Original Article

Analgesic and Anti-Inflammatory Effects of Hydroalcoholic and Hexane Extracts of *Smyrniopsis aucheri* in Animal Models

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Abstract

Background: The fruits of Apiaceae family have been widely used in traditional medicine for the treatment of pain and inflammation. In this study, we evaluated the analgesic and anti-inflammatory effects of wild celery (*Smyrniopsis aucheri*) seeds, as a member of the Apiaceae family. **Methods:** Hydroalcoholic and hexane extracts of seeds were prepared and for the evaluation of analgesic activity, acetic acid, formalin, and hotplate tests in male mice (20–30 g) and for anti-inflammatory assessment carrageenan-induced paw edema in rats and croton oil-induced ear edema in mice were used. **Results:** Hydroalcoholic and hexane extracts (100–400 mg/kg) significantly reduced abdominal spasms in the acetic acid test. In the formalin test, the hydroalcoholic extract at doses of 200 and 400 mg/kg reduced the pain of the chronic phase while hexane extract was effective in both acute and chronic phases. In the hot plate test, both extracts were ineffective. In the carrageenan and croton tests, both extracts at a dose of 400 mg/kg significantly reduced edema. **Conclusions:** The results revealed the analgesic and anti-inflammatory effects of plant seed extracts. Due to the lack of response of the extracts in the hot plate test, it seems that the plant mainly has a peripheral analgesic effect.

Keywords: *Acetic acid test, analgesics, anti-inflammatory agents, carrageenan test, croton oil, formalin test, nociception tests, Smyrniopsis aucheri*

Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) and opioids are widely used to control pain.[1-3] Gastrointestinal disorders, gastric bleeding, kidney damage, and cardiovascular problems are some of the complications of NSAIDs. The opioids also have side effects such as nausea, vomiting, respiratory depression, dependence, and tolerance.[4] For this reason, the use of herbal medications with anti-inflammatory and analgesic properties is increasing. Several plants of Apiaceae family including *Foeniculum vulgare*, *Heracleum persicum, Coriandrum sativum*, *Bunium persicum* and *Apium graveolens*, and *Carum copticum* have shown anti-inflammatory and analgesic effects.[1,5-9] *Kelussia odoratissima*, *A. graveolens*, and wild celery (*Smyrniopsis aucheri*) are the three species of celery.[10]

Anti-inflammatory effects were reported for *K. odoratissima* in the acute colitis induced by acetic acid and carrageenan-induced paw edema in rats.[11,12] This plant has also been used traditionally for colics and gastrointestinal disorders.[13] *Apium graveolens* is also effective in suppression of pain and inflammation.[14] Based on the analgesic and anti-inflammatory effects of *K. odoratissima* and *A. graveolens*, this study aimed to evaluate the analgesic and anti-inflammatory effects of wild celery (*S. aucheri*) in animal models.

Materials and Methods

Materials and chemicals

Carrageenan and croton oil (Sigma, USA), acetic acid and formalin (Merck, Germany) were used in this study. *Smyrniopsis aucheri* seeds were purchased from Pakan Bazr Company (Isfahan, Iran).

Preparation of the hydroalcoholic extract

Plant seeds weighed and then ground. Ethanol (80%) was added to the powder (with a 3 to 7 ratio), and the mixture was kept in the laboratory for 48 h. After that, the solution was filtered three times using a Buchner funnel, and the resulting extract was subsequently dried and condensed using a rotary device.[15,16]

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Preparation of the hexane extract

One hundred grams of powdered wild celery seeds was soaked in 600-mL hexane for 48 h. Then the solution was filtered by a Buchner funnel and evaporated using a rotary device.[17]

Animals

Male Swiss mice (20–30 g) and male Wistar rats (180–220 g) were used. Animals housed under standard conditions with 12/12 h light-dark cycles with free access to water and food. The animals were transferred to the laboratory 2 days before starting the experiments to acclimatize to the test environment. Animal procedures were approved by the Ethics Committee of Isfahan University of Medical Sciences (IR.MUI.REC.1396.3.506).

Animal tests

Acetic acid test

Five groups of male mice (n = 6) received vehicle (1% Tween 80 in normal saline), different doses (100, 200, and 400 mg/kg) of hydroalcoholic or hexane extracts, or indomethacin (10 mg/kg) intraperitoneally (i.p.). After 30 min, 1% acetic acid (v/v) was injected i.p. and 10 min later, the abdominal spasm was counted for 10 min.[7]

Formalin test

Formalin (20 μL) was injected in the animal's right paw subcutaneously 30 min after i.p. injection of vehicle (1% Tween 80 in normal saline), each extract (100, 200, and 400 mg/kg) or morphine (10 mg/kg). The paw licking time was measured at 0–5 and 20–40 min after the injection of formalin.[18]

Hot plate test

A hot plate (55°C) was used for the induction of pain in this test. At the beginning of the evaluation, the time it took for the animal to respond to pain was recorded as the control latency. Two groups of mice (n = 6) received hydroalcoholic, or hexane extract at a dose of 400 mg/kg i.p. The standard group received morphine (10 mg/kg, i.p.). After the injection of drugs, the reaction time of the animal was measured every 30 min for 2 h, and the analgesia was calculated.[19]

Carrageenan test

Vehicle (1% Tween 80 in normal saline, 1 mL/Kg, i.p.) was injected to animals of control group. Two groups of male rats (n = 6) received either hydroalcoholic or hexane extract (400 mg/kg, i.p.). The fourth group (reference group) received indomethacin (10 mg/kg, i.p.). Six animals were included in each group. Thirty minutes later, 100 μL of carrageenan (1% w/v) was injected into the right paw of the animals. The volume of the rat paw was measured using a plethysmograph (Ugo Basil, Italy) just before the injection of carrageenan and 4 h after that and the difference between the paw volumes was reported as an index of edema.[20]

Croton test

Control animals received (1% Tween 80 in normal saline, 10 mL/Kg, i.p.) and test animals received either hydroalcoholic or hexane extract (100, 200, and 400 mg/kg i.p.). Indomethacin (10 mg/kg, i.p.) was injected as the standard drug. Thirty minutes later, 20 μL of croton oil solution was applied to the inner part of the animal's right ear. After 6 h, the animals were sacrificed and disc samples with 6 mm diameter were prepared from both ears. The discs were weighed and the difference between the weights of the left and right disks was reported as ear edema.[8]

Statistical analysis

Data were expressed as mean ± SEM and group differences were analyzed by oneway ANOVA followed by Scheffe *post hoc*. A value of $P < 0.05$ was considered significant.

Results

Pharmacognosy

From 100 g of powdered wild celery seeds, 23 g of condensed hydroalcoholic extract and 19 g of condensed hexane extract were obtained.

Pharmacology

In the acetic acid test, both hydroalcoholic and hexane extracts of wild celery seed at all doses (100, 200, and 400 mg/kg) significantly reduced the number of abdominal spasms. A dose of 10 mg/kg of indomethacin reduced the number of abdominal spasms by 87.5% [Figures 1 and 2].

In the formalin test, the hydroalcoholic extract was ineffective in acute phase, but in the chronic phase, the doses of 200 and 400 mg/kg reduced the time of licking and reduced pain.

In this test, the hexane extract (100, 200, and 400 mg/kg) and morphine (10 mg/kg) significantly ($P < 0.001$) reduced paw licking time in both acute and chronic phases [Table 1].

In the hot plate test, neither hydroalcoholic nor hexane extracts demonstrated any significant analgesic effects (Data not shown).

In the carrageenan test, both hydroalcoholic and hexane extracts at a dose of 400 mg/kg decreased inflammation ($P < 0.01$). Indomethacin as the standard drug (10 mg/kg, i.p.) reduced paw inflammation by 80.2% [Figure 3].

In the croton test, both hydroalcoholic and hexane extracts significantly reduced inflammation and edema only at a dose of 400 mg/kg so that compared with control group, they inhibited ear edema by 44.3% and 74.6%, respectively ($P < 0.05$ for hexane extract and $P < 0.001$ for hydroalcoholic extract). Indomethacin also at a dose of

10 mg/kg reduced the inflammation and the edema of the animal's ear by 77.9% [Figure 4].

Discussion

In this study, *S. aucheri* seeds showed anti-inflammatory and analgesic effects. The acetic acid, formalin and hotplate tests were used to evaluate the analgesic effect.

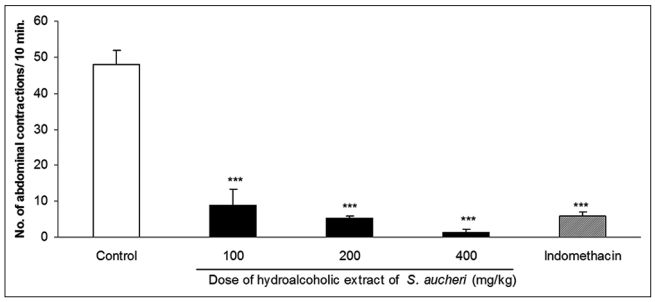

Figure 1: Effect of hydroalcoholic extract of *S. aucheri* on the abdominal spasm induced by acetic acid in mice. The vehicle (1% Tween 80 in normal saline), hydroalcoholic extract, and indomethacin (10 mg/kg) were injected intraperitoneally 30 min before the injection of 1% acetic acid (10 mL/kg). The values represent the mean ± SEM of abdominal spasms. ***$P < 0.001$ compared to the control group (oneway ANOVA followed by Scheffe *post hoc*)

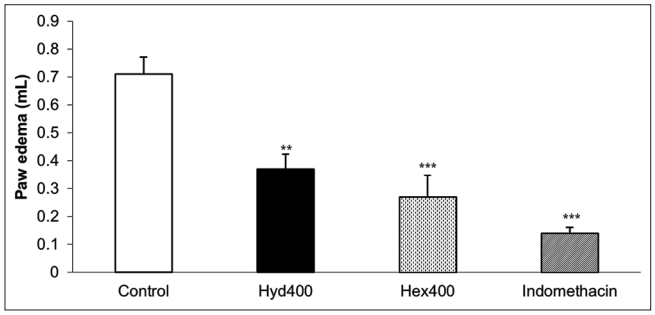

Figure 3: Effect of hydroalcoholic and hexane extracts of S. aucheri on carrageenan-induced paw edema in rat. The control group received the vehicle (1% Tween 80 in normal saline, 10 mL/kg), the test groups received a dose of 400 mg/kg of hydroalcoholic and hexane extract, and the standard group received indomethacin (10 mg/kg) by intraperitoneal injection. Thirty minutes later, 1% carrageenan (W/V) at a dose of 100 µL was injected subcutaneously into the paws of the rats and paw volume was measured at 0 and 4 h after the injection. Data show mean ± SEM of paw edema ($n = 6$). **$P < 0.01$; ***$P < 0.001$ compared to the control group (oneway ANOVA followed by Scheffe *post hoc*)

In the acetic acid test, both hydroalcoholic and hexane extracts of the plant's seed were able to reduce the number of acetic acid-induced abdominal spasms significantly.

In this non-specific test, many drugs such as opioids, antihistamines, NSAIDs, and clonidine display a positive response. This test is considered as a good animal model for visceral pain, and the results of this study demonstrate that the seeds of *S. aucheri* have a good potential for controlling visceral pain such as stomachaches, intestinal and renal colic.[19]

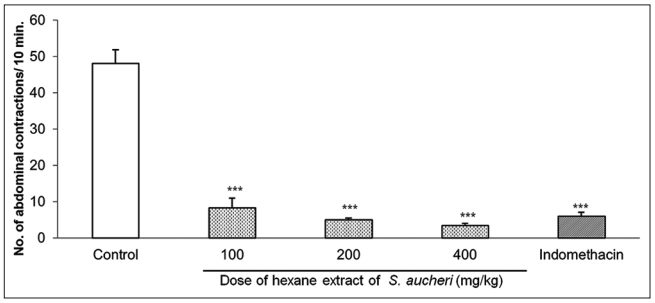

Figure 2: Effect of hexane extracts of *S. aucheri* on the abdominal spasm induced by acetic acid in mice. The vehicle (1% Tween 80 in normal saline), hydroalcoholic extract, and indomethacin (10 mg/kg) were injected intraperitoneally 30 min before the injection of 1% acetic acid (10 mL/kg). The values represent the mean ± SEM of abdominal spasms. ***$P < 0.001$ compared to the control group (oneway ANOVA followed by Scheffe *post hoc*)

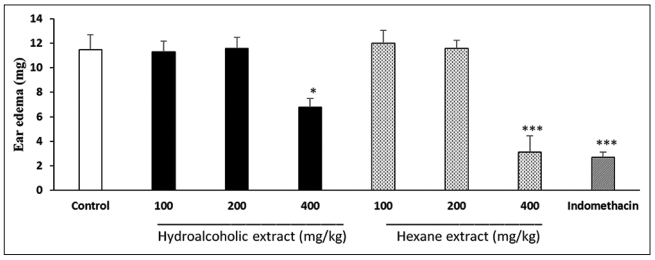

Figure 4: Effect of hydroalcoholic and hexane extracts of *S. aucheri* on croton oil-induced ear edema in mice. Thirty minutes after vehicle (1% Tween 80 in normal saline) or drug treatments, croton oil (5 mg/mL, 20 µL) was applied to the inner surface of the right ear of mice. After 6 h animals were sacrificed and discs with a diameter of 6 mm were cut from both ears and weighed. The values represent the mean ± SEM ($n = 6$). *$P < 0.05$; ***$P < 0.001$ compared to the control group (oneway ANOVA followed by Scheffe *post hoc*)

Table 1: Effect of hydroalcoholic and hexane extracts of *S. aucheri* on formalin-induced licking behavior

Group	Dose	Paw licking time (s)			
		First phase (0-5 min)		Second phase (20-40 min)	
		Mean±SEM	Inhibition (%)	Mean±SEM	Inhibition (%)
Control	---	27.5±5.1	---	128.4±13.1	---
Hydroalcoholic	100 mg/kg	26.8±6.6	2.5	104.6±22.7***	18.5
	200 mg/kg	20.3±6.3	26.2	38.8±7.8***	69.8
	400 mg/kg	24.3±13.7	11.6	2.6±0.4***	98.0
Hexane	100 mg/kg	7.3±1.1***	73.4	40.8±10.7***	68.2
	200 mg/kg	3.3±2.9***	88.0	36.3±8.4***	71.7
	400 mg/kg	7.0±5.4***	74.5	2.1±1.4***	98.4
Morphine	10 mg/kg	2.0±0.9***	92.7	2.8±1.1***	97.8

*** $P<0.001$ compared with control group (oneway ANOVA followed by Scheffe *post hoc*)

Formalin test, which is a model for chronic pain, is a biphasic test.[19] The initial phase (0–5 min) is induced by the stimulation of receptors and the transmission of signals by A delta fibers. The second phase is an inflammatory response and the pain is transmitted by C fibers.[21] Our results demonstrated that in the first phase, only hexane extract reduced formalin-induced neurogenic pain significantly ($P < 0.01$). In the second phase, both hexane and hydroalcoholic extracts reduced pain effectively ($P < 0.05$ and $P < 0.001$, respectively). Investigations have shown that drugs with central analgesic effects, such as opioids (morphine), can inhibit both phases of the formalin test, but medications that have peripheral analgesic effect only inhibit the second phase (inflammatory phase).[19,21] Based on the results of the second phase of the formalin test, which is an inflammatory phase, and considering the anti-inflammatory effect of these two extracts in the carrageenan test, it is likely that the analgesic effect occurs because of the inhibition of inflammation.

Hot plate test is a proper technique to evaluate drugs that have central analgesic effects.[19] Due to the lack of response of both extracts in this test it seems that the plant does not appear to have a central analgesic effect.

GC/MS analysis of the essential oil of the wild celery showed that its major components are alpha-bisabolol (19.91%), alpha-pinene (15.10%), and beta-pinene (6.58%).[22]

According to the study of Santos *et al.* (1998) alpha-pinene (400 mg/kg) has an analgesic effect in the formalin test.[23] The alpha-pinene content in the wild celery essential oil was reported to be 15.10% and probably has a crucial role in the analgesic effect of wild celery.[22]

The anti-inflammatory effect of the wild celery seeds was studied through carrageenan and croton tests. The increase in paw volume seen shortly after the administration of carrageenan is due to release of histamine.[24] The secretion of prostaglandins and leukocyte migration are involved later.[25] In carrageenan test both hydroalcoholic and hexane extracts had an anti-inflammatory effect. Croton test also confirmed the results of carrageenan test.

Drugs inhibit inflammation through many underlying mechanisms, including the inhibition of the arachidonic acid metabolism, inhibition of the prostaglandin synthesis, inhibition of the histamine release, etc.[26] However, the anti-inflammatory mechanism of the hydroalcoholic and hexane extracts of the wild celery seeds is unclear, and further studies should be undertaken to unveil the mechanism of action.

Some studies also suggest that flavonoids have been shown to have anti-inflammatory effects with the mechanism of inhibiting the enzyme producing eicosanoids and inhibiting the secretion of histamine from mast cells.[27] The presence of flavonoids in the hydroalcoholic extract of wild celery seeds was shown in a previous study[28] and the mechanism of anti-inflammatory action of the plant might be partly due to its flavonoids.

Conclusions

In general, it can be stated that hydroalcoholic and hexane extracts of wild celery seed have analgesic and anti-inflammatory effects, but understanding the underlying mechanisms of them requires further studies.

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Conflicts of interest

There are no conflicts of interest.

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