Pharmaceutical Biology

ISSN: 1388-0209 (Print) 1744-5116 (Online) Journal homepage: informahealthcare.com/journals/iphb20

Assessment of Eight Popularly Used Plant-Derived Preparations for Their Spasmolytic Potential Using the Isolated Guinea Pig Ileum

Dennis Mans, Jerry Toelsie, Zorana Jagernath, Kiran Ramjiawan, Andy van Brussel, Nawin Jhanjan, Sunil Orie, Marjory Muringen, Urvin Elliot, Simone Jurgens, Robert Macnack, Fernando Rigters, Shoba Mohan, Vikash Chigharoe, Sigmar Illes & Robbert Bipat

To cite this article: Dennis Mans, Jerry Toelsie, Zorana Jagernath, Kiran Ramjiawan, Andy van Brussel, Nawin Jhanjan, Sunil Orie, Marjory Muringen, Urvin Elliot, Simone Jurgens, Robert Macnack, Fernando Rigters, Shoba Mohan, Vikash Chigharoe, Sigmar Illes & Robbert Bipat (2004) Assessment of Eight Popularly Used Plant-Derived Preparations for Their Spasmolytic Potential Using the Isolated Guinea Pig Ileum, Pharmaceutical Biology, 42:6, 422-429, DOI: 10.1080/13880200490886012

To link to this article: https://doi.org/10.1080/13880200490886012

Published online: 29 Sep 2008.

Pharmaceutical Biology
2004, Vol. 42, No. 6, pp. 422–429

Taylor & Francis
Taylor & Francis Group

Assessment of Eight Popularly Used Plant-Derived Preparations for Their Spasmolytic Potential Using the Isolated Guinea Pig Ileum

Dennis R.A. Mans[1], Jerry R. Toelsie[2], Zorana Jagernath[2], Kiran Ramjiawan[2], Andy van Brussel[2], Nawin Jhanjan[2], Sunil Orie[2], Marjory Muringen[1], Urvin Elliot[2], Simone Jurgens[1], Robert Macnack[1], Fernando Rigters[2], Shoba Mohan[1], Vikash Chigharoe[2], Sigmar Illes[1] and Robbert Bipat[2]

[1]Department of Pharmacology and [2]Department of Physiology, Faculty of Medical Sciences, Anton de Kom University, Paramaribo, Suriname

Abstract

Aqueous extracts from eight plant species that are popularly used as spasmolytics have been evaluated for these presumed activities. The species included *Kalanchoë pinnata* (Lam.) Pers. (Crassulaceae), *Cymbopogon citratus* Stapf. (Gramineae), *Gossypium barbadense* L. (Malvaceae), *Caesalpinia pulcherrima* (L.) Schwartz (Caesalpiniaceae), *Tagetes erecta* L. (Compositae), *Bixa orellana* L. (Bixaceae), *Cassia alata* L. (Caesalpiniaceae), and *Phyllanthus amarus* Schum. & Thonn. (Euphorbiaceae). Potential spasmolytic activity of the extracts was judged by their ability to reduce forces of smooth muscle contraction of a 2-cm-long piece of guinea pig ileum induced by EC_{50} acetylcholine (27 ± 5 µg/l) or EC_{50} histamine (102 ± 13 µg/l). The dried extracts were used at concentrations of 0.01, 0.1, 1, and 10 mg/ml. Incubations were carried out in Tyrode buffer kept at a temperature of 37 °C and mixed with 5% CO_2 in air and were monitored for 30 s with 60-s intervals. Results (means ± SD; $n \geq 3$) were expressed relatively to forces of contraction due to EC_{50} acetylcholine or EC_{50} histamine alone. The extract from *K. pinnata* reduced the force of contraction due to histamine but not that due to acetylcholine progressively (40% to 95%) with concentrations increasing from 0.01 to 10 mg/ml. At 10 mg/ml, the *C. pulcherrima* and *B. orellana* extracts also counteracted only the histamine-induced force of contraction (by about 25% and 50%, respectively). The *C. citratus* extract decreased the acetylcholine-induced force of contraction by 20% to 60% at 0.1 to 10 mg/ml and that induced by histamine by 60% to 90% at 0.01 to 10 mg/ml. On the other hand, the *G. barbadense* extract potentiated rather than reduced forces of contraction due to both acetylcholine and histamine (1.2- to 2-fold at 0.01 to 10 mg/ml). The *T. erecta* extract had such an effect only on the acetylcholine-induced force of contraction (about 2-fold at 10 mg/ml). The use of the former but not the latter sample alone led to an increase in smooth muscle tone that was not reversed by atropine or chlorpheniramine. The *C. alata* and *P. amarus* extracts did not significantly modify forces of contraction due to either acetylcholine or histamine. Our results suggest that preparations from *K. pinnata*, *C. citratus*, *C. pulcherrima*, and *B. orellana*, but not from *G. barbadense*, *T. erecta*, *C. alata*, and *P. amarus*, may be useful against smooth muscle spasm. These actions were probably mediated by distinct mechanisms.

Keywords: Antagonism, guinea pig ileum, H_1 histaminergic receptor, muscarinic receptor, plant-derived spasmolytics, Surinamese folk medicine.

Introduction

Smooth muscle spasm plays an important role in the pathophysiology of diseases affecting hollow organs such as airways, intestines, urinary bladder, ureters, and uterus (Paton & Webster, 1985; Poon, 1991; Attili et al., 1995;

Accepted: August 5, 2004

Address correspondence to: Dennis R.A. Mans, Ph.D., Department of Pharmacology, Faculty of Medical Sciences, Anton de Kom University, Kernkampweg 5-7, Paramaribo, Suriname. E-mail: dennisra@sr.net

DOI: 10.1080/13880200490886012

Bonamico et al., 1995; Lemanske & Busse, 2003). Examples of such conditions are asthma, diarrhea, irritable bowel syndrome, kidney stones, and dysmenorrhea (Paton & Webster, 1985; Poon, 1991; Attili et al., 1995; Bonamico et al., 1995; Lemanske & Busse, 2003). Although rather common, these diseases often cause considerable discomfort and disability that can last from a few hours to several days and may even lead to death of the patient. Asthma, for instance, has not only a relatively high incidence throughout the world but also carries a significant risk of respiratory failure (Lemanske & Busse, 2003).

Treatment of these conditions usually involves relaxation of the smooth muscles in the walls of the affected organs. Physiologically, smooth muscle contraction can occur upon stimulation of plasma membrane–associated muscarinic or H_1 histaminergic receptors or through depolarization of the plasma membrane (Paton & Webster, 1985; Caulfield, 1993). Pharmacological interference with either of these processes will prevent the intracellular cascade of biochemical reactions that initiate smooth muscle contraction (Berridge, 1993; Wilson et al., 2002). The result is a decrease of muscle tone and relief of the disabling condition.

The group of drugs used to accomplish these antagonistic processes – spasmolytic or antispasmodic agents – includes, among others, butylscopolamine, papaverine, chlorpheniramine, and ipratropium bromide (Maitai et al., 1985; Milovanovic et al., 1997; Thirstrup, 2000; Giamberardino et al., 2001). Given the discomfort and disability resulting from colics, cramps, and airway constriction, it is not surprising that these drugs are in high demand. Butylscopolamine and ipratropium bromide, for instance, were in recent years among the 10 bestselling drugs, together accounting for worldwide annual sales of several hundred millions Euros (Krebs, 2002).

Today, many developing countries still rely almost entirely on plant products for their primary health care (De Smet, 1997). Because smooth muscle spasm is, as mentioned above, relatively common and often associated with considerable pain, plant-derived ethnopharmacological substances with spasmolytic properties, not surprisingly, also compose in these societies an important part of the medicinal armamentarium (De Smet, 1997; Hoareau & DaSilva, 1999). Unfortunately, there is in many cases no hard scientific evidence to support the therapeutic usefulness of such traditional preparations.

For these reasons, we decided to assess a series of plant-derived preparations that are popularly used as spasmolytics in various parts of the world (Titjari, 1985; Heyde, 1992; Sedoc, 1992; Raghoenandan, 1994) for such an activity. Thus, aqueous extracts were prepared from (parts of) these plant species and evaluated for their capacity to reduce forces of smooth muscle contraction of an isolated guinea pig ileum induced by acetylcholine or histamine. The results obtained are discussed against the background of available pharmacological, biochemical, and clinical information about the plants studied.

Materials and Methods

Plant-derived materials

The plant species *Kalanchoë pinnata* (Lam.) Pers. (Crassulaceae), *Cymbopogon citratus* Stapf. (Gramineae), *Gossypium barbadense* L. (Malvaceae), *Caesalpinia pulcherrima* (L.) Schwartz (Caesalpiniaceae), *Tagetes erecta* L. (Compositae), *Bixa orellana* L. (Bixaceae), *Cassia alata* L. (Caesalpiniaceae), and *Phyllanthus amarus* Schum. & Thonn (Euphorbiaceae) were collected in rural areas outside Paramaribo, the capital of the Republic of Suriname. The collection areas had been free of herbicides or pesticides for at least 6 months prior to collection. The plant species have been authenticated by Ms. S. Bipat from the Department of Agricultural Sciences of the Anton de Kom University, Paramaribo, Suriname. Amounts of 500 g of either whole plants or certain plant parts were collected, extracted with distilled water, evaporated to dryness under reduced pressure, and stored at –20 °C until testing. Information about the plant species collected for this study, the plant parts used, the extraction temperature applied, the periods of time of extraction, and their folk medicinal uses are given in Table 1.

Drugs and chemicals

The muscarinic receptor agonist acetylcholine, the specific muscarinic receptor antagonist atropine, the H_1 receptor agonist histamine, and the specific H_1 receptor antagonist chlorpheniramine were from Sigma Chemical Co (St. Louis, MO, USA). Shortly before experiments, the drugs were dissolved in and diluted with Tyrode buffer to the desired concentrations. All other chemicals used were from our laboratory stock and were of the highest grade available.

The isolated guinea pig ileum

Adult guinea pigs were obtained from the Animal Facility of our university. The animals were housed under standard conditions, food and water being available *ad libitum*. For experiments, they were anesthetized with chloroform, and the ileum was removed, cleaned, and left to stabilize overnight in Tyrode buffer at a temperature of 4 °C. The next day, a piece of ileum of about 2-cm length was placed in a glass tube containing 40 ml of Tyrode buffer that was kept at a temperature of 37 °C and mixed with 5% CO_2 in air.

Incubations

The ileum was exposed to acetylcholine, histamine, atropine, chlorpheniramine, or one of the extracts, either alone or at certain combinations. The resulting forces of smooth muscle contraction were detected by a FT-100 sensor (CB-Sciences, Dover, NH, USA) connected to the ileum, processed by an ETH-260 Bio Amplifier (CB-Sciences) and a Powerlab 400 E series digital/analog converter (ADInstruments, Castle

Table 1. Relevant information about the plant species used in the present study.

Scientific name (Popular name)	Family	Plant part(s) (extraction period and temperature)	Folk medicinal uses (literature references)
Kalanchoë pinnata (Lam.) Pers. (Mother of thousands)	Crassulaceae	Leaves (60 min, 45 °C)	Bronchospams (Titjari, 1985; Heyde, 1992; Sedoc, 1992; Raghoenandan, 1994); uterus cramps (Raghoenandan, 1994)
Cymbopogon citratus Stapf. (Lemon grass)	Gramineae	Leaves (30 min, 100 °C)	Bronchospams (Titjari, 1985; Heyde, 1992; Sedoc, 1992; Raghoenandan, 1994); uterus cramps (Titjari, 1985); urinary bladder spasms (Heyde, 1992); kidney colics (Heyde, 1992)
Gossypium barbadense L. (Sea island cotton)	Malvaceae	Leaves (30 min, 100 °C)	Uterus cramps (Heyde, 1992; Sedoc, 1992; Raghoenandan, 1994); intestinal cramps (Titjari, 1985; Heyde, 1992); urinary bladder spasms (Titjari, 1985); kidney colics (Sedoc, 1992)
Caesalpinia pulcherrima (L.) Schwartz (Bird of paradise, peacock flower)	Caesalpiniaceae	Leaves (30 min, 100 °C)	Intestinal cramps (Heyde, 1992); kidney colics (Heyde, 1992; Sedoc, 1992; Raghoenandan, 1994); kidney stones (Heyde, 1992; Sedoc, 1992; Raghoenandan, 1994)
Tagetes erecta L. (African marigold)	Asteraceae	Leaves (60 min, 45 °C)	Kidney stones (Raghoenandan, 1994)
Phyllanthus amarus Schun. et Thom (Black catnip)	Euphorbiaceae	Whole plant (60 min, 45 °C)	Uterus cramps (Heyde, 1992; Sedoc, 1992; Raghoenandan, 1994); intestinal cramps (Titjari, 1985; Heyde, 1992; Sedoc, 1992); kidney colics (Titjari, 1985; Heyde, 1992; Sedoc, 1992)
Cassia alata L. (Ringworm cassia)	Caesalpiniaceae	Roots (60 min, 100 °C)	Uterus cramps (Titjari, 1985; Heyde, 1992)
Bixa orellana L. (Annato)	Bixaceae	Leaves (30 min, 100 °C)	Uterus cramps (Titjari, 1985; Heyde, 1992)

Hill, Australia) to yield values in milligrams and were monitored for 30 s using the *Chart 4.2 for Windows* software (ADInstruments). Prior to each determination, the ileum was allowed to recover for 60 s in fresh, prewarmed Tyrode buffer alone.

Assessment of responses

Potential spasmolytic effects of the extracts were determined from their capacity to reduce forces of smooth muscle contraction of the ileum induced by acetylcholine and/or histamine at EC_{50} concentrations (effective drug concentrations causing 50% of the maximum achievable force of smooth muscle contraction). The extracts were used at the concentrations of 0.01, 0.1, 1, and 10 mg/ml. The EC_{50} values of acetylcholine and histamine were derived from dose-response curves, which were constructed by plotting forces of smooth muscle contraction against eight corresponding serial dilutions of the drugs between 2 and 500 µg/l. The antagonizing effects of atropine 1 µg/l and chlorpheniramine 0.4 µg/l (Paton & Webster, 1985; Caulfield, 1993) were taken to validate the usefulness of the ileum preparation to carry out the above-mentioned evaluations. In all cases, forces of smooth muscle contraction were corrected for basal tone (readings in the presence of Tyrode buffer alone).

Statistical analysis

Data presented are mean ± SD of at least three independent experiments performed in duplicate or triplicate, and p values < 0.05 were taken to indicate statistically significant differences according to analysis of variance (ANOVA).

Results

Responses of the guinea pig ileum to agonists and antagonists of smooth muscle contraction

Eight aqueous extracts from plant species that are popularly used as spasmolytics have been evaluated for such an activity using the isolated guinea pig ileum. To this end, the extracts were assessed for their ability to reduce forces of smooth muscle contraction of the ileum induced by EC_{50} acetylcholine or EC_{50} histamine.

Figures 1a and 1b show the plots of forces of smooth muscle contraction *versus* corresponding concentrations of acetylcholine or histamine. The EC_{50} values derived from these curves were 27 ± 5 µg/l for acetylcholine and 102 ± 13 µg/l for histamine (Table 2). At these concentrations, forces of smooth muscle contraction were 1735 ± 220 and 1181 ± 229 mg, respectively. The addition of either atropine

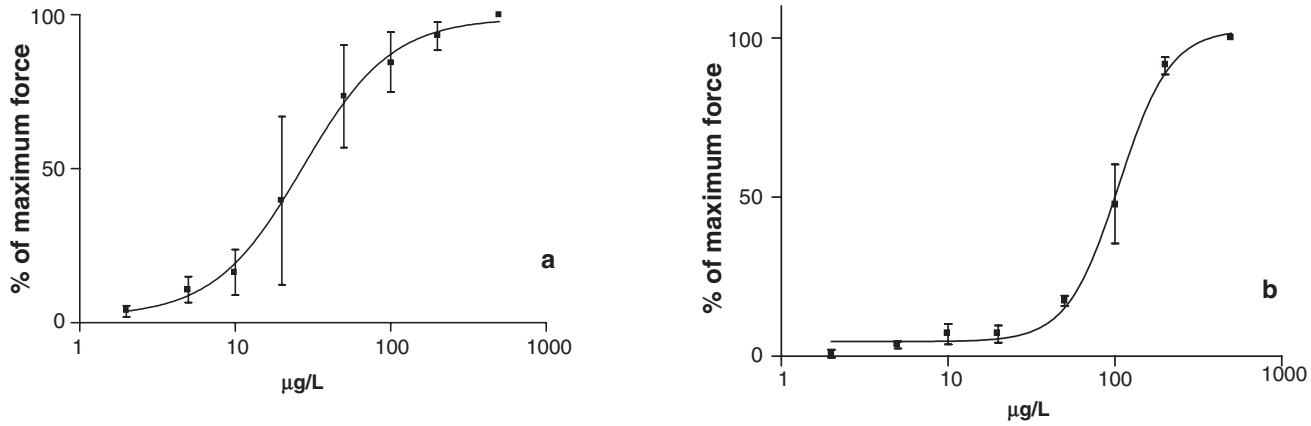

Figure 1. Effects of (a) acetylcholine and (b) histamine at concentrations of 2 to 500 µg/l on forces of smooth muscle contraction of the isolated guinea pig ileum. Results are means (data points) ± SDs (vertical bars) of at least three independent experiments performed in triplicate or duplicate.

Table 2. Forces of smooth muscle contraction (mg) of an isolated guinea pig ileum induced by EC_{50} acetylcholine (27 ± 5 µg/l) or EC_{50} histamine (102 ± 13 µg/l) in the absence or presence of atropine (1 µg/l) or chlorpheniramine (0.4 µg/l), respectively. Data are means ± SDs of at least three independent determinations performed in duplicate or triplicate.

Drug(s)	Forces of smooth muscle contraction (mg)
Acetylcholine alone	1735 ± 220
Acetylcholine + atropine	689 ± 379
Histamine alone	1181 ± 229
Histamine + chlorpheniramine	454 ± 158

1 µg/l or chlorpheniramine 0.4 µg/l reduced these values by approximately 60% (Table 2). Of note, at these concentrations, neither atropine nor chlorpheniramine elicited significant effects on smooth muscle tone. These observations are in agreement with expectations (Paton & Webster, 1985; Berridge, 1993; Caulfield, 1993; Wilson et al., 2002) and validate the suitability of the model to assess the plant extracts for their potential spasmolytic activity.

Effects of plant extracts on the force of smooth muscle contraction induced by acetylcholine

Subsequently, forces of smooth muscle contraction induced by EC_{50} acetylcholine in the presence of the extracts were determined and compared to those caused by EC_{50} acetylcholine alone. Data have been expressed relative to those found for the latter compound and are summarized in Table 3.

The *K. pinnata* extract did not significantly alter the acetylcholine-induced force of muscle contraction at 0.01 to 1 mg/ml but eradicated this effect almost completely at 10 mg/ml. Forces of muscle contraction found with the simultaneous use of EC_{50} acetylcholine and the *C. citratus* extract at 0.01 mg/l also did not differ significantly from that recorded for the former alone. However, the use of this extract at 0.1, 1, and 10 mg/ml led to a reduction in forces of muscle contraction to approximately 80%, 75%, and 40%, respectively, of that noted for EC_{50} acetylcholine alone. These findings indicated that the *K. pinnata* and *C. citratus* preparations can diminish contractions generated by stimulation of the muscarinic receptor.

The addition of the *G. barbadense* or *T. erecta* extracts to EC_{50} acetylcholine led to an increase (of about 2-fold) rather than a decrease in the force of muscle contraction caused by EC_{50} acetylcholine. The potentiating effect of the *G. barbadense* sample was already noticeable at 0.01 mg/ml and quite evident at higher concentrations. Furthermore, the use of this sample alone led to an appreciable increase in smooth muscle tone that did not respond to atropine (see footnote to Table 3). Thus, this sample seemed to act spasmogenic rather than spasmolytic through a mechanism that did not likely involve direct stimulation of the muscarinic receptor. The potentiating effect of the *T. erecta* extract was observed at 10 mg/ml, but this sample did not affect smooth muscle tone by itself (see footnote to Table 3), indicating that it lacked significant intrinsic spasmogenic properties.

Forces of smooth muscle contraction caused by EC_{50} acetylcholine simultaneously with the *C. pulcherrima*, *P. amarus*, *C. alata*, or *B. orellana* extracts were not significantly different from that due to EC_{50} acetylcholine alone.

Effects of plant extracts on smooth muscle contraction induced by histamine

Next, the samples were evaluated for their effects on the force of smooth muscle contractions induced by EC_{50} histamine. The results from these experiments have been expressed with respect to those found for EC_{50} histamine alone and are given in Table 4.

Table 3. Forces of smooth muscle contraction of the isolated guinea pig ileum induced by EC_{50} acetylcholine (27 ± 5 µg/l) in the presence of plant extracts, relative to that found for EC_{50} acetylcholine alone. The latter value was set at 100%. Data are expressed in %, and are means ± SDs of at least three independent experiments performed in duplicate or triplicate.

	Relative forces of smooth muscle contraction at extract concentrations of:			
	0.01 mg/ml	0.1 mg/ml	1 mg/l	10 mg/ml
K. pinnata	101 ± 4	108 ± 8	113 ± 7	4 ± 1[1]
C. citratus	84 ± 23	83 ± 13	75 ± 1[1]	41 ± 24[1]
G. barbadense[2]	125 ± 4[1]	162 ± 30[1]	183 ± 33[1]	190 ± 47[1]
C. pulcherrima	122 ± 25	118 ± 19	107 ± 5	114 ± 9
T. erecta[3]	117 ± 28	132 ± 34	134 ± 35	183 ± 47[1]
P. amarus	101 ± 9	113 ± 7	109 ± 22	100 ± 17
C. alata	117 ± 13	120 ± 12	127 ± 10	155 ± 64
B. orellana	107 ± 12	111 ± 3	106 ± 7	103 ± 8

[1] Significantly different from the force of smooth muscle contraction due to EC_{50} acetylcholine alone ($P < 0.05$, ANOVA).
[2] The force of smooth muscle contraction caused by the *G. barbadense* extract itself at 10 mg/ml was 2976 ± 943 mg. In the presence of atropine 1 µg/l, this value was 2754 ± 858 mg.
[3] The force of smooth muscle contraction recorded for the *T. erecta* extract itself at 10 mg/ml was 40 ± 5 mg.

Table 4. Forces of smooth muscle contraction of the isolated guinea pig ileum induced by EC_{50} histamine (102 ± 13 µg/l) in the presence of plant extracts, relative to that found for EC_{50} histamine alone. The latter value was set at 100%. Data are expressed in %, and are means ± SDs of at least three independent experiments performed in duplicate or triplicate.

	Forces of smooth muscle contraction at extract concentrations of:			
	0.01 mg/l	0.1 mg/l	1.0 mg/l	10.0 mg/l
K. pinnata	59 ± 13[1]	19 ± 0[1]	6 ± 3[1]	3 ± 1[1]
C. citratus	64 ± 19[1]	63 ± 19[1]	60 ± 4[1]	13 ± 1[1]
G. barbadense[2]	157 ± 55[1]	200 ± 29[1]	206 ± 31[1]	216 ± 38[1]
C. pulcherrima	102 ± 4	108 ± 6	111 ± 12	73 ± 18[1]
T. erecta	102 ± 10	104 ± 18	102 ± 17	102 ± 5
P. amarus	86 ± 19	115 ± 6	112 ± 14	91 ± 41
C. alata	97 ± 23	102 ± 30	93 ± 34	94 ± 32
B. orellana	99 ± 9	110 ± 9	102 ± 6	46 ± 27[1]

[1] Significantly different from the force of smooth muscle contraction due to EC_{50} histamine alone ($P < 0.05$, ANOVA).
[2] The force of smooth muscle contraction caused by the *G. barbadense* extract itself at 10 mg/ml was 3066 ± 1200 mg. In the presence of chlorpheniramine 0.4 µg/l, this value was 3052 ± 1251 mg.

The combination of EC_{50} histamine with the *K. pinnata* extract led to a progressive decrease in forces of smooth muscle contraction (from 59 ± 13% to 3 ± 1% of that induced by EC_{50} histamine) with extract concentrations increasing from 0.01 to 10 mg/ml. As this extract opposed the acetylcholine-mediated contractions only at 10 mg/ml (Table 3), its apparent antispasmodic activity might mainly involve antagonism of the H_1 receptor.

Forces of smooth muscle contraction produced by the simultaneous use of EC_{50} histamine and the *C. citratus* extract at 0.01–1 mg/ml on the one hand, and at 10 mg/ml on the other hand, were approximately 60% and 10%, respectively, of that determined for EC_{50} histamine alone. Because the use of this sample also led to a significant decrease in the force of muscle contraction caused by EC_{50} acetylcholine (Table 3), it may have the potential to counteract spasms

induced by stimulation of either the muscarinic or the H_1 receptor.

The use of the *C. pulcherrima* and *B. orellana* extracts at 10 mg/ml also led to reductions (of approximately 25% and 50%, respectively) in the histamine-induced force of muscle contraction. Because these samples did not affect the acetylcholine-induced force of muscle contraction (Table 3), their relaxant effects might thus be attributed to H_1 receptor antagonism.

On the other hand, the addition of the *G. barbadense* extract to EC_{50} histamine led, as found for its combination with EC_{50} acetylcholine (Table 3), to approximately a 2-fold increase in the force of smooth muscle contraction when compared to that induced by EC_{50} histamine alone. Furthermore, the increase in smooth muscle tone caused by this extract itself was not opposed by chlorpheniramine 0.1 µg/l (see footnote to Table 4). Because this phenomenon also did not respond to atropine (see footnote to Table 3), the spasmogenic action of this extract did not seem related to a direct interaction with either the muscarinic or the H_1 receptor.

The concomitant use of EC_{50} histamine and the *T. erecta*, *P. amarus*, or *C. alata* extracts did not yield forces of smooth muscle contraction that differed significantly from that induced by EC_{50} histamine alone (see footnote to Table 4). Thus, while the *T. erecta* sample intensified acetylcholine-generated smooth muscle contractions (Table 3), it did not affect those triggered by histamine. The *P. amarus* and *C. alata* extracts, on the other hand, did not exert detectable effects on smooth muscle contractions induced by either acetylcholine or histamine.

Discussion

To date, a significant number of plant species are popularly used for medicinal purposes throughout the world (De Smet, 1997; Hoareau & DaSilva, 1999), often without sufficient scientific proof of therapeutic efficacy. In this study, the aqueous extracts from (parts of) eight such species (*K. pinnata*, *C. citratus*, *C. pulcherrima*, *B. orellana*, *G. barbadense*, *T. erecta*, *C. alata*, and *P. amarus*) have been evaluated for their presumed antispasmodic activity. Using the isolated guinea pig ileum, our results support such an activity for the former four plant species.

The apparent spasmolytic activity of the *K. pinnata* extract was in accordance with previous data reporting that the leaf juice from this plant species had smooth muscle–relaxing activity in the isolated guinea pig ileum (Nassis et al., 1992). Furthermore, this sample appeared to protect laboratory animals from vascular permeability responses to and asphyxia by histamine (Nassis et al., 1992). The activity had been localized to a flavonoid-containing fraction (Nassis et al., 1992), which was consistent with the identification of flavonoid glycosides in water extracts from *K. pinnata* leaves (Gaind & Gupta, 1971). However, whereas the spasmolytic activity had solely been ascribed to competitive interaction with the H_1 receptor (Nassis et al., 1992), the current results suggest that antagonism of the muscarinic receptor may also play a role in this phenomenon. The latter effect was observed at the relatively high concentration of 10 mg/ml extract (Table 3). This may explain why it might have been overlooked in the earlier study with the leaf juice (Nassis et al., 1992), which likely contained lower amounts of the active ingredient(s) than the concentrated leaf extract used in the current study.

That the *C. citratus* leaf extract also may have useful antispasmodic activity was immediately clear from its counteracting effects on smooth muscle contractions caused by both acetylcholine and histamine. Such an activity is in line with the opposing effect of a *C. citratus* extract on castor oil–induced diarrhea in rats (Sadraei et al., 2003); its decreasing effect on the intestinal transit of a charcoal meal in mice and defecation scores of rats (Carlini et al., 1986); as well as the identification in the extract of flavonoids and terpenoids with suggested antispasmodic activity (Carlini et al., 1986). The partial reversal of the acetylcholine- as well as the histamine-induced contractions by the *C. citratus* extract may be attributed to interference with the muscarinic as well the H_1 receptor, as established for various first-generation antihistamines (Simons & Simons, 1994). This dual effect may be also explained by perturbation of biochemical processes common to both receptor types, for instance, transmembrane ion fluxes involved in plasma membrane depolarization (Paton & Webster, 1985; Caulfield, 1993). In this respect, a water extract from *C. citratus* leaves reportedly reversed the contractions of an isolated rat ileum induced by KCl (Sadraei et al., 2003). A third tentative explanation for our observations might be provided by the presence in *C. citratus* leaves of essential oils with potent analgesic properties such as citral, β-myrcene, and geraniol (Seth et al., 1976). The appreciable smooth muscle relaxant activity of these substances has been suggested to be mediated by a mechanism comparable to that established for certain opioids (Milovanovic et al., 1997). Whether, and which of these hypotheses holds true remains to be established.

The *C. pulcherrima* and *B. orellana* extracts had relatively modest, but significant, reducing effects on smooth muscle contractions caused by histamine, but not on those induced by acetylcholine. This suggests that the actions of these samples might be attributed to H_1 receptor antagonism, but this assumption needs to be confirmed in additional studies. Nevertheless, their apparent antispasmodic activities are in line with the presence in *C. pulcherrima* leaves of flavonoids that might be efficacious against gastrointestinal and genitourinary disorders (Haslam, 1996), as well as with the smooth muscle–relaxing activity of aqueous extracts from *B. orellana* roots and leaves observed in laboratory animals (Evans, 2000).

Unlike the above-mentioned extracts, that from *G. barbadense* leaves potentiated rather than counteracted smooth muscle contractions induced by both acetylcholine and histamine. Furthermore, this sample itself exhibited potent

spasmogenic activity that was not affected by atropine or chlorpheniramine, signifying that its apparent spasmogenic activity was not directly mediated by either the muscarinic or the H_1 receptor. Of note, in pilot experiments (data not shown), the use of papaverine 10 mg/ml (Maitai et al., 1985; Milovanovic et al., 1997; Thirstrup, 2000; Giamberardino et al., 2001) was also without a significant effect on the extract-induced smooth muscle contractions, which suggests that it also did not interfere with biochemical processes occurring immediately downstream from the receptors (Berridge, 1993; Wilson et al., 2002). At present, our observations cannot be satisfactorily explained. However, they are more consistent with the popular use of *G. barbadense* preparations to increase vaginal muscle tone postpartum or as an abortifacient (Heyde, 1992; Raghoenandan, 1994) than as an antispasmodic.

The extract from *T. erecta* leaves also augmented the force of smooth muscle contraction of the ileum induced by acetylcholine but not that caused by histamine. However, the fact that this extract failed to induce smooth muscle contractions on its own suggested that it did not have meaningful spasmogenic activity as found for the *G. barbadense* extract. These observations may tentatively be explained by those from a previous study (Bose et al., 1979), in which the contractions of an isolated guinea pig trachea induced by a *T. erecta* extract were not reversed by conventional blocking agents but by adrenaline (Bose et al., 1979). Thus, the extract might possess sympathicolytic rather than parasympathicomimetic properties, implying that its use may decrease sympathetic tone and intensify acetylcholine-induced smooth muscle contractions, as observed in the current study (Table 3). Obviously, this supposition must be verified in future studies.

Several studies support the ethnopharmacological uses of preparations from parts of *C. alata* and *P. amarus* against smooth muscle spasms. For instance, the results from a clinical study suggested that a water extract from *C. alata* leaves could effectively relieve constipation (Elujoba et al., 1989), presumably due to several anthraquinones with potent laxative properties (Thamlikitkul et al., 1990). Furthermore, water extracts from the leaves of *P. amarus* were observed to control frequent menstruation as well as kidney and urinary bladder disturbances (Calixto et al., 1999), which might be attributed to the interference of certain bioactive substances with key enzymes involved in the pathophysiology of these disorders (Calixto et al., 1999; Campos & Schor, 1998). Notwithstanding, the *C. alata* and *P. amarus* extracts did not elicit significant smooth muscle relaxant activity in the current study. These discrepancies cannot readily be explained but may be ascribed to differences in time of harvest, genotype, chemotype, geographical origin, and/or environmental and agronomic conditions (Svoboda et al., 1995).

Taken together, the results from this study combined with those from others (Carlini et al., 1986; Nassis et al., 1992; Haslam, 1996; Evans, 2000; Sadraei et al., 2003) speak in favor of the traditional uses of *K. pinnata*, *C. citratus*, *C. pulcherrima*, and *B. orellana* preparations as antispasmodics. Thus, these natural products may represent attractive alternatives for the treatment of bronchospams, uterus cramps, urinary bladder spasms, kidney colics, and intestinal cramps. Our data suggest further that these samples elicit their antispasmodic actions through distinct mechanisms. The challenge now is to establish definitely their roles in the treatment of such disorders through carefully designed animal toxicology and pharmacological studies, as well as well-controlled, double-blind clinical trials.

References

Attili AF, De Santis A, Capri R, Repice AM, Maselli S (1995): The natural history of gallstones: The GREPCO experience. The GREPCO Group. *Hepatology 21*: 655–660.

Berridge M (1993): Inositol trisphosphate and calcium signaling. *Nature 361*: 315–325.

Bonamico M, Culasso F, Colombo C, Giunta AM (1995): Irritable bowel syndrome in children: An Italian multicentre study. Collaborating Centres. *Ital J Gastroenterol 27*: 13–20.

Bose NB, Ahmed Q, Nandy M, Mohanty J, Sahoo G, Kar PK (1979): *Tagetes erecta*, its effect on smooth muscles (Abstract 99). In: *Proceedings of the Indian Pharmacological Society XII Annual Conference*, Raveendran R, Ed. Indian Pharmaceutical Society, Pondicherry, India.

Calixto JB, Santos AR, Cechinel Filho V, Yunes RA (1999): A review of the plants of the genus *Phyllanthus*: Their chemistry, pharmacology, and therapeutic potential. *Nephron 81*: 393–397.

Campos AH, Schor N (1998): *Phyllanthus niruri* inhibits calcium oxalate endocytosis by renal tubular cells: Its role in urolithiasis. *Med Res Rev 18*: 225–258.

Carlini EA, Contar J de DP, Silva-Filho AR, da Silveira-Filho NG, Frochtengarten ML, Bueno OF (1986): Pharmacology of lemongrass (*Cymbopogon citratus* Stapf). I. Effects of teas prepared from the leaves on laboratory animals. *J Ethnopharmacol 17*: 37–64.

Caulfield MP (1993): Muscarinic receptors – characterization, coupling and function. *Pharmacol Ther 58*: 310–379.

De Smet PAGM (1997): The role of plant-derived drugs and herbal medicines in health care. *Drugs 54*: 801–840.

Elujoba AA, Ajulo OO, Iweibo GO (1989): Chemical and biological analyses of Nigerian *Cassia* species for laxative activity. *J Pharmacol Biomed Anal 7*: 1453–1457.

Evans WC (2000): Annato: A natural choice. *Biologist* (London) *47*: 181–184.

Gaind KN, Gupta RL (1971): Flavonoid glycosides from *Kalanchoe pinnata*. *Planta Med 20*: 368–373.

Giamberardino MA, De Laurentis S, Affaitati G, Lerza R, Lapenna D, Vecchiet L (2001): Modulation of pain and hyperalgesia from the urinary tract by algogenic conditions of the reproductive organs in women. *Neurosc Lett 304*: 61–64.

Haslam E (1996): Natural polyphenols (vegetable tannins) as drugs: Possible modes of action. *J Nat Prod 59*: 205–215.

Heyde H (1992): *Geneesplanten van Suriname*. Paramaribo, Suriname, Offsetdrukkerij Westfort NV.

Hoareau L, DaSilva EJ (1999): Medicinal plants: A re-emerging health aid. *Electr J Biotechnol 2*: 56–70.

Krebs R (2002): Rolf Krebs discusses the pharma industry past, present and future. Interview by Joanne Clough. *Drug Discovery Today 7*: 990–991.

Lemanske RF Jr, Busse WW (2003): Asthma. *J Allergy Clin Immunol 111(Suppl 2)*: 502–519.

Maitai CK, Njoroge DK, Munenge RW (1985): A comparative study of two antispasmodic products: Buscopan and NO-Spa. *East African Med J 62*: 480–485.

Milovanovic DR, Dordevic S, Jankovic SM (1997): Treatment of spasms of visceral smooth muscles. *Med Pregl 50*: 194–200.

Nassis CZ, Haebisch EM, Giesbrecht AM (1992): Antihistamine activity of *Bryophyllum calycinum*. *Braz J Med Biol Res 25*: 929–936.

Paton DM, Webster DR (1985): Clinical pharmacokinetics of H_1-receptor antagonists (the antihistamines). *Clin Pharmacokin 10*: 477–497.

Poon FW (1991): Persistent colonic spasm. *Clin Radiol 44*: 363.

Raghoenandan UPD (1994): *Etnobotanisch onderzoek bij de Hindustaanse bevolkingsgroep in Suriname*. Paramaribo, Suriname, National Herbarium of Suriname.

Sadraei H, Ghannadi A, Malekshahi K (2003): Relaxant effect of essential oil of *Melissa officinalis* and citral on rat ileum contractions. *Fitoterapia 74*: 445–452.

Sedoc N (1992): *Afro-surinaamse Natuurgeneeswijzen*. Paramaribo, Suriname, NO Sedoc.

Seth G, Kokate CK, Varma KC (1976): Effect of essential oil of *Cymbopogon citratus* Stapf. on central nervous system. *Indian J Exp Biol 14*: 370–371.

Simons FER, Simons KJ (1994): The pharmacology and use of H_1-receptor-antagonist drugs. *New Engl J Med 330*: 1663–1670.

Svoboda KP, Gough J, Hampson J, Galambosi B (1995): Analysis of the essential oils of some *Agastache* species grown in Scotland from various seed sources. *Flav Fragr J 10*: 139–145.

Thamlikitkul V, Bunyapraphatsara N, Dechatiwongse T, Theerapong S, Chantrakul C, Thanaveerasuwan T, Nimitnon S, Boonroj P, Punkrut W, Gingsungneon V. (1990): Randomized controlled trial of *Cassia alata* Linn. for constipation. *J Med Assoc Thai 73*: 217–222.

Thirstrup S (2000): Control of airway smooth muscle tone: II–Pharmacology of relaxation. *Resp Med 94*: 519–528.

Titjari (1985): *Famiri Encyclopedia foe da Natoera Dresi Fasi. Gezinskruidenboek van de Natuurgeneeswijzen. Natuurgeneeswijzen uit het Zonnige Suriname.* Amsterdam, Sangrafoe.

Wilson DP, Sutherland C, Walsh MP (2002): Ca^{2+} activation of smooth muscle contraction. Evidence for the involvement of calmodulin that is bound to the triton-insoluble fraction even in the absence of Ca^{2+}. *J Biol Chem 277*: 2186–2192.