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PHARMACOLOGICAL STUDIES OF CARDAMOM OIL IN ANIMALS

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Cardamom seeds are widely used for flavouring purposes in food and as carminative. Little information has been reported on their pharmacological and toxicological properties or, for their volatile oil which constitutes about 5% of the seed's total weight.

A comparative study of the anti-inflammatory activity of the oil extracted from commercial Elettaria cardamomum seeds, in doses of 175 and 280 μl/kg and indomethacin in a dose of 30 mg/kg against acute carrageenan-induced planter oedema in male albino rats was performed, which proved to be marked.

Moreover, investigation of the analgesic activity using *p*-benzoquinone as a chemical stimulus proved that a dose of 233 μl/kg of the oil produced 50% protection against the writhing (stretching syndrome) induced by intraperitoneal administration of a 0.02% solution of *p*-benzoquinone in mice. In addition the antispasmodic activity was determined on a rabbit intestine preparation using acetylcholine as agonist, the results proving that cardamom oil exerts its antispasmodic action through muscarinic receptor blockage.

KEY WORDS: cardamom oil, anti-inflammatory, analgesic, anti-spasmodic effect.

INTRODUCTION

Medicines derived from plants formed a large part of the materia medica of earlier times. Moreover, many medical authorities and the general public are returning to the use of herbal medicine as many synthetic drugs have proved to exert side-effects [1].

Cardamom seeds were known to Discordies in AD 77 and were mentioned in the Arabian Nights. The principal constituent of the seeds is a volatile oil, of which they yield from 2–8 (average about 5) percent. The major components of cardamom oil are 1,8-cineole (20–60%) and alpha-terpinyl acetate (20–53%) [2]. The normal maximum contents of other principal components of the oil are linalyl acetate, linalol and borneol (each up to 8.0%), alpha-terpineol (4.3%), alpha-pinene, limonene and myrcene (each up to 3%) [3].

The volatile oil with cineole, limonene, terpineol and linalol form the active ingredient. It relieves wind and colic, increases the flow of saliva and stimulates the appetite.

Cardamom seeds are widely used for flavouring purposes in food. Medically, they are used for flatulent indigestion, and to stimulate the appetite in people with anorexia. Moreover, the seeds were prescribed in Ayurvedic medicine for coughs, colds, bronchitis, asthma and indigestion [4]. Furthermore, cardamom oil has antibacterial properties [5].

The volatile oils of many plants are known for their analgesic, anti-inflammatory [6, 7] and antispasmodic effects [8]. Therefore, it was of interest to study the action of cardamom oil on intestinal smooth muscle of rabbits and to investigate its effect on induced pain and inflammation in animals.

MATERIALS AND METHODS

Animals

(1) Adult male albino rats weighing 100–150 g were used for studying the anti-inflammatory action of the oil. The animals were uniformly hydrated by giving water (3 ml per rat) through gastric intubation to reduce variability to oedema response [9].

(2) Adult male mice weighing 25–30 g were also used for studying the analgesic action of the oil. The mice were first injected intraperitonealy with 0.25 ml of 0.02% *p*-benzoquinone solution and were observed for 20 min. Only animals which showed writhing within 20–60 min were used in the investigation of the analgesic action.

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(3) The antispasmodic action of the oil was investigated using adult male rabbits (1–2 kg).

Methods

Preparation of cardamom oil. The oil was prepared by steam distillation of crushed fruits of *Elettaria cardamomum* obtained from a recent harvest, which had not suffered excessive volatile-oil loss in order to obtain a good yield. At least 4 hours distillation was required to produce a full ester content of the oil [2].

Preliminary screening and acute toxicity studies [10]. Mice were divided into nine groups of four animals each. Cardamom oil was administered i.p. in doses of 50, 100, 200, 400, 600, 700, 800 and 1600 μl/kg body weight, while the last group, which received saline, served as control. The animals were observed continuously for 6 h for changes in autonomic or behavioural responses. Any mortality during the following 24 h was also noted.

Determination of the anti-inflammatory action.

Carrageenan-induced rat hind paw oedema was performed [11]. The animals were classified into four groups consisting of six rats each. The first group was injected with saline in volumes equivalent to cardamom oil and served as control. The second group received indomethacin (30 mg/kg, i.p.). The third group received cardamom oil (175 μl/kg, i.p.). The fourth group received cardamom oil (280 μl/kg, i.p.). One hour after drug administration, the rats were injected with carrageenan (0.05 ml, 1%, s.c. into the subplantar region of the paw). The animals were decapitated 3 hours following the induction of oedema. The right and left paws were cut at the tibio–tarsal articulation and weighed. The percentage increase in the weight of carrageenan-injected paw over the other paw for each animal was determined. The percentage inhibition of inflammation by the various treatments was calculated:

$$\text{Percent inhibition} = \frac{a - b}{a} \times 100.$$

where *a* represents the increase in paw weight in control group and *b* represents the increase in paw weight in the drug-treated group.

Determination of the analgesic activity

The analgesic activity of cardamom oil in mice was investigated by the *p*-benzoquinone-induced writhing method [12].

Animals showing writhing to *p*-benzoquinone were divided into groups of eight animals each. One group was given saline and served as control, while the other groups received aspirin in doses of 50, 75, 100, 125, 150 and 175 mg/kg i.p. and cardamom oil in doses of 133, 200, 233, 266, 333 and 400 μl/kg, i.p. 1 h before the injection of *p*-benzoquinone. The animals were observed for 1 h after the injection of the irritant (*p*-benzoquinone) during which the animals showing writhing were counted. The analgesic activity was expressed as percentage protection in comparison with control according to the following equation:

$$\% \text{ protection} = \frac{\text{number of animals which did not writhe}}{\text{total number of animals}} \times 100.$$

Determination of the antispasmodic action

The rabbits were killed by decapitation, segments of intestine about 2 cm long were dissected immediately and mounted in an organ bath of 50-ml capacity, filled with Tyrod's solution which was kept at 37°C by warm water. The perfusion solution was continuously bubbled with a 95% O_2 and 5% CO_2 gas mixture. Each preparation was allowed to equilibrate for one hour before addition of any dose of the oil.

Spontaneous movements and contractions of the intestine were recorded on a polygraph (physiograph MK-IV-P, Narco-Biosystems) using an isometric

Table I
Effect of indomethacin and cardamom oil on carrageenan-induced rat hind paw oedema

Drugs and doses	*Mean increase in carrageenan-induced paw weight*	*Percentage inhibition of control value*
Saline (control)	0.448±0.047	0
Indomethacin (30 mg/kg)	0.108±0.015*	76.0
Cardamom oil (175 μl/kg)	0.138±0.001*	69.2
Cardamom oil (280 μl/kg)	0.061±0.014*	86.4

All values are means±SE.
Number of animals (n=6).
*Significantly different from control group at $P<0.05$.

Table II
Analgesic activity of different doses of aspirin and cardamom oil using *p*-benzoquinone-induced writhing response in mice

Groups	*% Protection of control*	*Groups*	*% Protection of control*
Control	0.00	Control	0.00
Aspirin (mg/kg)		Cardamom oil (μl/kg)	
50	0.00	133	0.00
75	25.00	200	25.42
100	50.00	233	50.15
125	53.00	266	75.36
150	75.00	333	83.00
175	100.00	400	100.00

Number of animals in each group=8.

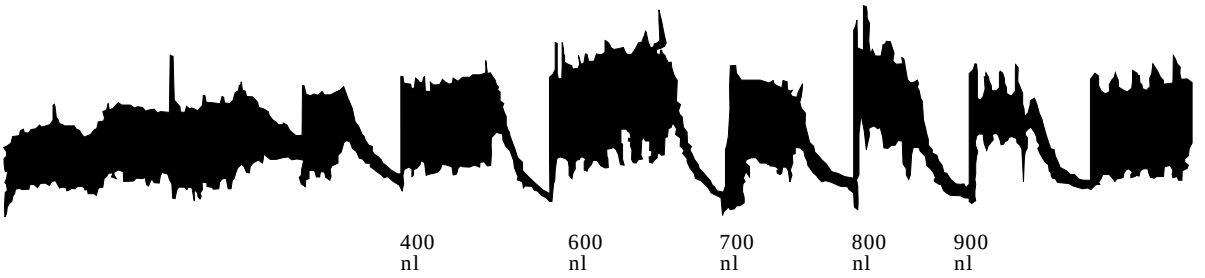

Fig. 1. Effect of cardamon oil on the spontaneous movements of isolated segments of rabbit intestine.

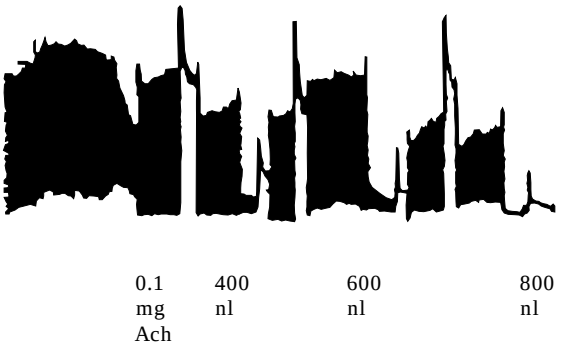

Fig. 2. Effect of cardamom oil on the intestinal contractions produced by 0.1 mg of acetylcholine.

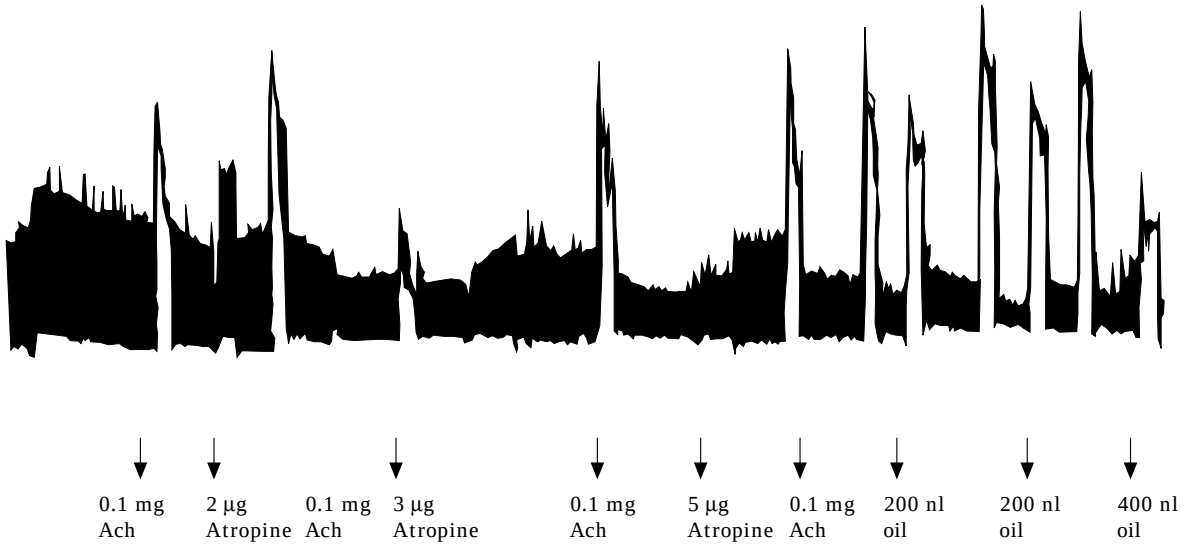

Fig. 3. Effect of atropine and cardamom oil on spasmogenic action of acetylcholine.

transducer (F-60 myograph, Narco-Biosystems). The composition of the Tyrod's solution was (mm): NaCl, 130; KCl, 2.60; $CaCl_2$, 1.36; $MgCl_2$, 0.98; $NaHCO_3$, 11.1; NaH_2PO_4, 0.36; and glucose, 5.55.

RESULTS

Intraperitoneal administration of cardamom oil in doses of 50–1600 μl/kg body weight did not produce any significant changes in the autonomic responses in mice. However, the animals showed mild behavioural changes in the form of drowsiness and staggering gait for a few minutes after injection of the oil followed by rapid recovery. The preliminary investigations also revealed complete absence of pain reflex in mice.

Table I shows the effect of indomethacin (30 mg kg) and cardamom oil (175 μl/kg; 280 μl/kg) on carrageenan-induced rat paw oedema. It is obvious that indomethacin significantly inhibited the carrageenan-induced paw oedema by 76% of the control value. Similarly, cardamom oil showed a remarkable reduction in the paw oedema weight. The percentage inhibition caused by the oil in doses of 175 and

280 μl/kg was 69.2 and 86.4 of the control values, respectively.

Table II summarizes the analgesic effect of different doses of aspirin and cardamom oil in mice. In the dosages studied, aspirin (175 mg kg) and cardamom oil (400 μl/kg) prevented the writhings in treated mice by 100% of control values.

The effects of cardamom oil on spontaneous intestinal movements was illustrated in Fig. 1. The addition of the oil to the organ bath in gradually increasing doses, namely, 200, 400, 600, 700, 800 and 900 nl, inhibited the spontaneous movements of the rabbit intestine in a dose-dependent manner.

In an attempt to identify the mode of action of the oil as antispasmodic, it was added to the organ bath after stimulating the rabbit intestine with 0.1 ml acetylcholine (0.1 mg). The results proved that the oil inhibited the stimulant action of acetylcholine in a dose-dependent manner (Fig. 2). In this study, it was shown that atropine (3 μg) and cardamom oil (400 nl) produced 50% reduction of the stimulant action of acetylcholine (Fig. 3).

DISCUSSION

Results of the present study have demonstrated an inhibitory action of cardamom oil on the isolated rabbit intestine (Fig. 1). The oil also attenuated the contractions induced by acetylcholine (Fig. 2). These data indicated that cardamom oil possesses a marked antispasmodic action.

Acetylcholine produces depolarization and contractions of non-vascular smooth muscle. The contractions are dependent on extracellular Ca^{2+} which gains access to the cytoplasm either via the opening of voltage-dependent Ca^{2+} channels (VDCs) or via receptor-operated Ca^{2+} channels (ROCs) [13]. The ability of cardamom oil to attenuate the spasmogenic action of acetylcholine could reside at the receptor site or at the level of Ca^{2+} influx via VDC or ROCs, but its dose-dependent inhibitory action of the spasmogenic effect of acetylcholine suggested that the oil has a direct muscarinic receptor antagonistic action. The present investigations also revealed that cardamom oil (400 nl) and atropine (3 μg) antagonized the response of rabbit intestine to acetylcholine to 50% (Fig. 3). A complete inhibition of the spasmogenic effect of acetylcholine was produced by doses of 800 nl and 5 μg of cardamom oil and atropine, respectively.

Results of the present study have clearly shown that cardamom oil (175 μl/kg) provoked a significant suppressive action on carrageenan-induced oedema but to a slightly lesser extent than indomethacin (30 mg/kg). However, the oil administered in a dose of 280 μl/kg exerts a more potent anti-inflammatory action than indomethacin (Table I). In addition, the test oil also possesses a promising significant analgesic activity (Table II).

In the laboratory, indomethacin is the most potent of the inhibitors of prostaglandin synthesis [14]. Hence it may be assumed that cardamom oil exerts its anti-inflammatory effect through reducing the synthesis of eicosanoid mediators of inflammation and it acts peripherally through its effects on inflammation as a potent analgesic drug. This oil thus possesses great potential therapeutic efficacy.

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