

SPASMOLYTIC ACTIVITY OF THE AQUEOUS AND ETHANOL CELERY LEAVES (*APIUM GRAVEOLENS* L.) EXTRACTS ON THE CONTRACTION OF ISOLATED RAT ILEUM

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Celery (*Apium graveolens* L.) is a plant species in the family Apiaceae, which has been used in traditional medicine for the treatment of gastrointestinal diseases. This study investigated the effects of aqueous and ethanol extracts from celery leaves on intestinal contractility.

Air-dried and powdered leaves were extracted with distilled water and 96% ethanol, respectively. The activities of the extracts on the smooth muscle contractions were evaluated using isolated rat ileum model. The isolated rat ileum was mounted in a 10ml tissue bath.

The results suggest that the cumulative concentrations of the extracts of celery statistically significantly inhibited spontaneous rat ileum contractions ($p < 0.01$). The extracts dose-dependently reduced the contractile effects of acetylcholine on the isolated ileum ($p < 0.05$). Ethanol extract exhibited significantly greater relaxant activity than the aqueous extract ($p < 0.05$). These results suggest that the aqueous and ethanol extracts of celery leaves can produce the inhibition of the spontaneous rat ileum contractions and contractions induced by acetylcholine.

These data indicate that celery extracts act as intestinal smooth muscle relaxants, which justifies their use in gastrointestinal disorders. *Acta Medica Medianae* 2015;54(2):11-16.

Key words: *Apium graveolens* L., intestinal motility, rat, ileum

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Introduction

Celery (*Apium graveolens* L.) is a plant species of the family Apiaceae, which has been used as a spice and as a traditional medicinal plant. It originates from the coasts of western and northern Europe. Nowadays, this plant is cultivated all over the world. Celery has been planted for over 3000 years. Based on historical evidence, celery was cultivated as a medicinal plant in China, ancient Egypt and Greece as well as in the Roman Empire. The extracts of celery have been used in traditional medicine for the treatment of diabetes, hypertension, bronchitis, asthma, liver and spleen diseases (1,2). Celery has been used as a homeopathic medicine for rheumatoid arthritis to relieve pain. The inhaled vapor of celery seed oil

was used in headaches treatment (3,4). Furthermore, celery seed has been used as a diuretic, digestive aid and emmenagogue (5).

Literature data report that celery has been used for a wide range of medicinal purposes: anti-oxidant (6-9), hypolipidemic (10,11), hepatoprotective (12), anti-inflammatory (13-15), anti-diabetic, vasodilatory, anticancer (16,17) and antiplatelet aggregation activity (18). Administration of celery seed extracts decreased blood pressure and increased the heart rate in hypertensive rats, but had no effect on normotensive rats (19). The celery leaves extract was reported to express negative inotropic and chronotropic effects on the isolated rat atria on spontaneous and noradrenalin-stimulated contractions (20). In recent experimental studies, extracts from celery have demonstrated the vasorelaxant effect in precontracted aortic rings with and without endothelium (21). Active substances from celery possessed the protective effect in the case of cerebral ischemia (22). Several studies have shown that pretreatment of celery extracts and oil produced a reduction in experimentally-induced gastric lesions (23-25).

Celery modulates reproductive toxicity in male rats (26,27), but this plant decreases

concentrations of follicle stimulating and luteinizing hormone and can reduce fertility (28). Essential oil and extracts from celery exhibited larvicidal, repellent, antifungal and antibacterial actions (29-32).

Previous studies suggest the beneficial effect of celery treatment for many gastrointestinal disorders. Thus, the aim of the present study was to investigate the effects of aqueous and ethanol extracts of celery leaves on the contractions of the isolated rat ileum.

Materials and methods

Acetylcholine chloride and atropine sulfate were obtained from Sigma Chemical Co. (St. Louis, MO, USA), and papaverine hydrochloride was obtained from Merck (Darmstadt, Germany). All drugs were dissolved in distilled water.

Dried and pulverized leaves of the celery were extracted in an ultrasonic bath with distilled water and 96% ethanol, respectively. After the separation of solvents, the extracts were concentrated in a rotary evaporator at a reduced pressure till a constant weight was achieved. The dry residues were dissolved in the distilled water in order to obtain 10% solution used for the experiments.

Wistar albino rats used in this study were obtained from the Animal Research Center of Faculty of Medicine, University of Niš, Serbia. The animals were housed in stainless steel cages under standard laboratory conditions. All animals were maintained at 20–24°C with a 12h light-dark cycle (light on 08:00 to 20:00h) at least one week before the experiment. The animals were fed with standard pellet and had free access to food and water. Experimental procedures were in compliance with the EU Directive 2010/63/EU for animal experiments and were also approved by the Local Ethics Committee (number 01-206-7). The Animal Care Committee of the Faculty of Medicine in Niš approved the present study, and all experiments were performed strictly according to the guidelines set by the Committee.

The experiments in rat ileum were conduc-

ted as previously described (33). The ileum fragments were isolated out and cleaned off mesenteries. Preparations of 2cm long parts of the ileum were mounted in 20ml tissue baths containing Tyrode's solution maintained at 37°C and aerated with a mixture of 5% carbon dioxide in oxygen. The composition of Tyrode's solution was (in mmol/l): NaCl 136.89, KCl 2.68, MgCl₂ 1.05, CaCl₂ 1.80, NaH₂PO₄ 0.42, NaHCO₃ 11.90 and glucose 5.5. The fragment were stretched to a sufficient tension and equilibrated for at least 30 min before starting experiments. The change of intestinal contractility was recorded using a transducer (TSZ-04-E, Experimetria Ltd, Budapest, Hungary), recorded and analyzed with a SPEL Advanced ISOSYS Data Acquisition System (Experimetria Ltd, Budapest, Hungary).

After stabilization period, the tissues were pretreated with the aqueous and ethanol extracts (0.01-3mg/mL). Papaverine (0.01–3µg/mL) was used as a control. In the second series of experiments, acetylcholine (5–1500nM) was added to the organ bath cumulatively to generate full concentration response curves. Then, concentration response curves were formed in the presence of the aqueous and ethanol extracts (1-3 mg/mL) in the organ bath. Atropine, a nonselective blocker of muscarinic receptors, was used as a positive control.

The results were expressed as mean ± standard deviation values of six determinations. Statistical evaluation was performed using the Student's t test. A value of probability, $p < 0.05$, was considered to be significant. The mean effective concentration (EC₅₀), the concentration that made 50% of maximal response, was calculated by a regression analysis.

Results

Relaxant effects of aqueous and ethanol extracts of celery leaves on spontaneous contractions in isolated rat ileum

The rat ileum exhibited spontaneous rhythmic contractions. The results indicated that

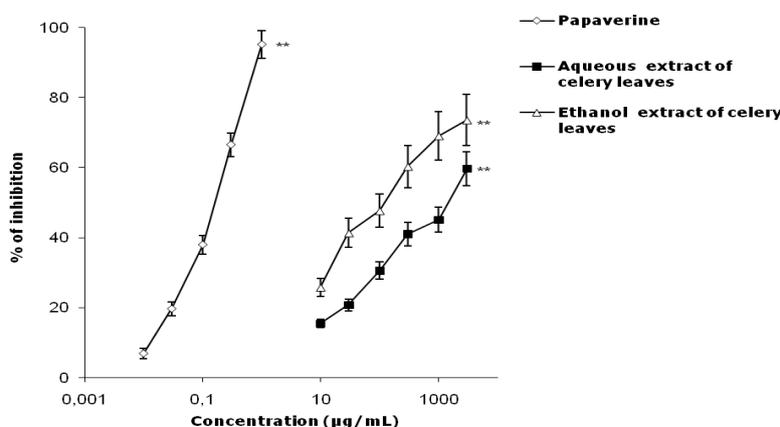


Figure 1. Inhibitory effects of the aqueous and ethanol extracts of celery leaves on spontaneous contractions in isolated rat ileum

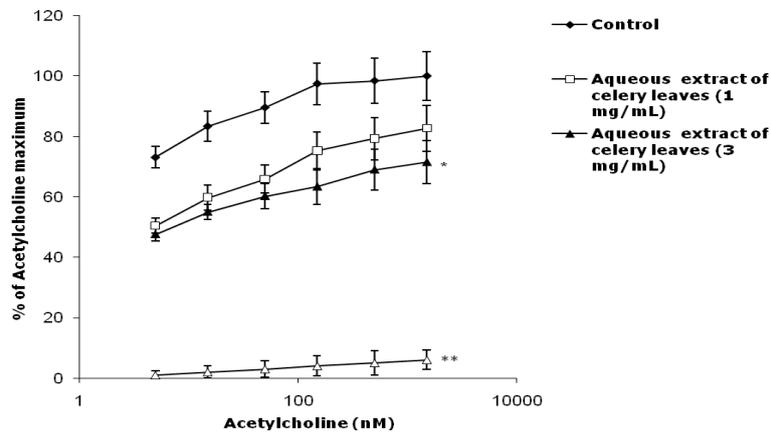


Figure 2. Inhibitory effects of the aqueous extract of celery leaves on acetylcholine-induced contraction in isolated rat ileum

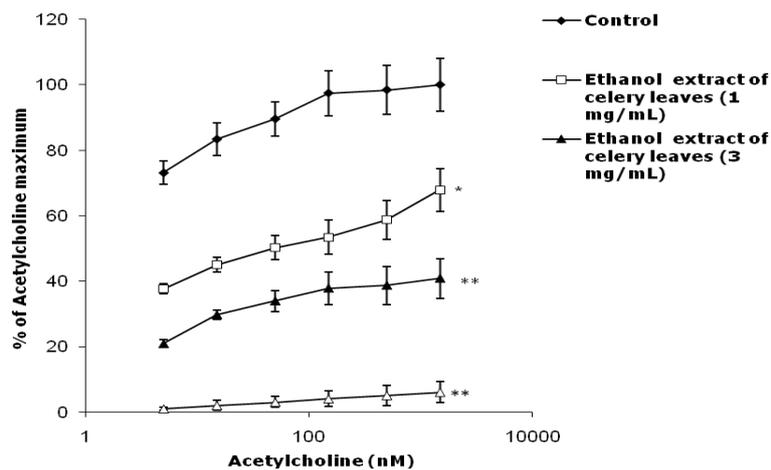


Figure 3. Inhibitory effect of the ethanol extract of celery leaves on acetylcholine-induced contraction in isolated rat ileum

the aqueous and ethanol extracts of celery leaves, at the concentrations of 0.01-3mg/mL, concentration-dependently decreased the amplitude and tension of ileal muscle contractions (Figure 1). Aqueous extract (at the concentration of 3mg/mL) caused significant inhibitory effects on spontaneous rat ileum contractions of $59.68 \pm 6.31\%$ ($p < 0.01$). Ethanol extract significantly inhibited spontaneous rat ileum contractions in a concentration-dependent manner ($p < 0.01$), with maximal inhibition of $73.58 \pm 8.17\%$. The ethanol extract (EC_{50} values of 0.12 ± 0.091 mg/mL) showed significantly higher activity than aqueous extract (EC_{50} values of 1.18 ± 0.39 mg/mL) ($p < 0.05$).

Relaxant effects of aqueous and ethanol extracts of celery leaves on contraction induced by acetylcholine

The aqueous extract of celery leaves (1-3 mg/mL) induced significant depression of the cumulative concentration response curve for acetylcholine ($p < 0.05$). Percentage of maximal response of acetylcholine alone (5–1500nM) was decreased in the presence of the aqueous extract in a concentration-dependent manner from 1

mg/mL ($82.65 \pm 6.58\%$) to 3mg/mL ($71.49 \pm 5.53\%$). The aqueous extract caused a modification of the EC_{50} of acetylcholine from 0.014 ± 0.0015 nM (in the absence of the extract) to 5.72 ± 0.64 nM, in the presence of the extract at a concentration of 3mg/mL (Figure 2).

The ethanol extract (1-3mg/mL) produced a significant and concentration dependent inhibition of acetylcholine-induced contraction in isolated rat ileum ($p < 0.05$). The ethanol extract (at the concentration of 3mg/mL) reduced the acetylcholine induced contractions to $40.83 \pm 5.36\%$ (Figure 3). The EC_{50} values of the acetylcholine (0.016 ± 0.002 nM) were affected by the ethanol extract (EC_{50} values of 13579.24 ± 1148.32 nM). The ethanol extract was more potent than aqueous extract ($p < 0.05$).

Discussion

The present study demonstrated that the aqueous and ethanol extracts of celery leaves exhibited relaxant activity in the isolated rat ileum, indicating that extracts are biologically active. The effect was reversible after washing, suggesting that the inhibition was not due to the damage of

the intestine by the extracts. These results are in good compliance with the traditional uses of this plant to treat intestinal disorders. Our studies indicated that spasmolytic activities of the aqueous extract of celery leaves were less potent than that of the ethanol extract.

In order to evaluate the potential mechanism of relaxant activity of celery leaves extracts, we performed experiments on the ileum contractions induced by acetylcholine. Gastrointestinal motility and secretory functions are regulated by the enteric nervous system (34). Acetylcholine, a neurotransmitter, is released by the parasympathetic nervous system, and plays an important physiological role in the regulation of gut movements. The interactions of acetylcholine with muscarinic receptors cause the depolarization and contraction of the intestinal smooth muscle. We established that the aqueous and ethanol extracts of celery significantly inhibited the contractions induced by acetylcholine in a concentration-dependent manner. The maximum inhibitions of the extracts were achieved at the concentration of 3 mg/mL. Inhibitory effects were less potent than that of atropine, a non-selective muscarinic receptor antagonist. These results indicated that the extract can modulate cholinergic neurotransmitter release and receptor functions. According to the experimental results, we found that celery leaves extracts produced stronger inhibition on spontaneous contractions than that on acetylcholine-induced contraction. We also demonstrated that the aqueous and ethanol celery extracts exhibited hypotensive and cardioinhibitory activities (35).

Phytochemical analysis of the celery indicated the presence of flavonoids apigenin, luteolin

and quercetin (14,36,37). Several researchers have reported that flavonoids exhibit a wide variety of biological effects such as antioxidant, anti-inflammatory, antispasmodic, and antidiarrheal activities (14,38-40). Apigenin exhibited vasodilatory (41,42) and antispasmodic activities, mainly caused by blockade of the calcium influx (43). Further studies are needed to identify the precise mechanism of spasmolytic action of celery extracts and chemical compounds that are responsible for this relaxant activity.

Conclusion

The results obtained in this work showed that the aqueous and ethanol extracts of celery leaves can inhibit spontaneous ileum contractions and contractions induced by acetylcholine. The relaxant effect of the extracts was reversible after washing the preparations, suggesting that the inhibition was not due to the damage of the intestine by the extracts. The inhibitory effect of ethanol celery leaves extract was significantly higher compared to the aqueous. Physiological activities of the celery may explain their traditional use for gastrointestinal disorders.

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SPAZMOLITIČKA AKTIVNOST VODENOG I ETANOLNOG EKSTRAKTA LISTA CELERA (*Apium graveolens L.*) NA KONTRAKCIJU IZOLOVANOG ILEUMA PACOVA

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Celer (*Apium graveolens L.*) je biljna vrsta iz familije *Apiaceae* koja se koristi u tradicionalnoj medicini za lečenje oboljenja gastrointestinalnog sistema. U ovom radu je praćen efekat vodenog i etanolnog ekstrakta lista celera na kontraktilnost tankog creva.

Osušeni i usitnjeni listovi su ekstrahovani destilovanom vodom i 96% etanolom. Uticaj vodenog i etanolnog ekstrakta lista celera na kontraktilnost glatkih mišića ispitan je na modelu izolovanog ileuma pacova. Segmenti ileuma pacova postavljeni su u kupatila za izolovane organe.

Dobijeni rezultati pokazuju da vodeni i etanolni ekstrakt lista celera statistički značajno inhibišu spontane kontrakcije ileuma pacova ($p < 0,01$). Ekstrakti proporcionalno primenjenoj koncentraciji redukuju kontrakcije ileuma indukovane acetilholinom ($p < 0,05$). Etanolni ekstrakt je pokazao značajno veću relaksantnu aktivnost u odnosu na vodeni ekstrakt ($p < 0,05$).

Dobijeni rezultati pokazuju da vodeni i etanolni ekstrakti lista celera inhibišu spontane kontrakcije ileuma pacova kao i kontrakcije indukovane acetilholinom, što objašnjava njihovu primenu u oboljenjima gastrointestinalnog sistema. *Acta Medica Mediana* 2015;54(2):11-16.

Ključne reči: *Apium graveolens L.*, ileum, pacov