Research Journal of Pharmacognosy (RJP) 11(1), 2024: 1–4
Received: 23 Aug 2023
Final revision: 10 Sep 2023
Accepted: 4 Oct 2023
Published online: 8 Oct 2023
DOI: 10.22127/RJP.2023.412266.2202

Short communication

Antinociceptive Effect of *Artemisia dracunculus* Essential Oil in Formalin Test and Possible Involvement of Serotoninergic Receptors and Nitric Oxide Pathway

Valiollah Hajhashemi*, Amir Hossein Eslami

Department of Pharmacology and Isfahan Pharmaceutical Sciences Research Center, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran.

Abstract

Background and objectives: *Artemisia dracunculus* (tarragon) essential oil has shown antinociceptive effect in some animal models. This study was aimed to find out its possible mechanism of action in formalin-induced pain behavior in mice. **Methods:** Essential oil of the plant was prepared by hydro-distillation method. Male Swiss mice (25–30 g) and formalin test were used in the pain model. First, the antinociceptive activity of three doses of *A. dracunculus* essential oil (50, 100 and 200 µL/kg) were examined and then the dose of 100 µL/kg was selected for mechanistic experiments. Different groups of mice (n=6) were pretreated with naloxone, prazocin, yohimbine, propranolol, ondansetron, cyproheptadine, sulpiride and haloperidol to evaluate contribution of opioid, adrenergic, serotoninergic and dopaminergic receptors in the essential oil effect. Besides arginine, N(G)-nitro-L-arginine methyl ester (L-NAME), methylene blue, tadalafil and glibenclamide were used to find out the possible involvement of nitric oxide pathway for the essential oil effect. **Results:** *Artemisia dracunculus* essential oil significantly ($p<0.001$) and in a dose-dependent manner demonstrated antinociception in both acute and chronic phases of the formalin test. Prazocin, yohimbine, propranolol, naloxone, ondansetron and sulpiride were ineffective in prevention of the induced antinociceptive effect. Cyproheptadine, arginine and tadalafil partially inhibited *A. dracunculus* essential oil antinociception while methylene blue and glibenclamide potentiated the effect. **Conclusions:** *Artemisia dracunculus* essential oil showed antinociceptive effect in formalin test and the observed effect was partially prevented by cyproheptadine, arginine and tadalafil indicating the possible role of serotoninergic and nitrergic pathways.

Keywords: acute pain; *Artemisia dracunculus*; mice; pain measurement; tarragon

Citation: Hajhashemi V, Eslami AH. Antinociceptive effect of *Artemisia dracunculus* essential oil in formalin test and possible involvement of serotoninergic receptors and nitric oxide pathway. Res J Pharmacogn. 2024; 11(1): 1–4.

Introduction

Several receptors including opioid, $5HT_{2A}$ and $5HT_3$ and dopamine (especially D_2) receptors are involved in the nociceptive system [1]. Also, contribution of nitric oxide/cyclic guanosine mono phosphate (NO / cGMP) pathway in pain signaling pathway is well-documented [2].

Artemisia dracunculus L. (tarragon) is a well-known plant of Asteraceae family with anti-inflammatory, antinociceptive, anti-microbial, antifungal, antioxidant, anticonvulsant, anti-diabetic, hepatoprotective, antiplatelet and immune-modulatory effects [3-5]. An ethanol extract of the aerial parts of *A. dracunculus* has demonstrated anti-inflammatory and antinociceptive effect in mice [4]. Maham et al. reported that of *Artemisia dracunculus* essential oil had antinociceptive effect in different animal models including hot-plate, formalin and acetic

*Corresponding author: Hajhashemi@pharm.mui.ac.ir

acid-induced writhing tests [5].
To the best of our knowledge, the mechanism of antinociceptive action of *Artemisia dracunculus* essential oil has not been studied yet and therefor this study was aimed to find out the possible mechanism of action.

Material and Methods

Ethical considerations

All the animal tests were conducted in accordance with the guidelines of Iranian National Research Council (Ethical code: IR.MUI.RESEARCH.REC.1400.467, Isfahan University of Medical Sciences).

Chemicals

Cyproheptadine was a gift from Raha Pharmaceutical companies (Iran). Prazosin, yohimbine, haloperidole, sulpiride, N(G)-nitro-L-arginine methyl ester (L-NAME), methylene blue and glibenclamide (Sigma, USA), propranolol and naloxone (Toliddaru, Iran), ondansetron (Tehran Chemie Pharmaceutical Co., Iran) were used in the study.

Plant material

Artemisia dracunculus essential oil was provided by Tabib Daru Company (Iran).

Animals

Male Swiss mice (weighing 25–30 g, 6 weeks old) were provided by the animal house of the School of Pharmacy (Isfahan, Iran) and kept in standard conditions of humidity, temperature, and the light/dark cycle. Both food and water were *ad libitium.*

Experimental design

In order to determine the optimal dosage for mechanistic research, a formalin test was conducted using three different doses of the essential oil (50,100 and 200 µL/kg). These doses were selected according to previous studies [4].
In the mechanistic experiments, thirteen groups of animals (n=6) were pretreated with prazocin (2 mg/kg), yohimbine (5 mg/kg), propranolol (2 mg/kg), cyproheptadine (2 mg/kg), ondansetron (2 mg/kg), naloxone (5 mg/kg), haloperidol (1 mg/kg), sulpiride (20 mg/kg) , arginine (100 mg/kg), L-NAME (20 mg/kg), methylene blue (5 mg/kg), tadalafil (2 mg/kg) or glibenclamide (10 mg/kg). Thirty minutes later, the essential oil (100 µL/kg) was injected i.p. and after 30 minutes, formalin (20 µL, 2.5% v/v) was injected into the hind paw of animals. Above doses were chosen based on previous reports [6].
After an injection of formalin, the amount of time that the paws were licked between 0 and 5 minutes and between 20 and 40 minutes was recorded and considered as the acute and chronic phases, respectively [7].

Statistical analysis

The results are presented as the mean ± standard deviation (SD). The results were analyzed by one-way analysis of variance (ANOVA), followed by the Tukey post hoc test. We considered the p values to be significant if they were < 0.05.

Results and Discussion

Formalin injection produced a rapid response characterized by paw licking in control group and the time spent for paw licking was 69.9 ± 14.9 seconds in acute (0-5 min) and 103.4 ± 19.1 in chronic (20-40 min) phases respectively. The essential oil decreased the paw licking time during both acute and chronic phases so that in the acute phase, the percent inhibition of nociception was 33.8, 62.1 and 94.6% for doses of 50, 100 and 200 µL/kg, respectively. In the chronic phase the percent inhibition of nociception for the mentioned doses were 56.9, 77.3 and 98.8%, respectively (Table 1).
Pretreatment of animals with naloxone, adrenergic receptor antagonists, sulpiride and ondansetron could not alter the antinociceptive effect produced by the essential oil (Table 1).
Cyproheptadine did not exert a significant alteration in the acute phase while it significantly ($p=0.042$) increased paw licking time in the chronic phase compared to the essential oil group (23.5 ± 5.9 s vs 61.5 ± 3.1 s).
L-Arginine and tadalafil significantly ($p<0.001$) reversed the antinociceptive effect of the essential oil while methylene blue (inhibitor of guanylyl cyclase) and glibenclamide (ATP-sensitive potassium channel closer) significantly ($p=0.006$ and $p=0.003$, respectively) potentiated essential oil-induced antinociception (Figure 1).
In our study, *Artemisia dracunculus* essential oil showed a dose-dependent antinociceptive effect in formalin-induced nociception model. It reduced pain behavior in both phases of formalin test. Our findings of the antinociceptive activity were in agreement with previous reports [4,5]. To

find out the possible mechanism, the mice were pretreated with several antagonists and enzyme inhibitors. Naloxone did not alter the essential oil antinociception indicating that the opioid receptors did not modulate the effect. Sulpiride could not exert a preventive effect against the essential oil antinociception. Besides, haloperidol significantly potentiated the oil-induced antinociceptive response that might be due to inhibition of other receptors such as adrenergic and cholinergic receptors [8]. 5-HT_3 receptors in the spinal dorsal horn and peripheral 5-HT_2 receptors contribute to transmission of nociceptive signals [9,10]. Our findings emphasized that 5-HT_3 receptors did not play any role in the antinociceptive effect of the essential oil while cyproheptadine exerted a preventive effect in the chronic phase of formalin test indicating that 5-HT_2 receptors are a probable target of the *A. dracunculus* essential oil. Experiments on NO pathway formed another part of our study. Several studies confirmed that the chronic phase of formalin test is inflammatory. On the other hand, it has been proved that nitric oxide is a pro-inflammatory agent and plays an important role in inflammation [11]. In inflammatory process, inducible nitric oxide synthase (iNOS) continuously synthesize NO, which results in expression of cyclooxygenase (COX-2). The later enzyme produces prostaglandins as well as reactive O_2 species which have detrimental role in vasodilation and other aspects of inflammation [12-14].

Table 1. The effect of pretreatment with antagonists on the antinociceptive effect of *Artemisia dracunculus* essential oil

Group	Paw licking time (s)	
	Acute phase	Chronic phase
Control	69.9 ± 14.9	103.4 ± 19.1
ADEO (50 µL/kg)	46.3 ± 3.4**	44.6 ± 3.1***
ADEO (100 µL/kg)	26.5 ± 5.3***	23.5 ± 5.9***
ADEO (200 µL/kg)	3.8 ± 1.4***	1.2 ± 1.2***
Naloxone (5 mg/kg) + ADEO100	22.3 ± 3.5***	20.3 ± 3.0***
Prazocine (2 mg/kg) + ADEO100	27.5 ± 3.5***	27.3 ± 3.6***
Yohimbine (5 mg/kg) + ADEO100	21.0 ± 2.3***	29.8 ± 2.4***
Propranolol (2 mg/kg)+ ADEO100	17.1 ± 2.6***	15.0 ± 4.2***
Cyproheptadine (2 mg/kg)+ ADEO100	35.3 ± 4.0***	61.5 ± 3.1***,#
Ondansetron (2 mg/kg)+ ADEO100	20.8 ± 2.1***	19.5 ± 4.9***
Haloperidol (1 mg/kg)+ ADEO100	9.3 ± 2.1***,#	1.0 ± 0.8***,#
Sulpiride (20 mg/kg)+ ADEO100	18.3 ± 5.1***	18.5 ± 3.7***

**p< 0.01 and ***p< 0.001 compared to control group; # p<0.05 compared to ADEO (100 µL/kg); ADEO: *Artemisia dracunculus* essential oil; (n=6)

Previous studies documented the anti-inflammatory and antioxidant effects of *A. dracunculus* essential oil [3,4] and therefor these effects might have some role in the antinociceptive response observed. In addition, our findings on NO pathway showed that arginine as a precursor of NO and also tadalafil as an inhibitor of phosphodiesterase prevented the essenstial oil-induced antinociceptive effect indicating that the essential iol had inhibited NO production. Additionally, L-NAME as an inhibitor of nitric oxide synthase and glibenclamide as an ATP-dependent potassium channel blocker potentiated the effect which confirms the contribution of NO pathway in the essential oil antinociception.

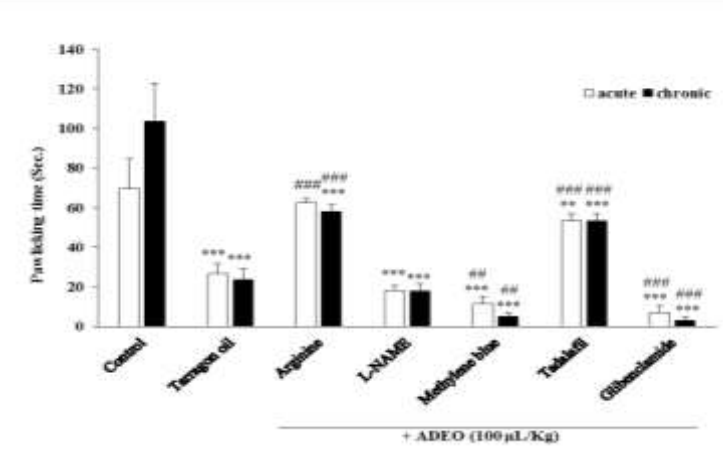

Figure 1. The effect of the drugs affecting NO/cGMP/K_{ATP} pathway on the antinociceptive effect of ADEO (n=6). Arginine (100 mg/kg), L-NAME (20 mg/kg), methylene blue (5 mg/kg), tadalafil (2 mg/kg) or glibenclamide (10 mg/kg) were injected (i.p.) thirty min before ADEO injection (100 µL/kg, i.p.); One group received only ADEO (100 µL/kg, i.p.) and control animals received vehicle (10 mL/kg); *** p< 0.001 vs control; ## p<0.01 and ### p< 0.001 vs ADEO alone; ADEO: *Artemisia dracunculus* essential oil

Conclusion

Artemisia dracunculus essential oil has antinociceptive effect in both phases of formalin test and NO/cGMP/K_{ATP} signaling seems to be the contributor to this effect. Also, according to the results obtained in cyproheptadine-pretreated animals, it seems that the role of serotonin receptors (especially $5HT_2$) should not be neglected.

Acknowledgment

This study was approved and financially supported by Isfahan University of Medical Sciences.

Author contributions

Valiollah Hajhashemi designed and supervised the research and prepared the manuscript; Amir Hossein Eslami performed the experiments.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

References

[1] Yam MF, Loh YC, Tan CS, Khadijah Adam S, Abdul Manan N, Basir R. General pathways of pain sensation and the major neurotransmitters involved in pain regulation. *Int J Mol Sci*. 2018; 19(8): 1–23.

[2] Molina-Ortega F, Lomas-Vega R, Hita-Contreras F, Plaza Manzano G, Achalandabaso A, Ramos-Morcillo AJ, Martínez-Amat A. Immediate effects of spinal manipulation on nitric oxide, substance P and pain perception. *Man Ther*. 2014; 19(5): 411–417.

[3] Obolskiy D, Pischel I, Feistel B, Glotov N, Heinrich M. *Artemisia dracunculus* L. (tarragon): a critical review of its traditional use, chemical composition, pharmacology, and safety. *J Agric Food Chem.* 2011; 59(21): 11367–11384.

[4] Eidi A, Oryan S, Zaringhalam J, Rad M. Antinociceptive and anti-inflammatory effects of the aerial parts of *Artemisia dracunculus* in mice. *Pharm Biol.* 2016; 54(3): 549–554.

[5] Maham M, Moslemzadeh H, Jalilzadeh-Amin G. Antinociceptive effect of the essential oil of tarragon (*Artemisia dracunculus*). *Pharm Biol*. 2014; 52(2): 208–212.

[6] Hajhashemi V, Salimian M, Hajihashemi O. Involvement of the NO/cGMP/K ATP pathway in the antinociceptive effect of rosemary (*Rosmarinus officinalis*) essential oil in mice. *Behav Pharmacol*. 2023; 34(1): 37–44.

[7] Hajhashemi V, Ghannadi A, Hajiloo M. Analgesic and anti-inflammatory effects of *Rosa damascena* hydroalcoholic extract and its essential oil in animal models. *Iran J Pharm Res*. 2010; 9(2): 163–168.

[8] Cendán CM, Pujalte JM, Portillo-Salido E, Baeyens JM. Antinociceptive effects of haloperidol and its metabolites in the formalin test in mice. *Psychopharmacology (Berl)*. 2005; 182(4): 485–493.

[9] Kidd EJ, Laporte AM, Langlois X, Fattaccini CM, Doyen C, Lombard MC, Gozlan H, Hamon M. 5-HT3 receptors in the rat central nervous system are mainly located on nerve fibres and terminals. *Brain Res.* 1993; 612(1-2): 289–298.

[10] Anjaneyulu M, Chopra K. Possible involvement of cholinergic and opioid receptor mechanisms in fluoxetine mediated antinociception response in streptozotocin-induced diabetic mice. *Eur J Pharmacol*. 2006; 538(1-3): 80–84.

[11] Sharma JN, Al-Omran A, Parvathy SS. Role of nitric oxide in inflammatory diseases. *Inflammopharmacology*. 2006; 15(6): 252–259.

[12] Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol*. 2007; 39(1): 44–84.

[13] Kröncke KD, Fehsel K, Kolb-Bachofen V. Inducible nitric oxide synthase in human diseases. *Clin Exp Immunol*. 1998; 113(2): 147–156.

Abbreviations

ADEO: *Artemisia dracunculus* essential oil; L-NAME: N(G)-nitro-L-arginine methyl ester