

Full Length Research Paper

Does essential oil of *Carum copticum* affect acetylcholine-induced contraction in isolated rat's Ileum?

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Our previous study demonstrated that aqueous extract of *Carum copticum* reduced contraction activity of rat's ileum; this study was conducted to examine the effect of essential oil from *C. copticum* on acetylcholine induced contraction in isolated rat's ileum. In experimental procedures, the tissue samples were suspended from the transducer lever, mounted in the organ bath containing Tyrode's solution and isotonic responses were recorded by using an isotonic transducer and the amplitude of contractions induced by cumulative log concentrations of acetylcholine after exposing tissue by saline, different concentrations of essential oil or aqueous extract. Our findings showed that essential oil derived from extract in concentrations more than 0.002% reduced acetylcholine -induced contractions. The relaxant effect observed in this study is probably due to the thymol which is the main constituent of the essential oil.

Key words: *Carum copticum*, essential oil, ileum, contraction, acetylcholine.

INTRODUCTION

Carum Copticum L. (Sprague ex Turrill) (CC) is a plant in Umbelliferae family with a white flower and small brownish seeds. Its major components are essential oil which is mainly composed of thymol and other substances such as γ -terpinene, p-t cymene, α -pinene and β -pinene (Chopra, 1982). CC is traditionally used in treatment of many gastrointestinal disorders such as indigestion, colic, dyspepsia and diarrhea (Avecina, 1985). The seeds of CC have several pharmacological effects including

cholinergic (Devasankaraiah et al., 1974), analgesic (Dashti-Rahmatabadi et al., 2007), anti-asthma and anti-dyspnea (Boskabady et al., 2003). Along with the availability of modern medications, the propensity towards the traditional medications is growing throughout the world (O'Malley, 2004) and promotes scientific investigations to evaluate the therapeutic effects of medicinal plants and their fractions. Some of these biological investigations have been focused on the fractions of extract. In our previous studies, the relaxant effect of CC on intestinal motility (Hejazian et al., 2007), and the inhibitory effect of CC extract on acetylcholine induced contraction have been demonstrated on isolated rat's Ileum (Hejazian et al., 2009). Considering the antispasmodic effects of its aqueous extract, this study was conducted to determine the spasmolytic role of its

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essential oil on acetylcholine induced contraction.

MATERIALS AND METHODS

Animals

In this study, adult male Albino rats living in the standard environmental and feeding conditions were used for isolation of ileum. For experimental procedure, permission of the animal ethics committee of Shahid Sadoughi Medical Science University (Yazd, Iran), in accordance with the internationally accepted principles for laboratory animal use and care mentioned by the European Community guidelines was obtained.

Tissue preparations

Experiments were performed as described in previous reports (Osinski et al., 1999; Fang et al., 2005). Briefly, adult male Albino rats weighing 200 to 250 g were sacrificed by cervical dislocation. Segments of ileum (2 cm in length), were obtained, flushed their contents and trimmed them of mesentery.

Extraction preparations and chemicals

The plant material was identified by a botanist in the herbarium of Yazd herbal medicine research center. 550 g of air dried seeds of CC were gently grounded and mixed with 500 ml of double distilled water. Then it was extracted by steam distilled apparatus. The essential oil was separated from aqueous extract by soxhlet apparatus. The concentration of essential oil in the aqueous extract was 1.5% V/v was purchase from Sigma Aldrich Chemie GmbH, Germany AE & EO concentrations.

Protocols

The tissue samples were suspended from the transducer lever in the axis of its longitudinal muscle with a piece of trade containing Tyrode's solution, it was maintained at 37°C and bubbled with 95% O₂ and 5% CO₂ (Osinski et al., 1999). Then, it was allowed to stabilize for 15 min prior to the addition of drug and washed out in 30 min intervals by a fresh Tyrode's solution. Isotonic responses were recorded using an isotonic transducer (T2) and an ocollograph recording system (the bioscience 400 Series Washington ocollograph).

Control contractions were obtained by adding acetylcholine (10 to 12 up to 10 to 2 M) to each tissue preparation in cumulative manner. In control records, normal saline was used as the vehicle.

Tissue preparations were washing out by Tyrode,s solution and incubated in the separate sets of experiments at least for 20 min. After exposing tissues to different concentrations of CC aqueous extract or it's essential oil, the contractile response of specimens induced by the cumulative acetylcholine were recorded.

Statistical analysis

The effect of different solutions on acetylcholine (Ach) induced contractions, were expressed as mean \pm SEM of the contraction amplitude. All statistical analysis and comparisons were made by means of the ANOVA followed by Tukey's test. The statistical significance was considered as $P < 0.05$.

RESULTS

Our findings showed that the CC aqueous extract in

concentrations of 0.4 and 0.8%, produces an inhibitory effect on acetylcholine induced contraction but its 0.2% had no effect on tissue contractions (Figure 1). The essential oil derived from extract in concentrations more than 0.002% (0.005 and 0.01%) reduced acetylcholine induced contractions (Figure 2).

DISCUSSION

In previous study, the relaxant and inhibitory effects of *C. copticum* aqueous extract (CCAЕ) on acetylcholine induced contraction of isolated rat's ileum have been demonstrated (Hejazian et al., 2007). In the present study, the effect of EO yield from CCAЕ on acetylcholine induced contraction of ileum smooth muscle was studied and compared with saline and CCAЕ

Various mechanisms are involved in gastrointestinal smooth muscle relaxation. These include the blocking action on excitatory such as cholinergic (Unnom et al., 2006) and histaminergic (Sá-Nunes, et al., 2003) or via agonistic action on inhibitory modulators such as adrenergic (Roberts, 1999), purinergic (Van Crombruggen et al., 2007), GABAergic (Zizzo et al., 2007) and/or nitric oxide (Kito and Suzuki, 2006). There are some evidences that CC ingredients influence the tracheal smooth muscle via their anti-cholinergic (Boskababy et al., 1996), anti histaminic (Boskababy et al., 2000) or calcium channel blocking activity (Gilani et al., 2005).

The results of this study were in accordance with our previous study in which CCAЕ showed a significant relaxant effect; the effect could be partly due to its EO ingredients that severely decreased Ach induced contractions as compared with saline.

The functional anti-cholinergic effect of CCAЕ and its EO observed in this study could be indirectly related to β -adrenergic stimulatory action or may be due to their inhibitory effect on cholinergic receptors. The inhibitory effect of extracts from this plant were, examined on isolated ileum preparations pretreated (Hejazian et al., 2009) and post treated with Ach (present study) indicates a functional antagonistic effect of EO on cholinergic receptors in rat's ileum.

The essential oil of *C. copticum* constitutes of pinene components (Prakash et al., 2006), thymol (Hisayama, 1983; Beer et al., 2007) and carvacrol (Aydin and Seker, 2005) which have potent relaxant effect (Boskabady et al., 2003). From these components, the anti cholinergic activity of thymol was also reported (Hisayama, 1983), on the basis of our previous studies in which CCAЕ showed a significant muscle relaxant effect and also regarding the present study that EO has had a potent relaxing effect; it could be concluded that observed relaxation effect of EO may be through the thymol action.

As a rule of thumb, smooth muscle contraction is a calcium and ATP dependent process and thymol exerting its relaxant effect through opposing this process. Thymol is used as potent agents for studying the mechanism of

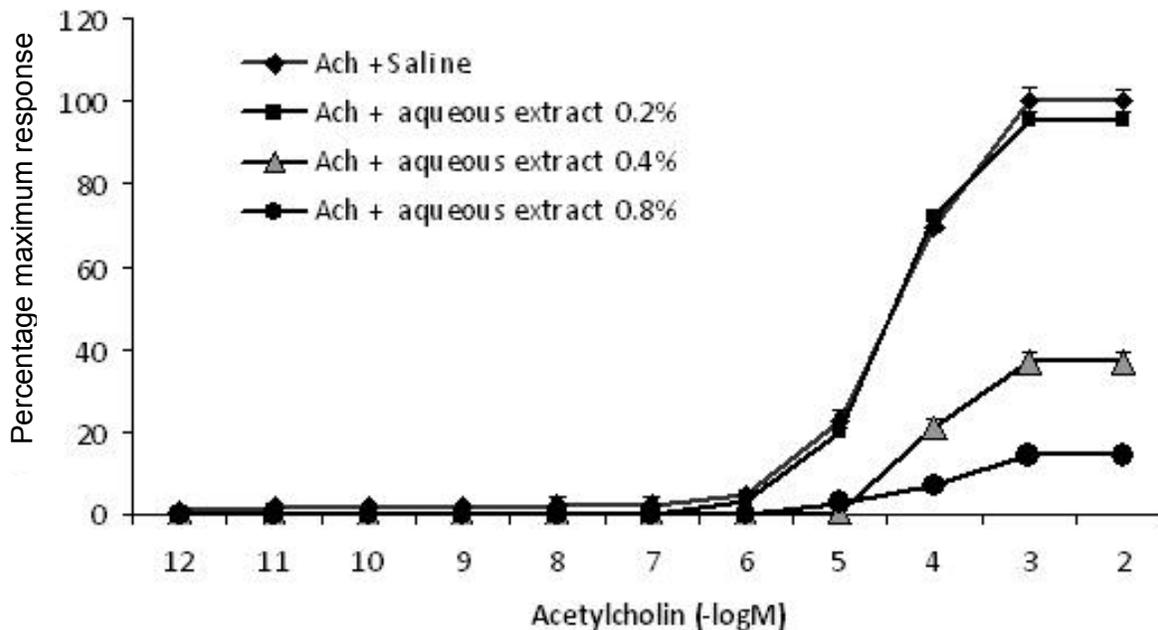


Figure 1. Mean inhibitory effect of extract of *C. Copticum* on acetylcholine-induced contractions in the isolated rat's ileum. Each point indicated the mean of 6 experiments and the vertical bars represent the SEM.

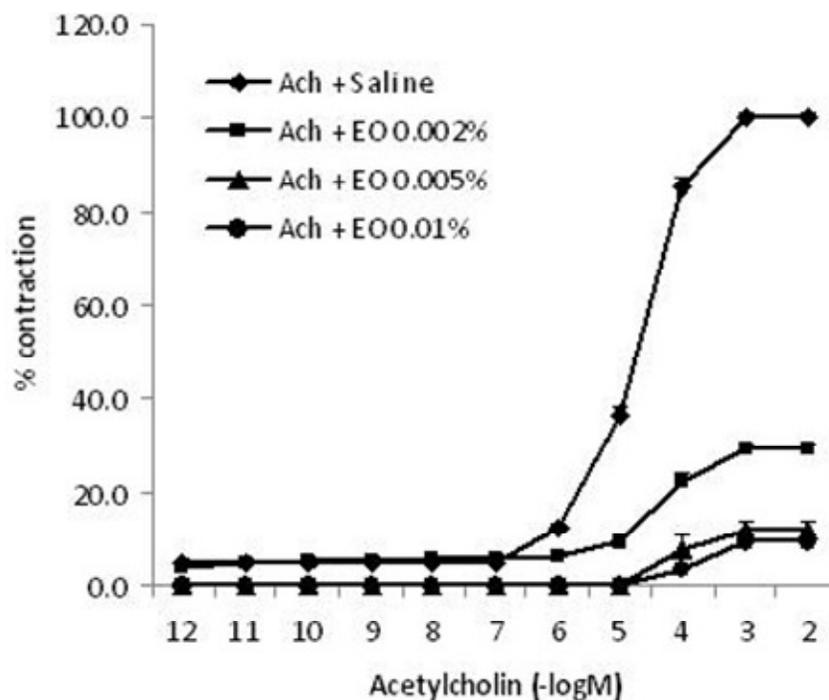


Figure 2. Mean inhibitory effect of essential oil of *C. Copticum* on acetylcholine-induced contractions in the isolated rat's ileum. Each point indicated the mean of 6 experiments and the vertical bars represent the SEM.

coupling between the ATPase reaction and contraction in muscle (Tomora and Iwamoto, 2004). It can also block the Ca^{2+} influx through the cell membrane (Ceccatto et al., 2009) and reduce the calcium content of the

sarcoplasmic reticulum (Szentandery et al., 2004). Since thymol is mainly deposited in the EO, the more potent spasmolytic effect of EO as compared with its aqueous extract may be related to the possible

mechanisms of actions depicted to this constituent who needs further investigations for more details.

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