The Effect of *Carum Copticum* Extract on Acetylcholine Induced Contraction in Isolated Rat’s Ileum

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**Abstract**

**Aims:** There are many biological investigations for determining an effective cure for the dysfunction of gastrointestinal tracts, using herbal medicine. It has been reported that *Carum Copticum* is a bactericidal agent and possesses anticholinergic, antihistaminic and β-adrenergic stimulatory effects in some tissues. However, these effects of *Carum Copticum* on mechanical activities of isolated intestine are not clearly identified yet. The present study has been designed to find out the specific effects of *Carum Copticum* on mechanical activity of isolated rat’s ileum.

**Materials and Methods:** In this study rat’s ileum contraction was recorded through an isolated tissue chamber in an organ bath by using isotonic transducer and oscillographic device. The effect of *Carum Copticum* extract on acetylcholine induced contraction in isolated rat’s ileum was evaluated.

**Results:** Our findings showed that 1% aqueous extract of *Carum Copticum* reduces the basal contractile activity of rat’s ileum. The extract also reduced acetylcholine induced contraction to 40% of its maximum response. The inhibitory action of *Carum Copticum* extract on acetylcholine induced contraction was similar but slower than that of atropine sulfate.

**Conclusion:** The results of this study showed an inhibitory effect of *Carum Copticum* extract on acetylcholine induced contraction in rat’s ileum.

1. **Introduction**

*Carum Copticum* (L.) is a plant in Umbelliferae family with a white flower and small brownish seeds. This plant commonly grows in Iran, India, Egypt and Europe. The major component of its fruit is essential oil which is composed of γ-terpinene, p-cymene, α-pinene, β-pinene, and other substances such as thymol and carvacol [1]. In the Iranian traditional medicine several therapeutic effects including...
expectorant, diuretic, anti-vomiting, analgesic, anti-
asthma, anti-dyspnea, and anti-spasm, is postulated
for Carum Copticum fruits [2]. Despite the availa-
bility of modern medications the propensity towards
the traditional medications is growing throughout
the world [3] which needs scientific investigations
for evaluating the therapeutic effects of medicinal
plants and their mechanisms of actions. Some of
these biological investigations have been focused
on gastrointestinal dysfunctions. One of the most
important abnormalities in gastrointestinal tract is
diarrhea, which may be produced by some infec-
tions or increase in motility [4,5]. Carum Copticum
is traditionally used in treating many gastrointesti-
nal disorders such as indigestion, colic, dyspepsia
and diarrhea [6] and is suggested to be anti nocic-
tive [7] as well. It has been found that Carum Copticum
is very effective against some bacteria and its effect on digestive tract may be due to its
antibacterial activity [8]. In other hand there are
evidences indicating the relaxant effects of Carum Copticum on smooth muscles [9,10] and in our pre-
vios study we found that Carum Copticum extract
reduces the motility of rat ileum [11]. Intestinal
motility is controlled by circulatory hormones, in-
trinsic and extrinsic nerves that release a variety
of transmitters. The most important excitatory
transmitter in gastrointestinal tract is acetylcho-
l ine which acts via muscarinic receptors in enteric
smooth muscle cell membranes [12]. This study
was conducted to understand the probable anti-
cholinergic effect of Carum Copticum fruit extract
on isolated rat’s ileum.

2. Materials and Methods

In this study 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 University (Yazd, Iran), in accord-
ance with the internationally accepted principles
for laboratory animal use and care mentioned by the
European Community guidelines were obtained.

For extraction preparation, 40g air-dried fruits
of Carum Copticum (identified by botanists in the
herbarium of Yazd herbal drug research center and
a sample specimen was stored and coded in this
center) were gently grounded and mixed with 250mL
of double distilled water (DDW) and subjected to
steam distillation for 3 hours at atmospheric pressure.

Experiments were performed as described in
previous reports [13,14]. Adult male rats weighing
200–250g were sacrificed by cervical dislocation.
Segments of ileum (2 cm in length), were obtained,
flushed of their contents and trimmed of mesentery.

Preparations were suspended from the transducer
lever in the axis of the longitudinal muscle with
fine thread, mounted in the 50mL internal chamber
of organ bath containing Tyrode’s solution, main-
tained at 37°C and bubbled with 95% O2 and 5% CO2
[13]. Then, it was allowed to stabilize for 60 min-
utes prior to drug addition. Isotonic responses were
recorded using an isotonic transducer (T2) and an
oscillograph recording system (the bioscience 400
Series Washington oscillograph). Acetylcholine (Sigma
Aldrich Chemie Gmbh, Germany) was used as a stan-
dard contractile control at the end of each assay.

After recording a base line as a control for ideal
mechanical activity, the specimens were influenced
by different concentrations of Carum Copticum ex-
tract in a cumulative manner to yield the minimum
effective concentration which was 1% of total ex-
tract diluted in Tyrode’s solution. The effects of 1%
Carum Copticum extract alone, after and before
the infusion of acetylcholine (5×10^{-4}M) was assessed
on the specimens.

The specimens were also influenced by 5×10^{-5}M
atropine sulfate (Sigma Aldrich Chemie Gmbh) fol-
lowing the acetylcholine infusion as a standard
cholinergic antagonist. For each drug assay five sets
of experiments were done.

The data were calculated as mean changes in
specimen length from base line due to changes in
contractile activity and analyzed by paired Student’s
t-test. The statistical significance was considered
as p≤0.05.

3. Results

Our findings showed that the Carum Copticum ex-
tact itself declines the amplitude of base line con-
tractions, in all five sets of experiments (3±0.57mm
vs. 4.28±1.11mm) which this relaxant effect was
significant (p=0.022). The effective dose of acetyl-
choline conversely significantly (p=0.000) elevated
the amplitude of base line contractions in all as-
sessments (19.57±4.6mm vs. 4.28±1.11mm). After
pretreating the specimens with 1% Carum Copticum
extract, the effective concentrations of acetylcho-
l ine only led to about 40% of its maximum response
(Figure 1).

The contractile effects of acetylcholine were
diminished by the infusion of atropine sulfate or
Carum Copticum extract.

The inhibitory effect of atropine sulfate (10^{-4}M)
on acetylcholine induced contraction was observed
after a few second and completed in 30 seconds,
while the inhibition due to 1% Carum Copticum ex-
tact was completed after 3.5 minutes (Figure 2).

Carum Copticum extract in concentrations less
than 1% (0.001, 0.002 and 0.004) could not produce
Effect of *Carum Copticum* on acetylcholine induced contraction in rat’s ileum

4. Discussion

According to our findings *Carum Copticum* extract induced a relaxant effect on ileal smooth muscle contraction induced by acetylcholine. Different mechanisms are involved in gastrointestinal smooth muscle relaxation. These include the blocking action on excitatory agents and inducing anticholinergic [15] or antihistaminic [16] activity or act via their agonistic action on inhibitory modulators such as adrenergic [17], purinergic [18], GABAergic [19] agents or due to their nitric oxide mimetic action [20]. There are some evidences that *Carum Copticum* ingredients insert their action on tracheal smooth muscles via anticholinergic [21] antihistaminic [9] and calcium channel blocking activity [6]. In the present study the action of *Carum Copticum* extract on rat’s ileal motility interfered with the contractile activity of acetylcholine on the specimens. The inhibitory effect of *Carum Copticum* extract on acetylcholine induced contraction seen in this study may be due to β-adrenergic stimulatory action or due to the inhibitory effect of this plant on cholinergic receptors. The inhibitory effect of the extracts of this plant were therefore, examined on isolated ileum preparations pretreated with acetylcholine, indicating a functional antagonistic effect of *Carum Copticum* extract on cholinergic receptors in rat’s ileum. The inhibitory action of *Carum Copticum* extract on acetylcholine induced contraction was similar but slower than that of atropine sulfate. The major constituents of *Carum Copticum* extract mediating its relaxant effect are pinene components [22–24], thymol [25,26] and carvacrol [27,28]. From these components the anticholinergic activity of thymol is reported [25], so the anticholinergic effect of *Carum Copticum* extract in this study may be through the action of its thymol ingredient which needs further investigations for more details.

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References


