Anethum graveolens L. is a member of the Apiaceae family and more commonly known as dill. Dill has been used for gastrointestinal ailments such as flatulence, indigestion, stomach ache and colic. It has therapeutic effects such as mucosal protective, antisecretory, antimicrobial, antispasmodic, antihypercholesterolaemic and antihyperlipidaemic. The aim of our study was to examine the effects of the dill methanol extract on spontaneous and acetylcholine-induced contractions on isolated rat ileum. Segments of the rat ileum were suspended in an organ bath. The isolated ileum had been treated with the methanol extract of dill in cumulative concentrations (0.003 - 1mg/mL). In the second series of experiments, acetylcholine (5 -1500nM) was cumulatively added to the bath in the absence and presence of methanol extract of dill (0.3-1mg/mL). Cumulative concentrations of methanol extract of dill significantly reduced the spontaneous rat ileum contractions (p<0.01) with EC50 value of 6.45 ±0.87mg/mL. The methanol dill extract concentration-dependently inhibited the contraction induced with acetylcholine (p<0.01), with an EC50 value of 0.41±0.057nM and 1.10±0.29nM (the EC50 value of acetylcholine was 0.06±0.0097nM). Our results showed the relaxant effect of the methanol dill extract on the isolated rat intestine. Extract of dill inhibited the spontaneous ileum contractions and contractions induced by acetylcholine. Acta Medica Medianae 2015;54(2):5-10.

Key words: dill, Anethum graveolens L., methanol extract, acetylcholine, rat, ileum

Introduction

Study of contractility of the small intestine is very important for investigating the mechanisms involved in intestinal physiological and pathophysiologial processes. Intestinal spasms are uncontrolled contractions in the muscles of the small and large intestines. The spasm of intestinal smooth muscle may produce symptoms such as abdominal pain, colic, flatulence, constipation and diarrhea. It is very important to find plants or plant products that relax the intestinal muscle and that may be used for treatment of intestinal cramps. Dill (Anethum graveolens L.) is an important member of the Apiaceae family. This plant originates from the southwest Asia and is cultivated in many areas worldwide (1,2). It is well-known as aromatic herb in cooking and as a medicinal herb (3). Dill has been used in traditional phyto-therapy for centuries to treat digestive disorders, convulsion, vomiting and menstrual problems (4,5). It is known that dill fruits have been given to increase milk secretion in nursing mothers (6). Treatment with seeds of dill may be effective in improving the symptoms of halitosis (7). Also, dill has therapeutic properties such as mucosal protective, antidiabetic, anti-cancer, antioxidant, antimicrobial, anti-inflammatory diuretic, antiinflammatory, antiinfective, antihypercholesterolaemic and antiinflammatory (8-23).

In experimental study, dill induces a stimulatory effect on the female reproductive system. Hekmatzade et al. and Mirmolaee et al. reported that boiled dill seeds reduced pain intensity and duration of labor stages (24,25). Dill reduces pain severity in primary dysmenorrhea in female students who received powder of dill seed (26). On isolated rat uterus, dill extract induced the spasmolytic effect (27).

Intravenous application of dill oil to cats increased respiratory volume and depressed arterial blood pressure (28). Aqueous extract of
Anethum graveolens seeds decreased food intake, body weight and increased the level of serotonin in brain and plasma of experimental animals (29). Dill extracts possess anticonvulsant activity (30, 31).

It is reported that dill has been used as a household remedy for gastrointestinal ailments such as flatulence, indigestion and stomachache. Dill is given for treating baby colics (32). Extract of dill fruit has antisecretory effect on mice gastric mucosa.

**Aims**

Although some studies have found that dill is an effective treatment for gastrointestinal diseases, yet more research is needed to clarify the underlying mechanism that mediates its action. The present study was aimed to evaluate the possible spasmolytic activity of methanol extract of dill on isolated rat ileum.

**Materials and methods**

Drugs

The drugs used in biological tests were: acetylcholine chloride (Sigma, USA), atropine sulphate (Sigma, USA) and papaverine hydrochloride (Merck, Germany). All drugs were dissolved in distilled water for each experimental protocol. The composition of Tyrode solution (in mM) was: NaCl (136), KCl (2.7), CaCl₂ (1.8), NaHCO₃ (12), NaH₂PO₄ (0.3) MgCl₂ (1.8) and glucose (5.6).

Plant material

The dill was purchased from a local grocery. Aerial parts of the dill were open-air dried in the shadow. Dried and pulverized aerial parts of the dill were extracted 10 minutes in ultrasonic bath with methanol. After the filtration, the extracts were concentrated in a rotary evaporator at reduced pressure till a constant weight was achieved. The obtained dry residue was dissolved in distilled water in order to get 10% solution used in the experiment. For experimental purposes, the plant extracts were dissolved in the Tyrode solution in the appropriate concentration. The volume of dill extracts added to the organ bath never exceeded 5% of its total volume.

Animals

In this study, Wistar albino rats aged 3-4 months, with body weight 200–300g, were obtained from the Animal Research Center of the Faculty of Medicine, University of Niš, Serbia. The rats were housed in stainless steel cages under standard laboratory conditions. These animals were maintained at 20–24°C with a 12h light-dark cycle at least 1 week before the experiment. They had free access to food and water. All experimental procedures with animals were in compliance with the European Council Directive of September 22th, Directive 2010/63/EU and were also approved by the Local Ethics Committee (number 01-206-7).

**Experimental protocol**

The ileum portions were isolated out and cleaned off mesenteries. Preparations of 2cm long parts of the ileum were mounted in 10ml tissue baths containing the Tyrode’s solution maintained at 37°C and aerated with a mixture of 5% carbon dioxide in oxygen. The fragments were stretched to a sufficient tension and equilibrated for at least 30min before starting experiments. The change of intestinal contractility was recorded using system TSZ-04-E; Spell Iso (Experimetria Ltd). After each assay, tissues were washed with fresh Tyrode and equilibrated for around 10min.

Rat ileum exhibits spontaneous rhythmic contractions. The isolated ileum had been treated with the methanol extract of dill in cumulative concentrations (0.003-1mg/mL). Papaverine (0.01–3μg/mL) was used as a control. Relaxant effect of the methanol extract of dill was expressed as a percentage of regular spontaneous ileum contractions.

In the second series of experiments, isolated intestinal segments were contracted by the agonists of muscarinic receptors acetylcholine (5-1500nM). Acetylcholine was cumulatively added to the bath in the absence and presence of methanol extract of dill (0.3-1mg/mL). Atropine (140nM) was used as a control. The contraction caused by maximal concentration of acetylcholine in the absence of the dill extract or atropine was considered as the 100% contraction.

**Statistical analysis**

Mean and standard error values were calculated for each group of results (n=6 for each set of experiments) and significance of differences between the means were determined by the Student’s t-test. A probability value of p<0.05 or less was taken as statistically significant. An EC₅₀ value (concentration of drugs causing half-maximal responses) was established by regression analysis.

**Results**

The experimental data indicated that methanol dill extract (0.003-1mg/mL), concentration-dependently decreased the amplitude and tension of ileal muscle contractions. Cumulative concentrations of methanol extract of dill significantly reduced the spontaneous rat ileum contractions (p<0.01) with EC₅₀ value of 6.45±0.87mg/mL (Figure 1.). Papaverine (0.015–5μg / mL) also relaxed the rat ileum in a concentration-dependent manner.
The methanol dill extract (0.3-1mg/mL) concentration-dependently inhibited the contraction induced with acetylcholine (p<0.01), with an EC50 value of 0.41±0.057nM and 1.10±0.29nM (the EC50 value of acetylcholine was 0.06±0.0097nM) (Figure 2.). Atropine (140nM) exerted inhibitory effect on the contractile response of acetylcholine in isolated rat ileum.

Discussion

In this paper, we studied the possible spasmylytic effect of the methanol extract of dill in the isolated rat ileum. The present results showed that methanol extract of dill is a potent relaxant of spontaneous rat ileum contractions. The spasmylytic effect was reversible after washing the
ileum with the fresh Tyrode solution, suggesting that the inhibition was not due to the damage of the intestine by the dill extract. Our findings are in agreement with Naseri et al. (27) study, in which they found that dill fruit hydro-alcoholic extract produced the spasmolytic effect on the isolated rat ileum.

We also wanted to investigate the mechanism underlying the action of methanol dill extract on muscle contractions of isolated rat ileum. In order to explain the possible mode of spasmylytic activity, we examined the influence of the dill extract on acetylcholine-induced intestinal smooth muscle contraction. In our study, we also found that methanol extract of dill reduced acetylcholine-induced contractions of the isolated rat ileum. Treatment of tissues with atropine, nonselective blocker of muscarinic receptors inhibited intestinal muscle contractions induced by acetylcholine. These findings suggest that the action of methanol dill extract on rat ileum motility is mediated by the influence on muscarinic receptors. The enteric nervous system regulated motor and secretory functions of the gastrointestinal system. Acetylcholine is a very widely distributed neurotransmitter that is released from excitatory cholinergic neurons and plays an important role in the stimulation of intestinal smooth muscle contractions. Activation of muscarinic receptor in the rat ileum by acetylcholine induces an increase in intracellular calcium concentration through the second messenger inositol triphosphate and by facilitating the influx of extracellular calcium (32-34).

Phytochemical analysis of the dill demonstrated the presence of flavonoids quercetin, rutin, isorhamnetin and their derivatives (35,36). Flavonoids as an important class of natural compounds exert physiological properties (37). Quercetin and rutin produced relaxation in the isolated ileum contraction (38,39). Also, isorhamnetin produced the spasmylytic effect on the isolated intestine of mice. Vizcaino et al. found

that quercetin and isorhamnetin induced endothelium-independent vasodilator effects in rat conductance and resistance arteries (40,41). Quercetin and isorhamnetin prevent angiotensin II-induced endothelial dysfunction in rat aorta (42).

According to the literary sources, monoterpenes have been isolated from dill (43). The monoterpenes carvone and limonene produced ileum relaxation (44) and have a protective effect against induced convulsion in experimental animals (45,46).

Spasmylytic effects of dill methanol extract in isolated rat ileum may be due to the presence of these physiologically active components. Our results may explain the traditional use of dill in traditional medicine to treat gastrointestinal diseases.

**Conclusion**

In the present study, we investigated the effects of methanol dill extract on spontaneous smooth muscle contractions and those induced by acetylcholine in isolated rat ileum. The results suggest that methanol dill extract exerted a spasmylytic activity. Our findings are in agreement with the use of dill in traditional medicine for the treatment of gastrointestinal disorders.

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**References**

Spasmolytic effect of anethum graveolens L. methanol extract... Marija Gočmanac Ignjatović et al.


