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Original Article

Antispasmodic effects of *Prangos ferulacea* acetone extract and its main component osthole on ileum contraction

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Abstract

Prangos ferulacea is a plant found in the Mediterranean and Middle-east regions used as carminative, anti-flatulent, emollient and antibacterial herb. It is believed that the coumarins are responsible for some of known effects of *Prangos*. In this research the relaxant effects of *P. ferulacea* coumrin rich extract as well as osthole as its main prenylated coumarins were investigated on rat ileum contraction *in vitro*. Relaxant effect of osthole and *P. ferulacea* extract were examined on contraction induced by KCl, acetylcholine (ACh) and electrical field stimulation (EFS) and compared with propantheline and nifedipine. The acetone extract of *P. ferulacea* concentration-dependently relaxed ileum contraction induced by KCl (IC_{50}=1.3 ± 0.25 μg/ml), ACh (IC_{50}=7.7 ± 1.1 μg/ml) and EFS (IC_{50}=8.8 ± 1.4 μg/ml), while, the extract at lower concentration (4 μg/ml) potentiated the ACh and EFS responses. Unlike the extract, osthole did not potentiate the ileum contraction but concentration-dependently inhibited ileum contractile responses to KCl (IC_{50}=2.2 ± 0.7 μg/ml), ACh (IC_{50}=2.5 ± 0.7 μg/ml) and EFS (IC_{50}=2.8 ± 0.24 μg/ml). Propantheline concentration dependently inhibited the ileum response to ACh, with IC_{50} value of 0.61 ± 0.09nM without affecting the KCl response. As expected, the EFS response was only partially reduced. Nifedipine (0.2-50 nM) inhibited tonic contraction induced by KCl with IC_{50} value of 2.5 ± 0.8 nM but only partially inhibited the response to ACh. However, the response to EFS was reduced only by 33%. These results confirmed both potentiatory and inhibitory action of *P. ferulacea* extract on rat ileum contractile activity. Osthole is responsible for the inhibitory effect but potentiating components are not yet known.

Keywords: *Prangos ferulacea;* Osthole; Ileum; EFS; KCl; Acetylcholine

INTRODUCTION

Smooth muscle spasms are among causes that adversely affect the life quality. Antispasmodic agents could be used for relieving spasm in the smooth muscles of gastrointestinal, biliary, urinary tract and female genital organs, especially the cervico-uterine plexus (1). They may also be used with radiology medicine to decrease bowel tonicity, to reduce spasm, and thus should make the examination more tolerable for the patient (2). Therefore, herbal remedies as a source of antispasmodic agents could be assessed (3). Apiaceaous plants are popular for gastero-intestinal disorders treatment especially in Middle East. Most of the pharmaceutical value of these plants lies in their secondary metabolites like coumarins, terpenoids (4,5), phthalides (6) and polyacetylenes (7) characteristically. Coumarins are benzo-α-pyrone derivatives which as potential pharmacological agents, found to have numerous therapeutic applications including anti-inflammatory, antioxidant (8), antitumor and anti-HIV therapy (9), and some are also active as neuroprotective agents (10). Osthole, 7 -methoxy -8 -(3- methyl -2 -butenyl) -1-benzopyran-2-one (Fig. 1), as a prenylated and methoxylated coumarin found in Apiaceaous plants including *Prangos* spp. (11-13) has reported to have inhibitory effects on rat

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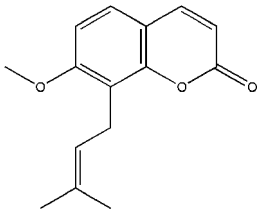

Fig. 1. The chemical structure of osthole

isolated uterus (14) and guinea pig ileum (15). It has also shown anticonvulsant (16), neuroprotective, antioxidant (8,17,18), hypo-lipidemic and anti-hypertensive effects (19). Osthole exists in *P. ferulacea* (Apiaceae) growing in Mediterranean and Middle-east regions like Iran remarkably in high amount (20). *P. ferulacea* is locally known as Jashir in Persian and is used as food and yogurt seasoning. Therefore, taking in mind the reported spasmolytic effects of cumarins (21-23) and based on previous studies on *P. ferulacea* proved it`s high amounts of coumarins (20), antispasmodic effects of coumarin rich extract and osthole from this plant were studied on rat isolated ileum.

MATERIALS AND METHODS

Plant material

Roots of *P. ferulacea* (L.) Lindl. were collected from Yasouj in Kohgiluyeh-Boirahmad province in June 2010, at an altitude of 1800 meter above sea level. The plant was identified at the Botany Department of Yasouj University and a voucher specimen (NO. 2408) was deposited at the Herbarium of the School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran. The air dried plant material was roughly cut and ground to the coarse powder.

Preparation of plant extract

500 g of aerial parts of the plant was extracted with acetone for two days (5L×4). The extract was concentrated to bear a viscous mass which was then kept at -20°C for two days and filtered chilled in which the filtrate resulted in a solid mass after drying.

Test compound

Osthole (Fig. 1) was extracted and purified from *P. ferulacea*. Full chemical characterization and purification was described earlier in the introduction (11).

Ileum contractile assessment

Experiments were conducted on male Wistar rats (180-230 g) bred in the School of Pharmacy animal house. All animals were handled in compliance with the principles of the guide for care and use of laboratory animals (24). On the day of experiment, the rat was killed by a blow on the head, followed by exsanguinations. A portion of ileum (2-3 cm long) was mounted for isotonic contraction under 1 g tension in 50 ml organ bath (Harvard, England) containing Tyrode's solution and continuously gassed with O_2 at 37°C. Ileum contraction was measured using a Harvard isotonic transducer and recorded on a Harvard Universal Oscillograph (England) pen recorder device. After calibrating the oscilograph, three successive washes was given to the tissue and allowed to relax to a stable baseline. Following a resting period of about 15-30 min, inhibitory effects of *P. ferulacea* extract and osthole as test compounds comparing with the standard drugs propanteline, nifedipine and lidocaine, were examined on isolated rat ileum contraction induced by KCl, and electrical field stimulation (EFS) as described before (25). In addition, ileum contraction was also induced by single concentration of acetylcholine (ACh, 200 nM) being in contact with the tissue for 30 sec before it was washed off with fresh Tyrode's solution. Initial contraction to ACh was determined (repeating twice at 15 min intervals), and then it`s responses in the presence of increasing concentration of test compounds were assessed in non-cumulative manner, until maximum compound responses was obtained. The same protocol was also repeated for vehicle treated time matched control tissues.

Drugs and solutions

Propantheline bromide, ACh hydrochloride, and nifedipine all from Sigma Aldrich company, lidocaine hydrochloride (Pasture, Iran) and other chemicals from Merk company were used in this research. The extract (10 mg/ml), osthole (10 mg/ml) and nifedipine (10 mM) as stock solutions were made up and

diluted in dimethyl sulphoxide (DMSO). Propantheline (10 mM), lidocaine (500 μM), ACh (100 mM) acidified by 1% acetic acid and tyrode's solution were prepared and further diluted with distilled water.

Measurements and statistical analysis

The contractile response to KCl, ACh and EFS were measured as maximum amplitude from pretreatment baseline and expressed as the percentage of the initial response in the absence of drugs or vehicle for each tissue. All the values are quoted as the mean ± standard error of the mean (SEM). The significance of differences (P<0.05) was calculated by one-way analysis of variance (ANOVA) for repeated measures or two tailed Student's *t*-test as appropriate. Sigma plot computer program was used for statistical analysis and constructing the graphs for calculation of IC_{50} values.

RESULTS

KCl caused a tonic contraction on rat ileum while ACh induced a rapid phasic response as described before (25). The EFS response was similar to EFS biphasic responses reported by Ekblad and Sundler (26). Nifedipine (0.2-50 nM) inhibited tonic contraction induced by KCl with IC_{50} value of 2.5 ± 0.8 nM (Fig. 2). However, nifedipine only partially inhibited the response to ACh and at concentration which removed the response to KCl, still 39% of initial contraction remained (Fig. 2). Nifedipine at concentration ranges which inhibited the KCl response (0.2 nM to 50 nM), concentration-dependently reduced the secondary contractile response to EFS but at its highest used concentration, still 13% of the initial response remained. Nifedipine had a weaker inhibitory effect on initial response of EFS and at concentration that nifedipine abolished the response to KCl, the response to EFS-1 was reduced only by 33% (see Fig. 2). Propantheline (50 pM- 50 nM) concentration-dependently inhibited the response to ACh, with IC_{50} value of 0.61 ± 0.09 nM (Fig. 3). At concentration of 50 nM, propantheline

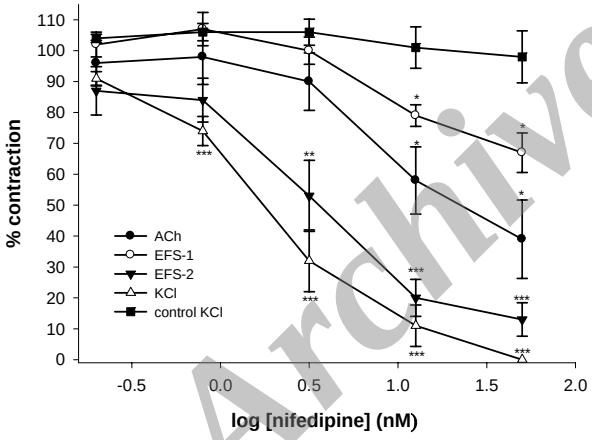

Fig. 2. Inhibitory effect of nifedipine on tension development in the isolated ileum of rat treated with KCl (80 mM), acetylcholine (ACh, 200 nM) and electrical field stimulation (EFS: 6V, 50 Hz, 1 sec duration). Contractile response was measured relative to the baseline. Ordinant scales: spasm remaining as a % of the contraction prior to compounds addition. Abscissa scales: log_{10} concentration of nifedipine. Each point is mean of six experiments and the vertical lines show the SEM. EFS-1=initial contractile response. EFS-2=secondary contractile response. Astrisks show statistical differences in comparison with the vehicle treated (DMSO) time-matched controls. **P*<0.05, ***P*<0.01, ****P*<.001 (*Student's t-test*). Maximum amount of DMSO in the bath was 0.004%.

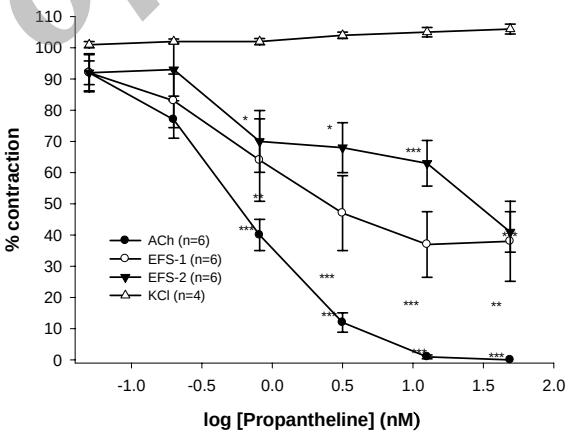

Fig. 3. Inhibitory effect of propantheline on tension development in the isolated ileum of rat treated with KCl (80 mM), acetylcholine (ACh, 200 nM) and electrical field stimulation (EFS: 6V, 50 Hz, 1 sec duration). Contractile response was measured relative to the baseline. Ordinant scales: spasm remaining as a % of the contraction prior to compounds addition. Abscissa scales: log_{10} concentration of propantheline. Each point is the mean of six experiments and the vertical lines show the SEM. EFS-1=initial contractile response. EFS-2=secondary contractile response. Except for KCl, the reduction in contractile response is statistically significant (P<0.001, ANOVA). Astrisks show statistically significant differences between KCl and EFS or ACh with the same propantheline concentration. **P*<0.05, ***P*<0.01, ****P*<0.001 (*Student's t-test*).

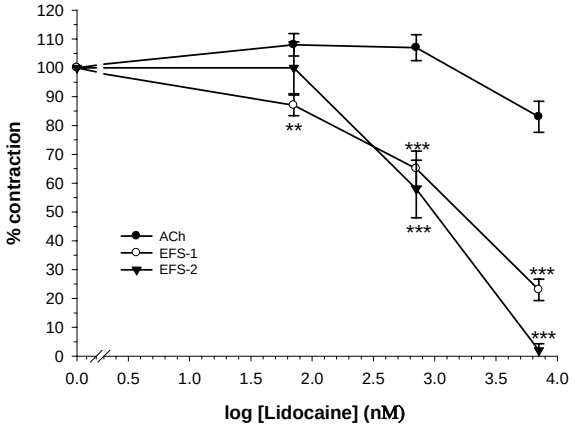

Fig. 4. Effect of lidocaine on tension development in the isolated ileum of rat treated with acetylcholine (ACh, 200 nM) and electrical field stimulation (EFS: 6V, 50 Hz, 1 sec duration). Contractile response was measured relative to the baseline. Ordinant scales: spasm remaining as a % of the contraction prior to compounds addition. Abscissa scales: $\log_{10}$ concentration of lidocaine. Each point is the mean of six experiments and the vertical lines show the SEM. EFS-1=initial contractile response. EFS-2=secondary contractile response. Astrisks show statistically significant differences between ACh and EFS with the same lidocaine concentration. ***P*<0.01, ****P*<0.001 (*Student's t-test*).

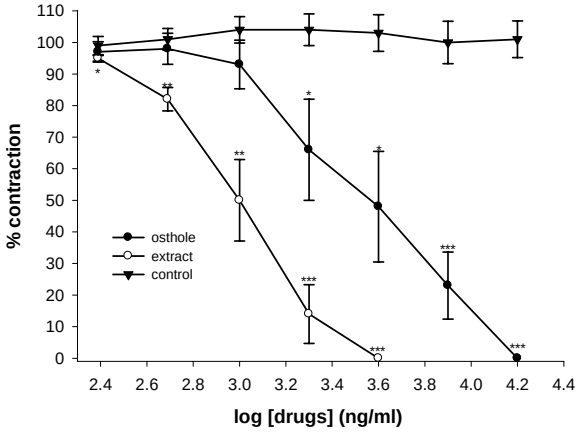

Fig. 5. Inhibitory effect of *P. ferulacea* extract and osthole on tension developments in the isolated ileum of rat treated with KCl (80 mM). Contractile response was measured relative to the baseline. Ordinant scales: spasm remaining as a % of the contraction prior to compounds addition. Abscissa scales: $\log_{10}$ concentration of compounds. Each point is the mean of six experiments and the vertical lines show the SEM. Astrisks show statistically significant differences between the test and the vehicle treated time-matched controls at corresponding points. **P*<0.05, ***P*<0.01, ****P*<.001 (*Student's t-test*).

abolished the response to ACh. Propantheline at concentrations which blocked ACh responses in rat ileum (50 pM to 50 nM), only partially attenuated the EFS responses (Fig. 3). Nevertheless, propantheline (50 pM to 50 nM) has no effect on tonic contraction induced by KCl (Fig. 3). Statistical analysis revealed no significant difference in contraction of vehicle-treated time-matched control tissues. Lidocaine (70 nM, 700 nM, and 7 µM) concentration-dependently reduced both EFS responses, while at 70 nM and 700 nM bath concentrations lidocaine had no inhibitory affect on ileum response to ACh. Nevertheless, with 7 µM lidocaine in the bath, ACh response was reduced by 17 ± 5.4% while the EFS-1 and EFS-2 responses were reduced by 77 ± 3.7% and 88 ± 2.3%, respectively (Fig. 4). The acetone extract of *P. ferulacea* (250 ng/ml - 4 µg/ml) and its major component osthole (0.5-7.8 µg/ml) applied cumulatively into the organ bath caused a concentration-dependent relaxation of rat ileum tonic contraction induced by KCl. Analysis of the concentration response curve revealed a mean IC_{50} value of 1.3 ± 0.25 µg/ml (n=6) and 2.2 ± 0.7 µg/ml (9.2 ± 2.7 µM, n=6), respectively (Fig. 5). On the other hand, the extract of *P. ferulacea* (250 ng/ml - 4 µg/ml) potentiated the response to ACh in comparison with vehicle treated time matched controls (Fig. 6a), but at bath concentration above 4 µg/ml, the extract concentration-dependently reduced the contractile response to ACh with IC_{50} value of 7.7 ± 1.1 µg/ml (Fig 6a).

P. ferulacea extract added into bath had no effect on ileum basal tension. However, depending on concentration used, both potentiated and inhibited the EFS responses (Figs. 6b,6c). The extract of *P. ferulacea* (4 µg/ml to 32 µg/ml) concentration-dependently inhibited contractile responses to EFS-1 (IC_{50}=8.8 ± 1.4 µg/ml) and EFS-2 (IC_{50}=5.2 ± 1.9 µg/ml, n=6). At its highest used concentration the extract totally removed both EFS responses. Unlike the extract, osthole did not potentiate the ACh response, but concentration-dependently attenuated contraction induced by ACh with IC_{50}=2.5 ± 0.7 µg/ml (10.3 ± 3.1 µM, n=5) (Fig. 7a).

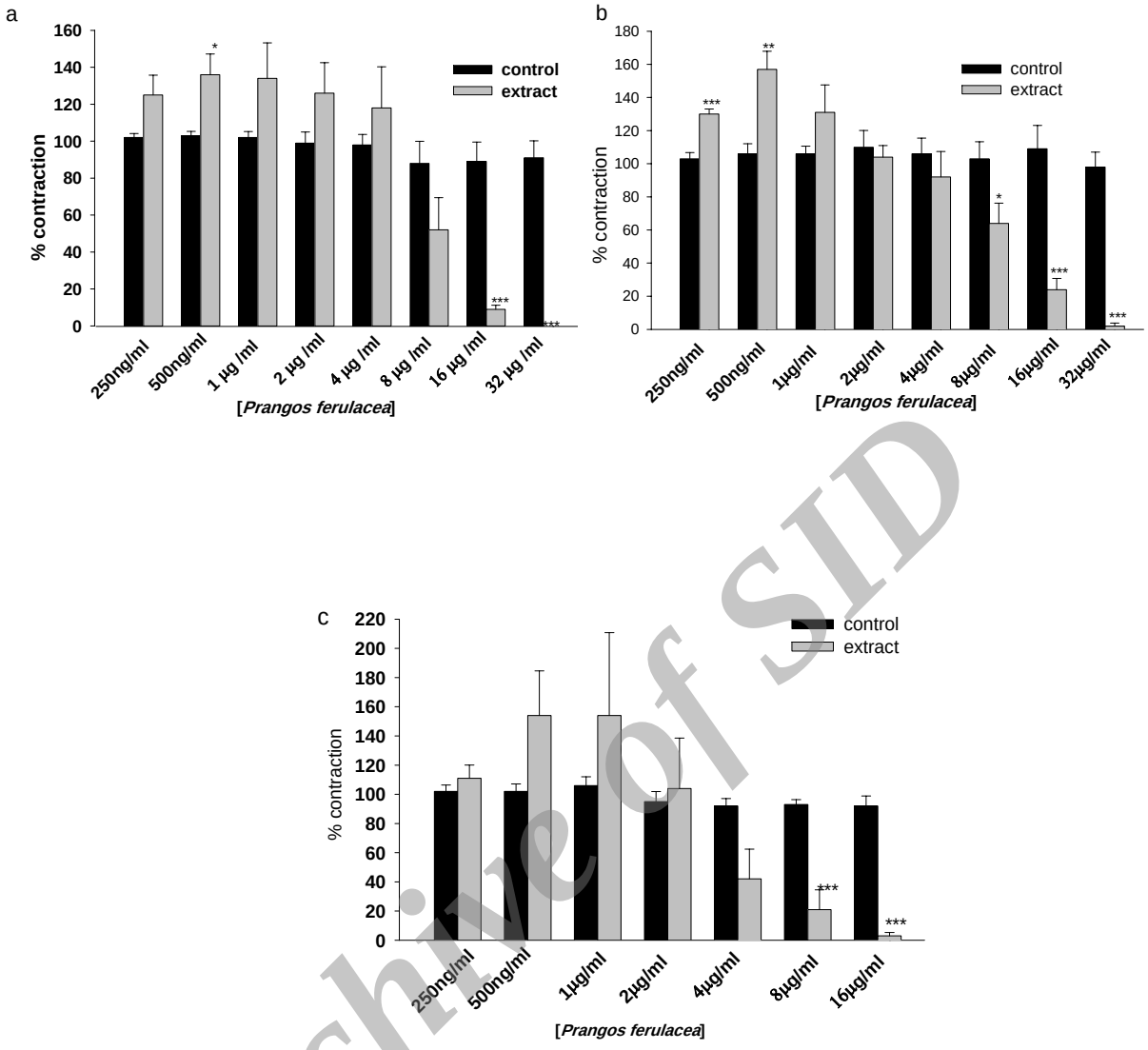

Fig. 6. Effects of *P. ferulacea* extract on tension developments in the isolated ileum of rat by **a**: acetylcholine (ACh, 200 nM, n=6), **b**: EFS-1 (n=5) and **c**: EFS-2 (n=6), (EFS= electrical field stimulation: 6V, 50 Hz, 1 sec duration). Contractile response was measured relative to the baseline. Ordinant scales: spasm remaining as a % of the contraction prior to extract addition. Abscissa scales: concentration of extract. Each point is the mean of six experiments and the vertical lines show the SEM. Astrisks show statistically significant differences between the extract and the vehicle treated (DMSO) time-matched control. **P*<0.05, ***P*<0.01, ****P*<.001 (*Student's t-test*). Maximum amount of DMSO in the bath was 0.32%.

Relaxation of the ileum began with 0.5 µg/ml osthole in the bath, and reduced to zero with 16 µg/ml concentration of osthole in the bath. Relaxant effect of and osthole at concentration ranges which inhibited the KCl and ACh responses were also examined on EFS responses (Figs. 7b,7c). Osthole at concentration ranges of 1-16 µg/ml concentration-dependently inhibited the EFS1 responses with IC_{50} value of 2.8 ± 0.24 µg/ml (IC_{50}=11.4 ± 1 µM, n=6). Inhibitory effect of osthole on EFS2 started at lower bath concentrations and complete inhibition was seen with 4 µg/ml (IC_{50}=0.8 ± 0.3 µg/ml; IC_{50}=3.5 ± 1.4 µM, n=5). No significant changes were observed in the time-matched control tissues treated with the vehicle (DMSO).

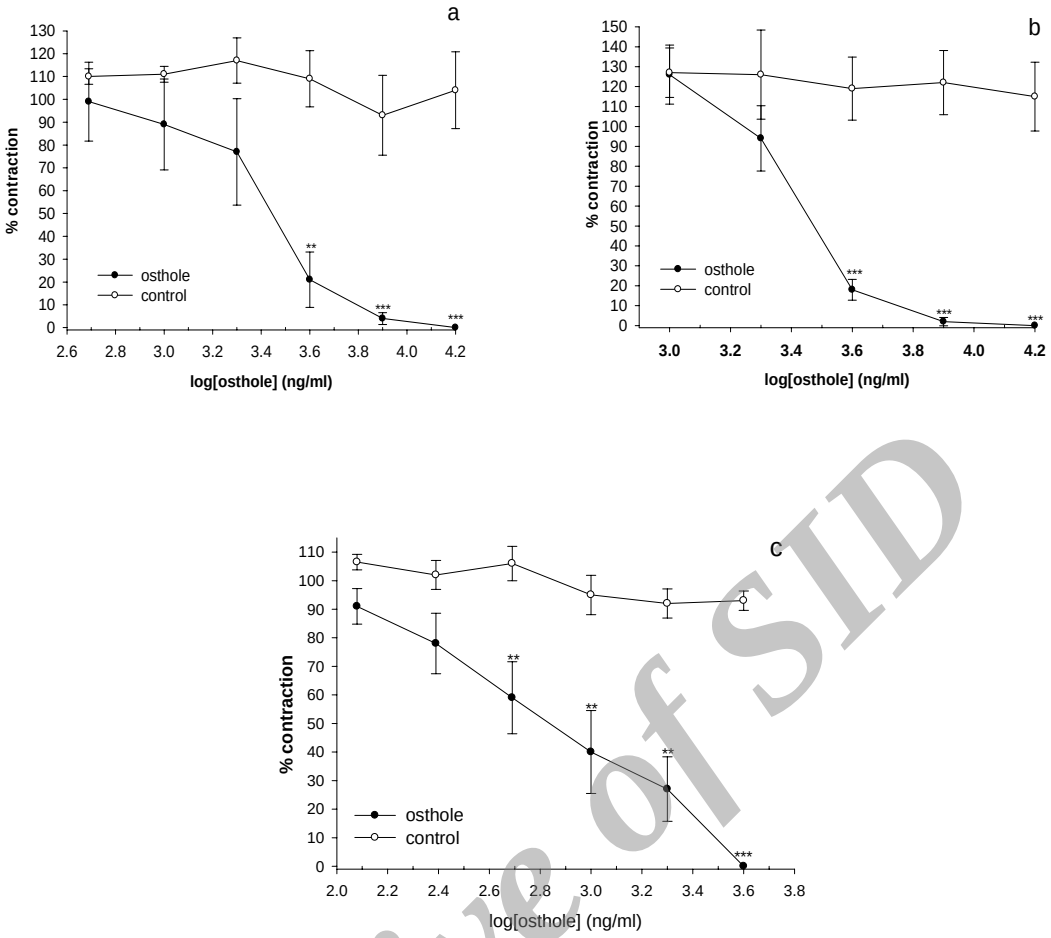

Fig. 7. Effects of osthole on tension developments in the isolated ileum of rat by **a**: acetylcholine (ACh, 200 nM, n=5), **b**: EFS-1 (n=6) and **c**: EFS-2 (n=5), (EFS= electrical field stimulation: 6 V, 50 Hz, 1 sec duration). Contractile response was measured relative to the baseline. Ordinant scales: spasm remaining as a % of the contraction prior to osthole addition. Abscissa scales: $\log_{10}$ concentration of osthole. Each point is the mean of six experiment and the vertical lines show the SEM. Astrisks show statistically significant differences between the extract and the vehicle treated (DMSO) time-matched control. $^{**}P<0.01$, $^{***}P<.001$ (*Student's t-test*). Maximum amount of DMSO in the bath was 1.6%.

DISCUSSION

In this study we have used an L-type Ca^{2+} channel blocker and a muscarinic receptor antagonist as standard drugs to compare their effect with that of *P. ferulacea* acetone extract and one of its component osthole. Nifedipine totally inhibited the KCl response indicating involvement of hydroperidine sensitive Ca^{2+} channels in contraction induced by KCl. At similar concentration nifedipine only partially inhibited the ACh response. This is because contraction induced by ACh is mediated through muscarinic M_3 receptors which are coupled to phospholipase C and the release of intracellular Ca^{2+} (27). Partial inhibition of EFS response by nifedipine, also indicate the involvement of second messenger system inositol triphosphate and release of internal Ca^{2+}. Propantheline at concentrations which totally blocked the response to ACh only partially attenuated the response to EFS and this is in consistence with other reports that muscarinic antagonists only partially remove contractile response to neuronal stimulation of ileum (27). The remaining response is due to the release of other neurotransmitter from enteric nervous system (28). Inhibition of EFS responses by selective concentration of lidocaine is in consistence with assumption

that parameters were used in this experiment is mainly stimulating the neuronal network embedded in the tissue. We have demonstrated that *P. ferulacea* extract is a relaxant of isolated rat ileum, inhibiting contraction induced by high concentration K^+ ions, ACh and EFS stimulation. Osthole is one of the components isolated from *P. ferulacea* extract. It had similar pattern of inhibitory effect on rat ileum contraction evoked by above stimulus. However, *P. ferulacea* extract was more potent than osthole at inhibiting KCl response. On the other hand, when inhibitory effects of the extract and osthele are compared on the contraction induced by either ACh or EFS, osthole was more potent than the *P. ferulacea* extract. Comparison of inhibitory concentrations of osthole and extract of *P. ferulacea* suggest that osthole is partially responsible for the inhibitory effect of the extract on KCl response and other inhibitory material also exists which contribute to the inhibitory effect of KCl. Total inhibition of KCl, ACh and EFS responses by extract and osthole indicates a more general inhibitory mechanism probably at contractile molecules levels which need to be investigated. For example, it has been suggested that inhibitory effect of osthole is due to the inhibition of phosphodiesterases enzyme on trachea (19) as well as Ca^{2+} channel blocking properties on vascular smooth muscle contraction (29). However, they have not made a comment that the blockade of Ca^{2+} channels might be a direct or indirect mechanism following increase in cAMP or cGMP levels. Although the dominant effect seen with *P. ferulacea* extract was an inhibitory response, nevertheless, there is some component in the extract that potentiated the response to ACh and EFS but not KCl. This might be due to an effect on enteric nervous system, since the para-sympathetic ganglia is embedded in the wall of gastrointestinal (30) and ACh could also stimulate these neurons through nicotinic receptors of parasympathetic ganglia.

Components other than osthole must be responsible for potentiating the EFS response since osthole did not potentiate the ACh or EFS response even at lower concentration than those which inhibited rat ileum.

CONCLUSION

Osthole is a major component of *P. ferulacea* acetone extract which is responsible for the relaxant effect of the extract on the rat ileum. On the other hand, the *P. ferulacea* extract had a potential to increase neuronal contractile response of rat ileum; therefore, if it is going to be used for gastrointestinal spasm, the stimulatory component should be separate. Nevertheless, oshtole has anti-spasmodic action on isolated rat ileum. It is necessary to investigate these effects *in vivo*. Furthermore, due to predictable anticoagulant properties of osthole as a cumarin derivative, its anticoagulant activity must be considered against its use as an antispasmodic drug and its acute and chronic toxicities should be passed before further pharmacological studies.

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