Anti-inflammatory activity of extracts and 11,13-dihydrozaluzanin C from Gochnatia polymorpha ssp. floccosa trunk bark in mice

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Aim of this study: Gochnatia polymorpha ssp. floccosa (Asteraceae), popularly known as “cambará”, is well recognized in Brazilian traditional medicine to treat the respiratory tract inflammatory diseases and rheumatism. However, no scientific data have been published to support this ethnomedicinal use. This work aimed to evaluate the anti-inflammatory action of its ethanol (EEGP) extract, ethyl acetate (EA), dichloromethane (DCM), petroleum ether (PE) butanolic (BT) fractions, and the isolated compounds (GPC1) and (GPC2). Materials and methods: The anti-inflammatory activities were evaluated in mice subjected to paw oedema and carrageenan-induced air pouch inflammation models. Results: The oral administration of EEGP (30, 100 and 300 mg/kg), DCM (50 mg/kg), BT (20 mg/kg) and GPC2 (10 and 30 mg/kg), but not EP and EA fractions (both at 30 mg/kg) and GPC1 (1 and 10 mg/kg), significantly inhibited the paw oedema induced by carrageenan (41 ± 13, 39 ± 5 and 60 ± 10% for EEGP at the three doses, respectively; 44.47 ± 12.8 and 70.19 ± 11.52% for DCM and BT, respectively; and 29.52 ± 4.8 and 31.67 ± 5.4% for 11,13-dihydrozaluzanin C at 10 and 30 mg/kg, respectively) compared to control group. The oral administration of EEGP (30, 100 and 300 mg/kg) inhibited the carrageenan-induced leukocyte migration in the air pouch model (37.2 ± 12.5, 62.6 ± 5.0 and 54.3 ± 6.8%, respectively), as well as protein extravasation (47.9 ± 12.5, 51.7 ± 15.2 and 60.9 ± 13.7%, respectively) compared to control group. In a similar way, DCM (50 mg/kg) or GPC2 (10 mg/kg), but not BT (20 mg/kg) given by oral route inhibited leukocyte infiltration into the pouch (29.5 ± 10.6 and 54.4 ± 21.8%, respectively). Also DCM and GPC2 significantly reduced the protein levels in the supernatants (52.4 ± 15.0 and 51.83 ± 16.9%, respectively). Conclusion: The results suggest that EEGP, and BT and DCM fractions from G. polymorpha possess anti-inflammatory activity and probably the compound 11,13-dihydrozaluzanin C was responsible, at least in part, for this action.

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Natural compounds with different mechanisms of action can be used to treat inflammatory diseases (Rios et al., 2009), especially from plants being used for such purposes. A number of plants traditionally used exhibit pharmacological properties with great potential in therapeutic applications. One important example is the willow tree and salicin that have been associated with salicylic acid, the key precursor molecule that has contributed to the discovery of acetylsalicylic acid, marketed as aspirin (Mahdi et al., 2006).

The genus Gochnatia (Asteraceae) comprises 70 species, which are found mainly in America, from Mexico to Argentina (Katina et al., 2008). Plants from Gochnatia genus are trees, which have been popularly considered as medicinal (Zardini, 1984; Rodrigues and Carvalho, 2007). Gochnatia polymorpha (Less) Cabr. is the most abundant species growing in Brazil, Paraguay, Uruguay, and Argentina, being recognized three subspecies, polymorpha, ceanothifolia, and floccosa (Cabrera and Klein, 1973). It is known as “cambará”, and flowers, leaves and trunk bark have been used in the traditional medicine for treating cough, bronchitis, colds, sore throat and respiratory diseases in general (Cabrera and Klein, 1973; Mentz and Schenkeln, 1989; Schmeda-Hirschmann and Bordas, 1990; Garlet and Irgang, 2001; Bueno et al., 2005; Araubbarri et al., 2008). Ethnopharmacology studies showed that a of infusion of the bark (1–10%) of this plant is used to sore throat in Kaiowá and Guarani indigenous populations in the Caarapó Reserve, Mato Grosso do Sul, Brazil (Bueno et al., 2005) while the leaves and trunk bark of G. polymorpha are used as tea and syrup in south of Brazil for inflammatory diseases from respiratory system (Garlet and Irgang, 2001).

Previous chemical studies addressing G. polymorpha (subspecies polymorpha or unspecified) have reported the occurrence of sesquiterpene lactones, dimeric guaianolides, bisabolones, diterpenes, triterpenes and coumarins (Farias et al., 1984; Bohmann et al., 1986; Sacilletto et al., 1997; Catalán et al. 2003). The leaves showed anti-inflammatory activity and flavonoids and phenolic compounds were identified in the active extracts, but they were not the active compounds responsible to this property (Moreira et al., 2000).

Screening for antimicrobial and cytotoxic activities of various parts of G. polymorpha ssp. floccosa showed that only dichloromethane extract of trunk bark had significant antibacterial and antifungal activities. Extracts were inactive in the brine shrimp lethality bioassay (Stefanello et al., 2006). There are no other studies with this subspecies.

The present work reports the results of anti-inflammatory evaluation of the ethanolic extract, DCM, and other fractions and isolated compounds, namely bauerenyl acetate (GPC1) and 11,13-dihydrozaluzanin C (GPC2) from trunk bark of G. polymorpha ssp. floccosa.

2. Materials and methods

2.1. Animals

The experiments were conducted using male Swiss mice (25–35 g), housed under 12 h light–dark cycle, controlled humidity (60–80%), and temperature (22 ± 1 °C) conditions. Food and water were freely available to the mice. The animals were acclimatized to the experimentation room for at least 2 h before testing and were used only once throughout the experiments. The studies reported in this manuscript were carried out in accordance with the current guidelines for the care of laboratory animals and following the guidelines set by the U.S. National Institute of Health and was approved by the ethics committee for research on laboratory-animal use of the institution (Nbr. 336). The number of animals was the minimum necessary to show consistent effects of the drug treatments.

2.2. Plant material

Trunk bark of G. polymorpha (Less) Cabr. ssp. floccosa Cabr. was collected in Curitiba, Paraná State, Brazil, in March 2004, and identified by Dr. Armando C. Cervi, Universidade Federal do Paraná. A voucher specimen (UPCB 30.100) is deposited at the herbarium of the Universidade Federal do Paraná, Brazil.

2.3. Preparation of extracts, fractions and isolation of compounds

Dried and powdered trunk bark (129 g) was extracted at room temperature with n-hexane and ethanol 95%, successively. The solvents were removed under vacuum, yielding the crude extracts in hexane (0.2%) and ethanol of G. polymorpha (EEGP, 1.8% or 2.322 g). EEGP was suspended in EtOH–H2O (1:1) and partitioned with dichloromethane (DCM), ethyl acetate (EA) and 1-butanol (BT), successively. The organic layers were separated and the solvents evaporated to give the respective residues (DCM 46.5% or 1.07973 g; EA 11.8% or 0.2739 g; BT 25% or 0.2739 g). The DCM residue (11.8 g) was submitted to silica gel VLC eluted with petroleum ether (PE), DCM, EA and MeOH. The fraction eluted with DCM (2.10 g) was submitted to silica gel chromatographic column (CC) eluted with increasing amounts of EA in DCM. The fractions were analyzed by thin-layer chromatography (TLC) using silica gel plates developed in several solvent systems and compounds were visualized by exposure under UV254/366 light and spraying with 5% (v/v) sulfuric acid (H2SO4) in ethanol solution, followed by heating on a hot plate. After this analysis, six fractions were obtained. Fraction II (85.2 mg) yielded G. polymorpha compound 1 (GPC1, 40.0 mg) or bauerenyl acetate after recrystallization in hexane. Fraction VII (1.1 g) was submitted to another silica gel CC, eluted with increasing amounts of acetone in hexane to give 10 fractions after TLC analysis. Fraction IV (350 mg) yielded G. polymorpha compound 2 (GPC2) or 11,13-dihydrozaluzanin C almost pure. An aliquot (25.0 mg) was recrystallized in n-hexane, yielding pure GPC2 (9.6 mg). Nuclear magnetic resonance spectra were recorded on a Bruker spectrometer (AC-200, operating at 200 MHz for 1H and at 50 MHz for 13C), using CDCl3 (deuterated chloroform) as solvent and tetramethylsilane as internal reference. The yielding of the fractions and isolated compounds were used to calculate the doses to be used.

2.4. Materials and reagents

α-Carrageenan (Cg) and dexamethasone were purchased from Sigma Chemical Co. (St. Louis, MO). Other drugs and reagents used were of analytical grade.

2.5. Carageenan-induced cell migration into the pouch

Air pouch model of inflammation was performed as described previously (Garcia-Ramallo et al., 2002), with some modifications. Mice were briefly anesthetized with ketamine and xylazine and 4 mL of sterile air was injected subcutaneously in the back of mice, on day 0. On day 3, a second injection of 2 mL of sterile air was performed into the preformed pouch. On day 6, animals were treated with vehicle, dexamethasone (0.5 mg/kg, s.c.) as positive control drug or EEGP (30, 100 and 300 mg/kg orally), DCM fraction (30 mg/kg orally), BT fraction (20 mg/kg orally), and 11,13-dihydrozaluzanin C (10 mg/kg, orally) and 1 h later the inflammatory response was induced by a injection of a solution of carageenan 0.1% (250 µl) directly into the pouch.
Four hours after the inflammatory stimulus mice were euthanized and the pouches were washed with 2 mL of phosphate-buffered saline (PBS) containing heparin (10 UI/mL). Exudates were collected and total cells were counted on Neubauer chamber under light microscopy. Exudates were then centrifuged at 1000 × g for 2 min at 4 °C and the pellet suspended in 1 mL of 3% albumin and added to previously prepared slides. Cells were stained with Rosenfeld and analyzed under light microscopy and 100 cells were counted.

2.5.1. Plasma leakage evaluation
Supernatant obtained after exudates centrifugation as described above was used to evaluate protein leakage to the inflammatory site. Protein levels were measured in the supernatants by Bradford’s reaction (Bradford, 1976), using a commercially available Bradford method (Bioagency, São Paulo, Brazil). Briefly, 10 μL of the samples were added to 200 μL of Bradford’s reactant and absorbance was measured at 595 nm. Results were interpolated to a standard curve and represented as mg/protein/100 μL.

2.6. Carrageenan-induced paw-oedema
Different groups of mice were orally treated with EEGP (30, 100 and 300 mg/kg), DCM fraction (50 mg/kg), other fractions such as BT (20 mg/kg), EA and PE (both at 30 mg/kg), GPC1 (1 and 10 mg/kg) or GPC2 (1, 3, 10 and 30 mg/kg) or vehicle. Another group of mice were treated subcutaneously with the anti-inflammatory drug dexamethasone (0.5 mg/kg). After 1 h, the animals received a 50 μL s.c. injection into the right hindpaw of carrageenan (300 μg/paw) suspended in sterile 0.9% saline. The contralateral paw received only saline and was used as control. The thickness of the paw oedema was measured using a digital micrometer (Great, MT-045B) 1 h before any treatment and at different time points (0.5, 1, 2, 4 and 24 h) after the injection of carrageenan. Results were expressed in μm and the difference between basal and post-injection values quantified as oedema (Kassuya et al., 2009).

2.7. Determination of myeloperoxidase (MPO) activity
To investigate whether oral treatment with extract, fractions and isolated compounds could affect the cellular migration induced by carrageenan the myeloperoxidase activity was measured into the mouse paw. For this purpose, animals were treated with GPC2 (1–30 mg/kg) or vehicle (solution with 0.1% Tween 80) 1 h prior to carrageenan injection, the tissues of the injected paws were removed and processed as described before (De Young et al., 1989). For MPO activity, the tissue was homogenized in 5% (w/v) of 80 mM phosphate buffer, pH 5.4, containing 0.5% of hexadecyltrimethylammonium bromide. The homogenate was centrifuged at 12,000 × g and 4 °C for 20 min. Aliquots (30 μL) of each supernatant were mixed with 100 μL of phosphate buffer 80 mM, 85 μL of phosphate buffer 0.22 M and 15 μL of 0.017% H2O2 on a 96-well plate. The reaction was triggered with 20 μL of 3,3,5-tetramethylbenzidine (dissolved in N,N-dimethylaniline). The plate was kept at 37 °C for 3 min, after which the reaction was stopped by adding 30 μL of sodium acetate 1.46 M, pH 3.0. The enzymatic activity was determined by measuring the optical density at 630 nm and was expressed as mOD per mg of protein.

2.8. Statistical analysis
Data are presented as mean ± S.E.M. Difference between groups was evaluated by analyses of variance (one-way ANOVA) followed by Newman–Keuls test. The number of animals per group is indicated in the legends. Statistical differences were considered to be significant at P < 0.05. Asterisks (*) denote significant differences compared to vehicle-treated group.

3. Results

3.1. Bio-guided isolation of compounds from G. polymorpha trunk bark
The crude extract in ethanol of trunk bark of G. polymorpha ssp. floccosa (EEGP) showed anti-inflammatory activity, which remained in the dichloromethane and 1-butanol fractions. Chromatographic fractionation of dichloromethane fraction yielded two major compounds, GPC1 and GPC2. Analysis of NMR spectra and comparison with reported data led to identification of GPC1 as bauerenyl acetate, a pentacyclic triterpene (Fig. 1A) previously reported in the bark of G. polymorpha ssp. unspecified (Farias et al., 1984). In the same way, GPC2 was identified as sesquiterpene lactone 11,13-dihydrozaluzanin C (Fig. 1B) (Bohlmann and Chen, 1982). This compound is being reported for the first time in G. polymorpha.

3.2. Effect of EEGP on carrageenan-induced leukocyte migration into the pouch
The injection of carrageenan in the pouch in the sixth-day-old promoted a plasma leakage and leukocyte migration comprised mainly by polymorphonuclear leukocytes (Fig. 2A–C). Analysis of variance showed significant difference between groups on leukocyte migration (F(5,46) = 20.76; P < 0.001) and protein exudation (F(5,26) = 6.713; P < 0.001). Analysis of the inflammatory activity of EEGP (30–300 mg/kg) indicated that this extract inhibited leukocyte migration (37.2 ± 12.5%, 62.6 ± 5.0% and 54.3 ± 6.8%, respectively) and plasma leakage (47.9 ± 12.5%, 51.7 ± 15.2% and 60.9 ± 13.7%, respectively). Dexamethasone, used as positive control, reduced both leukocyte infiltration into the pouch and protein exudation (76.7 ± 5.5% and 63.6 ± 12.9%, respectively) 4 h after carrageenan injection (Fig. 2A–C).

3.3. Effect of EEGP on carrageenan-induced paw oedema
The injection of carrageenan in the paw induced an oedema that started at 30 min and peaked at 2 h (Fig. 3A). Analysis of variance showed significant difference between groups (F(4,21) = 5.387; P < 0.01). The oral treatment with EEGP significantly inhibited Cg-induced paw oedema formation in a dose dependent manner (Fig. 3A and B), the inhibition were of 41 ± 13%, 39 ± 5% and 60 ± 10% at doses of 30, 100 and 300 mg/kg, respectively (Fig. 3A and B). Moreover, our results showed that the EEGP at dose of 100 mg/kg, presented its maximal inhibition 2 h after its admin-
Fig. 2. Effect of EEGP on carrageenan-induced leukocyte migration and plasma leakage into the air pouch. Mice (n = 8–10) were pretreated 1 h before with EEGP (30–300 mg/kg, p.o.), dexamethasone (0.5 mg/kg, s.c., diluted in saline) or vehicle. Pouches were washed with PBS containing heparin. Cells were counted and plasma leakage was analyzed. In (A), number of cells that migrates to the pouches 4 h after carrageenan. Results are expressed as cell 10^6/cavity. In (B), plasma leakage was measured by Bradford’s reaction. Results are expressed as mg of protein/ml. In (C), differential cell counting was performed. *P < 0.05, **P < 0.01, ***P < 0.001, compared to the vehicle group. Difference between groups were analyzed by analysis of variance (one-way ANOVA) followed by Newman–Keuls test.

Fig. 3. Effect of EEGP on carrageenan-induced paw oedema in mice. Animals received EEGP (30, 100 or 300 mg/kg, p.o.), dexamethasone (DEX – 0.5 mg/kg, s.c.) or vehicle and after 1 h, an intraplantar injection of carrageenan (300 μg/paw) was performed. In (A), the time-course of the inhibition induced by 100 mg/kg EEGP and dexamethasone is shown. In (B), bars show the effect of different doses of EEGP and dexamethasone in paw oedema (mm) 2 h after carrageenan injection. Each bar or point represents the mean ± SEM of 6 animals. *P < 0.05, **P < 0.01, ***P < 0.001, compared with vehicle-treated group.

3.4. Effect of fractions from G. polymorpha on carrageenan-induced paw oedema

The oral treatment of the animals with DCM fraction (50 mg/kg) and BT fraction (20 mg/kg), but not EA or PE fraction (30 mg/kg, Fig. 4A) significantly inhibited the Cg-induced paw oedema (Fig. 4A). Analysis of variance showed significant difference between groups (F(3,17) = 61.85; P < 0.0001). The percentage of inhibition at 120 min after carrageenan injection was 44.47 ± 12.8 and 70.19 ± 11.52% for DCM and BT fractions, respectively. Dexamethasone also inhibited oedema formation (88.24 ± 8.03%) in comparison to vehicle pre-treated group (Fig. 4A).

3.5. Effect of fractions from G. polymorpha on carrageenan-induced leukocyte migration into the pouch

When DCM (50 mg/kg), but not BT (20 mg/kg), was given to the animals, the leukocyte migration (including neutrophil and mononuclear cells) was inhibited and the maximal inhibition observed with DCM fraction was 29.53 ± 10.6% when compared to vehicle group (Fig. 4, panels B and D). However, both DCM (50 mg/kg) and BT fraction (20 mg/kg) inhibited plasma leakage with inhibition of 52.42 ± 15.0% for DCM fraction and 58.2 ± 11.2% for BT fraction (Fig. 4C). Difference between groups on leukocyte migration (F(4,29) = 27.69; P < 0.0001) and plasma leakage (F(4,30) = 13.08; P < 0.0001) was showed by analysis of variance.

3.6. Effect of 11,13-dihydrozaluzanin C and bauerenyl acetate on carrageenan-induced paw oedema and increase of MPO activity

Pre-treatment of the animals with GPC2 (10 and 30 mg/kg, p.o.), but not with GPC1 (1 and 10 mg/kg) reduced oedema forma-
Fig. 4. Effect of DCM, BT, EP or EA fractions on carrageenan-induced paw oedema and leukocyte migration and plasma leakage into the air pouch. Mice received DCM (50 mg/kg p.o.), BT (20 mg/kg, p.o.), PE or EA (both at 30 mg/kg, p.o.) dexamethasone (0.5 mg/kg, s.c.) or vehicle 1 h before carrageenan stimulus (n = 4–6 animals per group). In (A), oedema was observed at different 120 min (n = 4–6 animals per group). In (B), number of cells that migrates to the pouch 4 h after carrageenan stimulus. Results are expressed as cell 10^6/cavity. In (C), plasma leakage was measured by Bradford’s reaction. Results are expressed as mg of protein/ml. In (D), differential cell counting was performed. **P < 0.01, ***P < 0.001 compared to the vehicle group. Difference between groups was analyzed by analysis of variance (one-way ANOVA) followed by Newman–Keuls test.

Paw oedema inhibition produced by GPC2 (1–30 mg/kg) was dose dependent (Fig. 6A). Analysis of variance showed significant difference between groups (F(5,27) = 8.189; P < 0.0001). At doses of 10 and 30 mg/kg, but not lower doses, GPC2 was able to attenuate oedema formation at 120 min after carrageenan injection (29.52 ± 4.8 and 31.67 ± 5.4%, respectively). Dexamethasone inhibited oedema formation (45.49 ± 5.83%) at 120 min after inflammatory stimulus (Fig. 6A).

The subcutaneous injection of carrageenan (300 μg/paw) increased MPO activity about three-fold when compared with the paws of animals treated saline, resulting from carrageenan-induced leukocyte migration to the local of insult, thereby, it is a indicative of leukocyte presence (Fig. 6B). Analysis of variance showed significant difference between groups (F(6,11) = 24.85; P < 0.0001). The GPC2 was able to inhibit significantly (30.6 ± 9.44, 51.27 ± 3.38 and 54.56 ± 2.36%) the MPO activity with dose of 3, 10 and 30 mg/kg (Fig. 6B).

3.7. Effect of 11,13-dihydrozaluza and 11,13-dihydrozaluza on carrageenan-induced leukocyte migration into the pouch

The isolated compound GPC2 at 10 mg/kg, reduced significantly the leukocyte migration (mainly polymorphonuclear cells) to the cavity (54.37 ± 21.8% (F(3,20) = 25.87; P < 0.0001)) as illustrate in Fig. 7, panels A and C. Animals which were pre-treated with GPC2 (10 mg/kg), showed a decreased plasma leakage (51.83 ± 16.9% (F(3,19) = 12.90; P < 0.0001) Fig. 7, panel B) compared to vehicle pre-treated.

Fig. 5. Effect of bauerenyl acetate and 11,13-dihydrozaluza on carrageenan-induced paw oedema in mice. In the upper panels, animals received bauerenyl acetate (1 and 10 mg/kg, p.o.), 11,13-dihydrozaluza (10 mg/kg, p.o.), dexamethasone (Dex – 0.5 mg/kg, s.c.) or vehicle and after 1 h, an intraplantar injection of carrageenan (300 μg/paw). In (A), the time-course of the inhibition induced by bauerenyl acetate and 11,13-dihydrozaluza is shown. In (B), a dose response curves for bauerenyl acetate in paw oedema (mm) 2 h after carrageenan injection. Each bar or point represents the mean ± SEM of 6 animals. *P < 0.01, compared with vehicle-treated group.
Fig. 6. Effect of 11,13-dihydrozaluzanin C on carrageenan-induced paw oedema in mice. In the upper panels, animals were treated with 11,13-dihydrozaluzanin C (1–30 mg/kg, p.o.), dexamethasone (DEX – 0.5 mg/kg, s.c.) or vehicle and after 1 h, an intraplantar injection of carrageenan (300 μg/paw). In (A), bars show the effect of different doses of 11,13-dihydrozaluzanin C and dexamethasone in paw oedema (mm) 2 h after carrageenan injection. In (B), the inhibition induced by 11,13-dihydrozaluzanin C in increasing of myeloperoxidase (MPO) activity induced by local injection of carrageenan. Each bar or point represents the mean ± SEM of 6 animals.* P < 0.05, *** P < 0.001, compared with vehicle-treated group.

Fig. 7. Effect of 11,13-dihydrozaluzanin C on carrageenan-induced leukocyte migration and plasma leakage into the air pouch. Mice (n = 8–10) were pretreated 1 h before with 11,13-dihydrozaluzanin C (10 mg/kg, p.o.), dexamethasone (0.5 mg/kg, s.c., diluted in saline) or vehicle. Pouches were washed with PBS containing heparin. Cells were counted and plasma leakage was analyzed. In (A), number of cells that migrates to the pouches 4 h after carrageenan. Results are expressed as cell 10^6/cavity. In (B), plasma leakage was measured by Bradford’s reaction. Results are expressed as mg of protein/ml. In (C), differential cell counting was performed. * P < 0.05, *** P < 0.001, compared to the vehicle group. Difference between groups were analyzed by analysis of variance (one-way ANOVA) followed by Newman–Keuls test.

4. Discussion

The present study showed inhibitory properties of oral treatment of the ethanolic extract (EEGP), DCM and BT fractions obtained from G. polymorpha, a plant popularly used against inflammatory diseases in some countries, including Brazil. Furthermore, the anti-inflammatory effect, specially inhibition of leukocyte migration and plasma leakage, of the crude extract and DCM fraction is associated with the sesquiterpene lactone 11,13-dihydrozaluzanin C. G. polymorpha, which has been used by the population as a natural remedy to treat infectious or inflammatory conditions (Mors et al., 2000). These facts lead us to investigate the potential anti-inflammatory effect of this plant. Only the study of Moreira et al. (2000) has been published to support this ethnopharmacological use of G. polymorpha. Our study extended and corroborated the study of Moreira et al. (2000) showing the ability of the EEGP in inhibiting the oedema, myeloperoxidase activity and leukocyte migration induced by carrageenan in the paw and in the air pouch. The marked increase in the myeloperoxidase activity into the paw indicated an inflammatory process induced by carrageenan, instead of only oedema, and these results corroborated with others showing the anti-inflammatory efficacy induced by EEGP. We also evaluated the anti-inflammatory effects of semi-purified (BT, DCM, PE, EA) fractions obtained from EEGP. Our results demonstrated that the BT or DCM fractions, tested by oral route, were effective in reducing the paw oedema induced by carrageenan in mice and also inflammation into the pouch.

The potential anti-inflammatory or anti-oedematogenic properties of pharmacological substances experimentally induced in rodents uses the digital water plethysmometer or micrometer methodology. The bedding of plethysmometer is the water displacement produced by immersion of the animal paw in the measuring tube inducing a change in the conductance between electrodes. Sharma et al. (2004) compared plethysmometer and
micrometer methods to measure acute paw oedema for screening anti-inflammatory activity in mice and described and suggested that micrometer method is more sensitive to detect the lowest anti-inflammatory dose of indomethacin when compared with the plethysmometer method (Sharma et al., 2004).

Both DCM and BT fractions inhibited oedema formation and protein extravasation induced by carrageenan which suggests that both fractions possess compounds that potentially should be studied as anti-inflammatory. However, DCM fraction showed an inhibitory effect on leukocyte migration, an important additional activity which certainly contributes to its anti-inflammatory activity. For this reason, we decided to focus our studies, initially, in this fraction. Phytochemical studies showed that DCM fraction of G. polymorpha ssp. floccosa presents as major components the triterpene bauerenyl acetate (GPC1) and the sesquiterpene lactone 11,13-dihydrozaluzanin C (GPC2). In the present study, only the sesquiterpene lactone 11,13-dihydrozaluzanin C presented anti-inflammatory activity when tested in a DCM yielding-based dose. Moreira et al. (2000) have showed that ethanolic fraction of G. polymorpha leaves showed significant anti-oedemagenic activity with a dose of 150 mg/kg on carrageenan model in rats. The amino acid 4-hydroxy-N-methyl-proline isolated from G. polymorpha showed significant inhibition of oedema with a dose 200 mg/kg (Moreira et al., 2000) but not in equivalent doses of extract exhibiting activities. Probably the variation in composition of G. polymorpha according to the part of plant (leaves) and season of the collected species could explain previous results reporting the most activity in the more polar fraction (ethyl acetate), containing caffeic acid, chlorogenic acid, 3-O-methylquercetin, rutin and hyperoside (Moreira et al., 2000). Nevertheless, the anti-inflammatory effects of 11,13-dihydrozaluzanin C have never been described. The compound 11,13-dihydrozaluzanin C was able to inhibit Cg-induced oedema formation and decrease the MPO activity into the injured tissue in the Cg-induced paw oedema in mice. The MPO is an enzyme present, mainly, in neutrophils and the increase of in the MPO activity reflects the neutrophil migration into the tissue during inflammation. Thus, this is the first study showing that oral administration of 11,13-dihydrozaluzanin C reduces, in a dose-dependent manner, leukocyte migration and plasma leakage, suggesting that this sesquiterpene lactone is, at least in part, responsible for the anti-inflammatory effects of the G. polymorpha.

The pharmacological activities of some medicinal plants, especially those from the sunflower family Asteraceae, are attributed to their contents of sesquiterpene lactones such as mikanolide, helenalin, parthenolidine, artemisinin, bis(isoalantoliotil-B)-glutarate (Valerio et al., 2007). In fact, sesquiterpene lactones may present a range of pharmacological activities in vitro including anti-inflammatory, antiproliferative, and anti-microbial activities. Therefore, this study has been performed to investigate the anti-inflammatory activity of 11,13-dihydrozaluzanin C in combination with the anti-inflammatory activity of 8α-hydroxy-11α,13-dihydrozaluzanin C, zaluzanin-C and estafiatone, both isolated from Ainsliaea acerfolia which has been used as antipyretic, pain-relief, and anti-inflammatory in traditional herbal medicine in Korea (Shin et al., 2005), present anti-inflammatory activity which may be due to a pharmacophoric group in the molecule. The exocyclic α-methylene-γ-lactone was important for cytotoxic activity of analogs of sesquiterpene lactone helenalin (Lee et al., 1971, 1973, 1977; Lee and Furukawa, 1972; Hall et al., 1978).

The study of Shin et al. (2005) showed also that zaluzanin-C and estafiatone suppressed of NF-κB activation by inhibition of nuclear translocation of NF-κB resulting from blockade of the degradation of IκB, leading to suppression of the expression of iNOS and COX-2, which display important role in inflammatory signaling pathways. In addition, others analogs of 11,13-dihydrozaluzanin C, such as 8α-hydroxy-11α,13-dihydro-3β-O-β-d-glucozaluzanin C, 8α-hydroxy-11α,13-dihydrozaluzanin C, 3α-hydroxy-11β,13-dihydro-8α-O-β-d-glucozaluzanin C, 3β-hydroxy-11β,13-dihydro-8α-O-β-d-glucozaluzanin C showed inhibitory effects against COX-1 and COX-2 in vitro (Wang et al., 2009).

Indeed, it has been described that several extracts from plants rich in sesquiterpene lactones have gained considerable interest for treating human diseases such as inflammation, headache and infections (Ghantous et al., 2010). Few reviews have focused on sesquiterpene lactones extraction, analysis and characterization in biological systems, particularly for defining their anticance and anti-inflammatory activities (Zhang et al., 2005; Gurib-Fakim, 2006).

Thus it could lead as to formulate a hypothesis that 11,13-dihydrozaluzanin C could exert inhibitory effects in leukocyte migration and protein exudation through inhibition of NF-κB or inhibition of COX expression. However, further studies are necessary to verify the mechanism of action of this compound.

This is the first study showing that preparations obtained from G. polymorpha indicating the isolated 11,13-dihydrozaluzanin C are able to cause inflammatory parameters reduction in mice.

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