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Effect of Ferula Elaeochytris Root Extract on Smooth Muscle Contraction of Prostate Gland in Rat

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ABSTRACT

Background: Smooth muscle contraction and enlargement of the prostate are important targets for the treatment of lower urinary tract symptoms in patients with benign prostatic hyperplasia. The effect of Ferula elaeochytris (FE) root extract on tissues that play a role on fertility such as the prostate has not been demonstrated yet. The aim of this study is to investigate the effects of FE extract on isolated rat prostate tissue induced by electrical field stimulation (EFS) *in vitro*.

Methods: In this experimental study, 48 male rats were randomly divided into 6 groups (n = 8). Groups were consisting FE (20 µl), FE + Adenosine triphosphate (ATP; 50 µM), FE + prazosin (0.3 µM), FE + Calcium (Ca^{2+}; 3 mM and 6 mM), FE + suramin (100 µM), FE + phenylephrine and FE + carbachol.

Result: In our study, FE extract inhibited the neurogenic contractions induced by EFS on isolated rat anterior prostate tissue. The presence of suramin and prazosin were not significantly increase the inhibition caused by the FE extract, while Ca^{2+} and ATP significantly increased the inhibition by the FE extract. ($p<0.05$). In addition, FE extract significantly inhibited phenylephrine and carbachol contractions ($p<0.05$).

Key words: Electrical field stimulation (EFS), Ferula elaeochytris, Prostate, Smooth muscle.

INTRODUCTION

Prostate smooth muscle contraction and enlargement of the prostate contribute to the development of lower urinary tract symptoms in patients with benign prostatic hyperplasia (BPH) by disrupting bladder emptying due to proximal urethral obstruction (Hennenberg *et al*., 2014). Induction of prostate smooth muscle relaxation is an important goal for the medical treatment of voiding symptoms. α1-adrenoceptor antagonists (α1-blockers) are used to improve symptoms by inducing prostate smooth muscle contraction (Oelke *et al*., 2013). However, prostatic smooth muscle contraction is mediated by numerous other receptor systems, such as acetylcholine acting on muscarinic receptors or ATP acting on purinoceptors. For this reason, it has been postulated that blocking these receptors, particularly muscarinic receptors, is an appropriate additional target for a better pharmacological treatment for BPH. ATP causes smooth muscle contractions in rat and guinea pig prostates by activation of purinergic receptors. Purinergic contractions mediate contraction *via* P2X1 purinoceptors located on prostatic smooth muscle (Burnstock *et al*., 2011; Ralevic, 2015).

The genus *Ferula* L. (Apiaceae) includes approximately 180-185 species distributed from Asia, North Africa to North America. It is represented by 23 taxon in flora of Turkey, 13 of which are endemic and the mainly distributed Mediterranean, Southeast and Central Asia Innor Anatolia. Most of the species are known as “flint, calendula, and pulp” and are used in Anatolian traditional medicine as an aphrodisiac, tonic, antimicrobial, expectorant in hemorrhoids and urinary diseases (Altundag *et al*., 2011; Güzel *et al*., 2015; Sargin *et al*., 2015). The components and fatty acids in FE root extract were determined by GC/MS (Eser *et al*., 2019 and 2020). It has been shown that FE has a high capacity for total phenolic content and reduces glucose levels (Eser *et al*., 2020). Also, the corrective effects of FE on diabetes (Eser *et al*., 2020) and age (Eser *et al*., 2020) related erectile dysfunction have been demonstrated. Syriaca root extract has been used as a smooth muscle relaxant in the human corpus cavernosum. In addition, some substances found in this species have been shown to have potent antiproliferative

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and cytotoxic effects (Ozturk *et al*., 2012). There is limited data on the effects of FE root extract on prostate smooth. The aim of this study is to investigate the effects of FE extract on isolated rat prostate smooth muscle tissue induced by EFS *in vitro.*

MATERIALS AND METHODS

Animals

A group of healty male Wistar albino rats (200-250 g) with an age group of approximately 3 months obtained from Medicine Research Laboratories of Cukurova University, Turkey were used for present study. All experimental procedures were approved by the Cukurova University Animal Experiments Local Ethics Committee (Approval No: 02.10.2013 3/10). 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 photoperiod. They received standard food and tap water ad libitum.

Preparation of FE extract

FE was collected by the author in Engizek Plateau Kahramanmaraş (37°33′39″ N, 41°45′53″ E) in 2013. FE was defined and verified by Pr. Dr. Ilhan Uremis (taxonomist). FE extraction was carried out at Cukurova University. All plant material was dried under the shade and mechanically pulverized. Crude root extract was prepared using the Soxhlet extraction method (Bimakr *et al*., 2011). Twenty grams of the powdered flint root was taken and extracted in Soxhlet apparatus by keeping in 320 ml diethyl ether solvent at 60°C for 4 hours. At the end of the extraction, the balloon containing the solvent was removed and connected to the evaporator. The solvent was removed. The balloon was then kept in the oven set at 60°C for 1 hour, cooled to room temperature in the desiccator and weighed. The dried extract was kept at 4°C.

In vitro analysis of isolated prostate tissue

The rats were killed by cervical dislocation under isoflurane anesthesia, the abdominal part was opened and two lobes were removed from the prostate. The operation was carried out as previously described as in our previous study (Eser *et al.* 2020). Then, these tissues were isolated in a petri dish containing Krebs solution (119 mM NaCl, 4.6 mM KCl, 1.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, 15 mM $NaHCO_3$, 1.2 mM $NaHPO_4$ and 11 mM glucose) and made into 4 separate strips. Prostate strips were suspended between electrodes containing platinum under 1 gram of tension in 5 ml isolated organ baths ventilated with 5% CO_2 and 95% O_2 heated at 37°C containing Krebs solution. Tissues were left to incubate for 1 hour. The responses were recorded with isometric transducers (May FDT 10 A; Commat Ltd., Ankara, Turkey) on a two-channel recorder (Biopac MP36 Systems, Santa Barbara, CA, USA).

After incubation, progressively isometric contractions of the prostate tissues were induced using EFS (2, 4, 8 Hz, 50 V, 0.5 ms duration, 10 s trainler) sequences sent from a Grass S88 stimulator *via* two parallel platinum electrodes embedded in Perspex. Tissues were washed with Krebs solution. Aftewards, FE (20 µl as a result of our pilot study with different doses 2.5, 5, 10 and 20 µl) extract was added to the bath and kept for 10 minutes. For each study, Ca^{2+} (3 mM and 6 mM), ATP (purinergic receptor agonist; 50 µM), suramin (purinergic receptor antagonist; 100 µM) and prazosin (adrenergic receptor antagonist; 0.3 µM) was added to the bath and for kept for 10 minutes in the presence of FE extract. Neurogenic contractions were form with EFS. To examine the changes in the contractile length of tissues, phenylephrine (α1 adrenergic receptor agonist; 50 µM) and carboccol (cholinergic receptor agonist; 50 µM) were administered and the contraction length was observed. FE extract was given to examine the changes in the length of the contraction. After that, the tissue was washed with fresh Krebs solution and allowed to rest for 30 min. At the end of the study, the experiments were completed by contracting the tissues with Krebs solution containing 100 mM KCl.

Statistical analysis

The quantified by calculation the maximal amplitude (mg), Amplitudes of neurogenic contractions induced by EFS were expressed as a percentage of control EFS contractions (without drugs) at the each experiment. All data are expressed as mean ± S.E.m. All of the data were evaluated with the Bonferroni corrected t test that was 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 AND DISCUSSION

Effect of FE extract using ethyl alcohol as solvent on neurogenic contractions induced by EFS

In control experiments without any antagonist, EFS (2, 4, 8 Hz, 50 V, 0.5 ms duration, 15-30 seconds) produced a bimodal contraction in the prostate tissue consisting of a large amplitude transient response followed by a lower amplitude more sustained response. FE root extract (20 µl) significantly reduced EFS induced neurogenic contractions in isolated rat anterior prostate tissue (Fig 1). Ethyl alcohol (20 µl) was given in a 5 ml bath medium on neurogenic contractions induced by EFS in the prostate tissue and no change was observed on neurogenic contractions (Fig 2).

Effect of FE extract on neurogenic contractions induced by EFS in the presence of ATP, prazosin, suramin and Ca^{2+}

On isolated rat prostate tissue, FE extract significantly reduced neurogenic contractions induced by EFS (2, 4, 8 Hz, 50 V, 0.5 ms, 15-30 seconds) (Fig 3). In the presence FE extract, the prurinergic responses to EFS (4 Hz, 50 V, 0.15 ms) were significantly corrected by ATP in the prostate tissue (Fig 3). Further, α1-adrenergic receptor blocker, prazosin (0.3 µM) and purinergic receptor blocker suramin (100 µM) failed to modify the contractile response induced by EFS in the presence of FE extract (Fig 4 and 5). In additon, it was observed that 3 mM Ca^{2+} 2 Hz and 8 Hz frequencies except 4 Hz and all frequencies of 6 mM Ca^{2+} significantly corrected the inhibition in the presence of FE extract (Fig 6).

Effect of FE extract on the contractile response induced by phenylephrine and carbachol

In the presence phenylephrine, the adrenergic responses to EFS (2, 4, 8 Hz, 50 V, 0.5 ms duration, 15-30 seconds) were significantly inhibited by FE extract at the prostatate tissue. In additon, the cholinergic responses to EFS (2, 4, 8 Hz, 50 V, 0.5 ms duration, 15-30 seconds) were significantly inhibited by FE extract in the presence carbachol (Fig 7).

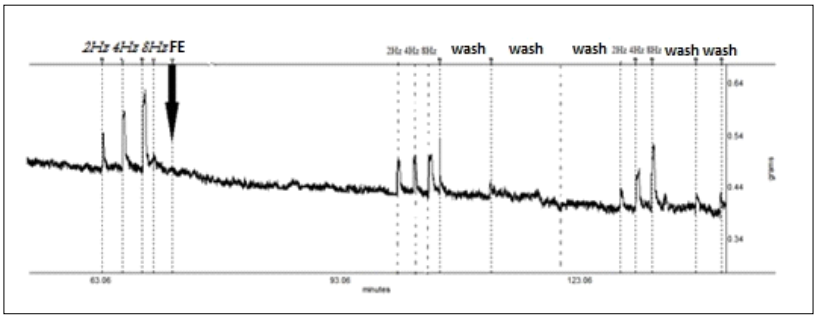


Fig 1: Effects of FE extract on neurogenic contractions induced by EFS (2, 4, 8 Hz, 50 V, 0.5 ms, 15-30 seconds) on isolated rat anterior prostate tissue.

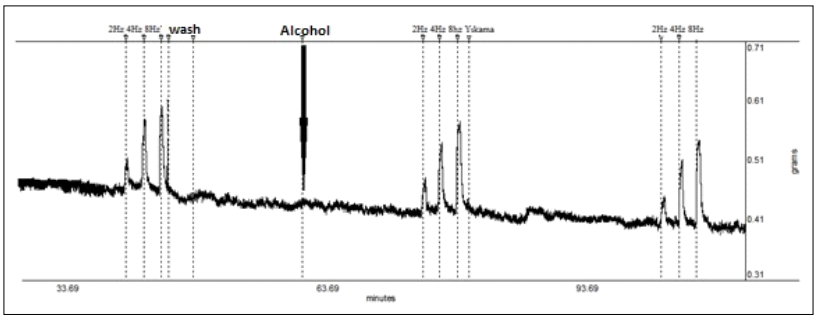


Fig 2: Effects of Ethyl alcohol on neurogenic contractions induced by EFS (2, 4, 8 Hz, 50 V, 0.5 ms, 15-30 seconds) on isolated rat anterior prostate tissue.

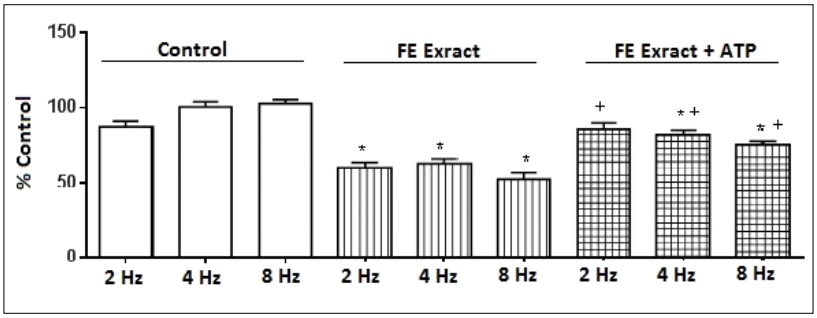


Fig 3: Effects of FE (20 µl) extract and purinergic receptor agonist ATP (50 µM) on neurogenic contractions induced by EFS (2, 4, 8 Hz, 50 V, 0.5 ms, 15-30 seconds) on isolated rat anterior prostate tissue. *,+ p<0.05.

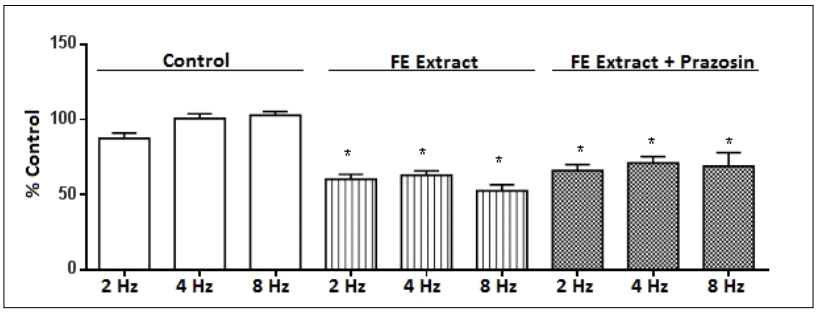


Fig 4: Effects of FE extract (20 µl) and α-adrenergic receptor blocker prazosin (0.3Mm) on neurogenic contractions induced by EFS (2, 4, 8 Hz, 50 V, 0.5 ms, 15-30 seconds) on isolated rat anterior prostate tissue. *p<0.05.

EFS application stimulates all neuron networks in the prostate wall and therefore stimulate smooth contraction both directly and indirectly. The nature of the contraction depends on the contribution of the types of neurotransmitters released during the stimulation periods (Yu *et al*., 2018). In our study, EFS induced smooth muscle contraction FE extract decreased rat prostate smooth muscle contractions in a dose-dependent and significant way. These inhibitions were reserved as a result of washing the medium with fresh Krebs solution and the contractions were restored. Since this reversible and dose-dependent inhibition, it has shown that the FE extract had a pharmacological effect on smooth muscle function.

Considering that α1 blockers represent the first line option for the medical treatment of voiding symptoms in BPH, α1-adrenergic and EFS induced contractions can be considered the gold standard of prostate smooth muscle contraction. α1 blockers can reduce prostate tone and BOO by inhibiting the effect of endogenously released noradrenaline on prostate smooth muscle cells (Ventura *et al*., 2011). Previous studies have reported purinergic smooth muscle contractions in rat prostates with contractile forces approaching the range of neurogenic and α1-adrenergic contractions (Oelke *et al*., 2013, Hennenberg *et al.,* 2013). In EFS induced muscle contractions, which are presumed to be mediated by the release of endogenous neurotransmitters and greater activation of postsynaptic α1-adrenoceptors on smooth muscle cells, the FE extract was inhibited contractions and the α1 adrenergic receptor blocker prazosin and purinergic receptor blocker suramin in the presence of FE extract was no effect on contractions. These results was not correlate the inhibitory effect of the inhibition of FE extract with adrenergic and purinergic pathways on the EFS induced contractile responses. However, inhibition of adrenergic

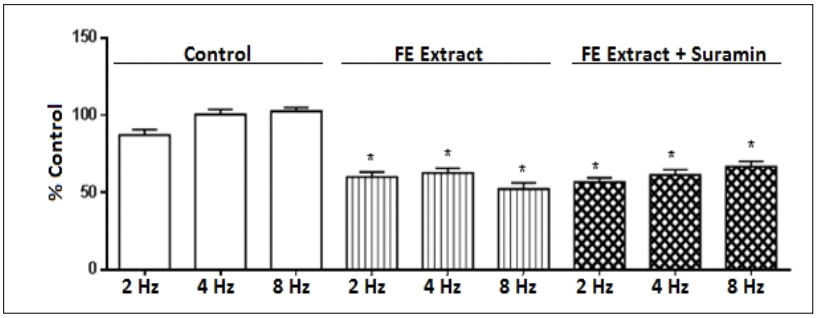


Fig 5: Effects of FE extract (20 µl) and purinergic receptor blocker suramin (100 µM) on neurogenic contractions induced by EFS (2, 4, 8 Hz, 50 V, 0.5 ms, 15-30 seconds) on isolated rat anterior prostate tissue. *p<0.05.

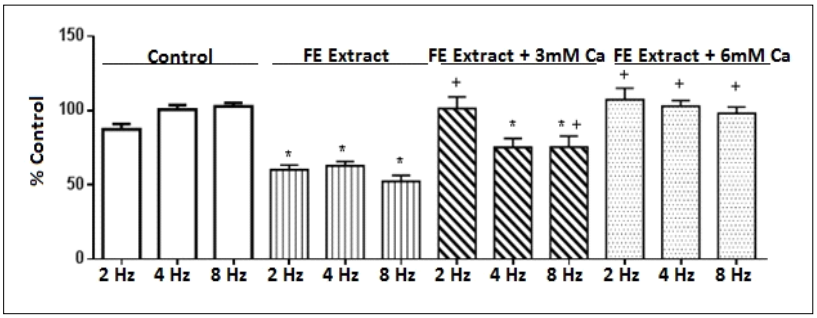


Fig 6: Effects of FE extract (20 µl) and Ca^{2+} (3 mM and 6 mM) on neurogenic contractions induced by EFS (2, 4, 8 Hz, 50 V, 0.5 ms, 15-30 seconds) on isolated rat anterior prostate tissue. *,+ p<0.05.

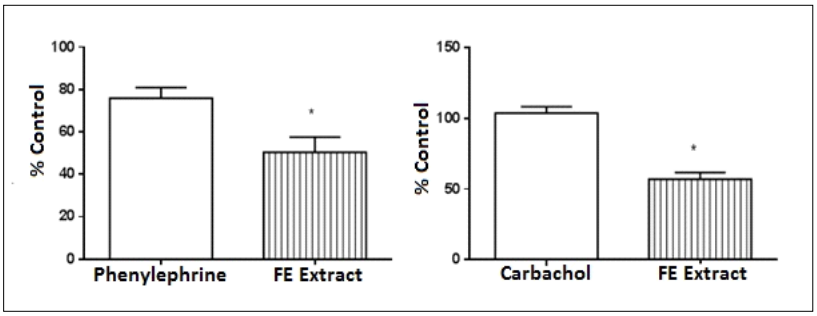


Fig 7: Effect of FE extract (20µl) on adrenergic contractions induced by the α-adrenergic receptor agonist phenylephrine and cholinergic contractions induced by the cholinergic receptor agonist carbachol on isolated rat anterior prostate tissue.

contraction by phenylephrine and cholinergic contraction by carbochol were occurred in the presence of FE extract.

It is widely accepted that the increase in free intracellular Ca^{2+} level is a prerequisite for activating smooth muscle contraction proteins (Webb *et al*., 2003). Ca^{+2} sensitivity is important for the ability of smooth muscle cells to maintain their contractile response in the presence of submaximal intracellular Ca^{+2} levels (Kong *et al*., 2006). Ca^{+2} channels play an important role in the control of contractile tone in many smooth muscle cells, including the prostate. Activation of Ca^{+2} channels causes smooth muscle relaxation through hyperpolarization of the membrane potential (Hughes *et al*., 2011). The most important finding of our study was that inhibition due to FE extract in the prostate was showed a dose-dependent and significant improvement with Ca^{+2} added to the environment from outside. Inhibition of the EFS response by the FE extract was indicated that activation of Ca^{+2} channels were contribute greatly to EFS induced contraction. This result showed that the inhibition of FE extract induced inhibition on neurogenic contractions induced by EFS in the rat prostate may be associated with Ca^{+2} channels.

In addition, inhibition in prostate tissue ATP is an important neurotransmitter that plays a versatile role in the regulation of smooth muscle contraction in the vasculature and various internal organs (Ford *et al.* 2011). ATP has been reported to cause both smooth muscle contraction and relaxation. It has been assumed that ATP induced contractions have a high importance for the composition of prostate smooth muscle tone (Wang *et al.* 2020). Various studies with non-human prostates have suggested that ATP can be secreted as a co-transmitter in adrenergic neurotransmission and contribute to the subsequent α1-adrenergic contraction of prostate smooth muscle (Hennenberg *et al*., 2018; Spek *et al*., 2021). In addition to ATP induced contractions, inhibition of EFS induced contractions by ATP has also been reported for rat prostate tissues. In our study, it was observed that in the presence of FE extract inhibition of EFS induced smooth muscle contractions and in the presence of FE extract, the purinergic receptor agonist ATP was significantly reversed contractions.

Conclusion

Our most important finding is that the inhibitory effects due to FE on the contractile responses induced by EFS were restored by external addition of Ca^{+2} into the organ baths these results indicate that the inhibitory effect of FE on neurogenic contractions due to EFS related with Ca^{+2} channels. In addition, we observed that the inhibitory effects of FE on neurogenic contractions are not related with adrenergic and purinergic pathways. According to our findings we can suggest that FE extract can be tried in the treatment of benign prostatic hyperplasia. So, it is possible this extract increase the cAMP or cGMP in the smooth muscle or this extract may has a possible effect on the PDE 4 or PDE 5 enzyms to induce a relaxation on the prostate tone. However we need some future studies to explain perfect direction of the inhibitory effect of this extract on the tone. FE root can cause infertility in men, as it reduces spontaneous contractions of prostate tissue, which plays a role in fertility, as well as these effects.

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