JPP Journal of Pharmacy and Pharmacology

JPP 2005, 57: 1493–1501

Received April 19, 2005
Accepted June 20, 2005
DOI 10.1211/jpp.57.11.0016
ISSN 0022–3573

Species differences in the gut stimulatory effects of radish seeds

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Abstract

This study describes the gastrointestinal (GI) prokinetic effects of the aqueous extract of radish seeds (Rs.Cr). Rs.Cr, which tested positive for terpenes, flavonoids, phenols, alkaloids and saponins, showed a spasmogenic effect in isolated rabbit jejunum and ileum, rat stomach fundus and ileum, and guinea-pig ileum and jejunum. Rs.Cr was around 10 times more potent in the guinea-pig tissues and this effect was resistant to atropine, pyrilamine or SB203186 while the spasmogenic effect in the rat and rabbit tissues was atropine sensitive. The extract exhibited atropine-sensitive GI prokinetic and laxative effects in vivo in mice. In the atropinized rabbit jejunum, Rs.Cr produced a spasmolytic effect independent of Ca^{++} or K^{+} channels, adrenergic or opioid receptor involvement. Activity-directed fractionation of Rs.Cr yielded four fractions, all showing effects similar to that of the parent extract. Rs.Cr and its fractions were found to be non-lethal up to 10 g kg^{-1} in mice for 24 h, except for the petroleum fraction, which showed 50% mortality at high doses. Some known radish compounds (spermine, spermidine, putrescine and sinigrin) were also tested and found to be devoid of any activity. The study shows species-specific spasmogenic effects of radish in rabbit, rat and mouse via muscarinic receptors but through an uncharacterized pathway in guinea-pig tissues. Additionally, a dormant relaxant effect was also seen, while the three polyamines and one glucosinolate from radish were found to be inactive, indicating that the compound(s) responsible for the activities reported remains to be isolated.

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Acknowledgements: The authors are extremely thankful to Mr Nauman Aziz, Department of Biological and Biomedical Sciences, for his help and guidance in the statistical analysis of the data. This study was supported with funds made available by the Higher Education Commission of Pakistan under the scheme of Distinguished National Professor Research Allowance.

Part of this work was presented at the Joint International Conference of SAAB & ISE, 7th to 11th Jan 2003, Pretoria, South Africa.

Introduction

Raphanus sativus Linn (family, Cruciferae), commonly known as radish, is a well-known salad plant cultivated all over the world for its culinary and medicinal properties. The various medicinal uses of the seeds, leaves and root of radish have been described in detail in the Chinese, Japanese, Unani and Ayurvedic systems of traditional medicine (Lust 1987; Chen & Keng 1990). Radish has been used especially in different gastrointestinal (GI) and hepatic disorders such as constipation, indigestion, dyspepsia, anorexia, nausea, flatulence, diarrhoea, dysentery, jaundice and hepatitis (Mahmood et al 1991; Gupta et al 1997; Duke 2002). The plant is also commonly used in many other conditions, such as hypertension, asthma, bronchosis, cough, kidney and bladder stone, cancer, cholera, edema, fever, headache, haemorrhoid, infection, rheumatism and lumbago (Duke 2002).

Reported activities for the plant (for review see Gutierrez & Perez 2004) show that it has antiurolithiatic (Vargas et al 1999), anti-inflammatory and antibleeding (Nagar 1993), influenza protective (Prahoveanu & Esanu 1987), antimicrobial (Ela et al 1996), antioxidant (Lugasi et al 1998), antitumour (Xiaoling et al 2001) and antiplatelet properties (Morimitsu et al 2000). Phytochemical studies revealed the presence of proteins; polyphenols; flavonoids; peroxidases; isoperoxidases; alkaloids, such as pyrrolidine, isoquinoline and phenethylamine; sulfuric compounds, such as glucoparin and sinigrin; and polyamines, such as spermine, spermidine and putrescine (Duke 1992; Vargas et al 1999). Different coumarins (Stoehr & Herrmann 1975), organic acids (Shyamala & Singh 1987) and polysaccharides (Matsuura & Hatanaka 1988) have also been identified.

There is no study in the literature reporting the GI effects of radish although it is used extensively in traditional medicine for conditions affecting this part of the body.

Thus in this investigation we report the species-specific GI stimulatory effects of the aqueous extract and fractions of radish seeds, mediated partially via activation of muscarinic receptors. Some of the commercially available polyamines (spermine, spermidine and putrescine) and a glucosinolate (sinigrin), known to be present in radish (Duke 1992), were also screened for biological activity.

Materials and Methods

Drugs and standards

The following reference chemicals were obtained from the sources specified: acetylcholine (ACh), atropine, carbachol (CCh), hexamethonium, histamine, 5-hydroxytryptamine (5-HT), nicotine, putrescine, pyrilamine, sinigrin, spermidine, spermine (Sigma Chemical Company, St Louis, MO, USA) and 1-piperidinylethyl-1H-indole-3-carboxylate (SB203186) (Tocris, Ballwin, MO, USA). The following chemicals were used to make the physiological salt solutions: potassium chloride (Sigma Chemical Company, St Louis, MO, USA), calcium chloride, glucose, magnesium chloride, magnesium sulfate, potassium dihydrogen phosphate, sodium bicarbonate, sodium chloride and sodium dihydrogen phosphate (Merck, Darmstadt, Germany). The chemicals used for the purpose of the charcoal meal transit test were acacia powder, hydrolysed starch and vegetable charcoal (BDH Laboratory Supplies, Poole, UK). Stock solutions of all the chemicals were made in distilled water and the dilutions were made fresh on the day of the experiment.

Animals

The experiments performed complied with the rulings of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (NRC 1996). Balb-C mice (20–25 g), Sprague–Dawley rats (170–200 g), local rabbits (1 kg) and guinea-pigs (500–600 g) of either sex used in the study were housed in the animal house of the Aga Khan University under a controlled environment (23–25°C). Animals were fasted for 24 h before the experiment but were given tap water ad libitum and a standard diet consisting of ($g\,kg^{-1}$): flour 380, fibre 380, molasses 12, NaCl 5.8, nutrivet L 2.5, potassium metabisulfate 1.2, vegetable oil 38, fish meal 170 and powdered milk 150.

Plant material and extract preparation

Radish seeds (972 g) were purchased from a wholesale market in Karachi. A sample of the plant material was deposited at the Herbarium of the Department of Biological and Biomedical Sciences, Aga Khan University, Karachi, with the voucher #RS-SE-08-99-19. The seed sample was cleaned of adulterants and soaked in 4 L of distilled water for a total of 3 days at 20°C. The plant material was then filtered through Whatman qualitative grade 1 filter papers and the filtrate was collected. This procedure was repeated twice and the combined filtrate was concentrated in a rotary evaporator to yield a thick, light brown extract (Rs.Cr) weighing 293.3 g (yield of 30.2%).

A part of this crude extract was kept for pharmacological testing while the rest (around 150 g) was used for fractionation. Activity-directed fractionation of the extract was carried out by standard phytochemical procedures using different organic solvents (Williamson et al 1998). A known quantity of Rs.Cr was dissolved in distilled water and partitioned with three successive portions of petroleum spirit (40–60° boiling rate). The combined petroleum layers were concentrated under vacuum to obtain the petroleum spirit fraction (Rs.Pt, 30.9 g). The extract was then treated in the same manner with chloroform (Rs.Cl, 27.5 g), followed by ethyl acetate (Rs.EtAc, 6.7 g). The remaining aqueous portion was freeze-dried to give Rs.Aq (83.6 g).

Preliminary phytochemical analysis

The radish crude extract (Rs.Cr) and all its fractions were screened for the presence of different classes of compounds by TLC using silica gel G (Merck) plates of 0.25 mm thickness (Wagner et al 1984). The extract was dissolved in methanol while the development of plates was carried out with chloroform:methanol (9:1 v/v). After development, the plates were sprayed with the following solvents and reagents for detection of the respective classes of compounds: water (lipophilic compounds); sulfuric acid and heating at 105°C for 5 min (organic compounds); 0.5% anisaldehyde in sulfuric acid, glacial acetic acid and methanol 5:10:85 v/v (terpenoids); 10% antimony trichloride in chloroform (flavonoids/terpenoids); 1% diphenylboric acid 2-aminoethyl ester in methanol followed by 5% polyethylene glycol 4000 in 96% ethanol (flavonoids); 0.5% ninhydrin in acetone (amino acids/peptides and secondary amines); 5% ethanolic sodium hydroxide (anthraquinones); 5% aqueous ferric chloride (tannins/phenols); 20% aqueous sodium carbonate followed by Folin–Ciocalteu reagent (phenols); 0.5% aqueous fast blue B salt followed by 0.1 M aqueous sodium hydroxide (phenols); Dragendorff reagent (alkaloids) and dilute sodium hydroxide (coumarins). Reagents were prepared according to Stahl (1969). Detection was carried out visually in visible light and under UV light ($\lambda = 365$ nm).

Isolated rabbit jejunum and ileum

All experiments on isolated tissues were carried out as previously described (Gilani et al 2004, 2005). After cervical dislocation of the animal, the abdomen was cut open and the jejunal and ileal portions isolated. Preparations 2-cm long were mounted in 10-mL tissue baths containing Tyrode's solution maintained at 37°C and aerated with a mixture of 5% carbon dioxide and 95% oxygen (carbogen). The composition of Tyrode's solution (mM) was KCl 2.7, NaCl 136.9, $MgCl_2$ 1.1, $NaHCO_3$ 11.9, NaH_2PO_4 0.4, glucose 5.6 and $CaCl_2$ 1.8 (pH 7.4). A preload of 1 g was applied to each tissue and

the tissues kept undisturbed for an equilibrium period of 30 min after which control responses to ACh (0.3 μM) were obtained. The tissues were presumed stable only after the reproducibility of the said responses. The radish extract, along with its fractions and the pure compounds spermine, spermidine, putrescine and sinigrin, were examined later for any spasmogenic or spasmolytic activity on the jejunal tissues.

Isolated rat stomach fundus

Rats were sacrificed by cervical dislocation. The stomach was removed and placed in Kreb's solution to isolate the fundus. The tissue was cut opened along the lesser curvature and divided into two longitudinal strips of 2 mm width and 15 mm length. Each strip preparation was mounted in a 10-mL tissue bath with Kreb's solution at 37°C and aerated with carbogen. The composition of Kreb's solution was (mM) NaCl 118.2, $NaHCO_3$ 25.0, $CaCl_2$ 2.5, KCl 4.7, KH_2PO_4 1.3, $MgSO_4$ 1.2 and glucose 11.7 (pH 7.4). Basal tension of 1 g was applied to each tissue and the responses recorded following an equilibrium period of 60 min. The sub-maximal doses of CCh (0.3 μM) were tested repeatedly to stabilize the preparation and the responses were recorded through isotonic Harvard transducers coupled with Harvard student oscillographs. Once stabilized, Rs.Cr was screened on the fundic preparations to note any activity.

Isolated rat ileum and guinea-pig ileum and jejunum

Guinea-pigs and rats were sacrificed by cervical dislocation. The abdomen was cut open and 2-cm long segments of ileum/jejunum were isolated and mounted in 10-mL tissue baths containing Tyrode's solution. One end of the tissue was attached to the metal tissue hook and the other was attached to an isotonic transducer and connected to a Harvard student oscillograph. Under these conditions, ileum behaves as a quiescent preparation. A preload of 1 g was applied to each tissue and kept constant throughout the experiment. The tissue was washed several times within a 5-min interval and was allowed to equilibrate for 30 min before isotonic contractions to a sub-maximal concentration of ACh (0.3 μM) were recorded. An agonist contact time of 20 s was used together with a 3-min interval between doses. Once the tissue was stabilized with reproducible effects from the doses of the standard, Rs.Cr was tested for any activity.

Charcoal meal GI transit test

The method of Croci et al (1997) was used with slight modifications. Mice were divided into different groups. Two of the groups were treated orally with increasing doses of Rs.Cr (100 mg kg^{-1} (n = 12) and 300 mg kg^{-1} (n = 8)) serving as the test groups. One group served as blank or negative control, treated with saline (10 mL kg^{-1}, p.o., n = 9) and the last group was administered CCh (1 mg kg^{-1}, p.o., n = 8) as the positive control. After 15 min the animals were given 0.3 mL of charcoal meal (distilled water suspension containing 10% gum acacia, 10% vegetable charcoal and 20% starch). After 30 min the animals were sacrificed and the abdomen immediately opened to excise the whole small intestine. The length of the small intestine and the distance between the pylorus region and the front of the charcoal meal was measured to obtain the charcoal transport ratio or percentage. To test for an ACh-like involvement in the prokinetic effect of the extract and CCh, separate sets of mice were pretreated with atropine (10 mg kg^{-1}, p.o.) 15 min before the administration of the extract or CCh.

Laxative activity test

The method of Haruna (1997) was followed for this activity. Mice (20–25 g) fasted for 6 h before the experiment were placed individually in cages lined with clean filter paper. The animals were divided into four groups with the first group acting as the control and administered saline (10 mL kg^{-1}, p.o., n = 8). The second and third groups received, orally, 100 and 300 mg kg^{-1} of Rs.Cr (n = 6) while the last group received CCh (1 mg kg^{-1}, i.p., n = 6). This served as the positive control. The faeces production (total number of normal as well as wet faeces) in all four groups was monitored for 18 h. To determine the mechanism of the laxative effect of the extract and CCh, separate sets of mice were pretreated with atropine (10 mg kg^{-1}, i.p.) 1 h before administration of the extract or CCh.

Acute toxicity test

Animals were divided into groups of five mice each. The test was performed using increasing oral doses of Rs.Cr and all its fractions (1, 5 and 10 g kg^{-1}) in 10 mL kg^{-1} volume to the test groups. Another group of mice was administered saline (10 mL kg^{-1}, p.o.) as the negative control. The mice were allowed food ad libitum and were kept under constant observation for 4 h to note any behavioural changes while mortality was observed for 24 h after drug administration.

Statistical analysis

All the data expressed are as mean ± s.e.m. (n = number of experiments) and the median effective concentrations (EC_{50} values) with 95% confidence intervals (CI). The statistical parameter applied was one-way (in the case of the in-vivo tests) and two-way (in the case of the in-vitro tests) analysis of variance (ANOVA) followed by Tukey's test, except for the laxative activity (Table 1) where Dunnett's test was applied using the GraphPAD program (GraphPAD, San Diego, CA, USA). A probability of less than 0.05 was considered statistically significant. Concentration–response curves were analysed by non-linear regression (GraphPAD program).

Table 1 Laxative effect of the crude extract of *Raphanus sativus* seeds (Rs.Cr) and carbachol in the absence and presence of atropine (10 mg kg^{-1}) in mice

Test samples	Dose (mg kg^{-1})	Total number of faeces	Total number of wet faeces	% wet faeces
Saline (p.o., mL kg^{-1})	10	1.8 ± 0.4	0.0	0.0
Rs.Cr (p.o.)	100	5.6 ± 1.0*	0.0	0.0
	+ atropine	1.5 ± 0.2	0.0	0.0
	300	8.6 ± 2.1**	0.3 ± 0.02	3.5
	+ atropine	1.9 ± 0.3	0.0	0.0
Carbachol (i.p.)	1	8.2 ± 1.5**	2.5 ± 1.0**	30.5
	+ atropine	2.1 ± 0.1	0.0	0.0

Values shown are mean ± s.e.m., n = 6–8. *P < 0.05 and **P < 0.01 vs saline, one-way ANOVA followed by Dunnett's test.

Results

Phytochemical analysis

Rs.Cr and all the fractions (Rs.Pt, Rs.Cl, Rs.EtAc and Rs.Aq) showed the presence of organic, double-bonded and fluorescent compounds along with the following classes of compounds: terpenoids, flavonoids, amino acids/peptides, secondary amines, phenols, alkaloids and saponins. Froth formation with the Rs.EtAc was minimal, indicating a low content of saponins. On critical evaluation of the TLC chromatograms it was noted that Rs.Cr showed a maximum number of spots for flavonoids when sprayed with a combination of diphenylboric acid 2-aminoethyl ester and polyethylene glycol. Rs.EtAc showed a concentration of organic compounds when sprayed with sulfuric acid, flavonoids, alkaloids (following spraying with Dragendorff) and also exhibited elaborated spots for amines and phenols when sprayed with two combinations of reagents: aqueous sodium carbonate + Folin–Ciocalteu reagent and aqueous fast blue B salt + aqueous sodium hydroxide. Rs.Aq showed minimum spots for terpenoids, amines and phenols.

Effect on rabbit jejunum

Rs.Cr caused a dose-dependent (10 μg mL^{-1} to 3 mg mL^{-1}) stimulatory effect in the spontaneously beating jejunal preparation (Figure 1) with an EC_{50} value of 0.18 mg mL^{-1} (0.13–0.26, 95% CI, n = 8). The spasmogenic effect was followed by relaxation at doses ≥3 mg mL^{-1} (Figure 1). The efficacy of the spasmogenic effect was 97.2 ± 0.9% of the ACh maximum effect (Figure 2). Pretreatment of the tissues with atropine (0.1 μM) blocked the spasmogenic effect (Figure 1) while hexamethonium (0.3 mM) had no effect. Following blockade of the spasmogenic component by atropine, a relaxant factor was unmasked (Figure 1), which was mediated in a dose-dependent (0.1–3.0 mg mL^{-1}) manner with an EC_{50} value of 0.71 mg mL^{-1} (0.49–1.02, n = 5). In order to determine the mode of this relaxant effect, the extract was tested on K^+-induced contractions but was found to be devoid of any inhibitory effect on both low (25 mM) and high (80 mM) K^+-induced contractions. The relaxant effect of Rs.Cr was also found to be resistant to blockade by phentolamine (1 μM), propranolol (1 μM) and naloxone (10 μM). The radish pure compounds, namely spermine, spermidine, putrescine and sinigrin, when tested on rabbit jejunum, were found to be devoid of any activity up to 10 mg mL^{-1}.

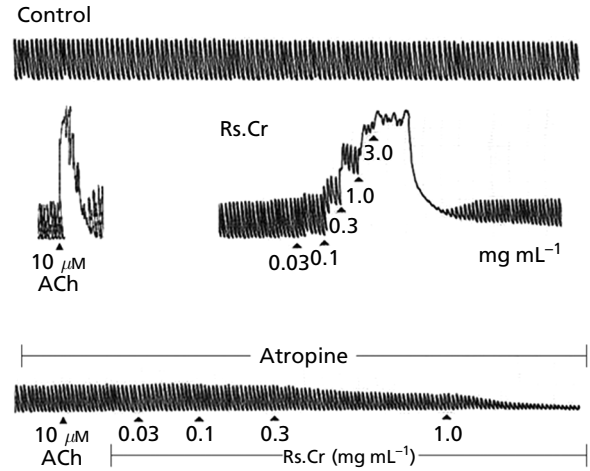

Figure 1 Typical tracing showing the effect of radish seed crude extract (Rs.Cr) and acetylcholine (ACh) in the absence and presence of atropine (0.1 μM) in isolated rabbit jejunum preparation.

Effect on rabbit ileum

Rs.Cr exhibited a dose-dependent (10 μg mL^{-1} to 5 mg mL^{-1}) stimulatory effect in spontaneously beating rabbit ileum (Figure 2) with an EC_{50} of 0.29 mg mL^{-1} (0.13–0.67, n = 5). The spasmogenic effect was followed by relaxation at doses ≥5 mg mL^{-1}. The efficacy of the spasmogenic effect was 96.0 ± 1.2% of ACh maximum effect (Figure 2). Pretreatment of the tissue with atropine (0.1 μM) blocked the spasmogenic effect while hexamethonium (0.3 mM) had no effect. Following blockade of the spasmogenic component, a relaxant factor was unmasked,

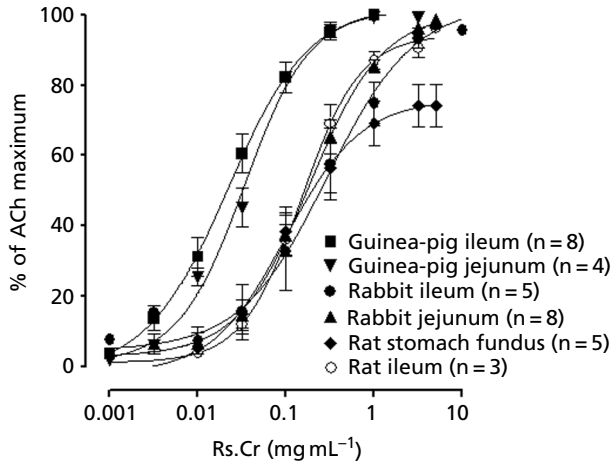

Figure 2 Dose–response curves showing the spasmogenic effect of radish seed crude extract (Rs.Cr) in different isolated GI tissue preparations. Values shown are mean ± s.e.m. and expressed as the percentage of acetylcholine (ACh) maximum response. There was a significant difference between the Rs.Cr-induced effects in guinea-pig tissues and those of rabbit and rat tissues, and between individual doses ($P < 0.01$); two-way ANOVA.

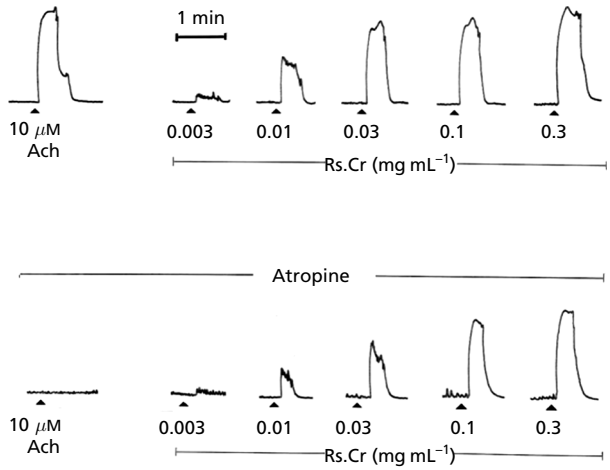

Figure 3 Typical tracing showing the atropine-resistant spasmogenic effect of radish seed crude extract (Rs.Cr) in comparison to acetylcholine (ACh) in the absence and presence of atropine (0.1 μM) in isolated guinea-pig ileum.

mediated in a dose-dependent (0.1–5.0 mg mL^{-1}) manner with an EC_{50} of 1.27 mg mL^{-1} (0.56–2.86, n = 3).

Effect on rat stomach fundus

In the rat stomach fundus, Rs.Cr produced a dose-dependent (10 μg mL^{-1} to 3 mg mL^{-1}) contractile effect (Figure 2) with an EC_{50} value of 0.11 mg mL^{-1} (0.04–0.23, n = 5). The efficacy of the spasmogenic effect was 74.2 ± 5.9% of CCh maximum effect (Figure 2). Pretreatment of the tissue with atropine (0.1 μM), not with hexamethonium (0.3 mM), completely blocked the effect of the radish extract in a similar way to CCh.

Effect on rat ileum

Rs.Cr exhibited an atropine-sensitive and hexamethonium-resistant dose-dependent (10 μg mL^{-1} to 5 mg mL^{-1}) stimulatory effect in this quiescent preparation (Figure 2) with an EC_{50} of 0.14 mg mL^{-1} (0.11–0.19, n = 3). The efficacy of the spasmogenic effect was 92.9 ± 3.3% of the ACh maximum (Figure 2). Pretreatment of the tissue with atropine (0.1 μM), not with hexamethonium (0.3 mM), completely blocked the effect of the radish extract.

Effect on guinea-pig ileum

Rs.Cr caused a dose-dependent (1 μg ml^{-1} to 1 mg mL^{-1}) stimulatory effect in this quiescent preparation (Figures 2 and 3) with an EC_{50} value of 0.02 mg mL^{-1} (0.01–0.03, n = 8). The efficacy of the spasmogenic effect was the same as for the ACh maximum (Figure 2) and remained unchanged in the presence of hexamethonium (0.3 mM), atropine (0.1 μM, Figure 3), pyrilamine (1 μM) and SB203186 (1 μM). The radish pure compounds, when tested on the resting baseline of ileum, were found to be devoid of any activity until a dose of 10 mg mL^{-1} was used.

Effect on guinea-pig jejunum

Rs.Cr caused a dose-dependent (1 μg ml^{-1} to 1 mg mL^{-1}) stimulatory effect in this quiescent preparation (Figure 2) with an EC_{50} value of 0.03 mg mL^{-1} (0.02–0.04, n = 4). The efficacy of the spasmogenic effect was the same as for the ACh maximum (Figure 2) but was resistant to blockade of ganglionic, muscarinic, histaminergic and serotonergic receptors (data not shown).

Effect on charcoal meal GI transit

The prokinetic effect of the radish extract was studied in mice. The extract dose dependently (100–300 mg kg^{-1}) propelled the charcoal meal through the small intestine of mice (Figure 4). The distance travelled by the vehicle control (saline) was 54.7 ± 2.6%. The plant extract at a dose of 100 mg kg^{-1} moved the charcoal meal to 62.6 ± 2.8% while at 300 mg kg^{-1} it moved it to 75.5 ± 2.1% ($P < 0.001$ vs saline control). CCh (1 mg kg^{-1}) was used as the positive control and moved the meal to 95.5 ± 2.6% ($P < 0.001$). This enhancement in the traverse of charcoal meal in comparison to saline-treated mice by the extract (100 and 300 mg kg^{-1}) and CCh (1 mg kg^{-1}) was absent in atropine (10 mg kg^{-1}) pretreated mice (Figure 4).

Laxative effect

The plant extract, when administered orally, showed a laxative effect in mice as reflected by the increase in the number of faeces in the test animals (Table 1). The laxative effect was dose-dependently mediated in the dose range of 100–300 mg kg^{-1}, showing an increase not only

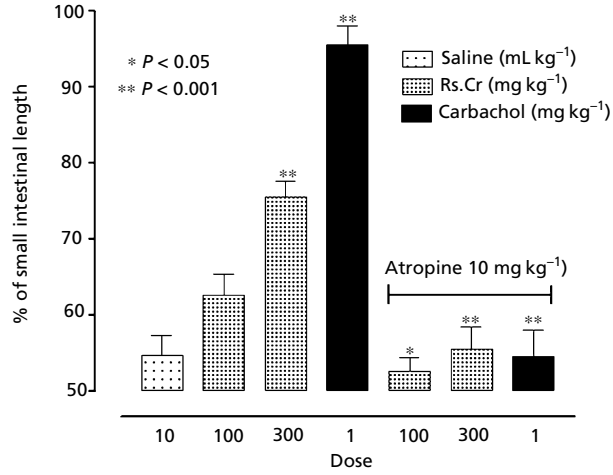

Figure 4 Bar diagram showing the in-vivo prokinetic effect of increasing doses of radish seed crude extract (Rs.Cr) and carbachol (CCh) in the absence and presence of atropine on GI transit of charcoal meal in mice (*$P < 0.05$ and **$P < 0.001$, vs saline for values in the absence of atropine and vs respective control values for values in the presence of atropine; one-way ANOVA followed by Tukey's test, n = 8–12).

in the total number of faeces but also in the number of wet faeces, and was comparable to CCh, which showed an effect at 1 mg kg^{-1} given i.p. (Table 1). The laxative effect seen with the extract (100 and 300 mg kg^{-1}) and CCh (1 mg kg^{-1}) in the form of an increase in dry and wet faeces was completely blocked in the atropine (10 mg kg^{-1}) pretreated mice (Table 1).

Effect of fractions on rabbit jejunum and guinea-pig ileum

The plant extract was subjected to activity-directed fractionation, yielding four fractions. Each fraction caused a dose-dependent (10 μg mL^{-1} to 3 mg mL^{-1}) contractile effect in spontaneously contracting rabbit jejunum with the same mechanism (Figure 5A), as the effect was blocked by atropine (0.1 μM). The maximum effects achieved by the petroleum, chloroform, ethyl acetate and aqueous fractions were 79.6 ± 6.1%, 92.0 ± 2.4%, 83.5 ± 3.8% and 90.1 ± 3.4% of the ACh maximum, respectively (Figure 5A). The spasmogenic effect of the fractions was obtained at similar doses without much difference in potency, with EC_{50} values of 0.16 mg mL^{-1} (0.07–0.33, n = 5), 0.18 mg mL^{-1} (0.12–0.28, n = 5), 0.28 mg mL^{-1} (0.19–0.42, n = 5) and 0.22 mg mL^{-1} (0.14–0.34, n = 5), respectively. After blockade of the spasmogenic effect of the fractions with atropine they all exhibited a dose-dependent (0.3–5.0 mg mL^{-1}) spasmolytic effect, similar to the parent crude extract (Figure 5A). This relaxant effect was mediated with EC_{50} values of 3.92 mg mL^{-1} (1.09–14.12, n = 5), 3.15 mg mL^{-1} (1.04–9.52, n = 5), 0.48 mg mL^{-1} (0.42–0.55, n = 5) and 0.79 mg mL^{-1} (0.50–1.23, n = 4) for the petroleum, chloroform, ethyl acetate and aqueous fractions, respectively.

All fractions were also tested on the guinea-pig ileum where they were found to have a dose-dependent

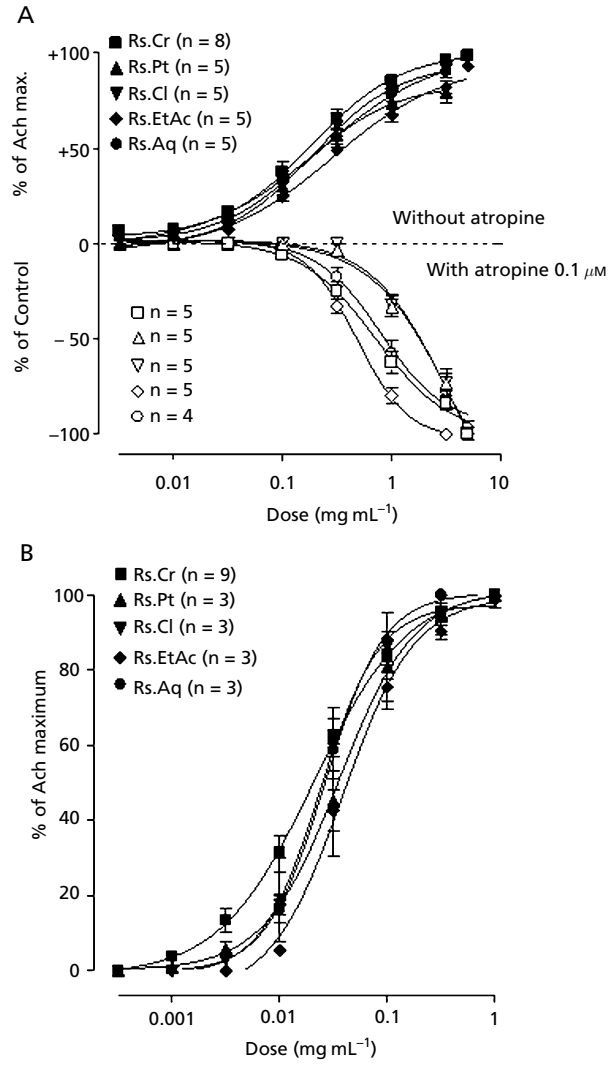

Figure 5 Dose–response curves showing (A) stimulant and relaxant effects of radish seed crude extract (Rs.Cr) and its fractions petroleum (Rs.Pt), chloroform (Rs.Cl), ethyl acetate (Rs.EtAc) and aqueous (Rs.Aq) in the absence and presence of atropine (0.1 μM) in rabbit jejunum. (B) shows the atropine-resistant stimulatory effect of Rs.Cr and its fractions in guinea-pig ileum preparations. Values shown are mean ± s.e.m. In (A) there is a significant difference between the curves in the presence and absence of atropine and between individual doses ($P < 0.01$). In (B) the difference is significant between individual doses ($P < 0.01$) while the curves are not different from each other ($P > 0.05$) except for Rs.EtAc, which was different from Rs.Cr ($P < 0.05$); two-way ANOVA.

(1 μg mL^{-1} to 1 mg mL^{-1}) stimulatory effect (Figure 5B) with EC_{50} values of 0.03 mg mL^{-1} (0.02–0.05, n = 3), 0.02 mg mL^{-1} (0.01–0.03, n = 3), 0.03 mg mL^{-1} (0.02–0.04, n = 3) and 0.03 mg mL^{-1} (0.02–0.04, n = 3), respectively, showing around 10 times higher potency compared to the effect obtained in jejunum. The efficacy of the spasmogenic effect was the same as that of the ACh maximum (Figure 5B) but was resistant to blockade by ganglionic, muscarinic, histaminergic and serotonergic blockers, similar to the parent crude extract.

Acute toxicity study

Different groups of five mice each were given graded doses of the plant extract and the resultant fractions (1, 5 and 10 g kg^{-1}, orally). The plant extract, along with all the fractions (except Rs.Pt), was found to be safe up to a dose of 10 g kg^{-1} with no mortality or any other apparent behavioural side-effects. Rs.Pt induced mortality in 50% of the animals; the dose of 10 g kg^{-1} through to the next lower dose of 5 g kg^{-1} caused no mortality.

Discussion

The aqueous extract of radish seeds showed a dose-dependent spasmogenic effect in gut preparations from different species. The stimulant effect in the rabbit and rat tissues was completely abolished in the presence of atropine, a muscarinic receptor blocker (Arunlakhshana & Schild 1959; Gilani & Cobbin 1986). Atropine is also known to block the effect (not the receptors) of nicotine as the end effect of nicotine in gut preparations is eventually due to release of acetylcholine from the myenteric plexus, which in turn activates muscarinic receptors at the end organ (Brown & Taylor 2001). To see whether the spasmogenic effect of the plant extract was mediated beyond the level of autonomic ganglia, the tissue was pretreated with hexamethonium, a ganglion blocker (Wien et al 1952). This treatment blocked the effect of nicotine but the effect of Rs.Cr remained unaltered, suggesting that it is devoid of any nicotinic effect and the spasmogenic effect observed is mediated through direct stimulation of muscarinic receptors.

Rs.Cr showed similar potency for its gut stimulatory effect in the rabbit and rat tissues; however, it was found to be around 10 times more potent in guinea-pig tissues. Unlike the atropine-sensitive stimulatory effect in rabbit and rat, the spasmogenic effect in guinea-pig was insensitive to ganglionic, muscarinic, histaminergic and serotonergic antagonists. It is possible that this non-cholinergic spasmogenic effect in guinea-pig is species specific, mediated at lower doses through an uncharacterized mechanism, hence dominating the muscarinic observed at higher doses in rabbit and rat tissues. Such species-dependent activities have also been observed in earlier studies (McLeod et al 1994; Nambi et al 1994; Ji et al 1994). Several other mechanisms are also known to be involved in imparting a stimulant effect in the GI, which could not be ruled out in this study, such as platelet-activating factor (Izzo et al 1998), nitric-oxide-donating or -releasing compounds (Mascolo et al 1994), cholecystokinin (Briejer et al 1999), dopaminergic antagonists (Briejer et al 1995), tachykinins (Severini et al 2002) and certain prostaglandins (Beubler & Kollar 1988). Although it might be premature to suggest how the radish seeds interact (through cholinergic or the uncharacterized receptors) when consumed in humans for their medicinal properties, as seen from this study Rs.Cr has a generalized spasmogenic effect in all the tissues tested from different species. A definite conclusion on the mode of stimulant effect of radish extract, in relation to its use in humans, can only be made after testing it on human tissues.

The in-vitro spasmogenic activity seen from the extract was confirmed in the in-vivo studies when it caused a dose-dependent increase in the traverse of charcoal meal in mice and also exhibited a laxative effect, similar to carbachol, a cholinergic agonist and intestinal stimulant (Brown & Taylor 2001). These in-vivo gut stimulatory activities of Rs.Cr and carbachol were blocked by atropine pretreatment, indicating an ACh-like effect. Earlier, Jung et al (2000) reported the GI motility-enhancing effects of another type of radish (*Brassica oleracea*, Cruciferae) in rats and mice via the activation of muscarinic receptors, thus indicating the widespread presence of muscarinic constituents in different kinds and species of radishes. The observed stimulant effect from the radish seed extract is in line with its traditional use in GI motility disorders as a laxative (Mahmood et al 1991; Duke 2002). Radish seeds are used to promote digestion and rectify food retention owing to their prokinetic ability. Seed powder (1–2 g) is consumed to improve the appetite, act as a digestive aid and produce laxation (Nadkarni 1976). The active doses (100 and 300 mg kg^{-1}) seen from the extract for imparting the GI stimulant effect may seem high but it should be noted that the ratio of metabolic rate to body weight in small animals is much greater (around 10 times) than in humans (Ganong 1991). Thus for an average weight individual (70 kg) a postulated dose (after seeing the results of this study) of the plant extract may be close to the dose usually consumed by people in traditional medicine practice (1–2 g).

In addition to the stimulant effect, Rs.Cr also exhibited a relaxant effect observed in the spontaneously contracting rabbit jejunum tissues, which was mediated independently of the Ca^{++} or K^{+} channels and the adrenergic or opioid receptors. All these mechanisms are known to relax intestinal tissues (Thompson 2002). Insensitivity of the extract to these interventions indicates that Rs.Cr might be acting through some other mechanism for its spasmolytic effect.

The crude extract was subjected to activity-directed fractionation, yielding three organic fractions and one aqueous fraction. The fractions were later screened on rabbit jejunum and guinea-pig ileum to determine their activity pattern. All the fractions, like the parent extract, showed a dose-dependent spasmogenic effect in rabbit jejunum that was atropine-sensitive but resistant to atropine in guinea-pig ileum. Following the blockade of spontaneous contractions in rabbit jejunum by atropine, a relaxant effect was seen; the ethyl acetate fraction was the most potent in exhibiting the relaxant effect. No clear separation or concentration of the spasmogenic factors was seen in any one of the fractions, indicating widespread distribution of the spasmogenic constituent(s) in the plant. None of the known constituents of radish studied (the polyamines spermine, spermidine and putrescine, and the glucosinolate sinigrin) were found to be active on the isolated rabbit jejunum and guinea-pig ileum, indicating that the active principle responsible

for the gut stimulatory or inhibitory effect of the radish seed extract is yet to be identified.

Phytochemical analysis of the extract and fractions revealed the presence of terpenoids, flavonoids, amines, phenols, alkaloids and saponins. The ethyl acetate fraction, which was found to be the most potent in exhibiting spasmolytic activity among all the fractions, was found with comparatively more spots for flavonoids, alkaloids, amines and phenols. Flavonoids and phenolic compounds are known to exhibit GI spasmolytic effects (Di-Carlo et al 1993), which could possibly give a clue to the greater potency of Rs.EtAc for GI relaxant activity. Saponins are known to have a gut stimulant action (Akah et al 1997) and as Rs.EtAc was found to be a minimum in the saponin content, could also result in its enhanced antispasmodic activity. The spasmogenic activity was found to be shared between all the fractions. Radish consists of a large quantity of soluble carbohydrates (Kapoor 1990), a reason why sinigrin was tested for any activity. Apart from that, different amines, phenols, flavonoids, organic acids, terpenes, saponins and volatile oils have also been isolated from radish (Mahmood et al 1991). Out of these we were able to screen some commercially available polyamines, which gave no activity. Either the activity lies with flavonoids, terpenes or saponins or the responsible compound for the spasmogenic and spasmolytic activities is yet to be isolated. More phytochemical work is needed in this respect.

Conclusion

The results showed that the radish seed extract exhibits a species-dependent gut stimulatory effect sensitive to atropine in rabbit, rat and mouse but is resistant to atropine in guinea-pig tissues. The extract also showed an antispasmodic effect in gut preparations, evident in the atropinized tissues, which could not be characterized. This study has provided a scientific basis for the traditional use of radish seeds in gut motility disorders with a species-specific mechanism of stimulatory effect.

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