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Antinociceptive Properties of Hydro Alcoholic Extracts of *Anethum graveolens* L. (dill) Seed and Aerial Parts in Mice

Masoume Rezaee-Asl[1], Azam Bakhtiarian[2], Vahid Nikoui[2], Mandana Sabour[1], Sattar Ostadhadi[2], Maryam-Sadat Yadavar-Nikravesh[1] and Mario Giorgi[3]*

[1]*Department of Pharmacology, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran*
[2]*Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*
[3]*Department of Veterinary Sciences, Veterinary Teaching Hospital, University of Pisa, Via Livornese (lato monte), San Piero a Grado, 56122 Pisa, Italy*

Abstract

Chronic pain and its treatment have always posed a significant challenge for medical practitioners and many attempts have been made to reduce and eliminate it, both in past and recent history. Research to discover new effective drugs with excellent safety profiles is ongoing. The aim of this study was to evaluate the suitability of the plant *Anethum graveolens* (dill) for use as an analgesic drug.

Forty-two mice were divided randomly into seven groups (n=6). In the formalin test, the first group received normal saline; the second group, extract of plant seed (300 mg/kg); the third group, extract of plant crops (300 mg/kg) and the forth group received morphine (1 mg/kg). For the hot plate test, the first group received normal saline; the second group, extract of plant seed (300 mg/kg) and the third group received extract of plant crops (300 mg/kg). All injections consisted of 0.5 ml given intraperitoneally.

In the early phase of formalin test, the animals treated with seed and crop extracts did not show analgesic effects compared to control group (P=0.386, P=0.284 respectively). In contrast, in the late phase of formalin test, seed and crop extracts significantly decreased indications of pain compared to the saline group with seed extracts showing stronger analgesic effects (P=0.004, P=0.023 respectively). In the hot plate test, crop and seed extracts showed hyperalgesic properties. This effect was stronger in animals treated with crop extracts as compared to seed extracts.

These findings indicate that *Anethum graveolens* can reduce inflammatory pain, probably by inhibiting inflammatory mediators. In contrast, this plant has no analgesic effects on spinal nociception and conversely may exacerbate it. This study provides a basis for the use of *Anethum graveolens* extracts in popular folk medicine, but further studies are necessary to elucidate the mechanism of its analgesic actions.

Keywords: *Anethum graveolens* L; Dill; Analgesic; Anti-inflammatory; Formalin test; Hot plate test; Pain; Mice

Introduction

Anethum graveolens L. is a member of the *Apiaceae* family, commonly known as dill. Dill is used both medicinally and as an aromatic herb in cooking. The fruit of *A. graveolens* has been used for medicinal purpose, particularly alleviation of indigestion and also to stimulate lactation in nursing mothers [1]. This plant is sparse in appearance with feathery leaves and tiny yellow flowers. It grows most abundantly in the Mediterranean region, Europe and in central and southern Asia [2]. It is a popular aromatic herb with a very long history of use going back more than 2000 years [3]. Dill has been shown to possess antihyperlipidaemic and antihypercholesterolaemic effects [2,4,5] and its seeds have been used as a non-pharmaceutical alternative for relief from digestive problems such as stomachache, indigestion and flatulence. It has been reported that chewing its seeds decreases the bad breath [6]. Other applications of this plant are for anticancer [7], antidiabetic [8], antioxidant [9,10], antisecretory [11] and anti-inflammatory [12] purposes, it has also been demonstrated to be cytotoxic to human lymphocytes [13]. It has also been reported as possessing insecticidal properties [14]. Due to the broad therapeutic effects of this plant, this study was designed to evaluate the suitability of *Anethum graveolens* L. for use as an analgesic drug.

Materials and Methods

Housing and handling of the animals

The animals were handled in accordance with the criteria outlined in the Guide for the Care and Use of Laboratory Animals (NIH US publication 86-23 revised 1985). NMRI mice (Pasteur Institute, Tehran, Iran), 6-8 weeks of age, were kept in a controlled environment (23 ± 2°C, 50 ± 5% humidity) under a 12 hour light/dark cycle (light on 08:00-20:00) and had free access to a standard pellet chow and tap water throughout the study. All experiments were conducted in Tehran University of Medical Sciences according to the recommendations of the ethics committee for animal welfare.

Preparation of hydro alcoholic extracts

Hundred grams of seeds and aerial parts were placed into a flask; one liter of 96% ethanol was then added. After 24 hours, when the solution had become clear, this was transferred into another flask. One liter of 70% ethanol was then added to the solid residue and after 12 hours the supernatant was again decanted into another flask. Both solutions were then combined and concentrated by vacuum distillation at a temperature of 50°C and 70 rpm rotation speed, until the volume decreased to 1/3 of initial volume.

In order to isolate proteins, fat and chlorophyll, chloroform

***Corresponding author:** Mario Giorgi, Department of Veterinary Sciences, Veterinary Teaching Hospital, University of Pisa, Via Livornese (lato monte), San Piero a Grado, 56122 Pisa, Italy, E-mail: mgiorgi@vet.unipi.it

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was added to clear solution. This process consisted of two steps, in each stage, chloroform phase was removed and the alcoholic phase was retained for subsequent stages. The solution was poured into a petridish and dried in the autoclave at temperatures below 50°C in sterile conditions.

Nociceptive behavioral tests

Formalin test: The formalin test is a valid and reliable model of nociception that produces two distinct periods of increased licking activity that can be attributed to different nociceptive mechanisms. The early licking phase lasts forthe initial 5 minutes and a late phase lasts from 15 to 45 minutes after the injection of formalin [15]. As previously described, formalin (20 µl of a 2.5% solution) was injected subcutaneously into the dorsal surface of the right hind paw. The animals were then placed under a glass funnel on a glass surface, under this; a mirror was angled at 45 degrees [16]. Immediately after the injection, the animals' movements were recorded every 15 seconds (0=injected paw is not favored, 1=injected paw has reduced/no weightbearing, 2=injected paw is elevated and not in contact with any surface, 3=injected paw is licked, bitten or shake). Animals were randomly divided into four groups (n=6). Animals in the negative control group received 0.5 ml of normal saline. One mg/kg of morphine (Temad Co., Iran) was injected into animals in the positive control group. The third and fourth groups received 0.5 ml (300 mg/kg) of the seed and crop extract of *Anethum graveolens* L. respectively. All injections were given via the intraperitoneal route.

Hot plate test: The hot plate method was used as a complementary test of pain. Hot plates are surfaces that can be heated by electricity. In our experiment before being injected, all mice were placed on the hot plate (Aratebfan Co., Iran) set at 55°C, and base tolerance levels were recorded when they started to lick their paws or demonstrated other ambulatory changes. The animals were then divided into four groups (n=6) and injected with normal saline, morphine and seed and crop extract at the doses and with same route of administration as the formalin test. Tolerance was assessed at 15, 30, 45 and 60 minutes after the injections. Tolerance was assessed for a maximum time of 40 seconds to prevent tissue damage [17].

Statistical analysis

Data were analysed using the statistical software SigmaPlot version 11. One way ANOVA testing was used to ascertain the significance of variations between the sum of the tolerance scores for the formalin test. Two way repeated measures ANOVA testing was used to assess differences in reaction time for the hot plate test. All data is shown as mean ± S.E.M. All data were considered significant at $P \leq 0.05$.

Results

Formalin test

In the formalin test, the sum of the tolerance scores for the positive control group (morphine) was the lowest and that for the negative control group (normal saline) was the maximum value. The sum of the scores from the treated animals was not significantly different to that of animals treated with saline in the early phase (P=0.284 for seed group and P=0.386 for crop group Figure 1). In the later phase of the formalin test, animals treated with seed and crop extracts demonstrated significant increases in their pain tolerance as compared to the saline group. Of the two groups, animals treated with seed extract indicated a stronger analgesic effect (P=0.004 and P=0.023 respectively) to the extent that no significant difference was noted between the analgesic effects of the seed extract and morphine (P=0.077, Figure 2).

Hot plate test

In contrast to results of the formalin test, for the hot plate test, the crop and seed extracts demonstrated hyperalgesic properties as compared to the control group and this effect was stronger for animals treated with crop as opposed to seed extract (P=0.006, P=0.008, P=0.062, P=0.384 for crop in 15, 30, 45 and 60 minutes respectively and P=0.033, P=0.013, P=0.003, P=0.292 for seed in 15, 30, 45 and 60 minutes accordingly). The crop extract showed hyperalgesic effects at all points tested, while there was no indication of this effect for the seed extract at the 45 minute time point. (There was a significant difference as compared with crop extract at this point (P=0.003, Figure 3).

Discussion

In this study, the analgesic properties of seed and crop extracts of *Anethum graveolens* were assessed. The formalin-induced pain response possesses two phases that involve distinct mechanisms. Signs observed during the first stage provide an indication of neurogenic

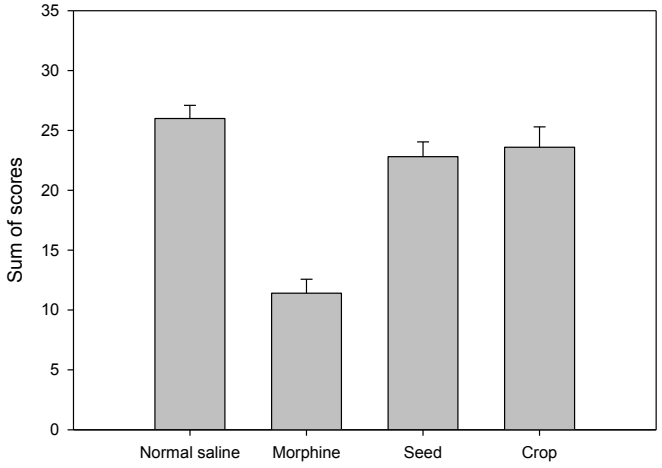
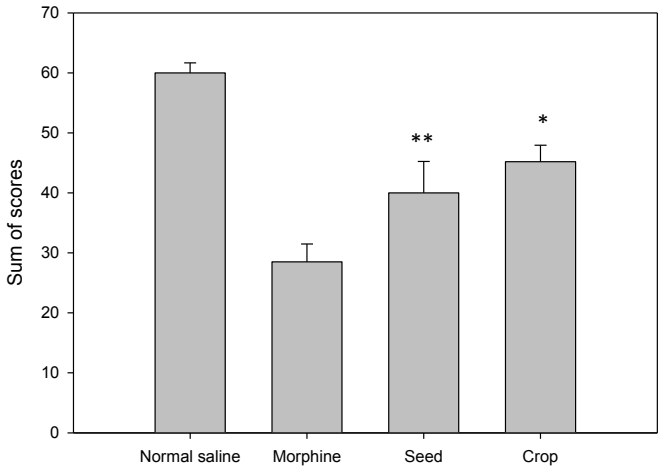


Figure 1: Nociceptive behaviour in the early phase (0-5 minutes) of formalin test.



*: Significantly different from saline group ($P \leq 0.05$)
**: Significantly different from normal saline group ($P \leq 0.01$)

Figure 2: Nociceptive behaviour in the late phase (15-45 minutes) of formalin test.

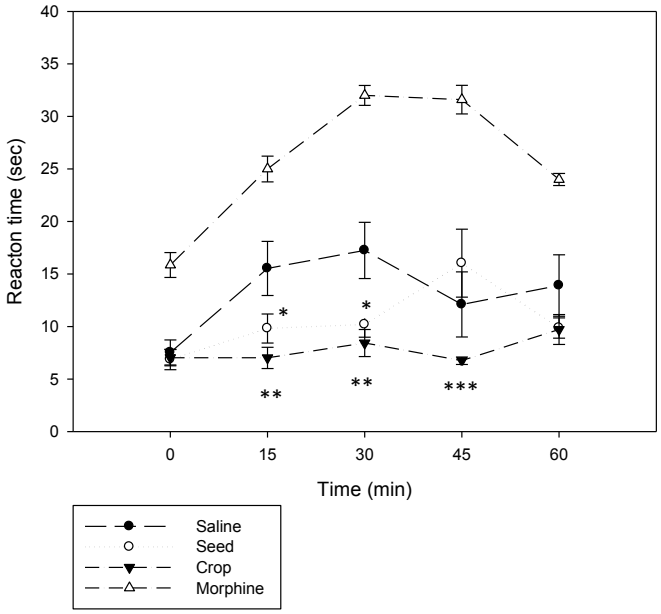


Figure 3: Nociceptive behaviour in hot plate test in different groups.

pain, and result from the direct chemical stimulation of myelinated and unmyelinated nociceptive afferent fibers, these are mainly C fibers [18]. The late phase assesses inflammatory pain which results from the action of inflammatory transmitters in peripheral tissues [19,20]. In acute phase of the formalin test, extract of seed and crop of *Anethum graveolens* provided no identifiable pain relief, but in late phase of formalin test, both forms of extract demonstrated an analgesic effect. This study demonstrates a difference in the quality of pain relief provided by the extracts at the early and late nociceptive phases of the formalin test with a superior effect during the second phase.

No analgesic effects were noted for either extract during the hot palate test. Yili et al. [21] reported that *Anethum graveolens* has been used traditionally to alleviate headaches and to reduce stomach, intestine and bladder inflammation. A previous study, somewhat similar to the present experiment, identified the anti-inflammatory effects of dill. It has been reported that topical administration of dill oil reduced formalin-induced paw edema in rats and that this effect was stronger than that following topical application of diclofenac [22]. In concurrence, the present study has also shown relief from inflammatory pain. Hekmatzadeh et al. [23] reported that boiled dill seeds reduced labor pain during childbirth. In addition, they demonstrated that the plant facilitated and accelerated the parturition process. Zagami et al. [24] reported beneficial aspects of this plant during the postpartum period.

Arachidonic acid and other lipid metabolites, serotonin, histamine, bradykinin and nerve growth factor can stimulate nociceptors and cause pain [25]. Prostaglandins also play a key role in causing pain. These substances have important intra and extra cellular physiological effects but in some pathological conditions, they may produce inflammatory responses and lead to sensations of pain. During tissue damage, cycloxygenase enzymes produce prostaglandins and these mediators, through binding to their receptors, can cause pain [26]. Earlier analysis of hydro-alcoholic extract of the plant essential oil identified the main constituents as carven, limonen and alpha flandrin (about 90 percent). Other compounds in the seed include flavonoids, coumarins, phenolic acids and steroids [27]. Based on the nature of constituents such as limonene and carven, the pharmacological properties of the seeds can be predicted to a degree. i) Limonene reduced the time the animals spent licking in the second phase of the formalin test but not in the first pain phase. Limonene reduces pain in this terminal phase probably by inhibiting synthesis or decreasing release of inflammatory mediators [28]. ii) The other important ingredient is carven. It is thought that carven shows analgesic effects through both central and peripheral mechanisms.

The hot-plate test can only evaluate the analgesic effect of drugs that act at the supraspinal level [29,30]. Extracts, at the studied dose, failed to demonstrate any significant influence in reducing the pain perception in the hot plate test, indicating a lack of potency in suppressing supraspinal nociception. At 15 and 30 minutes after injection of crop extract, and at 15, 30 and 60 minutes after injection of seed extract, results demonstrated a hypersensitivity to pain. A pervious study has demonstrated this effect for limonene [28]. Naseri and Heidari have reported that antispasmodic effects of *Anethum graveolens* may be due to blockade of voltage dependent calcium channels [31]. As these channels are involved in pain transmission [32], blockade of these channels by *Anethum graveolens* may partly account for the analgesic properties of this plant. In conclusion, our study demonstrates that *Anethum graveolens* L has anti-nociceptive properties. The mechanism for this may involve inhibition of the synthesis or action of inflammatory mediators.

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Declaration of Interest Statement

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

References

1. Stavri M, Gibbons S (2005) The antimycobacterial constituents of dill (Anethum graveolens). Phytother Res 19: 938-941.
2. Yazdanparast R, Bahramikia S (2008) Evaluation of the effect of Anethum graveolens L. crude extracts on serum lipids and lipoproteins profiles in hypercholesterolaemic rats. Daru 16: 88-94.
3. Ishikawa T, Kudo M, Kitajima J (2002) Water-soluble constituents of dill. Chem Pharm Bull (Tokyo) 50: 501-507.
4. Yazdanparast R, Alavi M (2001) Antihyperlipidaemic and antihypercholesterolaemic effects of Anethum graveolens leaves after the removal of furocoumarins. Cytobios 105: 185-191.
5. Hajhashemi V, Abbasi N (2008) Hypolipidemic activity of Anethum graveolens in rats. Phytother Res 22: 372-375.
6. Jana S, Shekhawat GS (2010) Anethum graveolens: An Indian traditional medicinal herb and spice. Pharmacogn Rev 4: 179-184.
7. Zheng GQ, Kenney PM, Lam LK (1992) Anethofuran, carvone, and limonene: potential cancer chemopreventive agents from dill weed oil and caraway oil. Planta Med 58: 338-341.
8. Panda S (2008) The effect of Anethum graveolens L. (dill) on corticosteroid induced diabetes mellitus: involvement of thyroid hormones. Phytother Res 22: 1695-1697.
9. Al-Ismail KM, Aburjai T (2004) Antioxidant activity of water and alcohol extracts of chamomile flowers, anise seeds and dill seeds. J Sci Food Agric 84: 173-178.

10. Satyanarayana S, Sushruta K, Sarma GS, Srinivas N, Subba Raju GV (2004) Antioxidant activity of the aqueous extracts of spicy food additives--evaluation and comparison with ascorbic acid in in-vitro systems. J Herb Pharmacother 4: 1-10.

11. Hosseinzadeh H, Karimi GR, Ameri M (2002) Effects of Anethum graveolens L. seed extracts on experimental gastric irritation models in mice. BMC Pharmacol 2: 21.

12. Tuntipopipat S, Muangnoi C, Failla ML (2009) Anti-inflammatory activities of extracts of Thai spices and herbs with lipopolysaccharide-activated RAW 264.7 murine macrophages. J Med Food 12: 1213-1220.

13. Lazutka JR, Mierauskiene J, Slapsyte G, Dedonyte V (2001) Genotoxicity of dill (Anethum graveolens L.), peppermint (Menthaxpiperita L.) and pine (Pinus sylvestris L.) essential oils in human lymphocytes and Drosophila melanogaster. Food Chem Toxicol 39: 485-492.

14. Seo SM, Kim J, Lee SG, Shin CH, Shin SC, et al. (2009) Fumigant antitermitic activity of plant essential oils and components from ajowan (Trachyspermum ammi), allspice (Pimenta dioica), caraway (Carum carvi), dill (Anethum graveolens), geranium (Pelargonium graveolens), and litsea (Litsea cubeba) oils against Japanese termite (Reticulitermes speratus Kolbe). J Agric Food Chem 57: 6596-6602.

15. Hunskaar S, Hole K (1987) The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. Pain 30: 103-114.

16. Khanavi M, Delnavazi MR, Nikoui V, Ostadhadi S, Bakhtiarian A (2012) Evaluation of analgesic effects of hydroalcoholic extract of Marrubium parviflorum by formalin test in mice. Asian J Plant Sci 11: 96-99.

17. Pourmohammadi N, Alimoradi H, Mehr SE, Hassanzadeh G, Hadian MR, et al. (2012) Lithium attenuates peripheral neuropathy induced by paclitaxel in rats. Basic Clin Pharmacol Toxicol 110: 231-237.

18. Hunskaar S, Fasmer OB, Hole K (1985) Formalin test in mice, a useful technique for evaluating mild analgesics. J Neurosci Methods 14: 69-76.

19. Tjølsen A, Berge OG, Hunskaar S, Rosland JH, Hole K (1992) The formalin test: an evaluation of the method. Pain 51: 5-17.

20. Rosland JH, Tjølsen A, Maehle B, Hole K (1990) The formalin test in mice: effect of formalin concentration. Pain 42: 235-242.

21. Yili A, Yimamu H, Maksimov VV, Aisa HA, Veshkurova ON, et al. (2006) Chemical composition of essential oil from seeds of Anethum graveolens cultivated in China. Chem Nat Compd 42: 491-492.

22. Naseri M, Mojab F, Khodadoost M, Kamalinejad M, Davati A, et al. (2012) The study of anti-inflammatory activity of oil-based dill (Anethum graveolens l.) extract used topically in formalin-induced inflammation male rat paw. Iran J Pharmaceut Res 11: 1169-1174.

23. Hekmatzadeh S, Mirmolaei S, Hoseini N (2012) The effect of boiled dill (Anethum graveolens) seeds on the long active phase and labor pain intensity. Armaghan Danesh 17: 50-59.

24. Zagami SE, Golmakani N, Kabirian M, Shakeri MT (2012) Effect of dill (Anethum graveolens Linn.) seed on uterus contractions pattern in active phase of labor. Indian J Traditional Knowledge 11: 602-606.

25. Dray A, Urban L, Dickenson A (1994) Pharmacology of chronic pain. Trends Pharmacol Sci 15: 190-197.

26. Zeilhofer HU (2007) Prostanoids in nociception and pain. Biochem Pharmacol 73: 165-174.

27. Jana S, Shekhawat GS (2010) Anethum graveolens: An Indian traditional medicinal herb and spice. Pharmacogn Rev 4: 179-184.

28. do Amaral JF, Silva MI, Neto MR, Neto PF, Moura BA, et al. (2007) Antinociceptive effect of the monoterpene R-(+)-limonene in mice. Biol Pharm Bull 30: 1217-1220.

29. Campos AR, Albuquerque FA, Rao VS, Maciel MA, Pinto AC (2002) Investigations on the antinociceptive activity of crude extracts from Croton cajucara leaves in mice. Fitoterapia 73: 116-120.

30. França DS, Souza AL, Almeida KR, Dolabella SS, Martinelli C, et al. (2001) B vitamins induce an antinociceptive effect in the acetic acid and formaldehyde models of nociception in mice. Eur J Pharmacol 421: 157-164.

31. Naseri MG, Heidari A (2007) Antispasmodic effect of Anethum graveolens fruit extract on rat ileum. Int J Pharmacol 3: 260-264.

32. Kim C, Jun K, Lee T, Kim SS, McEnery MW, et al. (2001) Altered nociceptive response in mice deficient in the alpha(1B) subunit of the voltage-dependent calcium channel. Mol Cell Neurosci 18: 235-245.

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