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The anti-inflammatory modulatory role of *Solidago chilensis* Meyen in the murine model of the air pouch

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Abstract

The aim of this study was to investigate the anti-inflammatory efficacy of an aqueous extract (AE), and its butanolic (BuOH) and aqueous residual (AR) fractions, derived from the rhizome of *Solidago chilensis* in inflammation caused by carrageenan in mice. *Solidago chilensis* Meyen rhizome was extracted using hot water at 90°C under infusion. The extract was filtered and lyophilized. Part of the aqueous extract was fractionated with n-BuOH, resulting in butanolic (BuOH) and aqueous residual (AR) fractions. Adult Swiss mice were used in the in-vivo experiments. We evaluated the effect of rhizome aqueous extract of *Solidago chilensis* and these two derived fractions on the inflammation induced by carrageenan in the mouse model of the air pouch. The aqueous extract and its derived fractions significantly inhibited leucocytes, neutrophils, exudation, myeloperoxidase and adenosine deaminase activity, as well as nitric oxide, interleukin-1 beta (IL-1β), neutrophil chemokine (KC) and tumour necrosis factor-alpha (TNF-α) levels ($P < 0.05$). Indometacin and dexamethasone inhibited all the studied inflammatory parameters ($P < 0.01$) with the exceptions that indometacin did not inhibit TNF-α levels and dexamethasone did not inhibit KC levels ($P > 0.05$). These results indicate that *Solidago chilensis* has a significant anti-inflammatory action on acute inflammatory responses and that its inhibitory activity may be due not only to the inhibition of pro-inflammatory mediators, but also to the inhibition of leucocyte infiltration.

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Introduction

Species of the genus *Solidago* are used in traditional medicine in several countries. Herbal remedies based on goldenrod (*Solidago virgaurea*) have been well used for centuries in the treatment of urinary tract diseases. The herbal preparations have a complex action spectrum (anti-inflammatory, antimicrobial, diuretic, antispasmodic, analgesic) and are especially recommended for the treatment of infection and inflammation (Melzig 2004). Studies in-vivo and in-vitro have also demonstrated that this species has important biological activity (Metzner et al 1984; Sampson et al 2000; Gross et al 2002; Choi et al 2005).

In South America the most abundant species is *Solidago chilensis* Meyen (Asteraceae), which is widely used in the popular medicine of several countries of this continent (Cabrera 1974). It is included in the first edition of the Brazilian Pharmacopea under the drug names erva-lanceta and arnica silvestre (Brazilian Pharmacopea 1929), the latter denomination being derived from its usage as a substitute for *Arnica montana*. Moreover, in Brazilian popular medicine it is recommended as a diuretic, analgesic and anti-inflammatory agent to treat burns and rheumatic disease, among other treatments (Mors et al 2000; Lorenzi & Matos 2002). However, there are few studies in-vivo or in-vitro to demonstrate the biological activity of this species, although one study has indicated that *Solidago chilensis (Solidago microglossa)* has antimicrobial activity (Morel et al 2006). Further, in the case of *Solidago chilensis*, notwithstanding its wide utilization and presence in the Brazilian Pharmacopoea, the pharmacological and chemical investigations are rather scarce. Previous studies in our laboratory have revealed that the aqueous extracts of rhizome, and also leaves, have important anti-inflammatory properties: the aqueous extract of rhizome of *Solidago chilensis* Meyen, and its derived fractions, significantly inhibited leucocytes, exudation and pro-inflammatory mediators in the mouse model of pleurisy induced by carrageenan (Goulart et al 2007).

From the chemical point of view, the flavonoid quercitrin has been reported to be one of the main components in the aerial parts (Gutierrez et al 1981; Sanchez de Medina 2002) and the labdane diterpene solidagenone is the main component in the rhizome (Schmeda-Hirschmann 1987).

Previous study in our laboratory revealed that rhizomes of *Solidago chilensis* Meyen demonstrate important anti-inflammatory properties in the mouse model of pleurisy induced by carrageenan (Goulart et al 2007). In this study we extend the effect of the aqueous extract and its derived fractions obtained from rhizomes of *Solidago chilensis* Meyen, aiming to evaluate the anti-inflammatory effect upon leucocyte migration, exudation level and myeloperoxidase/adenosine deaminase activity, as well as nitric oxide, interleukin-1 beta (IL-1β), neutrophil chemokine (KC) and tumour necrosis factor-alpha (TNF-α) levels in the inflammation caused by carrageenan in another inflammation model (air pouch).

Materials and Methods

Drugs

The following drugs and reagents were used: carrageenan (degree IV), human neutrophil myeloperoxidase, indometacin, (Sigma Chemical Co., St Louis, MO), dexamethasone, (Ache pharmaceutical laboratories S.A., São Paulo, SP, Brazil), enzyme-linked immunosorbant assay (ELISA) for quantitative determination of rat IL-1β and mouse KC (IBL-Immuno Biological Laboratories Co. Ltd, Fujioka-city, Gunma, Japan), mouse TNF-α (BD-Biosciences Pharmingen, San Diego, CA). Other reagents used were of analytical grade and were obtained from different commercial sources.

Plant materials

The rhizomes of *Solidago chilensis* Meyen were collected in Caibi, a town located in the state of Santa Catarina, Brazil. They were collected in March 2005, and were identified by the botanist Prof. Dr Daniel Falkenberg of the Department of Botany at the Federal University of Santa Catarina, Florianópolis, SC, Brazil, where a voucher specimen (FLOR34674) was deposited.

Preparation of the extract and its derived fractions and isolated procedures for compounds

Solidago chilensis Meyen rhizome was air-dried at room temperature for 7 days. Subsequently, the powdered rhizome was filtered and concentrated with a rota-evaporator to obtain the aqueous extract. The aqueous extract was extracted using hot water at 90°C (plant–solvent, 1:10, w/v) under infusion for 10 min, yielding 12%. Thereafter, the extract was filtered and a sample was lyophilized (Edward E-C Micromodulyo Freeze-Dryer, USA). Part of the aqueous extract of the rhizome was partitioned three times with 50 mL of n-butanol, resulting in the butanolic fraction (yield 12.9%) and aqueous residual fraction (yield 87.1%). These fractions were evaporated under reduced pressure at a temperature below 50°C, yielding dry residues, considered as the butanolic (BuOH) and aqueous residual (AR) fractions.

In accordance with results obtained in in-vivo studies from the BuOH fraction of *Solidago chilensis* some compounds were isolated. Thus, the BuOH fraction was submitted to successive chromatographic procedures to isolate the active compounds, one of them identified as chlorogenic acid. After acid and alkaline hydrolysis, we observed the predominant presence of caffeic acid.

Animals

Swiss mice, 18–25 g, were housed under standardized conditions (room at constant temperature (22 ± 2°C) with a 12-h light–dark cycle), humidity 50–60%, and were allowed free access to a standard mouse diet and water before use. This study was approved by the Committee for Ethics in Animal Research of our university (Protocol number-23080.007042/2006-28), and experiments were performed in accordance with the norms of the Brazilian College of animal experimentation.

Experimental protocol

Different groups of mice received air injection (1.5 mL) on three alternate days to induce the air pouch. On the sixth day, the mice received carrageenan 1% administered by the subcutaneous route, and 24 h later they were killed by an overdose of ether (Benincá et al 2007). The mice were fixed on a surgical table and an incision in the dorsal skin was made to perforate the air pouch. The cavity was washed with 1.0 mL of sterile phosphate-buffered saline (PBS; pH 7.6; composition in mmol: NaCl 137, KCl 2.7 and phosphate buffer salts 10) containing heparin (20 IU mL^{-1}). Leucocyte migration, exudation, myeloperoxidase (MPO) and adenosine deaminase (ADA) activity, as well as nitrate/nitrite concentration (NO^x), interleukin-1 beta (IL-1β), neutrophil chemokine (KC) and tumour necrosis factor-alpha (TNF-α) levels were evaluated 24 h after phlogogen administration.

Indometacin (5.0 mg kg^{-1}) and dexamethasone (0.5 mg kg^{-1}) were used as reference anti-inflammatory drugs.

Initially, the effect of the aqueous extract and its derived fractions (BuOH and AR), obtained from rhizome of *Solidago chilensis* Meyen, on the inflammation induced by carrageenan in the air pouch, in mice, was evaluated. In this protocol, different groups of mice were treated (0.5 h before) with different doses of aqueous extract (10–200 mg kg^{-1}), BuOH or AR fractions (10–50 mg kg^{-1}) administered by the intraperitoneal route. The inflammatory parameters (leucocyte migration and exudation) were analysed 24 h after carrageenan administration.

In another set of experiments employed to establish the time-course profile, different groups of mice were pre-treated with one dose of aqueous extract, BuOH fraction or AR fraction at different time points (0.5–4 h) and the same inflammatory parameters were evaluated 24 h after carrageenan administration.

According to this protocol, the aqueous extract (100 mg kg^{-1}), BuOH fraction (10 or 25 mg kg^{-1}) or AR fraction (25 or 50 mg kg^{-1}), administered 0.5 h before carrageenan induction,

were elected as the doses to be used in the following experiments.

The aqueous extract and its derived fractions, as well as indometacin and dexamethasone, were administered 0.5 h before inflammation induction.

Quantification of leucocyte migration and exudation

After killing the mice, samples of the air pouch cavity fluid were collected to determine the total and differential leucocyte contents, and exudation. Total leucocyte counts were determined in a Neubauer chamber, and cytospin preparations of exudates were stained with May–Grünwald–Giemsa for the differential count (Saleh et al 1996).

The degree of exudation was determined by measuring the amount of Evans blue dye extravasation in the exudate as previously described (Benincá et al 2007). Thus, in each experimental group, mice were challenged 0.5 h after the inflammation induction with a solution of Evans blue dye (25 mg kg^{-1}) administered by the intravenous route to evaluate the exudation in the air pouch cavity. On the day of the experiment, a batch of stored samples was thawed at room temperature and the amount of dye was estimated by colorimetry using an ELISA plate reader (Organon Teknika, Roseland, NJ) at 620 nm, by interpolation from a standard curve of Evans blue dye in the range of 0.01–50 μg mL^{-1}.

Quantification of myeloperoxidase activity

Standard samples with different concentrations of myeloperoxidase (from human neutrophils, Sigma: M6908; Sigma, St Louis, MO) were prepared to obtain a standard curve in the range of 0.07–140 mU mL^{-1}. Air pouch cavity fluid samples (40 μL) and standards were transferred to cuvettes and the reaction was initiated with the addition of 360 μL of assay buffer (0.167 mg mL^{-1} of *o*-dianisidine 2HCl and 0.0005% H_2O_2). The reaction was stopped with sodium azide 1%. Afterwards, the samples were centrifuged at 50 *g* for 5 min, the supernatants were separated and the rates of changes in absorbancy at 520 nm were determined. MPO activity was estimated by interpolation from the standard curve by means of colorimetric measurements in an ELISA plate reader (Organon Teknika, Roseland, NJ) (Rao et al 1993). Results were expressed as mU mL^{-1}.

Quantification of adenosine deaminase activity

Initially, standard samples (final volume of 500 μL) with different volume concentrations of $NaH_2PO_4.H_2O$ (35 mM), $Na_2HPO_4.H_2O$ (15 mM) and NH_3SO_4 (15 mM) were prepared to obtain a standard curve in the range of 10–50 U L^{-1}. Air pouch cavity fluid samples (20 μL) were transferred to cuvettes and the reaction was initiated by the addition of adenosine-phosphate-buffered solution (pH 6.5, 500 μL, composition in mM: $NaH_2PO_4.H_2O$ 35, $Na_2HPO_4.12H_2O$ 15 and adenosine 0.5). After incubation for 1 h at 37°C, the reaction was halted with the addition of a solution (1000 μL) of phenol (1 mM) and nitroprussiate (0.17 mM), plus alkaline buffer (1000 μL; NaOCl: 11 mM). This solution (final volume 2000 μL) was also added to the cuvettes with the different standard samples. Afterwards, the rate of change in absorbance at 620 nm was determined. Adenosine deaminase activity was estimated by interpolation from the standard curve by means of colorimetric measurements in an ELISA plate reader (Organon Teknika, Roseland, NJ) (Giusti & Galanti 1984). Results were expressed as U L^{-1}.

Quantification of nitrate/nitrite concentration

Nitric oxide and its breakdown products nitrite (NO_2^-) and nitrate (NO_3^-) were measured using the Griess method (Green et al 1982). Samples of exudate were collected, separated and stored at −20°C. Levels of nitrate/nitrite were determined as previously described by Saleh et al (1999); concentrations were estimated by means of colorimetric measurement at 450 nm in an ELISA plate reader (Organon, Teknika, Roseland, NY) by interpolation from a standard curve (0–150 μM). Results were expressed as μM.

Quantification of IL-1β, KC and TNF-α levels

Samples of exudate were collected and immediately prepared for the analysis of cytokine levels. In this protocol, commercially available kits were used with monoclonal specific antibodies for each cytokine. The cytokine levels were measured by enzyme-linked immunosorbant assay (ELISA), using the kits according to the manufacturers' instructions. The range of values detected by these assays were: IL-1β 100–6400 pg mL^{-1}, KC 23–1500 pg mL^{-1} and TNF-α 5–2000 pg mL^{-1}. The intra- and inter-assay coefficients of variation (CV) for IL-1β, KC and TNF-α were: intra CV: IL-1β = 6.2 ± 0.4%, KC = 9.7 ± 0.9% and TNF-α = 7.8 ± 0.9%; inter CV: IL-1β = 5.1 ± 0.6%, KC = 4.1 ± 0.9% and TNF-α = 9.6 ± 2.1%, with sensitivity values of IL-1β = 1.7 pg mL^{-1}, KC = 23.0 pg mL^{-1} and TNF-α = 5.0 pg mL^{-1}. All cytokine concentrations were estimated by means of colorimetric measurements at 450 nm in an ELISA plate reader (Organon Teknika, Roseland, NJ) by interpolation from a standard curve.

Data analysis

The data is reported as the mean ± s.e.m. Significant differences between groups were determined by analysis of variance complemented with Dunnett's or Student's *t*-tests (or both). $P < 0.05$ was considered significant.

Results

Effect of *Solidago chilensis*, indometacin and dexamethasone on leucocyte migration and exudation

The aqueous extract of the rhizome of *Solidago chilensis* (50–200 mg kg^{-1}) significantly decreased the leucocyte migration from 34.3 ± 7.4 to 88.3 ± 1.4% in comparison with the control group (carrageenan-treated mice) ($P < 0.05$) (Table 1). This extract (100 and 200 mg kg^{-1}) also significantly inhibited neutrophils from 73.8 ± 2.2 to 93.6 ± 1.1% ($P < 0.01$)

Table 1 Effect of the aqueous extract (and its butanolic and aqueous residual fractions) of *Solidago chilensis*, indometacin and dexamethasone on leucocyte migration and exudation in the inflammation induced by carrageenan in mice

Groups/doses	Leucocytes ($\times 10^6$)	Neutrophils ($\times 10^6$)	Mononuclear cells ($\times 10^6$)	Exudation (μg mL^{-1})
C[a]	12.4 ± 0.6	10.5 ± 0.8	1.9 ± 0.5	17.2 ± 0.7
AE (10 mg kg^{-1})[b]	15.2 ± 0.9	9.2 ± 0.8	6.0 ± 0.9**	15.6 ± 1.0
AE (25 mg kg^{-1})[b]	11.3 ± 1.9	9.9 ± 1.6	1.4 ± 0.4	13.5 ± 1.8
AE (50 mg kg^{-1})[b]	10.0 ± 1.0*	8.8 ± 0.8	1.2 ± 0.3	17.6 ± 1.8
AE (100 mg kg^{-1})[b]	4.7 ± 0.2**	2.8 ± 0.2**	1.9 ± 0.6	9.7 ± 0.7**
AE (200 mg kg^{-1})[b]	1.5 ± 0.2**	0.7 ± 0.1**	0.8 ± 0.1*	3.9 ± 0.3**
BuOH (10 mg kg^{-1})[b]	7.9 ± 0.4**	5.5 ± 0.4**	2.4 ± 0.4	8.2 ± 0.9**
BuOH (25 mg kg^{-1})[b]	10.3 ± 0.4*	7.4 ± 0.8**	2.9 ± 0.4	12.9 ± 1.2*
BuOH (50 mg kg^{-1})[b]	14.5 ± 0.6*	11.8 ± 0.8	2.7 ± 0.3	16.6 ± 0.6
AR (10 mg kg^{-1})[b]	12.5 ± 0.6	7.9 ± 0.3*	4.6 ± 0.3**	15.0 ± 0.7*
AR (25 mg kg^{-1})[b]	7.0 ± 0.6**	5.0 ± 0.6**	2.0 ± 0.2	10.8 ± 0.9**
AR (50 mg kg^{-1})[b]	7.9 ± 2.5**	5.2 ± 1.6**	2.7 ± 1.0	10.2 ± 2.2**
Indo (5 mg kg^{-1})[b]	4.9 ± 0.5**	3.2 ± 0.1**	1.7 ± 0.4	8.1 ± 0.5**
Dexa (0.5 mg kg^{-1})[b]	7.5 ± 1.1**	4.6 ± 0.4**	2.9 ± 0.9	11.2 ± 1.6**

Aqueous extract (AE: 10–200 mg kg^{-1}), butanolic fraction (BuOH: 10–50 mg kg^{-1}) or aqueous residual fraction (AR: 10–50 mg kg^{-1}) of *Solidago chilensis* were administered to different groups of mice 0.5 h before inflammation induction by carrageenan (1%). C, control = response in mice treated only with carrageenan; Indo, response in mice pre-treated with indometacin (5.0 mg kg^{-1}, i.p.); Dexa, response in mice pre-treated with dexamethasone (0.5 mg kg^{-1}, i.p.). [a]Administered by subcutaneous route (s.c.); [b]administered by intraperitoneal route (i.p.). *$P < 0.05$ and **$P < 0.01$, compared with control. Data are presented as means ± s.e.m, n = 5 mice.

and exudation from 43.7 ± 3.9 to 77.4 ± 1.9%, ($P < 0.01$) (Table 1). The dose of 200 mg kg^{-1} of this extract caused a significant decrease of mononuclear cells by 55.1 ± 1.1% ($P < 0.05$) (Table 1). Under the same conditions this extract at doses of 10 and 25 mg kg^{-1} did not modify the studied inflammatory parameters ($P > 0.05$), except the dose of 10 mg kg^{-1} that caused a significant increase in mononuclear cells by 216.0 ± 4.8% ($P < 0.01$) (Table 1).

The butanolic fraction (10 and 25 mg kg^{-1}) significantly inhibited leucocyte migration from 16.5 ± 3.6 to 36.6 ± 3.5% ($P < 0.05$) (Table 1), neutrophils from 29.5 ± 7.4 to 46.0 ± 3.7% ($P < 0.01$) and exudation from 25.4 ± 7.3 to 52.4 ± 5.3% ($P < 0.05$) in comparison with carrageenan-treated mice (Table 1). Nevertheless, this fraction did not modify mononuclear cells ($P > 0.05$) (Table 1). The BuOH fraction at the dose of 50 mg kg^{-1} significantly increased leucocyte migration by 16.4 ± 4.8% ($P < 0.05$), but did not inhibit neutrophils, mononuclear cells or exudation ($P > 0.05$) (Table 1).

The aqueous residual fraction (25 and 50 mg kg^{-1}) produced a significant decrease in leucocyte migration from 20.3 ± 7.4 to 44.2 ± 4.9% in comparison with the control group ($P < 0.01$) (Table 1). Moreover, this fraction (10–50 mg kg^{-1}) also significantly inhibited neutrophils from 38.9 ± 4.2 to 52.9 ± 5.5% ($P < 0.05$) and exudation from 35.2 ± 7.8% to 37.2 ± 5.3% ($P < 0.05$) (Table 1). The aqueous residual fraction at a dose of 10 mg kg^{-1} did not modify leucocyte migration ($P > 0.05$), but it caused a significant enhancement of mononuclear cells by 40.4 ± 16.6% ($P < 0.01$) (Table 1).

The time-course profile for the aqueous extract of the rhizome of *Solidago chilensis* showed that the pre-treatment periods of 0.5 and 2 h significantly inhibited leucocyte migration and exudation induced by carrageenan in the studied inflammation model ($P < 0.01$) (results not shown). However, its derived fractions (BuOH and AR) were effective in significantly inhibiting the same inflammatory parameters only after pre-treatment for 0.5 h (results not shown). The dose–response curve and time-course profile studied indicated that the inhibitory effects on leucocyte migration and exudation were optimal in mice injected 0.5 h before carrageenan administration with AE (100 mg kg^{-1}), BuOH (10 or 25 mg kg^{-1}) or AR (25 or 50 mg kg^{-1}). For this reason, these doses and the pre-treatment period of 0.5 h were chosen for the subsequent experiments.

As expected, indometacin (5.0 mg kg^{-1}, i.p.) or dexamethasone (0.5 mg kg^{-1}, i.p.) significantly inhibited leucocyte migration, neutrophils and exudation in the inflammation induced by carrageenan in the mouse air pouch model in comparison with carrageenan-treated mice ($P < 0.01$) (Table 1).

The inhibition of leucocyte migration and exudation of *Solidago chilensis* extract (AE) at the dose of 200 mg kg^{-1} was significantly (4-fold and 2-fold, respectively) higher than that of indometacin ($P < 0.01$). The inhibition of these inflammatory parameters of AE (200 mg kg^{-1}) were also significantly (5-fold for leucocyte migration and 3-fold for exudation) higher than that of dexamethasone ($P < 0.05$). On the other hand, indometacin was significantly (approximately 1.5-fold) more powerful than *Solidago chilensis*-derived fractions (BuOH and AR) in inhibiting leucocyte migration and exudation ($P < 0.05$). Further, *Solidago chilensis*-derived fractions and dexamethasone presented the same inhibitory effect upon leucocyte migration and exudation ($P > 0.05$) (results not shown).

Effect of *Solidago chilensis*, indometacin and dexamethasone on myeloperoxidase and adenosine deaminase activity and nitrate/nitrite concentration

As myeloperoxidase and adenosine deaminase activity are considered to be markers of leucocyte activation and migration

(Fröde & Medeiros 2001; Spicuzza et al 2006), in this study we decided to examine myeloperoxidase and adenosine deaminase activity since we were interested in evaluating whether *Solidago chilensis* Meyen was able to down-regulate these enzymes. Pre-treatment of mice with the aqueous extract of rhizome (100 mg kg^{-1}) and its BuOH (25 mg kg^{-1}) and AR (25 or 50 mg kg^{-1}) fractions caused a significant decrease in myeloperoxidase (% of inhibition: AE: 56.5 ± 2.1, BuOH: 57.0 ± 4.7 and AR: 48.4 ± 0.6) ($P < 0.01$) and adenosine deaminase activity (% of inhibition: AE: 58.4 ± 9.0, BuOH: 88.9 ± 2.4 and AR: 66.4 ± 3.3) in comparison with carrageenan-treated mice ($P < 0.01$) (Table 2).

Table 2 Effect of the aqueous extract (and its butanolic and aqueous residual fractions) of *Solidago chilensis*, indometacin and dexamethasone on myeloperoxidase (MPO), adenosine deaminase (ADA) and nitric oxide (NO^x) levels in inflammation induced by carrageenan in mice

Groups/doses	MPO (mU mL^{-1})	ADA (U L^{-1})	NO^x (μM)
C[a]	414.4 ± 55.2	108.4 ± 14.4	11.4 ± 1.1
AE (100 mg kg^{-1})[b]	179.1 ± 12.2**	72.0 ± 13.4**	7.9 ± 1.0**
BuOH (25 mg kg^{-1})[b]	178.3 ± 19.6**	12.0 ± 1.5**	4.9 ± 0.8**
AR (25 mg kg^{-1})[b]	—	36.4 ± 3.6**	—
AR (50 mg kg^{-1})[b]	213.9 ± 2.6**	—	7.8 ± 0.9**
Indo (5 mg kg^{-1})[b]	229.5 ± 15.8**	40.4 ± 3.4**	6.7 ± 1.2**
Dexa (0.5 mg kg^{-1})[b]	290.9 ± 23.8**	39.1 ± 15.2**	5.4 ± 0.1**

Aqueous extract (AE: 100 mg kg^{-1}), butanolic fraction (BuOH: 25 mg kg^{-1}) or aqueous residual fraction (AR: 25 or 50 mg kg^{-1}) of *Solidago chilensis* were administered to different groups of mice 0.5 h before inflammation induction by carrageenan (1%). C, control = response in mice treated only with carrageenan; Indo, response in mice pre-treated with indometacin (5.0 mg kg^{-1}, i.p.); Dexa, response in mice pre-treated with dexamethasone (0.5 mg kg^{-1}, i.p.). [a]Administered by subcutaneous route (s.c.); [b]administered by intraperitoneal route (i.p.). **$P < 0.01$, compared with control. Data are presented as means ± s.e.m., n = 5 mice.

Moreover, the aqueous extract of rhizome and its derived fractions also significantly inhibited nitrate/nitrite levels (% of inhibition: AE: 40.3 ± 4.4, BuOH: 57.0 ± 6.9 and AR: 31.4 ± 7.6) in comparison with the control group ($P < 0.01$) (Table 2).

Indometacin and dexamethasone pre-treatment at the studied doses had a significant inhibitory effect on myeloperoxidase and adenosine deaminase activity and nitrate/nitrite concentration in comparison with the control group ($P < 0.01$) (Table 2).

The inhibition of the myeloperoxidase activity of AE (100 mg kg^{-1}), BuOH (25 mg kg^{-1}) and AR (50 mg kg^{-1}) were significantly (approximately 1.5-fold) higher than those of indometacin and dexamethasone ($P < 0.05$). Further, the decrease in the adenosine deaminase activity of BuOH (25 mg/kg) was significantly (1.5-fold) higher than those of indometacin and dexamethasone ($P < 0.05$). No significant differences in the inhibition of nitric oxide levels were observed between the anti-inflammatory reference drugs and *Solidago chilensis* extract and its derived fractions ($P > 0.05$) (results not shown).

Effects of *Solidago chilensis*, indometacin and dexamethasone on cytokine levels

The aqueous extract of rhizome (100 mg kg^{-1}) and its BuOH (10 or 25 mg kg^{-1}) and AR (25 or 50 mg kg^{-1}) fractions caused a significant inhibition of IL-1β (% of inhibition: AE: 79.5 ± 4.6, BuOH: 50.3 ± 8.7 and AR: 27.8 ± 3.9) ($P < 0.01$), KC (% of inhibition: AE: 28.5 ± 9.5, BuOH: 71.9 ± 5.4 and AR: 64.7 ± 0.5) ($P < 0.05$) and TNF-α levels (% of inhibition: AE: 36.0 ± 2.8, BuOH: 49.8 ± 1.9 and AR: 81.3 ± 2.2) in comparison with carrageenan-treated mice ($P < 0.01$) (Table 3).

Indometacin and dexamethasone also significantly inhibited IL-1β levels ($P < 0.01$) (Table 3). On the other hand, dexamethasone, but not indometacin, inhibited TNF-α levels ($P < 0.01$) (Table 3). Indometacin, but not dexamethasone, inhibited KC levels ($P < 0.01$) (Table 3).

Table 3 Effect of the aqueous extract (and its butanolic and aqueous residual fractions) of *Solidago chilensis*, indometacin and dexamethasone on interleukin 1-beta (IL-1β), neutrophil chemokine (KC) and tumour necrosis factor-alpha (TNF-α) levels in inflammation induced by carrageenan in mice

Groups/Doses	IL-1β (pg mL^{-1})	KC (pg mL^{-1})	TNF-α (pg mL^{-1})
C[a]	2066.0 ± 4.0	40590.0 ± 3539.0	5283.0 ± 529.1
AE (100 mg kg^{-1})[b]	424.0 ± 94.1**	29013.7 ± 2000.0*	3364.0 ± 88.2**
BuOH (10 mg kg^{-1})[b]	1026.0 ± 179.7**	11400.0 ± 2209.0**	—
BuOH (25 mg kg^{-1})[b]	—	—	2650.0 ± 100.0**
AR (25 mg kg^{-1})[b]	1491.0 ± 80.9**	14320.0 ± 196.0**	—
AR (50 mg kg^{-1})[b]	—	—	988.7 ± 118.2**
Indo (5 mg kg^{-1})[b]	645.9 ± 297.3**	12460.0 ± 1679.0**	5225.4 ± 325.4
Dexa (0.5 mg kg^{-1})[b]	24.3 ± 5.2**	44830.0 ± 412.5	2799.0 ± 179.9**

Aqueous extract (AE: 100 mg kg^{-1}), butanolic fraction (BuOH: 10 or 25 mg kg^{-1}) or aqueous residual fraction (AR: 25 or 50 mg kg^{-1}) of *Solidago chilensis* were administered to different groups of mice 0.5 h before inflammation induction by carrageenan (1%). C, control = response in mice treated only with carrageenan; Indo, response in mice pre-treated with indometacin (5.0 mg kg^{-1}, i.p.); Dexa, response in mice pre-treated with dexamethasone (0.5 mg kg^{-1}, i.p.). [a]Administered by subcutaneous route (s.c.); [b]administered by intraperitoneal route (i.p.). *$P < 0.05$ and **$P < 0.01$, compared with control. Data are presented as means ± s.e.m., n = 5 mice.

AE (100 mgkg^{-1}), BuOH (10 mgkg^{-1}) and AR (25 mgkg^{-1}) were significantly (respectively 1.5-fold, 3.9-fold and 3.0-fold) more effective at inhibiting KC levels than dexamethasone ($P<0.05$). Dexamethasone was significantly more effective at inhibiting IL-1β than *Solidago chilensis* and its derived fractions ($P<0.01$). AE (100 mgkg^{-1}) BuOH (25 mg kg^{-1}) and AR (50 mgkg^{-1}) were significantly (respectively 1.5-fold, 1.9-fold and 5-fold) more effective than indometacin at inhibiting TNF-α levels. Only AR (50 mgkg^{-1}) was significantly (5-fold) more effective at inhibiting TNF-α than dexamethasone ($P<0.01$) (results not shown).

Discussion

Inflammation may be defined as a condition or state into which tissues enter as a response to injury or insult. This event is caused, among other factors, by the release or activation of pro-inflammatory cytokines, such as IL-1β, TNF-α, nitric oxide and other inflammatory mediators, as well as different types of cells (Schmid-Schönbein 2006). Furthermore, neutrophils are the most important and extensively studied cells involved in the acute inflammatory response. As the principal circulating phagocyte, neutrophils are the first and most abundant leucocyte to be delivered to the site of infection or inflammation (Seely et al 2003; Nathan 2006). Another point to consider is that the air pouch model has been reported to have histological similarity to synovial membranes and when challenged via an injection of carrageenan the inflammatory reaction is characterized by a significant enhancement of neutrophils that is histologically similar to that observed in the chronic synovial inflammation (Sedgwick & Lees 1986).

First of all, our results showed that *Solidago chilensis* and its two derived fractions significantly inhibited pro-inflammatory mediators, such as NO, TNF-α and IL-1β, as well as activated leucocytes, at the site of inflammation. These results are in agreement with those described by Goulart et al (2007) who demonstrated that the rhizome of *Solidago chilensis* significantly inhibits leucocytes, exudation and pro-inflammatory mediators and enzymes in the mouse model of pleurisy induced by carrageenan.

In this study, *Solidago chilensis* inhibited leucocytes, for the most part neutrophils, in the mouse air pouch cavity.

It is interesting to note that BuOH demonstrated a stronger inhibitory effect on leucocyte migration and exudation at the lower dose (10 mgkg^{-1}) than the higher dose (50 mgkg^{-1}). According to our data, it is reasonable to speculate that BuOH has a narrow inhibitory window since lower doses (10 mgkg^{-1}) caused a significant inhibition of either leucocyte migration (36.6 ± 3.5%) or exudation (52.4 ± 5.2%), whereas higher doses (50 mgkg^{-1}) did not present the same efficacy. This property is common to immunosuppressive drugs (Yocum et al 2003).

The inhibitory effect upon leucocyte migration was also associated with a decrease in myeloperoxidase activity. The myeloperoxidase system of neutrophils is important as a potent bactericidal oxidant (Klebanoff 2005) and it is directly proportional to chemotaxis and activated neutrophil infiltration into inflamed tissues in the inflammatory process (Smith 1994; Gaut et al 2001). In parallel, an important inhibition of adenosine deaminase activity was also observed, an enzyme considered to be a lymphocyte activation marker (Fröde & Medeiros 2001; Spicuzza et al 2006).

Solidago chilensis aqueous extract, and its BuOH or AR fractions also significantly decreased IL-1β, KC and TNF-α levels, as well as nitric oxide concentration.

Tumour necrosis factor-alpha (TNF-α) is a prototypical inflammatory and immunomodulatory cytokine expressed by activated macrophages, monocytes, neutrophils, T-cells and NK cells (Tian et al 2005). On the other hand, interleukin-1 beta (IL-1β) is produced and released predominantly by macrophages (Chang et al 2004). Furthermore, IL-1β, along with TNF-α, is a central mediator of the host inflammatory response with the ability to activate adhesion molecule expression, promoting the enhancement of leucocyte trafficking to the site of inflammation (Seely et al 2003; Ruth et al 2005). IL-1β and TNF-α can also play an important role in releasing specific inflammatory cytokines (C-X-C chemokines) from activated cells (Seely et al 2003; Ruth et al 2005).

Neutrophils play an essential role in the formation of inducible nitric oxide synthase (iNOS)-derived NO and nitrated proteins in inflammation. The NO-derived iNOS also has a role in stimulating further neutrophil accumulation at the site of inflammation (Greenacre et al 2002).

The neutrophil cell membrane also mediates the interaction of the neutrophil with the extracellular environment; it expresses a complex array of adhesion molecules and receptors for various ligands, including mediators, cytokines, immunoglobulins and membrane molecules in other cells. The neutrophil recruitment is dependent on the expression of adhesion molecules, such as selectin and, particularly, the intercellular adhesion molecule (ICAM-1), in endothelial cells that serve as ligands for both CD11a/CD18 and CD11b/CD18 on the neutrophil membrane (Seely et al 2003). In this context, the hypothesis that *Solidago chilensis* can inhibit adhesion molecules cannot be discarded.

Further, in the case of *Solidago virgaurea*, flavonoids such as quercitrin and hyperoside are considered to be the main active constituents with anti-inflammatory activity. For the European goldenrod, the presence of flavonoids has also been reported and, additionally, a phenolic acid derivative named leiocarposide, which is also considered to be an active component in relation to anti-inflammatory activity, according to in-vivo assays (Metzner et al 1984). In the case of *Solidago chilensis*, we have isolated from its BuOH fraction some compounds identified as chlorogenic acid and caffeic acid. Some of the anti-inflammatory activity presented here may be attributed to these compounds but further analysis must be done to clarify other compounds involved in this effect.

Conclusions

Our results showed that *Solidago chilensis* Meyen has significant inhibitory activity in the experimental animal model of acute inflammation and inhibits not only inflammatory responses caused by carrageenan, but also acts in the suppression of activated leucocyte infiltration. All anti-inflammatory effects of *Solidago chilensis* were similar to those observed

with animals pre-treated with either indometacin or dexamethasone. It is possible that *Solidago chilensis* has a similar anti-inflammatory mechanism of action to that of indometacin or dexamethasone.

References

Benincá, J. P. Montanher, A. B., Zucolotto, S. M. Schenkel, E. P., Fröde, T. S. (2007) Evaluation of the anti-inflammatory efficacy of *Passiflora edulis. Food Chem*. **104**: 1097–1105

Brazilian Pharmacopea (1929) Companhia Editora Nacional, São Paulo, Brazil

Cabrera, A. L. (1974) *Flora ilustrada de entre rios*. Parte VI, Tomo VI. INTA, Buenos Aires, Argentina

Chang, D. M., Shyue, S. K., Liu, S. H., Chen, Y. T., Yeh, C. Y., Lai, J. H., Lee, H. S., Chen, A. (2004) Dual biological functions of an interleukin-1 receptor antagonist–interleukin-10 fusion protein and its suppressive effects on joint inflammation. *Immunology* **112**: 643–650

Choi, S. Z., Choi, S. U., Bae, S. Y., Pyo, S., Lee, K. R. (2005) Immunobiological activity of a new benzyl benzoate from the aerial parts of *Solidago virgaurea* var. gigantean. *Arch. Pharm. Res*. **28**: 49–54

Fröde, T. S., Medeiros, Y. S. (2001) Myeloperoxidase and adenosine-deaminase levels in the pleural fluid leakage induced by carrageenan in the mouse model of pleurisy. *Mediators Inflamm*. **10**: 223–227

Gaut, J. P., Yeh, G. C., Tran, H. D., Byun, J. Henderson, J. P., Richter, G. M., Brennan, M. L., Lusis, A. J., Belaaouai, A., Hotchkiss, R. S., Heinecke, J. W. (2001) Neutrophils employ the myeloperoxidase system to generate antimicrobial brominating and chlorinating oxidants during sepsis. *Proc. Natl Acad. Sci*. USA **98**: 11961–1196

Giusti, G., Galanti, B. (1984) Adenosine deaminase: colourimetric method. In: Bergmeyer, H. U. (ed.) *Methods of enzymatic analyses*. 3th edn, Verlag Chemie, Weinheim, Germany, pp 315–323

Goulart, S., Moritz, M. I. G., Lang, K. L., Liz, R., Schenkel, E. P., Fröde, T. S. (2007) Anti-inflammatory evaluation of *Solidago chilensis* Meyen in a murine model of pleurisy, *J. Ethnopharmacol*. **113**: 346–353

Green, I. C., Wagner, D. A., Glowski, J, Skipper, P. L., Wishnok, J. S., Tannenbaum, S. B. (1982) Analysis of nitrate, nitrite and [15N] nitrate in biological fluids. *Anal. Biochem*. **126**: 131–138

Greenacre, S. A., Rocha, F. A., Rawlingson, A., Meinerikandathevan, S., Poston, R. N., Ruiz, E., Halliwell, B., Brain, S. D. (2002) Protein nitration in cutaneous inflammation in the rat: essential role of inducible nitric oxide synthase and polymorphonuclear leukocytes. *Br. J. Pharmacol*. **136**: 985–994

Gross, S. C., Goodarzi, G., Watabe, M., Bandyopadhyay, S., Pai, S. K., Wataben K. (2002) Antineoplastic activity of *Solidago virgaurea* on prostatic tumor cells in an SCID mouse model. *Nutr. Cancer* **43**: 76–81

Gutierrez, A. B., Oberti, J. C., Juliani, H. R. (1981) Constituents of *Solidago chilensis* (Compositae). *An. Asoc. Qmic. Arg*. **69**: 27–31

Klebanoff, S. J. (2005) Myeloperoxidase: friend and foe. *J. Leuk. Biol*. **77**: 1–28

Lorenzi, H., Matos, F. J. A. (2002) *Plantas medicinais no Brasil-Nativas e exóticas*. Instituto Plantarum de Estudos da Flora, São Paulo, Brazil

Melzig, M. F. (2004) Goldenrond – a classical exponent in the urological phytotherapy. *Wien. Med. Wochenschr*. **154**: 523–527

Metzner, J., Hirschelmann, R., Hiller, K. (1984) Antiphlogistic and analgesic effects of leiocarposide, a phenolic bisglucoside from *Solidago virgaurea* L. *Pharmazie* **39**: 869–870

Morel, A. F., Dias, G. O., Porto, C., Simionatto, E., Stuker, C. Z., Dalcol, I. I. (2006) Antimicrobial activity of extractives of *Solidago microglossa. Fitoterapia* **77**: 453–455

Mors, W. B., Rizzini, C. T., Pereira, N. A. (2000) *Medicinal plants of Brazil*. Reference Publications, Algonac, USA

Nathan, C. (2006) Neutrophils and immunity: challenges and opportunities. *Nat. Rev. Immunol*. **6**: 173–182

Rao, T. S., Curie, J. L., Shaffer, A. F., Isakson, P. C. (1993) Comparative evaluation of arachidonic acid (AA)-and tetradecanoylphorbol acetate (TPA)-induced dermal inflammation. *Inflammation* **17**: 723–741

Ruth, J. H., Amin, M. A., Wood, J. M., He, X. Samuel, S., Yi, N., Haas, C. S., Koch, A. E., Bullard, D. C. (2005) Accelerated development of arthritis in mice lacking endothelial selectins. *Arthritis Res. Ther*. **7**: 959–970

Saleh, T. S. F., Calixto, J. B.; Medeiros, Y. S. (1996) Anti-inflammatory effects of theophylline, cromolyn and salbutamol in a murine model of pleurisy. *Br. J. Pharmacol*. **118**: 811–819

Saleh, T. S. F., Calixto, J. B., Medeiros, Y. S. (1999) Effects of anti-inflammatory drugs upon nitrate and myeloperoxidase levels in the mouse pleurisy induced by carrageenan. *Peptides* **20**: 949–956

Sampson, J. H., Phillipson, J. D., Bowery, N. G., O'Neill, M. J., Houston, J. G., Lewis, J. A. (2000) Ethnomedicinally selected plants as sources of potential analgesic compounds: indication of in vitro biological activity in receptor binding assays. *Phytother. Res*. **14**: 24–29

Sanchez de Medina, F., Vera, B., Galvez, J., Zarzuelo, A. (2002) Effect of quercitrin on the early stages of hapten induced colonic inflammation in the rat. *Life Sci*. **70**: 3097–3108

Schmeda-Hirschmann, G. (1987) A labdan diterpene from *Solidago chilensis* roots. *Planta Med*. **2**: 179–180

Schmid-Schönbein, G.W. (2006) Analysis of inflammation, *Annu. Rev. Biomed. Eng*. **8**: 93–151

Sedgwick, A. D., Lees, P. A. (1986) Comparison of air pouch, sponge and pleurisy models of acute carrageenan inflammation in the rat. *Agents Actions* **18**: 439–446

Seely, A. J. E., Pascual, J. L., Cristou, N. V. (2003) Science review: cell membrane expression (connectivity) regulates neutrophil delivery, function and clearance. *Crit. Care* **7**: 291–307

Smith, J. A. (1994) Neutrophils, host defense, and inflammation: a double-edged sword. *J. Leuk. Biol*. **56**: 672–686

Spicuzza, L., Di Maria, G., Polosa, R. (2006) Adenosine in the airways: implications and applications. *Eur. J. Pharmacol*. **533**: 77–86

Tian, B., Nowak, D. E., Brasier, A. R. (2005) A TNF-induced gene expression program under oscillatory NF-Kappa B control. *BMC Genomics* **6**: 137

Yocum, D. E., Furst, D. E., Kaine, J. L., Baldassare, A. R., Stevenson, J. T., Borton, M. A., Mengle-Gaw, L. J., Schwartz, B. D., Wisemandle, W., Mekki, Q. A. (2003) Efficacy and safety of tacrolimus in patients with rheumatoid arthritis: a double-blind trial. *Arthritis Rheum*. **48**: 3328–3337