Peppermint Aqueous Extract Counteracts Smooth Muscle Contraction in Rat Ileum

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ABSTRACT

Peppermint is widely used for gastrointestinal disorders; however, its pharmacological action on isolated rat ileum has not yet been evaluated. In this study, the effect of peppermint aqueous extract was investigated for its ability to reduce rat ilial smooth muscle contractility induced by acetylcholine (Ach) and potassium chloride (KCl). The experiments were conducted using an organ bath apparatus. A section of rat ileum was suspended in an organ bath containing Tyrode’s solution. The tissue was stimulated with either Ach or KCl. Iliial smooth muscle contractility was studied in the absence and presence of peppermint. Peppermint aqueous extract prevented ilial smooth muscle contractility induced by both Ach and KCl. The aqueous extract of peppermint (1g/ml) caused a rightward shift of the Ach dose response curve and brought about a decrease in the maximum response. This extract also produced a rightward shift in the KCl dose response curve but without affecting the maximum response. These results indicate that peppermint aqueous extract counteracts Ach and KCl induced smooth muscle contractility in rat ileum. The results also suggest that the peppermint effect on Ach induced contractility was non competitive in nature. The reduction in KCl induced contractility also indicates that peppermint aqueous extract was at least partially mediated by its effect on ilial smooth muscle calcium channels.

Keywords: Peppermint, Rat, Iliial Smooth Muscle, Acetylcholine, KCl, Dose Response.

INTRODUCTION

As the smooth muscle contractile activity is a major regulator of functions of the gastrointestinal (GIT) system, malfunction of contractility in this system leads to a host of clinical disorders. Because of this there is a great deal of interest in smooth muscle physiology and the mechanisms that manipulate it. Specific disorders of GIT smooth muscles include spasm, constipation, diarrhea and intestinal cramps. Their underlying causes and mechanisms of treatment involve smooth muscles1,2. Treating such disorders will involve medications targeting smooth muscle contractility. There are a good number of pharmaceutical agents manipulating muscle activity that have been derived from plant sources such as Papavarine, Anabesine, Cissampeline and Tubocurarine3.

Several metabolic and physical disorders related to gastric motility disturbances have been treated by using synthetic and herbal medicines. Medicinal plants have been used for thousands of years, and more recently there has been increased interest towards herbal remedies because of their effectiveness and lower cost.

In Jordan, the use of herbal medicine is very...
common. 60% of the Jordanian population utilizes traditional medicines with around 485 plant species used therapeutically in traditional medicine.

Peppermint is one of the most common plants used in Jordan, obtained from the over-ground parts of the flowering plant Mentha x Piperita L, it contains a large amount of essential oil. This oil is characterized by the presence of menthol and monoterpenes which are responsible for many of the activities of this herb. It also contains flavonoid compounds and tannins. Peppermint oil has been used traditionally as a carminative agent, in the relief of flatulence and colic pain, and more recently in therapy of the irritable bowel syndrome.

Peppermint oil has been shown to relax isolated gastrointestinal smooth muscle and its mechanism of action has been studied in several pharmacological preparations. In the latter, it has been suggested that peppermint oil influenced a reduction in the availability of calcium in gastrointestinal smooth muscles. Evidence for the calcium antagonistic properties of peppermint oil were demonstrated when menthol and peppermint oil reduced the binding of specific calcium channel ligands in smooth muscle and also inhibited depolarization-induced Ca⁺ uptake into neuronal preparations.

In this study, we have investigated the effect of peppermint aqueous extract rather than oil on isolated rat ileum, where the majority of Jordanians utilize peppermint as an aqueous extract or tea.

MATERIALS AND METHOD

Materials
Ach, KCl and all tyrode solution constituents; NaCl, KCl, CaCl₂, NaHCO₃, NaH₂PO₄, MgCl₂ and glucose were obtained from Sigma-Aldrich. Peppermint aqueous extract was prepared by collecting leaves of fresh whole peppermint plant (Mentha x Piperita L, 100g) and then chopping and soaking them in 100 ml boiling distilled water, the mixture was then filtered and the filtrate was used in the conducted experiments. The two different concentrations used (2.5% v/v and 3.75% v/v) were representative of the amount in volume of peppermint filtrate divided by the final volume of Tyrode's solution in the organ bath. The extraction method used here was based on the common method reported by Jordanian traditional medicine providers where they either prepared mint in this manner, or suggested doing so to patients. Although this may differ from common phytochemical extraction methods, the goal here was to mimic common local practices and investigate their pharmacological effectiveness and thus therapeutic value.

Animals
Sprague Dawley rats (190 ± 10 g) were purchased from the animal facility from Jordan Applied University and housed at 20-24°C with access to food and water. The rats were deprived of food (not water) for 24 h before the experiment. All procedures concerning animals were carried out according to Jordanian regulations for animal experimentation and care, and in compliance with the United Kingdom Home Office animal care guidelines.

Ileum Preparation
On the day of experiment, rats were sacrificed by cervical displacement and one or two pieces (1.5-2 cm) of ileum were dissected out. The piece of ileum was cleaned from its luminal contents by flushing its lumen gently with a stream of Tyrode's solution by using a pipette then mounted between two stainless steel hooks in tissue bath (40mL) containing Tyrode's solution (37°C, bubble of oxygen and pH 7.4). The lower hook was fixed at the bottom of the tissue bath and the upper one was connected to an isotonic transducer (Harvard Transducer, UK). Each tissue was placed under 1 g resting tension and equilibrated for 60 min prior to the execution of
experimental protocols. During this period, the tissue was washed with Tyrode's solution every 15 min and the tension was readjusted to 1 g. Ileum contractions were displayed and recorded on Universal Harvard Oscillograph, (UK). The ileum contractions were induced initially by Ach (15mM) or KCl (10 mM) and then dose response curves were established. Further dose response curves were obtained after the crude peppermint aqueous extract was added to the tissue bath.

**Measurements and Statistical Analysis:**

All data are expressed as mean ± standard error of the mean (SEM). Results were analyzed using non linear regression (curve fit) with an extra sum-of squares F test comparison method for logEC50 and hillslope values using GraphPad Prism® software.

**RESULTS**

**Ach Dose Response Curve**

Figure (1) indicates the contractile effect of different concentrations of Ach (5 x 10^{-10} to 5 x 10^{-9} M) on isolated rat ileum. As shown, an increase in Ach concentration caused increasing illial smooth muscle contraction and a maximum response was observed at a concentration of 5 x 10^{-9} M. Ileum contraction to Ach was computed based on the percentage and maximal effect induced by concentration of 5 x 10^{-9} M which was considered to be 100% response and other responses were observed as percentages of this maximal response. Log EC_{50} value for Ach was -8.818.

![Figure 1. Dose response curve achieved by Ach on contraction of isolated rat ileum.](image)

Each point represents mean ± SEM of 3 observations. LogEC_{50} = -8.818.

**Effect of Atropine on Ach Induced Contraction on Rat Ileum**

The use of atropine in conjunction with Ach produced a parallel rightward shift of the Ach dose response curves without affecting the maximum response (fig.2).
Figure 2. Figure illustrate the inhibitory effect of atropine on Ach dose response curve.

Each point represents mean ± SEM of 3 observations. Atropine caused a concentration-dependent rightward shift of the Ach dose response curve where LogEC₅₀ for Ach alone was -7.555 and in the presence of 1.3 nM atropine was -6.619, and with 2.6 nM of atropine was -5.686. LogEC₅₀ was significantly different for each data set (P < 0.0001). HillSlope was not significantly different for any of the data sets (P < 0.0001).

Analysis of this data utilizing a Schild plot showed a slope of -0.9824 ± 0.02419, which was not significantly different from unity, and a pA2 (pKB) value of - 9.612 (fig.3).

Figure 3. Figure illustrates the schild plot for atropine.

Where dr = EC₅₀ for Ach in the presence of atropine divided by EC₅₀ for Ach. Slope = -0.9824 ± 0.02419 and pA2 value = 9.612.
Effect of Peppermint Aqueous Extract on Iliial Contraction Induced by Ach

The dose response curve for Ach was evaluated in the presence and absence of peppermint aqueous extract. Here, two different concentrations of the extract were used, 2.5 % and 3.75 %, where there was a concentration-dependent rightward shift of the Ach dose response curve. Also peppermint aqueous extract brought about a significant concentration-dependent reduction in the maximal response (fig. 4).

![Figure 4. Effect of peppermint extract on rat ileum contraction induced by Ach.](image)

Each point represents mean ± SEM of 3 observations. Peppermint aqueous extract caused a concentration dependent rightward shift in the Ach dose response curve where LogEC$_{50}$ for Ach was -7.734 for Ach alone, -7.130 in the presence of 2.5% peppermint and -6.820 in the presence of 3.75% peppermint. This extract also brought about a concentration dependant reduction in the maximal response where Hill Slope for Ach was 7.593 for Ach alone, 2.571 in the presence of 2.5% peppermint and 2.321 in the presence of 3.75% peppermint. LogEC$_{50}$ was significantly different for each data set (P < 0.0001). HillSlope was also significantly different for each data set (P < 0.0001).

Effect of Peppermint Aqueous Extract on Iliial Contraction Induced by KCl:

Peppermint extract caused a dose-dependent relaxant effect on the contraction of rat’s ileum. Figure 5 illustrates the dose response curve obtained from using KCl as an inducer of ilial muscle contractility in the presence of two different concentrations of mint aqueous extract. Here, a concentration-dependant inhibition of ilial muscle contractility was observed and the dose response curve of KCl suffered a rightward shift without affecting the maximal response.
DISCUSSION

The results of this study confirm the ability of peppermint extract to directly relax gastrointestinal smooth muscles. These results are in accordance with relevant findings on the effect of peppermint oil on smooth muscle contractility in vitro and in vivo. In the latter, peppermint oil was examined for its clinical viability in patients suffering GIT disorders and proved clinically effective in managing irritable bowel syndrome and colonic spasms during endoscopy.8,9

Using Ach in vitro on illial tissue to induce contraction serves as a model where excessive enteric nervous system activation would be responsible for GIT spasms. Agents which counteract these spasms are considered antispasmodic drugs which exert their effects by either blocking muscarinic receptors, relaxing smooth muscles, or blocking calcium-channels.13,14,15 The results obtained in this study indicate that peppermint aqueous extract reduced the Ach induced contractions in rat ileum and shifted the dose response curves downward and to the right. And since the scenario did not follow the competitive muscarinic receptor behavior as seen with atropine (fig.2 and fig.3) it is safe to assume that ability of peppermint to antagonize Ach induced contraction was due to its interference with intracellular calcium availability in illial smooth muscle cells. Rat illial smooth muscle contractions induced by KCl were antagonized by the peppermint aqueous extract, and the dose response curve suffered rightward without affecting the maximum response. Agents which inhibit contractions induced by KCl are considered calcium channel blockers, where exposure of smooth muscle cells to K+ stimulates contractions through the opening of voltage-dependent L-type Ca2+ channels and consequent influx of extra cellular Ca2+.16 The previous suggests that the peppermint extract used in this study may have at least partially conveyed its effects in this manner. The obtained data also illustrate that peppermint aqueous extract antagonized KCl induced contractions without affecting maximum response. This is supported by Taylor et
al. where similar antagonism was reported after using menthol, a major constituent of peppermint oil. The authors of the previous study suggested that the produced antagonism was a result of interference with cellular Ca\(^{2+}\) influx. The alterations in the dose response curve produced by KCl in this study suggest a similar mode of action. The interference of peppermint aqueous extract with Ca\(^{2+}\) influx could be attributed to direct action on voltage gated calcium channels preventing their natural response to alterations in potential. On the other hand, peppermint extract could be indirectly hyperpolarizing smooth muscle cells through interfering with either cyclic adenosine monophosphate levels or the intracellular calcium pool. This would then hinder Ca\(^{2+}\) influx through voltage gated channels which normally respond to muscle cell depolarizing. In any case, the consequent outcome would be reduced smooth muscle contractility.

When other research had proven the usefulness of peppermint oil in counteracting intestinal spasms and hyper motility, it remained not completely clear if peppermint aqueous extract would be of value in such situations. In particular, plant oil extracts usually do not harbor similar compounds as water extracts would. This would have previously questioned the value of peppermint tea in such situations and that it merely had a psychologically induced relaxing effect. In this study it was clearly established that peppermint aqueous extract and hence peppermint tea is of value in treating GIT symptoms, such as spasms and cramps, secondary to excessive illial smooth muscle stimulation.

**CONCLUSION**

From the results obtained in this study we conclude that peppermint aqueous extract is a useful herbal remedy for treating intestinal spasms. While many herbal remedies used in Jordan have questionable benefits, mint aqueous extract was actually one that has proven to have significant effects on GIT motility and thus, valuable in treating intestinal spasms and related GIT symptoms.

The mechanism of action of the peppermint aqueous extract is presumed to be achieved through interfering with calcium channel natural responses and not via directly blocking muscarinic receptors. The effects of peppermint aqueous extract on the calcium current resembles those caused by menthol, suggesting that menthol, which is the major constituent of peppermint, maybe implicated in mediating these actions.

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مستخلص النعناع المائي يثبط تقلص العضلات الملساء في لقائف الأمعاء الدقيقة للفرائن

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ملخص

يستخدم النعناع على نطاق واسع لإعدادات الجهاز الهضمي، ولم يتم حتى الآن دراسة على لقائف الأمعاء الدقيقة، ففي هذا البحث، تم دراسة تأثير مستخلص النعناع المائي من حيث قدرته على تثبيت انقباضات الأمعاء الناتجة عن الاستيتيز كولين وكليوريد البوتاسيوم. أجريت التجارب باستخدام جهاز حمام الأغشية، حيث عُلقت قطعة من لقائف الأمعاء الدقيقة المعزولة من الفأر في الجهاز الذي تحتوي على محلول الملح التأريدي. وقد تم تحسين لقائف الأمعاء الدقيقة بوساطة مادة الاستيتيز كولين أو مادة كليوريد البوتاسيوم. يبقى النسيج تحت درجة حرارة 37 درجة مئوية مع استمرار تدفق غاز الأكسجين في الجهاز. وقد درست الارتباطات التي تحصل لقائف الأمعاء الدقيقة في غياب مستخلص النعناع وجودة. واستطاع مستخلص النعناع المائي منع الارتباطات الناجمة عن الاستيتيز كولين وكليوريد البوتاسيوم. وقد عمل مستخلص النعناع المائي على زيادة منحنى الاستجابة للاستيتيز كولين لجهة اليمين وحدث انخفاضاً على الحد الأقصى للإانتظام. وازعج منحنى الاستجابة لوكليوريد البوتاسيوم إلى جهة اليمين ولكن من غير أن يؤثر في الحد الأقصي للإانتظام. وهذه النتائج تشير إلى أن مستخلص النعناع المائي للنعناع يعمل على انسحاب العضلات في لقائف الأمعاء الدقيقة، ويشير تأثير مستخلص النعناع على الارتباطات بسبب الاستيتيز كولين إلى أنه غير تنافسي في طبيعته على مستقبلات الأشريكي. أما تأثيره على كليوريد البوتاسيوم، فيشير إلى أن ارتخاء العضلات ناجم عن تأثيره على قوات الكالسيوم الموجودة في العضلات الملساء.

الكلمات المفتاحية: النعناع، فانار، العضلات الملساء في لقائف الأمعاء، الاستيتيز كولين، كليوريد البوتاسيوم، منحنى الاستجابة.

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