



The anti-allergic activity of the acetate fraction of *Schinus terebinthifolius* leaves in IgE induced mice paw edema and pleurisy

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Abstract

Schinus is a genus of the Anacardiaceae family and contains *Schinus terebinthifolius*, the Brazilian pepper tree that is widely used in folk medicine. We investigate the anti-allergic activity of the ethyl acetate fraction of *S. terebinthifolius* Raddi (ST fraction). HPLC analysis revealed that gallic acid, methyl gallate and 1,2,3,4,6-pentagalloylglucose are the major aromatic components of the fraction. Oral pre-treatment with the ST fraction (100 mg/kg) significantly inhibited paw edema induced by compound 48/80 (100 ng/paw) and to a lesser extent, the allergic paw edema (OVA, 3 µg/paw). The ST fraction (100 and 200 mg/kg) also inhibited the edema induced by histamine (100 µg/paw), preventing mast cell degranulation and, consequently, histamine release in Wistar rat peritoneal mast cells induced by C 48/80 (5 µg/mL). This histamine inhibition was also observed after mast cell pre-treatment with both methyl gallate and 1,2,3,4,6-pentagalloylglucose (100 µg/mL), the isolated compounds from the ethyl acetate fraction. Pre-treatment with the ST fraction (100 mg/kg) significantly inhibited total leukocyte and eosinophil accumulation in pleural cavities 24 h after the intrathoracic injection of OVA (12.5 µg/cavity). This effect was related to the inhibition of CCL11/eotaxin and CCL5/RANTES in pleural lavage fluid. Pre-treatment with this fraction (100 mg/kg) failed to reduce the cell influx that was observed after LPS-injection into pleural cavity (250 ng/cavity). These findings demonstrate the anti-allergic effect of the ST

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fraction, which includes the inhibition of edema formation and histamine release caused by mast cell degranulation and eosinophil influx into the pleural cavity probably reflected by the decreased levels of chemokines in recovered pleural lavage fluid.

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1. Introduction

The worldwide prevalence of allergic diseases has increased during the last two decades [1,2]. Acute allergic reactions result from the release of preformed granule-associated histamine and tryptase, membrane-derived lipid mediators (leukotrienes, prostaglandins and platelet activating factor), cytokines and chemokines; release of these signals occurs when an allergen interacts with IgE bound to the membranes of mast cells and basophils, which leads to events such as smooth muscle contraction, vasodilatation, increased vascular permeability and mucous hypersecretion [3]. Thus, when allergic disease occurs, affected tissue is infiltrated by cells with a Th2-type cytokine profile (such as IL-4, IL-5, IL-9 and IL-13 [4–6], which favors the synthesis of IgE) and mast cells, lymphocytes and eosinophils are activated, all of which leads to inflammation and disease [7,8].

As mentioned above, during this initial phase, recruitment and activation of eosinophils also occurs. The recruitment of this cell type into the airways after allergen challenge is a complex process, involving several cytokines, lipid mediators and chemokines like CCL11/eotaxin, CCL5/RANTES and macrophage inflammatory protein (MIP)-1 α , [9,10]. Once in the allergic site, eosinophils play an important role in the pathophysiology of allergic diseases by releasing numerous mediators such as cationic protein, major basic protein, PAF, leukotrienes (LT) C4, LTD4, prostaglandins and eosinophil-derived neurotoxin, which induce tissue damage [11]. On the other hand, eosinophils may also function as repair cells since they produce fibrogenic growth factors and matrix metalloproteinase, which is involved in airway tissue remodeling in asthma [12].

Schinus is a genus of trees from the Anacardiaceae family that includes the pepper trees, most notably *Schinus molle*, the Peruvian pepper tree, and *Schinus terebinthifolius*, the Brazilian pepper tree. This plant is indigenous to the coast of tropical Brazil and is distributed from northeast to south of Brazil. In folk medicine, it is used as anti-inflammatory, febrifuge, analgesic, depurative, and to treat illnesses of the urogenital system and others. The phytochemical study of the *Schinus* genus revealed the presence of terpenoids and fatty acids in both *S. molle* [13,14] and *S. terebinthifolius* [15].

In the present study, we investigate the capacity of an acetate fraction of *S. terebinthifolius* Raddi to inhibit allergic edema formation and eosinophil accumulation in mouse allergic pleurisy and the mechanisms implicated in this phenomenon.

2. Materials and methods

2.1. Materials

Ovalbumin (OVA), Compound 48/80 (C48/80), histamine, disodium cromoglycate, phosphate-buffered saline (PBS),

Tween 20, *o*-phenylenediamine dihydrochloride (OPD), ethylenediaminetetracetic sodium salt (EDTA), Hank's balanced salt solution (HBSS), bovine serum albumin (BSA) and LPS (from *Escherichia coli* serotype 0127-B8) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Aluminum hydroxide was purchased from EMS Sigma Pharma (São Paulo, Brazil). Purified anti-murine eotaxin and RANTES, biotinylated anti-eotaxin and RANTES and recombinant eotaxin and RANTES were all obtained from R&D Systems (Minneapolis, MN). Streptavidin-conjugated horseradish peroxidase was purchased from Caltag Laboratories (Burlingame, CA). 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).

2.2. Animals

Male BALB/c mice (18–20 g), Swiss Webster mice (20–25 g) and Wistar rats (180–200 g), provided by the Oswaldo Cruz Foundation breeding unit (CECAL-FIOCRUZ), were used. The animals were provided with a standard pellet diet and tap water *ad libitum* in a room with the temperature ranging from 22 to 24 °C under a cycle of 12 h light/darkness. All experiments were performed in accordance with the Committee on Ethical Use of Laboratory Animals of Fundação Oswaldo Cruz (CEUA 0384/07) and the ethical guidelines of the International Association for the Study of Pain [16].

2.3. Plant material and fraction preparation

Entire leaves of *S. terebinthifolius* were collected in July 2000 at Rio de Janeiro, Brazil, with a voucher specimen (RB-444.626) deposited at Rio de Janeiro Botanical Garden Herbarium. The plant material was ground and exhaustively extracted with methanol by static maceration. The solution was filtered and concentrated in a rotatory evaporator, and the extract was suspended in water-methanol (9:1) and partitioned in turn with dichloromethane (20 mL $\times$ 3) and ethyl acetate (20 mL $\times$ 3). The ethyl acetate fraction was evaporated from methanol extract under reduced pressure to 9.36% (w/w). The fraction was analyzed by HPLC (Shimadzu LC-10 ADVP) with a diode array detector (SPD-M10AVP) and an auto injector (SIL-10ADVP). Analysis was performed on a supelcosil (LC18) column, and the following solvent system conditions were used: (A) acetonitrile/methanol (75:25) and (B) TFA 0.05%, flow rate 1.0 mL/min, detection at 220 nm. The spectrometric analysis (^1H and ^{13}C NMR, UV, IR), according to the literature data [22,23] indicated the presence of three aromatic compounds identified as gallic acid, methyl gallate and 1,2,3,4,6-pentagalloylglucose (Fig. 1).

These compounds were isolated using counter-current chromatography (Pharma-Tech, CCC1000). Finally, the

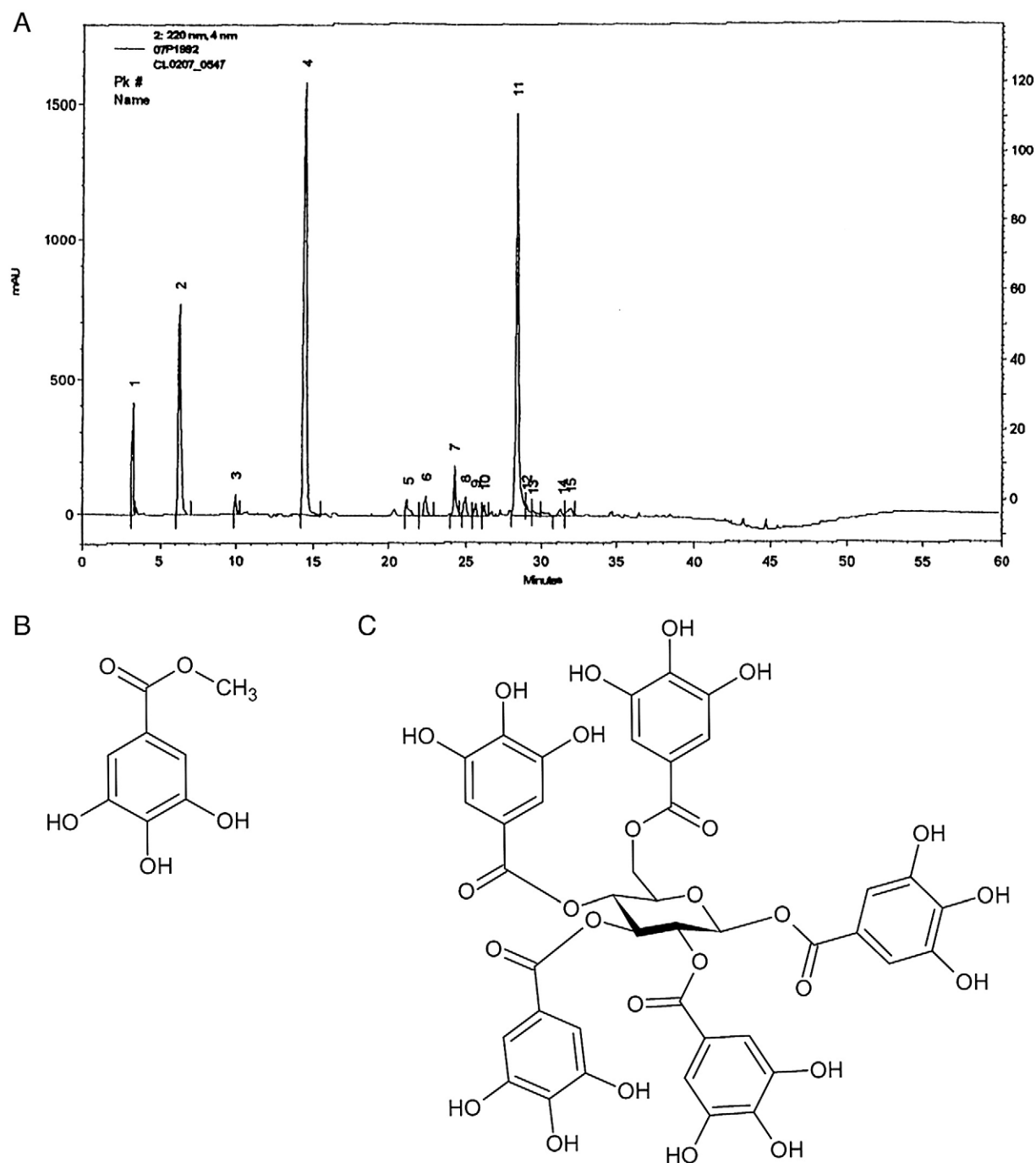


Figure 1 (A) HPLC chromatograms of the ethyl acetate fraction from *Schinus terebinthifolius*. The spectrometric analysis indicated the presence of Gallic acid (2), Methyl gallate (4) and 1,2,3,4,6-pentagalloylglucose (11). (B) The structure of Methyl gallate and (C) 1,2,3,4,6-pentagalloylglucose.

compound's quantification, carried out in HPLC, indicated that the ethyl acetate fraction presents 4% of gallic acid, 28% of methyl gallate and 26% of 1,2,3,4,6-pentagalloylglucose. The standard working solution were prepared individually in methanol/water (75:25 v/v) and was injected in triplicate.

2.4. Ovalbumin sensitization

The mice were actively sensitized by a subcutaneous (s.c.) injection of 0.2 mL of a mixture of ovalbumin (OVA, 50 µg)

and aluminum hydroxide ($\text{Al}(\text{OH})_3$, 5 mg). The experiments were performed 14 days thereafter.

2.5. Treatments

In paw edema experiments, mice received the anti-histamine agent, the H1-receptor antagonist promethazine (10 mg/kg), or aqueous ST fraction (25–200 mg/kg) orally (p.o.) 1 h before stimulation. For pleurisy experiments, dexamethasone was given intraperitoneally (i.p., 10 mg/kg), whereas the

aqueous extract was given orally (p.o., 100 mg/kg), both 1 h before challenge with OVA or induction by LPS. In both experiments, 12 h fasted animals received 200 μ L of treatment orally, and the control groups were treated with the same volume of vehicle alone.

2.6. Induction of paw edema

Paw edema was induced as previously described [17]. Briefly, naïve Swiss mice received an intraplantar (i.pl.) injection of histamine (100 μ g/paw) or Compound 48/80 (C48/80, 100 ng/paw) into one hind paw. The final volume was 50 μ L/paw and the contralateral paw received the same volume of sterile saline and was used as control. The volumes of each hind paw were measured by using a plethysmometer (Ugo Basile, Italy), 30 min after stimulation.

In another set of experiments, sensitized Swiss mice received an i.pl. injection of OVA (3 μ g/paw) and the induction of paw edema was evaluated 30 min after stimulation. In both moments, Paw edema was expressed in μ L as the difference between stimulated and non-stimulated paws.

2.7. Mast cell purification and histamine measurement

Isolation of mast cells was performed using a continuous Percoll gradient as previously reported [18]. Briefly, cells were recovered from the peritoneal cavity of normal Wistar rats after rinsing with 20 mL of heparinized (10 IU/mL) calcium and magnesium-free Hank's solution (HBSS⁻). After centrifugation of the cells at 150 g for 10 min, the supernatant was discarded and the pellet resuspended in HBSS⁻ containing 0.1% bovine serum albumin (BSA). Cells were pooled, mixed with isotonic Percoll (72%) and overlaid with 1 mL of HBSS⁻. Purified mast cells were resuspended in Hank's solution containing Ca²⁺ and Mg²⁺ (HBSS⁺). Purity (95%) and viability (98%) were evaluated by Toluidine blue and Trypan blue exclusion staining, respectively. The cells were added to a 96-well plate (10⁵ cells/well) and preincubated for 1 h in a 5% CO₂ atmosphere at 37 °C with 50 or 500 μ g/mL of dried ST fraction and, in another set of experiments, with 100 μ g/mL of dried ST fraction, 1,2,3,4,6-pentagalloylglucose or methyl gallate. All treatments were dissolved in HBSS⁺, and in both experiments, disodium cromoglycate (10.2 μ g/mL) was used as a reference inhibitor. After this period, cells were incubated for 30 min with 5 μ g/mL C48/80. The reaction was stopped on ice and the supernatants were recovered and stored (-20 °C).

To perform the *in vitro* IgE mast cell sensitization, the cells were added to a 96-well plate (10⁵ cells/well) and incubated with IgE at 1 μ g/mL overnight at 5% CO₂ atmosphere and 37 °C. Then, sensitized mast cells were preincubated with 10 or 100 μ g/mL of dried ST fraction and 100 μ g/mL of 1,2,3,4,6-pentagalloylglucose or methyl gallate. One hour later, these cells were challenged with DNP-BSA (1 μ g/mL) for 4 h, and the supernatants were stored (-20 °C). Disodium cromoglycate (10.2 μ g/mL) was used as a reference inhibitor.

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).

2.8. Pleurisy

After previous sensitization against OVA, mice were challenged with an intrathoracic (i.t.) injection of OVA (12.5 μ g/cavity) as described elsewhere [20,21]. Briefly, an adapted needle was inserted into the right side of the thoracic cavity of animals to enable the intrapleural administration of OVA diluted in sterile, pyrogen-free saline (50 μ L). Sensitized BALB/c mice challenged with saline vehicle alone were used as a negative control group. In another set of experiments, naïve Swiss Webster mice were injected via i.t. with LPS (250 ng/cavity) in the same final volume (50 μ L). The control group received an i.t. injection of vehicle. In both instances, 24 h after stimulus, mice were killed by excess carbon dioxide, and their thoracic cavities were rinsed with 1 mL of PBS containing EDTA (10 mM), pH 7.4. Total leukocyte counts were performed in an automatic particle counter Z2: Counter (Coulter, Beckman Coulter, Fullerton, California, USA). Differential cell counts were done using stained cytospin smears (Shandon, Pittsburgh, Pennsylvania, USA) by the May-Gruenwald-Giemsa method under light microscopy (100 $\times$). Counts are reported as numbers of cells ($\times 10^6$) per cavity.

2.9. Enzyme-linked immunosorbent assay

Levels of CCL11/eotaxin and CCL5/RANTES in the cell-free pleural fluid were evaluated by sandwich enzyme-linked immunosorbent assay (ELISA), using matched antibody pairs from PharMingen (San Diego, CA), according to the manufacturer's instruction. Results are expressed as ng/mL, and values were obtained by comparison with a standard curves (0.031–1 ng/mL for CCL11/eotaxin and 0.002–0.5 ng/mL for CCL5/RANTES).

2.10. Statistical analysis

All data are presented as mean $\pm$ S.E.M. and 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 to be statistically significant.

3. Results

3.1. Effect of the *S. terebinthifolius* fraction on paw edema

Histamine is a major inflammatory mediator involved in acute allergic reactions. We evaluated a possible anti-histaminic effect of The ST fraction using a paw edema model induced by histamine. As observed in Fig. 2A i.pl. injection of histamine (100 μ g/paw) into the hind paw of naïve mice induced a significant paw edema; 30 min later, it was inhibited by promethazine treatment (10 mg/kg, p.o., 46.4% inhibition) and also by oral treatment with the ST fraction at doses of 100 mg/kg (79.6% inhibition) and 200 mg/kg (86.1% inhibition).

The next step was to evaluate the effect of the ST fraction on allergic reactions; the model of anaphylaxis edema triggered by the mast cell degranulator C48/80, as well as by OVA injection, in sensitized animals was used. As observed in Fig. 2B the i.pl.

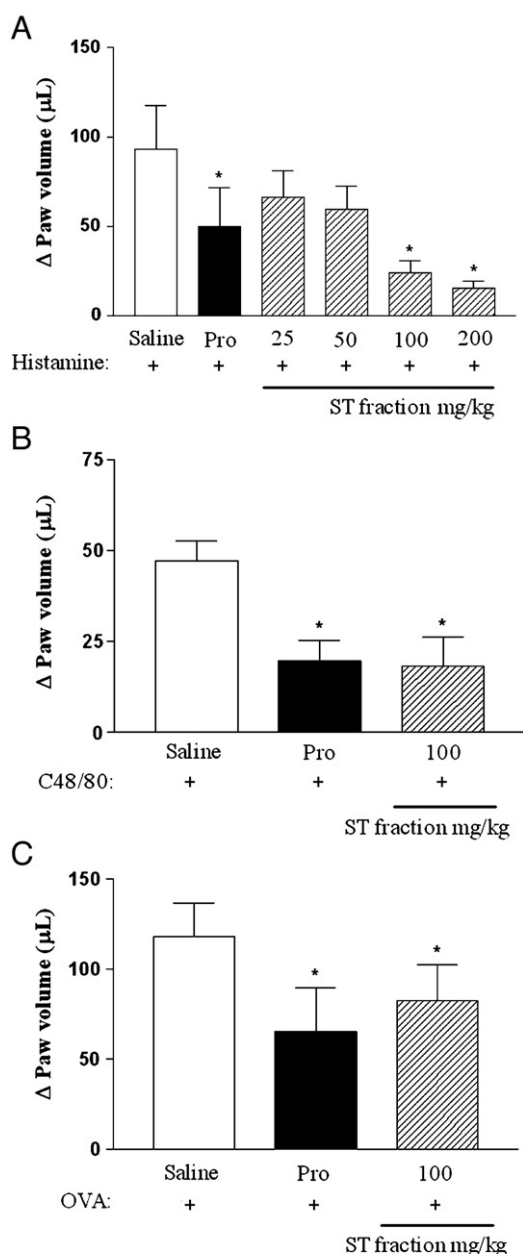


Figure 2 Effect of oral pre-treatment with *Schinus terebinthifolius* fraction (ST fraction) on paw edema induced by histamine (100 μ g/paw, ipl; A) compound 48/80 (C48/80, 100 ng/paw, ipl; B) or ovalbumin (OVA, 3 μ g/paw, ipl; C). Promethazine (10 mg/kg, p.o.) was used as reference inhibitor. Results are expressed as the mean $\pm$ S.E.M. for at least 5 animals per group. * $P \leq 0.05$ compared to saline control.

administration of C48/80 into the naïve mouse hind paw induced a significant paw edema 30 min after the injection. Oral pre-treatment with the ST fraction at a dose of 100 mg/kg (61.1% inhibition) inhibited the edema formation. This inhibition was also observed after promethazine treatment (10 mg/kg, p.o., 58.4% inhibition). The anaphylactic edema caused by OVA (50 μ g) triggered paw swelling within 30 min in sensitized mice. The oral administration of the ST fraction (100 mg/kg, p.o.) inhibited

edema formation as did promethazine (30.2% and 44.9% inhibition, respectively), as shown in Fig. 2C.

3.2. Effect of the *S. terebinthifolius* fraction on histamine release from peritoneal mast cells

Mast cell degranulation, followed by the release of mediators (mainly histamine) is a major component of allergic edema. Thus, we investigated the effect of the ST fraction on the histamine released during mast cell degranulation. Fig. 3A shows that stimulation with C48/80 (5 μ g/mL) induced the release of histamine in rat mast cells. This phenomenon was significantly suppressed by treatment with disodium cromoglycate, a classic mast cell membrane stabilizer (27.2% inhibition). Pre-treatment with the ST fraction (50 and 500 μ g/mL, 1 h before C48/80) also

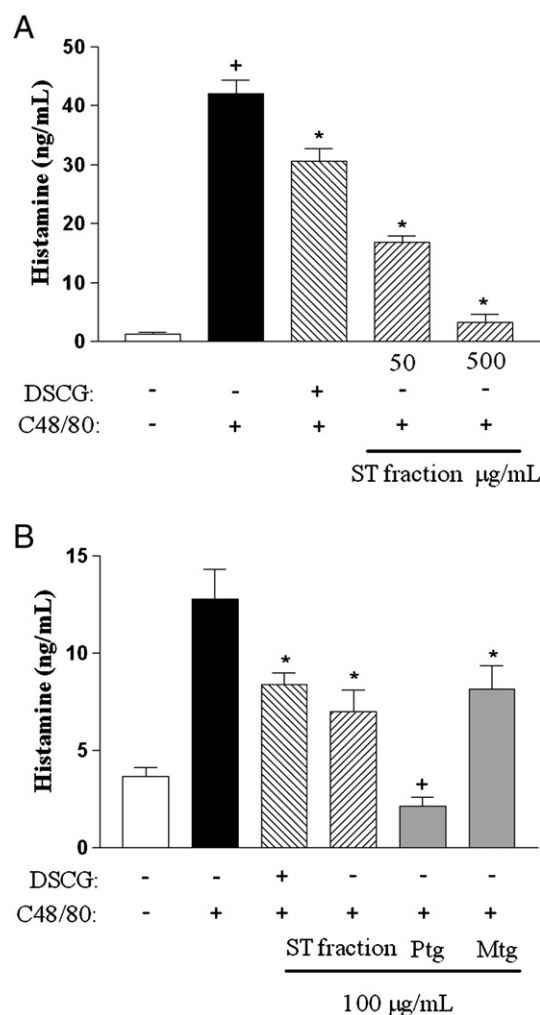


Figure 3 Effect of *Schinus terebinthifolius* fraction (ST fraction 50–500 μ g/mL; A) and *Schinus terebinthifolius* fraction, 1,2,3,4,6-pentagalloylglucose and methyl gallate (ST fraction, Ptg and Mtg respectively 100 μ g/mL, B) on histamine release from rat peritoneal mast cells challenged *in vitro* with compound 48/80 (C48/80, 5 μ g/mL). Mast cells were incubated with DSCG (10.2 μ g/mL) as control. Histamine was quantified by fluorimetric assay. Results are expressed as the mean $\pm$ S.E.M. of one experiment in triplicate. * $P \leq 0.05$ and * $P \leq 0.01$ compared to C48/80 stimulated group.

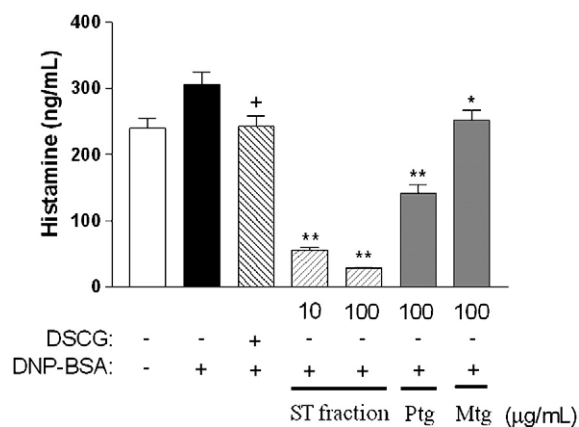


Figure 4 Effect of *Schinus terebinthifolius* fraction, 1,2,3,4,6-pentagalloylglucose or methyl gallate (ST fraction, Ptg and Mtg respectively) on histamine release from IgE sensitized rat peritoneal mast cells challenge with DNP-BSA (1 µg/mL). Histamine was quantified by fluorimetric assay from supernatant collected 4 h post antigen challenge. Results are expressed as the mean $\pm$ S.E.M. of one experiment in triplicate. * $P \leq 0.05$, * $P \leq 0.01$ and ** $P \leq 0.001$ as compared to sensitized and challenged mast cells.

significantly inhibited the release of histamine (59.9% and 92.2% inhibition, respectively). A similar result was observed with substances isolated from the ST fraction; both 1,2,3,4,6-pentagalloylglucose and methyl gallate (100 µg/mL, 1 h before C48/80) were able to inhibit histamine release (83.1% and 36.15%,

respectively; Fig. 3B). In Fig. 4 it is observed that histamine release after the passive sensitization of IgE mast cells is significantly inhibited with the pre-treatment, 1 h before antigen challenge, with the ST fraction (10 and 100 µg/mL), 1,2,3,4,6-pentagalloylglucose (100 µg/mL) and methyl gallate (100 µg/mL) (81.68%, 90.63%, 53.8% and 17.3% of inhibition, respectively).

3.3. Effect of the *S. terebinthifolius* fraction on allergic pleurisy

Pleural washes were recovered 24 h after saline or OVA (12.5 µg/cavity) challenge. As shown in Fig. 5, the i.t. injection of OVA into previously sensitized mice induced an intense accumulation of leukocytes (Fig. 5A), especially eosinophils (Fig. 5D), whereas a small number of neutrophils remain in the pleural cavity of the animals (Fig. 5C). Oral pre-treatment with the ST fraction (100 mg/kg) 1 h before stimulation significantly inhibited the total leukocyte and eosinophil influx into the pleural cavity, as did dexamethasone (10 mg/kg, i.p.).

3.4. Inhibition of CCL11/eotaxin and CCL5/RANTES production by the *S. terebinthifolius* fraction

As observed in Fig. 6, intrathoracic antigen challenge significantly increased the levels of CCL11/eotaxin and CCL5/RANTES in pleural lavage fluid of sensitized animals 24 h after the challenge. The oral pre-treatment of mice with the ST fraction (100 mg/kg) induced a significant reduction in the levels of both chemokines (from 0.21 ± 0.004 to 0.10 ± 0.008 ng/mL CCL11/eotaxin and 0.51 ± 0.004 to 0.019 ± 0.004 ng/mL CCL5/RANTES). A similar result was observed after dexamethasone

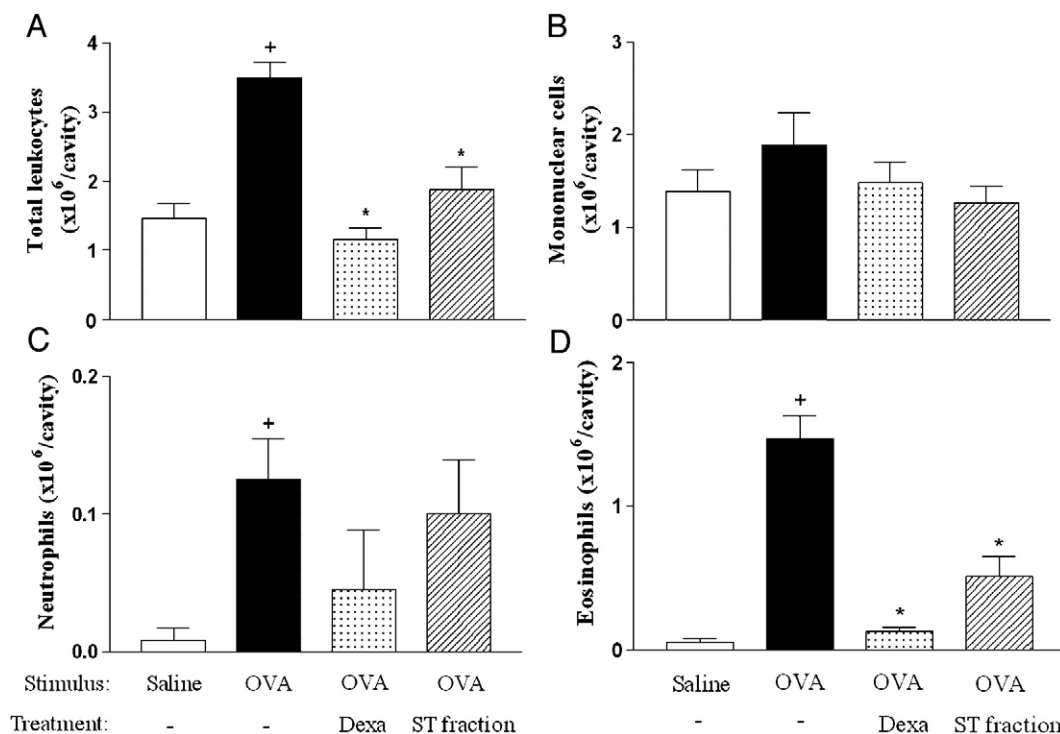


Figure 5 Effect of oral pre-treatment with *Schinus terebinthifolius* fraction (ST fraction, 100 mg/kg) on leukocytes (A), mononuclear cell (B), neutrophil (C) and eosinophil (D) accumulation into pleural cavity 24 h after OVA i.t. injection (12.5 µg/cavity). Dexamethasone (Dexa, 10 mg/kg, i.p.) was used as reference inhibitor. Results are expressed as the mean $\pm$ S.E.M. for at least 5 animals per group. * $P \leq 0.01$ compared to saline group; * $P \leq 0.01$ compared to untreated group.

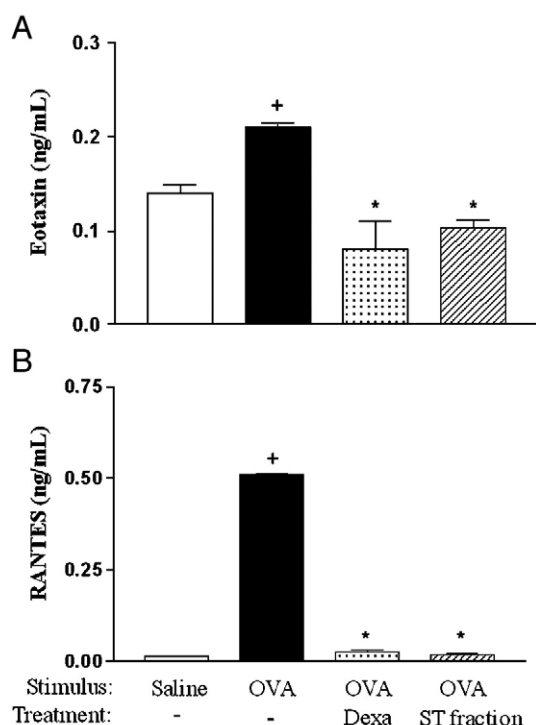


Figure 6 Effect of pre-treatment with *Schinus terebinthifolius* fraction (ST fraction, 100 mg/kg, p.o.) on CCL11/eotaxin (A) and CCL5/RANTES (B) generation on OVA-stimulated mice. Protein levels were determined by ELISA in pleural washes recovered 24 h after saline or ovalbumin (OVA, 12.5 µg/cavity, i.t.) challenge, compared with pleural washes recovered from mice pre-treated with dexamethasone (Dexa, 10 mg/kg, i.p) or ST fraction and injected with OVA. Results are expressed as the mean ± S.E.M. for at least 5 animals per group. ⁺ $P \leq 0.05$ compared to saline group; ^{*} $P \leq 0.05$ compared to untreated group.

treatment (from 0.21 ± 0.004 to 0.08 ± 0.03 ng/mL CCL11/eotaxin and 0.51 ± 0.004 to 0.027 ± 0.004 ng/mL CCL5/RANTES).

3.5. Effect of the *S. terebinthifolius* fraction on pleurisy induced by LPS

In order to study if ST fraction pre-treatment was acting specifically on allergic pleurisy eosinophilia, we investigated its effects on LPS-induced pleurisy. As shown in Fig. 7, the i.t. injection of LPS into the pleural cavity of mice induced a significant increase in the number of total leucocytes in the pleural cavity 24 h after stimulation (Fig. 7A). This increase was due mainly to neutrophil migration (Fig. 7C). Dexamethasone pre-treatment (10 mg/kg, i.p.) inhibited the leukocyte accumulation. However, oral administration of the ST fraction (100 mg/kg) 1 h before stimulation was not able to reduce the cell influx observed after LPS-injection into pleural cavity.

4. Discussion

The present study demonstrates that the ST fraction and the aromatic compounds 1,2,3,4,6-pentagalloylglucose or methyl gallate isolated from the ST fraction exhibit important anti-allergic properties, which are marked by inhibition

of edema formation, mast cell degranulation, and eosinophil influx as a result of decreased production of eosinophilotactic mediators. Treatment with the ST fraction inhibited the paw edema triggered by C48/80 to the same extent as promethazine, the reference anti-histaminic compound tested. This treatment also inhibited the paw edema triggered by antigen challenge, although this effect was not similar to that observed on C48/80-induced edema. It is well established that mast cell activation may be initiated upon interaction of an allergen with its specific IgE antibody attached to the cell membrane through high-affinity receptor FcεRI; this causes the release, in the tissues, of several inflammatory mediators such as histamine [24], serotonin (5-HT) [25] and platelet activating factors (PAF) [26] from mast cells. The literature shows that both 5-HT and PAF, when injected into the hind paw of naïve mice, triggered paw swelling within 30 min after stimulus. In addition, prostaglandin (PG) D2 is generated after the immunologic activation of mast cells, and it can contribute significantly to inflammatory and allergic reactions, playing a key role in the generation of edema and hyperalgesia [27]. Moreover, histamine [28] and PAF [29] are potent stimulators of PG production. Thus, it is possible to understand, at least in part, the decreased inhibition of ST fraction treatment observed on allergic paw edema when compared to C48/80-induced edema.

Once produced and stored, histamine is liberated in large quantities during the immediate phase of allergic reactions, evoking features such as plasma leakage and edema [30,31]. The involvement of this mediator has been suggested to be not only an early-phase mediator producing clinical allergic symptoms through binding specific cell receptors, H1 to H4 [32–34], but also a participant during the late-phase allergic response, activating neutrophils and eosinophils and being a chemoattractant for these cells [35]. Moreover, histamine increases IL-8 levels and evokes leukocyte rolling in endothelial cells [36,37]. The anti-histaminic effect of the ST fraction was evaluated against histamine-induced hind paw edema in mice. It is noteworthy that the extent of inhibition the ST fraction provided against histamine-induced paw edema was better than that obtained with promethazine, the reference anti-histaminergic compound tested. Nonetheless, the mechanisms by which the ST fraction inhibits allergic-induced edema might differ from those underlying the actions of promethazine (i.e., histamine H₁ receptor blockade) as the ST fraction also effectively inhibited *in vitro* histamine released during mast cell degranulation that was induced by C48/80 or after antigen challenge with DNP-BSA. In addition, the isolated aromatic compound 1,2,3,4,6-pentagalloylglucose was much more efficient than methyl gallate at inhibiting mast cell degranulation caused by both stimuli and seems to be one of the major component responsible for this effect.

In light of such considerations, it appears that the anti-allergic properties of the ST fraction can be ascribed to the inhibition of pro-inflammatory mechanisms triggered via histamine as well as direct action on mast cell membrane.

Blood and tissue eosinophilia are characteristic features of allergic inflammation disease conditions associated with prominent production of Th2 cytokines IL-4, IL-5, and IL-13. It is known that IL-5 plays an important role in their differentiation, proliferation and maturation in the bone marrow [38]. Moreover, the chemokines released by the

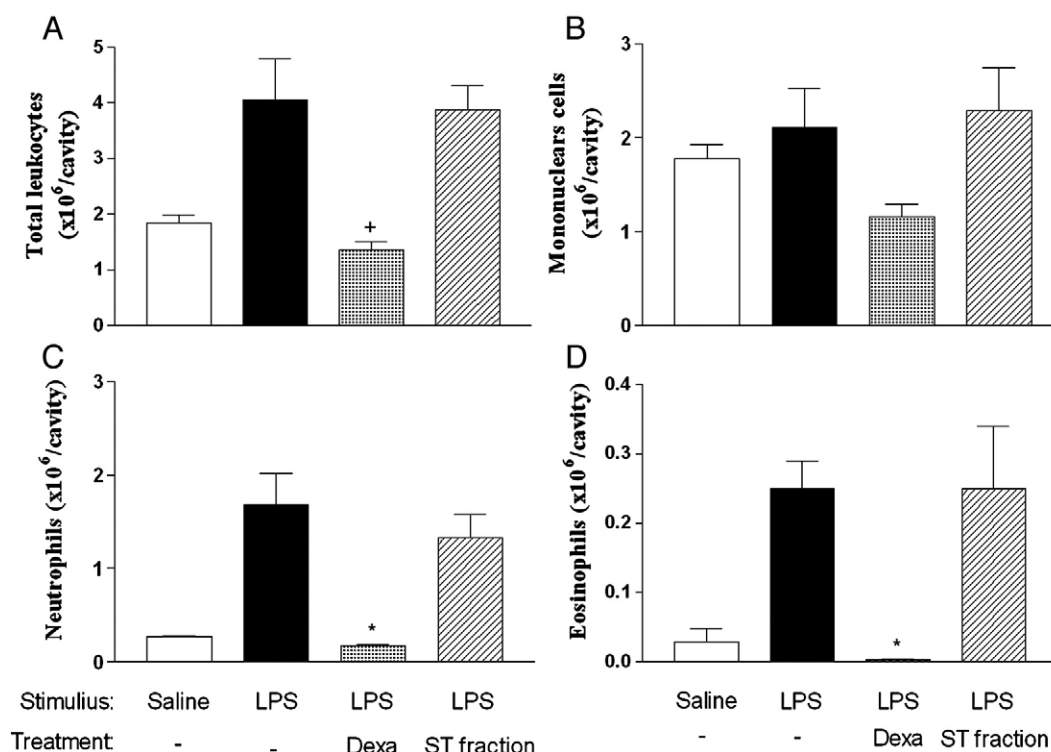


Figure 7 Effect of oral pre-treatment with *Schinus terebinthifolius* fraction (ST fraction, 100 mg/kg) on leukocytes (A), mononuclear cell (B), neutrophil (C) and eosinophil (D) accumulation into pleural cavity 24 h after LPS i.t. injection (250 ng/cavity). Dexamethasone (Dexa, 10 mg/kg, i.p.) was used as reference inhibitor. Results are expressed as the mean $\pm$ S.E.M. for at least 5 animals per group. ^{*} $P \leq 0.05$ compared to saline group; ^{*} $P \leq 0.01$ compared to untreated group.

epithelium are important to eosinophil accumulation and survival. Among the C-C chemokines, CCL11/eotaxin, in addition to IL-5, diffuses into the circulation and acts synergistically to induce eosinophil release from the bone marrow into the blood. CCL5/RANTES, some of the monocyte chemoattract proteins (MCPs) and MIP-1 α are other CC chemokines that are also known to stimulate eosinophil responses [39–41]. Based on that, we examined the leukocyte influx into the pleura cavity, and our results show that the treatment with the ST fraction inhibited eosinophil accumulation in allergic pleurisy without a significant change in mononuclear cell or neutrophil recruitment. This treatment also inhibited the increase in CCL11/eotaxin and CCL5/RANTES levels in pleural lavage fluid, explaining, at least in part, the lower influx of eosinophil to pleural cavity observed after OVA's challenge.

Taken together, our results suggest an anti-allergic property for the ST fraction that inhibits histamine release during mast cell degranulation, diminishing edema formation and inhibiting eosinophil influx and CCL11/eotaxin and CCL5/RANTES production. The precise molecular mechanisms underlying the activity of The ST fraction, however, remain to be elucidated.

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References

- [1] Holgate ST. The epidemic of allergy and asthma. *Nature* 1999;402:B2–4.
- [2] Williams H, Robertson C, Stewart A. World variations in the prevalence of symptoms of atopic eczema in the International Study of Asthma and Allergies in Childhood. *J Allergy Clin Immunol* 1999;103:125–38.
- [3] Kay AB. Overview of allergy and allergic diseases: with a view to the future. *Br Med Bull* 2000;56:843–64.
- [4] Bochner BS, Udem BJ, Lichtenstein LM. Immunological aspects of allergic asthma. *Annu Rev Immunol* 1994;12:295–335.
- [5] Busse WW, Lemanske Jr RF. Asthma. *N Engl J Med* 2001;344:350–62.
- [6] Umetsu DT, McIntire JJ, Akbari O, Macaubas C, DeKruyff RH. Asthma: an epidemic of dysregulated immunity. *Nat Immunol* 2002;3:715–20.
- [7] Miescher SM, Vogel M. Molecular aspects of allergy. *Mol Aspects Med* 2002;23:413–62.
- [8] Hamelmann E, Gelfand EW. IL-5-induced airway eosinophilia—the key to asthma? *Immunol Rev* 2001;179:182–91.
- [9] Luster AD. Chemokines: chemotactic cytokines that mediate inflammation. *N Engl J Med* 1998;338:436–45.
- [10] Pease JE, Williams TJ. Eotaxin and asthma. *Curr Opin Pharmacol* 2001;1:248–53.
- [11] Adamko DJ, Odemuyiwa SO, Vethanayagam D, Moqbel R. The rise of phoenix: the expanding role of the eosinophil in health and disease. *Allergy* 2005;60:13–22.

- [12] Levi-Schaffer F, Garbuzenko E, Rubin A. Human eosinophil regulate human lung-and skin derived fibroblast properties *in vitro*: a role for transforming growth factor beta (TGF- β). *Proc Natl Acad Sci USA* 1999;96:9660–5.
- [13] Pozzo-Balbi T, Nobile L, Scapini G, Cini M. The triterpenoid acids of *Schinus molle*. *Phytochemistry* 1978;17:2107–10.
- [14] Terhune SJ, Hogg JW, Lawrence BM. β -Spathulene: a new sesquiterpene in *Schinus molle* oil. *Phytochemistry* 1974;13:865–6.
- [15] Lloyd HA, Jaouni TM, Evans SL, Morton JF. Terpenes of *Schinus terebinthifolius*. *Phytochemistry* 1997;16:1301–2.
- [16] Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. *Pain* 1983;16:109–10.
- [17] Henriques MG, Silva PM, Martins MA, Flores CA, Cunha FQ, Assreuy-Filho J, et al. Mouse paw oedema. A new model for inflammation. *Braz J Med Biol Res* 1987;20:243–9.
- [18] De Oliveira BE, De Frias Carvalho V, Diaz BL, Balduino A, Cordeiro RS, Martins MA. Adoptive transfer of mast cells abolishes the inflammatory refractoriness to allergen in diabetic rats. *Int Arch Allergy Immunol* 2003;131:212–20.
- [19] Shore PA, Burkhalter A, Cohn Jr VH. A method for the fluorometric assay of histamine in tissues. *J Pharmacol Exp Ther* 1959;127:182–6.
- [20] Sampaio ALF, Rae GA, Henriques MGO. Role of endothelins on lymphocyte accumulation in allergic pleurisy. *J Leukoc Biol* 2000;67:189–95.
- [21] Henriques MG, Weg VB, Martins MA, Silva PM, Fernandes PD, Cordeiro RS, et al. Differential inhibition by two hexazepine PAF antagonists of acute inflammation in the mouse. *Braz J Pharmacol* 1990;99:164–8.
- [22] Makato N, Takashi Y, Gen-ishi N, Itsuo N. Tannins and related compounds Part 9. isolation and characterization of polygal-loulglucose from Turkish galls (*Quercus infectoria*). *J Chem Soc Perkin Trans I* 1983;961–5.
- [23] Sheetal A, Honnegowda S, Mandapati R. Isolation and TLC densitometric quantification of gallicin, gallic acid, lupeol and b-sitosterol from *Bergia suffruticosa*, a hitherto unexplored plant. *Chromatographia* 2007;66:725–34.
- [24] Rivera J, Gilfillan AM. Molecular regulation of mast cell activation. *J Allergy Clin Immunol* 2006;117:1214–25.
- [25] Brito FA, Lima LA, Ramos MFS, Nakamura MJ, Cavalher-Machado SC, Siani AC, et al. Sampaio ALF. Pharmacological study of anti-allergic activity of *Syzygium cumini* (L.) Skeels. *Braz J Med Biol Res* 1990;99:164–8.
- [26] Penido C, Costa KA, Pennaforte RJ, Costa MFS, Pereira JFG, Siani AC, et al. Anti-allergic effects of natural tetranortriterpenoids isolated from *Carapa guianensis* Aublet on allergen-induced vascular permeability and hyperralgia. *Inflamm Res* 2005;54:295–303.
- [27] Carey MA, Germolec DR, Langenbach R, Zeldin DC. Cyclooxygenase enzymes in allergic inflammation and asthma. *Prostaglandins Leukot Essent Fat Acids* 2003;69:157–62.
- [28] Juan H, Sametz W. Histamine-induced release of arachidonic acid and of prostaglandins in the peripheral vascular bed: mode of action. *Naunyn Schmiedebergs Arch Pharmacol* 1980;314:183–90.
- [29] Goggle R, Hoffman S, Nusing S, Narumiya R, Uhlig S. Platelet-activating factor-induced pulmonary edema is partly mediated by prostaglandin E(2), E-prostanoid 3-receptors, and potassium channels. *Am J Respir Crit Care Med* 2002;166:657–62.
- [30] Metcalfe DD, Baram D, Mekori YA. Mast cell. *Physiol Rev* 1997;7:1033–78.
- [31] MacGlashan D. Histamine: a mediator of inflammation. *J Allergy Clin Immunol* 2003;112:S53–9.
- [32] Ash AS, Schild HO. Receptors mediating some actions of histamine. *Br J Pharmacol* 1997;120:302–14.
- [33] Lovenberg TW, Roland BL, Wilson SJ, Jiang X, Pyati J, Huvar A, et al. Cloning and functional expression of the human histamine H3 receptor. *Mol Pharmacol* 1999;55:1101–7.
- [34] Oda T, Morikawa N, Saito Y, Masuho Y, Matsumoto S. Molecular cloning and characterization of a novel type of histamine receptor preferentially expressed in leukocytes. *J Biol Chem* 2000;275:36781–6.
- [35] He S, Peng Q, Walls AF. Potent induction of a neutrophil and eosinophil-rich infiltrate *in vivo* by human mast cell tryptase: selective enhancement of eosinophil recruitment by histamine. *J Immunol* 1997;159:6216–25.
- [36] Repka-Ramirez MS, Baraniuk JN. Histamine in health and disease. *Clin Allergy Immunol* 2002;17:1–25.
- [37] Xie H, He SH. Roles of histamine and its receptors in allergic and inflammatory bowel diseases. *World J Gastroenterol* 2005;11:2851–7.
- [38] Williams TJ. The eosinophil enigma. *J Clin Invest* 2004;113:507–9.
- [39] Palframan RT, Collins PD, Williams TJ, Rankin SM. Eotaxin induces rapid release of eosinophils and their progenitors from bone marrow. *Blood* 1998;91:2240–8.
- [40] Rothenberg ME, Luster AD, Leder P. Murine eotaxin: an eosinophil chemoattractant inducible in endothelial cells and in interleukin 4-induced tumor suppression. *Proc Natl Acad Sci U S A* 1995;92:8960–4.
- [41] Weller CL, Jose PJ, Williams TJ. Selective suppression of leucocyte recruitment in allergic inflammation. *Mem Inst Oswaldo Cruz* 2005;100:153–60.