Short Communications

COMPOSITION OF THE ESSENTIAL OIL OF *ASA-FOETIDA* AND ITS SPASMOLYTIC ACTION

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رغم أن راتنج الصمغ الزيتي لنبات الحلتيت يستعمل في الطب الشعبي كعلاج مضاد للتقلصات أثناء المغص في بعض أجزاء إيران ، إلا أن تأثيراته الفارماكولوجية على القناة الهضمية لم يتم دراستها من قبل. تهدف هذه الدراسة إلى تحليل مكونات الزيت العطري للنبات بالإضافة إلى تأثيره الإسترخائي على انقباضات المعي اللفائفي المعزول للجرذان المستحثة بكلوريد البوتاسيوم، والأسيتايل كولين، و -5هيدروكسي تريبتامين. وقد تبين أن زيت نبات الحلتيت العطري قد ثبط الاستجابة لكلوريد البوتاسيوم (80 ميلي مولار)، وللأستيل كولين (320 نانومولار)، ولـ 5-هيدروكسي تريبتامين (1.28 ميكرومولار) بصورة معتمدة على التركيز. لقد تحقق التأثير المثبط للزيت العطري على الانقباضات المستحثة بكلوريد البوتاسيوم عند تركيز أدنى (تركيز مثبط 50 = 5.8±1.1 نانولتر/مل) بالمقارنة مع التأثير على الانقباضات بالمواد الأخرى (اسيتايل كولين و 5-هيدروكسي تريبتامين). وقد أظهر هذا البحث أن الزيت العطري هو مرخي للمعي اللفائفي للجرذ، حيث أن تثبيط فرط النشاط الانقباضي للمعي اللفائفي هو أساس العلاج لبعض الاعتلالات المعوية مثل الإسهال. هذه الدراسة تؤكد الفاعلية الاسترخائية المحتملة لهذا العلاج النباتي.

Oleo-gum resin of *Ferula assa-foetida* L. has been used in folk medicine as an spasmolytic remedy for abdominal cramps and spasms in some parts of Iran. However, so far its pharmacological effects on gastrointestinal tract has not been studied. The aim of this study was to analyse the components as well as to look for relaxant effect of *F. assa-foetida* essential oil (FAEO) on rat isolated ileum contractions induced by KCl, acetylcholine (ACh) and 5-hydroxytryptamine (5-HT). FAEO inhibited the response to KCl (80mM), ACh (320nM) and 5-HT (1.28μM) in a concentration-dependent manner. The inhibitory effect of FAEO on KCl contractions was achieved at a lower concentration (IC50=5.8±1.1nl/ml) in comparison with the effect on ACh and 5-HT contractions. This research shows that FAEO is a relaxant of rat ileum. As the inhibition of contractile over-activity of the ileum is the base of the treatment of some gastro-intestinal disorders such as diarrhoea, this study support the potential spasmolytic activity of this herbal remedy.

Keywords: *Ferula assa-foetida,* essential oil compsition, spasmolytic, ileum, KCl, ACh, 5-HT.

Introduction

Ferula assa-foetida L. (Apiaceae) is one of the most important of thirty species of Iranian *Ferula* growing wild or recently cultivated in several areas of the country (1,2). Asafetida or anghouzeh (in Persian) is an oleo-gum resin obtained by incising the living rhizome and roots of *F. assa-foetida* (2,3). Asafetida has been reported in Iranian folk medicine to be antispasmodic, anodyne, carminative, expectorant and stomachic and is a folk remedy for colic, and an essential oil and it seems that the essential oil has been partly associated with the pharmacological actions of the plant (2,3,5). The chemical composition of the essential oil of the oleo-gum resin of *F. assa-foetida* has also been studied and the major constituents were sulphur containing compounds with disulfides as major components and various monoterpenes (2, 3, 5 - 8).

To the best of our knowledge, there is no report on the anti-spasmodic activity of *F. assa-foetida* essential oil (FAEO) on ileum. Therefore, in this research we have studied the effect of FAEO on contraction of isolated ileum *in vitro* in order to look for its possible anti-spasmodic activity.

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Materials and Methods

Preparation of essential oil

Oleo-gum resin of *F. assa-foetida* was collected from plants cultivated in Iranian Research Institute of Forests and Rangelands in Isfahan, in August 1997. The essential oil was isolated by hydrodistillation of the hardened oleo-gum resin of the plant for 3h according to the method recommended in European Pharmacopoeia (9).

Analysis of essential oil

The oil was analysed by GC and GC/MS. GC analysis was carried out on a Perkin-Elmer 8500 gas chromatograph with a FID detector and a BP-1 capillary column (30m x 0.25mm; film thickness 0.25 μm). The operating conditions were as follows: carrier gas, helium with a flow rate of 2 ml/min; Column temperature, 60-275°C at 4°C/min; injector and detector temperature, 280°C; volume injected, 0.1μl of the oil; split ratio, 1:25.

GC/MS analysis was performed on a Hewlett Packard 6890 MS selective detector coupled with Hewlett Packard 6890 gas chromatograph equipped with a cross linked 5% PHME siloxane HP-5MS capillary column (30m x 0.25mm; film thickness 0.25 μm) and operating under the same conditions as described above. The MS operating parameters were as follows: ionization potential 70eV, ionization current 2A, ion source temperature 200°C and resolution 1000.

Identification of components in the oil was based on GC retention indices relative to *n*-alkanes and computer matching with the Wiley 275. L library as well as by comparison of the fragmentation patterns of mass spectra with those reported in the literature (10-12). The relative percentage of the oil constituents was calculated from the GC peak areas.

Drugs and Solutions

The following drugs were used in the experiments: acetylcholine chloride (ACh, Sigma), 5-hydroxytryptamine (5-HT, Sigma) and *Ferula assa-foetida* essential oil. 5-HT was made up as 10mM stock solution in distilled water. ACh was made up as 100mM stock solution in distilled water and acidified with a drop of acetic acid, dilution being made in distilled water. KCl was made up as 2M stock solution in distilled water. Essential oil was made up as 100μl/ml stock solution in 70% ethanol, further dilutions were made in distilled water. Tyrode's solution was composed of (mM): NaCl, 139.9; KCl, 2.68; $CaCl_2$, 1.8; $MgCl_2$, 1.05; $NaHCO_3$, 11.9; NaH_2PO_4, 0.42 and glucose 5.55 made up in distilled water and gassed with O_2. All chemicals, unless stated, were purchased from Merck.

Antispasmodic study

Male Wistar rats (200-250g) bred in Isfahan, were killed by a blow on the head followed by exsanguinations. A portion of ileum was removed and placed in oxygenated Tyrode's solution at room temperature. The tissue was then suspended in Tyrode's solution at 37°C and bubbled with oxygen. From a resting tension of 1g, isotonic contractions, elicited by KCl, 5-HT and ACh were recorded using Harvard isotonic transducer and displayed on a Harvard Universal Oscillograph pen recorder device. Drugs and essential oil were added directly to the organ bath in volumes usually not exceeding 5% of bath volume (20ml organ bath). A concentration-response curve was obtained by cumulative addition of the FAEO at 15min intervals after addition of 80mM KCl. The effect of ACh (320nM) and 5-HT (1.28μM) were studied using a single dose regimen with a contact time of 30s and time cycle of 3min. Each concentration of FAEO was at least 10min in contact with the tissue before its effect being evaluated. All experiments were conducted in parallel with time-matched controls using the tissue from same animal and adding equivalent volume of vehicle (ethanol).

Measurements and statistical analysis

Contractions were measured as maximum changes in tension from pre-drug baseline within the contact time or as area under the curve produced by tissue contraction at 5min intervals just before addition of the next concentration of FAEO (or vehicle) and expressed as percentage of control response for each tissue. Mean and standard error of mean (S.E.M.) values were calculated for each group of results and significance of differences between the means was calculated by two-tailed unpaired Student's t-test or by one way analysis of variance (ANOVA). Differences were considered statistically significant when $P<0.05$. Origin computer program was used for fitting non-linear curve and calculation of IC_{50} value (IC_{50}=drug concentration causing 50% of maximum response).

Results

Analysis of the essential oil

The oleo-gum resin of *F. assa-foetida* yielded 6.5% yellowish essential oil with an acrid odour. Thirty-three identified components in the essential oil, representing for 97.5% of the total detected oil components, are listed in Table 1 with their percentage composition and retention indices.

Antispasmodic activity

Rat ileum suspended in Tyrode's solution under 1g tension after 15min had a stable tension. KCl (80mM) produced a sustained tonic contraction. ACh (320nM) and 5-HT (1.28μM) induced a phasic contraction in the tissue, reaching to their maxima within 30s of contact. FAEO in a concentration-dependent manner (0.6-20nl/ml) inhibited the ileum contraction induced by 80mM KCl with an IC_{50} value of 5.8±1.1nl/ml (n=6, Figure 1). With 20nl/ml bath concentration FAEO abolished the response to KCl. These inhibitory effects of FAEO could be seen within few minutes contact with the tissue and were maintained as long as FAEO was present in the bath and persisted 20min after washing. Then, normal contractile response to KCl was restored. During the course of experiments there was small fluctuation in the tonic responses of the tissues treated with equivalent volume of the vehicle (ethanol) but these changes were not statistically significant (ANOVA). FAEO (10-80nl/ml) had a significant inhibitory effect on ACh response ($IC_{50=}$ 20.1±2.8nl/ml), abolishing the response at its maximum used concentration (Figure 1). FAEO (2.5-80nl/ml) also reduced the response to 5-HT with an IC_{50} value of 14.3±0.9nl/ml (Figure 1). Equivalent amount of the maximum volume of the vehicle (ethanol) used in this research caused no significant changes on ACh or 5-HT responses.

Discussion

The objective of this research was to study the effect of FAEO on ileum contraction to find pharmacological evidence for use of oleo-gum resin of *Ferula-assa-foetida* as spasmolytic plant materials. FAEO inhibited the ileum contractions due to KCl, ACh or 5-HT in a relatively low concentrations. The inhibitory effect was probably due to different components acting separately or together. About sixty percent of oil components of FAEO are disulfides and it seems that they have been partly associated with these pharmacological actions of the essential oil. From the identified components of FAEO, α-pinene (12.2%) and β-pinene are two components with known antispasmodic effect on ileum contraction induced by KCl and ACh (13). However, their inhibitory actions appear at relatively higher concentrations than those seen with FAEO. Therefore, other components are also involved in the observed inhibitory effects. β-caryophyllene is a component with known local anaesthetic activity at concentration ranging from 1 to 1000μg/ml (14). However, its contribution in the observed relaxant action of the essential oil is unlikely, because it only accounted for 0.1% of the essential oil constitutes. Furthermore, KCl, ACh and 5-HT in our experiment mainly cause contraction by direct action on smooth muscle cells rather than through neuronal stimulation. Nevertheless, further experiments are required to exclude any contribution of local anaesthetic activity of the essential oil components. FAEO has potent inhibitory effect on contraction of ileum induced by KCl, 5-HT and ACh. As it can be seen from Figure 1 at equal bath concentrations the inhibitory effect of FAEO on KCl response is far better than the effect on ACh and 5-HT. This differences may suggest that either different components acting by different mechanism of actions or FAEO has selective inhibitory effect on contraction induces by spasmogens with distinct mechanism of actions. The ileum contraction induced by KCl is a direct affect on ileum smooth muscle, as high concentration of KCl added extracellulary results in cell depolarisation and consequently activation of voltage-dependent calcium channels and Ca^{2+} influx into smooth muscle cell. Therefore, agents that inhibit contraction induced by KCl should somehow inhibit the entry of Ca^{2+} ions or otherwise inhibit the intercellular contraction mechanism. ACh, on the other hand, interact with muscarinic receptors on ileum smooth muscle cell membrane (15) and thereby increases the activity of membrane bound phospholipase-C enzymes and generation of inositol triphosphate (IP_3). IP_3 being released in the cytoplasm will interact with the receptors on intracellular Ca^{2+} store sites and causes the release of intracellular Ca^{2+} stores. Competitive antagonists of muscarinic receptors, like atropine and dicyclomine, antagonised the response to ACh by blocking the

muscarinic receptors and therefore, without altering the maximum response they shift ACh concentration-response curve to the right (16). Although, atropine can completely abolish the effects of ACh on the gastrointestinal tract, it inhibits only incompletely the effects of vagal impulses on motility of the gut. There are evidences, which suggest that 5-HT is partly responsible for the remaining response (17). FAEO relaxes the ileum contraction due to depolarisation (KCl) and activation of muscarinic or serotonergic receptors. The 5-HT contraction is mediated by release of ACh from the cholinergic neurone as well as activation of serotonergic receptors on the smooth muscles of ileum (18). Inhibitory effect of FAEO on KCl, ACh and 5-HT induced contractions, may indicate a general mechanism of action but more potent inhibitory effect of KCl induced contraction may indicate a blocking action on calcium channels. There is a suggestion that the inhibitory effect of *Teucrium polium* essential oil on smooth muscle is due to an effect on Ca^{2+} ion channels (19). In addition, by means of electrophysiological technique it has been shown that peppermint oil can block L-Type Ca^{2+} channels in the ileum (20). However, as the inhibitory effect of FAEO are due to a number of different components, further studies are needed to understand the mechanism of action of this plant material.

This study showed the relaxant effect of FAEO on the ileum contractions by three different spasmogens. Thus, the antispasmodic activity of FAEO in this *in vitro* model, supports a rational suggesting basis for folk and traditional use of the asafetida in gastrointestinal cramps, spasms and colic.

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Table 1: Composition of essential oil of oleo-gum resin of *Ferula assa-foetida*

Compounds	***Percentage***	Retention Indices
tricyclene	0.1	925
alpha-pinene	12.2	937
camphene	1.7	951
beta-pinene	3.4	979
myrcene	0.2	990
alpha-phellandrene	0.5	1005
delta-3-carene	0.2	1012
para-cymene	0.6	1026
limonene	0.1	1033
(Z)-beta-ocimene	2.3	1041
(E)-beta-ocimene	0.8	1051
dipropyl disulfide	0.1	1098
(Z)-1-propenyl sec-butyl disulfide	35.1	1142
(E)-1-propenyl sec-butyl disulfide	22.1	1149
methyl propyl disulfide	0.5	1168
dimethyl tetrasulfide	0.4	1224
vanillin	0.1	1397
2-propenyl propyl trisulfide	0.7	1413
beta-caryophyllene	0.1	1425
dipropyl trisulfide	0.2	1429
(Z)-1-propenyl propyl trisulfide	2.8	1435
(E)-1-propenyl trisulfide	1.6	1441
aromadendrene	0.4	1449
dodecanol	0.6	1476
methyl pentyl tetrasulfide	7.1	1508
guaiol	1.0	1599
2-tridecanone	1.8	1692
guaiol acetate	0.1	1727
dipropyl tetrasulfide	0.1	1762
octadecane	0.1	1797
nonadecane	0.2	1900
(Z)-1-propenyl propyl tetrasulfide	0.2	2115
(E)-1-propenyl propyl tetrasulfide	0.1	2121

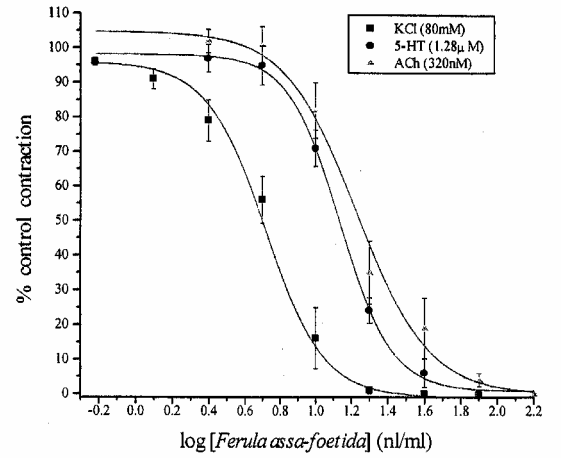

Figure 1. Effect of *Ferula assa-foetida* essential oil on tension development to KCl, acetylcholine (ACh) and 5-hydroxytryptamine (5-HT) in isolated ileum of rats. Sigmoidal curve fitted through the points in presence of drugs using 2 fold increments in concentration. Ordinate scale: drug effect expressed as % inhibition of ileum contraction. Abscissa scale: $\log_{10}$ concentration of the essential oil. The points are mean and the vertical bars show the S.E.M. (n=6).

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