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Anti-Spasmodic and Anti-Nociceptive Effects of *Teucrium polium* Aqueous Extract

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ABSTRACT

Background: *Teucrium polium* has been known as an important traditional medicinal plant and is used for different therapeutic purposes such as gastrointestinal disorders. Therefore, the anti-spasmodic and anti-nociceptive activities of aqueous extract of *Teucrium polium* w as examined. **Methods:** Anti-spasmodic effect of different concentrations (47-470 mg/l) of *Teucrium polium* extract was assessed on acetylcholine (220 nM) precontracted guinea pig isolated ileum. The anti-cholinergic effect of the plant was also examined by obtaining concentration-response curves in the absence and presence of *Teucrium polium* extract (470 mg/l) and atropine (10 nM). Anti-nociceptive effect of different doses (30-240 mg/kg) of *Teucrium polium* aqueous extract was determined by hot-plate test on mice and compared with the effect of morphine (10 mg/kg) as positive control. **Results:** Maximum inhibition response induced by *Teucrium polium* extract on contraction induced by acetylcholine (220 nM) was 93.5%. In the absence and presence of *Teucrium polium* extract (470 mg/l) and atropine (10 nM) the EC50 (the effective concentration causing 50% of maximum response) of Ach were 28.3 ± 2.1, 55.4 ± 3.7 and 208.1 ± 9.2 nM respectively. There was also a parallel right-ward shift in the log concentration-response curve of acetylcholine in the presence of atropine, but a non-parallel shift in the presence of *Teucrium polium* extract. The *Teucrium polium* extract increased reaction time dose-dependently (P<0.01 for all doses). However the anti-nociceptive effect of extract was significantly less than that of morphine (P<0.001). **Conclusion:** These results show that *Teucrium polium* aqueous extract have anti-nociceptive and anti-spasmodic effects and may have some clinical benefits for gastrointestinal disorders. *Iran. Biomed. J. 10 (3): 145-149, 2006*

Keywords: Anti-spasmodic, Anti-nociceptive, Aqueous extract, Lamiaceae, *Teucrium polium*

INTRODUCTION

Teucrium polium (TP) is a member of the Lamiaceae family which is widely grown in Iran (named in persian "*Kalpooreh*"). Aerial parts of TP have been used in traditional medicine for various purposes such as anti-hypertensive, anti-bacterial, carminative, anti-nociceptive, anti-inflammatory, anti-diarrhea, anti-diabetes and anti-convulsant (Persian books**). Phytochemical analyses of TP have identified several constituents including mainly flavonoids, sesquiterpenoids, neo-clerodane diterpe-noids [1] tannin and alkaloids [2-4].

Previous studies have demonstrated some of the pharmacological effects of TP such as anti-bacterial [5], anti-inflammatory [6], anti-ulcero-genic [7], anti-nociceptive [8, 9], anti-diabetes [10], anti-hypertensive [11], hypo-lipidemic [12] and calcium antagonist [13]. A related species, *Teucrium chamaedrys*, has been used in France for the treatment of obesity [14]. The goal of this study was to investigate the anti-spasmodic and anti-nociceptive effects of aqueous extract of TP.

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MATERIALS AND METHODS

Preparation of plant extract. Aerial parts of TP were collected from hills around Ferdous (south of Khorasan province, Iran) in June and identified by botanists in Herbarium of Pharmacy School, Mashhad University of Medical Sciences (voucher No. 152-2016-4). The samples were dried under shade and ground to powder. Powder (100 g) was extracted with 500 ml distilled water by maceration at 32°C for 72 h and shaked intermitantly and then the solution was filtered twice and evaporated under reduced pressure at 35°C. The concentrated plant extract (a yield of 23%) was maintained at -20°C until being used and freshly dissolved in distilled water just before experiments.

Evaluation of anti-spasmodic effect:

Tissue preparation. Guinea pigs, weighing 250-400 g, were killed by a blow to the base of the skull and a piece (2 cm) of ileum was dissected and placed in gass-aeriated (5% CO_2 and 95% O_2) Tyrode's solution (composition in mM: NaCl 136, KCl 5, $CaCl_2$ 2, $MgCl_2$ 0.98, NaH_2PO_4 0.36, $NaHCO_3$ 11.9 and Glucose 5.5) at room temperature. All chemicals were purchased from Merck (Germany). The connective tissue was carefully removed and the tissue mounted in 50-ml chamber of organ bath containing Tyrode's solution (pH 7.4) bubbled with a mixture of 5% CO_2 and 95% O_2 at 37°C. Tissues were washed with Tyrode's solution every 10 min.

Measurement of contractile activity. The tissues were allowed to equilibrate for 1 h with a resting tension of 1 g. The ileum contractions elicited by acetylcholine were recorded using kymograph. Drugs were added directly to the organ bath and responses expressed as percentage of the initial contractions. The tissues were first exposed to each concentration of TP extract or atropine and after 4 min, acetylcholine was added to the organ bath (n = 6-8). The percent inhibition of acetylcholine-induced contractions was regarded as inhibitory effect of TP extract or atropine.

Examination of anti-cholinergic effects. The anti-cholinergic effect of the TP extract (470 mg/l) and atropine (10 nM) was examined by recording cumulative log concentration-response curve of acetylcholine (5-5000 nM) induced contraction of the ileum 4 min after exposing tissue to each solution. The maximum response to acetylcholine and the EC50 of acetylcholine in the absence or presence of TP extract and atropine was then compared (n = 8). Acetylcholine and atropine were purchased from Sigma (UK).

Evaluation of anti-nociceptive effect. Swiss Albino mice, weighing 30 ± 3 g of either sex, were used. They were kept in an animal house provided with a 12-h light/dark cycle at ambient temperature of 22°C and free access to water and food. The pain sensitivity of mice was tested with a hot-plate adjusted at 52 ± 0.2°C. The latency to a discomfort reaction (licking paws or jumping) was determined before and after drug administration. The cut-off time was 30 s. The animals were randomly divided into 6 groups (n = 12 for each group). The different doses of aqueous extract (30, 60, 120 and 240 mg/kg), morphine (10 mg/kg, as a positive control) and solvent (as a negative control) were injected intraperitoneally and the mice were tested at different times (0, 10, 25, 40 and 60 min) after injection.

Statistical analysis. The results were expressed as the mean ± SEM for each group of experiments. The results of different concentrations of extract were compared using ANOVA test. The results of plant extract was compared with those of control using paired student's *t*-test for relaxant effect and unpaired student's *t*-test for anti-nociceptive effect. The correlation between concentration and inhibitory effect of the extract was calculated using least squares regression. Statistical significance was considered at $P<0.05$.

RESULTS

Anti-spasmodic effect. All concentrations of the TP aqueous extract (47, 94, 188, 376 and 470 mg/l) significantly inhibited the contraction induced by 220 nM acetylcholine ($P<0.01$ to $P<0.001$ for all cases, Fig. 1). The final concentration of the extract (470 mg/l) almost completely inhibited the ileum contraction (93.5%). There was a significant correlation between the different concentrations of extract and their inhibitory effect (r = 0.991, Fig. 1). The final concentration of the extract (470 mg/l) had a significant inhibitory effect on the acetylcholine concentration-response curve (Fig. 2). The EC50 of acetylcholine in the presence of TP extract (55.4 ± 3.7 nM) was significantly greater than the EC50 in the absence of TP extract (28.3 ± 2.1 nM, $P<0.01$).

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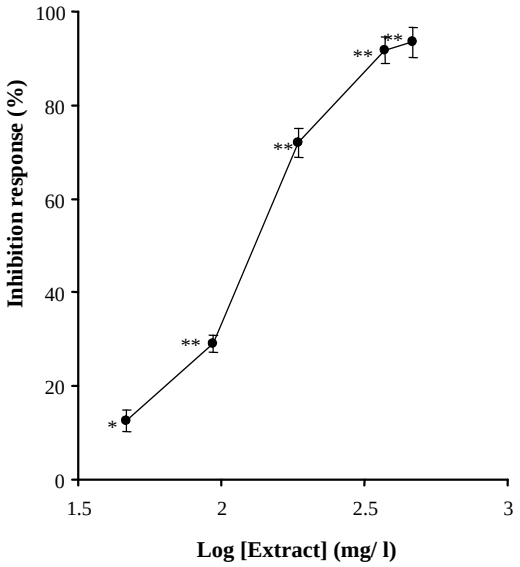

Fig. 1. Concentration-dependent inhibitory effect of *Teucrium polium* extract on acetylcholin-induced contraction! Different concentrations (47-470 mg/l) of *Teucrium polium* aqueous extract significantly inhibited contraction induced by acetylcholine (220 nM) on isolated guinia pig ilium (**P*<0.01, ***P*<0.001). Each point represents the mean ± SEM of the inhibitory effect of the extract (n = 6).

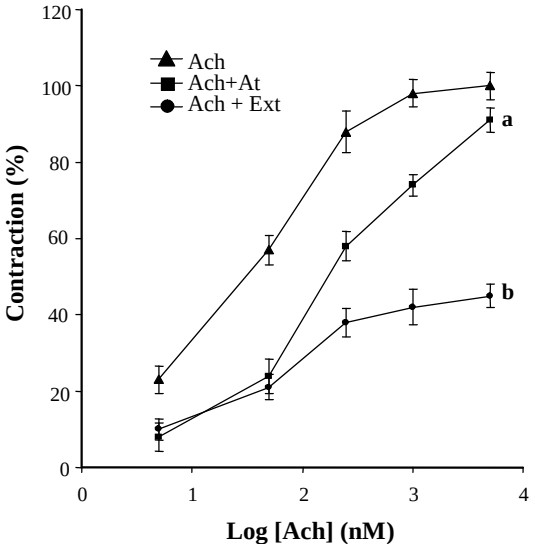

Fig. 2. Cumulative log concentration-response curves of acetylcholine (Ach)-induced contraction on isolated guinea pig ileum in the absence and presence of 10 nM atropine (At) and 470 mg/l *Teucrium polium* aqueous extract (Ext). Each point represents the mean ± SEM (n = 8). The concentration-response curve in the presence of atropine showed a parallel rightward shift and in the presence of extract, a non-parallel rightward shift compared to acetylcholine alone. The EC50 of acetylcholine in the presence of atropine and *Teucrium polium* extract was significantly greater compared to EC50 of acetylcholine alone (a *P*<0.001, b *P*<0.01).

However, log concentration-response curve of acetylcholine in the presence of TP extract showed non-parallel rightward shift. Atropine as a positive control increased the EC50 of acetylcholine from 28.3 ± 2.1 nM to 208.1 ± 9.2 nM (*P*<0.001). Log concentration-response curve of acetylcholine in the presence of atropine showed parallel rightward shift compared to the curve obtained in the absence of atropine.

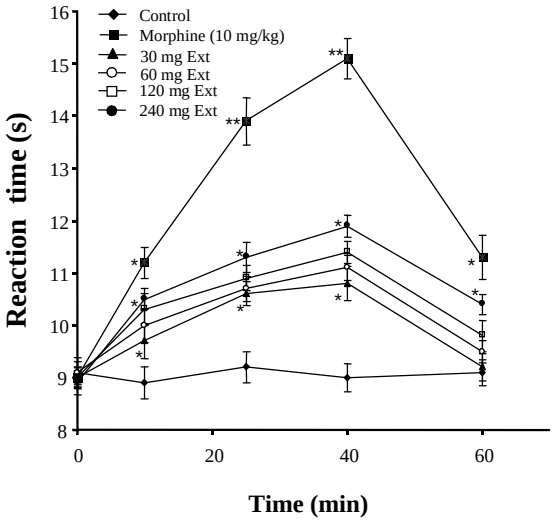

Fig. 3. Anti-nociceptive effect of different doses of *Teucorium polium* aqueous extract (30, 60, 120 and 240 mg/kg) on mice by Hot-plate test at different times (0,10, 25, 40 and 60 min). Each point represents the mean ± SEM of the reaction time (n = 12). Ten min after administration, the effect of all doses of the extract and morphine were statisticaly significant compared to that of control (* *P*<0.01, ** *P*<0.001).

Anti-nociceptive effect. The anti-nociceptive effect of different doses of aqueous extract (30, 60, 120 and 240 mg/kg) was significantly greater compared to that of negative control (*P*<0.01 for all doses). The maximum responses were reached after 40 min. The time latency of different doses of the extract was about 10 min. The anti-nociceptive effect of extract was dose-dependent with about 60 min duration of action (Fig. 3). Aqueous extract at dose of 240 mg/kg increased reaction time from 9 ± 0.08 s to 11.9 ± 0.47 s at 40 min (an increase of 32%, *P*<0.001). However, the anti-nociceptive effect of the extract was significantly less than that of morphine (*P*<0.001).

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DISCUSSION

The results of this study indicate that the aqueous extract of TP has anti-spasmodic and anti-nociceptive activities. TP extract (47-470 mg/l) induced significant relaxation in isolated guinea pig ileum precontracted with acetylcholine. The relaxation induced by TP extract was concentration-dependent (Fig. 1). The final concentration of the extract (470 mg/l) produced 93.5% inhibition of acetylcholine-induced contraction. However, as shown in Figure 2, the extract caused a non-parallel rightward shift in acetylcholine concentration-response curve and the maximum response to acetylcholine was not achived in the presence of extract. Therefore, the mode of inhibitory effect of TP aqueous extract on acetylcholine concentration-response seems to be non-competetive antagonism at muscarinic receptor which attenuates the maximum response, to acetylcholine.

The results of the present study were supported by the study of Sadraei *et al.* [15] which have reported the anti-spasmodic effect of *Teucrium polium* essential oil on rat isolated ileum. Although the potency of *Teucrium polium* essential oil seems to be higher than that of TP aqueous extract but this difference may be due to several factors including different type of components of TP (essential oil and aqueous extract) and different animal tissues (rat isolated ileum and guinea pig isolated ileum).

As shown in Figure 3, the anti-nociceptive activity of TP extract was significant and dose-dependent and the hot-plate test is a specific central anti-nociceptive test [16, 17]. Therefore, these results suggest that the extract may exert it's effect through central opioid receptors or promoted release of endogenous opiopeptids. However, the aqueous extract is more soluble in water and low penetration to the central nervous system is predictable, but similar to some water soluble drugs (like morphine sulfate), and some other published studies on plant aqueous extract [18, 19], practically this extract can induce central effects. Baluchnejadmojarad *et al.* [9] have reported that anti-nociceptive effect of *Teucrium polium* extract in the diabetic rat using formalin test and suggested central and peripheral mechanisms. In another study, the anti-nociceptive effect of *Teucrium polium* was evaluated using Writhing test, a visceral pain model [8]. Therefore, central and also peripheral mechanisms may involve in its anti-nociceptive effect. The maximum responses to different doses of TP extract and morphine were similarly reached after 40 min, but the potency of the extract was less than morphine (Fig. 3). Identification and isolation of active constituents of the extract may incraese the potency, which require further studies.

In conclusion, the aqueous extract of the aerial parts of TP showed anti-spasmodic and anti-nociceptive effects. Therefore, this plant may have some clinical benefits for gastrointestinal disorders such as colic. The results of this study also support it's use in folk medicine for gastrointestinal disorders. However, further studies are needed to determine possible mechanisms of action, establish safety profiles of the extract, and evaluate the potential value of TP for the management of gastrointestinal disorders.

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