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Original article

Analgesic and Anti-inflammatory Activity of *Teucrium chamaedrys* Leaves Aqueous Extract in Male Rats

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Abstract

Objective(s)
Current study was undertaken to investigate the analgesic and anti-inflammatory effects of the aqueous extract of *Teucrium chamaedrys* in mice and rats.
Materials and Methods
For evaluating of analgesic and anti-inflammatory activity, we used the carrageenan- and dextran-induced paw oedema, acetic acid-induced writhing, tail flick and formalin pain tests.
Results
The extract of *T. chamaedrys* (50–200 mg/kg) and acetylsalicylic acid (100 mg/kg) produced a significant ($P< 0.01$) inhibition of the second phase response in the formalin pain model, while only the high dose (200 mg/kg) of the extract showed an analgesic effect in the first phase. The extract also inhibited acetic acid-induced abdominal writhes in a dose-dependent manner. The tail flick latency was dose dependently enhanced by the extract but this was significantly ($P< 0.05$) lower than that produced by morphine (10 mg/kg). The extract (25–250 mg/kg) administered 1 hr before carrageenan-induced paw swelling produced a dose dependent inhibition of the oedema. No effect was observed with the dextran-induced oedema model. Results of the phytochemical screening show the presence of alkaloids, flavonoids and triterpenoids in the extract.
Conclusion
The data obtained also suggest that the anti-inflammatory and analgesic effects of the extract may be mediated via both peripheral and central mechanisms. The role of alkaloids, flavonoids and triterpenoids will evaluate in future studies.

Keywords: Analgesic, Anti-inflammatory, Formalin test, Tail flick, *Teucrium chamaedrys*

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Introduction

The use of natural products is growing in the world especially in developing countries such as China, India, Arabic countries and Iran. The chemical diversity of plants has made them one of the main sources for the isolation of bioactive organic compounds (1). Analgesic therapy is dominated by two major classes of analgesic drugs; namely opioids and non steroidal anti-inflammatory drugs (NSAIDs). Both classes of analgesic drugs produce serious side effects, such as gastrointestinal disturbances, renal damages (with NSAIDs drugs), respiratory depression and possibly dependence (with opioids) (2, 3). The Mediterranean flowering plant known as *Teucrium chamaedrys* (Germander) is a member of Lamiaceae family and useful in herbal medicine for its anti-inflammatory, anti-rheumatic, digestive and diuretic effects (4). It is used externally as an astringent infusion on the gums and also in the treatment of wounds (4). *T. chamaedrys*, which is one of the most common and highly investigated species in the *Teucrium* genus, is marketed for use in weight control (5). In the present study, we evaluated the anti-inflammatory effects of the aqueous extract of *T. chamaedrys*, using the carrageenan-and dextran-induced rat paw oedema test and its analgesic activity, using the formalin test, tail flick latency and mouse writhing assays.

Materials and Methods

Preparation of plant extract

Fresh *T. chamaedrys* was collected at the flowering stage (July 2008) and authenticated by Ms. Jalilian, member of the School of Agricultural Sciences, Razi University, Kermanshah, Iran. Fresh leaves of *T. chamaedrys* were cut into pieces and macerated in distilled water. The liquid was decanted after 4 days and filtered. The filtrate was evaporated to dryness in an oven set at 40 °C. The dried extract was weighed and dissolved in distilled water to a concentration of 200 mg/ml. The extract was maintained at 4 °C throughout experiments.

Drugs

The following drugs were used in our experiments: indomethacin (Zahravi Pharmaceutical Company, Tabriz, Iran), carrageenan (Sigma Chemical Company, St. Louis, USA), acetylsalicylic acid (Darou Pakhsh Pharmaceutical Company, Tehran, Iran), methysergide (Sandoz, Basle, Switzerland), dextran (Sigma Chemical Company, St. Louis, USA) and morphine (Darou Pakhsh Pharmaceutical Company, Tehran, Iran).

Animals

Male Wistar rats (150–200 g) and male NMRI mice (25–30 g), kept at the Laboratory Animal Centre of the School of Pharmacy, Kermanshah U0niversity of Medical Sciences, Iran, were used. The animals were housed in standard cages with free access to food (standard laboratory rodent's chow) and water. The animal house temperature was maintained at 23±3 °C with a 12 hr light/dark cycle (light on from 06:00 to 18:00 hr). The ethical guidelines for the investigation of experimental animals were followed in all tests (6). All efforts were made to minimize animal suffering and to reduce the number of animals used.

Analgesic activity

Mouse writhing assay

This was carried out according to the method described previously (7). The extract of *T. chamaedrys* (50–200 mg/kg, i.p.) or distilled water (10 ml/kg) was administered to the separated groups of mice, 1 hr before intraperitoneal injection of acetic acid (0.6% v/v in normal saline, 10 ml/kg). Another group received acetylsalicylic acid (100 mg/kg, sc) as the reference drug. The number of writhes was counted for 15 min.

Formalin test

The method used was similar to that described previously (8, 9). Twenty microlitres of 1% formalin was injected subcutaneously into the right hind paw of mice. The time (in sec) spent in licking and biting responses of the injected paw was taken as an indicator of pain response. Responses were measured for a period of 5 min (first phase) and 15–30 min (second phase) after formalin injection. Extract of *T. chamaedrys* (50–200 mg/kg, i.p.) or acetylsalicylic acid (100 mg/kg, SC) was administered 30 min before formalin injection.

Control animals received the same volume of distilled water.

Tail flick test (TFT)

Tail-flick to radiant heat (Tail-Flick Apparatus Model P-162, Pouyaye Armaghan Co., Iran) was used to measure acute nociceptive responses in mice. The intensity of the thermal stimulus was adjusted to produce 5-6 sec latency in tail-flick response. Five millimeter of the tail was submitted to noxious heating. The trial was automatically terminated at 12 sec if a response did not occur (cut off time) to avoid damage to the tail. The TFT was measured 30 min before and 30 min after administration of extract (50–200 mg/kg, i.p. injection) or morphine (10 mg/kg, sec) in different groups of animals. Control animals received distilled water (10 ml/kg). The percentage of maximal possible effect (MPE%) for each animal was calculated, using the following formula:

MPE%= [(pre treatment TFT–post treatment TFT)/ (12−pre treatment TFT)] ×100 (10).

Anti-inflammatory activity

Rats (n=10 in each group) were allotted to different experimental groups. Oedema was induced in the rats by injection of carrageenan (0.1 ml, 1% w/v in normal saline) or dextran (0.1 ml, 1% w/v in normal saline) into the sub-plantar tissue of the right hind paw (11). Extract (25–250 mg/kg), distilled water (0.1 ml/100 g rat), indomethacin (10 mg/kg) or methysergide (1 mg/kg) were administered intraperitoneally 1 hr before injection of phlogistic agents. The paw volume (ml) was measured with a volume-differential meter (Model S-79, Electronic Industry Development, Iran). Measurements were made immediately before injection of the phlogistic agent (T_0) and again 6 h later (T_6) in the animals injected with dextran or carrageenan. The paw swelling was calculated as the difference between T_6 and T_0.

Preliminary phytochemical screening

The *T. chamaedrys* extract was screened for alkaloids, flavonoids, triterpenoids and saponins, using thin layer chromatography (12). In order to chemically screen the extract, Dragendorff's reagent (potassium bismuth iodide) was used for alkaloids, Mg^{2+} and HCl for flavonoids, Liebermann–Burchard method for terpenoids, and the ability to produce foam for saponins. The presence of phenolic carboxylic acids in the extract was also determined, using anisaldehyde-sulfuric acid and vanillin-sulfuric acid reagents (13).

Acute toxicity

Doses of 0.5-4 g/kg of the extract was administered to mice (n= 10 per group). The animals were observed for any abnormal behavior such as sedation, motor impairment and hyperexcitability for 3 hr. Furthermore, the incidence of mortality was noted up to 24 hr after administration. LD_{50} was estimated by log dose–probit analysis (14).

Statistical analysis

Results are expressed as mean±SEM. Statistical analysis was performed, using one way analysis of variance (ANOVA) followed by Tukey's test for multiple comparisons. $P< 0.05$ was the critical criterion for statistical significance.

Results

Mouse writhing assay

In control mice, the number of writhes during the 15 min test period was 73.3±3.4 (n= 10). The treatment of animals with *T. chamaedrys* aqueous extract (50-200 mg/kg) produced a significant (P= 0.009) and dose dependent inhibition of the control writhes (Table 1). There was not any significant difference (P= 0.066) between the effect of extract 200 mg/kg and acetylsalicylic acid (76.4% and 71.1%, respectively).

Formalin test

Results obtained from animals received *T. chamaedrys* extract, showed a dose-dependent relationship in both phases of formalin induced pain. A significant inhibition (14.6%) was produced only with the dose of 200 mg/kg extract compared to control, in the first phase (P= 0.041) (Table 2). However, all the doses inhibited the second phase significantly (P= 0.008), similar to acetylsalicylic acid.

Table 1. Effect of *T. chamaedrys* aqueous extract on acetic acid-induced writhing in mice.

Group	Dose (mg/kg)	Number of writhings (per 15 min)	%Inhibition
Control	-	73.3±3.4	-
T. chamaedrys	50	36.2±2.1*	50.6±3.2
	100	34.6±3.2*	52.7±4.1
	200	17.3±3.4*	76.4±4.5
Acetylsalicylic acid	100	21.2±1.3*	71.1±2.5

Values are mean±SEM (n=10 animals per group). *P< 0.01 vs. control.

Table 2. Effect of *T. chamaedrys* aqueous extract on formalin-induced pain.

Group	Dose (mg/kg)	0-5 min	%Inhibition	15-30 min	%Inhibition
Control	-	118.2±5.3	-	97.2±6.6	-
T. chamaedrys	50	111.3±6.9	5.8	49.3±8.9* *	49.2
	100	101.5±5.9	14.1	25.6±4.9**	73.6
	200	100.9±4.6*	14.6	21.7±6.3***	77.6
Acetylsalicylic acid	100	116.9±7.6	-	33.4±9.2**	65.6

Values are mean±SEM (n=10 animals per group). *P< 0.05, **P< 0.01, ***P< 0.001 vs. control.

Tail flick test

Table 3 shows the effect of the extract on TFT response in mice. All doses of the extract significantly (P= 0.033) increased the TFT time compared to the control. The effect of morphine (10 mg/kg) was significantly higher (P= 0.035) than that produced by the highest dose of the extract.

Anti-inflammatory activity

The extract administered 1 hr before carrageenan showed a dose dependent inhibition of the induced oedema. Carrageenan produced a swelling of the rat paw which reached a peak (0.96±0.08 ml) (Figure 1). The extract showed a significant inhibition of oedema at the doses of 100 mg/kg (P= 0.044), 200 mg/kg (P= 0.009) and 250 mg/kg (P= 0.0007) compare to the control. Indomethacin (10 mg/kg) produced a less swelling of the rat paw and there was not any significant difference (P= 0.082) between the effect of extract 250 mg/kg and indomethacin (0.20±0.05 and 0.16±0.04 ml, respectively).

Dextran produced a rapid paw swelling, which reached a peak in 0.89±0.1 ml. Pre-treatment of rats with extract (25-250 mg/kg) did not suppress the dextran oedema but methysergide (1 mg/kg) inhibited the same paw oedema at 0.08±0.04 ml. There was not any significant difference (P= 0.061) between the effect of extract and control (P= 0.071) (Figure 2).

Phytochemical tests

Preliminary phytochemical analysis revealed the presence of alkaloids (Dragendorff's indicator became orange), flavonoids (7, 8 dimethoxyflavone because the indicator became orange) and triterpenoids (Liebermann–Burchard indicator became green blue).

Acute toxicity

The LD_{50} of the extract when administered intraperitoneally was 3500 mg/kg.

Table 3. Effect of *T. chamaedrys* aqueous extract on tail flick test

Group	Dose (mg/kg)	Pre-treatment (sec)	Post-treatment (sec)	% Inhibition
Control	-	5.5±0.3	5.67±0.1	2.6
T. chamaedrys	50	5.3±0.1	7.01±0.2	25.3*
	100	6.1±0.7	9.67±0.6	60.5* *
	200	5.7±0.9	9.82±0.7	65.4* *
Morphine	10	5.2±0.4	10.8±0.5	82.3* *

Values are mean±SEM (n=10 animals per group). *P< 0.05, **P< 0.01 vs. control.

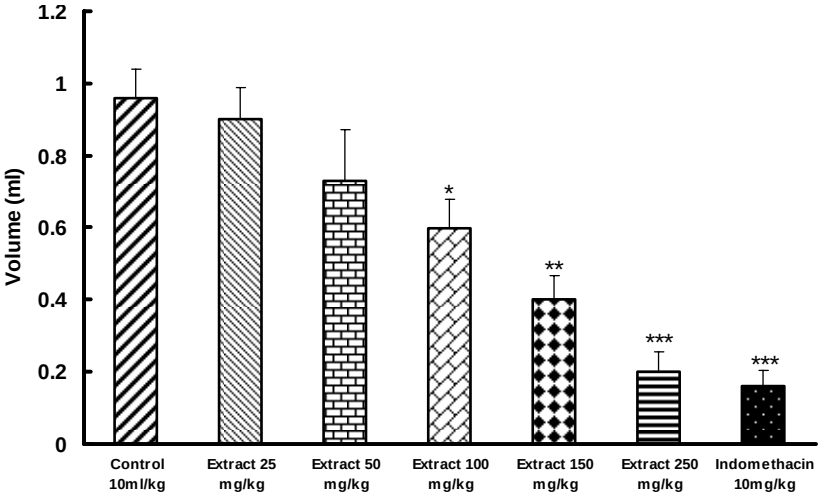

Figure 1. Effect of *T. chamaedrys* aqueous extract on rat paw swelling induced by carrageenan. Vertical bars are mean±SEM (n=10 animals per group). **P*< 0.05, ***P*< 0.01, ****P*< 0.001 vs. control.

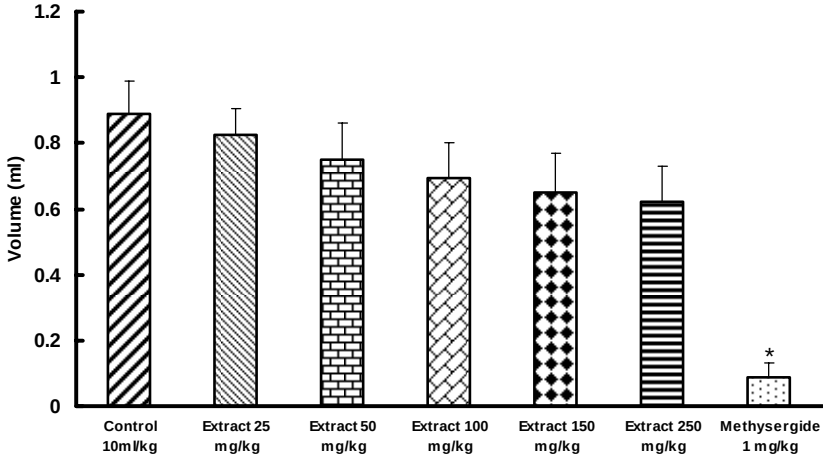

Figure 2. Effect of *T. chamaedrys* aqueous extract on rat paw swelling induced by dextran. Vertical bars are mean±SEM (n=10 animals per group). **P*< 0.001 compared to control.

Discussion

The data obtained from the present study indicate that *T. chamaedrys* aqueous extract produced a dose dependent anti-inflammatory effect on carrageenan-induced paw oedema. At the dose of 200 mg/kg, this effect was similar to that produced by the standard anti-inflammatory drug; indomethacin. The carrageenan-induced paw oedema as an *in vivo* model of inflammation is a screening procedure in which the involvement of the cyclooxygenase products of arachidonic acid metabolism and the production of reactive oxygen species are well established (15). It is reported that the carrageenan oedema shows three distinct phases, namely an initial release of histamine and 5- hydroxytryptamine, a second phase mediated by kinins and a third phase (about 5 hr of oedema) in which the mediator is suspected to be prostaglandin (16). The effect of the extract was most pronounced at the later stages of the inflammatory response, which corresponds to the phase of prostaglandin release. The extract was however, ineffective in the dextran model showing that it does not inhibit inflammation by blocking the release of histamine and 5HT, two mediators which are released by dextran (17, 18). In this study, it was shown that the extract inhibited both phases of formalin-induced pain, a model which is very useful for elucidating the mechanism of pain and analgesia (19). Drugs which act mainly centrally, such as narcotics, inhibit both phases of formalin-induced pain while peripherally acting drugs, such as aspirin, only inhibit the

late phase (20). The effect of the extract on tail flick response provides a confirmation of its central effect since the assay is specific for opioid induced analgesic effect (21). In the present study, *T. chamaedrys* like morphine, a centrally acting analgesic drug, produced an inhibitory effect on the nociceptive response in tail-flick test. The extract also inhibited acetic acid-induced writhing in mice hence it can be suggested that the analgesic effect of the extract is also peripherally mediated. Preliminary phytochemical analysis performed in this study shows the presence of alkaloids, flavonoids and triterpenoids in the extract of *T. chamaedrys*. The anti-inflammatory action of triterpenoids has been reported by many researchers (22-24). Even more the suppression of inducible nitric oxide synthase (iNOS) and inducible cyclooxygenase-2 (COX-2) enzymes has been shown for triterpenoids (9, 19). Flavonoids influence many biological functions including protein synthesis, cell proliferation differentiation and angiogenesis for the benefit of mankind (25). Detailed information on the absorption features of flavonoids is not yet available. Various flavonoids, both glycosides and aglycones were previously reported having potent anti-inflammatory and analgesic activity. Simoes *et al* (26) reported anti-inflammatory activity of flavonoids against carrageenan-induced paw edema model and was noted a more pronounced protective effect on the earlier stages of the oedematogenic response. It is suggested that some flavonoids blocked both the cyclooxygenase and lipoxygenase pathways of the arachidonate cascade at relatively high concentrations, while at lower concentrations only the lipoxygenase pathway (25) is blocked. Although, the absorption peculiarity of flavonoids has not yet fully understood, it has been claimed that only the flavonoids might be able to pass through the gut wall, largely depending on the chemical structure (25). Previous studies suggested that alkaloid extract involves at least partially the opioid system in analgesic action (27). Therefore, it seems that analgesic and anti-inflammatory profile of *T. chamaedrys* might be related to the triterpenoids, flavonoids and alkaloids present in the leave.

The ability of the extract, in this study, to suppress abdominal writhes, increase tail flick latency, inhibit both phases of formalin induced pain, as well as suppressing the carrageenan-induced inflammation confirm the analgesic and anti-inflammatory activities of the extract.

Conclusion

It is concluded that the aqueous extract of *T. chamaedrys* possesses analgesic and anti-inflammatory properties, which are probably mediated via inhibition of prostaglandin synthesis as well as central inhibitory mechanisms. The extract will, therefore, be of potential benefit in the management of pain and inflammatory disorders.

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References

1. Basso LA, da Silva LH, Fett-Neto AG, de Azevedo WF Jr, Moreira Ide S, Palma MS, *et al*. The use of biodiversity as source of new chemical entities against defined molecular targets for treatment of malaria, tuberculosis, and T-cell mediated diseases – A Review. Mem Inst Oswaldo Cruz 2005; 100:475-506.
2. Domaj MI, Glassco W, Aceto MD, Martin BR. Antinociceptive and pharmacological effects of metanicotina, a selective nicotine agonist. J Pharmacol Exp Ther 1999; 291: 390-398.
3. Farshchi A, Ghiasi G, Malek Khatabi P, Farzaee H, Niayesh A. Antinociceptive Effect of Promethazine in Mice. Iran J Basic Med Sci 2009; 12:140-145.
4. Chiej R. Enyclopedia of Medicinal Plants. MacDonald, London: UK, 1984; p 252.
5. Dao T, Peytier A, Galataeau F, Valla A. Chronic active hepatitis progressing to cirrhosis after Germander administration Gastroenterol. Clin Biol 1993; 17: 609.
6. Bowd AD. Ethics and animal experimentation. Amer Psychol 1980; 35: 224-225.
7. Koster R, Anderson M, DeBeer EJ. Acetic acid analgesic screening. Fed Proc 1959; 18:418–420.

8. Shibata M, Ohkubo T, Takahashi H, Inoki R. Modified formalin test; characteristic biphasic pain response. Pain 1989; 38:347–352.
9. Vianna GSB, doVale TG, Rao VSN, Matos FJA. Analgesic and antiinflammatory effects of two chemotypes of *Lippia alba*: a comparative study. Pharm Biol 1998; 36: 347–351.
10. Xiaohong C, Geller BE, Adler MW. Nociceptin/orphanin FQ blocks the antinociception induced by mu, kappa and delta opioid agonists on the cold water tail-flick test. Eur J Pharmacol 2007; 557:32-36.
11. Winter C, Risley E, Nuss O. Carrageenin-induced inflammation in the hind limb of the rat. Fed Proc 1962; 46: 118-126.
12. Trease GE, Evans WC. Pharmacognosy. Bailliere Tindall Press: London; 1983.
13. Wagner H, Bladt S. Plant Drug Analysis. Springer: Berlin; 1996.
14. Miller LC, Tainter ML. Estimation of the ED_{50} and its errors by means of logarithmic–probit graph paper. Proc Soc Exp Biol Med 1944; 57:261–264.
15. Smith MJH, Ford-Hutchinson AW, Elliot PNC, Bolam J. Prostaglandin in the anti-inflammatory activity of a humanplasma fraction in carrageenan-induced paw oedema in the rat. J Pharm Pharmacol 1974; 26: 692.
16. Wang LM, Mineshita S. Preventive effects of Unsei-in and Oren-gedoku-to, Chinese traditional medicines, against rat paw oedema and abdominal constriction in mice. J Pharm Pharmacol 1996; 48:327-331.
17. Nishida S, Kagawa K, Tomizawa S. Dextran-induced paw oedema and 5-hydroxytryptamine release. Biochem Pharmacol 1979; 28:3149–3150.
18. Pearce FL. On the heterogeneity of mast cells. Pharmacology 1986; 32:61–71.
19. Tjolsen A, Berge OG, Hunskaar S, Rosland JH, Hole K. The formalin test: an evaluation of the method. Pain 1992: 51:5-17.
20. Santos ARS, Filho VC, Niero R, Viana AM, Moreno FN, Campos MM, *et al*. Analgesic effects of callus culture extracts from selected species of Phyllantus in mice. J Pharm Pharmacol 1994; 46:755-759.
21. Clark SJ, Follenfant RL, Smith TW. Evaluation of opioid-induced antinociceptive effects in anaesthetized and conscious animals. Br J Pharmacol 1988; 95:275–283.
22. Huss U, Ringbom T, Perera P, Bohlin L, Vasange M. Screening of ubiquitous plant constituents for COX-2 inhibition with a scintillation proximity based assay. J Nat Prod 2002; 65:1517-1521.
23. Suh N, Honda T, Finaly HJ, Barchowsky A, Williams C, Benoit NE, *et al*. Novel triterpenoids suppress inducible nitric oxide synthase (iNOS) and inducible cyclooxygenase (COX-2) in mouse macrophages. Cancer Res 1998; 58:717-723.
24. Vazquez B, Avila G, Segura D, Escalante B. Antiinflammatory activity of extracts from Aloevera gel. J Ethnopharmacol 1996; 55:69-75.
25. Carlo DiG, Mascolo N, Izzo AA, Capasso F. Flavonoids, old and new aspects of a class of natural therapeutic drugs. Life Sci 1999; 65:337–353.
26. Simoes CM, Schenkel OE, Bauer P, Langeloh LA. Pharmacological investigations on *Achyrocline satureioides* (Lam.) DC. compositae. J Ethnopharmacol 1988; 22: 281-293.
27. Farouk L, Laroubi A, Aboufatima R, Benharref A, Chait A. Evaluation of the analgesic effect of alkaloid extract of *Peganum harmala* L.: Possible mechanisms involved. J Ethnopharmacol 2008; 115:449-454.