

ANTISPASMODIC EFFECT OF AERIAL PART OF *TEUCRIUM POLIUM L.* ESSENTIAL OIL ON RAT ISOLATED ILEUM *IN VITRO*

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ABSTRACT

Teucrium polium L. (*Lamiaceae*) is used in the traditional medicine of Iran for the treatment of disorders of the gastrointestinal tract. The essential oil from the aerial parts of *T. polium* was assessed for antispasmodic activity and compared with the effect of atropine and dicyclomine. Acetylcholine and KC1 were used for induction of contraction on rat isolated ileum. *Teucrium polium* essential oil (TPEO) at concentrations of 8.6 to 34.4 µg/mL attenuated the maximum inducible response to acetylcholine concentration-response curve. It also inhibited the response to 80 mM KC1 in a concentration dependent manner ($pD_2 = 1.2 \pm 0.13$). Dicyclomine (3.46 & 34.6 ng/mL) also reduced the response to acetylcholine on rat isolated ileum and inhibited KC1-induced contractions while atropine only inhibited the response to acetylcholine. This study shows that TPEO is a relaxant of rat isolated ileum and may have some clinical benefits for gastrointestinal disorders such as colic.

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INTRODUCTION

Teucrium polium L. (*Lamiaceae*) is a herb widely growing in Iran.¹ Aerial parts of this plant have been used in Iranian traditional medicine for various indications in internal use such as the treatment of indigestion, flatulence, pain and fever.^{2,3} Several constituents of *T. polium* have been identified, mainly flavonoids,^{4,5} iridoids⁴ and sesquiterpenoids.⁶ The composition of the essential oil has also been described.⁷ Although *T. polium* has been known in Iran for many centuries, few publications regarding the pharmacology, anti-inflammatory activity,⁸ antipyretic and antibacterial actions,⁹ hypoglycemic effects¹⁰ and calcium antagonistic effects¹¹ are available. The goal of this investigation was to determine whether the essential oil of *T. polium* possesses antispasmodic activity.

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MATERIAL AND METHODS

Plant material

Aerial parts of *T. polium* were collected in June 1997, on hills near Kashan (Iran) and identified by Dr. M.R. Rahiminejad (Department of Biology, Isfahan University, Isfahan, Iran). A voucher specimen of this plant material was deposited in the herbarium of the Faculty of Pharmacy & Pharmaceutical Sciences, Isfahan, Iran.

Essential oil isolation

The samples were dried in the shade at ambient temperature (25-30°C) and ground to powder. The weighed powder (20 g) was submitted to hydrodistillation for 3 hours, according to the standard procedure reported in the European Pharmacopoeia.¹² The oil content was 1.2% (v/w) on a dry weight basis.

Experimental procedure

Non-pregnant female Wistar rats (200-250 g) bred in Isfahan, were killed by a blow on the head followed by ex-

sanguination. A portion of ileum was removed and placed in oxygenated Tyrode's solution (see solutions) at room temperature. The connective tissue was carefully trimmed from the tissue and then suspended in Tyrode's solution at 37°C and bubbled with oxygen. From a resting tension of 1g, isotonic contractions, elicited by KCl and acetylcholine were recorded using a Harvard isotonic transducer and displayed on a Harvard Universal Oscillograph pen recorder device. Drugs were added directly to the organ bath in volumes usually not exceeding 5% of bath volume (20 mL organ bath). The effect of acetylcholine was studied using a single dose regimen with a contact time of 30 s and time cycle of 4 min. Four consecutive full concentration-effect curves were obtained in absence, vehicle treated and then in the presence of two concentrations of testing drugs (essential oil). A concentration-response curve was also obtained by cumulative addition of drugs at 10 min intervals after addition of 80 mM KCl. When appropriate, experiments were conducted in parallel with time-matched controls adding equivalent volumes of vehicle in lieu of drug. Each drug concentration was at least 10 min in contact with the tissue before their effects were evaluated.

Drugs and solutions

Tyrode's solution, composed of (mM): NaCl, 139.9; KCl, 2.68; CaCl₂, 1.8; MgCl₂, 1.05; NaHCO₃, 11.9; NaH₂PO₄, 0.42 and glucose 5.55 was made up in double distilled water and bubbled with CO₂ until the pH was adjusted to 7.4; thereafter the pH remained constant. KCl was made up as a 2 M solution in double distilled water.

The following drugs were used for the experiments: acetylcholine chloride; atropine sulphate; dicyclomine hydrochloride; TPEO. Acetylcholine was made up as 100 mM stock solution in double distilled water and acidified with acetic acid. Dicyclomine and atropine were made up as 100 mM stock solution in double distilled water, and further dilution was made in Tyrode's solution. TPEO was made up in 1% tween 20 in distilled water, dilution being made in distilled water or Tyrode's solution as appropriate. All chemicals were purchased from Merck. Drugs were from Sigma and TPEO was prepared as above.

Measurements and statistical analysis

Contractions were measured as maximum changes in tension from pre-drug baseline within the contact time or as the area under the curve produced by tissue contraction at 5 min intervals just before addition of the next concentration of the test drug and expressed as percentage of control or maximum induced 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 paired 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 pD_2 value.

RESULTS

Rat ileum suspended in Tyrode's solution shows irregular spontaneous contractile activity which attenuates by changing of bath fluid. KCl (80 mM) produced a sustained tonic contraction which was maintained during the course of experiments. Acetylcholine at nanomolar concentration caused a concentration-dependent contraction of tissue, reaching its maximum within 30 s of contact. TPEO in a concentration-dependent manner inhibited the ileum contraction induced by 80 mM KCl with a pD_2 value of $1.2 \pm 0.13 \mu\text{g/mL}$ ($n = 6$, Fig. 1,2). With 70 $\mu\text{g/mL}$ bath concentration TPEO completely abolished the response to KCl. These inhibitory effects of TPEO could be seen within 10 min of contact with the tissue and were maintained as long as it was present in the bath and persisted 30 to 60 min after

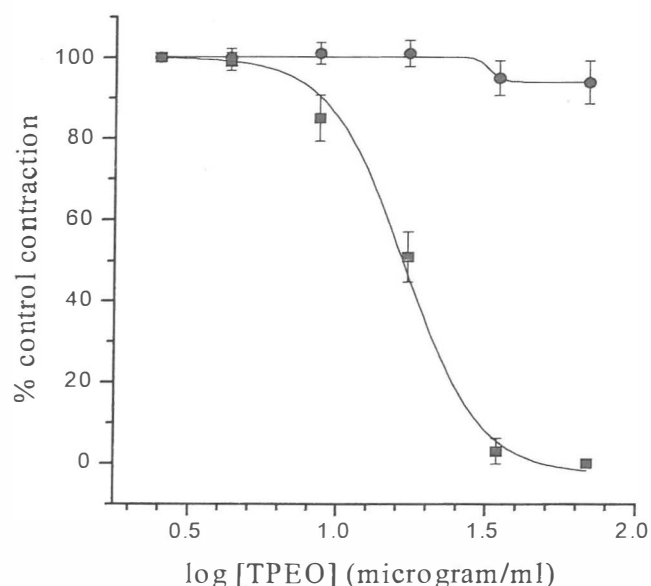


Fig. 1. Effect of TPEO on tension development to 80 mM KCl in isolated rat ileum. Sigmoidal curve fitted through the points in the presence of TPEO using 2 fold increments in concentration (square; $n = 6$). The line through the circles shows the response of parallel time-matched controls treated with vehicle in equivalent volume ($n = 6$). Ordinate scale: response expressed as % of the control response to KCl (80 mM) prior to addition of TPEO. Abscissa scale: \log_{10} concentration of TPEO. The points are mean and the vertical bars show the S.E.M.

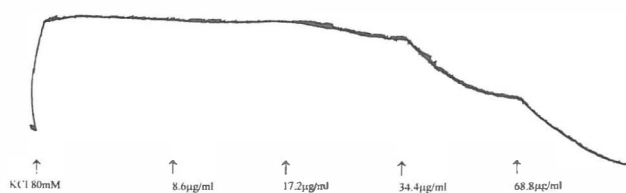


Fig. 2. Inhibitory effect of cumulative addition of TPEO on tonic contraction of rat ileum induced by KCl.

washing. Then the normal contractile response to KCl was restored. As shown in Fig. 3, TPEO (8.6-34.4 $\mu\text{g}/\text{mL}$) had a significant inhibitory effect on the ACh concentration response curve, reducing the maximum induced contraction. With 34.4 $\mu\text{g}/\text{mL}$ TPEO concentration almost a complete inhibition of ileum contraction was achieved. Atropine and dicyclomine also antagonised the response to acetylcholine without altering the maximum response. In the presence of atropine 0.695 ng/mL (1 nM) and 6.95 ng/mL (10 nM) there was 4 and 16 fold rightward shifts in the concentration-response curve of acetylcholine, respectively (see Fig. 4). A similar shift in the concentration response curve was also seen with 3.46 ng/mL (10 nM) and 34.6 ng/mL (100 nM) dicyclomine bath concentration.

The muscarinic cholinergic antagonist 'dicyclomine' at 34.6 ng/mL to 1.04 $\mu\text{g}/\text{mL}$ bath concentrations also significantly reduced the response to KCl in such a way that the response to 80 mM KCl was attenuated from 101 ± 1.7 to 24 ± 10.5 at 216 ng/mL (625 nM) bath concentration, while atropine up to 434 ng/mL (625 nM) had no significant effect on any of the responses produced by 80 mM KCl. There was no significant change in any of the tissues treated with the vehicle.

DISCUSSION

The objective of this work was to study the action of TPEO on contractile activity induced by two different

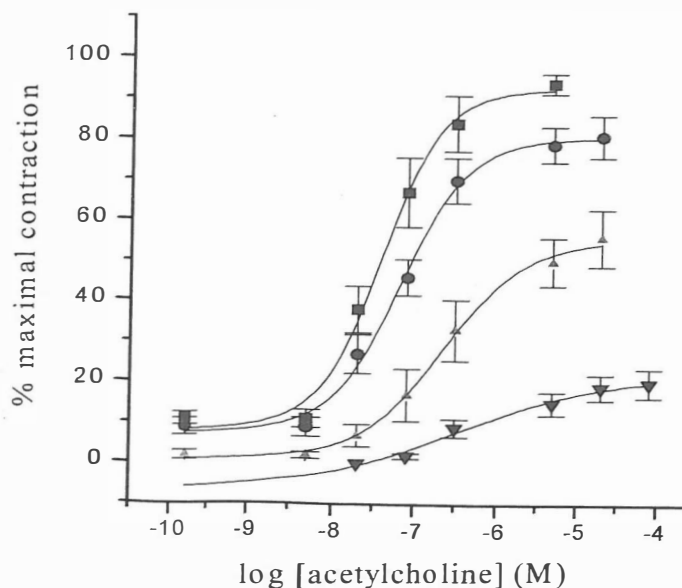


Fig. 3. Effect of TPEO on tension development to acetylcholine in isolated ileum of rats. Sigmoidal curve fitted through the points in the absence (square) and presence of TPEO 8.6 (circle), 17.2 (triangle up) and 34.4 $\mu\text{g}/\text{mL}$ (triangle down). Ordinate scale: response expressed as % of the maximum response to acetylcholine in each tissue. Abscissa scale: \log_{10} concentration of acetylcholine. The points are mean and the vertical bars show the S.E.M. ($n=6$).

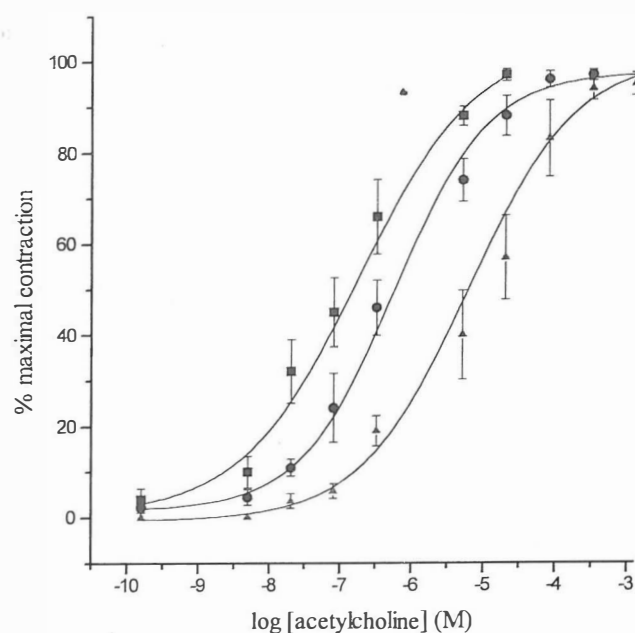


Fig. 4. Effect of atropine on tension development to acetylcholine in isolated ileum of rats. Sigmoidal curve fitted through the points in the absence (square) and presence of atropine 1 nM (circle) and 10 nM (triangle). Ordinate scale: response expressed as % of the maximum response to acetylcholine in each tissue. Abscissa scale: \log_{10} concentration of acetylcholine. The points are mean and the vertical bars show the S.E.M. ($n=6$).

spasmogens in rat ileum in comparison with two other agents to seek for scientific evidence for beneficial use of this drug in gastrointestinal disorders. Current therapy for some of these disturbances is directed towards inhibition of smooth muscle contractions. Antagonists of muscarinic receptors are used in the control of such conditions. However, they have undesired adverse effects.¹³ Although dicyclomine has a weaker antimuscarinic action, it is used more than atropine for gastrointestinal spasm, perhaps because it also has a direct muscle relaxant effect as our experiment shows that it inhibits the response to KCl. Nevertheless, the direct inhibitory effect of dicyclomine is only seen at concentrations higher than those needed for antagonism of muscarinic receptors.

TPEO (8.6-34.4 $\mu\text{g}/\text{mL}$) is a relaxant of rat isolated ileum *in vitro* and its relaxant effect is qualitatively similar to that of dicyclomine (34.6 ng/mL). The inhibitory effect of TPEO on acetylcholine concentration-response is like non-competitive antagonism attenuating the maximum response. Furthermore, TPEO relaxes the ileum contraction due to depolarisation (KCl) and activation of muscarinic receptors which mediate the response to acetylcholine.¹⁴

Salhab and co-workers have reported the antispasmodic effect of *T. polium* on rabbit jejunum¹⁵ and our results are consistent with them. There are also reports about the antispasmodic activity of other plants of the *Lamiaceae* family

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such as peppermint, rosemary, balm and sage oil.^{16,17} TPEO contains several components⁷ and some of them such as guaiol¹⁵ and carvacrol¹⁸ may contribute in spasmolytic activity. The inhibitory effect of *T. polium* qualitatively and quantitatively is very similar to the inhibitory effect of *Satureja hortensis* L essential oil.¹⁹ *Thymus vulgaris* essential oil also has antispasmodic effect on rat ileum and similar to *T. polium* inhibits the response to 80 mM KCl in a concentration-dependent manner,²⁰ although, at a different concentration. Further studies using the individual active constituents of essential oil are needed to be done to see which component(s) is (are) responsible for the inhibitory effect of *T. polium* essential oil.

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