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Gut modulatory, blood pressure lowering, diuretic and sedative activities of cardamom

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Abstract

Ethnopharmacological relevance: Cardamom (Elettaria cardamomum) is traditionally used in various gastrointestinal, cardiovascular and neuronal disorders.

Aim of the study: To rationalize cardamom use in constipation, colic, diarrhea, hypertension and as diuretic.

Materials and methods: Cardamom crude extract (Ec.Cr) was studied using in vitro and in vivo techniques.

Results: Ec.Cr caused atropine-sensitive stimulatory effect in isolated guinea-pig ileum at 3–10 mg/ml. In rabbit jejunum preparations, Ec.Cr relaxed spontaneous and K+ (80 mM)-induced contractions as well as shifted Ca++ curves to right, like verapamil. Ec.Cr (3–100 mg/kg) induced fall in the arterial blood pressure (BP) of anaesthetized rats, partially blocked in atropinized animals. In endothelium-intact rat aorta, Ec.Cr relaxed phenylephrine (1 μM)-induced contractions, partially antagonized by atropine and also inhibited K+ (80 mM) contractions. In guinea-pig atria, Ec.Cr exhibited a cardio-depressant effect. Ec.Cr (1–10 mg/kg) produced diuresis in rats, accompanied by a saluretic effect. It enhanced pentobarbital-induced sleeping time in mice. Bio-assay directed fractionation revealed the separation of spasmogenic and spasmyloytic components in the aqueous and organic fractions respectively.

Conclusion: These results indicate that cardamom exhibits gut excitatory and inhibitory effects mediated through cholinergic and Ca++ antagonist mechanisms respectively and lowers BP via combination of both pathways. The diuretic and sedative effects may offer added value in its use in hypertension and epilepsy.

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Keywords: Elettaria cardamomum; Cardamom; Gastrointestinal motility; Hypotensive; Diuretic; Sedative

1. Introduction

Elettaria cardamomum Maton (cardamom, family; Scitaminaceae) locally known as “elaichi” is a perennial herb, indigenous to India, Pakistan, Burma and Sri Lanka (Nadkarni, 1976). In addition to its wide use for culinary purpose, cardamom has folkloric repute as carminative, stomachic, diuretic, abortifacient, antibacterial, antiviral, antifungal and is considered useful in treatment of constipation, colic, diarrhea, dyspepsia, vomiting, headache, epilepsy and cardiovascular diseases (Khan and Rahman, 1992; Duke et al., 2002).

Phytochemical studies revealed the presence of multiple chemicals, such as α-terpineol, myrcene, heptane, subinene, limonene, cineol, α-phellandrene, menthone, α-pinene, β-pinene (Shaban et al., 1987), linalol, nerolidol (Okugawa et al., 1988), β-sitostenone, γ-sitosterol, phytol, eugenyl acetate (Gopalakrishnan et al., 1990), bisabolene, borneol, citronellol, p-cymene, geraniol, geranyl acetate, stigmasterol and terpinene (Duke, 1992).

Cardamom in combination with some other plants was found to reverse the liver damage induced by CCl4 (Shirwaikar et al., 1992). The aqueous and methanolic extracts of eight Zingiberaceae herbs including cardamom were examined in rabbits for their effect on gastric secretion and the oral administration of either extract caused a significant decrease in gastric secretion and pepsin output (Sakai et al., 1989). In another study, cardamom was found to increase the gastric acid secre-
tion (Vasudevan et al., 2000). Clinical studies with herbal eye drop preparation containing cardamom was found useful in cases of cataract refractive errors and some infective conditions of the eye (Ghosh et al., 1985; Mukherji and Sahai, 1985; Mitra et al., 1986). Two commonly used Ayurvedic medicines consisting of Myristica fragrans, Syzygium aromaticum and cardamom exhibited antifertility activity (Sethi et al., 1987). Its volatile oil was found to exhibit analgesic, antiinflammatory, antimicrobial and antispasmodic properties (Al-Zuhair et al., 1996; Elgayar et al., 2001; Daswani and Bohra, 2003).

In this study, we report that cardamom exhibits combination of spasmycity, spasmyolytic, blood pressure (BP)-lowering, vasodilator, cardio-suppressant, diuretic and sedative activities. The activity-directed fractionation of the crude extract resulted in the separation of gut excitatory and relaxant components in the aqueous and organic fractions respectively.

2. Materials and methods

2.1. Plant material, preparation of crude extract and fractions

Dried fruits of Elettaria cardamomum were purchased from a local market in Karachi and the sample voucher (EC-SE-07-04-54) was submitted to the Department of Biological and Biomedical Sciences herbarium, Aga Khan University, Karachi. After cleaning of adulterant material, the fruits were ground with an electric grinder into a coarse powder. Extraction and fractionation was carried out as described previously (Williamson et al., 1998). About 986 g of ground material was soaked in aqueous-methanol (70%) at room temperature (23–25°C) for 3 days with occasional shaking. It was filtered through a muslin cloth and then through a Whatman qualitative grade 1 filter paper. This procedure was repeated twice and the combined filtrates were evaporated on rotary evaporator under reduced pressure (–760 mmHg) to a thick, semi-solid pasty mass of dark brown color; i.e. the crude extract (Ec.Cr), yielding approximately 10.81%. Ec.Cr was completely solubilized both in the distilled water and saline for use in in vitro and in vivo experiments.

Activity-guided fractionation was carried out, using solvents of increasing polarity. Ec.Cr was dissolved in 300 ml of distilled water. Petroleum spirit was added to it and shaken vigorously in a separating funnel. The petroleum spirit layer (upper) was collected thrice and evaporated on rotary evaporator to give the petroleum spirit fraction (Ec.Pt). The lower layer was taken in a separating funnel, chloroform was added. The chloroform layer (lower) was collected thrice and evaporated on rotary evaporator to obtain the chloroform fraction (Ec.Cl). The other layer (upper) was again taken into a separating funnel, ethyl acetate was added into it, separated and was also evaporated in rotary evaporator to give the ethyl acetate fraction (Ec.EtAc). The remaining lower layer was collected and evaporated to yield the aqueous fraction (Ec.Aq).

2.2. Phytochemical screening

Preliminary screening of the plant extract for various phytochemical classes was carried out following the reported methods (Harborne, 1984; Akinremi et al., 2005). Alkaloids were tested by using Dragendorf’s reagent. Appearance of yellow color with AlCl3 reagent and green or black with aqueous FeCl3 detects flavonoids and tannins respectively. Plant material treated with petroleum ether and subsequently extracted with CHCl3 was noted for green to pink or purple color after reaction with acetic anhydride and HCl in succession to detect sterols and terpenes respectively. Saponins were detected on the basis of froth upon vigorous shaking. The observation of yellow florescence under UV light on filter paper impregnated with the vapors from boiling extract indicates the presence of coumarins. Benzene extract prepared from acidified plant material was treated with NH4OH for anthraquinones based on the appearance of pink, violet or red color.

2.3. Drugs and animals

Acetylcholine chloride (ACh), atropine sulphate, furosemide, histamine, pentobarbital sodium, phenylephrine hydrochloride (PE), potassium chloride and verapamil hydrochloride were purchased from Sigma Chemicals Company, St. Louis, MO, USA. Diazepam, pentothal sodium and heparin injections were obtained from F. Hoffmann-La Roche, Basel, Switzerland, Abbott Laboratories, Karachi, Pakistan and Rotex Medica, Trottau, Germany respectively. Chemicals used for making physiological salt solutions were: potassium chloride (Sigma Chemicals Co.), calcium chloride, glucose, magnesium chloride, magnesium sulfate, potassium dihydrogen phosphate, sodium bicarbonate, sodium dihydrogen phosphate (Merck, Darmstadt, Germany) and sodium chloride from BDH Laboratory supplies, Poole, England. All chemicals used were of the analytical grade available. Stock solutions of the drugs were made in distilled H2O/saline and the subsequent dilutions were prepared fresh on the day of experiment.

Animals used in this study such as adult rabbits (1–1.5 kg), guinea-pigs (450–500 g), Sprague–Dawley rats (200–220 g) and Swiss albino mice (20–25 g) of either sex and local breed were housed at the Animal House of the Aga Khan University, maintained at 23–25°C. Food was withdrawn from rabbits and guinea-pigs 24 h prior to the experiment and rats were deprived of water during the collection of urine. Guinea-pigs were sacrificed by cervical dislocation and rabbits by blow on back of the head. Experiments performed complied with the rulings of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (NRC, 1996) and approved by the Ethical Committee of the Aga Khan University.

2.4. Isolated tissue experiments

2.4.1. Guinea-pig ileum

The guinea-pig abdomen was cut, ileum was dissected out and kept in Tyrode’s solution (Gilani and Aftab, 1992). Segments, each of about 2 cm length, were mounted individually in a 10 ml
tissue bath, filled with Tyrode’s solution, at 37°C and aerated with a mixture of 95% O2 in CO2 (carbogen). The composition of the Tyrode’s solution in mM was: KCl 2.68, NaCl 136.9, MgCl2 1.05, NaHCO3 11.90, NaH2PO4 0.42, CaCl2 1.8 and glucose 5.55, with of pH 7.4. A preload of 1 g was applied to each tissue and isotonic contractions were recorded with a Bioscience transducer coupled to a Harvard oscillograph. After equilibration period of 30 min, the tissues were repeatedly treated with sub-maximal concentration (0.3 μM) of ACh with 3 min interval until constant responses were recorded. The contractile effect of the test material was assessed as percent of the maximum effect produced by the control drug, ACh.

2.4.2. Rabbit jejunum

The jejunum was dissected out after surgical opening of rabbit abdomen, kept in Tyrode’s solution and cleaned off mesenteries (Gilani et al., 2005a). Each segment of about 2 cm length was suspended in a 10 ml tissue bath containing Tyrode’s solution, maintained at 37°C and aerated with carbogen. Intestinal responses were recorded isotonically using Bioscience transducers and oscillograph. Each tissue was allowed to equilibrate for at least 30 min before the addition of any drug and then stabilized with a sub-maximal concentration of ACh (0.3 μM). Under these conditions, rabbit jejunum exhibits spontaneous rhythmic contractions, allowing to test the relaxant (spasmolytic) effect directly without use of an agonist.

For the determination of calcium channel blocking (CCB) activity, high K+ (80 mM) was used to depolarize the preparations as described by Farre et al. (1991). K+ was added to the tissue bath, which produced a sustained contraction. Test materials were then added in a cumulative fashion to obtain concentration-dependent inhibitory responses (Van Rossum, 1963).

To confirm the Ca++ antagonist action of the test substance, the tissue was allowed to stabilize in normal Tyrode’s solution, which was then replaced with Ca++-free Tyrode’s solution containing EDTA (0.1 mM) for 30 min in order to remove Ca++. This solution was further replaced with K+-rich and Ca++-free Tyrode’s solution, having the following composition (mM): KCl 50, NaCl 91.04, MgCl2 1.05, NaHCO3 11.90, NaH2PO4 0.42, glucose 5.55 and EDTA 0.1. Following an incubation period of 30 min, control concentration–response curves (CRCs) of Ca++ were obtained. When the control Ca++ CRCs was found super-imposable (usually after two cycles), the tissue was pretreated with the plant extract for 60 min to test the possible CCB effect. The CRCs of Ca++ were reconstructed in the presence of different concentrations of the test material.

2.4.3. Rat aorta

Thoracic aorta from the rats was set up by following the procedure of Furchgott and Zawadzki (1980) with slight modifications. Care was taken in isolating the tissue to avoid any damaging of endothelium. It was then carefully cleaned of fats and then made into rings. Individual rings were mounted in 5 ml tissue bath with Kreb’s solution maintained at 37°C and aerated with carbogen. Composition of the Kreb’s solution was (mM): NaCl 118.2, NaHCO3 25.0, CaCl2 2.5, KCl 4.7, KH2PO4 1.3, MgSO4 1.2 and glucose 11.7 (pH 7.4). Each tissue was incubated for 30 min under the resting tension of 1 g. Changes in tension were recorded and analyzed isometrically through force transducer (Fort-10, WPI, UK) coupled to a bridge amplifier (Transbridge TBM 4M, WPI) and PowerLab ML 845 data acquisition system (ADInstruments, Sydney, Australia). The tissues were then stabilized with PE (1 μM). After stabilization, an induction contraction was obtained with PE (1 μM). Once plateau was achieved, ACh (0.3 μM) was than tested on PE-induced contraction to observe the endothelium integrity. The tissues were pre-incubated with atropine (1 μM) for 20 min to explore for a cholinergic receptor mediated vasodilator effect. The possibility of Ca++ antagonist effect involvement was also ruled out by administering the extract on high K+ (80 mM)-induced contractions.

2.4.4. Guinea-pig atria

Right atria from guinea-pigs were dissected out, cleaned of fatty tissue and mounted individually in 20 ml tissue bath containing Kreb’s solution, at 32°C and aerated with carbogen. The tissues were allowed to beat spontaneously (due to the presence of pacemaker cells) under the resting tension of 1 g and an equilibrium period of 30 min was given (Tanaka et al., 1996). Tension changes in the tissue were recorded via a Grass force–displacement transducer (model FT-03) coupled with a Grass model 7 Polygraph (Grass instrument company, Quincy, MA, USA).

2.5. In vivo experiments

2.5.1. Measurement of BP

These experiments were performed according to the method previously described (Gilani et al., 2005b). Rats were anaesthetized with thiopental sodium (70–90 mg/kg, i.p.) and the arterial BP was recorded through a pressure transducer (P23 XL) coupled with a Grass model 7 Polygraph. Drugs were injected through a cannula inserted into the jugular vein. After a 20 min period of equilibrium, the rats were injected intravenously with 0.1 ml saline (NaCl 0.9%) or with the same volume of test substance. Arterial BP was allowed to return to the resting level between injections. Changes in BP were recognized as the difference between the steady-state values before and the lowest readings after injection. Mean arterial blood pressure (MABP) was calculated as the diastolic BP plus one-third pulse width.

2.5.2. Diuretic assay

The protocol of Ratnasooriya et al. (2004) was followed with some modifications. Rats were divided into five groups of five animals each. The control group received saline (50 ml/kg, i.p.) and another group of animals was given furosemide (10 mg/kg, i.p.) as reference diuretic. The other groups were treated with the different doses of extract. Immediately after treatment, each animal was housed in its metabolic cage. The urine was collected in graduated vials and volume was measured every hour for 6 h. Total urine excreted out was collected and volume was calculated. The assay was repeated at intervals of 1 week on the same
group of rats. Treatments for each rat were randomized. The electrolyte concentration was measured using Corning flame photometer 410, UK.

2.5.3. Pentobarbital-induced sleeping time

Mice were divided into five groups of five animals each. One group received normal saline (10 ml/kg, i.p.), another group was given diazepam (5 mg/kg, i.p.) while to the rest of the groups, extract was administered in increasing doses (Gilani et al., 2000a). The pentobarbital (75 mg/kg, i.p.) was then given after 1 h to all the animals. The prolongation of animals sleeping time was noted.

2.5.4. Acute toxicity test

Animals were divided in groups of five mice each. The test was performed using increasing doses of the extract, given orally in 10 ml/kg volume to different groups serving as test groups (Sanmugapriya and Venkataraman, 2006). Another group was administered saline (10 ml/kg) as negative control. The mice were allowed food ad libitum and kept under regular observation for 6 h while the lethality was recorded after 24 h.

2.6. Statistical analysis

Data expressed as mean ± standard error of mean (S.E.M., n = number of experiments) and the median effective concentrations (EC50 values) with 95% confidence intervals (CI). The statistical parameter applied is Student’s t-test. p < 0.05 was considered statistically significant. CRCs were analyzed by non-linear regression using GraphPad program (GraphPAD, San Diego, CA, USA).

3. Results

3.1. Phytochemical screening

Ec.Cr was found to contain alkaloids, flavonoids, saponins, sterols and tannins while tested negative for the rest of classes.

3.2. Effect on ileum

In guinea-pig ileum, Ec.Cr (3–10 mg/ml) caused a concentration-dependent contractile effect. The efficacy of the stimulant effect was 9.3 ± 2.36, 49.48 ± 5.53 and 70.61 ± 4.4% (n = 5) at the concentrations of 3, 5 and 10 mg/ml respectively when compared to ACh maximum response. Pretreatment of the tissue with atropine (1 mM) completely blocked the effect of Ec.Cr, similar to that of ACh, while the histamine response remained unchanged (Figs. 1 and 2).

3.3. Effect on jejunum

Ec.Cr caused a concentration-dependent inhibition of spontaneous and K+ (80 mM)-induced contractions of rabbit jejunum with respective EC50 values of 2.96 (2.61–3.36, 95% CI, n = 5) and 3.37 mg/ml (2.38–4.78, n = 5). Similarly, verapamil relaxed both types of contractions with EC50 values of 0.46 (0.25–0.55, n = 4) and 0.04 μM (0.03–0.05, n = 4) respectively (Fig. 3). Ec.Cr (0.3–1.0 mg/ml) shifted the Ca++ CRCs to right (Fig. 4A), like that caused by verapamil (Fig. 4B).

3.4. Effect on BP

Ec.Cr at the doses of 3, 10, 30 and 100 mg/kg caused a respective fall of 6.81 ± 1.07, 16.49 ± 2.39, 36.78 ± 4.89 and 52.6 ± 5.67% (n = 4) in MABP of anaesthetized rats. When this BP-lowering effect was challenged with atropine (1 mg/kg), partial blockade was observed. Fig. 5 shows tracing from a typical
3.5. Effect on aorta

When tested on vascular preparations at resting tension, the extract was found devoid of any vasoconstrictor effect up to 10 mg/ml. Ec.Cr caused concentration-dependent relaxation of PE (1 μM)-induced contractions in endothelium intact rat aortic preparations with EC50 value of 2.94 mg/ml (1.99–4.33, n = 4). In the presence of atropine (1 μM), the inhibitory effect against PE was partially blocked with significant change at 1 mg/ml (p < 0.05) and 3–5 mg/ml (p < 0.01), shifting the curve to the right, with EC50 value of 7.39 mg/ml (4.63–11.82, n = 4). Ec.Cr also inhibited the K+ (80 mM)-induced contractions with EC50 value of 3.66 mg/ml (2.24–5.98, n = 4) as shown in Fig. 7.

3.6. Effect on atria

Ec.Cr caused concentration-dependent suppression of the atrial contractions with EC50 value of 2.94 mg/ml (1.67–5.16, n = 4). The cardio-depressant effect of Ec.Cr was found resistant to atropine (1 μM), while pretreatment of tissue with atropine completely blocked the inhibitory response of ACh (Fig. 8).

3.7. Effect on urine output

The urinary output of rats/100 g of the body wt./6 h in saline treated group was 3.4 ± 0.17 ml while with furosemide (10 mg/kg) was 6.93 ± 0.17 ml (p < 0.001 vs. saline). Ec.Cr at the doses of 1, 3 and 10 mg/kg increased the urinary volume to 4.13 ± 0.23, 5.05 ± 0.1 and 5.54 ± 0.26 ml (p < 0.001 vs. saline) respectively indicating diuretic effect (Fig. 9A). In addition to the increase in urinary volume, Ec.Cr also enhanced the Na+ and K+ excretion. Total Na+ and K+ contents (mmol/100 g body wt./6 h) in pooled urine samples were 0.63 ± 0.03, 0.69 ± 0.06, 0.84 ± 0.08 (p < 0.05) and 0.16 ± 0.01, 0.14 ± 0.01, 0.20 ± 0.01 (p < 0.01) respectively at the doses of 1, 3 and 10 mg/kg of Ec.Cr, as compare to the respective Na+ and K+ contents of 0.44 ± 0.13 and 0.11 ± 0.03 mmol in saline group. Similarly, furosemide (10 mg/kg) increased the Na+ and K+ excretion to 0.88 ± 0.03 (p < 0.01 vs. saline) and 0.19 ± 0.01 mmol (p < 0.05 vs. saline) respectively (Fig. 9B).

3.8. Effect on sleeping time

Ec.Cr (30–300 mg/kg) prolonged the pentobarbital-induced sleeping time in mice. The sleeping time in the saline treated group was 117.25 ± 2.1 min, while with diazepam (5 mg/kg)
Fig. 4. Concentration–response curves of Ca ++ in the absence and presence of different concentrations of (A) *Elettaria cardamomum* crude extract (Ec.Cr) and (B) verapamil in isolated rabbit jejunum preparations. Values shown are mean ± S.E.M., n = 4.

Fig. 5. Typical tracing showing the blood pressure (BP)-lowering effect of *Elettaria cardamomum* crude extract in comparison to acetylcholine (ACh) in absence and presence of atropine in an anaesthetized rat. Small triangles show the times at which the drugs were administered.

was 348.01 ± 13.27 min (p < 0.001 vs. saline). Ec.Cr at the doses of 30, 100 and 300 mg/kg prolonged the sleeping time to 162.5 ± 2.47 (p < 0.01 vs. saline), 202.75 ± 8.54 (p < 0.001 vs. saline) and 277.4 ± 4.91 min (p < 0.001 vs. saline) respectively (Fig. 10).

3.9. Acute toxicity

Ec.Cr did not cause any mortality up to the dose of 10 g/kg.

3.10. Effect of fractions

The aqueous fraction (Ec.Aq) caused atropine-sensitive marked stimulatory effect in the guinea-pig ileum. The effi-
Fig. 8. Typical tracing showing the cardio-depressant effect of *Elettaria cardamomum* crude extract and acetylcholine (ACh) in the absence and presence of atropine in isolated guinea-pig atrial preparations.

The contractile effect was 34.05 ± 7.9, 52.52 ± 7.16 and 83.93 ± 6.12% (*n* = 5) at 3, 5 and 10 mg/ml respectively as compared to ACh maximum response (Fig. 2). In rabbit jejunum, Ec.Aq was found devoid of any spasmolytic activity, while the organic fractions, i.e. Ec.Pt, Ec.Cl and Ec.EtAc caused concentration-dependent relaxation of both spontaneous and K+ (80 mM)-induced contractions with respective EC50 values of 0.02 (0.01–0.03, *n* = 4) and 0.08 (0.06–0.09, *n* = 5), 0.23 (0.15–0.32, *n* = 4) and 0.79 (0.52–1.21, *n* = 4), 0.10 (0.06–0.17, *n* = 3) and 1.02 mg/ml (0.56–1.88, *n* = 3) as shown in Fig. 3.

4. Discussion

In view of the well-known use in indigestion, the cardamom was tested for its possible stimulatory effect in guinea-pig ileum, a quiescent preparation, considered useful for this purpose (Gilani and Aftab, 1992), where it produced excitatory effect, like that caused by ACh and histamine. Pretreatment of the tissues with atropine, a muscarinic receptor antagonist (Arunlakhshana and Schild, 1959) abolished the stimulatory effect of extract, similar to that of ACh, without altering the response of histamine which indicates that the cardamom causes gut stimulation via cholinergic pathway. ACh is a neurotransmitter released by the parasympathetic nervous system, mediates its action in the gut by activation of M3 muscarinic receptor subtypes and hence plays an important physiological role to regulate the peristaltic movements of the gut (Brown and Taylor, 1996). This may explain the medicinal use of cardamom in dyspepsia and constipation.

The cardamom has also been used in overactive diseases of the gut, such as abdominal spasm and diarrhea. To see whether it also contains some spasmolytic constituent(s), it was tested on rabbit jejunum, a spontaneously contracting hyperactive preparation considered more responsive to spasmolytics (Ghayur and Gilani, 2005). Ec.Cr inhibited the spontaneous contractions, thus showing antisypmodic action. In earlier studies we observed that the relaxant effect of medicinal plants is usually mediated through blockade of Ca++ channels (Gilani et al., 1994a, 1999, 2005b). To investigate whether the spasmolytic effect of cardamom is also mediated via similar mechanisms, the extract was tested on high K+ induced contractions. High K+ (>30 mM) is known to cause smooth muscle contractions through opening of...
voltage-dependent L-type Ca++ channels, thus allowing influx of extracellular Ca++ causing a contractile effect (Bolton, 1979) and the substance causing inhibition of high K+-induced contraction is considered as inhibitor of Ca++ influx (Godfraind et al., 1986). Ec.Cr relaxed the high K+-induced contractions, like that caused by verapamil, a standard Ca++ antagonist (Fleckenstein, 1977), indicating its CCB action. The Ca++ antagonist effect of cardamom was further confirmed when it shifted the Ca++ CRCs to the right, like verapamil. The ability of the extract to inhibit the high K+-induced contraction, followed by rightward displacement of Ca++ CRCs strongly suggest the presence of Ca++ antagonist(s) in cardamom.

Both cholinergics and Ca++ antagonists are known to effectively reduce the BP (Ghayur et al., 2005). When injected intravenously to the normotensive rats under anesthesia, cardamom produced a dose-dependant fall in the arterial BP. Partial blockade of hypotensive responses with atropine indicate that cardamom lowers BP due to the presence of cholinergic and an additional (Ca++ antagonist) component(s), as identified in the gut preparations. BP is the product of peripheral resistance and cardiac output (Johansen, 1992), hence the extract was further studied in aorta and heart for the possible vasodilator and cardiac inhibitory effects.

In endothelium-intact rat aorta, cardamom caused relaxation of PE-induced contractions, partially blocked in the presence of atropine, as expected. Cholinergic receptor (M3) mediated vasodilator activity in the vasculature is brought about by the release of nitric oxide from the endothelium (Furchgott and Zawadzki, 1980). Ec.Cr also relaxed the contractions induced by high K+, due to the presence of Ca++ antagonist component(s). When tested in spontaneously beating guinea-pig atria, the cardamom caused suppression of the atrial contractions, unaffected by atropine pretreatment. The lack of atropine resistance to the cardamom effect in heart, as opposed to the gut and vessels, indicate the absence of cholinergic component in its effect on the heart. It is possible that the observed cholinergic component(s) interacts only with M3 subtype without any effect on M2 receptors located in the heart (Eglen and Watson, 1996) and the cardio-depressant effect of the extract is possibly due to CCB. However, further studies are required involving the identification of active constituents to get a clear answer of this interesting scenario.

The diuretic effect of cardamom was confirmed, when it caused significant increase in the urine volume (diuresis) in rats, like furosemide, a standard diuretic (Ives, 1998). In parallel with increase in the volume of urine, Ec.Cr also enhanced the urinary excretion of Na+ and K+, similar to that caused by furosemide. Loop diuretics in addition to increase in urinary flow rate are also known to strongly enhance the urinary electrolyte excretion, considered as saluretic (Jackson, 2001). Diuretics are commonly prescribed in combinations with antihypertensive drugs for the control and management of moderate to severe hypertension (Shah et al., 2004) and the presence of diuretic constituents is likely to complement the BP-lowering effect of the cardamom.

Sedatives are routinely used as adjunct therapy for the management of epilepsy (McNamara, 2006). The extract was tested for its possible sedative effect as a component which can add to its overall potential in such condition. Ec.Cr caused prolongation of pentobarbital-induced sleeping time in mice, similar to that observed with diazepam, a standard sedative agent (Hobbs et al., 1996). This may also explain the medicinal use of cardamom in epilepsy, though further studies are required to see whether it has direct antiepileptic effect.

Activity-directed fractionation revealed that the stimulant component (cholinomimetic) was separated in the aqueous fraction as it produced atropine-sensitive contractile effect in ileum, but found devoid of any relaxant effect in spontaneously contracting rabbit jejunum preparations. The efficacy of the aqueous fraction for its stimulatory effect was more pronounced than that of the parent crude extract, apparently due to the shift of inhibitory component(s) to the organic fractions. All the three organic fractions, i.e., petroleum ether, chloroform and ethyl acetate were found devoid of any stimulant effect, while caused concentration-dependent inhibition of both spontaneous and high K+-induced contractions of rabbit jejunum. The petroleum spirit fraction was found to be the most potent for its spasmylic action, being 150, 10 and 5-times more potent than the parent extract, chloroform and ethylacetate fraction respectively. This pattern of separating gut stimulatory and inhibitory constituents respectively in aqueous and organic fractions is in accordance with our previous findings, showing that the cholinergic component is concentrated in the aqueous fraction, while Ca++ antagonist in organic fractions (Gilani et al., 2000b, 2006).

The results of phytochemical analysis showed that cardamom contains alkaloids, flavonoids, saponins, sterols and tannins. The chemical structures, properties and pharmacological activities of saponins and flavonoids are so complex. But the observed cholinomimetic and CCB effects of the extract may be due to the presence of saponins and flavonoids, as such phytochemicals have been reported to exhibit cholinergic and Ca++ antagonist activities respectively (Gilani et al., 1994b; Revuelta et al., 1997). Among the constituents of the plant, α-pinene possesses spasmodic, antispasmodic and sedative effects, Geraniol and myrcene are antispasmodic and hypotensive and citronellol exhibits sedative action (McMahon, 2002).

In acute toxicity testing, the extract was found safe at the dose as high as 10 g/kg, which is in line with the wide therapeutic and dietary use of cardamom.

5. Conclusion

This study showed that cardamom possess gut stimulatory and inhibitory effects mediated through cholinomimetic and Ca++ antagonist mechanisms respectively and lowers BP via combination of both cholinergic and CCB pathways, thus provide sound mechanistic background for its folkloric use in constipation, colic, diarrhea and hypertension. The diuretic activity observed is likely to compliment the antihypertensive use of the cardamom. Sedative action might be a contributing factor to the use of cardamom in the management of epilepsy.
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References


