

EFFECTS OF THE ETHANOLIC LEAF EXTRACT OF *ANETHUM GRAVEOLENS* L. ON CONTRACTILE ACTIVITY OF ISOLATED RAT ILEUM AND TRACHEA

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Dill (*Anethum graveolens* L., Apiaceae) has been used for centuries as a spice, as well as a remedy for gastrointestinal problems in traditional medicine. The aim of this study was to evaluate the effects of the ethanolic leaf extract of *Anethum graveolens* L. (AGEE) on the smooth muscle contractile activity of rat isolated ileal and tracheal strips. AGEE was obtained by using ultrasonic extraction from air-dried and powdered leaves of cultivated *Anethum graveolens* L. This study examined the effects of AGE on the spontaneous, KCl (80 mM), Acetylcholine and CaCl₂-induced smooth muscle contraction of isolated rat ileum, as well as on the contractile activity of isolated rat trachea induced by Carbachol and KCl (80 mM). Results showed that AGEE produced significant ($p < 0.01$) concentration-dependent relaxation of spontaneous and induced contractions of ileal smooth muscle. Addition of AGEE significantly ($p < 0.01$) reduced in a dose dependent manner the contractile effects of the carbachol and KCl on the isolated rat trachea. Our findings indicate that AGEE decreased contractile response of rat ileal and tracheal smooth muscle. *Acta Medica Medianae* 2023; 62(2): 23-30.

Key words: dill, *Anethum graveolens* L., extract, rat, ileum, trachea.

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Introduction

Herbal medicine research is very extensive because medicinal plants represent a great therapeutic potential for the prevention and treatment of wide range of diseases. Dill (*Anethum graveolens* L.) is a plant of the Apiaceae family, and has a long history of use, both as a medicinal and aromatic plant (1). In Ayurvedic medicine, preparations of *A. graveolens* are well known as carminative, stomachic and diuretic agents (2). Dill has been used in herbal medicine

for the treatment of mental disorders, convulsions, asthma, thyroid diseases (3, 4), to regulate the menstrual cycle, to reduce labour pain, to increase milk production in lactating women, to prevent colic in babies and alleviate pain effects (5, 6). Numerous studies demonstrated that dill might also have hepatoprotective, anti-inflammatory, antithrombotic, analgesic, apoptogenic, antioxidant, antibacterial, antifungal, larvicidal, anti-hyperlipidaemic, antidiabetic, and cerebroprotective properties (7-12).

It is known that the fruit hydroalcoholic extract of *A. graveolens* inhibited the contractions of the isolated rat ileum (13). Also, Jafarzade et al. (14) showed relaxant effect of seed hydroalcoholic extract of *A. graveolens* on isolated rat trachea. However, studies on effects of the *A. graveolens* leaf extract in the isolated rat intestine and trachea were not found in the available literature.

The Aim

The aim of our study was to investigate the effects of the ethanolic leaf extract of *A. graveolens* on the spontaneous and induced contractions of isolated rat ileal and tracheal strips.

Material and methods

Drugs and Reagents

The following drugs were used: Acetylcholine chloride (Sigma Chemical Co-USA), Carbamoylcholine chloride (Carbachol, Sigma Chemical Co-USA), Verapamil (Sigma Chemical Co-USA), Atropine sulphate (Sigma Chemical Co-USA) and Papaverine hydrochloride (Merck, Darmstadt, Germany).

Preparation of the Extract

The plant material (leaves) was collected in the surrounding area of Niš, and a voucher specimen for *A. graveolens* was deposited in the Herbarium of the Faculty of Science and Mathematics, Department of Biology and Ecology, University of Niš, Serbia, with the accession number 16420. The leaves were then air-dried, pulverized and extracted in an ultrasonic bath with 96% ethanol. The extract was concentrated in a rotary evaporator. The obtained dry residues were dissolved in the distilled water.

Animals

Male Wistar albino rats were kept under standard laboratory conditions. All the experimental procedures with the animals were in compliance with the European Council Directive of September 22nd, Directive 2010/63/EU and were approved by the Animal Ethical Committee of the Faculty of Medicine in Niš (No: 01-206-7).

Tissue Preparation

Overnight fasted animals were sacrificed by cervical dislocation. The trachea was dissected and immediately placed in a Krebs solution, whereas the ileum was placed in a Tyrode's solution. The change of tissue segments contractility was recorded using a TSZ-04-E Spell Iso system (Experimetria Ltd., Budapest, Hungary).

Ileum

After a dissection of the abdominal cavity, the ileum segments were isolated and cleaned off mesenteries. Ileal strips were mounted in an organ bath containing a Tyrode's solution (37°C, aerated with carbogen). The rat ileum was cumulatively treated with AGEE (0.02-6 mg/mL). Papaverine (0.1-30 µM) was used as a control substance. The relaxant effect was expressed as a percentage change of the basal tone compared with baseline values. The potential anticholinergic activity was examined by the cumulative addition of acetylcholine (5-1500 nM), and then concentration response curves were obtained in the presence of AGEE (6 mg/mL), or atropine (140 nM). To evaluate the possible calcium channel blocking activity of AG, the effects of AGEE or

verapamil on the contractions of the rat ileum induced by KCl (80 mM) or CaCl₂ (0.01-3 mM) were recorded. In the calcium-induced contractions of the isolated rat ileum, a calcium-free Tyrode's solution was used, as described earlier (15, 16). The relaxation of the isolated ileum, which was precontracted with acetylcholine or calcium ions, was calculated as a percent of the agonists-induced contractions.

Trachea

The trachea was cleaned of connective tissue and tracheal rings containing 2-3 cartilage were prepared. Tracheal strips were mounted by inserting two stainless steel hooks in the lumen and placed in to organ baths containing oxygenated Krebs solution at 37 °C. The tracheal smooth muscle were contracted by KCl (80 mM) or carbachol (1 µM), and then AGEE was added. Verapamil was tested on KCl (80 mM) and atropine on carbachol-induced contractions (17, 18).

Statistical analysis

All results were expressed as mean±standard deviation (SD) of six determinations. A statistical significance of differences between two means was performed using the Student's t-test. A probability value of P < 0.05 was considered to be significant. The half maximal effective concentration (EC₅₀), the concentration which elicited 50% of maximal response, was established by a regression analysis. The data was analyzed using the SPSS statistical software package (v.20.0; SPSS, Chicago, IL, USA).

Results

AGEE in cumulative concentrations (0.02-6 mg/mL) produced a significant and concentration-dependent relaxation of rat ileum spontaneous contractions, with the EC₅₀ values of 5.76±3.28 mg/mL, respectively (Table 1). Papaverine (0.1-30 µM) decreased the spontaneous rat ileum contractions with EC₅₀ value of 2.01±0.01 µM (Figure 1).

The high concentration of K⁺ induced a tonic contraction in the rat ileum smooth muscle. AGEE caused a significant and concentration-dependent relaxation of the KCl induced contractions in the isolated rat ileum (EC₅₀ values 6.38±0.49 mg/mL) (Table 1). The EC₅₀ value of verapamil was 0.36±0.028 µM (Figure 2).

AGEE dose dependently reduced the rat ileum contractions stimulated by acetylcholine. The EC₅₀ of acetylcholine in the presence of AGEE (6 mg/ml, 489.02±27.25 nM) was significantly higher than the EC₅₀ of acetylcholine alone (0.54±0.08 nM; P < 0.001) (Table 2). The value

of EC50 in the presence of atropine was 445.32 ± 28.57 mM (Figure 3).

AGEE inhibited the CaCl_2 induced contractions in a Ca^{2+} -free medium. The EC50 values of calcium ions alone (0.005 ± 0.0009 mM) were significantly increased in the presence of AGEE (6 mg/ml, $\text{EC}_{50} = 0.94 \pm 0.01$ mM, $P < 0.001$) (Table 2). The EC50 value for calcium ions was affected by verapamil (8.67 ± 0.61 mM) (Figure 4).

AGEE caused a significant and concentration-dependent relaxant effect on the KCl and carbachol induced precontractions of the isolated rat tracheal rings, with EC50 values of 6.24 ± 0.49 mg/mL and 10.88 ± 0.97 mg/mL, respectively (Figure 5). Verapamil produced inhibition of KCl-induced contractions with EC50 value of 0.22 ± 0.01 μM . Atropine, as a positive control, abolished carbachol-induced contractions (EC_{50} value was 0.004 ± 0.0003 μM) (Table 3).

Table 1. Half maximal effective concentration (EC_{50}) values of the *Anethum graveolens* L. ethanolic leaf extract (AGEE), as well as of papaverine and verapamil on spontaneous and KCl-induced contractions in isolated rat ileum

Drug	EC50 values for spontaneous contractions	EC50 values for KCl-induced contractions
AGEE	5.76 ± 3.28 mg/mL	6.38 ± 0.49 mg/mL
Papaverine/Verapamil	2.01 ± 0.01 μM	0.36 ± 0.028 μM

*Results are expressed as mean \pm SD (n=6).

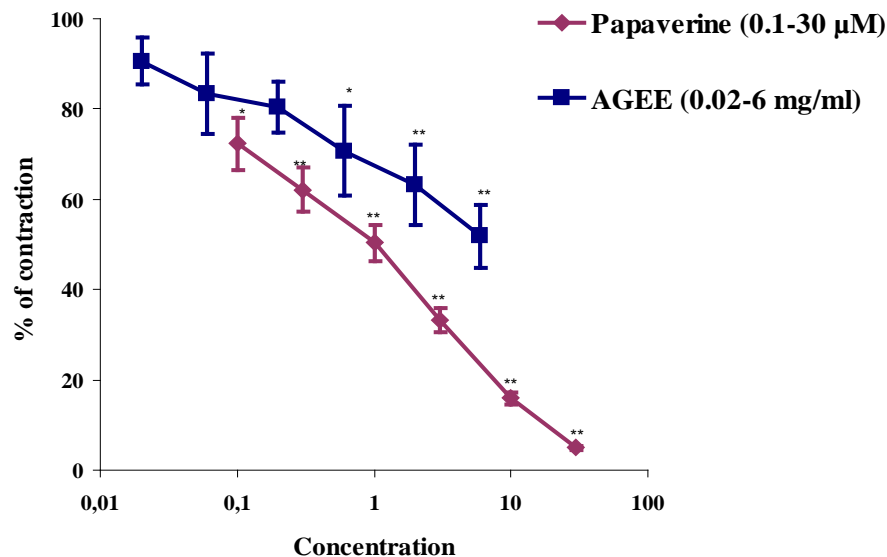


Figure 1. Relaxant effect of the *Anethum graveolens* L. ethanolic leaf extract (AGEE) and papaverine on spontaneous contractions in isolated rat ileum. Each data point represents the mean \pm SD of the percentage values with respect to the spontaneous contractions in Tyrode solution (control). * $p < 0.05$, ** $p < 0.01$ versus Tyrode (n=6)

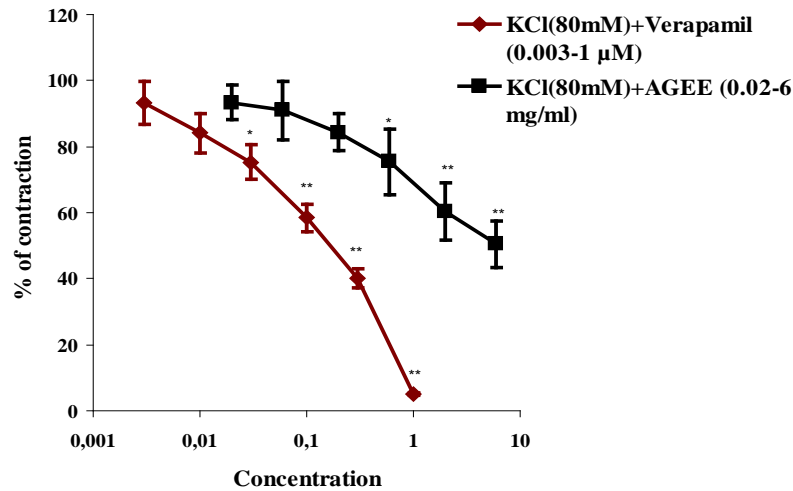


Figure 2. Relaxant effects of the *Anethum graveolens* L. ethanolic leaf extract (AGEE) and verapamil on ileum contraction induced by KCl (80 mM). Each data point represents the mean values in percent of maximal response \pm SD. * $p < 0.05$, ** $p < 0.01$ versus control (n=6)

Table 2. Half maximal effective concentration (EC₅₀) values of the *Anethum graveolens* L. ethanolic leaf extract (AGEE), as well as of atropine and verapamil on acetylcholine and CaCl₂-induced contractions in isolated rat ileum

Drug	EC ₅₀ values for Acetylcholine -induced contractions	EC ₅₀ values for CaCl ₂ -induced contractions
Control	0.54 \pm 0.08 nM	0.005 \pm 0.0009 mM
AGEE	489.02 \pm 27.25 nM**	0.94 \pm 0.006 mM**
Atropine/Verapamil	445.317 \pm 28.57 mM	8.67 \pm 0.61 mM

*Results are expressed as mean \pm SD (n=6). ** P < 0.01 significantly different when compared with control group.

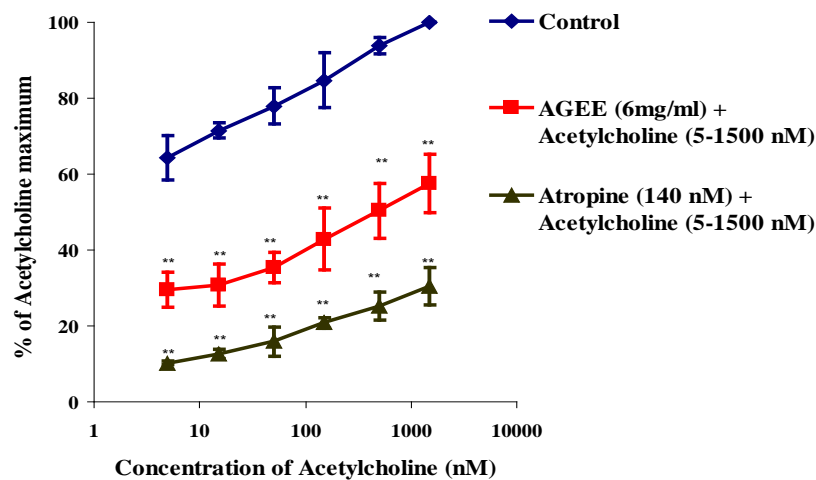


Figure 3. Relaxant effects of the *Anethum graveolens* L. ethanolic leaf extract (AGEE) and atropine on ileum contraction induced by acetylcholine. Each data point represents the mean values in percent of maximal response \pm SD. * $p < 0.05$, ** $p < 0.01$ versus control (n=6)

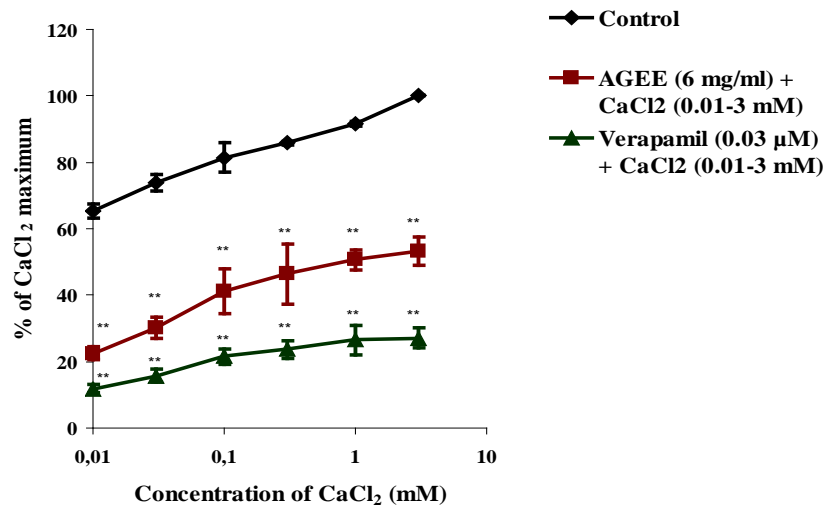


Figure 4. Relaxant effects of the *Anethum graveolens* L. ethanolic leaf extract (AGEE) and verapamil on contractions of the isolated rat ileum induced by CaCl_2 . Each data point represents the mean values in percent of maximal response \pm SD. ** $p < 0.01$ versus control ($n=6$)

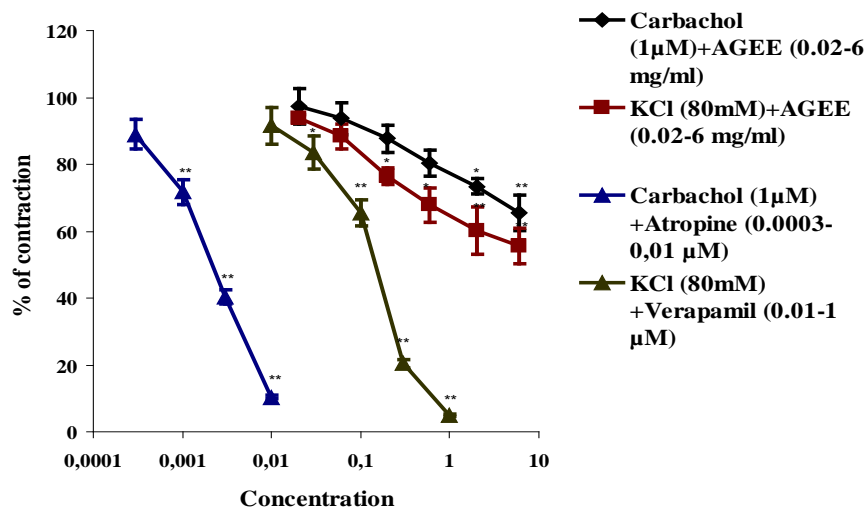


Figure 5. Relaxant effect of the *Anethum graveolens* L. ethanolic leaf extract (AGEE), verapamil and atropine on contractions of the isolated rat trachea induced by KCl and carbachol. Each data point represents the mean values in percent of maximal response \pm SD. * $p < 0.05$, ** $p < 0.01$ versus control ($n=6$)

Table 3. Half maximal effective concentration (EC_{50}) values of the *Anethum graveolens* L. ethanolic leaf extract (AGEE), as well of verapamil and atropine on KCl and carbachol-induced contractions in isolated rat trachea

Drug	EC_{50} values for KCl-induced contractions	EC_{50} values for Carbachol-induced contractions
AGEE	6.24 ± 0.49 mg/mL	10.88 ± 0.97 mg/mL
Verapamil/Atropine	0.22 ± 0.01 μM	0.004 ± 0.0003 μM

*Results are expressed as mean \pm SD ($n=6$).

Discussion

The results of this study showed that AGEE induced a significant relaxant effects on the isolated rat ileum and rat trachea contractions. AGEE inhibited spontaneous and acetylcholine, KCl and CaCl₂-induced contractility of the rat ileum strips and relaxed the carbachol and KCl-contracted trachea. The muscle relaxant responses of AGEE were similar to a smooth muscle relaxant such as papaverine, atropine, a nonselective muscarinic antagonist and verapamil, a calcium channel blocker.

The neuroendocrine mediator, acetylcholine, is an important regulator of gastrointestinal motility (19). AGEE induced a significant depression of the cumulative concentration response curve for acetylcholine in the isolated rat ileum. Atropine, an antagonist of the muscarinic receptors, inhibited acetylcholine induced contractions. The interactions of acetylcholine with muscarinic receptors in the intestinal smooth muscle induce a G protein-mediated signal transduction that activates phospholipase C, resulting in an increase of intracellular calcium, depolarization and the contractions of the smooth muscle (20). Muscarinic acetylcholine receptors present on the smooth muscle cells are potential therapeutic targets for intestinal motility disorders. AGEE significantly inhibited acetylcholine stimulated rat ileum contractions, indicating a possible anticholinergic effect. These results may explain the traditional use AG for digestive system disorders. A relaxant potential of the methanol extract (21) and the hydroalcoholic fruit extract of dill (13) was also reported.

Many studies have shown that the activation of actin and myosin contractile filaments of the smooth muscle occurs due to increased sarcoplasmic free calcium (15, 16, 22, 23). The rise in the concentration of calcium could result from the influx of calcium through voltage-dependent ion channels and the secondary release from intracellular stores. A possible relaxant mechanism of the AGEE may be mediated through an inhibition of calcium channel pathways. High concentration of KCl induced both a depolarization and a tonic contraction of ileum smooth muscle through the activation of voltage operated L-type calcium channels (24). In this study, AGEE inhibited ileal contractions induced by KCl. The spasmolytic effect was similar but milder compared to those of the calcium antagonist verapamil. Also, this study found that the pretreatment of the isolated rat ileum with the

AGEE reduced CaCl₂ induced smooth muscle contractions in the calcium-free medium, similar to the verapamil calcium channel blocker. The obtained results indicate that the spasmolytic effect of the AGEE is possibly mediated through the calcium influx reduction from the extracellular fluid.

Our results on the spasmolytic effect of AGEE are confirmed by a study with clinical application of the dill powder of the crude herb in patients with irritable bowel syndrome (25). Inhibited acid secretion and lesions in the rats stomach in pretreated rats with dill extracts has also been noted in the literature (26).

When looking at the use of AG to treat respiratory diseases, AGEE was studied in isolated rat trachea to reveal its underlying mechanisms of relaxant activity. AGEE caused a significant relaxation of tracheal smooth muscle contractions induced with KCl and carbachol. As expected, verapamil relaxed the tracheal contractions induced with KCl. Atropine, a muscarinic receptor antagonist also relaxed the carbachol-induced contractions. These results suggested that the relaxant effect of AGEE on isolated rat trachea might be related to combined reduction of calcium influx and the inactivation of muscarinic receptors. The obtained results of bronchodilatory activity of AGEE are in agreement with the already reported study that found the relaxant activity of dill hydroalcoholic extract (14).

Conclusion

The present results showed that AGEE significantly decreased contractions of the isolated rat ileum and rat trachea. AGEE inhibited spontaneous and acetylcholine, KCl and CaCl₂-induced contractility of the rat ileum and relaxed the carbachol and KCl-contracted trachea. The data we obtained suggested the possibly of an anticholinergic and calcium channel-blocking AGEE activity and may, at least partially, account for the traditional use of *A. graveolens* for stomach and respiratory disorders.

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UTICAJI ETANOLNOG EKSTRAKTA LISTA *ANETHUM GRAVEOLENS* L. NA KONTRAKTILNU AKTIVNOST IZOLOVANOG ILEUMA I TRAHEJE PACOVA

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Mirođija (*Anethum graveolens* L., Apiaceae) se vekovima koristi kao začin, ali i kao lekovito sredstvo za gastrointestinalne probleme u tradicionalnoj medicini. Cilj ove studije jeste da proceni efekte etanolnog ekstrakta lista *Anethum graveolens* L. (AGEE) na kontraktilnu aktivnost izolovanog ileuma, kao i traheje pacova. AGEE je dobijen ultrazvučnom ekstrakcijom iz osušenih i usitnjenih listova kultivisane mirođije. Ova studija pratila je efekte AGEE na spontane, KCl (80 mM), acetilholinom i CaCl₂ indukovane kontrakcije izolovanog ileuma pacova, kao i kontraktilnu aktivnost izolovane traheje pacova indukovane karbaholom i KCl (80 mM). Rezultati pokazuju da AGEE indukuje statistički značajnu ($p < 0,01$) i kontrakciono zavisnu relaksaciju spontanih i indukovanih kontrakcija izolovanog ileuma. Takođe, AGEE statistički značajno ($p < 0,01$) umanjuje, proporcionalno primenjenoj koncentraciji, kontraktilne efekte karbahola i KCl na izolovanu traheju pacova. Naši rezultati pokazuju da AGE smanjuje kontraktilnost glatke muskulature izolovanog ileuma i traheje pacova. *Acta Medica Medianae* 2023;62(2):23-30.

Ključne reči: mirođija, *Anethum graveolens* (L.), ekstrakt, pacov, ileum, traheja

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