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Spasmolytic and Anti-Spasmodic Action of *Trachyspermum ammi* Essence on Rat's Ileum Contraction

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Abstract

Background:

Trachyspermum ammi is a plant of umbelliferae family and has been traditionally used in the treatment of many gastrointestinal disorders such as indigestion, colic, and diarrhea. Our previous study demonstrated that aqueous extract of *Trachyspermum ammi* reduced the contractile activity of rat's ileum.

Aims:

This study was designed to examine the effect of *Trachyspermum ammi* essence on acetylcholine-induced contraction in isolated rat's ileum.

Materials and Methods:

In this experimental procedure, the tissue samples were suspended from the transducer lever and mounted on the organ bath containing Tyrode's solution. Isotonic responses were recorded by using an isotonic transducer and the amplitude of contractions. This process was induced by cumulative logarithmic concentrations of acetylcholine before and after exposing tissue by saline and different concentrations of essence.

Results:

The chemical constituents of the essence from distillate extract of *Trachyspermum ammi* seeds were studied by gas chromatography mass spectrometry (GC-MS) and the chemical analysis showed many components in which thymol was the main constituent. Our findings showed that the essence derived from the extract in all concentrations used in this study (0.002, 0.005, and 0.01% V/V) significantly reduced acetylcholine-induced contractions (47.34, 60.46, and 86%, respectively, $P < 0.05$). The same concentrations of the essence also exhibit a significant anti-spasmodic action on acetylcholine-induced contractions (0.1,88.3, and 90.7%, respectively, $P < 0.05$).

Conclusion:

Since thymol was the major constituent of our samples of *Trachyspermum ammi*, the relaxant effect observed in this study is probably due to this agent which can exert an anti-cholinergic property.

Keywords: Acetylcholine, Contraction, Essence, ileum, Smooth muscle, *Trachyspermum ammi*

Introduction

Trachyspermum ammi is a plant of umbelliferae family with a white flower and small brownish seeds. Its major component is essence which is mainly composed of thymol (49.0%), γ-terpinene (30.8%), p-cymene (15.7), β-pinene (2.1%), myrcene (0.8%), and limonene (0.7%).[1] *Trachyspermum ammi* has been traditionally used in the treatment of many gastrointestinal disorders such as indigestion, colic, and diarrhea.[2] The extracts obtained from the seeds of carum copticum have several pharmacological effects including anti-cholinergic,[3] analgesic,[4] anti-asthmatic,[5] and anti-tussive[6] activity. Along with the availability of modern medications, the propensity toward the traditional medications is progressively growing throughout the world[7] and promotes the scientific investigators to evaluate the therapeutic effects of medicinal plants and their fractions. In our previous studies, it has been demonstrated that the relaxant effect of carum copticum extract on intestinal motility,[8] and its inhibitory effect on acetylcholine-induced contraction[9] have been demonstrated on isolated rat's ileum. Since the majority of pharmacologically active constituents of carum copticum have accumulated in its essential oil,[1] this study was conducted to examine the spasmolytic and anti-spasmodic effect of *Trachyspermum ammi* Essence (TAE) on acetylcholine-induced contraction in isolated rat's ileum.

Materials and Methods

In this study, adult male albino rats weighing 200-250 g, living in the standard environment and feeding conditions, were used for isolation of ileum. All experimental procedures were carried out by permission of the Animal Ethics Committee of Shahid Sadoughi University of Medical Sciences (Yazd, Iran), which was in accordance with the internationally accepted principles for laboratory animal use and care mentioned by the European Community guidelines. *Trachyspermum ammi* seeds were provided by Agricultural Research Center (Yazd,Iran) and identified by a botanist in this center. One hundred grams of air-dried seeds of *Trachyspermum ammi* were gently grounded and mixed with 500 ml of double-distilled water. Then, its essence was prepared by Soxhlet apparatus. The concentration of essence was 1.5% V/V. A sample of the yielded essence was analyzed by Agilent Technologies (Delaware, USA) 6890N network GC system. In this study, 0.002, 0.005, and 0.01% concentrations of essence were examined for their spasmolytic and anti-spasmodic action. Acetylcholine chloride, as a standard stimulant of gastrointestinal smooth muscle was purchased from Sigma Aldrich Chemie Gmbh, Germany. Experiments were performed as described in our previous report.[7,8] Briefly, adult male albino rats were sacrificed by cervical dislocation. Segments of ileum (2 cm in length), were excised, flushed of their contents, and trimmed of their mesentery. The specimens were conserved in Tyrode's solution until the onset of experimental procedure. The tissue sample was fixed at the bottom of the internal chamber of an organ bath containing 50 ml Tyrode's solution in the axis of its longitudinal muscle and its opposite end was tightly tied to the isotonic transducer lever with a piece of thread, the chamber was maintained at 37°C and bubbled with 95% O_2 and 5% CO_2. Isotonic responses were recorded by using an isotonic transducer (T2) and an oscillograph recording system (the Bioscience 400 Series Washington Oscillograph). Then, it was allowed to stabilize for 15 min prior to the addition of drug, and washed out in 30-minutes intervals by a fresh Tyrode's solution.

To investigate the spasmolytic action of essence, first we obtain the maximum contraction of ileum using acetylcholine 10^{-4} M. Then, we study the effect of different concentrations of essence on maximal contraction. Furthermore, to determine the anti-spasmolytic action of essence, we apply different concentrations of essence to the tissues under study 7 min before the creation of contraction; then, we apply the cumulative concentrations of acetylcholine (10^{-12} up to 10^{-2} M) on tissues to see the effect of the essence on the induced contraction.

Statistical Analysis: The effect of different experimental solutions were expressed as Mean ± standard deviation (SD) of percentage inhibition of contraction amplitude and compared to maximum effect induced by acetylcholine. All statistical analyses and comparisons were made by means of the analysis of variance (ANOVA), followed by Tukey's test. The statistical significance was considered as $P < 0.05$.

Results

The chemical constituents of the essence from distillate extract of *Trachyspermum ammi* seeds were studied

by GC-MS. The results showed that there are numerous ingredients in the sample, and thymol was its main constituent [Table 1]. Our findings showed that the essence derived from *Trachyspermum ammi* seeds in concentrations of 0.002, 0.005, and 0.01% reduced acetylcholine (10^{-4} M)-induced contractions significantly by 52.66 ± 4.12, 39.44 ± 4.46, and 14 ± 2.4, respectively vs. 100 ± 0.00 ($P < 0.05$, [Figure 1]. The inhibitory effect of TAE (0.01 %) on acetylcholine-induced contraction was immediately initiated upon its addition to the organ bath, when it reached to its maximum within 0.5 min and persisted at least for 5 min [Figure 2]. On the other hand, the same concentrations of *Trachyspermum ammi* (0.002, 0.005, and 0.01%) showed a significant anti-spasmodic action against cumulative concentrations of 10^{-5} up to 10^{-2} M acetylcholine [$P < 0.05$, Figure 3]. In the presence of 10^{-3} M acetylcholine, the maximum (96.16%) and minimum (88.17%) inhibition of contraction were induced by 0.01 and 0.002% of *Trachyspermum ammi*, respectively, and the difference between the anti-spasmodic effects of these two concentrations was statistically significant [$P < 0.05$, Figure 4]. Due to the high concentration of thymol in TAE, the anti-spasmodic effect of TAE and thymol on isolated rat's ileum was compared [Figure 5]. Each point indicates the mean of six experiments, the vertical bars represent the SD, and *indicates the significant difference between the acetylcholine-induced contractions in the presence of different concentrations of thymol and TAE ($P < 0.05$).

Discussion

The results of the present study showed that different concentrations of TAE can inhibit a potent spasmolytic and anti-spasmodic effect on isolated rat's ileum. This finding was consistent with our previous studies in which the aqueous and ethanolic extracts showed a significant relaxant effect on ileal smooth muscle.[8,9] However, TAE had more potent inhibitory effect on Ach-induced contractions as compared to aqueous extracts. According to our previous work, only distillate concentrations of more than 1% could inhibit the maximum contractile response of the effective concentration (10^{-4} M) of acetylcholine by about 60%,[9] whereas in the present study, the minimum concentration of TAE (0.002%) suppressed the contractile activity of 10^{-3} M acetylcholine up to 88.17%. The time course for essence in exerting its anti-spasmodic action was also shorter than that of aqueous extracts. The concentration of 0.01% essence exerted its inhibitory effect immediately and reached to its maximum rate within 30 seconds, whereas its aqueous extracts (1%) initiated the inhibitory action on acetylcholine-induced contraction after one minute and completed its action within 2 min.[9]

The essence of *Trachyspermum ammi* in our sample mainly consisted of thymol, cymene, gamma-terpinene, carvacrol, and pinenes. These compounds have also been previously reported as the major constituents of carum copticum extracts;[1,10] however, the percentage of thymol extracted in our sample was much higher than the previous reports. All of the above-mentioned components have been reported to have potent relaxant effect on smooth muscle in various organs.[11,12,13,14]

Various mechanisms are involved in gastrointestinal smooth muscle relaxation. These include the blocking action on excitatory pathways, such as cholinergic[15] and histaminergic[16] or via agonistic action on inhibitory modulators such as adrenergic,[17] purinergic,[18] GABAergic,[19] and/or nitric oxide.[20] Of the components of the extract and its essence, the anti-cholinergic activity of thymol has been reported.[21] On the basis of our previous studies in which the extract showed a significant muscle relaxant effect and also regarding the present study in which the essence has had more potent anti-spasmodic effect, it could be concluded that the observed inhibitory effect of essence on smooth muscle contraction may be through the thymol action. There have also been reported that carum copticum ingredients influence the tracheal smooth muscle via their anti-cholinergic,[22] anti-histaminic,[23] or calcium channel blocking activity.[24] These reports have mentioned that the functional anti-cholinergic effect of its extract and essence could be indirectly related to β-adrenergic stimulatory action or may be due to their direct inhibitory effect on cholinergic receptors.[23]

The inhibitory effect of extracts of this plant on isolated ileum preparations which were pre-treated and post-treated with Ach, indicates their functional antagonistic effect on cholinergic receptors in rat's ileum. As a rule of thumb, smooth muscle contraction is a calcium- and adenosine triphosphate (ATP)-dependent process, and thymol exerted its relaxant effect through opposing this process.[25] It can also block the Ca^{+2}

influxes through the cell membrane[26] and reduction of the calcium content of the sarcoplasmic reticulum.[27] On the other hand, carvacrol which is another pharmacological active constituent of the essence has been shown to possess a significant relaxant effect on smooth muscles through its blocking action on muscarenic receptors and/or β-adrenergic stimulation.[11] It has been demonstrated that Pinene compounds which are present in essence, exhibit a relaxant effect on the tonic contractions induced by either KCl or Ach.[28] Since these inhibitory compounds are mainly deposited in the essence, the more potent spasmolytic effect of essence as compared to extract may be related to the possible mechanisms of actions ascribed to these constituents. Finally, more details of the issue demands further investigation.

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Footnotes

Source of Support: Nil

Conflict of Interest: None declared.

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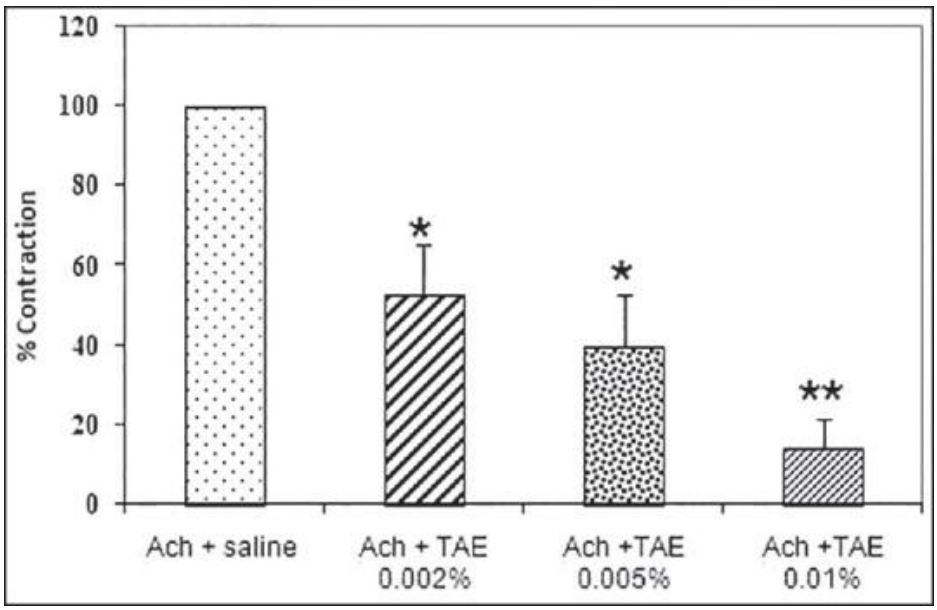
Figures and Tables

Table 1

Compound	Composition (%)	Retention index
Thymol	86.265	416
Carvacrol	0.470	416
α-terpinyl propionate	0.051	416
Piperitenone	0.071	416
Dimethyl phthalate	0.699	811
2,4-Di-tert-butylphenol	0.610	811
Dillapiole	0.022	342
α-thujene	0.009	769
α-pinene	0.020	619
β-pinene	0.078	619
β-myrcene	0.010	662
α-terpinene	0.013	662
o-Cymene	1.370	662
Eucalyptol	0.024	662
Limonene	0.005	662
γ-terpinene	0.526	662
4-terpineol	0.041	684
α-terpineol	0.032	678

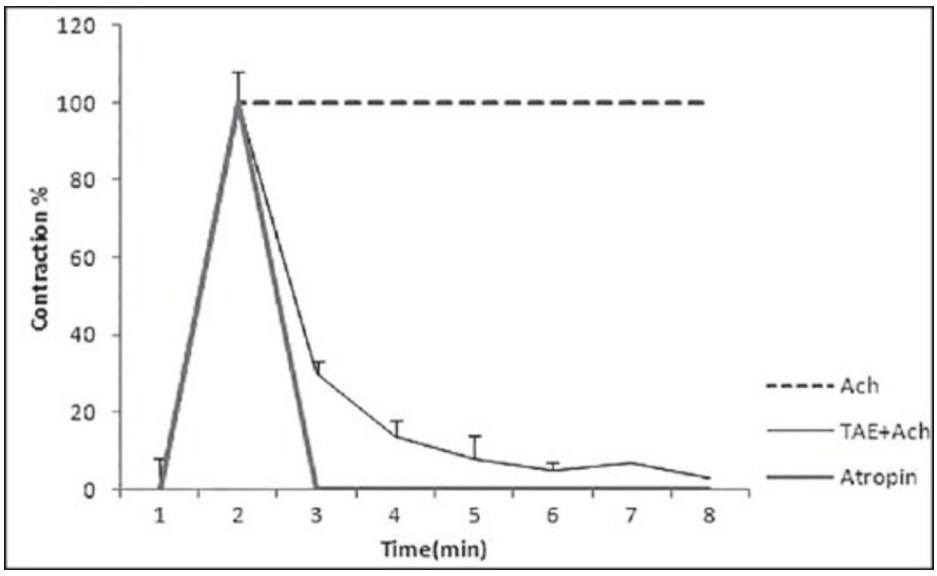
Chemical constituents of the essence from *Trachyspermum ammi*

Figure 1



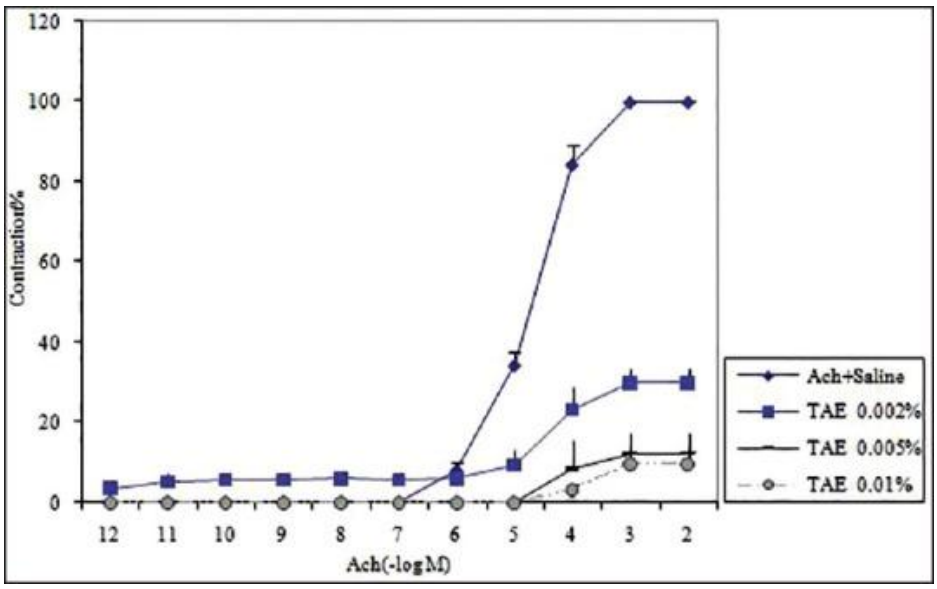
Spasmolytic effect of *Trachyspermum ammi* Essence (TAE) on acetylcholine-induced contractions (10^{-4} M) in the isolated rat's ileum ($n = 6$). *indicates the significant difference ($P < 0.05$) as compared to the acetylcholine-induced contraction according to the one-way analysis of variance (ANOVA), followed by Tukey's post-test

Figure 2



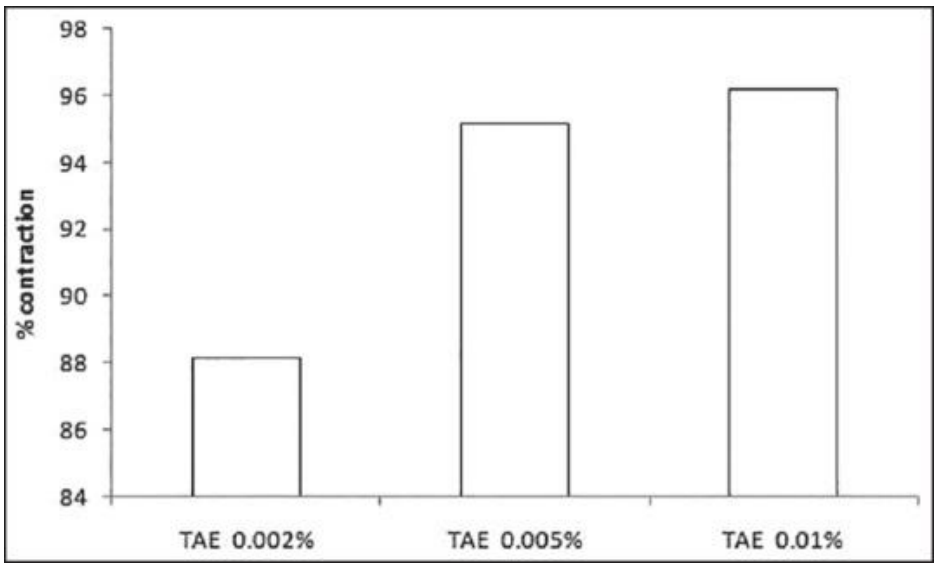
The time-course inhibitory effect of atropine (10^{-6} M) and *Trachyspermum ammi* Essence (TAE) (0.01%) on acetylcholine-induced contractions (10^{-4} M) in the isolated rat's ileum ($n = 6$)

Figure 3



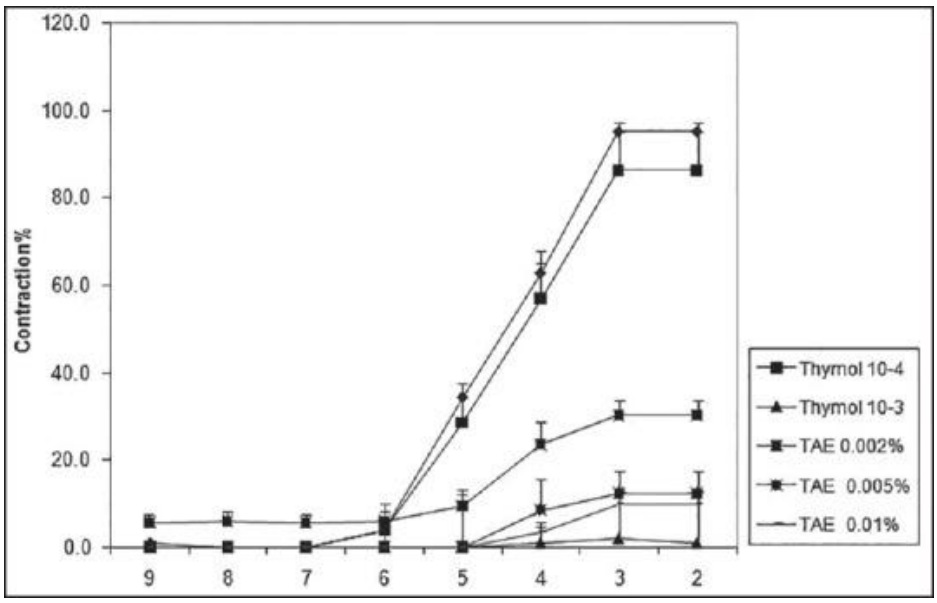
Anti-spasmodic effect of *Trachyspermum ammi* Essence (TAE) on isolated rat's ileum. Each point indicates the mean of six experiments and the vertical bars represent the standard deviation (SD). * indicates the significant difference between the acetylcholine-induced contractions in the presence of saline and three different concentrations of TAE ($P < 0.05$). According to the oneway analysis of variance (ANOVA), followed by Tukey's post-test

Figure 4



The percentage of contraction inhibition induced by different concentrations of *Trachyspermum ammi* Essence (TAE) in the presence of 10^{-3}M acetylcholine ($N = 6$). *indicates the significant difference ($P < 0.05$) as compared to the 0.002% TAE according to the one-way analysis of variance (ANOVA), followed by Tukey's post-test

Figure 5



The comparison between anti-spasmodic effect of *Trachyspermum ammi* Essence (TAE) and thymol on isolated rat's ileum. Each point indicates the mean of six experiments and the vertical bars represent the standard deviation (SD). *indicates the significant difference between the acetylcholine-induced contractions in the presence of different concentrations of thymol and TAE ($P < 0.05$)