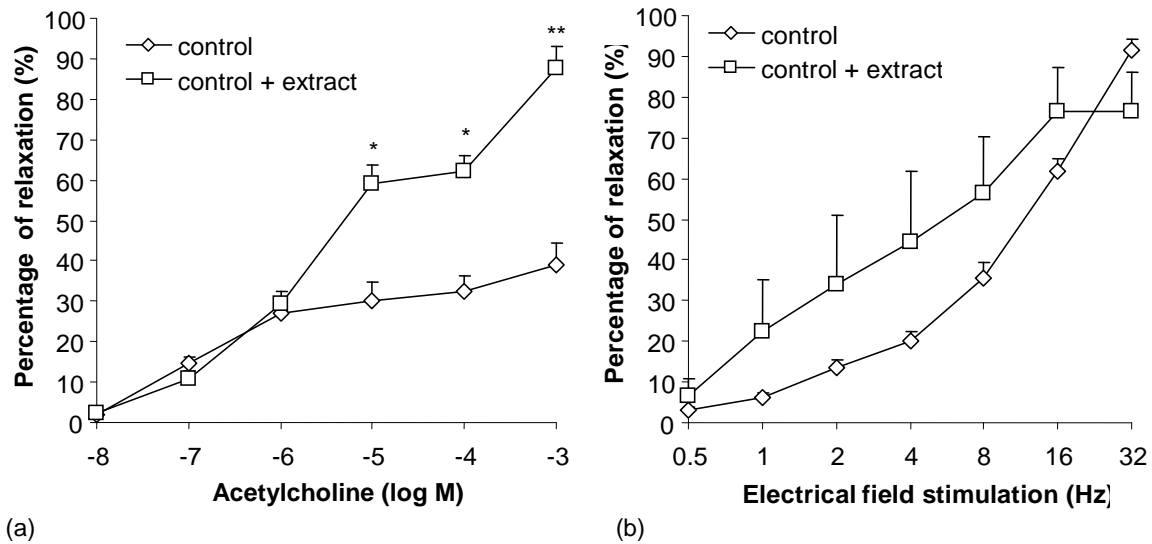


Figure 3. Effects of *F. syriaca* plant extract on the human corporal relaxation evoked by (a) acetylcholine or (b) electrical field stimulation in human corpus cavernosum precontracted with phenylephrine. Data are shown as mean \pm S.E.M. for five preparations. * $P < 0.05$, ** $P < 0.01$ versus control.

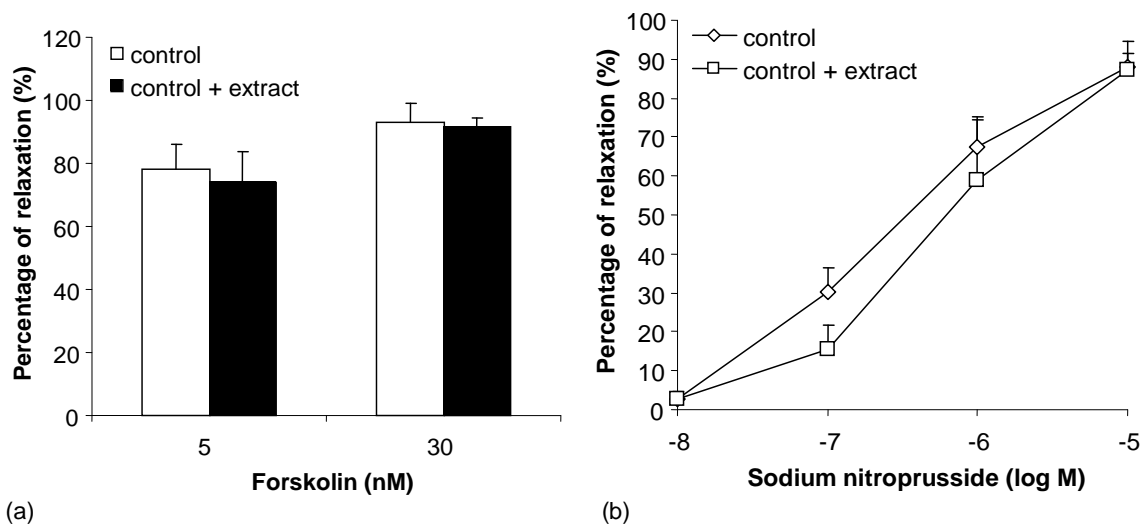


Figure 4. Effects of water extract of *F. syriaca* roots on human corporal relaxation evoked by (a) forskolin or (b) sodium nitroprusside in human corpus cavernosum precontracted with phenylephrine. Data are shown as mean \pm S.E.M for five preparations.

by extract in human cavernosal tissue.

Furthermore, in the presence of plant extract, the ACh-induced relaxations significantly increased (maximum response $39.04 \pm 5.3\%$ before and $87.5 \pm 11.2\%$ after, $p < 0.01$), indicating that extract may relax this preparation by activating cholinergic receptors which release NO from the endothelium (Trigo-Rocha et al., 1993).

Electrical stimulation of corporal smooth muscle under NANC condition produced frequency-dependent relaxations which were confirmed to neurogenic in origin

and entirely NO mediated and nitergic in nature (Burnett, 1995). In erectile tissues, nitric oxide (NO) derived mainly from nitergic inhibitory nerve fibres is clearly implicated in the relaxation of the corpus cavernosum, a key step in penile erection (Kim et al., 1991). Stimulation induced relaxation was not affected in tissues which is incubated with plant extract. Thus, our data indicate that relaxation is not preceded by nerve stimulation and subsequent NO release. We may further suggest that extract had no additive effect of extract on EFS-evoked

relaxation.

Erdurak et al. (2006) investigated the root oil of *F. syriaca* and they reported that the main components were characterized as bornyl acetate (69.4%) and terpinolene (12.5%) for the root oil of *F. syriaca*. Bornyl acetate is a bicyclic monoterpene with an anti-inflammatory activity that presents in essential oils (Matsubara et al., 2011; Tung et al., 2008). In a previous study, it is reported that high-dose bornyl acetate suppressed sympathetic nerve activity in human (Matsubara et al., 2011). The similar mechanism might play a role on relaxation of corpus cavernosum incubated with *F. syriaca* root's extract by impairing the equilibrium of sympathetic and parasympathetic systems. On the other hand, EFS induced relaxation responses in tissues with incubation of plant extract were not affected in our study and these results did not support the relaxation mechanism.

In cavernosal tissues, forskolin activate adenylate cyclase, and mediate cyclic adenosine monophosphate (cAMP) formation by non-receptor mechanism led to relaxation (Andersson and Stief, 1997). cAMP is an important second messenger in mediating the relaxation of diverse types of smooth muscle (Murray, 1990). Our results also demonstrated that extract had no enhancing effect on the forskolin induced relaxation. These findings indicate that extract could not play like a non-selective phosphodiesterase (PDE) inhibitor. Furthermore, we also suggest that *F. syriaca* plant extract did not enhance sodium nitroprusside induced cGMP production.

Conclusion

Our results show that water extract of *F. syriaca* root can play a role which is similar to a soluble guanylate cyclase activator and this effect may synergize with endogenous NO. On the other hand, it could be affected by Bornyl acetate with the impaired equilibrium autonomic nervous system control on corpus cavernosum. Thus constituents of extract may be of partial and transient benefit to the erectile function of impotent men. This is the first study that evaluated the relaxant effect of *F. syriaca* on human corpus cavernosum. However, this study showed a relaxation with a similar role of soluble guanylate cyclase activator in *in vitro* studies, it should be investigated and supported with *in vivo* studies in animal and human models.

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