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European Journal of Pharmacology 522 (2005) 108–115
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Isoquercitrin from *Argemone platyceras* inhibits carbachol and leukotriene D_4-induced contraction in guinea-pig airways

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Received 21 July 2005; received in revised form 16 August 2005; accepted 29 August 2005
Available online 3 October 2005

Abstract

Argemone platyceras is used in Mexico as a remedy for cough, bronchitis and pneumonia. The present study was performed to investigate the pharmacological anti-asthmatic properties of *Argemone platyceras* on airways and to identify its active principles. Methanol extracts of leaves and flowers, subsequent organic and aqueous extraction phases, and silica gel chromatography fractions were assayed on the carbachol-induced response, and/or on ovalbumin antigenic challenge, and on leukotriene D_4-induced response of tracheae from sensitized and non-sensitized guinea-pigs. Methanol extracts, ethyl-acetate phase, and its fractions 6 and 7 inhibited the carbachol-induced contractile response. Isoquercitrin and rutin were the main compounds found in fractions 6 and 7 respectively. Isoquercitrin (fraction 6) abolished the response to ovalbumin, and decreased the contractile response to leukotriene D_4. Because of its effect on carbachol-induced contractile response, on the late-phase response to ovalbumin, and on leukotriene D_4-induced contractile response, isoquercitrin might be highly useful in treatment of asthma.

Keywords: Isoquercitrin; Rutin; Asthma; Carbachol; *Argemone platyceras*; Antigen challenge; Anti-asmathic properties; Anti-leukotriene action

1. Introduction

Asthma is a disease of the airways with an underlying inflammatory component. The prevalence and healthcare burden of asthma are rising and it is predicted that this rise will continue throughout the current century (Belvisi et al., 2004). Recent asthma research is focused on chronic inflammation and remodeling of the airways as manifested by airway thickening, airway smooth muscle hypertrophy, subepithelial fibrosis and matrix protein deposition, epithelial cell damage and hyperplasia of goblet cells, infiltration of eosinophils in epithelium and submucosa, and cytokine production.

Cysteinyl leukotrienes (C_4, D_4, and E_4) can be generated by eosinophils, mast cells and alveolar macrophages. The actions of cysteinyl leukotrienes are mainly due to interaction with the cysteinyl leukotriene-1 receptor, which can lead to airway smooth muscle contraction, leukocyte chemotaxis, and vascular permeability increase (Lemanske and Busse, 2003). Cysteinyl leukotrienes may take part in the asthmatic remodeling process by their direct effects on smooth muscle cells, eosinophils, epithelial cells, and likely other cell types (Hui and Funk, 2002).

Leukotriene production is increased in asthma, and blood eosinophils from asthmatic patients synthesize 5- to 10-fold greater amounts of leukotrienes than those from normal subjects. Studies in guinea pigs suggest that these mediators can potentiate the release of acetylcholine and tachykinins from vagal nerve endings and capsaicin-sensitive afferent C fibers, respectively (Salvi et al., 2001). Therefore it is expected that

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doi:10.1016/j.ejphar.2005.08.046

leukotriene synthesis inhibitors or cysteinyl leukotriene-1 receptor antagonists may have additional anti-inflammatory properties for asthma management. Currently, clinical studies have demonstrated patient improvement from leukotriene-modifying drugs in the management of all grades of asthma (Lin and Casale, 2002). Consequently, leukotriene antagonists and lipoxygenase inhibitors have provided an attractive option for drug development.

Despite the information available on several plants used in traditional medicine for treatment of asthma, natural sources have been neglected in the search for anti-asthmatic compounds (Dorsch and Wager, 1991). In Mexican traditional medicine it is a common practice to use herbal infusions or decoctions for treatment of respiratory ailments. One of the most commonly used herbs for this purpose pertains to the genus *Argemone*. Particularly, *A. platyceras* Link and Otto (Papaveraceae), which is a spiny annual herb widely distributed throughout Mexico, is commonly known as "chicalota" and used in the form of flower infusion by several Mexican ethnic groups as a remedy for cough, bronchitis and pneumonia (Emes et al., 1994). Previous chemical investigations have revealed the isoquinoline alkaloids contained in this plant (Atta-ur-Rahman, 1994). However, there are no systematic studies regarding *A. platyceras* pharmacological properties.

The aims of the present study are: 1) to investigate the pharmacological anti-asthmatic properties of *A. platyceras* in guinea-pig airways, and 2) to identify active principles through bioassay-guided fractionation. To achieve these aims, we investigated the in-vitro activity of methanol extracts (flowers and leaves), active phases, and active fractions from *A. platyceras* on contractile responses induced by agonists (carbachol, histamine and leukotriene D_4), and by antigenic challenge (ovalbumin) on tracheal smooth muscle of non-sensitized and sensitized guinea pigs.

2. Materials and methods

2.1. Plant material and extract preparation

During the summer of 2000, the fresh leaves and flowers of *Argemone platyceras* Link and Otto (Papaveraceae) were collected at Santa Maria Village on the Toluca-Cuernavaca Freeway near Mexico City, Mexico. The material was authenticated in the Herbarium of the Mexican Institute of Social Security in Mexico City, and a voucher was stored for reference under code number IMSSM-14496. Leaves and flowers were dried at room temperature during two weeks and precautions were taken to avoid exposure to direct sunlight. Afterward, plant material was milled to obtain 2–5 mm size particles that were extracted exhaustively at room temperature with methanol during 3 days. The methanol extract was then filtered through a Whattman #1 paper and evaporated to dryness in a rotary evaporator under reduced pressure. The weights of the crude dried extracts were 131.5 g (18% yield) and 88.4 g (14.7% yield) for flowers and leaves, respectively. Extracts were stored under refrigeration at −4 °C and protected from light until their utilization.

2.2. Fractionation and isolation

Part of the methanol crude extract of the *leaves* (32.4 g) was dissolved in methanol and partitioned with dichloromethane, ethyl acetate, and water. The organic phases were evaporated using a rotary evaporator under reduced pressure, and the aqueous phase was freeze-dried. Dried residues from the three phases were redissolved in a mixture of ethanol : dimethyl sulfoxide (5 : 2) and tested in tracheal-ring assay. Only ethyl-acetate phase showed activity and was subjected to subsequent fractionation.

The ethyl-acetate phase (2 g) was subjected to silica gel column (40 g) chromatography using a dichloromethane–methanol gradient. Seven fractions were collected, but only fractions 6 and 7 eluted with dichloromethane–methanol (8 : 2) showed activity in the biological assay. These fractions were kept at room temperature to allow evaporation of the solvent. Subsequently, they were macerated with ethyl acetate, obtaining an orange gum (78 mg) and a yellow powder (116 mg), respectively. These were analyzed by thin-layer chromatography using Silica Gel plates (Merck, 0.25 mm) developed with dichloromethane-methanol (3 : 1). Thin-layer chromatography plates were observed under ultraviolet light and revealed using two different spray reagents (ferric chloride (5%) in ethanol and Cerium IV dehydrate sulphate-Baker (2%) in 2 N sulfuric acid). Fractions 6 and 7 exhibited one main spot with Rf = 0.57, and 0.34, respectively, with both sprays, which were identified by spectroscopic methods ([^{1}H] and [^{13}C] nuclear magnetic resonance [NMR], infrared, ultraviolet) and mass spectroscopy as isoquercitrin and rutin, respectively. The electrospray ionization mass spectroscopy apparatus used for this identification was a Quattro II (Micromass, UK). Nuclear magnetic resonance spectra were recorded with a Varian spectrometer (Unity 300) and Chemagetics Infinity 300, both at 300 MHz, with 5-mm Nalorac probes; all spectra were recorded in methanol-d4. Detailed spectra are available on request from jankowc@umoncton.ca.

Extract from *flowers* (6.4 g) was partitioned with dichloromethane, ethyl acetate, and water. Only the ethyl-acetate phase inhibited contraction induced by carbachol in guinea-pig tracheae. Fractions were analyzed as previously described, rendering the same compounds obtained previously from the leaves.

2.3. Agonist experiments

Male guinea pigs (400–500 g) bred under conventional conditions were used. Animals were killed by an i.p. injection of 95 mg/kg sodium pentobarbital and exsanguinated. Animal experiments were performed following the recommendations of the Policies on Animal Experimentation of the Scientific Committee of the Instituto Mexicano del Seguro Social. Trachea was removed and connective tissue cleaned off. Four tracheal rings composed of four cartilages each were obtained from each guinea pig and suspended in a 5-ml organ bath containing Krebs bicarbonate solution of the following composition (mM): NaCl 120; KCl 4.7; KH_2PO_4 1.2; $MgSO_4$

Table 1
Carbachol- and histamine-induced response of isolated guinea-pig tracheae in the presence of methanol extracts of *A. platyceras*

Extract (μg/ml)	Carbachol		Histamine	
	pD_2	Maximal response (%)	pD_2	Maximal response (%)
Flowers				
Control	6.90±0.06	208±11	5.83±0.23	105±16
10	6.75±0.06	101±5	5.45±0.02	65±12
30	6.39±0.01[a]	143±11	5.08±0.28	42±8[a]
100	6.41±0.13[a]	131±9	4.61±0.47	28±8[a]
Leaves				
Control	7.13±0.06	150±9	4.81±0.43	112±80
10	6.82±0.02[a]	145±6	5.10±0.21	117±10
30	6.53±0.10[b]	141±3	4.56±0.28	100±20
100	6.23±0.03[b]	158±7	4.72±0.33	62±50

Data are mean±S.E.M. (n=4–5). [a] $P<0.05$, [b] $P<0.001$ as compared with control group.

1.2; $NaHCO_3$ 25; $CaCl_2$ 2.5, and glucose 11. This solution was maintained at 37 °C, pH 7.4 and bubbled with 5% CO_2–95% O_2. Isometric tension was recorded by means of a Grass polygraph model 7B via a Grass FT03C transducer. Tissues were placed under a resting tension of 1 g (maintained throughout the experiments) and allowed to equilibrate for 60 min; during this time, tissues were washed with fresh Krebs bicarbonate solution at 15-min intervals. To further standardize tissue responses, two consecutive electric stimuli (16 Hz, 100 mV, 10 ms for 10 s) were applied. At the end of the equilibration period, tissues were stimulated twice with 60 mM KCl. Only tissues with two similar consecutive responses to KCl were included in the study.

Cumulative concentration-response curves to the agonists carbachol (1×10^{-8}–1×10^{-6} M) and histamine (1×10^{-6}–1×10^{-4} M) were constructed (Van Rossum, 1963). Extracts, phases and fractions of leaves and flowers of *A. platyceras* (10, 30 and 100 μg/ml) or the vehicle were added 30 min prior to the first dose of agonist. Extracts, phases and fractions were dissolved in dimethyl sulfoxide : ethanol (5 : 2) in such a way that final vehicle concentration in the organ bath was ≤0.15%, which proved to be innocuous. Only one concentration-response curve was obtained from each tissue. At the end of experiments, a maximally effective concentration of KCl (60 mM) was added to each bath to assess tissue viability.

2.4. Antigen challenge experiments

One group of animals was sensitized at day 0 with 120 μg of ovalbumin mixed with 2 mg $Al(OH)_3$ dissolved in 2 ml of saline solution which were administered i.p. and s.c. (1 ml each way). On day 8, guinea pigs were exposed to an aerosol generated from a solution of 3 mg/ml ovalbumin in saline delivered by an ASPEN nebulizer (particle diameter 1–7 μm, flow 0.6 ml/min) for 7 min. On day 15, animals were exposed (aerosol) to 0.5 μg/ml ovalbumin in saline solution for 3 min. On day 25, animals were killed and tracheae were obtained and processed as described. Tracheae were pre-incubated with extracts, phases or fractions of *A. platyceras* flower (1, 3, and 10 μg/ml) or leaf (3, 10, and 30 μg/ml) or vehicle during 30 min; then, tissues were challenged with 3.74 μg/ml ovalbumin and responses recorded over 60 min. To distinguish the contribution of cyclooxygenase products to contractile response, a series of experiments was carried out in the absence or in the presence of 1×10^{-6} M indomethacin, previously added to the Krebs bicarbonate solution.

2.5. Anti-leukotriene effects

Because fraction 6 completely inhibited late response to ovalbumin, which is caused primarily by leukotrienes (Yamada et al., 1992), the likely antileukotriene effect of this fraction was investigated. For this, cumulative concentration-response curves for leukotriene D_4 (1×10^{-11} to 1×10^{-7} M) in guinea-pig tracheal rings were constructed in presence of indomethacin (1×10^{-6} M). To inhibit conversion of leukotriene D_4 into leukotriene E_4, tissues were incubated with 6 mM cysteine (inhibitor of amino peptidase); subsequently, fraction 6 was added 30 min before elaboration of the cumulative concentration-response curve to leukotriene D_4. A positive anti-

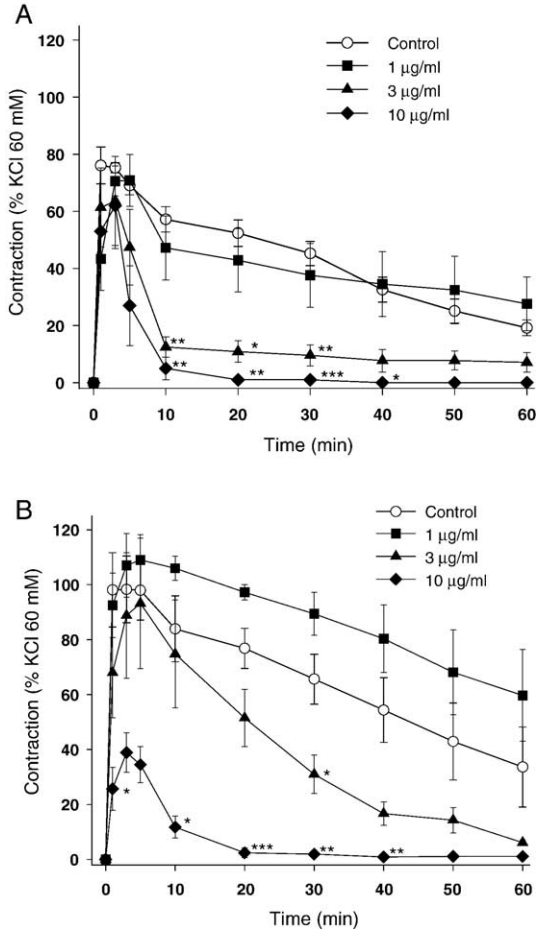

Fig. 1. Temporal course of the contractile response induced by antigen (ovalbumin) in tracheae from sensitized guinea pigs after 30-min incubation with methanol extract of the flower of *Argemone platyceras* in the absence (A) or presence (B) of indomethacin. Each point represents mean value±S.E.M. (vertical lines) of four experiments. *$P<0.05$, **$P<0.01$, ***$P<0.001$.

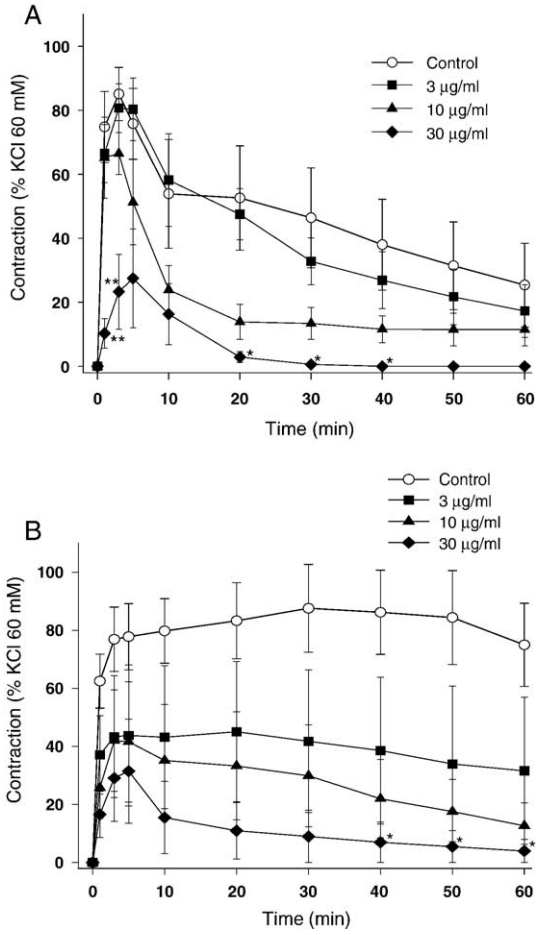

Fig. 2. Temporal course of the contractile response induced by antigen (ovalbumin) in tracheae from sensitized guinea pigs after 30-min incubation with methanol extract of the leaf of *Argemone platyceras* in the absence (A) or presence (B) of indomethacin. Each point represents mean value±S.E.M. (vertical lines) of four experiments. *$P<0.05$, **$P<0.01$.

leukotriene control group of tissues was incubated with zafirlukast (0.1 μg/ml).

2.6. Chemicals

Agonists and salts required to prepare Krebs-bicarbonate solutions were supplied by Sigma Chemical Co. (St. Louis, MO, USA).

2.7. Data analysis

Results are expressed as percentage of the contraction produced by 60 mM KCl and are mean±S.E.M. of at least four experiments. Responses to ovalbumin were calculated for nine time points over the 60-min period (at 1, 3, 5, 10, 20, 30, 40, 50, and 60 min). Responses to histamine and carbachol were assessed by maximal response and pD_2 (the negative logarithm of EC_{50}) (Tallarida et al., 1989). Values were calculated from straight-line regression analysis. These results were statistically analyzed by analysis of variance (ANOVA) and Bonferroni test for multiple comparisons, with a probability value of $P<0.05$ regarded as significant. Relative potency was determined from the horizontal distance d between the lines, as described by Tallarida and Murray (1981).

3. Results

3.1. Effects of extracts on agonist experiments

The flower methanol extract of the *A. platyceras* (30 and 100 μg/ml) produced a significant concentration-dependent rightward shift of the carbachol-induced response as demonstrated by pD_2 values ($P<0.05$) (Table 1). Maximal response values did not display differences. With respect to histamine-induced activity, maximal response was decreased by incubation with 30 and 100 μg/ml of the flower extract ($P<0.05$) (Table 1). The three concentrations assayed of the leaf methanol extract diminished the pD_2 values of carbachol-induced response (10 μg/ml, $P<0.05$, and 30 and 100 μg/ml, $P<0.001$) and did not modify the histamine-induced response (Table 1).

3.2. Effects of extracts con antigen challenge experiments

The flower methanol extract inhibited the contractile response to ovalbumin in tracheae from sensitized guinea pigs. In the absence of indomethacin, the flower extract (3 and 10 μg/ml) significantly inhibited late phase of the contractile response between 10 and 30 or 40 min, respectively (Fig. 1A). In the presence of indomethacin, the flower methanol extract (10 μg/

Table 2
Effect of flower and leaf ethyl-acetate phases, and fractions 6 and 7 of *A. platyceras* on carbachol-induced response of isolated guinea-pig tracheae

Treatment (μg/ml)	pD_2	Maximal response (%)
Ethyl acetate phase of flowers		
Control	7.29±0.08	110±6
10	7.07±0.02	100±1
30	6.77±0.09	105±4
100	6.59±0.06[b]	101±1
Ethyl acetate phase of leaves		
Control	7.09±0.09	115±9
10	6.80±0.15	120±13
30	6.51±0.09	109±5
100	6.05±0.08[b]	99±10
Fraction 6		
Control	6.93±0.16	100±6
10	6.47±0.07	105±4
30	6.20±0.08	100±3
100	6.00±0.07[a,c]	101±3
Fraction 7		
Control	7.35±0.05	110±4
10	7.17±0.06	105±3
30	7.05±0.04	104±3
100	6.83±0.08[a]	108±9

Data are means±S.E.M. ($n=4$).
[a] $P<0.05$ and [b] $P<0.01$ as compared with control group.
[c] $P<0.05$ as compared with fraction 7.

ml) significantly inhibited both early and late phases (3–40 min) of the contractile response to ovalbumin (Fig. 1B) (*P* values as in Fig. 1).

Regarding the leaf methanol extract, in the absence of indomethacin 30 μg/ml decreased early and late response to ovalbumin ($P<0.01$ and $P<0.05$, respectively) (Fig. 2A). In the presence of indomethacin, only the highest concentration of the leaf extract (30 μg/ml) significantly inhibited contractile response to ovalbumin from 40 min onward ($P<0.05$) (Fig. 2B). As methanol extracts from flowers and leaves did not display any effect on the pD_2 values of histamine-induced response, further experiments on histamine response were discarded.

3.3. Effects of ethyl-acetate phase on agonist experiments

Three extraction phases were obtained from methanol extract of *A. platyceras* leaves and flowers: ethyl-acetate; dichloromethane, and water phases. Only the ethyl-acetate phase (100 μg/ml) of leaves and flowers significantly decreased pD_2 of contractile response induced by carbachol ($P<0.01$) (Table 2).

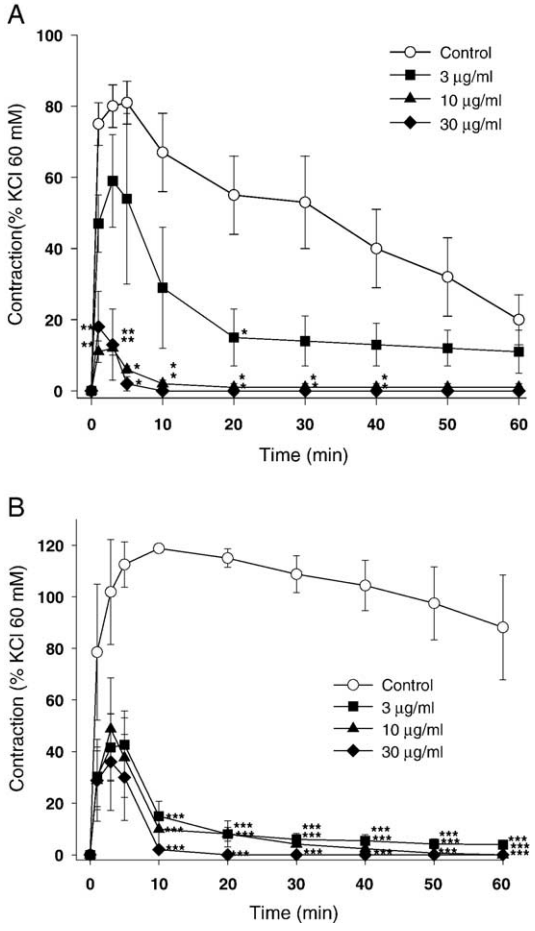

Fig. 3. Temporal course of the contractile response induced by antigen (ovalbumin) in tracheae from sensitized guinea pigs after 30-min incubation with fraction 6 in the absence (A) or presence (B) of indomethacin. Each point represents mean value±S.E.M. (vertical lines) of four experiments. *$P<0.05$, **$P<0.01$, ***$P<0.001$.

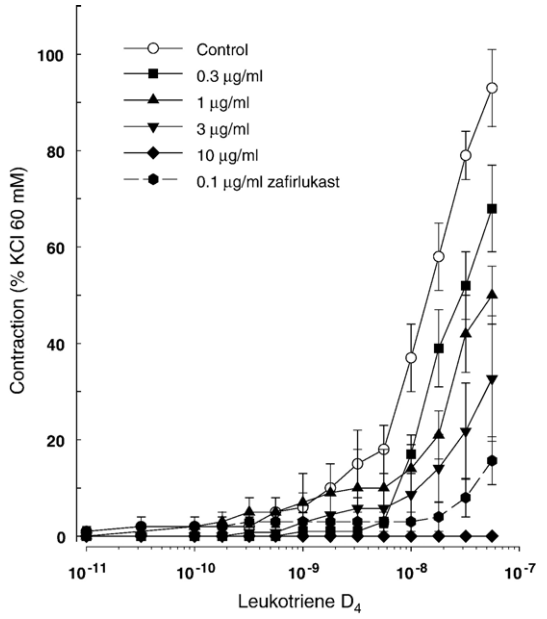

Fig. 4. Effect of fraction 6 and zafirlukast on concentration–response curves produced by leukotriene D_4 in the presence of indomethacin in guinea-pig isolated tracheae. Results are expressed as percentage of response to 60 mM KCl and are mean±S.E.M. (vertical lines) of four observations. *P* values as indicated in Table 3.

3.4. Effects of fractions on agonist experiments

The fractionation of the ethyl-acetate phase from leaves and flowers rendered seven fractions on column chromatography. Only fractions 6 and 7 (100 μg/ml) inhibited contractile response induced by carbachol, response in guinea-pig tracheal rings, but fraction 6 was more potent than fraction 7 ($P<0.05$) (Table 2). Therefore, fraction 6 was selected for further analysis.

3.5. Effects of fractions on antigen challenge experiments

Fraction 6 inhibited contractile response to ovalbumin of tracheae from sensitized guinea pigs similarly to the whole methanol-extract effect. Whereas in the absence of indomethacin, fraction 6 (10 and 30 μg/ml) significantly inhibited both phases of contractile response to ovalbumin (Fig. 3A, *P* values indicated in figure), in the presence of indomethacin fraction 6 significantly inhibited contractile response to ovalbumin at all concentrations assayed ($P<0.001$) (Fig. 3B).

Table 3
Leukotriene D_4-induced response of isolated guinea-pig tracheae in the presence of fraction 6 of *A. platyceras*

Fraction 6 (μg/ml)	pD_2	Maximal response (%)
Control	8.70±0.05	93±8
0.3	8.86±0.04	68±9
1	7.20±0.50[a]	50±6
3	7.01±0.25[b]	33±13[a]
10	0[c]	0[c]
Zafirlukast (0.1)	6.52±0.29[c]	16±5[a]

Data are means±S.E.M. ($n=4–5$).
[a] $P<0.05$, [b] $P<0.01$, and [c] $P<0.001$ as compared with control group.

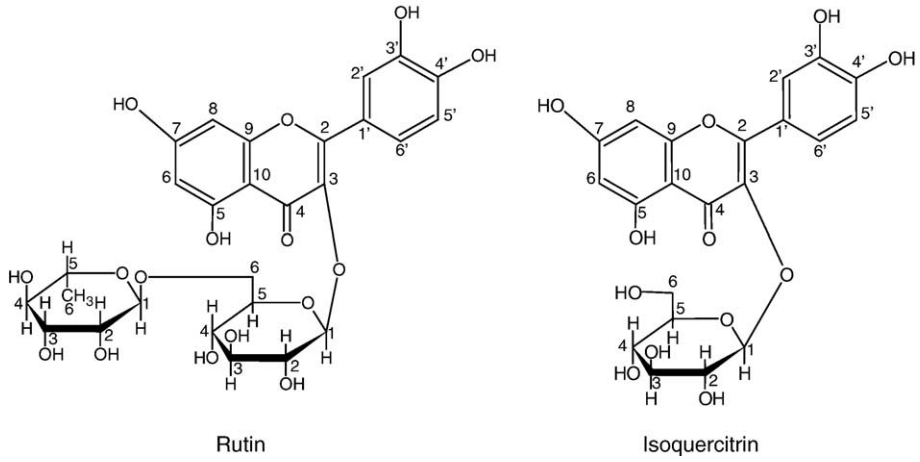

Fig. 5. Glycoside flavonoids isolated from *Argemone platyceras* leaves.

3.6. Anti-leukotriene effects

Incubation with fraction 6 (1, 3 and 10 μg/ml) produced pD_2 values and maximal response decrease to leukotriene D_4 similar to those produced by zafirlukast (0.1 μg/ml), which was used as anti-leukotriene positive control. This fraction completely inhibited contractile response at 10 μg/ml (Fig. 4, Table 3). The relative potency of 3 μg/ml of fraction 6 and 0.1 μg/ml zafirlukast was 1.

3.7. Identification of compounds

Fraction 6 was identified from its spectroscopic data as the glycosilated flavonoid isoquercitrin (Markham et al., 1978; Price and Rhodes, 1997; Lin and Lin, 1999) (Fig. 5).

Unambiguous [^{1}H]-NMR, [^{13}C]-NMR assignments were established using HMBC, HMQC, COSY and HETCOR, and DEPT. Isoquercitrin: orange gum, mp 150–154 °C; UV (methanol) λ_{max} 349, 305, 208 nm; ESI-MS m/z 464 $[M]^+$, 463(35), 301(100); [^{1}H]-NMR (methanol, 300 MHz); [^{13}C]-NMR (methanol, 75 MHz), (Tables 4 and 5).

Fraction 7 was identified as the glycosilated flavonoid rutin (Batterham and Highet, 1964; Lin et al., 2000) (Fig. 5). Unambiguous [^{1}H]-NMR, [^{13}C]-NMR assignments were established for the first time, using HMBC, HMQC, COSY and HETCOR, and DEPT.

Several assignations were corrected based on HMBC (H_6 and H_8, as well as for C_2, C_3, C_6, $C_{1'}$, $C_{6'}$, and G_2). Rutin: yellow

Table 4
[^{1}H] Nuclear magnetic resonance spectral data for glycoside flavonoids

[^{1}H]	Rutin	Isoquercitrin
	ppm	ppm
6	6.40 d(J=2.0)	6.20 d(J=1.4)
8	6.21 d(J=2.0)	6.39 d(J=1.4)
2′	7.67 d(J=2.1)	7.71 d(J=2.1)
5′	6.87 d(J=8.5)	6.87 d(J=8.5)
6′	7.63 dd(J=8.5, 2.1)	7.58 dd(J=8.5, 2.1)
G_1	5.10 d(J=8.1)	5.23 d(J=2.3)
G_2	3.48 dd(J=9.0, 8.1)	3.48 t(J=9.5, 8.2)
G_3	3.42 d(J=4.2)	3.43 t(J=9.5, 8.5)
G_4	3.27 s	3.35 t(J=9.8, 8.5)
G_5	3.33 s	3.22 ddd(J=9.8, 5.4, 2.3)
Ga_6	3.80 d(J=10.6)	3.71 d(J=11.9, 2.3)
Gb_6	3.39 d(J=4.2)	3.57 d(J=11.9, 5.4)
R_1	4.52 d(J=1.5)	
R_2	3.63 dd(J=3.4, 1.5)	
R_3	3.53 dd(J=9.6, 3.4)	
R_4	3.28 s	
R_5	3.45 d(J=3.4)	
CH_3	1.12 d(J=6.2)	

G=glucose, R=rhamnose. Chemical shifts are refereed to TMS.

Table 5
[^{13}C] Nuclear magnetic resonance spectral data for glycoside flavonoids

[^{13}C]	Rutin		Isoquercitrin	
	ppm	HMBC	ppm	HMBC
2	166.1	2′(^3J), 6′(^3J)	159.1	2′(^3J), 6′(^3J)
3	104.8	G_1(^3J)	135.7	G_1(^3J)
4	179.5		179.6	
5	163.1	6(^2J)	163.2	6(^2J)
6	130.5		100.0	8(^3J)
7	159.5	8(^2J), 6(^2J)	166.1	8(^2J), 6(^2J)
8	95.0	6(^3J)	94.8	6(^3J)
9	158.6	8(^2J)	158.6	8(^2J)
10	105.7	8(^3J), 6(^3J)	105.8	8(^3J), 6(^3J)
1′	123.2	2′(^2J), 5′(^3J)	123.2	2′(^2J), 5′(^3J)
2′	117.8	6′(^3J)	117.1	6′(^3J)
3′	145.9	2′(^2J), 5′(^3J)	146.0	2′(^2J), 5′(^3J)
4′	149.9	2′(^3J), 6′(^3J), 5′(^2J)	150.0	2′(^3J), 6′(^3J), 5′(^2J)
5′	116.2		116.1	
6′	123.7	2′(^3J)	123.3	2′(^3J)
G_1	100.1	8(^3J)	104.4	G_2(^2J)
G_2	78.3	G_2(^2J), G_4(^2J)	75.8	G_3(^2J)
G_3	77.3	Gb_6 (^2J), G_4(^2J)	78.2	G_2(^2J), G_4(^2J)
G_4	72.3	R_1(^3J), R_2(^2J)	71.3	G_3(^2J)
G_5	75.8	G_3(^2J)	78.5	G_4(^2J)
G_6	68.7	R_1(^3J)	62.7	
R_1	102.5	Ga_6 (^3J)		
R_2	72.25	R_1(^2J)		
R_3	71.5	G_3(^2J)		
R_4	74.0	R_2(^3J), R_3(^2J), R_6(^3J)		
R_5	69.8	R_1(^3J), R_4(^2J), R_6(2J)		
CH_3	18.0			

G=glucose, R=rhamnose. Chemical shifts are refereed to TMS; ^2J, and ^3J indicates ^{13}C-^{1}H couplings to two and three bonds, respectively.

powder, mp 182–184 °C (reported 184–188 °C, Bhandari, 1964); UV (methanol) λ_{max} 358, 303, 209 nm; ESI-MS m/z 610 $[M]^+$, 609(100), 301(40); [^{1}H]-NMR (methanol, 75 MHz) (Table 4); [^{13}C]-NMR (methanol, 300 MHz) (Table 5).

3.8. Assessment of cytotoxicity

To evaluate cytotoxicity of the extraction phases and chromatography fractions of *A. platyceras* (100 μg/ml), contractile responses of guinea-pig tracheae to 60 mM KCl at the end of incubation were compared with those recorded prior to incubation. No change was observed.

4. Discussion

The present data show the following:

1) Methanol extracts of flowers and leaves of *A. platyceras* as well as ethyl-acetate phase, and subsequent fractions 6 and 7 displaced concentration–response curves to carbachol in a parallel rightward manner in tracheae isolated from non-sensitized guinea pigs.
2) Fraction 6 abolished response to antigen challenge with ovalbumin in tracheae from sensitized guinea pigs, and caused a decrease of both pD_2 and maximal response values of leukotriene D_4-induced response in tracheae from non-sensitized animals.
3) Isoquercitrin and rutin are the main glycosilated flavonoids identified in fractions 6 and 7, respectively.

Isoquercitrin and rutin have not been reported previously in *A. platyceras*, but have been found in other species such as *Humulus lupulus* (Bhandari, 1964), *Psidium guajava* (Lozoya et al., 1994), *Conyza filaginoides* (Mata et al., 1997), *Theobroma cacao* (Sanchez-Rabaneda et al., 2003), *Hypericum perforatum* (Zou et al., 2004).

The present data on inhibitory actions of fractions 6 and 7 on carbachol-induced response of guinea-pig tracheae are in agreement with previous results showing that isoquercitrin and rutin are able to inhibit carbachol-induced contractile response in isolated rat and guinea-pig ileum (Mata et al., 1997; Lozoya et al., 1994). Other studies have reported vasorelaxant effects on isolated rat thoracic aorta (Ajay et al., 2003), as well as anti-inflammatory activity in different experimental models (Calixto et al., 2003; Morikawa et al., 2003). The fraction 6-induced inhibition of carbachol response in tracheae from guinea pigs suggests a likely interaction between isoquercitrin and muscarinic (M_3) receptors.

Antigen-challenge guinea-pig model has proved to be a good asthma model as it displays some similarities with human asthma in response to several contractile and relaxant substances (Campos and Church, 1992). Allergen challenge causes bronchoconstriction characterized by early and late phases, the latter associated with the lipoxygenase pathway and leukotrienes.

Cysteinyl leukotrienes play a central role in promotion of airway inflammation and modulation of airway smooth muscle cell function (Holgate et al., 2003), and are implicated in bronchoconstriction induced by antigen challenge and in specific bronchial hyperreactivity in both intrinsic and extrinsic asthma (Breschi et al., 2002).

In the allergic asthma model assayed, fraction 6 completely inhibited late response induced by antigen challenge, suggesting that this fraction exerts its effect mainly on the late inflammatory reaction which involves leukotriene release. Tracheae were incubated with indomethacin to avoid contribution of cyclooxygenase products to contractile response and make leukotrienes the main contractile mediators in the late response. When compared with the effect of zafirlukast, fraction 6 displayed similar activity on antigen response late phase. However, zafirlukast, a well known cysteinyl leukotriene receptor antagonist, inhibits both early and late phases of antigen-induced bronchoconstriction as well as decreases airway sensitivity to methacholine (Aharony, 1998). The selective and total inhibition of fraction 6 (10 μg/ml) on concentration–response curve to leukotriene-D_4, as well as the relative potency between zafirlukast (0.1 μg/ml) and fraction 6 (3 μg/ml) might be related to an interaction of isoquercitrin with leukotriene-D_4 receptors. Due to the dual effect on carbachol-and leukotriene-induced responses, fraction 6 might be useful in treatment of asthma, and the reputation of *Argemone platyceras* as an anti-asthmatic remedy seems to be justified.

Our data present the first pharmacological evidence of the action of compounds isolated from *Argemone platyceras* on an allergic asthma model. Further investigations on natural sources for anti-asthmatic drugs should be encouraged.

Ackowledgments

We are grateful to CONACyT-Mexico for providing a graduate scholarship to Jacquelina Fernandez Vargas, and for project funding No. 3476M; to Jaime Tortoriello, MD, Ph.D., for the kind gift of plant material, and to Rocio Patiño Maya, Luis Velasco Ibarra, and Javier Perez Flores for recording IR, UV, and first NMR, and EM spectra.

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