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Original article

Evaluation of Anti-Nociceptive and Anti-Inflammatory Activities of *Apium graveolens* L. Roots Extract in Mice

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Abstract

Background and objectives: Pain is an unpleasant feeling which affects the quality of life and relieving from pain is an important goal in many treatment protocols. *Apium graveolens* L. (celery) has been considered as sedative, analgesic, carminative, antispasmodic and diuretic in Iranian traditional medicine. The aim of present study was to evaluate the anti-nociceptive and anti-inflammatory effect of celery root in mice. **Method:** Repeated maceration was employed as extraction method. Hot plate and acetic acid writhing test were conducted to assess analgesic effect of celery root. The extract was also evaluated for anti-inflammatory probable effect by formalin induced ear edema and xylene induced paw edema tests. **Results:** Total flavonoid content determined by aluminum chloride colorimetric method was 0.0625 mg quercetin/g extract. No significant difference was observed between the positive control group which received morphine and test group in hot plate test and the most effective dose of celery root extract was 200 mg/kg, while the frequency of writhings was significantly different in all test groups in comparison with control group (p value< 0.05), the extract (100, 200 and 400 mg/kg) significantly suppressed inflammation in formalin induced edema assay, 60 and 120 min after injection. The results of xylene test also demonstrated notable antiedematogenic effect in applying 200 and 400 mg/kg of extract. **Conclusion:** Celery root has analgesic and anti-inflammatory effects which might be related to the flavonoids and resins present in the species.

Keywords: *Apium graveolens*; hydroalcoholic extract; inflammation; mice; nociception

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Introduction

More than 26% of Americans experience a painful problem of any sort which lasts for 24 hours. The estimated cost of chronic pain is 100 billion dollar in a year including lost income and lost productivity [1].

In many pathologic conditions such as wounds and trauma, pain is a common sensation which is one of the most frequently observed symptom and as a consequence, relieving of pain and pain related phenomenon, inflammation, is one of the

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major goals in treatment procedures. Pain management is a big challenge especially in patients with chronic and sub-acute pain; therefore, there is a requirement for an approach which contains different aspects [2] where principal target of effective pain control is to increase threshold of pain sensation to improve quality of life. Interest to natural products which derived from medicinal plants as alternatives for present therapies has been growing [3]. *Apium graveolens* L., Apiaceae, is commonly known as celery which is native to Mediterranean region [4]. Celery has been used as an aphrodisiac, anthelmintic, antispasmodic, carminative, diuretic, emmenagogue, laxative, sedative, stimulant, and detoxifying agent [5]. The extract and essential oil of celery leaves have demonstrated anti-inflammatory properties as well as relieving pain and swelling in human joints [6,7]. Hydroalcoholic extract of celery fruit has showed antinociceptive effects that partially can be explained via N-Methyl- D- Aspartate (NMDA) receptors in acute phase and nitric oxide (NO) synthesis pathways in chronic phase [8]. Other studies demonstrated broad range of biological activities for celery including antioxidant [9] antimicrobial [10], hepatoprotective [11,12], anti-gastric ulcers [13]. Celery leaves juice is effective in decreasing lipid peroxidation in doxorubicin treated animals [14]. *Apium graveolens* L. and some other plants are considered as antinociceptive agents in folk medicine [15]. This can be related to the high level of several bioactive substances such as polyphenols, flavonoids, phenolic acids, coumarins, furanocoumarins and sterols [16]. The aim of present study was to investigate in the effect of celery hydroalcoholic root extract on pain and inflammation in mice.

Material and Methods

Ethical consideration

All animal experiments were in accordance with the NIH Guide for Care and Use of Laboratory Animals (NIH Publications No. 80-23). The animal studies in this research were approved by ethic committee of Shahid Sadoughi University of medical sciences, Yazd, Iran. (Letter NO.72199; 7/5/2014)

Plant collection

The celery roots were purchased from farmlands of Isfahan province, Iran, during august 2015 and a voucher specimen was deposited at the Herbarium of Faculty of Pharmacy, Shahid Sadoughi University of Medical Sciences, Yazd, Iran (No. SSU0033).

Extraction

The roots were shade-dried and milled to prepare coarse powder by using a grinder, followed by extraction using maceration method. One hundred g of dried powder was soaked in 1.4 L of ethanol 80% for two days and this procedure was repeated for 3 times. The extract was dried by vacuum evaporator and diluted to prepare 200, 300 and 400 mg/kg using normal saline as solvent.

Animals

One hundred and forty male albino mice (25-30 g) were purchased from animal house of Medical School, Shahid Sadoughi University of Medical Sciences, Yazd, Iran. They were kept at controlled temperature (22±2 °C) with a 12:12 hour light-dark cycle and with standard lab chow and tap water ad libitum. The mice were divided into 20 groups where each group contained 7 mice. The experiments reported in the study were carried out in accordance with current ethical guidelines for the investigation of experimental pain in conscious animals [17]. The number of animals and intensities of noxious stimuli were limited to the minimum necessary for demonstration of the consistent effects of the drug treatments.

Total flavonoid content

Total flavonoid was determined by aluminum chloride colorimetric method with some modifications [18]. One mL of the extract with 4 mL of double distilled water was poured into a 10 mL volumetric flask. Then, 0.3 mL of 5% $NaNo_2$ was added to the mixture. After 5 min, 0.3 mL of AlCl3 (10%) was also added to the flask. Finally, 2 mL of NaOH (1M) was added at 6^{th} min. All of components were mixed completely and the absorbance was measured at 510 nm. The result was expressed as mg quercetin equivalent per gram of the extract ($y = 14.603x-0.0035$, $r^2 = 0.9993$).

Hot-plate test

The test was carried out according to the hot plate test routine procedure with some modifications**.** Briefly, before the initial of experiment, the mice

were habituated to a Plexiglas cylinder for 5 min. Then hot-plate apparatus was set at 54±0.1 °C and the mice were placed into an acrylic cylinder (20 cm in diameter) on the heated surface. The time in seconds between placement and licking of mice hind paws or jumping (whichever occurred first) was recorded as the response latency or reaction time. Cut off time was set at 45 seconds to prevent tissue damage [19]. After baseline record, the mice were immediately administered with tests and control substances. They intraperitoneally (i.p.) received injections of the vehicle (normal saline, 10 mL/kg), celery root extract at three doses (200, 300, 400 mg/kg) and morphine 8 mg/kg^{-} as the negative, test and positive control groups, respectively. The reaction time of each mouse was observed at 0, 15, 30, 45 and 60 min after injection. The results were presented as mean ± SD in table 1 and the maximum possible effect (%MPE) was calculated according to the equation described below to find the time with maximum nociceptive effect.

%MPE = (Test latency- Control latency) × 100/ (cut off-control latency)

Acetic acid-induced writhing test

The test was performed based on Collier et al. study [20] where test groups were injected with the extract (200, 300 and 400 mg/kg) followed by intraperitoneal injection of 0.3% acetic acid after 15 min to cause a typical stretching response. After 5 min, the mice were kept in separated cages and stretching or writhing of each mouse was counted for a period of 30 min by an observer who was blinded to the groups. The analgesic effect was determined by calculating the mean reduction in the frequency of constrictions for each concentration versus the normal saline control group. The percentage inhibition of writhing was calculated by using the following equation:

%inhibition: 100× (control mean writhings- treated mean writhings)/control mean writhings

Xylene-induced ear edema

Three groups of mice received celery root extract (100, 200 and 400 mg/kg, i.p.) and 2 groups received normal saline and dexamethasone (15 mg/kg, I.P.) as the negative and positive groups, respectively. After 30 min, xylene (0.03 mL) was applied to the both anterior and posterior surfaces of the right ear. Then the mice were sacrificed 2 h after xylene application by performing cervical dislocation and a circular section from each ear was cut using a cork borer (7 mm diameter) and weighted immediately. The weight gain was measured by subtracting the weight of the untreated left ear section from the treated right ear section [21].

% inhibition :(Wc-Wt)/Wt × 100

Where Wt is the weight of treated right ear and Wc is the weight of untreated left ear.

Formalin induced inflammation

The mentioned concentrations (100, 200 and 400 mg/kg, i.p.) of the extract were injected intraperitoneally to the mice and after 30 min, 0.02 mL of formaldehyde 2.5% was injected subcutaneously into the mice right hind paw to induce inflammation. Dexamethasone (15mg/kg) and normal saline were consumed as the positive and negative control groups, respectively. The changes in paw thickness (mm) were measured by caliper every hour for 3 times [22].

% Inhibition: (Tc-Tt)/Tt × 100

Where Tt is paw thickness of mice treated with the extract and Tc is the thickness of paw of control group at the same time.

Data analysis

Data were expressed as mean± standard deviation (S.D) and the result were analyzed using one-way analysis of variance (ANOVA) followed by Turkey's- post hoc test where p value<0.05 was considered as significant. Graph Pad Prism 5 program was used to analyze the whole behavioral studies.

Results and Discussion

Total flavonoid content of the extract was 0.0625 mg quercetin/g extract. The latencies after injection (base line latency) were statistically analyzed by the test of ANOVA and no significant difference was seen between the test and control groups. The maximum efficacy was observed when 200 mg/kg of root extract was injected (after 30 min). Latency of response in different groups has been shown in table 1. In the case of maximum possible effect (MPE), the most effective dose was observed at 30 min after extract injection (figure 1).

Table1. The latency time observed by hot plate test in mice groups (n=7)

Group (mg/kg)	Latency time (s)				
	0	**15**	**30**	**45**	**60**
Control	9.64 ±1.60	11.20±2.40	11.6±7.40	10.3±2.54	8.1±2.50
C 200	9.42±1.59	13.53±3.37*	18.33±4.56**	13.92±3.90*	10.41±2.60
C 300	9.73±2.75	14.16±1.72*	14.36±1.12*	14.04±2.27*	10.8±2.27
C 400	9.9±2.66	14.16±1.72*	14.36±1.12*	14.04±2.27*	12.3±2.27
Morphine 8	10.12±1.90	14.25±3.70*	16.73±4.40**	15.99±2.70**	14.1±4.00*

C: celery root extract; *: p<0.05 (compared to negative control); **: p< 0.01 (compared to negative control)

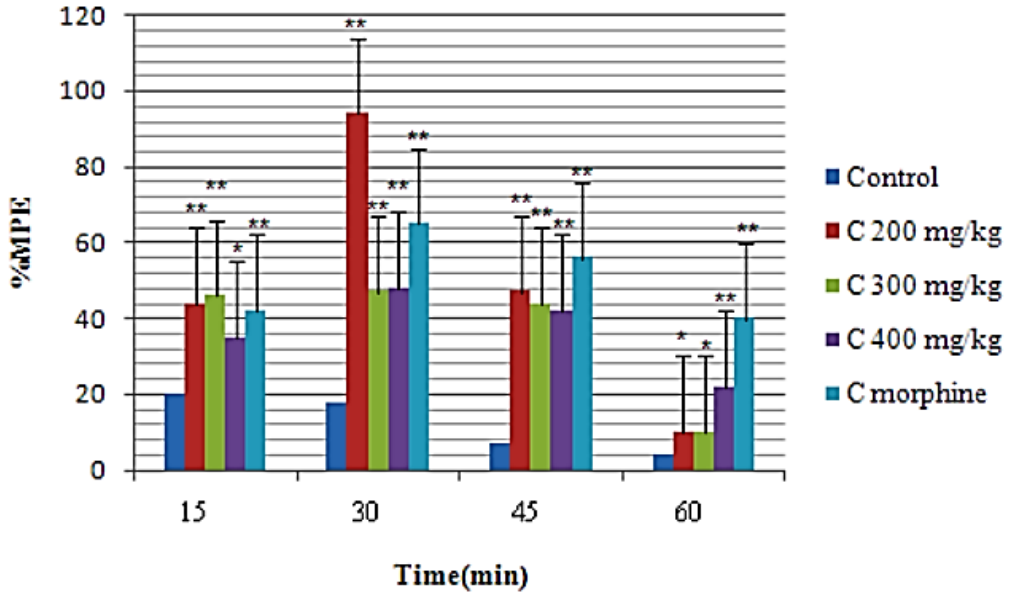

Figure 1. The percentage of maximum possible effect (%MPE) of different treatments on acute pain inhibition at different time points in hot plate test (n=7); *: p<0.05 (compared to negative control); **: p< 0.01 (compared to negative control)

Table 2. The effect of celery root extracts on number of writhings and percentage of inhibition on acetic acid-induced writhing test in groups (n=7)

Group (mg/kg)	Number of writhing	Percentage of inhibition (%)
Control	82.4±2.3	-
C 200	57.8±1.9*	29.4
C 300	35.1±2.8**	57.1
C 400	44.5±2.6*	45.6
Diclofenac 30	30.4±2.7**	62.8

C: celery root extract; *: p<0.05(compared to negative control); **: p< 0.01(compared to negative control)

The abdominal constriction test described by Collier et al. [20] was used to determine the analgesic activity against chronic pain.

The frequency of writhings was significantly different in all test groups in comparison with the control group (p<0.05) where the concentration of 300 mg/kg resulted in the least writhing number (35.1±2.8) with 57.1% inhibition. The results have been presented in (table 2).

The results were expressed as change of paw thickness (mm) of right hind paw in comparison with the un-injected left hind paw.

Significant inhibitory effect was observed in different extract concentrations at 60 and 120 min subsequent to injection (p<0.05). The most reduction in changes of paw thickness compared to control group was demonstrated by 200 and 400 mg/kg at the time of 60 min respectively (table 3).

Injection of celery root extract suppressed ear edema (200 and 400 mg/kg) and significant difference was observed between treated and untreated group, ears weight after injection of 200 and 400 mg/kg of extract were 0.025±0.005 and 0.018±0.005 while the difference was not observed for the lower concentration (100 mg/kg) significantly (ear weight: 0.036±0.005, ear weight of control: 0.038±0.005, p< 0.05). The results have been presented in table 4.

The present study evaluated the anti-inflammatory and anti-nociceptive effects of celery root extract. According to the findings, the extract showed anti-nociceptive effect in both related tests where the maximum effectiveness was achieved by the concentration of 200 mg/kg, ip.

Table 3. The effect of celery root extracts on formalin-induced inflammation in hind paw of in mice

	Diameter of paw(mm)				
Concentrations (mg/kg)	**Control**	**C 100**	**C 200**	**C 400**	**Dexamethasone 15**
Time(min)					
30	0.54±0.1	1.09±0.4	0.7±0.3	0.76±0.2	0.07±0.01**
60	0.71±0.2	0.45±0.1*	0.45±0.1*	0.41±0.1*	0.21±0.05**
120	0.72±0.2	0.48±0.1*	0.48±0.1*	0.45±0.1*	0.31±0.04**

C: celery root extract; *: p<0.05(compared to negative control); **: p< 0.01(compared to negative control)

The observed anti-nociceptive activity in both tonic (writhing test) and phasic (hot plat) nociceptive models indicated that the celery extract can affect central and peripheral anti-nociceptive activities [23]. The mentioned effect of celery leaves extract could be relevant to the flavonoid such as apigenin, which is playing an important role in cyclooxygenase pathway of inflammation at the peripheral route [22]. The mentioned anti-inflammatory effect was less observed in high concentration of the extract, according to SAR (structure-activity relationship) of flavonoids, presence of some functional groups such as ortho-dihydroxy groups at the B ring and OH at C-5 position of the A ring contributes in this effect. In high concentration of root extract these functional groups are involved in intermolecular bonds and exit from monomer state. So the anti-inflammatory effect is less than lower concentration [24]. Anti-inflammatory effect of celery root extract was investigated by xylene and formalin-induced inflammation tests where the formalin induced edema assay includes two stages containing the acute phase in which histamine and serotonin are released and the chronic phase which is mostly related to the release of prostaglandins. The xylene test also induces the release of P substance as a neurogenic inflammatory mediator from the sensory neurons which is related to the acute phase of inflammation [25,26].

Table 4. The effect of celery root extracts on xylene-induced ear edema in mice

Group (mg/kg)	**Weight gain of ear(g)**	**Percentage of inhibition (%)**
Control	0.038±0.005	-
C 100	0.036±0.005	5.2
C 200	0.025±0.005*	34.2
C 400	0.018±0.005*	52.6
Dexamethasone 15	0.015±0.005**	60.5

C: celery root extract; *: p<0.05(compared to negative control); **: p< 0.01(compared to negative control)

Therefore, the findings demonstrated that celery root may induce the ability of decreasing the inflammatory mediators both in the acute and chronic phases of the mentioned tests. Although the results showed anti-inflammatory effect in both tests, it was more considerable in xylene induced ear edema assay (p< 0.05). The findings of formalin-induced paw edema test demonstrated notable difference at 60 and 120 min after injection of the extract that are consistent with other study [25].

There are many reports describing the anti-nociceptive and anti-inflammatory activities of different parts of celery. According to the study done by Nasri et al., hydroalcoholic extract of celery fruit demonstrated anti-nociceptive activity in acute and chronic phase. Moreover, it was mentioned that a part of anti-nociceptive effect could be via NMDA receptors in acute phase and inhibition of NO synthesis pathways in chronic phase of pain [8]. The aqueous and hexane extracts obtained from celery fruit were evaluated by Ramazani et al. and the result noted that both extracts showed significant anti-inflammatory activity while only the hexane extract reduced the nociception induced by formalin solution [22].

Anti-inflammatory activity of the aqueous extract of celery stem has been illustrated by Lewis and Tharib in two animal models. It has been concluded that the major anti-inflammatory effect was due to unidentified polar substances [27]. The composition of celery stalk consists of different kind of pectic polysaccharides which have anti-inflammatory activity [28] that could be investigated in root extract. It is also rich in flavonoids including apigenin and quercentin that have presented anti-inflammatory effects in many medicinal plants [29]. Presence of the same mentioned components in the root of celery may elucidate the mechanism of anti-nociceptive and anti-inflammatory in the present study. In conclusion, the results of this research reinforce the hypothesis of analgesic activity of celery hydroalcoholic root extract which is conformed to its traditional use as an anti-nociceptive and anti-inflammatory agent. However, more detailed animal experiments, including immunologic studies, should be conducted to determine the

inflammatory mediators releasing in the inflammatory process. It is also recommended that future researches focus on the analgesic and anti-inflammatory effects of other fractions of the celery root extract via certain mechanism of action.

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Author contributions

Ali Mohammad Ranjbar designed the study, performed data analysis and supervised the project; Alireza Vahidi was involved in designing the study; Mohammad Ebrahim Rezvani and Vahid Ramezani revised the method of study; Minoo Boroumand reviewed and analyzed data, drafted the paper and finalized the manuscript; Yadollah Jahani implemented the project and data analysis.

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.

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Abbreviations

NMDA: N-Methyl-D-Aspartate; NO: Nitric Oxide; TLC: Thin Layer Chromatography; MPE: Maximum Possible Effect; NIH: National Institute of Health; ANOVA: one-way analysis of variance