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Pharmacological study of anti-allergic activity of *Syzygium cumini* (L.) Skeels

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Abstract

Myrtaceae is a plant family widely used in folk medicine and *Syzygium* and *Eugenia* are among the most important genera. We investigated the anti-allergic properties of an aqueous leaf extract of *Syzygium cumini* (L.) Skeels (SC). HPLC analysis revealed that hydrolyzable tannins and flavonoids are the major components of the extract. Oral administration of SC (25-100 mg/kg) in Swiss mice (20-25 g; N = 7/group) inhibited paw edema induced by compound 48/80 (50% inhibition, 100 mg/kg; $P \leq 0.05$) and, to a lesser extent, the allergic paw edema (23% inhibition, 100 mg/kg; $P \leq 0.05$). SC treatment also inhibited the edema induced by histamine (58% inhibition; $P \leq 0.05$) and 5-HT (52% inhibition; $P \leq 0.05$) but had no effect on platelet-aggregating factor-induced paw edema. SC prevented mast cell degranulation and the consequent histamine release in Wistar rat (180-200 g; N = 7/group) peritoneal mast cells (50% inhibition, 1 µg/mL; $P \leq 0.05$) induced by compound 48/80. Pre-treatment of BALB/c mice (18-20 g; N = 7/group) with 100 mg/kg of the extract significantly inhibited eosinophil accumulation in allergic pleurisy (from 7.662 ± 1.524 to 1.89 ± 0.336 x 10^6/cavity; $P \leq 0.001$). This effect was related to the inhibition of IL-5 (from 70.9 ± 25.2 to 12.05 ± 7.165 pg/mL) and CCL11/eotaxin levels (from 60.4 ± 8.54 to 32.8 ± 8.4 ng/mL) in pleural lavage fluid, using ELISA. These findings demonstrate an anti-allergic effect of SC, and indicate that its anti-edematogenic effect is due to the inhibition of mast cell degranulation and of histamine and serotonin effects, whereas the inhibition of eosinophil accumulation in the allergic pleurisy model is probably due to an impairment of CCL11/eotaxin and IL-5 production.

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Key words
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- Allergy
- Paw edema
- Pleurisy
- Cytokine

Introduction

Myrtaceae is a plant family widely used in folk medicine in different countries and *Eugenia* and *Syzygium* are among its most important genera. Species of this family are often used for several medicinal purposes, including the treatment of diarrhea (1) and pain. Experimental data also suggest the action of these species on inflammatory pro-

cesses, respiratory diseases (2), and allergic disorders (3). The seeds of *Syzygium cumini* (L.) Skeels (SC; Myrtacea, syn., *Eugenia jambolana* Lamk) have been reported to be useful as astringents in diarrhea as well as dysentery (4). Other parts of the plant have been reported to possess anti-diabetic (5), bactericidal (6) and anti-mutagenic (7) properties. The ethanolic bark extract has been reported to have anti-inflammatory activity in carrageenan and formaldehyde paw edema (2). The same extract was also shown to inhibit histamine-, serotonin (5-HT)- and prostaglandin 2-induced paw edema (8).

The allergic process has an important inflammatory component in which mast cell activation and degranulation are the first phenomena observed. During this process, mast cells release several inflammatory mediators including histamine, 5-HT, platelet-aggregating factor (PAF), leukotrienes, and a variety of cytokines which can elicit many events associated with allergic inflammation, such as edema formation and cellular infiltration (9). Eosinophil accumulation is the main feature of allergic inflammation, these being some of the most abundant leukocytes present at the site. The triggering and regulation of eosinophil accumulation in allergic inflammation depend on the release of cytokines and chemokines such as interleukin-4 (IL-4), IL-5 and CCL11/eotaxin in response to an antigen challenge (10,11). Once they reach the allergic site, eosinophils degranulate and release several mediators, including leukotrienes, major basic protein, PAF, cationic protein, and eosinophil-derived neurotoxin that contribute to extensive tissue damage (12). The modulation of eosinophil accumulation is one of the main targets for the discovery of anti-allergic compounds because of its potential tissue damaging effects.

In the present study, we investigated the ability of an aqueous leaf extract of SC to inhibit mouse allergic edema formation and eosinophil accumulation in mouse allergic pleurisy and the mechanisms involved in such phenomena

Material and Methods

Material

Compound 48/80 (C48/80), ovalbumin (OVA, grade V), histamine, PAF, 5-HT, dexamethasone, disodium cromoglycate, 3% PBS/milk, PBS, *o*-phtaldialdehyde, HBSS, complete Freund's adjuvant, and all other highest grade reagents were purchased from Sigma (St. Louis, MO, USA). WEB2170 was obtained from Boehringer-Ingelheim (Ingelheim, Germany), Percoll was from Amersham Pharmacia Biotech AB (Uppsala, Sweden); May-Gruenwald-Giemsa, Toluidine blue and Trypan blue were purchased from Merck (Rio de Janeiro, RJ, Brazil).

Animals

Male Swiss Webster (20-25 g) and BALB/c mice (18-20 g) and Wistar rats (180-200 g) from our own colony (CECAL-FIOCRUZ) were housed in a room with controlled temperature and lighting, with free access to lab chow and tap water. All experiments were conducted in accordance with the ethical guidelines of the International Association for the Study of Pain (13) and the institutional guidelines for animal use (CEUA 00050/00).

Plant material and extract preparation

Entire branches of SC were collected in Rio de Janeiro in January 1999. The plant material was identified by Dr. Graziela M. Barrozo (*in memoriam*), Botanic Garden of Rio de Janeiro, and a voucher was deposited in the Herbarium of the Botanic Garden of Rio de Janeiro under number HB-83016. The aqueous extract of fresh leaves of SC was obtained by decoction of leaves in distilled water (100 g/L) for 3 to 5 min. The

extract was filtered, lyophilized, stored at room temperature, and dissolved in distilled water immediately before use.

HPLC characterization of the extract

The separation of the components of the mixture was dependent on the pH of the mobile phase, with pH near 4 being satisfactory. Thus, 0.1% phosphoric acid was added to the mobile phase to adjust its pH to 4.1. The mobile phase eluted all the phenolic compounds within 30 min at 0.75 mL/min. Analysis was performed on a Supelcosil LC 18 (250 mm x 4.6 mm, I.D., 5 µm particle size) column.

A 40.0-mg sample of the extract was accurately weighed and transferred to a 3.00-mL microcentrifuge tube, suspended in 2.00 mL methanol and cooled in an ice bath. The suspension was sonicated (Odontrobrás, Ribeirão Preto, SP, Brazil) for 20 min and then centrifuged for 10 min at 3000 rpm, at 4ºC (Beckman Coulter, Fullerton, CA, USA). The supernatant was decanted and stored at -20ºC. Before injection into the column, the samples were filtered in an UltraFree-MC 0.22-µm filter unit (Millipore, São Paulo, SP, Brazil) with a Durapore membrane (Millipore) with 0.5-mL capacity.

HPLC data were collected and processed with a Shimadzu Class-VP 6.12 model apparatus (Kyoto, Japan) equipped with a diode-array detector and SP3 chromatographic software. The gradient of mobile phase elution was programmed for solvent A (acetonitrile: water, 5:95, v/v) and solvent B (acetonitrile: water, 90:10, v/v). The column was previously equilibrated with the mobile phase (100% solvent A) for 30 min at a flow rate of 0.75 mL/min. The mobile phase was adjusted to pH 4.0 with phosphoric acid. The flow rate was 0.75 mL/min and the injection volume 20 µL. The column temperature was maintained at 25ºC during analysis. The gradient program started with 0% of solvent B and increased linearly to 100% of solvent B in 30 min or 3.33%/min. Injections were performed in triplicate. After 44 min, the elution program was returned to the initial condition and held there for 10 min in order to recondition the column.

Treatments

Mice fasted overnight, received the antihistamine agent, the H_1-receptor antagonist promethazine (10 mg/kg) or aqueous extract (25-100 mg/kg) orally (*po*) in a final volume of 200 µL, 1 h before stimulation. The control groups were similarly treated with vehicle alone. In some experiments of allergic pleurisy, dexamethasone was given intraperitoneally (2 mg/kg) 24 and 1 h before stimulation (N = 7/group).

Induction of paw edema

Paw edema was induced as previously described (14). Briefly, Swiss mice received an intraplantar (*ipl*) injection of C48/80 (100 ng/paw), histamine (100 µg/paw), PAF (1 µg/paw), or 5-HT (100 µg/paw) into one hind paw. The final volume was 50 µL and the contralateral paw received the same volume of sterile saline and served as control (N = 7/group). The volumes of each hind paw were measured with a plethysmograph (Ugo Basile, Varese, Italy) 30 min after stimulation.

Swiss mice sensitization and allergic paw edema were induced as described (15). Briefly, animals were sensitized with a subcutaneous injection of 100 µL of a mixture of OVA (50 µg), and aluminum hydroxide (5 mg). Fourteen days later, mice were challenged by an *ipl* injection of OVA (3 µg/paw) and the induction of paw edema was evaluated 30 min after stimulation (N = 7/group).

Allergic pleurisy

Active sensitization of BALB/c mice was achieved with a subcutaneous injection of

Freund's complete adjuvant emulsion (100 µL) containing OVA (100 µg). Fourteen days later, mice were challenged with an intrathoracic injection of OVA (12.5 µg/cavity, N = 7/group) as described elsewhere (16,17). Briefly, an adapted needle was inserted into the right side of the thoracic cavity of OVA-sensitized animals to permit the intrapleural administration of OVA diluted in sterile pyrogen-free saline (50 µL). Sensitized mice challenged with saline vehicle alone were used as negative controls.

At 24 h after the stimulus, mice were killed with excess carbon dioxide and their thoracic cavities were rinsed with 1 mL PBS containing 10 mM EDTA, pH 7.4. Total leukocyte counts were made with an automatic Coulter counter (Beckman Coulter, Fullerton, CA, USA). Differential cell counts were made using stained cytospin (Shandon, Pittsburgh, PA, USA) by the May-Gruenwald-Giemsa method under light microscopy (100X). Counts are reported as numbers of cells (x 10^6) per cavity.

Enzyme-linked immunosorbent assay

Levels of eotaxin and IL-5 in the cell-free pleural fluid were evaluated by sandwich enzyme-linked immunosorbent assay using matched antibody pairs from Pharmingen (San Diego, CA, USA), according to manufacturer instructions. Results are reported as picograms per cavity of two experiments in triplicate and values were obtained by comparison with a standard curve (0.015-15 ng/mL for eotaxin and IL-5).

Mast cell purification and histamine measurement

Rat peritoneal mast cells were isolated as previously described (18). Briefly, male Wistar rats (N = 7/group) were killed with excess carbon dioxide and the peritoneal cavity was rinsed with 20 mL of heparinized (10 IU/mL) calcium- and magnesium-free Hank's solution ($HBSS^-$). The fluid was collected, centrifuged at 150 *g* for 10 min at 4ºC, the pelleted cells were resuspended in $HSSB^-$ containing 0.1% bovine serum albumin and submitted to a continuous isotonic Percoll gradient (72%) for mast cell isolation. Purified mast cells were resuspended in HSSB containing Ca^{2+} and Mg^{2+}. Purity (95%) and viability (98%) were evaluated by Toluidine blue and Trypan blue exclusion staining, respectively. The cells were added to a 24-well plate (10^5 cells/well) and preincubated for 1 h with 1 µg/mL of dried SC extract dissolved in saline or with disodium cromoglycate at 10.2 µg/mL in a 5% CO_2 atmosphere, at 37°C. After this period, cells were incubated for 30 min with 5 µg/mL C48/80. The reaction was stopped in ice. Histamine was quantified in the supernatant by a fluorimetric assay as previously described (19). The fluorescence intensity was measured at 450 nm (excitation at 360 nm) with a Spectra Max Gemini EM spectrofluorometer (Molecular Devices, Sunnyvale, CA, USA). Percent inhibition of histamine release was calculated as follows: % inhibition = 100 - {(histamine release with SC x 100)/ histamine release without SC}.

Statistical analysis

Data are reported as means ± SEM and were analyzed statistically by one-way ANOVA, and differences between groups were assessed using the Student-Newman-Keuls post-test. A P value <0.05 was considered significant.

Results

Chemical characterization of the extract

The yield of the crude aqueous extract was 6.5% based on fresh leaves (g/g leaves). HPLC fingerprinting of the aqueous SC leaf extract showed an elution diagram consistent with the presence of tannins and flavonoids (Figure

1A). The peaks were grouped into three regions based on the UV absorption profile, and these regions showed the typical patterns of UV absorption, supporting the presence of ellagitannin (Figure 1B), gallotannin (Figure 1C) and flavonoids (Figure 1D) in SC. The presence of flavonoids was also observed by TLC using NP/PEG (data not shown).

Effect of *Syzygium cumini* extract on paw edema triggered by compound 48/80 or ovalbumin

To assess the effect of SC extract (crude extract obtained from fresh leaves by decoction in water) on allergic reactions, we first used the model of anaphylaxis edema caused by the mast-cell degranulator C48/80 or by OVA in sensitized animals. The *ipl* administration of C48/80 into the mouse hind paw triggered a significant edema 30 min after the injection, as shown in Figure 2A. Oral pre-treatment with SC extract inhibited edema formation at doses of 25, 50, and 100 mg/kg (maximal inhibition of 50% at 25 mg/kg) to almost the same extent as promethazine, an anti-histaminic compound (65% inhibition at the dose of 10 mg/kg; Figure 2A, inset). It is noteworthy that oral treatment with 200 or 400 mg/kg SC extract inhibited edema formation at the same intensity (64 and 58%). Figure 2B shows that OVA (50 µg) triggered paw swelling within 30 min in sensitized mice. Oral pre-treatment with SC extract (25-100 mg/kg) led to a slight (20%) inhibition of allergic paw edema with no differences observed between the doses tested, even when we used the doses of 200 or 400 mg/kg of oral SC extract (25 and 32.8% inhibition, respectively; data not shown). Conversely, pre-treatment with promethazine (10 mg/kg; *po*) was able to

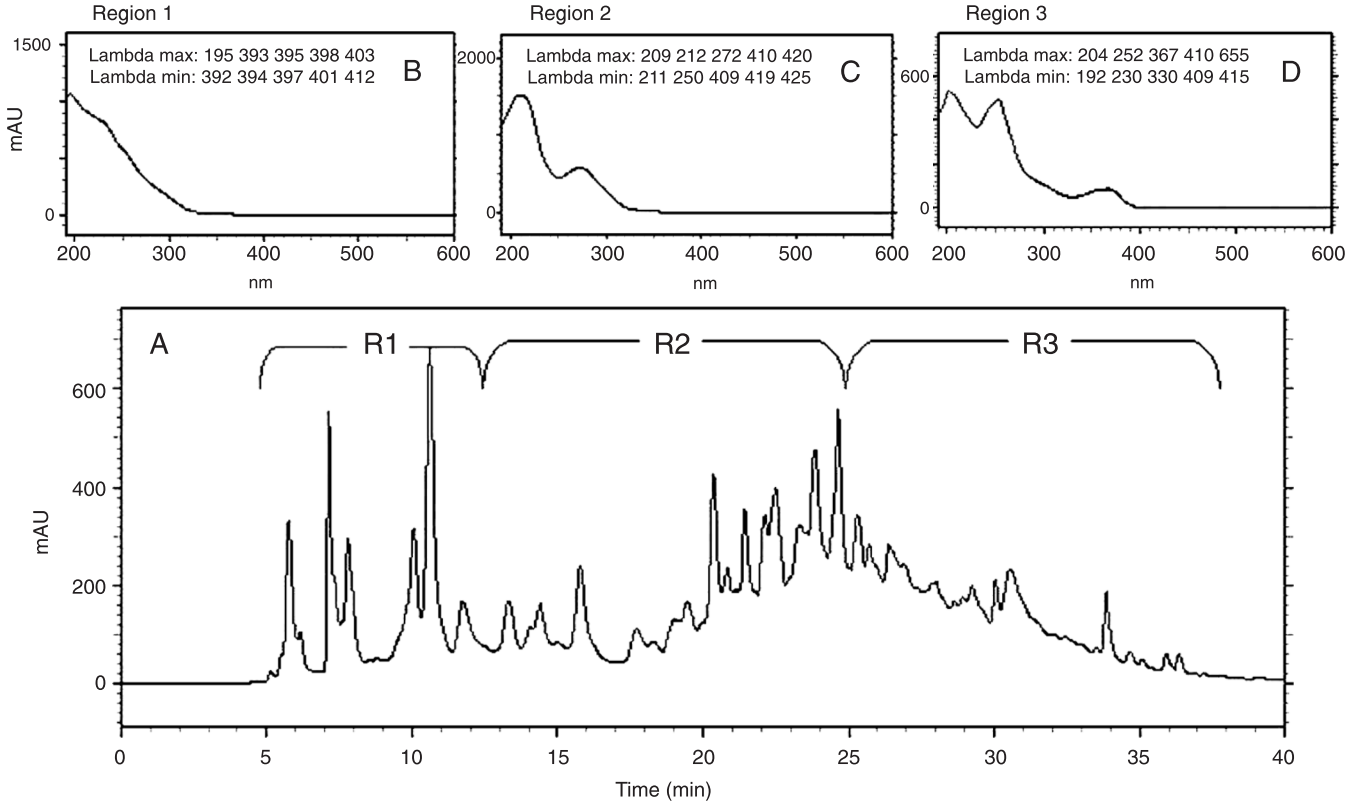

Figure 1. HPLC fingerprint of the *Syzygium cumini* aqueous leaf extract (λ = 280 nm; A) showing typical patterns of ellagitannins (B), gallotannins (C), and flavonoids (D) in the UV-visible absorption spectra.

inhibit edema by 50% (Figure 2B, insert).

Effect of *Syzygium cumini* extract on paw edema triggered by different inflammatory mediators

Histamine, 5-HT and PAF are the major inflammatory mediators involved in allergic edema formation. In an attempt to understand the effect of SC, we analyzed the effect of the extract against the edema induced by each of these mediators. As observed in Figure 3A, *ipl* injection of histamine (100 µg/paw) into the hind paw of naive mice induced a significant paw edema 30 min after the injection which was inhibited by oral treatment with SC extract, with a maximal inhibition of 58% achieved at 50 mg/kg ($P \leq 0.05$). Figure 3B shows that the *ipl* injection of 5-HT (100 µg/paw) into the hind paw induced a significant paw edema 30 min after the stimulus, which was inhibited by treatment with SC at the dose of 100 mg/kg (51% inhibition; $P \leq 0.05$). Conversely, the mouse paw edema induced by PAF (1 µg/paw; 30 min) was not affected by oral pre-treatment with SC extract at the doses of 25, 50, or 100 mg/kg (Figure 3C). These results suggest that the anti-edematogenic effect of oral SC extract on allergen-induced paw swelling was due to an anti-histamine and anti-serotonin effect.

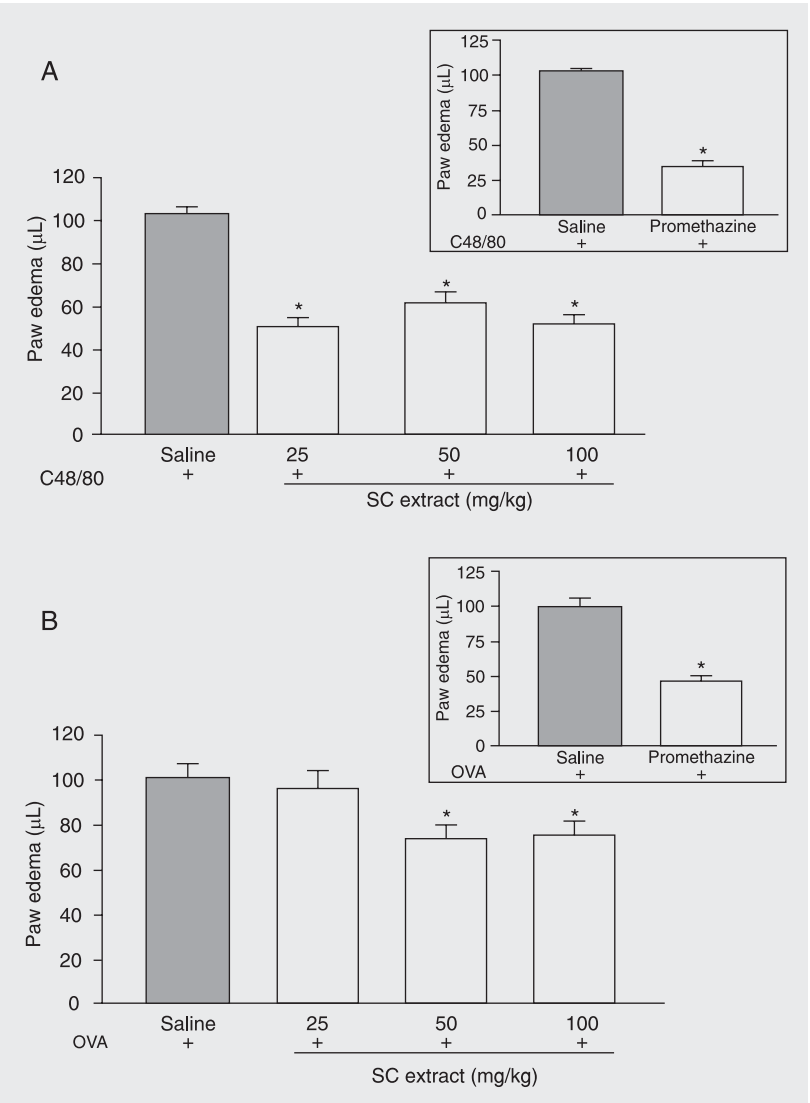

Figure 2. Effect of oral pre-treatment with different doses of aqueous extract of *Syzygium cumini* (SC) leaves on paw edema induced by compound 48/80 (C48/80, 100 ng/paw; Panel A) or ovalbumin (OVA, 3 µg/paw; Panel B). Promethazine (10 mg/kg, *po*) was used as reference inhibitor (inset). Data are reported as the mean ± SEM for at least 7 animals. *$P \leq 0.05$ compared to saline control (Student-Newman-Keuls post-test).

Effect of *Syzygium cumini* extract on histamine release from rat peritoneal mast cells

Mast cell degranulation followed by the release of vasodilating mediators (mainly histamine) is the major component of allergic edema. In this set of experiments we investigated the effect of the SC extract on mast cell degranulation by means of histamine release. As shown in Table 1, stimulation with C48/80 (5 µg/mL) induced the release of 20 ng/mL histamine in rat peritoneal mast cells that was inhibited by treatment with disodium cromoglycate, a classic mast cell membrane stabilizer (31% inhibition). Pre-treatment with SC extract (1 µg/mL, 1 h before C48/80) significantly inhibited the release of histamine from mast cells (49.5%).

Effect of *Syzygium cumini* extract on allergic pleurisy

Twenty-four hours after the intrathoracic

injection of OVA (12.5 µg/cavity), an intense accumulation of total leukocytes (Figure 4A), mononuclear cells (Figure 4B) and eosinophils (Figure 4D) was observed, while remaining numbers of neutrophils were present in the pleural cavity of BALB/c mice (Figure 4C). Dexamethasone pre-treatment (2 mg/kg, intraperitoneally) significantly inhibited the influx of total leukocytes (76%), mononuclear cells (62%) and eosinophils (99% inhibition). The oral administration of SC (100 mg/kg) 1 h before stimulation markedly inhibited the eosinophil accumulation (75% inhibition, $P \leq 0.001$) in the pleural cavity. It is important to note that this inhibition was selective, with no effect on the numbers of total leukocytes, mononuclear cells or neutrophils.

Inhibition of CCL11/eotaxin and IL-5 production by *Syzygium cumini* extract

In order to understand the effects of the SC extract on eosinophil mobilization during an allergic inflammation, we investigated the effect of *in vivo* oral pre-treatment with SC on CCL11/eotaxin and IL-5 levels in pleural wash fluid. As observed in Figure 5, intrathoracic antigen challenge significantly increased the levels of CCL11/eotaxin and IL-5 in pleural lavage fluid of sensitized animals 24 h after the challenge ($N = 7$/group; $P \leq 0.05$). Oral treatment with the SC extract led to a decrease in the levels of IL-5 (from 70.9 ± 25.2 to 12.05 ± 7.165 pg/mL; two experiments in triplicate; $P \leq 0.05$) and CCL11/eotaxin (from 60.4 ± 8.54 to 32.8 ± 8.4 ng/mL; two experiments in triplicate; $P \leq 0.05$) in pleural wash fluid. This effect was similar to the inhibition observed after dexamethasone treatment (from 70.9 ± 25.2 to 4.29 ± 2.60 pg/mL IL-5 and from 60.4 ± 8.54 to 13.40 ± 3.91 ng/mL CCL11/eotaxin).

Discussion

The results of the current study demon-

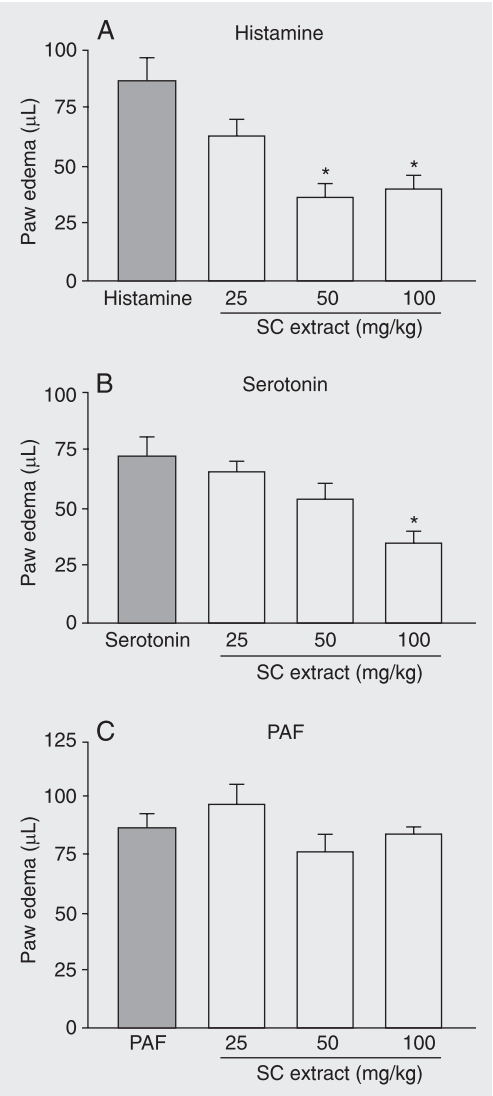

Figure 3. Effect of oral pre-treatment with different doses of aqueous extract of *Syzygium cumini* (SC) leaves on paw edema induced by histamine (100 µg/paw; A), serotonin (100 µg/paw; B) and platelet-aggregating factor (PAF; 1 µg/paw; C). Data are reported as mean ± SEM for at least 7 animals. *$P \leq 0.05$ compared to control (Student-Newman-Keuls post-test).

Table 1. Effect of *Syzygium cumini* extract and disodium cromoglycate on histamine release from rat peritoneal mast cells challenged *in vitro* with compound 48/80.

Groups	Histamine release (ng/mL)	% Inhibition
Mast cells + medium	2.1 ± 1.4	
Mast cells + C48/80	20.0 ± 5.0	0
DSCG + C48/80	13.8 ± 2.6	31.0
SC extract + C48/80	10.1 ± 6.1	49.5

Data regarding histamine release are reported as the mean ± SD of two experiments in triplicate. Rat peritoneal mast cells were incubated with *S. cumini* extract (SC; 1 µg/mL) or disodium cromoglycate (DSCG; 10.2 µg/mL) and challenged *in vitro* with compound 48/80 (C48/80; 5 µg/mL) 1 h later. Histamine present in the supernatant was quantified by fluorimetric assay.

strate that the leaf extract of SC displays a marked anti-allergic property. Treatment with the SC extract inhibited the paw edema induced by C48/80, a potent mast cell degranulator, to an extent comparable to the effect of promethazine, a classical anti-histaminic used to relieve symptoms of allergic reactions. Treatment with the SC extract also inhibited the paw edema triggered by antigen challenge, although this effect on allergic paw edema was not equivalent to the effect observed on C48/80-induced edema. Moreover, these results indicate a different mechanism of inhibition of C48/80 and antigen-induced paw edema by the SC extract, suggesting an action on specific targets.

Histamine, 5-HT and PAF have been extensively reported to be the major mediators involved in allergic edema formation. Supporting these observations, treatment with SC displayed an inhibitory effect on edema induced by histamine and 5-HT, but failed to inhibit PAF-induced paw edema. The participation of PAF in edema forma-

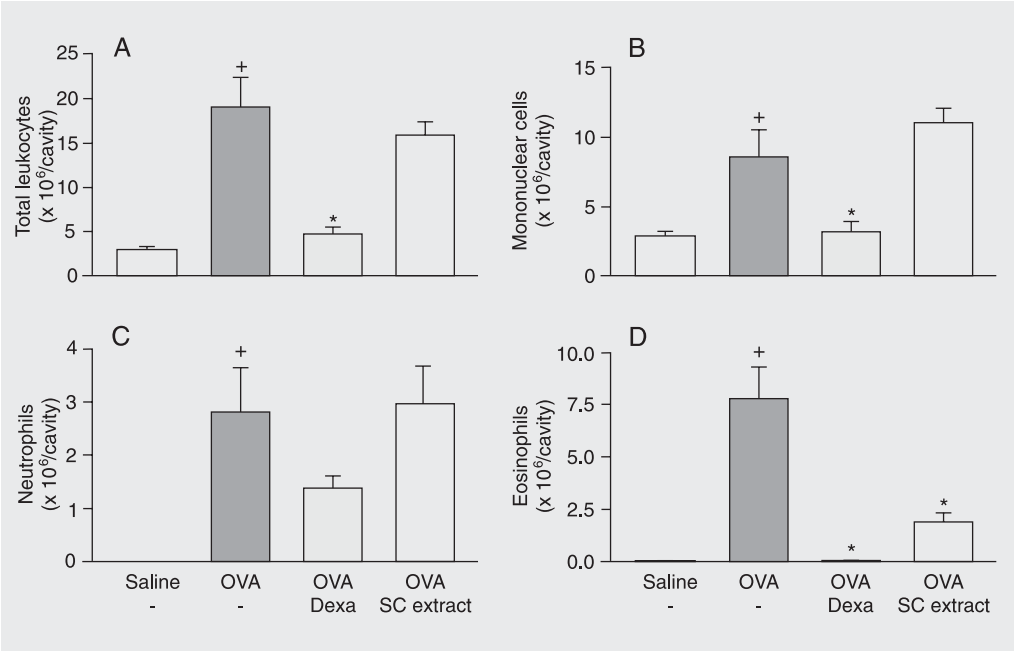

Figure 4. Inhibition of allergic pleurisy by oral pre-treatment (1 h) with the aqueous extract of *Syzygium cumini* (SC) leaves (100 mg/kg). Pleurisy was induced in 14 day-sensitized mice by antigen challenge (ovalbumin, OVA: 12.5 µg/cavity). Total leukocyte (A), mononuclear cell (B), neutrophil (C), and eosinophil (D) counts were performed 24 h after challenge. Data are reported as the mean ± SEM for at least 7 animals. Dexa = dexamethasone. $^{+}P \leq 0.05$ compared to saline group; $^{*}P \leq 0.05$ compared to untreated group (Student-Newman-Keuls post-test).

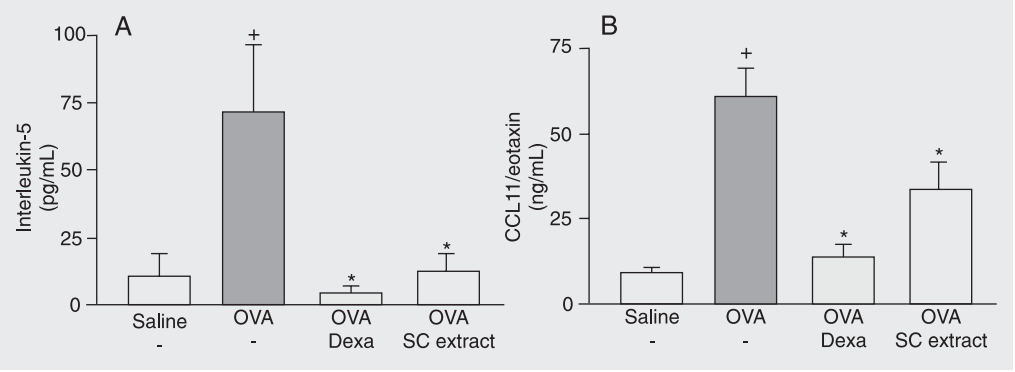

Figure 5. Effect of oral pre-treatment (1 h) with the aqueous extract of *Syzygium cumini* (SC) leaves (100 mg/kg) on interleukin-5 (A) and CCL11/eotaxin (B) generation in ovalbumin (OVA)-stimulated mice. Protein levels were determined by ELISA in pleural washes recovered 24 h after saline or OVA (12.5 µg/cavity) stimulation, compared with pleural washes recovered from mice pre-treated with dexamethasone (Dexa, 2 mg/kg; *ip*) or SC (100 mg/kg, *po*) and injected with OVA. $^{+}P \leq 0.05$ compared to saline group; $^{*}P \leq 0.05$ compared to untreated group (Student-Newman-Keuls post-test).

tion and eosinophil mobilization in allergic inflammation has been reported (20,21) and the lack of effect of the SC extract on PAF-induced edema and the participation of the other mediators in the triggering of allergic paw edema may account for discrete effect of the SC extract on allergic paw edema. These results suggest that the SC extract can be much more effective in inhibiting reactions whose mechanism depends on the release of histamine and of 5-HT.

Kim and colleagues (3) showed that treatment with an aqueous extract of *S. aromaticum* (L.) Merr. et Perry (Myrtaceae) flower buds had an inhibitory effect on C48/80-induced systemic anaphylaxis and IgE-mediated passive cutaneous anaphylaxis reaction. These results were due to an inhibitory action of this extract on histamine release from mast cells. In agreement with this report, we observed that the SC extract also had a direct effect on mast cell degranulation, inhibiting *in vitro* the histamine release induced by C48/80. This result suggests that the anti-edematogenic effect of SC on C48/80 or antigen-induced paw edema may be due to an action on the mast cell degranulation process. However, the effect of SC leaf extract on the edema triggered by exogenous histamine and serotonin also suggests that the SC extract has a direct effect on the inhibition of these mediators.

The presence of polyphenol, gallic acid, ellagic acid derivatives (22,23), tannins (24, 25), and glycosylated flavonoids (23,26,27) has been reported in *Syzygium* species. We extended the previous observation that SC leaf extracts contain flavonoids (23,27). Ramirez and Roa Jr. (28) showed a correlation between the anti-inflammatory activity and the content of total phenolic compounds in the extracts of SC. Our results on the anti-edematogenic effect of the SC extract also support the earlier observation of Slowing and colleagues (29) that the presence of flavonoid glycosides may be associated with the anti-inflammatory activity of a methanol extract of *E. jambos* leaves. The presence of phenolic compounds, especially flavonoids, in the aqueous extract of SC leaves and its anti-edematogenic activity justify the use of aqueous extracts and infusions of the plant in folk medicine.

Treatment with the SC extract inhibited eosinophil accumulation in allergic pleurisy, without a significant change in mononuclear cell or neutrophil recruitment. This treatment also inhibited the rise of IL-5 and CCL11/eotaxin levels in pleural lavage fluid in allergic pleurisy.

Eosinophils play an important role in the pathophysiology of allergic diseases (30,31). The accumulation and survival of these cells are regulated by IL-5 secreted by activated T lymphocytes, as well as by chemokines released by the epithelium. Among the C-C chemokines, CCL11/eotaxin, an eosinophil-specific chemoattractant, is one of the most important mediators of allergic inflammation, with a potent and selective effect in mobilizing eosinophils from bone marrow to the blood (11,32-34). The effect of treatment with the SC extract on IL-5 and CCL11/eotaxin levels may explain the specific inhibition of eosinophil accumulation induced by SC.

These activities of the SC extract may probably be due to the presence of flavonoids in the extract, since these substances isolated from Myrtaceae species, including SC, are known to exert a potent inhibitory effect on a variety of enzymes related to cell activation and to the production of inflammatory mediators (35,36). Some isolated flavonoids possess anti-inflammatory (37), anti-allergic (38) and analgesic (39) activities; however, few flavonoids from SC leaves (23,27) and flowers have been isolated or identified (40).

Our findings indicate an anti-allergic activity of the SC extract observed by the inhibition of edema formation, mast cell degranulation and histamine release as well as the inhibition of eosinophil accumulation

and CCL11/eotaxin and IL-5 production. Taken together, the present results suggest the potential of SC as a herbal-based therapy for the treatment of allergic diseases.

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