

## Antispasmodic Effect of *Anethum graveolens* Fruit Extract on Rat Ileum

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**Abstract:** The aim of the present study was to investigate the effect of Dill Fruit Hydroalcoholic Extract (DFHE) on the rat ileum contractions induced by some known spasmogens and also to study the possible mechanism(s) involved. Dill fruit extract was prepared by macerated with alcohol (70%). A piece of ileum (2 cm) was removed from male Wistar rats and mounted in an organ bath containing air bubbled Tyrode solution with 0.5 g initial tension and contractions were recorded by an isotonic transducer. The precontracted ileum by KCl (60 mM), ACh (1  $\mu$ M) and BaCl<sub>2</sub> (4 mM) were relaxed by the cumulative concentrations (0.5-4 mg mL<sup>-1</sup>) of DFHE ( $p < 0.0001$ ). The relaxatory effect of the extract on the BaCl<sub>2</sub>-induced ileum contractions was greater than the other spasmogens. The spasmolytic effect of the extract (1 mg mL<sup>-1</sup>) was not reduced after tissue incubation (20-30 min) with phentolamine (1  $\mu$ M), propranolol (1  $\mu$ M), naloxone (1  $\mu$ M) and L-NAME (100  $\mu$ M). In high-potassium (120 mM) Ca<sup>2+</sup>-free Tyrode solution, cumulative concentrations of CaCl<sub>2</sub> (0.225-3.6 mM) induced ileal contractions, however, the extract (0.5-2 mg mL<sup>-1</sup>) reduced these contractions dose-dependently ( $p < 0.001$ ). Present results suggest that the  $\alpha$ - and  $\beta$ -adrenoceptors, opioid receptors and NO generation are not involved in the DFHE inhibitory effect. Furthermore, the results suggest that the relaxatory effect of DFHE on the ileum may be due to blockade of voltage dependent calcium channels.

**Key words:** Antispasmodic, *Anethum graveolens*, rat, Ileum

### INTRODUCTION

Dill (*Anethum graveolens*) from Umbelliferae has been cultivated in Europe since antiquity and used as popular aromatic herb and spice (Ishikawa *et al.*, 2002). Its fruits have been used for medicinal purposes in the relief of digestive problems and to stimulate milk for nursing mothers (Norman, 1990; Zargari, 1991). It is believed that dill water has a soothing effect on the digestive system. In Iran, is given to babies to relieve hiccups and colic (Zargari, 1991). Moreover, it is reported that dill fruit extract has a significant mucosal protective and antisecretory effect on mice gastric mucosa (Hosseinzadeh *et al.*, 2002). The anticancer (Zheng *et al.*, 1992), antimicrobial (Delaquis *et al.*, 2002), antihyperlipidaemic and antihypercholesterolaemic (Yazdanparast and Alavi, 2001) effects of dill have been also reported. The fruit contains essential oil rich in *d*-carvone, *trans*- and *cis*-dihydrocarvone, *trans*- and *cis*-carveol, limonene, *d*-dihydrocarveol, *l*-dihydrocarveol,  $\alpha$ -phellandrene and  $\beta$ -terpineol (Ishikawa *et al.*, 2002). In Iran, it is given to babies to relieve hiccups and colic pain (Zargari, 1991). However, there is little information regarding the underlying mechanism that mediated its spasmolytic action. The aim of the present study was,

therefore, to investigate the possible mechanism(s) that mediate the effects of Dill Fruit Hydroalcoholic Extract (DFHE) on rat ileum precontracted with various agents.

### MATERIALS AND METHODS

**Chemicals:** Acetylcholine (ACh), propranolol, L-NAME and phentolamine were purchased from Sigma (USA), naloxone from Tolidaru Company (Iran) and all solutes were purchased from Merck (Germany). The composition of Tyrode solution (in mM) was: NaCl (136), KCl (2.7), CaCl<sub>2</sub> (1.8), NaHCO<sub>3</sub> (12), NaH<sub>2</sub>PO<sub>4</sub> (0.3), MgCl<sub>2</sub> (1.8) and glucose (5.6).

**Preparation of extract:** Dill fruits were purchased from local grocery in Ahwaz and taxonomically identified by Dr. M. Heidari from Ahwaz Ramin University of Agriculture and Natural Sources. The seeds were powdered by an electric grinder and powder was extracted by maceration using 70% alcohol for 72 h at room temperature. The mixture was then filtered (Whatman No.1) and the solvent evaporated at 30°C by blowing air to the extract. The extract was stored at 4°C till further use. All the concentrations are the final concentrations in the organ bath.

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**Animals and tissue preparation:** Male Wistar rats (200-270 g) were purchased from animal house of Jundishapur Ahwaz University of Medical Sciences and kept at 20-24°C under 12/12 h light/dark cycle and had free access to food and water. All of the animals used in this study were treated in accordance to the guidelines on Animals Care of Ahwaz Jundishapur University of Medical Sciences. The rats were housed in individual cages with wire-mesh bottoms for 24 h before experiment and were deprived of food but had free access to drinking water. Rats were sacrificed by a blow to the skull and cervical dislocation. After laparotomy, a segment (2 cm) of the distal part of the ileum (the terminal 2 cm was discarded) was dissected out and rinsed intraluminally with cold oxygenated Tyrode solution. The ileum was suspended in an organ bath (10 mL) containing Tyrode solution (37°C, pH 7.4) between two stainless steel hooks and solution was bubbled with air continuously (Madeira *et al.*, 2002). The lower hook was fixed at the bottom of the organ bath and the upper one was connected to an isotonic transducer (Harvard Transducer). Under 0.5 g resting tension, the ileal contractions were recorded (Universal Harvard Oscillograph, UK) after 60 min equilibrium period. During this period, the organ bath solution was refreshed every 15 min. The ileum contractions were induced by KCl (60 mM), acetylcholine (ACh, 1  $\mu$ M) or BaCl<sub>2</sub> (4 mM) and once the plateau of contraction elicited by spasmogens was achieved, extract was added to the organ bath cumulatively (0.5, 1, 2 and 4 mg mL<sup>-1</sup>). In order to study the receptors that may mediate the extract activity, after recording the inhibitory effect of the extract (1 mg mL<sup>-1</sup>), on the KCl-induced contraction, in the separate experiments, the same procedure was repeated in the presence of phentolamine, propranolol, naloxone and L-NAME ( $\alpha$ - and  $\beta$ - adrenoceptors, opioid antagonists and nitric oxide synthase inhibitor, respectively). The role of the extracellular calcium was evaluated by depolarizing the tissue with KCl (120 mM) in Ca<sup>2+</sup>-free solution and then calcium chloride was applied to the organ bath cumulatively (0.225, 0.45, 0.9, 1.8 and 3.6 mM). Then, the same procedure was repeated in the presence (3 min) of the extract (0.5, 1 or 2 mg mL<sup>-1</sup>). One ileal preparation was used for each of the antagonist applied. The extract was dissolved in Tyrode solution and the volume added to the organ bath never exceeded 5% of its total volume.

**Statistical analysis:** All values in text and figures are expressed as mean $\pm$ SEM for *n* number of animals. Statistical significance of differences between two means was assessed by Student's *t*-test. Multiple means were compared by one-way analysis of variance

(ANOVA). *p*-values of less than 0.05 were considered to present significant differences. The plateau of the contraction caused by each spasmogen (KCl, ACh and BaCl<sub>2</sub>) in the absence of the extract or applied antagonists was considered as the 100% contraction.

## RESULTS

**Effect of extract on ileum contraction induced by KCl, ACh and BaCl<sub>2</sub>:** Cumulative concentrations of the dill fruit extract (0.5, 1, 2 and 4 mg mL<sup>-1</sup>) reduced the ileum contractions induced by KCl (60 mM), acetylcholine (ACh, 1  $\mu$ M) and BaCl<sub>2</sub> (4 mM) dose-dependently (ANOVA, *p*<0.0001, *n*=8)(Fig. 1). The spasmolytic effect of the extract on the BaCl<sub>2</sub>-induced contraction was greater than the relaxant effects on the ACh-induced contractions (*p*<0.05). The IC<sub>50</sub> of these curves were 0.96, 1.12 and 1.23 mg mL<sup>-1</sup>, respectively.

**The spasmolytic effect of extract in presence of alpha-adrenoceptor antagonist:** The spasmolytic effects of dill fruit extract (1 mg mL<sup>-1</sup>) on KCl-induced contraction in the present and in the absence of phentolamine (1  $\mu$ M, for 30 min, *n*=8) were not significantly different (Fig. 2).

**The spasmolytic effect of extract in presence of beta-adrenoceptor antagonist:** The inhibitory effect of the dill fruit extract (1 mg mL<sup>-1</sup>) on the KCl-induced contractions was not reduced, rather was increased significantly (*p*<0.001, *n*=7) in the presence of the propranolol (1  $\mu$ M, for 30 min)(Fig. 3).

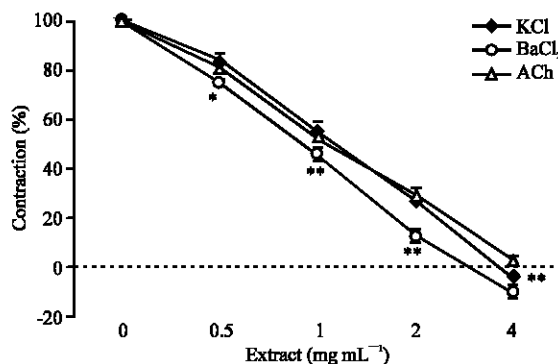


Fig. 1: Effect of cumulative concentrations of dill fruit extract on the rat ileum contractions induced by KCl (60 mM), ACh (1  $\mu$ M) and BaCl<sub>2</sub> (4 mM). The contraction induced by each spasmogens (in the absence of extract) has been taken as 100%. The number of rats in each group was 8 and \* and \*\* indicate the significant differences (*p*<0.05 and *p*<0.01, respectively) between BaCl<sub>2</sub> group and ACh group

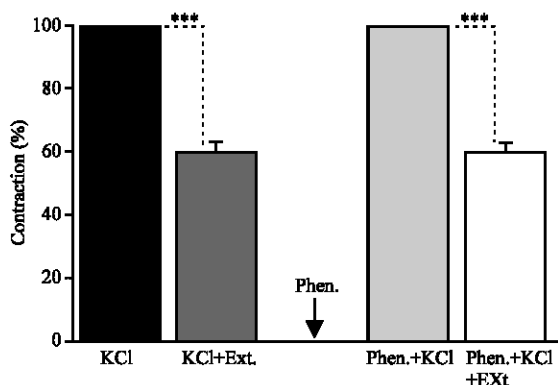


Fig. 2: Effect of dill fruit extract on the KCl-induced rat ileum contraction in the absence and in the presence of the phentolamine. KCl; 60 mM, Phen.: Phentolamine; 1  $\mu$ M, Ext.: Extract; 1 mg mL<sup>-1</sup>, n = 8, \*\*\*p<0.0001. The tissue preparations were incubated with phentolamine for 30 min

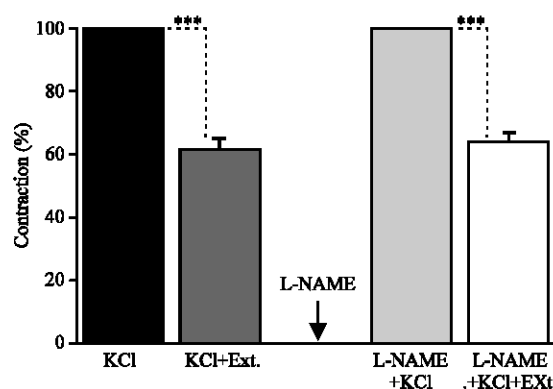


Fig. 4: Effect of dill fruit extract on the KCl-induced rat ileum contraction in the absence and in the presence of the L-NAME (100  $\mu$ M). KCl; 60 mM, Ext.: Extract; 1 mg mL<sup>-1</sup>, n = 7, \*\*\*p<0.0001. The tissue preparations were incubated with L-NAME for 20 min

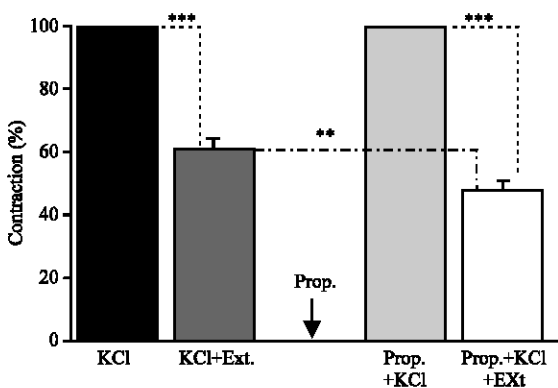


Fig. 3: Effect of dill fruit extract on the KCl-induced rat ileum contraction in the absence and in the presence of the propranolol. KCl; 60 mM, Prop.: Propranolol; 1  $\mu$ M, Ext.: Extract; 1 mg mL<sup>-1</sup>, n = 7, \*\*p<0.001 and \*\*\*p<0.0001. The tissue preparations were incubated with propranolol for 30 min

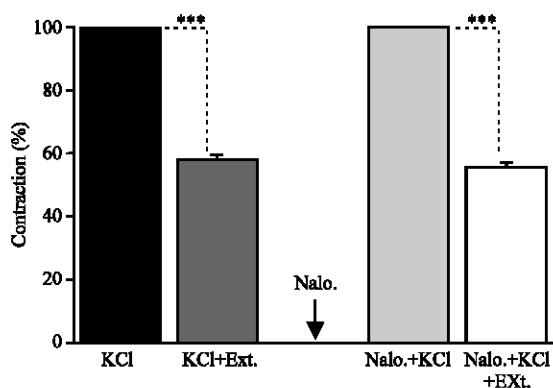


Fig. 5: Effect of dill fruit extract on the KCl-induced rat ileum contraction in the absence and in the presence of the naloxone. KCl; 60 mM, Nalo.: Naloxone; 1  $\mu$ M, Ext.: Extract; 1 mg mL<sup>-1</sup>, n = 7, \*\*\*p<0.0001. The tissue preparations were incubated with naloxone for 30 min

**Effect of nitric oxide synthase inhibitor (L-NAME) on spasmolytic effect of extract:** The spasmolytic effect of the dill extract (1 mg mL<sup>-1</sup>) on KCl-induced contractions in the absence and in the presence of nitric oxide synthase inhibitor (L-NAME, 100  $\mu$ M for 20 min, n = 7) was not significantly different (Fig. 4).

**The spasmolytic effect of extract in presence of opioid receptors antagonist:** The inhibitory activities of the dill extract (1 mg mL<sup>-1</sup>) on KCl-induced contractions in the absence and in the presence of naloxone was applied on

the KCl-induced ileum contractions in the absence and in the presence of naloxone (1  $\mu$ M for 30 min, n = 7) were not significantly different (Fig. 5).

**Spasmolytic effect of extract on Ca<sup>2+</sup>-induced contraction in KCl-depolarized ileum:** In the calcium free Tyrode solution, cumulative concentrations of calcium chloride (0.225-3.6 mM) induced concentration dependent ileum contractions in the rich-potassium Tyrode solution. However, in the presence of the dill fruit hydroalcoholic extract (0.5, 1 and 2 mg mL<sup>-1</sup>) these contractions were reduced in a dose dependent manner (p<0.0001, n = 7-9) (Fig. 6).

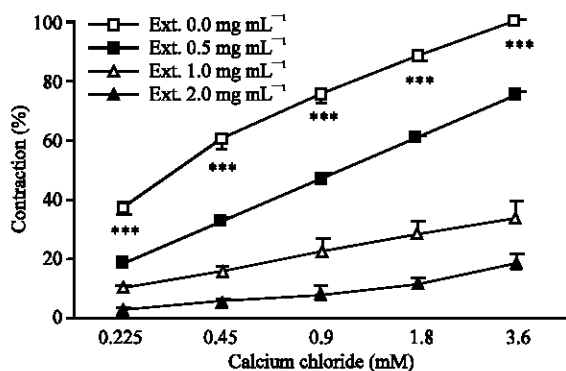


Fig. 6: Spasmogenic effects of the cumulative concentrations of calcium chloride on high potassium Tyrode solution-depolarized ileum in the absence and in the presence of different concentrations of dill fruit extract. Statistical analysis was performed between extract (Ext.) at 0.0 mg mL<sup>-1</sup> as the control and extract at lowest concentration (0.5 mg mL<sup>-1</sup>). The maximum contraction induced by calcium chloride (3.6 mM) in the absence of the extract was considered as 100% (\*\*\*)p<0.0001, n = 8-10)

## DISCUSSION

The present study showed that dill fruit hydro-alcoholic extract is a potent relaxant of contractions induced by a variety of spasmogens in rat ileum. It reversed the KCl-induced contractions, a calcium channel mediated spasmogen, as well as those produced by acetylcholine, a receptor mediated agent and BaCl<sub>2</sub> (a non-selective smooth muscle agonist). Furthermore, following washing, this inhibitory activity was totally reversible, suggesting that these effects are membrane mediated. On the other hand, this inhibitory effect could not be related to reduction of smooth muscle myofilaments sensitivity to calcium, otherwise, washing the tissue was unable to reverse the inhibitory effect. The depolarization induced by high potassium concentration activates the L-type voltage dependent calcium channels (VDCCs) (Karaki *et al.*, 1997). The common pathway for all these agents is via an increase in the intracellular calcium ion concentration. Therefore, dill fruit extract, seem to induce its spasmolytic effect through calcium channels. Acetylcholine induces contraction by activation of muscarinic receptors (Elorriaga *et al.*, 1996) which in turn, increases the intracellular calcium through inositol triphosphate (IP<sub>3</sub>) (Eglen *et al.*, 1996) and also by facilitating the inflow of extracellular calcium through the receptor-operated calcium channel (Zhang *et al.*, 2005). On the other hand, BaCl<sub>2</sub>, as a non-selective potassium channel blocker (Liu *et al.*, 2001), induces

depolarization and contraction in ileal smooth muscles possibly by promoting calcium release from intracellular pools (Rahwan *et al.*, 1977). Furthermore, it has been suggested that substances, which inhibit the KCl-induced contraction, act by blocking L-type voltage dependent calcium channels (VDCCs) (Gilani *et al.*, 2001). This suggestion is supported by the existence of L-type VDCCs in the rat intestine (El Bardai *et al.*, 2004).

The existence of  $\alpha$ -adrenoceptor in the ileum and its inhibitory effect have been shown (Stebbing *et al.*, 2001) and the ineffectiveness of phentolamine on the spasmolytic activity of the extract indicates the noninvolvement of these receptors. Moreover, the presence of  $\beta$ -adrenoceptors and their inhibitory activity are also shown in rat ileum (Roberts *et al.*, 1999) and we showed that the inhibitory effect of the extract was unaffected by propranolol, a non-selective  $\beta$ -adrenoceptor antagonist. In fact, a greater relaxation observed in the presence of propranolol suggests that either the components in the extract or actions that are unrelated to the  $\beta$ -blocking action of propranolol could be the mediators of this action. Nitric oxide relaxes the rat ileum (Ekblad and Sundler, 1997) but, since the inhibitory effect of the extract was unaffected by L-NAME as a nitric oxide synthase inhibitor, it suggests that the extract dose not act through NO pathway. Furthermore, the gastrointestinal motility can be suppressed by opioid receptors activation (Gray *et al.*, 2005) but, since naloxone (as a non-selective opioid receptor antagonist) did not reduce the inhibitory effect of the extract the involvement of these receptors is negated.

In order to confirm the role of VDCCs as a possible mechanism that mediated the relaxatory effects of DFHE, depolarization of the ileac preparation in Ca<sup>2+</sup>-free with high-K<sup>+</sup> Tyrode solution was undertaken. Results showed that this extract produced highly significant inhibition of the contractile responses produced by cumulative addition of calcium chloride. These observations suggest that the relaxation observed was primarily mediated via inhibition of VDCCs. However, the greater reduction of BaCl<sub>2</sub>-induced contractions observed can not be explained fully from these findings and suggest that other mechanisms may be involved in the relaxation produced by this mixture. However, the inhibitory effect clearly indicates the involvement of VDCCs in spasmolytic effect of dill fruit extract. It has been reported that dill contains flavonol and flavonoid derivatives of quercetin (Gebhardt *et al.*, 2005; Moehle *et al.*, 1985). Furthermore, *in vitro* and *in vivo* studies have shown that quercetin inhibits the small intestine motility (Zhang *et al.*, 2003). Therefore, it is possible to assume that the spasmolytic effect of dill fruit extract may be due to these compounds. The present results support the use of dill fruit in traditional medicine for gastrointestinal disorders.

## ACKNOWLEDGMENT

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