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Original Research Article

Effect of *Ferula elaeochytris* root extract on smooth muscle contraction of vas deferens gland in rat

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Abstract

Purpose: *To evaluate the effect of Ferula elaeochytris (FE) at the prostatic and epididymal ends of rat vas deferens.*
Methods: *The effects of cumulative concentrations of FE (10 µL; 31.25 mg/µL and 20 µL; 62.5 mg/µL) were investigated on prostatic and epididymal ends of rat vas deferens in the presence of prazosin (0.3 µM), suramin (100 µM), atropine (10 nM) and nitric oxide synthase inhibitor (L-NOARG; 100 µM). The muscle contractions were induced by electrical field stimulation (EFS; 4 Hz, 50 V, 0.15 ms). Calcium (3 and 6 mM) was added into the bath medium while electrical field stimulation (EFS) was in progress.*
Results: *Ferula elaeochytris significantly inhibited the muscle contractions induced by electrical field stimulation (EFS) in a concentration-dependent manner. In the presence of prazosin or suramin, the contractile responses to EFS were significantly inhibited by FE at the prostatic and epididymal ends of vas deferens ($p < 0.05$). However, this inhibition was not affected by atropine and L- NOARG, suggesting that there is no direct interaction of FE with cholinergic and nitrergic responses. However, in the presence of prazosin or suramin, Ca^{2+} addition to the organ bath significantly reversed the inhibitory effect of FE at the prostatic and epididymal ends of vas deferens ($p < 0.05$).*
Conclusion: *These results show an inhibitory effect for the extract of FE on neurogenic contractile activity of prostatic and epididymal ends of vas deferens. This effect of FE may be associated with Ca^{2+} channels.*

Keywords: *Contractile activity, Electrical Field Stimulation (EFS), Ferula elaeochytris, Rat, Vas deferens*

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INTRODUCTION

"*Ferula* L. *umbelliferous* is a genus of flowering plants with 185 species. It is distributed in the eastern Mediterranean region and Central Asia [1]. This plant is found in arid climates and 23 different varieties have been reported in Turkey [2]. *Ferula* has not only an aphrodisiac effect, but also anti-oxidant, anti-proliferative, anti-inflammatory, cytotoxic, antidiabetic, antimicrobial and antifungal effects [3-5]. It is used as an expectorant in the treatment of neurological and

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vascular disorders, osteoporosis and infertility, hemorrhoids and urinary diseases in Anatolia in Asia [6]. The components and fatty acids in FE root extract showed that FE has a high capacity for total phenolic content and reduces glucose levels [7,8]. Furthermore, the corrective effects of FE on diabetes and age-related erectile dysfunction have been demonstrated [9,10]. In addition, it has been shown that *Ferulago syriaca* root extract can be used as a smooth muscle relaxant in human corpus cavernosum tissue [11].

There is very limited data on *Ferula elaeochytris* root extract effects on the contractile activity of the vas deference tissue. The effects of FE on erectile dysfunction in diabetic rats, *in vitro* and *in vivo* has been reported in a previous study [9]. It was shown that FE restored neurogenic and endothelial dysfunction and also increased glucose levels in diabetic rats. The effects of this extract on the contractile activity of rat ventral prostate smooth muscle was also reported [9].

There is no data on *Ferula elaeochytris* root extract effects on contractile responses of vas deferens tissue. Therefore, in this study, the aim was to investigate the effects of FE extract on smooth muscle activities of rat vas deferens tissue, *in vitro*. Hence the possible positive or negative effects of FE used for aphrodisiac purposes on the vas deferens tissue in male fertility will be demonstrated.

EXPERIMENTAL

Animals

Healthy ~ 3 months old male Wistar albino rats (200 - 250 g) were obtained from the Health Sciences Experimental Application and Research Center of Cukurova University in Turkey. All experimental procedures were approved by the Cukurova University Animal Experiments Local Ethics Committee (approval no: 02.10.2013 3/10). All experiments were carried out in accordance with the Principles of Laboratory Animal Care [12]. A total of 48 male rats were housed in a room with controlled temperature (24 ± 2 °C), humidity (45 – 55 %) and a 12-hour light to 12-hour dark period. They received food and tap water *ad libitum*.

Drugs

Drugs (DMSO, Atropine, Prazosin, Suramin and L- NOARG) were obtained from Sigma Chemical Co. (St Louis, MI, USA). Stock solutions of L – NOARG and suramin were prepared using distilled water. Prazosin was dissolved in dimethyl sulfoxide (DMSO) (0.1 %). All other agents were dissolved in distilled water. However, FE extract was dissolved in ethyl alcohol.

Preparation of FE extract

Whole plant including roots of FE was collected from the Engizek Plateau Kahramanmaraş located along the eastern meridian of (37°) N, and (41°) E. *Ferula elaeochytris* was identified and verified by Pr. Dr. Ilhan Uremis (taxonomist). A voucher specimen was kept in the herbarium of the Department of Plant Protection, Faculty of Agriculture, Hatay Mustafa Kemal University, Turkey. The extraction methods were carried out at Cukurova University. All plant material was dried under the shade and the root was separated and mechanically pulverized. Crude root extract was prepared using the Soxhlet extraction method [13]. At the end of the extraction, a flask containing the solvent was attached to an evaporator and the solvent was discarded. The extract was incubated for 1 h at 60 °C, cooled to room temperature in a desiccator and weighed. Dried extract was kept at 4 °C. In the present study, the effect of ethyl alcohol on contractions was also investigated because the extraction solvent was ethyl alcohol.

Preparation of the isolated vas deferens tissues

The rats were sacrificed by cervical dislocation. Both vas deferens tissues were removed and placed in a dish containing Krebs solution (composition: NaCl 119 mM, KCl 4.6 mM, $CaCl_2$ 1.5 mM, $MgCl_2$ 1.2 mM, $NaHCO_3$ 15 mM, $NaHPO_4$ 1.2 mM, and glucose 11 mM). They were freed from connective and adipose tissues, and then prostatic or epididymal ends of vas deferens were subsequently separately mounted in a 5 mL jacketed organ bath containing Krebs solution maintained at 37 ºC and bubbled with a mixture of 95 % O_2 and 5 % CO_2 (pH 7.4). Tissues were allowed to equilibrate for 1 h, during which the preparation was washed with fresh Krebs solution at 15 min intervals. The responses were recorded with isometric force displacement transducer (MAY, FDT 10-A). Data were recorded and stored using data acquisition software (BIOPAC MP30 Systems, Inc.).

At the end of the equilibration period, neurally-evoked isometric contractions of vas deferens tissues were induced using trains of Electrical Field Stimulation EFS (4 Hz, 50 V, 0.5 ms duration, 10 s trains) delivered from a Grass S88 stimulator via two parallel platinum electrodes embedded in Perspex. In the control experiment,

EFS evoked responses were recorded for a 1 h time period (no drug was added) to examine the stability of the nerve-evoked responses. After the control responses to EFS were recorded for approximately 20 min, FE 5 μL (15.62 mg/μL) was administered four times to the same tissue consecutively at intervals ranging from 10 min to the last concentration (20 μL; 62.5 mg/μL) in the organ bath). In the preliminary studies, suitable concentrations to obtain reproducible inhibitory effects on the nerve-evoked contractions of rat vas deferens were 10 and 20 μL (31.25 and 62.5 mg/μL). After the first administration of the extract, the tissue was washed with Krebs solution. In these preliminary experiments recorded for 3 h, no drug was added to examine the stability of the EFS- induced neurogenic contractions of vas deferens. In addition, the α_1-adrenoceptor antagonist, prazosin (0.3 μM), cholinergic receptor antagonist, atropine (1 μM), a purinergic antagonist, suramin (100 μM), nitric oxide synthase inhibitor, L-nitroarginine (L-NOARG; 100 μM), and Ca^{2+} channel agonist, Ca^{2+} (3 and 6 mM) were used to isolate the adrenergic, cholinergic, purinergic, and nitrergic responses of the vas deferens tissues to EFS respectively [14]. The EFS evoked responses were recorded for 20 min. Then, 10 μL (31.25 mg/μL) of FE extract was added to the organ bath and left for 10 min and the resulting effect was observed. Another 10 μL (31.25 mg/μL) of FE was added to the bath for another 10 min (FE was in the bath for 20 min in total). Then, the organ bath was flushed with Krebs solution.

Statistical analysis

Amplitudes of neurogenic contractions induced by EFS in the presence of FE and other drugs were expressed as a percentage of the control EFS contractions (without drugs) for each experiment. All data are expressed as mean ± SEM. All the data were evaluated with the Bonferroni corrected *t*-test used in analysis of variance (ANOVA). *P*-values of less than 0.05 were considered significant. Statistical analysis was performed with GraphPad Prism software (San Diego, CA, USA).

RESULTS

Contractions elicited by EFS

In the control experiments in the bath medium without any antagonist, EFS (4 Hz, 50 V, 0.5 ms train stimulation) elicited a bimodal contraction (Figure 1). The amplitude of the EFS induced contractions was stabile for 3 h duration (457.2 ± 11.3 mg at the beginning of the experiment; 436, 2 ±17.9 mg at the end of the experiments). The shape or appearance of the contraction to EFS was similar to those of control responses. But, the amplitude of the contractions to EFS decreased by suramin and prazosin. This decrease was not statistically significant ($p > 0.05$).

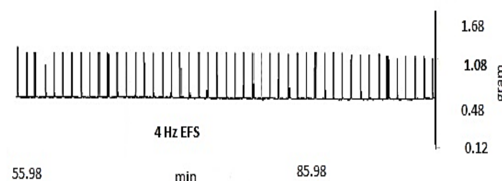

Figure 1: Contractions elicited by EFS

The effect of FE in prostatic and epididymal ends of vas deferens

The FE treatment caused a significant dose-dependent decrease on the contractions to EFS ($p < 0.05$) in the prostatic and epididymal ends of vas deference (Figure 2).

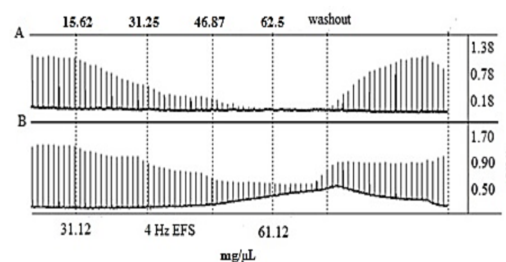

Figure 2: Representative trace showing the effects of FE on the contractile activity of vas deferens. A) Prostatic B) Epididymal end

Effect of the solvent of FE (ethyl alcohol) on the neurogenic contractions induced by EFS

The effects of 20 μL of ethyl alcohol (96 %) introduced in a 5 mL organ bath medium on neurogenic contractions induced by EFS in both parts of the isolated rat vas deferens tissue were examined and no change was observed on neurogenic contractions (Figure 3).

Effects of FE on the contractile responses to EFS in the presence of prazosin or suramin at the prostatic and epididymal ends of vas deferens

The FE treatment caused a decrease on the contractions to EFS in the presence of both antagonists. However, this decrease was not significant statistically ($p > 0.05$). Also, no significant difference was observed in the inhibitory effect of FE in both ends of vas deferens ($p > 0.05$). In the presence of both

antagonists, the contractions to EFS (4 Hz, 50 V, 0.15 ms) were significantly inhibited by FE at the prostatic and epididymal ends of vas deferens ($p < 0.05$). However, the contractions to EFS (4 Hz, 50 V, 0.15 ms) significantly inhibited by FE initially (31.25 mg/µL) at the epididymal ends of vas deferens was insignificant in the presence of prazosin. In addition, on second administration of FE, its inhibitory effect was more pronounced compared to the first administration (Figure 4).

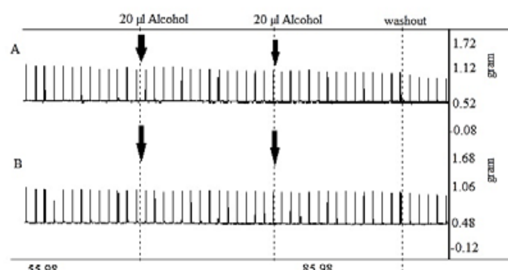

Figure 3: Representative trace showing the effects of 20 µL alcohol on neurogenic contractions induced by EFS (4 Hz, 50 V, 0.5 ms duration, 10-s train) in the vas deferens. A) Prostatic B) Epididymal end

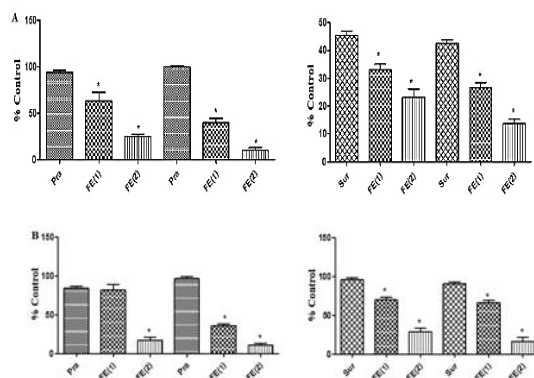

Figure 4: Effect of FE on the contractile responses to EFS in the presence of prazosin or suramin in vas deferens. (A) Prostatic (B) Epididymal end. (significant inhibition = *$p < 0.05$) FE (1): 31.25 mg/µL, FE (2): 62.5 mg/µL

Effects of Ca^{2+} addition on the inhibitor effect of FE

Ca^{2+} (3 and 6 mM) addition to the organ bath significantly ($p<0.05$) reversed the inhibitory effect of FE at both ends of vas deferens (Figure 5).

Effect of atropine and L-NOARG on the inhibitory effect of FE

The muscarinic receptor antagonist, atropine's (1 µM) treatment did not affect the inhibition due to FE on EFS-induced neurogenic contractions in the presence of prazosin or suramin at both ends (Figure 6). A nitric oxide synthase inhibitor L-NOARG (100 µM) in the presence of prazosin or suramin did not affect the inhibition due to FE on the EFS-induced neurogenic contractions in both ends of vas deferens.

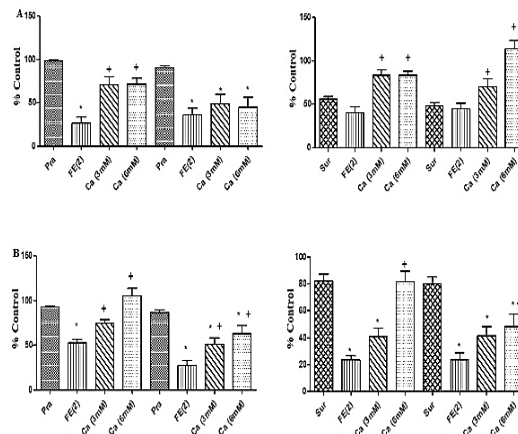

Figure 5: Effect of Ca^{2+} (3 mM and 6 mM) on the inhibitory effect due to FE at vas deferens (A) Prostatic (B) Epididymal end. (significantly reversed = $^{*,+}p < 0.05$). FE (2): 62.5 mg/µL

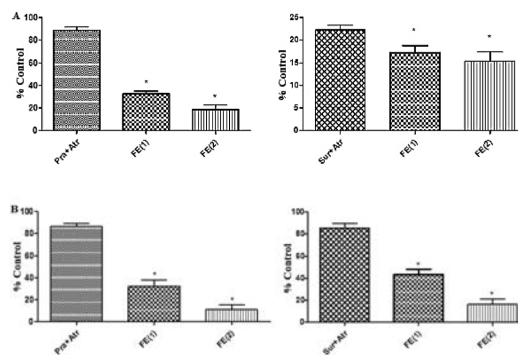

Figure 6: Effect of atropine on the inhibitory effect of FE on EFS-induced contractions in the presence of prazosin or suramin at the (A) Prostatic and (B) Epididymal end ($^{*}p < 0.05$). FE (1): 31.25 mg/µL, FE (2): 62.5 mg/µL

DISCUSSION

The contractile activity of the vas deferens is associated with the movement of sperm from epididymis to urethra, and the dysfunction of this activity results in various ejaculation disorders like premature ejaculation [15]. The present experiment revealed that FE root extract exhibited a significant inhibitory activity on the EFS-induced neurogenic contractions at the prostatic or epididymal ends of the rat vas deferens. This inhibitory effect was observed in the treatment of suramin and prazosin

separately. However, this inhibition rate was similar at both the prostatic and epididymal ends.

Although adrenergic mechanisms have an important role for smooth muscle contraction of vas deferens, many substances can alter the contraction of smooth muscle layers by regulating the releasing activity of neurotransmitter or the basal tone. Metabolic disorders and drugs, used for lower urinary system disorders, can affect the contractile functions, and may cause ejaculation disorder [16].

Besides pharmacological and developmental studies, molecular evidences showed that P2X1 receptors, presumably forming homomeric channels, are mainly responsible for managing the fast purinergic transmission in the vas deferens of mouse [17].

In the current study, the effect of FE at the prostatic or epididymal parts separately was investigated. Although, at these parts purinergic and adrenergic responses were seen, the extent of inhibition due to FE was observed as similar. Also, it was observed that there was no significant difference between the purinergic or adrenergic responses obtained from this both ends. Cholinergic pathway signaling in the epididymis part could be disturbed by the drugs that have anticholinergic adverse effects like anti-depressant agents. These types of drugs are mostly used over longer periods of time. Inhaled atropine, an anticholinergic agent, is used as a bronchodilator, and may have more local effects to respiratory system [18]. The effects of anti-muscarinic agents were related with decreased fertility in rat, but effects on epididymis part were not much different from the other organs in the male reproductive tract. Nevertheless, side effects are regressed with the discontinuation of the treatment [19].

Some studies demonstrated that vas deferens contractions generated by EFS were inhibited by atropine, a muscarinic receptor blocker, whereas some studies demonstrated that atropine had little or no effect [20,21]. This current experiment showed that the muscarinic blocker atropine was inefficient in affecting inhibitory effect of FE. The findings thus far indicate that the inhibitory effect of FE on the contractile activity of vas deferens is not mediated via an anticholinergic, adrenergic or purinergic pathways. It is well-known that the increment in free $(Ca^{2+})_i$ levels are a prerequisite for activating smooth muscle contraction proteins. The Ca^{2+} sensitivity is significant for the capability to retain the contractile activity of smooth muscle cells in the presence of submaximal $(Ca^{2+})_i$ levels [21]. The Ca^{2+} channels have a significant role to control the contractile activity in many smooth muscle cells, including the vas deferens. Activated Ca^{2+} channels lead to smooth muscle relaxation by hyperpolarization of membrane potential [22].

One of the striking results of this study is that the inhibition due to FE extract in the vas deferens showed a dose-dependent and significant improvement on Ca^{2+} addition to the organ bath. Inhibition of the EFS response by FE extract indicates that activation of Ca^{2+} channels contribute greatly to the extract's EFS-induced contraction. This result showed that the inhibition of FE extract-induced inhibition on EFS-induced neurogenic contractile activity in vas deferens may be associated with Ca^{2+} channels. It is well known that Nitric oxide (NO) activate the soluble guanylate cyclase (sGC), resulting in increasing cGMP in smooth muscle cells [23]. Nitric oxide-induced generation of cGMP can decrease vascular responses of smooth muscle to exogenous catecholamines. It has been proposed that the specific PKG substrate required for smooth muscle relaxation by inducing cGMP is absent in rat vas deferens. This may indicate that the contraction of rat vas deferens by exogenous noradrenaline (NA) is unaffected by nitric oxide synthase (NOS) enzyme inhibitor agents [24]. This finding shows that L-NOARG, a NOS enzyme inhibitor, did not alter the inhibitory effects due to FE, suggesting that this inhibitory effect was not due to a possible NO pathway. Vas deferens is defined as a muscular tube that acts as a channel to transport mature sperm from epididymis to urethra in preparation for ejaculation. So, the contractile activity is very important for this transport of the sperm [24,25].

The findings from this present study may suggest that FE can affect this sperm transport by reducing the contractile activity of the vas deferens and hence male fertility. Furthermore, it has been shown that FE corrected neurogenic and endothelial dysfunction in diabetic rats [9]. Hence FE would be used an aphrodisiac, however further studies such as investigating the effect of FE on the sperm count, motility and quality will need to be carried out.

CONCLUSION

Ferula elaeochytris extract inhibits the neurogenic contractions of rat vas deferens. This effect of FE may be associated with Ca^{2+} channels. This inhibitory effect due to FE may affect the male fertility. It can therefore be suggested that FE might be used carefully as an aphrodisiac.

However, there would be need to carry out some future studies to investigate the effect of FE on sperm motility.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Ozge Ozturk Cimentepe, Cemil Gocmen, Nadire Eser and Hacer Sinem Buyuknacar designed the study and supervised the data collection Ozge Ozturk Cimentepe and Mehmet Cimentepe prepared the manuscript for publication and reviewed the draft of the manuscript; and Ozge Ozturk Cimentepe and Cemil Gocmen analyzed and interpreted the data. All authors have read and approved the manuscript

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