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Antinociceptive effect of *Teucrium polium* leaf extract in the diabetic rat formalin test

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Abstract

This study was designed to evaluate the analgesic effect of *Teucrium polium* leaf extract in the diabetic rat formalin test. For this purpose, streptozotocin (STZ)-diabetic rats received intraperitoneal injection of this extract (100 and 200 mg/kg per day) for a period of 2 weeks. It was found out that *Teucrium polium*-treated diabetic rats exhibited a lower nociceptive score as compared to untreated diabetics. The results may suggest therapeutic potential of *Teucrium polium* extract for the treatment of diabetic hyperalgesia.

Keywords: *Teucrium polium*; Pain; Formalin test; Diabetic rat

1. Introduction

1.1. Plant

Teucrium polium, a wild-growing flowering plant belonging to the family Labiatae, was obtained from mountainous regions of Hamedan (Iran) in mid-July and authenticated by Mr. Ghamkhar (Department of Botany, Shahrekord University, Shahrekord). The voucher number 2003-46 was specified by Shahrekord University Herbarium.

1.2. Reported activities

Teucrium polium is a medicinal plant with antinociceptive (Abdollahi et al., 2003), antioxidant (Couladis et al., 2003), hypolipidemic (Rasekh et al., 2001), anti-inflammatory, anti-rheumatoid (Tariq et al., 1989), and hypoglycemic (Gharaibeh et al., 1988) properties. Therefore, this study was carried out to evaluate the antinociceptive effect of aqueous *Teucrium polium* leaf extract in streptozotocin (STZ)-diabetic rats using formalin test.

2. Materials and methods

2.1. Extraction

Fresh leaves of *Teucrium polium* were separated, cleaned, and dried at room temperature. Thereafter, 150 g of dried leaves was grounded and the obtained powder was mixed with 1000 ml of distilled boiling water for a period of 15 min under continuous stirring. The obtained mixture was filtered twice through a mesh and then one time through a filtered funnel, and the obtained liquid was dried on a magnet stirrer until a concentrated residue (69%, w/w) was obtained. This stock extract was maintained at −20 °C until being used. Lower concentrations of the extract were prepared by dilution of the stock with cold and sterile 0.9% saline solution.

2.2. Experimental procedure

Male albino Wistar rats (Pasteur's institute, Tehran, Iran) weighing 200–250 g (7–9 weeks old) were housed

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in an air-conditioned colony room on a light/dark cycle at 21 ± 3 °C and supplied with standard pelleted diet and tap water ad libitum. Procedures involving animals and their care were conducted in conformity with the institutional guidelines of Iran University of Medical Sciences (Tehran, Iran) and in accordance with the NIH guidelines for the care and use of laboratory animals.

The animals were randomly divided into control ($n = 15$) receiving 0.9% saline, *Teucrium polium*-treated control ($n = 12$), sodium salicylate (SS; Sigma Chemical)-treated control ($n = 9$), vehicle-treated diabetic ($n = 13$), SS-treated diabetic ($n = 7$), and *Teucrium polium*-treated diabetic ($n = 24$ in two equal-sized parts) groups. Diabetes was induced by a single intraperitoneal injection of STZ (60 mg/kg/l; Upjohn) dissolved in cold 0.9% saline immediately before use. After 2 weeks, *Teucrium polium* extract was intraperitoneally administered to diabetic rats at doses of 100 and 200 mg/kg per day for 2 weeks. Control rats received the *Teucrium polium* extract only at a dose of 200 mg/kg and SS (200 mg/kg, i.p.) was administered 1 h before conducting the formalin test. Serum glucose level and body weight were monitored at the start and end of the experiment. Diabetes was verified by a serum glucose level higher than 250 mg/dl (glucose oxidase kit, Zistchimie, Tehran).

For assessment of pain, formalin test was used according to the previously described method (Dubuisson and Dennis, 1977). Briefly, each animal was acclimatized to the observation box before any testing began. Then, it was given a subcutaneous injection of 50 μl of 2.5% formalin into the plantar surface of one hind paw. It was then immediately placed in a Plexiglas box. Observations continued for the next 60 min. A nociceptive score was determined for 5 min blocks by measuring the amount of time spent in each of the four behavioral categories: 0, the position and posture of the injected hind paw is indistinguishable from the contralateral paw; 1, the injected paw has little or no weight placed on it; 2, the injected paw is elevated and is not in contact with any surface; 3, the injected paw is licked, bitten, or shaken. Then, a weighted nociceptive score, ranging from 0 to 3 was calculated by multiplying the time spent in each category by the category weight, summing these products and dividing by the total time for each 5 min block of time. The first 10 min post-injection was considered as the early phase, and the time interval 15–60 as the late phase.

2.3. Statistical analysis

All values were given as mean ± S.E.M. Statistical analysis was carried out using student's paired *t*-test and one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Statistical *P*-value less than 0.05 was considered significant.

3. Results and discussion

Regarding body weight and serum glucose level (Table 1), there was no significant differences between the groups before the experiment. At the end of 4 weeks, the body weight of the untreated ($P < 0.01$) and *Teucrium polium*-treated diabetic ($P < 0.05$) rats was found to be significantly lower as compared to control rats. In addition, untreated ($P < 0.001$) and *Teucrium polium*-treated ($P < 0.005$) diabetic rats also had elevated serum glucose level over those of control rats. In this respect, treatment of diabetic rats with *Teucrium polium* extract caused a significant reduction in the latter parameter in comparison with untreated diabetics ($P < 0.01$).

Hind limb formalin injection produced a marked biphasic response in the rats of all groups. Hyperalgesia was significantly ($P < 0.05$) greater in untreated diabetic than in control rats in both phases of the test (Fig. 1). Treatment of control and diabetic rats with sodium salicylate (200 mg/kg, i.p.) caused a significant reduction ($P < 0.05$) in nociceptive score only in the second phase of the formalin test. In addition, treatment of control rats with *Teucrium polium* extract (200 mg/kg) caused lower nociceptive scores in both phases of the formalin test ($P < 0.05$). On the other hand, diabetic animals receiving *Teucrium polium* extract showed also a less intensive nociceptive behavior, especially in the first phase of the test, as compared to untreated diabetic rats.

There are two main conclusions to be drawn from the obtained results as follows: first, the results clearly demonstrated that there is an intensified nociceptive response in both phases of the formalin test in diabetic rats. It is a well-established fact that diabetic rats display exaggerated hyperalgesic behavior in response to noxious stimuli that may model aspects of painful diabetic neuropathy (Freshwater et al., 2002) and for this reason, STZ-diabetic rats have been increasingly used as a model of painful diabetic neuropathy

Table 1
Body weight and serum glucose level of control, diabetic, and *Teucrium polium* extract-treated rats

	Body weight (g)		Serum glucose (mg/dl)	
	Week + 0	Week + 4	Week + 0	Week + 4
Control	211.1 ± 3.7	234.5 ± 4.8	103.6 ± 4.3	107.6 ± 5.1
Control + *Teucrium polium* (200 mg/kg)	225.4 ± 3.9	233.2 ± 4.9	98.9 ± 4.7	90.7 ± 6.2*
Diabetic	217.4 ± 4.5	196.4 ± 4.3**	105.1 ± 4.5	389.1 ± 14.9****
Diabetic + *Teucrium polium* (100 mg/kg)	231.1 ± 4.2	209.4 ± 5.1$^{*,\$}$	97.1 ± 5.6	280.7 ± 13.1$^{***,\$\$}$
Diabetic + *Teucrium polium* (200 mg/kg)	220.1 ± 5.4	213.5 ± 6.1$^{*,\$}$	108.2 ± 4.8	256.3 ± 12.3$^{***,\$\$}$

$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$, and $^{****}P < 0.001$ (as compared to control group); $^{\$}P < 0.05$ and $^{\$\$}P < 0.01$ (as compared to diabetic group).

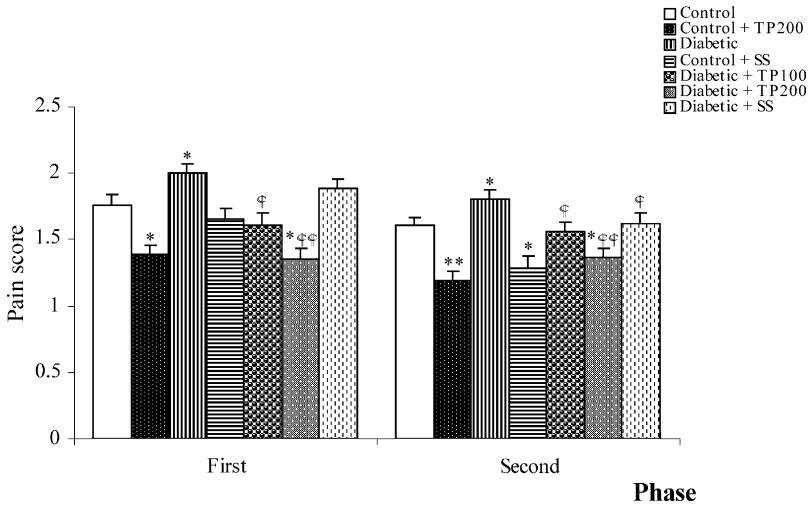

Fig. 1. The effect of *Teucrium polium* extract (100 and 200 mg/kg, i.p.) and sodium salicylate (SS, 200 mg/kg) on nociceptive scores in the first (early) and second (late) phases of the formalin test. All data represent mean ± S.E.M. $^{*}P < 0.05$ and $^{**}P < 0.01$ (as compared to control group). $P < 0.05$ and $P < 0.01$ (as compared to diabetic group).

to assess the efficacy of potential analgesic agents (Fox et al., 1999). Although evaluation of mechanisms causing these symptoms is complicated because of the overlap between the systemic effects of hyperglycemia and its toxic effects within the peripheral nervous system, but direct functional toxicity of hyperglycemia in the peripheral nervous system (Dobretsov et al., 2001), an increased activity of primary afferent fibers leading to an increased excitatory tone within the spinal cord, increased release of glutamate and activation of the NMDA receptor, reduced activity of both opioidergic and GABAergic inhibitory systems (Malcangio and Tomlinson, 1998), decreased activity of nNOS–cGMP system in neurons of dorsal root ganglion (Sasaki et al., 1998), altered sensitivity of the dopaminergic receptors and altered responsiveness of the dopaminergic system, possibly through the enhancement and/or deactivation of the endogenous Met-enkephalinergic system (Takeshita and Yamaguchi, 1998; Rutledge et al., 2002), and alterations in L-type Ca^{2+} channels (Gullapalli et al., 2002) could be involved in the modulation of nociception in diabetic rats. Secondly, it was demonstrated that intraperitoneal administration of aqueous *Teucrium polium* extract at a dose of 200 mg/kg for a period of 2 weeks could produce a significant analgesic effect in both phases of the formalin test in control and diabetic rats. On the other hand, sodium salicylate significantly reduced the nociceptive score only in the second phase of the formalin test in control and diabetic rats. It has been known that central acting drugs like narcotics inhibit both phases of the formalin test equally (Shibata et al., 1989), while peripheral acting drugs like aspirin only inhibit the late phase (Rosland et al., 1990). Therefore, the effect of *Teucrium polium* extract in this study could be mediated possibly through a central and/or a peripheral mechanism. One of the possible mechanisms that could partially explain the beneficial analgesic property of *Teucrium polium* extract may be attributed to its hypoglycemic (Gharaibeh et al., 1988) and antioxidant (Couladis et al., 2003) effects. Since hyperglycemia in diabetic state could induce some functional alterations in the nervous system (Dobretsov et al., 2001), this extract may have attenuated the hyperalgesic condition.

To conclude, administration of aqueous *Teucrium polium* extract could attenuate the hyperalgesic state of diabetic rats and this may be of potential benefit in painful diabetic conditions.

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