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Teucrium polium Inhibits Nerve Conduction and Carrageenan Induced Inflammation in the Rat Skin

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Abstract: The effects of acute and chronic perineural treatment of the rat saphenous nerve with *Teucrium polium* (L.) extract on the conduction property of primary afferent nerve fibers and carrageenan-induced skin inflammation were studied. Direct application of 2.0% *T. polium* extract to the nerve trunk caused immediate complete inhibition of compound action potentials (CAPs) of all types of primary afferent nerve fibers. Partial recovery of CAPs was seen in up to 37.5% of Aαβ-fibers, 30.4% of Aδ-fibers and 32.4% for C-fibers over one hour after removal of the extract. Fifteen to twenty days after a single perineural application of 2% *T. polium* extract, the CAPs of primary afferent nerve fibers were reduced to 60.78% ± 7.54 (n = 14) for Aαβ-fibers, 67.39% ± 9.34 (n = 14) for Aδ-fibers and 62.2% ± 8.25 (n = 14) for C-fibers. Carrageenan-induced acute skin inflammation was reduced to 56.86% ± 12.81 (n = 9) after local subcutaneous injection of the herb extract. These results indicate that *T. polium* contains one or more potent non-selective neurotoxic agents with anti-inflammatory activity

Key Words: *Teucrium polium*, Sensory nerve, Neurogenic inflammation

Introduction

Teucrium polium L. (Labiatae) has long been recognized in folk medicine in the treatment of many pathophysiological implications, such as gastrointestinal disorders, inflammations, diabetes and rheumatism. Its extract has been shown to induce hypotensive [1], anti-inflammatory [2-3], hypoglycemic [2,4-5], antispasmodic [6-7], antibacterial and antipyretic [8] activities. Chemical analyses of *T. polium* have shown it to contain various compounds such as iridoids, flavonoids and cirsiliol [3,9]. The present study was carried out to examine the immediate and long-term effects of *T. polium* on the afferent conduction property of sensory nerve fibers and their peripheral effector response of skin inflammation.

Material and Methods

Preparation of *T. polium* extract

The flowering tops and leaves of *T. polium* L. were collected locally during May 1999, dried at room temperature and ground to a fine powder. Extraction was done with distilled water under reflex for 4 hours, followed by filtration. The dry weight was determined in an amount of 50 ml dried at room temperature using a shallow container.

Experimental preparation

Experiments were carried out on male albino rats (*Rattus norvigicus*) weighing 200-430g deeply anesthetized by urethane (1.8 g/kg, i.p.). Catheters were inserted into the trachea to allow artificial ventilation as needed and into the left common carotid artery for monitoring systemic arterial blood pressure, which was around 105/80 mm Hg. Body temperature was monitored with a rectal thermistor probe and maintained close to 37°C by a feedback-controlled heating blanket system (Harvard Apparatus, USA). The saphenous nerve in the medial aspect of the right hindlimb was exposed, cut in the upper thigh, and covered with a pool of liquid paraffin made from skin flaps sutured to a brass ring. For electrical stimulation, a small segment (about 5 mm) of the nerve above the knee was freed and placed on a pair of platinum electrodes. Electrical stimulation of Aαβ- and Aδ-fibers was done at 0.20 to 0.45 mA, 0.05 msec pulse duration and 10 Hz frequency, and that of C-fibers at 1.5 to 3.5 mA, 0.5 msec pulse duration and 1 Hz frequency.

For electrical recording, the proximal cut end of the nerve in the upper thigh was placed on a pair of similar platinum electrodes. The conduction distance between the stimulating electrodes and recording electrodes ranged from 22 to 26 mm. Signals were amplified and filtered using conventional means, monitored by a loudspeaker and displayed simultaneously on a digital storage oscilloscope (Tektronix 2232) and digitized on a personal computer using Labview software (National Instruments, USA).

Acute perineural application of *T. polium*

The acute effect of *T. polium* extract was examined on a group of rats (n = 5). For perineural application of the herb extract, a small segment (about 5 mm) of the nerve was freed between the stimulating electrodes and recording electrodes and placed on a small sheet of parafilm. A small patch of cotton thread soaked with 0.1 ml of freshly prepared 2.0% *T. polium* extract in sterile olive oil was placed around the exposed segment of the nerve for 5 min. Recording of the CAPs of primary afferent nerve fibers was done before application of the extract, during the 5 minutes of application, and 10 min, 30 min and 60 min after removal of the extract and washing with normal saline. For comparison with the vehicle, a similar recording procedure was carried out using only sterile olive oil.

Chronic perineural treatment with *T. polium*

The chronic effect of *T. polium* extract was tested on a group of rats (n = 14) anesthetized by injection (i.p.) of chloral hydrate (160 mg/kg) and pentobarbitone sodium (35 mg/kg). The level of anesthesia was adjusted such that areflexia was monitored throughout the operations. The hair was clipped from the innervation zone of the saphenous nerve in the medial surface of the right thigh. Under sterile conditions, a small incision was made along the mid thigh and a small segment (about 5 mm) of the saphenous nerve was freed and placed on a small patch of parafilm. Perineural treatment was done by placing a small piece of sterile cotton thread soaked with 0.1 ml of 2.0% *T. polium* extract around the exposed segment of the nerve for 30 minutes. The extract was then removed, the nerve washed with sterile normal saline and the wound closed in layers. In another control group of rats (n = 3), the saphenous nerve was exposed in the same manner, but treated with sterile olive oil. The animals from both groups were left for 15-20 days until the final acute experiment. Recordings of CAPs of primary afferent nerve fibers were made from the ipsilateral nerve treated with *T. polium* extract or olive oil in the right operated limb and from contralateral nerve in the untreated left limb in the same animal.

Carrageenan-induced inflammation

The anti-inflammatory activity of *T. polium* was tested on a group of rats (n = 9). Under brief ether anesthesia, a small area of skin (about 10 mm diameter) in both the ipsilateral right hindlimb and the equivalent site in the contralateral left hindlimb were injected (s.c.) with 0.15 ml of 4% of carrageenan in saline [10-11]. After 30 minutes, the above inflamed skin area in the ipsilateral site was injected with 0.1 ml of 2.0% *T. polium* extract, while that in the contralateral site was injected with the same volume of normal saline. Three hours later, the animals were deeply anesthetized by urethane (1.8 g/kg, i.p.), and the trachea and external left jugular vein were cannulated. Evans blue (50 mg/kg) in normal saline was infused (i.v.) followed by the placing of both hindlimbs in a water bath at 50°C for 10 min. Small pieces of inflamed skin (180 to 350 mg) were dissected from the sites of injection with *T. polium* extract or normal saline. Background determination of dye content was done by dissecting similar pieces of skin from areas outside the inflamed zones. The tissues were "blotted" dry, weighed and placed in 4 ml dimethylformamide at 37°C for 24 hours. Dye content in the skin (μg/g) was determined spectrophotometrically at the absorbance maximum of 620 nm calculated from a standard dilution curve of Evans blue.

Carrageenan-induced paw edema

Carrageenan-induced acute paw edema was examined in a group of rats (n = 18). Under brief ether anesthesia, the plantar aspects of both limbs were injected (i.pl.) with 0.15 ml of 4% carrageenan. Thirty minutes later, the inflamed right plantar surface received 0.1 ml of 2.0% *T. podium* extract, while the inflamed left plantar surface was injected with the same volume of normal saline. After 3 hours, paw edema was determined by measuring the dorso-plantar paw width with a Vernier caliper.

Statistical analysis

Results are expressed as means ± S.E.M. Statistical significance of the differences between control and test groups was determined using unpaired Student's *t-test.* P< 0.05 was taken as the significance level.

Results

Effect of acute perineural application of *T. polium* on CAPs

Direct perineural application of 2.0% *T. polium* extract solution in sterile olive oil to the rat saphenous nerve induced immediate (within less than 5 minutes) complete disappearance of compound action potentials (CAPs) of Aαβ-, Aδ- and C-fibers (Figs. 1, 2 and 3 respectively). One hour after removal of the extract, the amplitudes of CAPs were still significantly below pre-treatment levels, being 37.5% ± 8.8 (n = 5) for Aαβ-fibers (P < 0.01), 30.4% ± 6.2 (n = 5) for Aδ- fibers (P<0.005) and 32.4% ± 6.9 (n = 5) for C-fibers (P<0.005). The amplitudes of CAPs of all the above afferent nerve fiber types were unaffected by application of the vehicle (olive oil) alone for more than 3 hours of recording.

Effect of chronic perineural treatment of *T. polium* on CAPs

Fifteen to twenty days after a single chronic perineural treatment of the saphenous nerve with 2.0% *T. polium* extract solution in sterile olive oil, the amplitudes of CAPs of Aαβ-, Aδ- and C-fibers in ipsilateral treated nerves were lower than those in contralateral unoperated nerves (Fig. 4). The mean amplitude of CAPs of Aαβ-, Aδ- and C-fibers in ipsilateral chronic treated nerves were significantly lower than those in contralateral untreated nerves (Table 1) (P < 0.025). However, the thresholds, maximum strength of excitation and conduction velocities of these nerve fibers were significantly unaffected by *T. polium* extract treatment. The amplitudes, conduction velocities, thresholds and maximum strength of excitation of CAPs of the nerve fibers in ipsilateral nerves which had been chronic treated with olive oil were similar to those in contralateral untreated nerves.

The anti-inflammatory effect of *T. polium* on carrageenan-induced skin inflammation

Local injection (s.c.) of 2.0% *T. polium* extract at the site of carrageenan-inflamed skin caused high reduction in neurogenic plasma extravasation (Fig. 5). The amount of the extracted Evans blue from inflamed skin areas treated with *T. polium* extract was 79.7 µg/g ± 13.7 (n= 9), which is significantly less than that extracted from inflamed skin areas injected with normal saline (149.8 µg/g ± 13.5, (n = 9) (P < 0.005). The anti-inflammatory effect of 2.0% of *T. polium* extract against carrageenan-
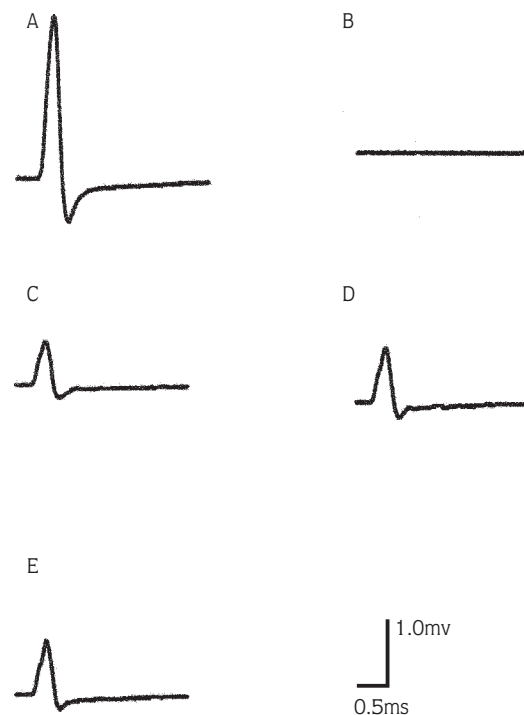

Fig. 1. Effect of 2.0% *T. polium* extract on CAPs of Aαβ-fibers in rat saphenous nerve: before (A), 5 min of application (B), and after 10 min (C), 30 min (D) and 60 min (E) of removal of the extract.

Table 1. Amplitudes, conduction velocities, thresholds and maximum strength of excitation of CAPs of Aαβ-, Aδ- and C-fibers of rat saphenous nerve after chronic (15-20 days) perineural treatment of ipsilateral nerve with 2.0% T.polium extract or olive oil. Means ±S.E.M. (n). *P<0.025.

|	Aαβ-fibers				Aδ-fibers				C-fibers			
	Experimental group		control group		Experimental group		control group		Experimental group		control group	
	T. polium treated	contralat. untreated	olive oil treated	contralat. untreated	*T. polium* treated	contralat. untreated	olive oil treated	contralat. untreated	*T. polium* treated	contralat. untreated	olive oil treated	contralat. untreated
Amplitude (mv)	1.86±0.27 (14)*	2.95±0.39 (14)	2.4±0.73 (3)	2.39±0.77 (3)	0.132±0.02 (14) *	0.219±0.04 (14)	0.186±0.09 (3)	0.183±0.05 (3)	0.115±0.02 (14) *	0.204±0.04 (14)	0.179±0.07 (3)	0.160±0.03 (3)
Cond. vel. (m/sec)	53.4±2.0 (14)	55.3±1.6 (14)	47.0±4.5 (3)	51.6±4.1 (3)	10.3±0.4 (14)	10.9±0.4 (14)	11.3±1.9 (3)	13.8±1.9 (3)	1.15±0.02 (14)	1.19±0.03 (14)	1.19±0.06 (3)	1.16±0.07 (3)
Threshold (mA)	0.22±0.007 (14)	0.22±0.008 (14)	0.22±0.003 (3)	0.21±0.012 (3)	0.28±0.013 (14)	0.27±0.013 (14)	0.27±0.009 (3)	0.26±0.016 (3)	1.93±0.052 (14)	1.84±0.017 (14)	1.84±0.028 (3)	1.82±0.03 (3)
Max excit. (mA)	0.29±0.029 (14)	0.27±0.011 (14)	0.28±0.011 (3)	0.26±0.01 (3)	0.39±0.027 (14)	0.41±0.027 (14)	0.41±0.017 (3)	0.41±0.026 (3)	2.70±0.101 (14)	2.57±0.12 (14)	2.51±0.05 (3)	2.49±0.135 (3)
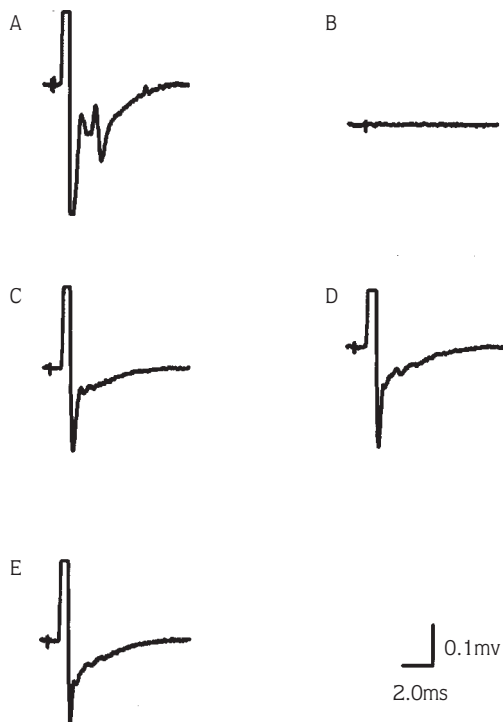
Fig. 2. Effect of 2.0% *T. polium* extract on CAPs of Aδ-fibers in rat saphenous nerve: before (A), 5 min of application (B), and after 10 min (C), 30 min (D) and 60 min (E) of removal of the extract.
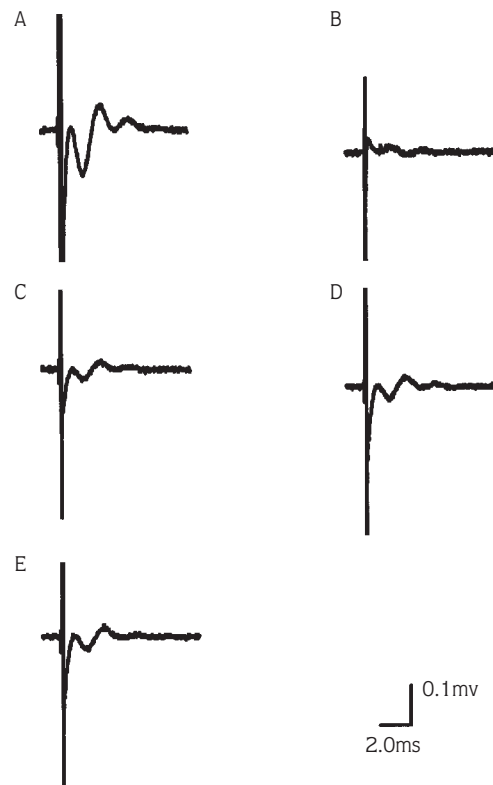

Fig. 3. Effect of 2.0% *T. polium* extract on CAPs of C-fibers in rat saphenous nerve: before (A), 5 min of application (B), and after 10 min (C), 30 min (D) and 60 min (E) of removal of the extract.

induced skin inflammation is characterized by severe inhibition of plasma protein extravasation localized at the site of injection (Fig. 6).

Effect of *T. polium* on carrageenan-induced paw edema

Intraplantar injection of 2.0% *T. polium* extract at the site of carrageenan-induced paw edema enhanced paw swelling. The mean paw thickness of ipsilateral inflamed paws treated with *T. polium* extract was 9.19 mm ± 0.14 (n = 18), which is significantly higher than that of contralateral inflamed paws injected with normal saline (8.11 mm ± 0.279, n = 18) (P < 0.005).

Discussion

The immediate complete non-selective inhibition of conduction of primary afferent nerve fibers induced by *T. polium* shown in the present study may explain the wide use of the herb in folk medicine for treatment of many neural-based disorders. It was only rather recently confirmed that sensory nerve fibers participate in many pathophysiological implications of the stomach [12-16], intestine [17-18], respiratory tract [19-21], eye [22] urogenital system [23-27], as well as in rheumatoid arthritis [28-32]. Therefore, the ability of the herb extract to inducea completely none-selective block of nerve conduction might be responsible for relief from neural-origin disorders and inflammatory responses, because it is known that neurogenic inflammation is due to excitation of a subgroup of primary afferent C-fibers, notably the capsaicin-sensitive C-polymodal nociceptors [33] which contain proinflammatory neuropeptides such as substance P, neurokinin A, calcitonin gene-related peptide and vasoactive intestinal peptide as neurotransmitters [34-38].

The present study shows that *T. polium* induced immediate complete unspecific inhibition of nerve conduction, with all the components of compound action potentials (CAPs) of sensory nerve fibers being equally affected. This may indicate that the mechanism of action of the herb is unlikely to be mediated through specific-mediated membrane receptors. In this regard, it differs
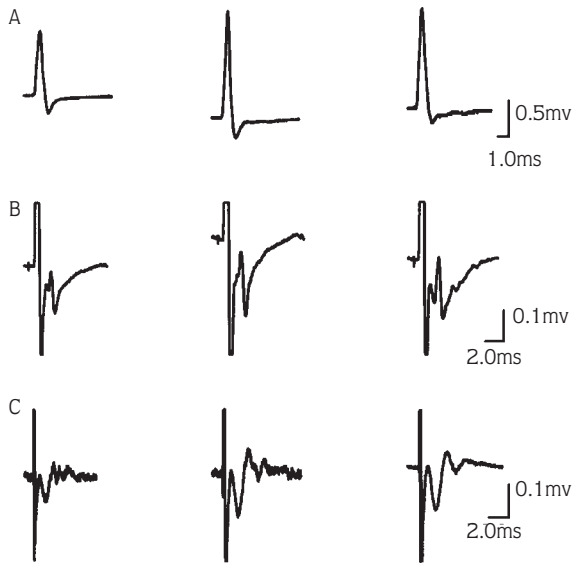
Fig. 4.   Compound action potentials of A$\alpha\beta$-fibers (A), Aδ-fibers (B) and C-fibers (C) in rat saphenous nerve after 15 days of perineural treatment of ipsilateral nerve either with 2.0% *T. polium* extract (left) or with olive oil (right) compared to that in contralateral nerve in untreated hindlimb (middle).
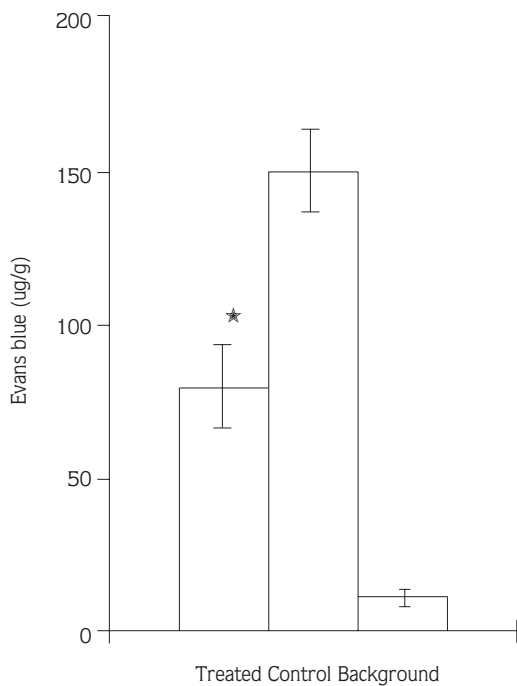

Fig. 5.   The anti-inflammatory effect of 2% *T. polium* on carrageenan-induced acute neurogenic plasma extravasation in the rat skin (treated) compared to normal saline (control). * P < 0.005.

Fig. 6.   The anti-inflammatory effect of 2% *T. polium* on carrageenan-induced acute neurogenic plasma extravasation in the rat skin (left) compared to normal saline (right).

from that of capsaicin, the permanent C-fiber neurotoxic agent found in red pepper [39-41], which acts through a common vanilloid membrane receptor [42-43]. The non-selective pharmacological activity of *T. polium* might be responsible for its diffuse effects on many normal physiological functions. This could be a major limitation in using it against specific medical treatment. The phytochemical screening of the herb has shown that it contains several compounds of iridoids, flavonoids and cirsiliol [3,9]. However, it is not possible to tell from the present study which of these components is responsible for its neural conduction failure. Further biochemical, anatomical and physiological studies are in progress to identify the active ingredient(s) in the herb and their pathophysiological implications.

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