June 2012

Gut and airways relaxant effects of Carum roxburghianum

Munasib Khan
Aga Khan University

Arif-ullah Khan

Najeeb-ur-Rehman
Aga Khan University

Anwar Gilani
Aga Khan University

Follow this and additional works at: http://ecommons.aku.edu/pakistan_fhs_mc_bbs

Part of the Pharmacology Commons

Recommended Citation
Available at: http://ecommons.aku.edu/pakistan_fhs_mc_bbs/81
Gut and airways relaxant effects of *Carum roxburghianum*

Munasib Khan, Arif-ullah Khan, Najeeb-ur-Rehman, Anwarul-Hassan Gilani

---

**Abstract**

Ethnopharmacological relevance: *Carum roxburghianum* is traditionally used in hyperactive gastrointestinal and respiratory disorders. The present study was carried out to investigate the possible gut and airways relaxant potential of *Carum roxburghianum* to rationalize its folk uses.

**Materials and methods:** Crude extract of *Carum roxburghianum* (Cr.Cr) was studied in vivo and in vitro techniques.

**Results:** Cr.Cr exhibited protective effect against castor oil-induced diarrhea in mice at 100–1000 mg/kg. In rabbit jejunum preparations, Cr.Cr (0.03–3.0 mg/mL) caused relaxation of spontaneous and K⁺ (80 mM)-induced contractions at similar concentrations, like papaverine. Pretreatment of tissues with Cr.Cr (0.1–1.0 mg/mL) shifted Ca²⁺ concentration–response curves (CRCs) to right, like verapamil. Cr.Cr (0.03 and 0.1 mg/mL) caused leftward shift of isoprenaline-induced inhibitory CRCs, similar to papaverine. In isolated guinea-pig ileum, Cr.Cr (0.01 and 0.03 mg/mL) produced rightward parallel shift of acetylcholine-curves, like atropine. Cr.Cr (1.0–30 mg/kg) caused suppression of carbachol (CCh, 100 μg/kg)-induced increase in inspiratory pressure of anaesthetized rats. In guinea-pig trachea, Cr.Cr (0.03–1.0 mg/mL) relaxed CCh and high K⁺-induced contractions, shifted isoprenaline-induced inhibitory CRCs to left at 0.1 and 0.3 mg/mL and CCh-curves parallel to right (0.01 and 0.03 mg/mL). Cr.Cr did not cause any mortality of mice up to 10 g/kg dose.

**Conclusion:** These results indicate that *Carum roxburghianum* possess combination of antidiarrheal, anti-spasmodic and bronchodilatory effects, which provides pharmacological basis to its traditional use in the disorders of gut and airways hyperactivity, like diarrhea, colic and asthma.

© 2012 Elsevier Ireland Ltd. All rights reserved.

---

**1. Introduction**

*Carum roxburghianum* (DC) Craib & Benth. (family: Apiaceae/ Umbelliferae), commonly known as “Wild celery/Ajmadu” and locally as “Ajmud” is native to tropical Asia, Africa and is cultivated as spice and medicinal plant in Bangladesh, India, China, Pakistan and Indonesia (Nadkarni, 1976; Papini et al., 2007; Mishra, 2009). *Carum roxburghianum* is used in traditional system of medicine to treat diarrhea, abdominal spasm (colic), asthma, bronchitis cough, common cold, dyspepsia, lethargy, loss of consciousness, palpitation, vomiting, pain in bladder and kidneys (Lis-Balchin and Deans, 1997; Saroja et al., 1998; Babu and Madhavi, 2003; Wyk and Wink, 2004; Lokhande et al., 2006) as well as considered useful as anthelmintic, antigout, antimicrobial, cardiotonic, carminative, condiment, digestive, diuretic, emmenagogue, stimulant and stomachich (Hanelt et al., 1986; Khare, 2004; Deshpande et al., 2008).

Phytochemical studies on the plant revealed presence of limonene, sabine, terpinen-4-ol, (Z)-ligustilide, γ-terpinene, menthol, citronellol, 3,6-dimethyl-2,3,3a,4,5,7a-hexahydrobenzofuran citronellol, geraniol, benzene, 1-methoxy-4-(1-propenyl) phenol, 2-methoxy-4-(1-propenyl)-Neryl propionate, β-lemene, apio, γ-eudesmol, camphor, patchulic alcohol, α-pinene, β-pinene, β-myrcene, α-heliladrene, ocimene, α-4-dimethylstyrene, decane, 3-methyl-cis-p-mentha-2,8-diene-1-ol, dihydrocarvone, trans-carveol, eugenol, myristic and elemicin (Chowdhury et al., 2009). Total lipids (seed) include hydrocarbons, wax esters, sterol esters, triacylglycerols, free fatty acids, diacylglycerols, lysophosphatidylethanolamines and phosphatidylinositols. Oleic acid was found as major component.

---

**Abbreviations:** ACh, acetylcholine; ANOVA, analysis of variance; CaCl₂, calcium chloride; CCh, carbachol; CI, confidence interval; COil, castor oil; CRCs, concentration–response curves; EC₅₀, median effective concentration; EDTA, ethylenediaminetetra acetic acid; Cr.Cr, *Carum roxburghianum*; KCl, potassium chloride; KH₂PO₄, potassium dihydrogen phosphate; KPK, Khyber Pakhtun Khwa; MgCl₂, magnesium chloride; MgSO₄, magnesium sulfate; mmHg, millimeter of mercury; n, number of experiments; NaCl, sodium chloride; NaH₂PO₄, sodium dihydrogen phosphate; NaHCO₃, sodium bicarbonate; p.o., Persia ora (oral); SEM, standard error mean.

* Corresponding author. Tel.: +92 21 3484571; fax: +92 21 493 4294/494 2095.

E-mail address: anwar.gilan@aku.edu (A.-H. Gilani).

0378-8741/ – see front matter © 2012 Elsevier Ireland Ltd. All rights reserved.

doi:10.1016/j.jep.2012.03.039
in all lipid classes, whereas palmitic, linoleic and linolenic acids were present in lesser quantities. Arachidic acid was identified as minor component in only seven out of twelve lipid classes. Major constituents of the essential oil from seed are: α-limolene, α-terpinene, β-linalool and β-piperitone (Waheed et al., 2003; Deshpande et al., 2008). Other constituents are bergapten, β-sitosterol, cuminol, 7-methoxy-6-methylcoumarin, thymol, thymoquinol and umbelliferone (Khare, 2004).

Despite the fact that extensive phytochemical research has been carried out on *Carum roxburghianum*, reports related to pharmacological investigation are limited, only citing its antitumor activity (Alluri et al., 2005). In this study, we provide evidence that *Carum roxburghianum* exhibits antidiarrheal, antispasmodic and bronchodilatory activities, which justify *Carum roxburghianum* usefulness in overactive gut and Airways disorders.

2. Materials and methods

2.1. Plant material and extraction

Seeds were purchased from grocery shop in Peshawar, Khyber Pakhtunkhwa (KPK), Pakistan. The plant material was identified with help of a taxonomist, Dr. Ilyas Iqbal, Department of Botany, University of Malakand, KPK, Pakistan. A voucher specimen (UOM/BGU/148) has been submitted to the herbarium of same university. The seed were clean up adulterants and approximately 909.09 gm of the seeds were soaked in 4.0L aqueous-methanol (70%) at room temperature for three days with occasional shaking. The soaked material was filtered through a muslin cloth and then through Whatman qualitative grade 1 filter paper (Williamson et al., 1998). This procedure of soaking and filtration was repeated twice more. All the filtrates were combined and evaporated to dryness on rotary evaporator under reduced pressure (−760 mmHg) at 35−40 °C to obtain crude extract of *Carum roxburghianum* (Cr.Cr) having 23.25% yield. Cr.Cr was completely soluble in normal saline (0.9%, w/v) and distilled water for in vivo and in vitro experiments.

2.2. Chemicals

Acetylcholine chloride (ACh), atropine, carbachol (CCh), dicyclomine, isoprenaline, loperamide, papaverine and verapamil were purchased from Sigma Chemical Co., St. Louis, MO, USA. Aminophylline, pentothal sodium (thiopental) and castor oil were respectively obtained from GlaxoSmithKline, Abbot Laboratories and KCL Pharma, Karachi, Pakistan. Chemicals used for making physiological salt solutions were: potassium chloride (KCl, Sigma Chemical Co., St. Louis, MO, USA), calcium chloride (CaCl₂), Ethylenediaminetetra acetic acid (EDTA), glucose, magnesium chloride (MgCl₂), magnesium sulfate (MgSO₄), potassium dihydrogen phosphate (KH₂PO₄), sodium bicarbonate (NaHCO₃), sodium dihydrogen phosphate (NaH₂PO₄, Merck, Darmstadt, Germany) and sodium chloride (NaCl) from BDH Laboratory supplies, Poole, England. All chemicals used were of analytical grade and solubilized in distilled H₂O/saline.

2.3. Animals

Rabbits (1–1.2 kg), guinea-pigs (500–550 g), Sprague-Dawley rats (200–250 g) and Balb-C mice (20–25 g) of local breed and either sex were used for this study housed at the Animal House of the Aga Khan University, maintained at 23−25 °C and were given a standard diet and tap water. Rabbits and guinea-pigs starved for 24 h were sacrificed by blow on back of head and cervical dislocation. Experiments performed complied with rulings of Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (1996) and approved by the Ethical Committee of the Aga Khan University.

2.4. In vivo experiments

2.4.1. Castor oil-induced diarrhea

Mice were fasted for 24 h before the experiment. Animals were housed in individual cages and divided in five groups, each containing 10 mice. The first group received saline (10 mL/kg, p.o.) and served as negative control. The doses of the test extract were selected on trial basis and three increasing doses of extract, 100, 300 and 1000 mg/kg were given to three different groups. A group of mice was treated with loperamide (10 mg/kg, p.o.), as positive control. One hour after treatment, each animal received 10 mL/kg of castor oil (Gilani et al., 2008a). The aforementioned test material was administered orally to mice through feeding needle, fitted to U-100 insulin syringe. Afterward, the cages were inspected for the presence of diarrhea droppings, their absence was noted as a positive result, indicating protection from diarrhea at that time.

2.4.2. Bronchodilatory activity

Rats were anaesthetized with sodium thiopental (Pentothal, 80–100 mg/kg, i.p.), than incubated with 5 cm long tracheal tube (Intramedic Polyethylene Tubing, ID 1.77 mm. OD 2.80 mm, Cl Adams, Division of Becton Dickinson and Company, Parsippany, NJ, USA) and ventilated with volume ventilator (Miniature ideal pump, Bioscience, UK) adjusted at rate of 70–80 strokes/min to deliver 7–10 mL/kg of room air. A polylethylene catheter was inserted into jugular vein for drugs administration. Changes in the inspiratory pressure (mmHg) were measured by pressure transducer (MLT-1199) connected to side arm of tracheal cannula and recorded by PowerLab 4/25 with running chart software via Quad bridge amplifier (AD Instruments, Bella Vista, NSW, Australia). Bronchoconstriction was induced with CCh (100 μg/kg), which was reversed within 7−10 min (Khan et al., 2012). Test drug was given to animals 5−8 min prior to administration of CCh. The responses were expressed as percent reduction of CCh-induced bronchospasm.

2.4.3. Acute toxicity test

Mice were divided in groups of five mice each. The test was performed using increasing doses of the plant extract, given orally in 10 mL/kg volume to different groups serving as test groups. Another group of mice was administered saline (10 mL/kg, p.o.) as negative control. The mice were allowed food ad libitum and kept under regular observation for lethality recorded after 24 h (Gilani et al., 2005a).

2.5. Isolated tissue preparations

2.5.1. Rabbit jejunum

The rabbit abdomen was opened and jejunum was dissected out, kept in normal Tyrode’s solution and cleaned of mesenteries. Each segment of about 2 cm length was suspended in 10 mL tissue bath containing Tyrode’s solution (pH 7.4), maintained at 37 °C and aerated with a mixture of 95% O₂ and 5% CO₂ (carbogen). The composition of Tyrode’s solution was (mM): NaCl: 136.9, KCl: 2.7, MgCl₂·6H₂O: 0.5, NaHCO₃: 11.9, NaH₂PO₄·2H₂O: 0.32, CaCl₂: 1.8 and glucose: 5.05. One end of the segment was attached to metal tissue hook and other was attached by cotton thread to an isotonic Bioscience transducer, connected to Student Oscillograph (Harvard Apparatus, Holliston, MA, USA). An initial load of 1 g was applied to each tissue and was allowed to equilibrate for 30 min before the addition of any drug. Following equilibration period, each preparation was then stabilized with sub-maximal concentration of ACh (0.3 μM) at 3 min interval until constant responses were recorded (Gilani et al., 2005a). The inhibitory effects of test
substances were measured as percent change in jejunum spontaneous contractions. High K+ (80 mM) was used to depolarize the preparations. Once plateau of the induced contraction was achieved (usually within 7–10 min), the test material was then added in cumulative fashion to obtain concentration-dependent inhibitory responses. To construct Ca++ concentration–response curves (CRCs), 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++ from tissue. This solution was further replaced with K+-rich and Ca++-free Tyrode’s solution, having composition (mM): NaCl: 91.03, KCl: 50, MgCl₂·6H₂O: 0.50, NaHCO₃: 11.9, NaH₂PO₄·2H₂O: 0.32, glucose: 5.05 and EDTA-Na₂·2H₂O: 0.1. Following an incubation period of 30 min, control CRCs of Ca++ were obtained. When control Ca++-CRCs were found super-imposable (usually after two cycles), the tissue was pretreated with test drug for 1 h. The CRCs of Ca++ were reconstructed in the presence of different concentrations of test material. The isoprenaline-induced inhibitory CRCs against CCh-induced contractions were constructed in absence and presence of test substance (Gilani et al., 2005b).

2.5.2. Guinea-pig ileum

The ileum was dissected out and kept in Tyrode’s solution. The 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 carbogen. An initial load of 0.7 g was applied to the tissue and isometric contractions were recorded with a Bioscience transducer coupled to Harvard Oscillograph. After equilibration period of 30 min, each tissue preparation was repeatedly treated with sub-maximal concentration (0.3 μM) of ACh (Gilani et al., 2008a) until constant responses were recorded. Control bolus curves to ACh were then constructed by the addition of increasing concentrations of agonist. The ACh–CRCs were then re-determined in presence of increasing concentrations of crude extract (0.01 and 0.03 mg/mL) and atropine (0.003 and 0.01 μM), as described earlier (Choo and Mitchelson, 1978).

2.5.3. Guinea-pig trachea

Trachea was dissected from guinea-pig and kept in normal Kreb’s solution. The tracheal tube was cut into rings, 2–3 mm wide, each containing about two cartilages. Each ring was opened by longitudinal cut on ventral side opposite the smooth muscle, forming a strip with smooth muscle in center and cartilaginous portions on edges. The preparation was mounted in 20 mL tissue bath, containing Kreb’s solution (pH 7.4), at 37 °C and aerated with carbogen. The composition of Kreb’s solution was (mM): NaCl: 118.2, NaHCO₃: 25.0, CaCl₂: 2.5, KCl: 4.7, KH₂PO₄: 1.2, MgSO₄·7H₂O: 1.2 and glucose: 11.7. A tension of 1 g was applied to tracheal strips continuously throughout experiment. Each tissue was equilibrated for 1 h before addition of any drug. In some preparations, carbachol (1 μM) or K+ (80 mM) were used to stabilize respective preparations (Gilani et al., 2008b), until constant responses of each agonist were achieved. When sustained contractions were obtained, relaxant effect of test substance was assessed by adding in cumulative fashion. Like in jejunum, isoprenaline-CRCs were constructed in trachea, as described previously (Shah and Gilani, 2010). Cumulative curves to CCh were constructed using increasing concentration of agonist. When a 3-fold increase in concentration produced no further increment in response, the tissue was washed to re-establish base-line tension. The CCh-CRCs were repeated in presence of increasing concentrations of test drug. Isometric responses were recorded on Grass model 7 Polygraph (Grass instrument company, Quincy, MA, USA).

<table>
<thead>
<tr>
<th>Table 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effect of the crude extract of Curamin roxburghianum (Cr.Cr) on castor oil (C.Oil, 10 mL/kg)-induced diarrhea in mice.</td>
</tr>
<tr>
<td>Treatment (p.o.)</td>
</tr>
<tr>
<td>------------------</td>
</tr>
<tr>
<td>Saline (10 mL/kg) + C.Oil</td>
</tr>
<tr>
<td>Cr.Cr (100 mg/kg) + C.Oil</td>
</tr>
<tr>
<td>Cr.Cr (300 mg/kg) + C.Oil</td>
</tr>
<tr>
<td>Cr.Cr (1000 mg/kg) + C.Oil</td>
</tr>
<tr>
<td>Dicyclicline (50 mg/kg) + C.Oil</td>
</tr>
<tr>
<td>Dicyclicline (100 mg/kg) + C.Oil</td>
</tr>
<tr>
<td>Loperamide (10 mg/kg) + C.Oil</td>
</tr>
</tbody>
</table>

p < 0.05 compared to saline group, Chi–square test. **p < 0.01 compared to saline group, Chi–square test.

2.6. Statistical analysis

The data expressed are mean ± standard error of mean (SEM, n = number of experiment) and median effective concentrations (EC₅₀) with 95% confidence intervals (CI). The statistical parameters applied were Chi-square-test for antidiarheal assay, one way analysis of variance (ANOVA) followed by Dunnott’s test for bronchodilatory activity. Difference of p < 0.05 was considered statistically significant. The CRCs were analyzed by non-linear regression and two-way ANOVA followed by Bonferroni’s post-test correction was used for multiple comparisons of CRCs with respective control. All the graphing, calculations and statistical analysis were performed using GraphPAD program (GraphPAD, San Diego, CA, USA).

3. Results

3.1. Effect on castor oil-induced diarrhea

Cr.Cr exhibited dose-dependent (100, 300 and 1000 mg/kg) protective effect against castor oil-induced diarrhea in mice. The negative control group (saline treated) did not show any protection against castor oil-induced diarrhea. Pretreatment of animals with Cr.Cr, showed 10% protection from diarrhea at 100 mg/kg, 20% at 300 mg/kg and 50% protection at 1000 mg/kg (p < 0.05). Dicyclicline caused protection of 30% (p < 0.05) and 90% (p < 0.01) at respective doses of 50 and 100 mg/kg. Loperamide (10 mg/kg) showed 100% protection from diarrhea (p < 0.01) in the positive control group (Table 1).

3.2. Effect on jejunum

Cr.Cr caused concentration-dependent (0.03–1.0 mg/mL) relaxation of jejunum spontaneous contractions (Fig. 1). Fig. 2 shows the comparative inhibitory effect of Cr.Cr, papaverine and verapamil against spontaneous and K+ (80 mM)-induced contractions. Cr.Cr was found to be equally effective against spontaneous and K+ (80 mM)-induced contractions with EC₅₀ values of 0.37 (0.32–0.48, 95% CI, n = 4) and 0.34 mg/mL (0.28–0.42, n = 3) respectively as shown in Fig. 2A. Papaverine also showed similar pattern of non-specific inhibitory response (Fig. 2B) with respective EC₅₀ values of 3.4 (2.9–4.8, n = 4) and 3.6 μM (3.1–4.7, n = 4), whereas, verapamil was found more potent against K+ (80 mM)-induced contractions with EC₅₀ value of 0.045 μM (0.04–0.06, n = 3), as compared to spontaneous contractions [0.30 μM (0.2–0.4, n = 3)] as shown in Fig. 2C. Pretreatment of tissue with Cr.Cr (0.1–1.0 mg/mL) caused rightward shift in the Ca++ CRCs accompanied by suppression (p < 0.05 and p < 0.001) of the maximum contractile effect (Fig. 3A), similar to that caused by verapamil (0.03–0.3 μM, p < 0.01 and p < 0.001) as shown in Fig. 3B. When tested for possible interaction with isoprenaline, pretreatment of tissues with
Cr.Cr (0.03 and 0.1 mg/mL) shifted isoprenaline-induced inhibitory CRCs to left (p < 0.05 and p < 0.01), showing potentiating effect (Fig. 4A). Papaverine caused similar concentration-dependent (0.3 and 1.0 μM) leftward shift (p < 0.05 and p < 0.01) in the CRCs of isoprenaline (Fig. 4B).

3.3. Effect on ileum

Cr.Cr at 0.01 and 0.03 mg/mL caused rightward parallel shift of ACh-curves without suppressing (p > 0.05) maximum response (Fig. 5A). Similarly, atropine (0.003 and 0.01 μM) caused rightward parallel shift, without suppression (p > 0.05) of maximum stimulant effect (Fig. 5B).

3.4. Effect on carbachol-induced bronchoconstriction

Cr.Cr at the doses of 1, 3, 10 and 30 mg/kg (n = 6) caused 16.8 ± 4.6, 27.3 ± 5.54 (p < 0.01), 36.8 ± 7.7 (p < 0.01) and 43.3 ± 7.1% (p < 0.01), respective suppression of the control (CCh, 100 μg/kg)–induced increase in the inspiratory pressure of anaesthetized rats (Fig. 6A). Aminophylline was used as control, inhibited the CCh (100 μg/kg)–mediated bronchoconstriction at 1, 3, 10 and 30 mg/kg (n = 4) by 10 ± 7.4, 13.2 ± 4.15, 28.0 ± 7.7 (p < 0.05) and 37.0 ± 9.3% (p < 0.01) respectively (Fig. 6B).

3.5. Effect on trachea

Cr.Cr was found devoid of any stimulant action, when screened on tracheal resting baseline. When tested against CCh (1 μM) and K⁺ (80 mM)–induced contractions, Cr.Cr caused concentration-dependent inhibition with respective EC50 values of 0.09 (0.04–0.12, n = 4) and 0.50 mg/mL (0.26–0.96, n = 3) as shown in Fig. 7A. Papaverine relaxed the CCh (1 μM) and K⁺ (80 mM)–induced contractions at similar concentration (Fig. 7B) with respective EC50 values of 2.9 (2.6–3.6, n = 5) and 3.1 μM (2.9–3.9, n = 4), whereas verapamil was found more potent in its inhibitory effect against K⁺ (80 mM)–induced contractions with EC50 value of 0.027 μM (0.02–0.03, n = 4), when compared with CCh–induced contractions [0.57 μM (0.38–0.85, n = 3)] as shown in Fig. 7C. Pretreatment of tracheal preparations with Cr.Cr shifted the isoprenaline–induced inhibitory CRCs to left (Fig. 8A) in concentration–dependent manner (0.1 and 0.3 mg/mL, p < 0.01 and p < 0.001), similar to that caused by papaverine (1.0 and 3.0 μM, p < 0.05, p < 0.01 and p < 0.001), showing potentiating effect (Fig. 8B). Cr.Cr at 0.01 and 0.03 mg/mL caused rightward parallel shift of CCh-curves, without suppressing (p > 0.05) maximum contractile response (Fig. 9A). Similarly, atropine (0.01 and 0.03 μM) caused rightward parallel shift without suppression (p > 0.05) of maximum contractile effect (Fig. 9B).
3.6. Acute toxicity

The three different groups of mice were given Cr.Cr in graded doses of 1.5 and 10 g/kg respectively and animals were observed for mortality after 24 h of drug administration. The extract did not cause any mortality up to the dose of 10 g/kg.

Fig. 2. Concentration-dependent inhibitory effect on spontaneous and K⁺ (80 mM)-induced contractions of (A) crude extract of Carum roxburghianum (Cr.Cr), (B) papaverine, and (C) verapamil in isolated rabbit jejunum preparations. Values shown are mean ± SEM, n = 3–4. * p < 0.05 and ** p < 0.01 compared to respective concentrations values in high K⁺-curve (Cr.Cr) and spontaneous contractions (verapamil), two-way analysis of variance followed by Bonferroni’s post-test.

4. Discussion

In view of traditional use of Carum roxburghianum in hyperactive gut disorder, diarrhea, its extract was evaluated for the possible antidiarrheal action in mice. In castor oil-induced diarrhea model, Carum roxburghianum extract in dose-dependent fashion showed protective effect, like that caused by dicyclomine and loperamide, standard antidiarrheal drug (Reynolds et al., 1984). Castor...
oil induces diarrhea as a results of the action of ricinoleic acid formed during the hydrolysis of oil, which produces changes in transport of electrolytes and water, leading to generation of giant contractions of intestine (Iwao and Terada, 1962; Croci et al., 1997). Thus, a potential antidiarreheal agent may exhibit its antidiarreheal effect by inhibiting bowel contractions. The antidiarreheal activity of Cr.Cr following oral administration appears to be virtue of gastrointestinal relaxant component(s) presence in Carum roxburghianum.

For investigation of possible spasmodlytic effect, isolated rabbit jejunum preparation was used, which is known to exhibit spontaneous rhythmic contractions, thus allowing the testing of relaxant activity. In jejunum, Cr.Cr inhibited both spontaneous and high K⁺-induced contractions with similar potency. Papaverine, a phosphodiesterase and Ca++ influx inhibitor (Rang et al., 1999) caused similar pattern of inhibitory effect with comparable potency against spontaneous and K⁺-induced contractions, while verapamil, a standard Ca++ antagonist (Fleckenstein, 1977) was relatively selective in its inhibitory effect on the K⁺-induced contractions. Pretreatment of tissue with plant extract shifted the Ca++-CRCs to right, with suppression of maximum contractile effect, similar to that caused by verapamil. The extract potentiated isoprenaline-induced relaxant effect, similar to that caused by papaverine. The ACh-CRCs in presence of different concentrations of extract were constructed in guinea pig ileum, as this preparation is considered more useful to quantify contractile responses of an agonist in presence of an inhibitor (Daniel et al., 2001). The plant extract caused rightward parallel shift in ACh-curves, without suppressing maximum stimulant response, a characteristic of competitive or specific antagonist (Arunlakhshana and Schild, 1959). Atropine, a standard antimuscarinic agent (Eglen and Harris, 1993) also caused similar rightward parallel shift of ACh-curves. The observed

Fig. 5. Concentration–response curves of acetylcholine (ACh) in the absence and presence of (A) crude extract of Carum roxburghianum (Cr.Cr) and (B) atropine in isolated guinea-pig ileum preparations. Values shown are mean ± SEM, n=5–7. The “ns” shows non-significant difference (p>0.05) at the maximum response level of each ACh curve, two-way analysis of variance followed by Bonferroni’s post-test.

Fig. 6. Dose-dependent suppressant effect of (A) crude extract of Carum roxburghianum (Cr.Cr) and (B) aminophylline on carbachol (CCh)-mediated bronchoconstriction in anaesthetized rats. Values shown are mean ± SEM, n=4–6. *p<0.05 and **p<0.01 vs. control CCh group, one way analysis of variance followed by Dunnett’s test.
antidiarrheal and antispasmodic effects of *Carum roxburghianum* justify its traditional use in diarrhea and abdominal colic.

Based on the folkloric reputation of *Carum roxburghianum* application in asthma, it was tested for possible bronchodilatory effect in anaesthetized rats. Cr.Cr dose-dependently suppressed the CCh-evoked bronchospasm, like that caused by aminophylline.

Fig. 7. Concentration–response curves showing inhibitory effect of (A) crude extract of *Carum roxburghianum* (Cr.Cr), (B) papaverine, and (C) verapamil on carbachol (CCh) and K⁺ (80 mM)-induced contractions in isolated guinea-pig tracheal preparations. Values shown are mean ± SEM, n = 3-5. *p < 0.05, **p < 0.01 and ***p < 0.001 compared to respective concentrations values in high K⁺-curve (Cr.Cr) and CCh-contractions (verapamil), two-way analysis of variance followed by Bonferroni’s post-test.

Fig. 8. Inhibitory concentration–response curves of isoprenaline against carbachol (CCh)-induced contractions in the absence and presence of different concentrations of (A) crude extract of *Carum roxburghianum* (Cr.Cr) and (B) papaverine in isolated guinea-pig tracheal preparations. Values shown are mean ± SEM, n = 4–5. *p < 0.05, **p < 0.01 and ***p < 0.001 compared to respective concentrations values in the isoprenaline control curves, two-way analysis of variance followed by Bonferroni’s post-test.
(soluble salt of theophylline), a well-known bronchodilator (Evans et al., 1980). Carum roxburghianum was further studied in isolated tracheal preparations for underlying tracheo-relaxant effect. The plant extract, papaverine and verapamil all caused tracheal relaxation, pre-contracted with CCh and high K⁺ with varying potencies. Unlike the papaverine and verapamil, plant extract was found comparatively more potent against CCh than K⁺-induced contractions. Pretreatment of tracheal tissues with plant extract, like papaverine shifted the isoprenaline-induced inhibitory CRCs to left, similar to that observed in gut preparations. The extract displaced CCh-curves to right in parallel fashion, without suppression of maximum response, like that caused by atropine. Thus, owning airways relaxant potential, might account for Carum roxburghianum medicinal use in congestive respiratory disease, asthma. In acute toxicity test, Cr.Cr was found safe up to the dose of 10 g/kg, which indicate that Carum roxburghianum is relatively safe in acute toxicity study, though further studies are required to know the precise safety profile.

In conclusion, these results reveal that Carum roxburghianum possesses antidiarrheal, antispasmodic and bronchodilator effects. Thus, this study provides scientific rationale to Carum roxburghianum traditional use in hyperactive gut and airways disorders, like diarrhea, spasm and asthma.

Acknowledgments

This study was supported by Higher Education Commission of Pakistan. The author, Munasib Khan is on leave from University of Malakand for the PhD study.

References


Fig. 9. Concentration–response curves of carbachol (CCh) in the absence and presence of (A) crude extract of Carum roxburghianum (Cr.Cr) and (B) atropine in isolated guinea-pig tracheal preparations. Values shown are mean ± SEM, n = 4. The “ns” shows non-significant difference (p > 0.05) at the maximum response level of each CCh curve, two-way analysis of variance followed by Bonferroni’s post-test.


