Vol 6 | Issue 2| 2015 | 69-77.

e - ISSN 2249-7544
Print ISSN 2229-7464

INTERNATIONAL JOURNAL
OF
PHYTOPHARMACY RESEARCH
www.phytopharmacyresearch.com

EVALUATION OF ANTI-INFLAMMATORY POTENTIAL OF *AGERATUM CONYZOIDES* (AC) EXTRACTS *IN VIVO* AND *IN VITRO* MODELS: CONFIRMATION OF POPULAR USE

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ABSTRACT

Ageratum conyzoides (AC), known as "Mentrasto", is a medicinal plant used for the treatment of several inflammatory processes. The aim of this study was to evaluate the anti-inflammatory effects of hydroalcoholic, methanol and ethanol extracts of AC. The activity was evaluated using paw oedema induced by carrageenan, ear oedema induced by croton oil and pleurisy methods using carrageenan, bradykinin (BK), histamine (HIS) and substance P (SP) as flogistic agents. AC was effective in reducing paw oedema, the dose of 300mg/kg was the one that best inhibited oedema. The IMs (maximum inhibitions) were calculated for 69% MeOH, 65% EtOH and 76% for EHA. Both extracts, when incorporated into a cream (1, 3 and 5%), were effective in inhibiting ear oedema. The IMs were calculated as 60% MeOH, 72% EtOH and 77% EHA at a concentration of 5%. EAH (500 mg/kg) inhibited leukocytes by 57.82±1.25%, neutrophils by 58.57±2.22%, mononuclear cells by 49.49±3.52%, PGE2 by 48.16±2.12%, LTB4 activities by 46.67±1.45 and NO levels by 39.00±1.53% in Cg induced pleurisy. EHA also inhibited leukocytes in pleurisy induced by BK (48.80±0.32%), HIS (71.13±0.26%) and SP (56.73±2.26%). *Ageratum conyzoides* (AC) showed an important anti-inflammatory profile in the experimental models used, moreover, several mechanisms including the inhibition of mediator release and action, appear to account for the anti-inflammatory effect of AC.

Keywords: *Ageratum conyzoides*, Popular use, Inflammation, Mice, Pleurisy, Oedema, Carrageenan.

INTRODUCTION

Ageratum conyzoides (Asteraceae) is a herba ceous perennial plant, popularly known as mentrasto, erva-de são-joão, catinga-de-bode among others [1, 2]. It is native to America and adapts to various environmental conditions [3, 1]. It is widely used for medicinal purposes by the populations of Brazil and other countries, and in Brazilian folk medicine it is used to treat various diseases including fever, dermatitis, rheumatism, diarrhea, diuresis, and inflammation, and for wound healing [3, 4]. Studies have shown that the species contains essential oils in the leaves, as well as phenolic compounds [5].

The first pharmacological trials evaluating anti-inflammatory activity of the plant were carried out by [6] but more recently, the hydroalcoholic extract (HAE) of this plant was studied for its anti-inflammatory effect on subacute (cotton pellet-induced granuloma) and chronic (formaldehyde-induced arthritis) models of inflammation in rats [7, 8].

Several chemical components present in the plant (coumarins, saponins, tannins, flavonoids, alkaloids, flavones, chromones and terpenes) may be responsible for the pharmacological effects of plant [9, 10]. In this context, this study was designed to widen knowledge on the anti-inflammatory property of the plant, by evaluating the potential mechanisms involved in this property.

MATERIALS AND METHODS

Plant material

The whole plant of *A. conyzoides* (L.) (AC) was collected in 2007 in Penha at Santa Catarina State, Brazil. The collected specimens were analyzed by botanist Msc. Oscar Benigno Iza, and deposited at the Herbarium

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Barbosa Rodrigues (HBR), Itajaí, SC (number 47.102).

Drugs and treatments

The following drugs and reagents were used: distilled water, lambda carrageenan (grade IV), bradykinin (BK), substance P (SP), histamine (HIS), croton oil, captopril, dexamethasone and indomethacin (IND) from Sigma Chemical Co., St Louis, MO, USA. Heparin ™ (Roche, Brazil), Evans blue dye (Merck, Brazil), Türk solution and May-Grunwald and Giemsa (Laborclin, Pinhais, PR Brazil). PGE2, LTB4, NO ELISA kits (R&D Systems). The drugs were prepared each day (NaCl 0.95%). The other reagents used were also of analytical grade and were obtained from different commercial sources. The doses and routes of administration of *A. conyzoides* materials were selected in accordance with previously established protocols based either on data from the literature [10,11] or on previous tests carried out in our laboratory (unpublished results).

Extract preparation

Fresh aerial parts (50 g) *A. conyzoides* were macerated with a mixture of methanol and water at a ratio of 1:1, in methanol 100% and ethanol 100% at room temperature for 7 days each, The different extracts were then submitted to concentration using a rotary evaporator at reduced pressure, at a maximum temperature of 50°C, to obtain concentrated extracts. The extracts used in the biological assays were hydroalcoholic (methanol:water 1:1), methanol and ethanol. The yields of the hydroalcoholic, methanolic and ethanolic extracts obtained were 4.8%, 4.7 and of 4.5%, respectively and were calculated from the dried leaves.

Animals

Non-fasted adult male Swiss mice (2/5-30 g) were used in the experiments. The animals were maintained in a controlled temperature environment (22 ± 3°C), with daylight supplemented with electric light from 7:00 a.m. to 7:00 p.m., and free access to food and water. The experiments were performed after approval of the protocol by the Institutional Ethics Committee of UNIVALI (037/2009-CEP/UNIVALI) and conducted in accordance with the international standards for study with laboratory animals [12], where the number of animals by experimental group was the minimum necessary to obtain statistical results.

Effect of A. conyzoides in paw oedema induced by carrageenan

The paw oedema experiments were carried out as previously described [13, 14] is the phlogistic agents was used (Carrageenan-Cg). Under ether anesthesia, the animals received 0.05mL intraplantar injections in one hind paw, containing carrageenan (300 µg/paw). The contralateral paw received 0.05mL of PBS which was used as a control.

Oedema was measured using a plethysmometer (Ugo Basile-Italy) at several time-points, after injection of inflammatory mediator or the irritant. Oedema was expressed in millimeters as the differences between the tests in the control paws. Hydroalcoholic, methanol and ethanol extracts, at different concentrations (100, 300 and 500 mg/kg), were administered by the systemic route 30 min prior to testing. Separate groups of animals were treated with dexamethasone (1 mg/kg, i.p.), used as a reference anti-inflammatory drug, 3 hours before the tests.

Effect of A. conyzoides in ear oedema induced by croton oil

The edema was expressed as the increase in ear thickness due to the inflammatory challenge. The ear thickness was measured before and after induction of the inflammatory reaction using a micrometer (Mitutoyo Series 293). The micrometer was applied near the tip of the ear just distal to the cartilaginous ridges and the thickness was recorded in µm. To minimize variation due to the technique, a single investigator performed the measurements throughout any one experiment.

The oedema was induced in the right ear by application topical of 20 µl of croton oil (1 µg/ear). After 1 hour, was applied topically to the right ear of the animals, the cream with the extracts hydroalcoholic, methanol and ethanol at different concentrations (1%, 3% and 5%). Two other groups of animals received dexamethasone cream (0.1%), used as positive control, or the base only (cream without active), used as negative control. The ear thickness was measured before and 6 hours after induction of inflammation [15].

Pleurisy

Experimental pleurisy was induced first by intravenous administration of a solution of Evans blue (25 mg/kg, 0.2 ml, iv), to indirectly determine the degree of exudation in the pleural cavity [16, 17]. After 30 minutes, the injection of Evans Blue, groups of animals were treated only with the hydroalcoholic extract of *A. conyzoides* (100, 300 and 500 mg/kg), and indomethacin (10 mg/kg) administered by the intraperitoneal route, was used as the reference product.

After 1 hour of treatment, the animals were lightly anesthetized with ethyl ether and then 0.1 ml of sterile saline (NaCl 0.9%) containing carrageenan (1%), SP (20 nmol/ cav), BK (10 nmol/cav), histamine (100 µg/cav) or only saline (control group), was administered directly into the pleural cavity (injection intrapleural), through the intercostal space [18]. In experiments with BK, the animals were pretreated 30 minutes before with captopril (5 mg/kg via i.p.) to prevent the degradation of BK by kinases [19]. After 4 hours of administration of the phlogistic agent, the animals were killed with an overdose of ethyl ether, the thorax was opened and the pleural cavity was washed with 1 mL of solution heparinized saline (PBS) (20 UI/mL). The pleural fluid was collected for subsequent determination of total and differential cell exudate (leukocytes). An aliquot of 1 mL was stored in a freezer (-20 ºC) for subsequent determination of levels of Evans blue.

Total leukocyte counts were determined in a Neubauer chamber after diluting the pleural fluid (10 µL)

in 200 µL of Turk solution (2% acetic acid), were stained with May-Grunwald-Giemsa for the differential leukocyte counts, which were performed under magnification of 400 or 1000 X. The degree of exudation was determined by measuring the amount of Evans blue dye (25 mg/kg, i.v.) extravasation in the exudate. The spectrophotometer reading optical density at a wavelength of 600 nm was used to estimate the amount of dye by interpolation from a standard curve of Evans blue in the range of 0.3-100 µg/mL [20].

Suppression of arachidonic acid metabolites (PGE2 and LTB4) and nitric oxide (NO)

This experiment was based on [21] with minor modifications. In the cells free supernatant of pleural fluid by a standard sandwich ELISA procedure to assess the pharmaceutical effect on inflammatory mediator's quantity in vivo model, the production of PGE2, LTB4 and nitrite/nitrate concentrations were measured [22]. The assay for prostaglandin E2 (PGE2) and leukotriene B4 (LTB4) was performed according to the manufacturer's instructions using kits from R&D systems. Nitrite/nitrate production, an indicator of nitric oxide (NO) was measured in the exudate as previously described [23]. Briefly, the nitrate in the exudate was first reduced to nitrite by incubation with nitrate reductase (670 mU/ml) and NADPH (160 µM) at room temperature for 3 h. The nitrite concentration in the samples was then measured by the Griess reaction, by adding 100 µl of Griess reagent (0.1% naphthylethylenediamide dihydrochloride in H_2O and 1% sulfanilamide in 5% concentrated H_2PO_4 (vol. 1:1) to 100 µl samples. Absorbance results were assessed using an ELISA microplate reader set (Meglan, USA).

Statistical analysis

The data were expressed as mean and standard deviations, and statistically assessed by one-way analysis of variance (ANOVA). The difference between the groups was evaluated by the Bonferroni or Dunnet test, where applicable, $p < 0.05$ was considered significant.

RESULTS

Experiment 1: effects of A. conyzoides on paw oedema induced by carrageenan

Figure 1 shows the antioedematogenic activity of *A. conyzoides* by the method described previously [24]. It should be noted that the hydroalcoholic extract (100 to 500 mg/kg) revealed an antioedematogenic effect by inhibiting the formation of paw oedema. It is observed that the inhibition of oedema at the dose of 300 mg/kg was superior to that caused by dexamethasone (1 mg/kg); IMs were calculated, showing 76% and 68% respectively. Methanolic extract in all doses was effective in inhibiting the oedema after 2 hours, and the dose that best inhibited oedema was 300mg/kg, with IMs of 76% for the extract and 68% for dexamethasone. The ethanolic extract significantly inhibited oedema formation, since there 1 hour the dose that inhibited the oedema was better than 300mg/kg, with IMs of 65% for the extract and 73% for dexamethasone.

Experiment 2: effects of A. conyzoides on ear oedema induced by croton oil

In the biological assays shown in figure 2, it was observed that treatment with cream containing hydroalcoholic extract of *AC* significantly inhibited oedema at all the concentrations, compared with the control (pure cream). For the experiment, the IMs calculated for the doses of 1, 3 and 5% were 63.63±2.45%, 77.18±1.15% and 76±3.05%, respectively ($p<0.01$). The results showed that there is no statistical difference between the effect of the antioedematogenic extract and of the anti-inflammatory agent used as positive control, and that both treatments where effective in reducing oedema. It is observed that the methanolic and ethanolic extract also significantly inhibited oedema formation ($p<0.01$) compared with the control, with effect comparable as dexamethasone. The IMs calculated for the methanol extract were 58±0.94%, 56.7±0.45% and 65.4±0.34% respectively, and the IMs for the ethanolic extract, 69±1.34%, 60±0.45% and 72±1.12%.

Experiment 3: Carrageenan-induced pleurisy

Effects of A. conyzoides on cellular infiltration and exudation

The treatment with hydroalcoholic extract of *AC* (100, 300 and 500 mg/kg) caused a significant dose-dependent decrease in leukocyte migration (Fig. 3A) when administered 1 hours before carrageenan with inhibition of 39.09±2.11%, 57.82±1.25%, and 71.93±0.89% ($p<0.01$ or $p<0.001$), respectively. This reduction was attributed to inhibition of neutrophil influx (24.09±2.44%, 41.52±1.89%, 58.57±2.22%) ($p<0.05$, or 0.01) (Fig. 3B). Also reduced mononuclear cells (16.49±1.14%, 37.03±2.52%, 49.49 ±3.52%) ($p< 0.05$) (Fig. 3C). Under the same conditions, exudation was also inhibited by AC at a dose of 300 mg/kg (55.42±2.44%; $p<0.05$) and 500 mg/kg (49.49±1.74%; $p<0.05$) (Fig. 3D). Treating the animals with indomethacin (10 mg/kg) significantly decreased the leukocyte (74.03±0.98%, $p<0.001$), neutrophil (58.39±0.78%, $p<0.01$), and monocyte (47.47±0.28%, $p<0.05$) counts, and exudate (5.45±1.02%, $p<0.01$).

Effects of A. conyzoides on Pleurisy induced by substance P, bradykinin and histamine

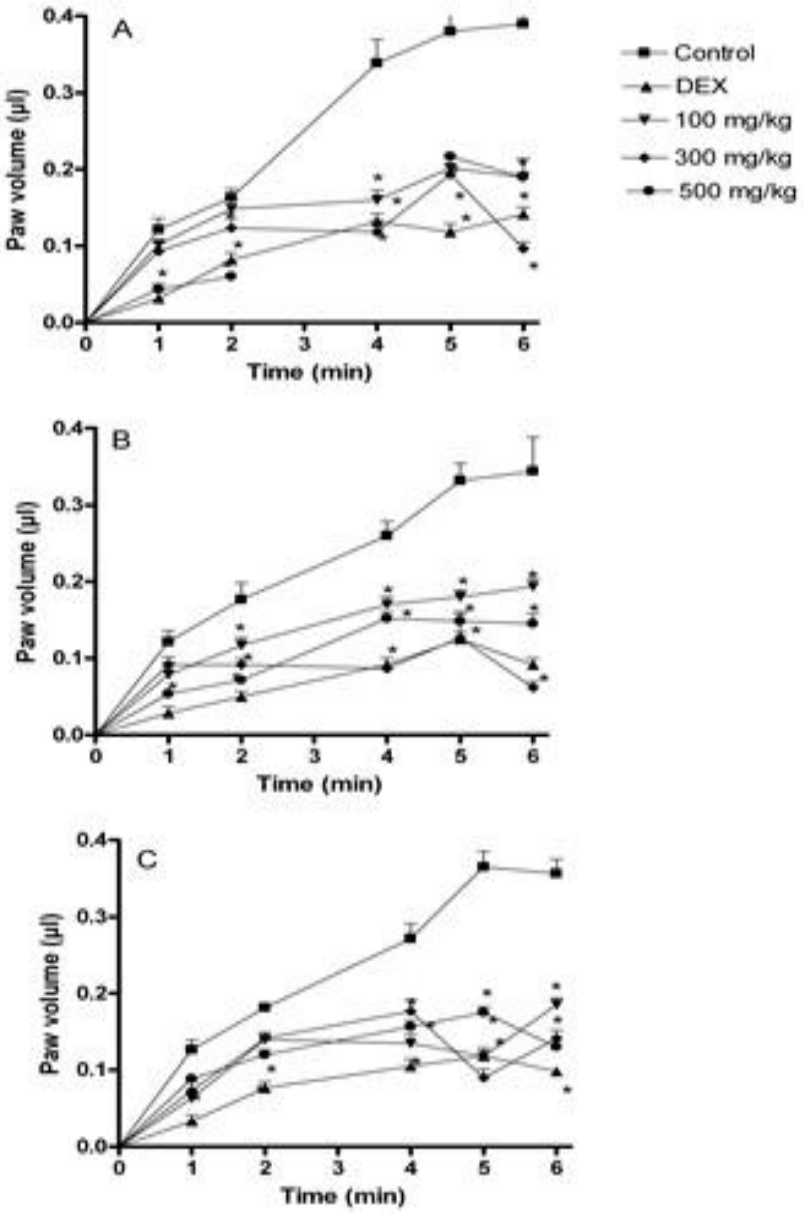
The hydroalcoholic extract of *AC* (100 to 500 mg/kg) significantly reduced the leukocyte count in pleurisy induced by BK (19.68±0.48% to 48.8±0.32%, $p<0.01$), by SP (22.68±2.31% to 71.13±0.26%, $p<0.01$), (Table 1) and mononuclear cells by BK (28.15±1.54% to 57.14±0.26%, $p<0.01$) and SP (30.45±1.35% to 56.73±2.26%, $p<0.01$). *AC* also inhibited neutrophil infiltration from 100 to 500 mg/kg in pleurisy induced by SP (37.09±1.37% to 61.29±0.29%, $p<0.01$) (Table 1). The levels of exudate in inflammation induced by BK (51.23±0.27% to 63.54±1.45%, $p<0.01$) and SP (38.23±1.45% to 42.81±2.35%, $p<0.01$) were also significantly reduced by treatment with the extract at 100 to 500 mg/kg. The animals treated with indomethacin (10mg/kg) significantly inhibited in almost all

inflammatory parameters ($p<0.01$), with the exception of neutrophil induced HIS ($p>0.05$).

Experiment 4: suppression of arachidonic acid metabolites (PGE2 and LTB4) and nitric oxide

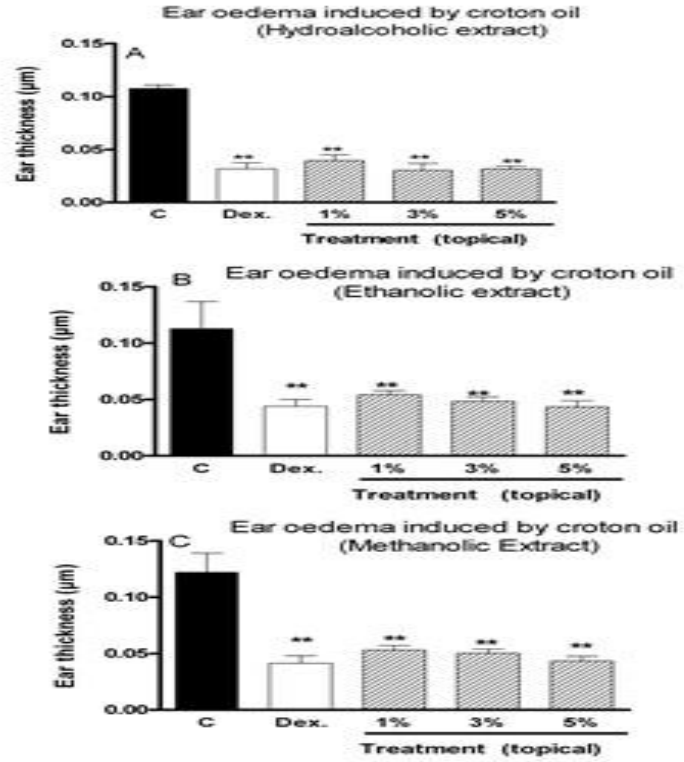
As can be seen in figure 04 A and B, in the acute model of inflammation induced by carrageenan injection into the pleural cavity, highly elevated levels of PGE2 and LTB4 were observed. However the group of animals treated with *AC* at doses ranging from 100-500 mg/kg, showed highly significant inhibition of PGE2 (15.44±2.10%, 28.37±3.14% and 48.16±2.12%) $p<0.05$, $p<0.001$ (Fig.4A) and LTB4 (45.30±2.00%), $p<0.0001$, (Fig. 4B) at the dose of 500 mg/kg. A decrease in NO level (39.00±1.53) was also observed. The group of animals treated with indomethacin also showed highly significant inhibition of PGE2 (55.80±2.32%, $p<0.0001$) and LTB4 (46.67±1.45%, $p<0.001$) at the dose of 10.0 mg/kg. As can be seen in Fig. 4C, oral treatment with AC 1 h before and 4 h after carrageenan injection also significantly ($p<0.01$) attenuated the NO levels (39.71±2.14). At the higher dose, levels of 300 and 500 mg/kg, *AC* demonstrated an effect that was almost comparable to the standard drug-indomethacin, which shows that AC extract displays a potent anti-inflammatory effect.

Fig 1. Effects of different doses of hydroalcoholic, methanol (A) and ethanol extracts (B) of AC (100-500mg/kg, i.p.) on the inflammation induced by carrageenan in the paw oedema.



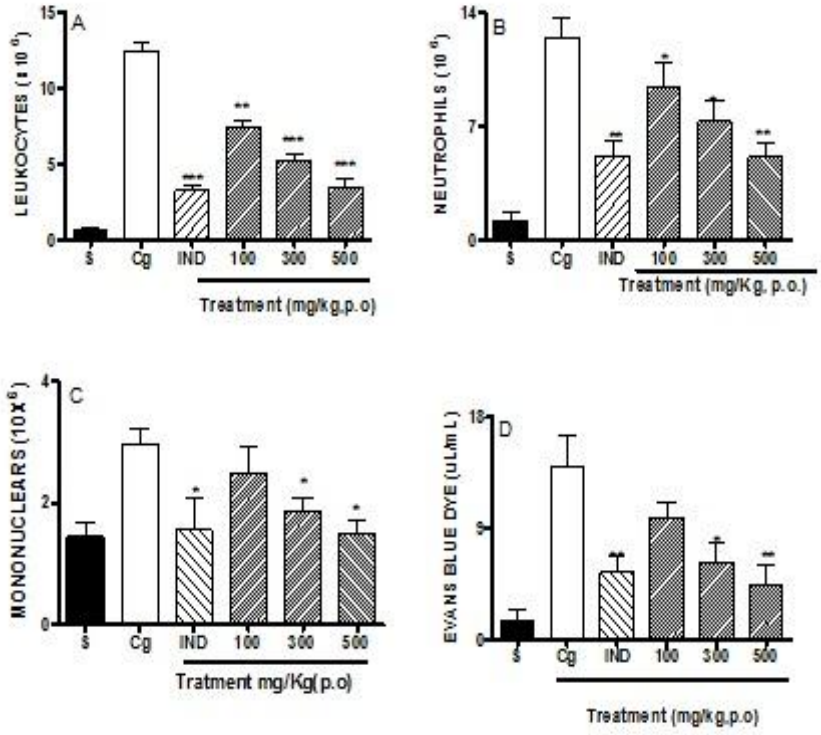
Control (animals treated only with sterile saline), DEX= animals treated with dexamethasone (1mg/kg, i.p.) plus carrageenan. Each point represents the mean of 6-8 animals and the vertical bars the SEM. Significant differences determined by ANOVA complemented by Dunnett's tests. $^{**}p< 0.01$ compared with control group.

Fig 2. Inhibitory effects of the cream base of the hydroalcoholic, methanol and ethanol extract of AC (1, 3, 5 %) on inflammation induced by croton oil in the ear oedema



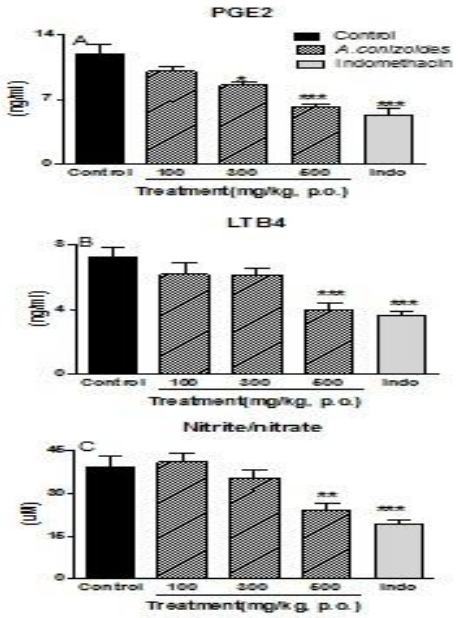
Control (animals treated only with sterile saline), DEX= animals treated with dexamethasone (0.1%). Each point represents the mean of 6-8 animals and the vertical bars the SEM. Asterisks denote statistical differences compared with controls (*p<0.05, **p<0.01). ANOVA was performed, followed by the Dunnett's test.

Fig 3. Effects of different doses of hydroalcoholic extract of AC (100-500mg/kg, v.o.) on the inflammation induced by carrageenan (Cg, 1%) in the mouse model of pleurisy



Effects of AC upon leukocytes (A), neutrophils (B), mononuclear (C) and exudation (D). S= control (animals treated only with sterile saline), Cg= animals treated with carrageenan, IND= animals treated with indomethacin (10mg/kg, i.p.) plus carrageenan. Each group represents the mean of 6-8 animals and the vertical bars the SEM. Significant differences determined by ANOVA complemented by Dunnett's test ** $p < 0.01$.

Fig 4. Effect of graded doses (100-500 mg/kg, p.o.) of hydroalcoholic extract of AC and indomethacin (10.0 mg/kg,i.p.) on PGE2 (Fig. 4A), LTB4 (Fig. 4B) and nitrite/nitrate (Fig.4C) levels in cells free supernatant of pleural fluid after pleurisy induced by carrageenan



Results are represented as mean±S.E.M with n=6 in each group. *p* value; ***p<0.001; **p<0.01; *p<0.05; The statistical test used is ANOVA followed by post Bonferroni test.

Table 1. Effect of hydroalcoholic extract of AC and indomethacin (10.0 mg/kg,i.p.) on pleurisy induced by different agents phlogistic

Group/dose	Absolute values		
	Bradykinin	Histamine	Substance P
	(10 nmol/cav.)	(1 µg/cav.)	(20 nmol/cav.)
Leukocytes (x 10^6)			
C[a]	2.54 ± 0.16	1.98 ± 1.45	2.91 ± 0.14
AC (100 mg/kg)	2.04 ± 0.14*	1.94 ± 0.02	2.25 ± 0.18**
AC (300 mg/kg)	1.55 ± 0.12**	1.87 ± 0.07	0.96 ± 0.16**
AC (500 mg/kg)	1.30 ± 0.11**	1.89 ± 0.09	0.84 ± 0.08**
IND (10 mg/kg)	1.25 ± 0.08**	1.08 ± 0.05**	0.93 ± 0.05**
Neutrophils (x 10^6)			
C[a]	0.36 ± 0.08	0.44 ± 0.01	0.62 ± 0.02
AC (100 mg/kg)	0.36 ± 0.04	0.37 ± 0.01	0.39 ± 0.03**
AC (300 mg/kg)	0.34 ± 0.03	0.35 ± 0.01	0.29 ± 0.04**
AC (500 mg/kg)	0.29 ± 0.04	0.41 ± 0.05	0.24 ± 0.01**
IND (10 mg/kg)	0.18 ± 0.09**	0.35 ± 0.02	0.23 ± 0.01**
Mononuclears (x 10^6)			
C[a]	2.38 ± 0.12	1.52 ± 0.04	1.41 ± 0.14
AC (100 mg/kg)	1.71 ± 0.16**	1.49 ± 0.03	0.98 ± 0.09**
AC (300 mg/kg)	1.07 ± 0.08**	1.45 ± 0.08	0.72 ± 0.11**
AC (500 mg/kg)	1.02 ± 0.10**	1.42 ± 0.05	0.61 ± 0.05**
IND (10 mg/kg)	1.00 ± 0.06**	0.70 ± 0.04**	0.63 ± 0.06**
Exudation (µg/mL)			
C[a]	2.03 ± 0.32	3.09 ± 0.12	3.06 ± 0.19
AC (100 mg/kg)	0.99 ± 0.17**	2.47 ± 0.31	1.89 ± 0.12**
AC (300 mg/kg)	0.82 ± 0.11**	2.56 ± 0.30	1.74 ± 0.28**
AC (500 mg/kg)	0.74 ± 0.14**	2.54 ± 0.35	1.75 ± 0.13**
IND (10 mg/kg)	0.68 ± 0.11**	1.54 ± 0.17**	1.75 ± 0.12**

AC animals pretreated (0.5 h) with different doses of hydroalcoholic extract (100-500 mg/kg), C= control (animals treated only with sterile saline), Ca = animals treated with carrageenan, IND= animals treated with indomethacin (10mg/kg, i.p.) plus carrageenan. Each group represents the mean of 6-8 animals and the vertical bars the SEM. Significant differences determined by ANOVA complemented by the Dunnett's or Student t tests. *$P < 0.05$ and ** $P < 0.01$. a = administered by the intrapleural route.

DISCUSSION

Today, there are a number of inflammatory disorders that cannot be cured, however efforts to develop safer and more effective treatments based on an improved understanding of the role of inflammatory mediators and the use of natural products are beginning to bear fruit [25]. Specifically in Brazil, although the biodiversity is large and different plant species are used in folk medicine as anti-inflammatory medications, only *Cordia verbenacea,* a medicinal plant known as "erva-baleeira", has been transformed into a medication – Acheflan® [26]. Thus the interest in plants with anti-inflammatory property has intensified in recent years.

In the present study we observed that *AC* exerts an acute anti-inflammatory effect in three models of inflammation caused by different phlogistic agents. Furthermore, it was demonstrated that the capability of the extract to promote the suppression of arachidonic acid metabolites (PGE2 and LTB4) and nitric oxide are responsible for the anti-inflammatory activity of the plant.
Paw oedema induced by carrageenan in mice has a biphasic inflammatory response. The first phase (0-2.5 hours) is associated with the release of several mediators, such as histamine, serotonin and kinins, as well as TNFα, IL-1β, IL-2, IFN-γ with the release of vascular permeability [27]. The second phase (4 hours) is correlated with increased prostaglandin derived from free radicals and induction of cyclooxygenase [28]. In this study the results show that hydroalcoholic, methanol and ethanol extracts of *AC* (100-500mg/kg, i.p.). Effectively inhibited carrageenan-induced oedema, contradicting the results obtained by [29] who reported that treatment with water extract of this plant did not influence paw edema induced by carrageenan or dextran, nor did it reduce chronic paw edema induced by complete Freund's adjuvant or formaldehyde in rats. It can be inferred that the anti-inflammatory action of the extract studied may be due to an inhibition of inflammatory mediators involved in the events.

In folk medicine, this plant is also used as a topical anti-inflammatory. To this end, the plant is macerated manually and mixed with lard to form an ointment, which is placed over the wound [30]. Oladejo demonstrate that histologically, in models of wound healing in rats, *AC* showed fewer inflammatory cells compared with control. We evaluated the effects of plant extracts in a model of cutaneous inflammation in mice (ear oedema induced by croton oil) obtaining significant results. Croton oil is a phlogistic agent extracted from *Croton tiglium* L., Euphorbiaceae, and it has an irritant and vesiculant effect on the skin. Croton oil contains phorbol esters, TPA being the predominant phorbol ester. Topical application of croton oil or TPA promotes an acute inflammatory reaction characterized by vasodilatation, polymorphonuclear leukocyte infiltration to the tissue and edema formation. These changes are triggered by PKC activation, which promotes an increase in the activity of PLA2. The events of inflammation in this model (cutaneous vasodilation and erythema formation) occur in the first two hours, followed by increased thickness of the ear as a result of cell leakage, which reaches a peak in the sixth hour and then tends to decrease, reaching basal values after 24 hours [10].

The adherence of PMN in the vessel wall and degranulation of mast cells is observed between the 4^{th} and 6^{th} hours. However, the maximum infiltration of PMN in the tissue is reached just 24 hours after topical application of croton oil [18]. The results of this study demonstrate that *AC* produces a reduction in ear oedema induced by croton oil. Although initial studies, anti-inflammatory effect with the cream with the extracts obtained from the plant under study, validate and demonstrate its popular use its efficacy when topical is used.

In view of these results, we have used several pharmacological tools to define the pro-inflammatory potentials involved in the inflammation process, and which were inhibited by *A. conyzoides.* The results enabled us to characterize a marked inhibitory profile on cell migration, for both neutrophil and mononuclear cells, as well as an important and significant inhibition of exudation. Furthermore, these anti-inflammatory actions were also demonstrated by the inhibition of leucocytes and/or exudation in pleurisy induced by carrageenan, bradykinin, histamine or substance P, phylogens considered mediators involved in the acute inflammatory response [9, 12, 14]. The study showed that *A. conyzoides* has selective affinity to the endogenous neuropeptide bradykinin (BKII) involved in mediating pain, an activity exerted by phenolic compounds.

In this study, we demonstrate for the first time that *AC* extract has a potent capacity to reduce the migration of neutrophils at the site of inflammation. LTB4 is a strong chemoattractant for neutrophils. We observed highly significant inhibition of PGE2 and LTB4, which is possibly one of the mechanisms of anti-inflammatory actions of *AC.*
In the present study, we also observed decreased NO production, which might be responsible for the decreased production of PGE2 at the site of inflammation. Our results demonstrate that *AC* significantly inhibits NO levels. This effect may be reflect to the anti-inflammatory effect of this plant, which can be associated with inhibition of reactive oxygen species, including the superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and the hydroxyl radical (OH•).

Besides the essential oil of this plant, other metabolites with pharmacological activity have been identified. However, there is a wide spectrum of pharmacological activities from the classes of compounds obtained from this plant. Compounds such as flavonoids have a broad range of biological activities. The list includes effects on the central vascular system, and diuretic, spasmolytic, antiviral and anti-inflammatory properties [31]. Although the biological activities of flavonoids from *AC* have not been well investigated, it is important to highlight four polymethoxiflavones isolated from citrus juices that have been shown to be important candidates for cancer prevention. Two of these are the same polymethoxiflavones isolated from *AC* (Okunade, 2002). A review of literature indicates the presence of pyrrolizidine alkaloids in plants as *AC*, which may explain the anti-inflammatory effect, because the alkaloids inhibit

the synthesis of prostaglandins by blocking the activity of COX-2, the enzyme responsible for the inflammatory process [32].

CONCLUSION

In conclusion, our study confirms and extends the previous work on the anti-inflammatory effect of *A. conyzoides* in hydroalcoholic, methanol and ethanol extracts by inhibiting the inflammation induced by different phlogistic agents in three major models of inflammation used to study the anti-inflammatory properties of natural products. In addition, the inhibition of liberation of pro-inflammatory mediators (PGE2, LTB4) and nitrite/nitrate concentrations appears to account for the actions of *A. conyzoides*.

ACKNOWLEDGEMENTS

This study was supported by grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and FAPESC¸ Fundação de Amparo a Pesquisa do Estado de SC, Brazil. This work represents a part of the Master of Science Dissertation of Liliane Trivellato Grassi, carried out for the Postgraduate Program in Pharmacy of the University of Vale do Itajaí – UNIVALI (Brazil). We are grateful to Oscar Benigno Iza (Universidade Federal de SC) for identifying the plant materials.

CONFLICT OF INTEREST

The authors report no declarations of interest

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