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RESEARCH ARTICLE

Antinociceptive and anti-inflammatory effects of isolated fractions from *Apium graveolens* seeds in mice

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Abstract

The antinociceptive and anti-inflammatory effects of the aqueous and hexane extracts obtained from *Apium graveolens* L. (Apiaceae) seeds were evaluated. Formalin and xylene-induced ear edema tests were used in mice. The fractions were administered intraperitoneally at doses of 100-500 mg/kg body weight (BW). Both extracts with the xylene-induced ear edema test showed significant anti-inflammatory activity at all doses as compared with control. Only the hexane fraction reduced the nociception produced by formalin solution in the first phase (0-5 min) at 300, 400, and 500 mg/kg BW, and in the second phase (20-30 min) at 500 mg/kg BW. It is concluded that the hexane fraction has major contribution to the overall antinociceptive activity. Both fractions showed remarkable anti-inflammatory effect which supported the traditional use of *Apium graveolens* in diseases associated with inflammation.

Keywords: *Anti-inflammatory activity; antinociceptive activity; Apiaceae;* Apium graveolens

Introduction

Apium graveolens L. (Apiaceae), commonly known as celery, is native to Southern Europe. This plant is now grown and consumed as a vegetable in many parts of the world (Duke, 2001). Celery seed is small, about 1-2 mm in length, oval, dark brown, and ribbed, with a characteristic odor. All parts of the plant, especially seeds, are spicy, carminative, diuretic, appetizer, stimulant, hypotensive, aphrodisiac, anti-inflammatory, and laxative (Satyavati & Rina, 1976). In Iranian traditional medicine, celery seeds are used to treat rheumatoid arthritis, gout, and kidney complaints (Zargari, 1990). The seeds are reported to possess antinociceptive, anti-inflammatory properties (Lewis, 1985; Al-Hindawi et al., 1989; Atta & Alkofahi, 1998) and antioxidant properties (Lewis et al., 1985; Momin et al., 2002). A previous study in our laboratory has revealed that hydroalcohol extract of celery seeds has anti-inflammatory and antinociceptive activity (Nasri et al., 2007). This study investigated the anti-inflammatory and antinociceptive effects of aqueous and hexane extract of celery seeds in order to find which part of the extract (hydrophilic or hydrophobic) is effective.

Materials and methods

Seeds of *A. graveolens* were purchased from a herbal store. The sample was botanically identified by Gh. Amin, from the Department of Pharmacognosy, Tehran University of Medical Science. The cultivated plant from these seeds was deposited at the Herbarium of the Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran (Voucher No. THE-6686).

Preparation of plant fractions

The seeds of *A. graveolens* were cleaned and ground to a fine texture. Then the powder (100 g) was extracted

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exhaustively by percolation at room temperature with a hydroalcohol solution (ethanol/water 75% v/v). The ethanol extract was concentrated under vacuum and then completely extracted with hexane. The residue was used in the experiments as the aqueous fraction because it was soluble in water. Hexane solution was evaporated using a rotary evaporator and the hexane fraction was obtained. For injections, the aqueous and hexane fractions were freshly dissolved in (DMSO/Normal saline 20% v/v).

Animals

Male NMRI mice weighing 20-25 g were obtained from Razi institute (Karaj, Iran). The animals were housed in standard laboratory conditions and allowed access to water and food *ad libitum.* They were maintained under constant temperature and in a 12h light-dark cycle and an environmental temperature of 21° ± 2°C. The experimental protocol was approved by the animal care review committee of TUMS (Tehran University of Medical Sciences).

Selection of doses

On the basis of Atta and Alkofahi's findings (1998) and our preliminary experiments on hydroalcohol extract of *A. graveolens* seeds, doses of 200 and 400 mg/kg BW, were found to be more effective. Therefore we examined doses of 100–500 mg/kg BW.

Xylene-induced ear edema

The method described previously by Atta and Alkofahi (1998) was used with slight modifications. Male mice were divided into groups of eight mice each. Twenty minutes after the i.p. injection of the aqueous and hexane extracts at doses of 100, 200, 300, 400, and 500 mg/kg BW, 0.03 mL of xylene was applied on the anterior and posterior surfaces of the right ear. The left ear was considered as control. Control animals received (DMSO/normal saline 20% v/v) or dexamethasone (15 mg/kg). Two hours after xylene application the mice were sacrificed and both ears were removed. Circular sections of both treated and untreated ears were taken using a cork borer with a diameter of 7 mm and weighed. The difference in weight between left untreated ear sections and right treated ear section was calculated.

Formalin test

The method described previously by Hunskaar and Hole (1987) was used with slight modifications. Briefly, pain was induced by injecting 0.02 mL of 2.5% formalin (40% formaldehyde) in distilled water in the subplantar of the right hind paw. Male mice were divided into groups of seven mice each. Aqueous and hexane extracts were administered intraperitoneally at doses of 100, 200, 300, 400, and 500 mg/kg BW, 20 min before formalin injection. The control group received the same volume (DMSO/normal saline 20% v/v). Morphine was used as positive control (10 mg/kg i.p.).

The animals were observed to evaluate the licking time (an index of nociception) during the first phase, neurogenic (0-5 min), and the second phase, inflammatory (20-30 min), after formalin injection.

Phytochemical screening

Phytochemical screening of the extracts was performed with thin layer chromatography on TLC aluminum sheets (Merck $60F_{254}$). Chloroform/methanol (2.5:1) and diethyl ether/toluene (1:1) saturated with acetic acid (10%) were used as solvents for the aqueous and hexane extracts, respectively. The spots were visualized under UV light at 254 and 365 nm.

Statistical analysis

Results were analyzed using one way ANOVA followed by Tukey-Kramer multiple comparison test. The data were expressed as mean values ± SEM and difference between the means of treated and control groups in the two phases was considered significant at $P < 0.05$.

Results

Topical application of the aqueous and hexane extracts (100–500 mg/kg BW) to the mouse ear resulted to potent suppression ($P < 0.001$) of acute edema induced by xylene (Table 1). The percentage inhibition offered by 200 mg/kg of hexane extract was the same as that of dexamethasone (15 mg/kg) 76.06 and 76.74%, respectively.

In the formalin test, aqueous extract showed no effect in the two phases (Table 2). These phases corresponded to neurogenic and inflammatory pains respectively. Hexane extract at doses of 300, 400, and 500 mg/kg significantly inhibited licking time in the first phase of the response by 50.7, 62.7, and 55.2 ($P < 0.01$), respectively, as compared with that of control group.

In the second phase of the response, only 500 mg/kg of hexane extract significantly inhibited licking time (92.5%, $P < 0.05$). Morphine was used as a positive control and acted throughout the phases (Table 2).

TLC has demonstrated that aqueous extract consists of flavonoid and phenolic acid. The hexane extract contains fatty acid, phthalid, and coumarins.

Table 1. Effect of *Apium graveolens* aqueous and hexane extract on xylene-induced ear swelling in mice.

Treatment (dose)	Ear swelling (mg)	Inhibition (%)
Control	13.2 ± 1.56	–
Dexamethasone (15 mg/kg)	3.07 ± 0.73 ***	76.74
Aqueous extract (100 mg/kg)	6.12 ± 1.13 ***	53.63
Aqueous extract (200 mg/kg)	5.67 ± 0.92 ***	57.04
Aqueous extract (300 mg/kg)	4.03 ± 1.11***	69.46
Aqueous extract (400 mg/kg)	4.31 ± 1.18 ***	67.34
Aqueous extract (500 mg/kg)	5.52 ± 1.07 ***	58.18
Hexane extract (100 mg/kg)	3.22 ± 0.77 ***	75.6
Hexane extract (200 mg/kg)	3.16 ± 0.83 ***	76.06
Hexane extract (300 mg/kg)	5.96 ± 0.74 ***	54.84
Hexane extract (400 mg/kg)	4.38 ± 1.21 ***	66.81
Hexane extract (500 mg/kg)	4.98 ± 1.05 ***	62.27

Values are the mean ± SEM ***P < 0.001, compared to control. Differences between groups were statistically analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test (n = 8).

Table 2. Effect of *Apium graveolens* aqueous and hexane extract on formalin-induced pain test in mice.

	Licking times			
Treatment (dose)	0–5 min	Inhibition	20–30 min	Inhibition (%)
Control	140.6 ± 19.6	–	54.7 ± 9.4	–
Morphine (10 mg/kg)	43.1 ± 14.6***	69.3	7.8 ± 7*	85.7
Aqueous extract (100 mg/kg)	117.5 ± 9.8	16.4	47.5 ± 13.4	13.1
Aqueous extract (200 mg/kg)	144 ± 26.7	-2.4	45 ± 13.4	17.7
Aqueous extract (300 mg/kg)	140 ± 0.4	0.44	26.2 ± 7.9	52
Aqueous extract (400 mg/kg)	92 ± 10.5	34.6	31.25 ± 12.7	42.8
Aqueous extract (500 mg/kg)	165 ± 11.6	-17.3	37.5 ± 14	31.4
Hexane extract (100 mg/kg)	83.6 ± 13	40.6	44.3 ± 18	18.9
Hexane extract (200 mg/kg)	67.5 ± 10.8	52	38.7 ± 6.8	29.2
Hexane extract (300 mg/kg)	69.3 ± 13.9**	50.7	24.3 ± 9.4	55.4
Hexane extract (400 mg/kg)	52.5 ± 12**	62.7	22.5 ± 12.7	58.8
Hexane extract (500 mg/kg)	61.6 ± 12.8**	55.2	4.2 ± 4.2*	92.5

Values are the mean ± SEM *P < 0.05, **P < 0.01, ***P < 0.001, compared to control. Differences between groups were statistically analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test (n = 7).

Discussion

In the present study the aqueous extract did not show significant effect on the first and second phase of formalin test. In the formalin test, the hexane extract significantly inhibited licking times in the first phase (at doses of 300, 400, and 500 mg/kg BW) and in the second phase (at dose of 500 mg/kg BW). The inhibition percentage of 500 mg/kg BW of hexane extract was comparable to morphine (centrally acting analgesic) as a positive control. The hexane extract showed significant effect in both phases, which indicates the central analgesic effects of this fraction. On the other hand, Atta and Alkofahi (1998) showed a dose-dependent analgesic protective effect of ethanol extract of *A. graveolens* in the hot-plate and acetic acid writhing test, which indicates the central and peripheral effects.

Our findings in this and previous research have revealed the acute anti-inflammatory effects of total and isolated fractions of *A. graveolens* seeds that are in agreement with Al-Hindawi et al. (1989), but Atta and Alkofahi (1998) did not observe an anti-inflammatory effect against acute inflammation after administration of *A. graveolens* total extract. This result may be due to the existence of various secondary metabolites in plant seed due to different environmental condition.

As preliminary phytochemical analysis indicated, the aqueous extract contains phenolic acids and flavonoids, which are documented anti-inflammatory constituents of some plants (Silvan et al., 1998; O'Leary et al., 2004). Investigations of celery have revealed the presence of apigenin and apiin as major flavonoids, which are known for anti-inflammatory properties (Perry, 1980; Mencherini et al., 2007). Recently, some studies have been done on the mechanism of the action of celery flavonoids. Apiin as a major constituent of *A. graveolens* leaf extract has shown significant inhibitory activity on nitrite production *in vitro* (Mencherini et al., 2007). It was reported by Sultana et al. (2005) that apigenin is an antioxidant and suppresses the generation of hydrogen peroxide and anti-immunoglobin E-induced histamine release. Also, there are reports demonstrating that flavonoids inhibit cyclooxygenase-2 (COX-2) activity. COX-2-catalyzed synthesis of prostaglandin E2 plays a key role in inflammation and its associated diseases (O' Leary et al., 2004).

From phytochemical analysis, it could be suggested that the anti-inflammatory and antinociceptive effects of the hexane extract may be due to their content of phthalids and coumarins. Other studies have demonstrated that various butylphthalids in

celery such as sedanolide and sedanenolide (Woods et al., 2001; Liu et al., 2005; Kurobayashi et al., 2006) and some furocoumarins (such as osthol, xanthotoxin, and isoimperation) produce significant anti-inflammatory and/or antinociceptive activities (Chen et al., 1995; Kontogiorgis et al., 2006; Peroutka et al., 2007). Hoult et al. (1994) reported that polyphenols such as coumarins and flavonoids act like non-steroidal-anti-inflammatory drugs (NSAIDs) in an acute inflammatory test (carrageenan test).

Clearly, there is a need for further studies to understand the mechanisms of the action of *A. graveolens* active ingredients. The results of the present study show that both aqueous and hexane extracts have potent anti-inflammatory effects, but only hexane extract has antinociceptive activity. Although in most research, major focus is on the hydrophilic substances of celery seeds (flavonoids), these findings show that hydrophobic substances are probably more effective in anti-inflammatory and antinociceptive activity.

In conclusion, this study can affirm the traditional usage of this extract for treating pain in swelling, rheumatoid arthritis, kidney complains and many diseases.

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