Effects of *Cissampelos sympodialis* Eichl. and its Alkaloid, Warifteine, in an Experimental Model of Respiratory Allergy to *Blomia tropicalis*

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Abstract: Asthma is one of the most prevalent chronic diseases worldwide. Medicinal plants are historically used in its treatment. The plant *Cissampelos sympodialis*, known in Northeastern Brazil as "Jarrinha"or "Milona", is used to treat some inflammatory conditions, including asthma. The objective of this study was to evaluate the potential of *Cissampelos sympodialis* EICHL. extract (CsE) and its isolated alkaloids, especially warifteine (Wa) on a *Blomia tropicalis* extract (BtE)-induced experimental model of allergy. The respiratory allergy was induced in AJ mice by the administration of BtE. Mice were orally treated with the 400 mg/kg of CsE or 8 mg/kg of total alkaloids fraction (TAF) or 4 mg/kg of Wa and the following parameters were analyzed: (a) total cell numbers in bronchoalveolar fluid (BAF); (b) differential cell numbers in BAF; (c) eosinophil peroxidase (EPO) activity in BAF; (d) IgE serum levels by passive cutaneous anaphylaxis; (e) IL-5, IL-13, IL-10, and IFN-γ levels in BAF; (f) histopathological alterations in the lung. The treatment of the animals with CsE, Wa or TAF led to a reduction in the numbers of total cells and eosinophils in BAF. The same reduction was observed in EPO levels in the BAF. The levels of IL-5 and IL-13 were also reduced in animals treated with *Cissampelos sympodialis*, while IL-10 levels were significantly increased in the BAF of CsE-treated animals. The treatment also decreased the density of inflammatory cells in the lung by histopathological examinations demonstrating the potential of this medicinal plant as new agent for asthma treatment.

Keywords: Asthma, *Blomia tropicalis*, *Cissampelos sympodialis*, natural products, warifteine, cytokines.

1. INTRODUCTION

Asthma affects approximately 350 million people world-wide and occupies the third place in public health costs. The prevalence in Brazil is the eightth highest in the world [1].

A survey by the National Asthma Program found that 60% of people with moderate asthma and 70% with severe asthma had used complementary and alternative medicine to treat their condition [2].

Historically, herbal medicine has a great importance in the treatment of asthma. Four of the five classes of drugs currently used to treat asthma - namely, α_2 agonists, anti-cholinergics, methylxanthines and cromones - have origins in herbal treatments going back at least 5,000 years [3].

Particularly in Brazil, with its enormous biodiversity, the search for new natural products and bioactive molecules is an important goal. *Cissampelos sympodialis* Eichl. (Menispermaceae) is a plant species found in the northeast and southeast of Brazil. A hot water infusion of *C. sympodialis* root bark is largely used in the indigenous and folk medicine to treat several inflammatory disorders, including asthma [4-6]. Phytochemical analysis of *C. sympodialis* root extracts identified a group of alkaloids, including milonine, methylwarifteine, roraimine and warifteine [7].

In vitro immunological studies showed that the leaf hydroalcoholic extract of CsE increased the production of interleukin-10 (IL- 10) and inhibited the T-cell proliferative response of mouse spleen cells stimulated *in vitro* with concanavalin A [8]. CsE also induced an IL-10-dependent inhibition of *Trypanosoma cruzi* killing by macrophages and modulated B-cell responses [9]. Studies *in vivo* demonstrated that the intraperitoneal injection of CsE has an anti-inflammatory effect on carrageenan- or capsaicin-induced mouse ear edema [10]. In addition, Bezerra-Santos *et al.* (2004) [4] showed that the oral treatment with CsE not only reduced the plasma levels of total and ovalbumin (OVA)-specific IgE, but also induced INF-γ production in OVA-sensitized BALB/c mice. A recent study, conducted by the same group, demonstrated that warifteine is at least in part responsible for the inhibition of immediate hypersensitivity and thermal hyperalgesic reactions in OVA-sensitized animals [11].

However, despite the fact that OVA-sensitized mice constitute a well studied model of respiratory allergy [6], its immunological response pattern is not the same elicited by *bona fide* aeroallergens, such as those from *Blomia tropicalis*

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and *Dermatophagoides pteronissinus* mites, which are the most important indoor allergens associated with asthma and rhinitis [12].

The objectives of this study is to evaluate the effect of CsE and its alkaloids, especially warifteine, in a murine model of respiratory allergy to *B. tropicalis* extract (BtE), and to investigate some of the immunological phenomena modulated by CsE in order to elucidate the mechanism by which it may be exerting its effect on experimental allergy.

2. MATERIAL AND METHODS

2.1. Animals

Female AJ mice (25-30 g) and Wistar rats (250-300 g) were used throughout the study. Animals were maintained with free access to food and water. They were obtained from the animal facilities of the Fundação Oswaldo Cruz, Bahia, Brazil. Groups of 5 animals were used in each experiment. All the experimental procedures were approved by the Ethical Committee for Use of Experimental Animals of the Faculdade de Odontologia, Universidade Federal da Bahia, Brazil (protocol number: 02/09).

2.2. Obtention of *Blomia tropicalis* Antigens

B. tropicalis (BtE) were cultivated in a fish food-containing standardized environment, purified with saturated NaCl and lysed in 0.15 M phosphate-buffered saline, pH 7.4 (PBS), in a blender (51BL30; Waring Commercial, Torrington, CO, USA). After centrifugation with ether for removal of lipids (4500 RPM / 10min), the protein content was determined by Lowry's method [13] and the extract was subsequently stored at -70° C until use. The amount of *Bt*E used in the experiments was standardized by measuring its content in *B.tropicalis* Blo t 5 allergen, measured by a commercially available capture ELISA kit (INDOOR Biotechnologies, Charlottesville, VI, USA). All used batches contained 30-40 ng of this allergen per µg of protein.

2.3. *Cissampelos sympodialis* Extract and Alkaloids

Leaves from *C. sympodialis* were obtained from the Botanical Garden of the Laboratory of Pharmaceutical Technology, University Federal of Paraíba (voucher specimen Agra 1456). The leaves were dried at 50° C and pulverized. The powder was extracted with 70% ethanol in water at 70° C for 5 days. The dried extract was dissolved in water and filtered in water. Known volumes were dried to determine the final concentration of the water-soluble components. The yield of these components has been shown to be 22% on average [8].

Warifteine was isolated using a standard alkaloid acid-base extraction procedure. Following extraction, warifteine was purified by silica gel column chromatography, and recristalized by hot ethanol [14]. Warifteine was quantified in the leaf extract (CsE) by means of High Performance Liquid Chromatography (HPLC) with ultraviolet detection. Calibration curves to Wa were constructed by using the standard addition method. The separation of warifteine was achieved using a Luna C18 column (250 x 4.6 mm I.D., 5 µm of particle size) from Phenomenex (Phenomenex, Torrance, USA) fitted with a C-18 pre-column. The mobile phase consisted of water:acetonitrile:triethylamine (57:43: 0.05. v/v) delivered isocratically at a flow rate of 1.3 mL/ min. The injection volume was 20 µL and detection was at 246 nm. The extract (CsE) used in all experiments described in this paper had a nominal concentration of 0.95% of warifteine. The used dose was expressed as mg of soluble components per kg of mouse body weight. The warifteine solution had its pH adjusted to 7.0 with 1 N NaOH. Dilutions were made in endotoxin free saline for *in vivo* experiments [11].

To obtain the total alkaloids fraction (TAF), the plant hydroalcoholic extract was dissolved in 3% HCl and extracted several times with $CHCl_3$. The aqueous fraction was basified with NH_4OH to pH 9 and again extracted with $CHCl_3$. The $CHCl_3$ extract was washed with H_2O, dried ($MgSO_4$) and the solvent evaporated to afford the TAF [11]. The concentration of Wa in TAF was estimated to be 54.1% by HPLC analysis.

2.4. Sensitization and Challenge with *Blomia tropicalis*

A/J mice (n=5) were sensitized with two subcutaneous injections (day 0 and day 7) of BtE (10 µg of protein) adsorbed to 4 mg/mL of $Al(OH)_3$ in saline. One day after the second injection, the animals were challenged intranasally (i.n.) every 24 hours with BtE (10 µg of protein), in 50 µL of saline, for four days [15]. Twenty-four hours later, the animals were sacrificed using xilazine and ketamine (40 mg/kg/body weight, intraperitoneally). The bronchoalveolar lavage fluid was collected after blood collection.

2.5. Treatment with *Cissampelos sympodialis* Extract and Alkaloids

The dose (400mg/Kg) and route (v.o.) of CsE were determined based on previous studies carried out by our collaborators [6, 8]. One hour before each *Blomia tropicalis* (BtE) sensitization, the animals were treated with 400 mg/kg of CsE, 4 mg/kg of warifteine (Wa), 8mg/kg of total alkaloid fraction (TAF) or, as positive control, 3mg/kg of dexametasone (Dx). After the last sensitization, the animals were treated every day until the last challenge. The groups of animals were defined as: **Control,** non-sensitized and saline-treated mice; **BtE,** BtE-sensitized mice; **BtE/CsE,** BtE-sensitized and CsE-treated mice; **BtE/TAF**, BtE-sensitized and TAF-treated mice; **BtE/Wa**, BtE-sensitized and Wa-treated mice; **BtE/Dx**, BtE-sensitized and Dx-treated mice.

2.6. Bronchoalveolar Lavage Fluid

The trachea was canulated and the lungs were washed four times with 0.5 mL of HBSS. About 1.5 mL of BAF was obtained from each mouse**.** The count of total leukocytes in the BAF was immediately performed in a hemocytometer. Differential cell counts were obtained by using May–Grunwald–Giemsa - stained cytospin preparations. A differential count of at least 100 cells was made in a blind fashion and according to standard morphologic criteria.

2.7. Eosinophil Peroxidase Activity

The EPO activity in the cells obtained from bronchial alveolar lavage was measured according to a previously

described method [16]. Briefly, cell suspensions were frozen and thawed three times in liquid nitrogen. After centrifugation at 4° C for 10 min at 1000 g, the cell lysates were placed in 96-well plates (75 µL/well), followed by the addition of 1.5 mmol/L of o-phenylenediamine and 6.6 mmol/L of H_2O_2 in 0.05 mol/L Tris-HCl, pH 8.0. After 30 min at room temperature, the reaction was stopped with the addition of 75 µL of 0.2 mol/L citric acid, and the absorbance of the sample determined at 492 nm in an ELISA plate reader.

2.8 Histopathological Analysis and Quantification of Lung Inflammation

The histopathological alterations and quantification of lung inflammation were performed as described previously [17]. Briefly, lung tissues were fixed by inflation with freshly prepared 4% paraformaldehyde in PBS for 24 h and embedded in paraffin. Tissue sections (5 µm) were stained with haematoxylin and eosin and the histopathological alterations were analyzed using microscope with 20X magnification. The data on quantification of lung inflammation were acquired using the software Image-Pro Plus Version 6.1 (Media Cybernetics, San Diego, CA, USA) using the amount of total inflammatory cells per mm^2. After that we used the average from each animal/slide.

2.9. Determination of Anti-*B. tropicalis* IgE Antibody Titer

The determination of IgE titers was done by passive cutaneous anaphylaxis (PCA). Different dilutions of mouse sera were inoculated intradermally on the shaved backs of Wistar rats. After 48 h, the rats were intravenously injected with BtE (4 mg of protein/kg) in Evans blue solution (1%). Thirty min afterwards the rats were euthanized and the dorsal skins removed. The highest serum dilution giving a 5-mm diameter flare or bluing reaction was taken as the PCA titer [18].

2.10. Cytokine Profile in Bronchoalveolar Lavage Fluid

The concentrations of IL-5, IL-10, IL-13, and IFN-γ were quantified in the BAF by standard ELISA kits as recommended by the manufacturer (BD Pharmingen, USA).

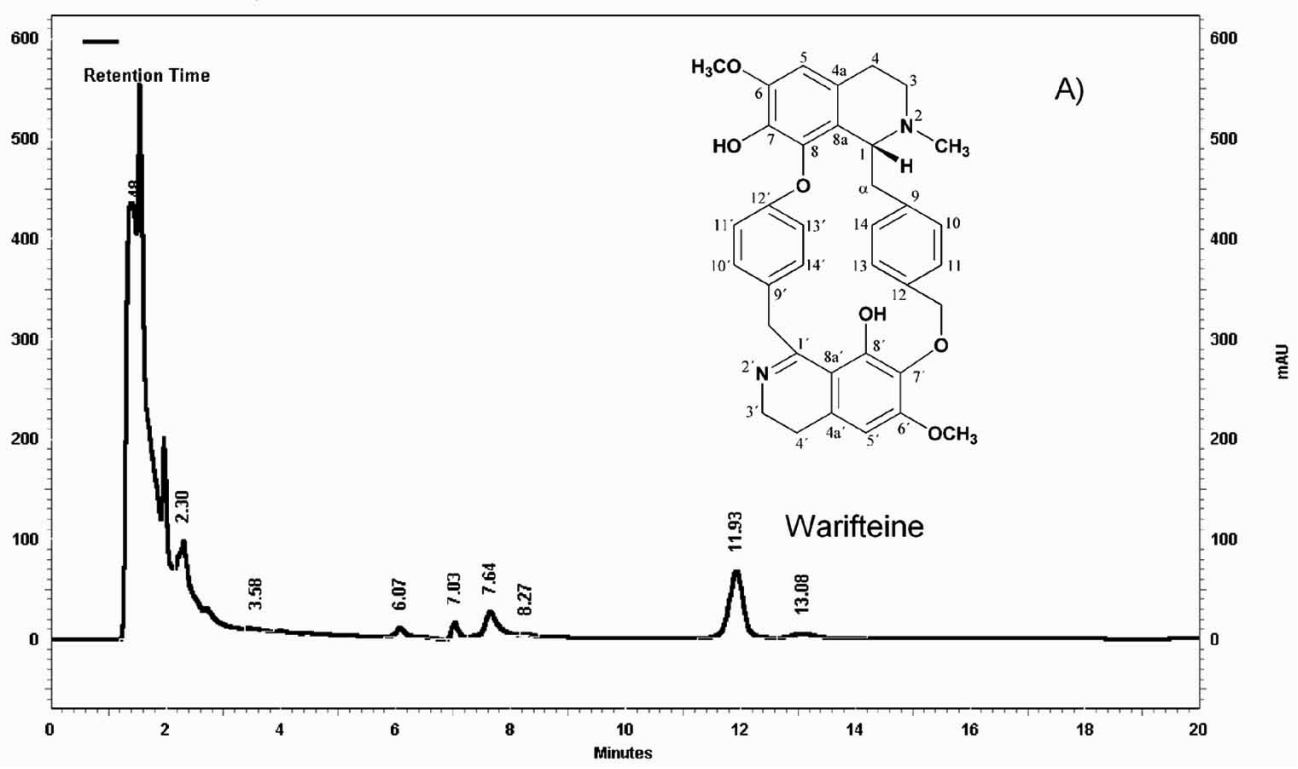
2.11. Statistical Analysis

The One-way analysis of variance and Tukey as post-test (for data with normal distribution) were used to determine statistical significance between groups. Differences in P values ≤ 0.05 were considered statistically significant. Each experiment was repeated at least two times.

3. RESULTS

3.1. Determination of Warifteine in the Leaf Ethanolic Extract and Total Alkaloid Fraction of *C. sympodialis*:

Fig. (**1**) shows the chromatogram of the ethanolic leaf extract (CsE) (Fig. **1a**), a solution of warifteine (Wa) (Fig. **1b**) and of the total alkaloid fraction (Fig. **1c**), demonstrating that the separation of warifteine was achieved without any interferents from the extract at the same retention time of the alkaloid (Fig. **1a**). This chromatographic method was used for quantification of Wa in the ethanolic extract used in all subsequent experiments as described in section 2.3.



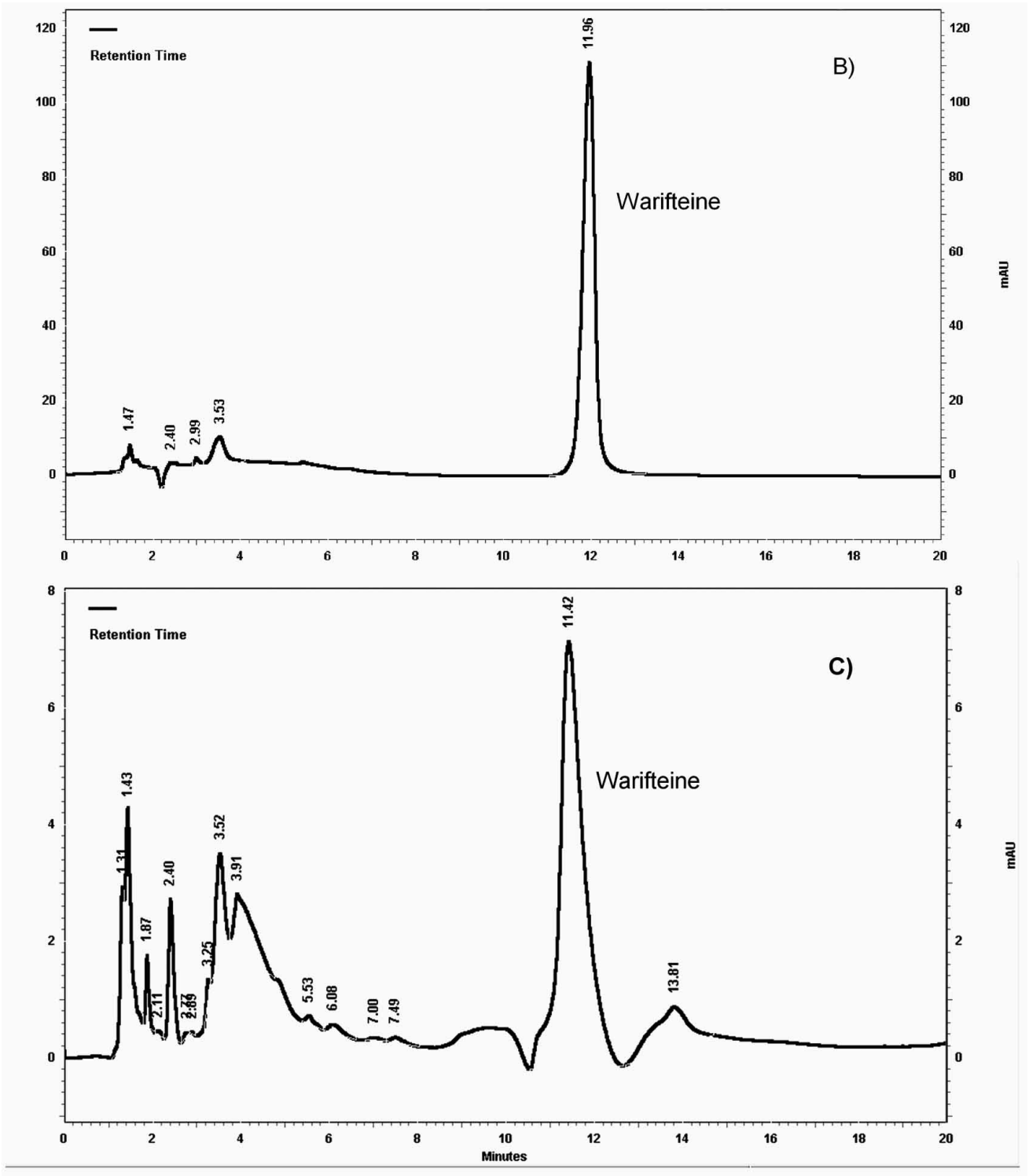


Fig. (1). Chromatogram of a sample of *Cissampelos sympodialis* (CsE) hydroalcoholic extract (**A**), a sample of warifteine at 80 ug/mL (**B**) and TAF at 5 µg/mL (**C**), showing the peak of the alkaloid warifteine (Wa) with a retention time of 11.9 minutes.

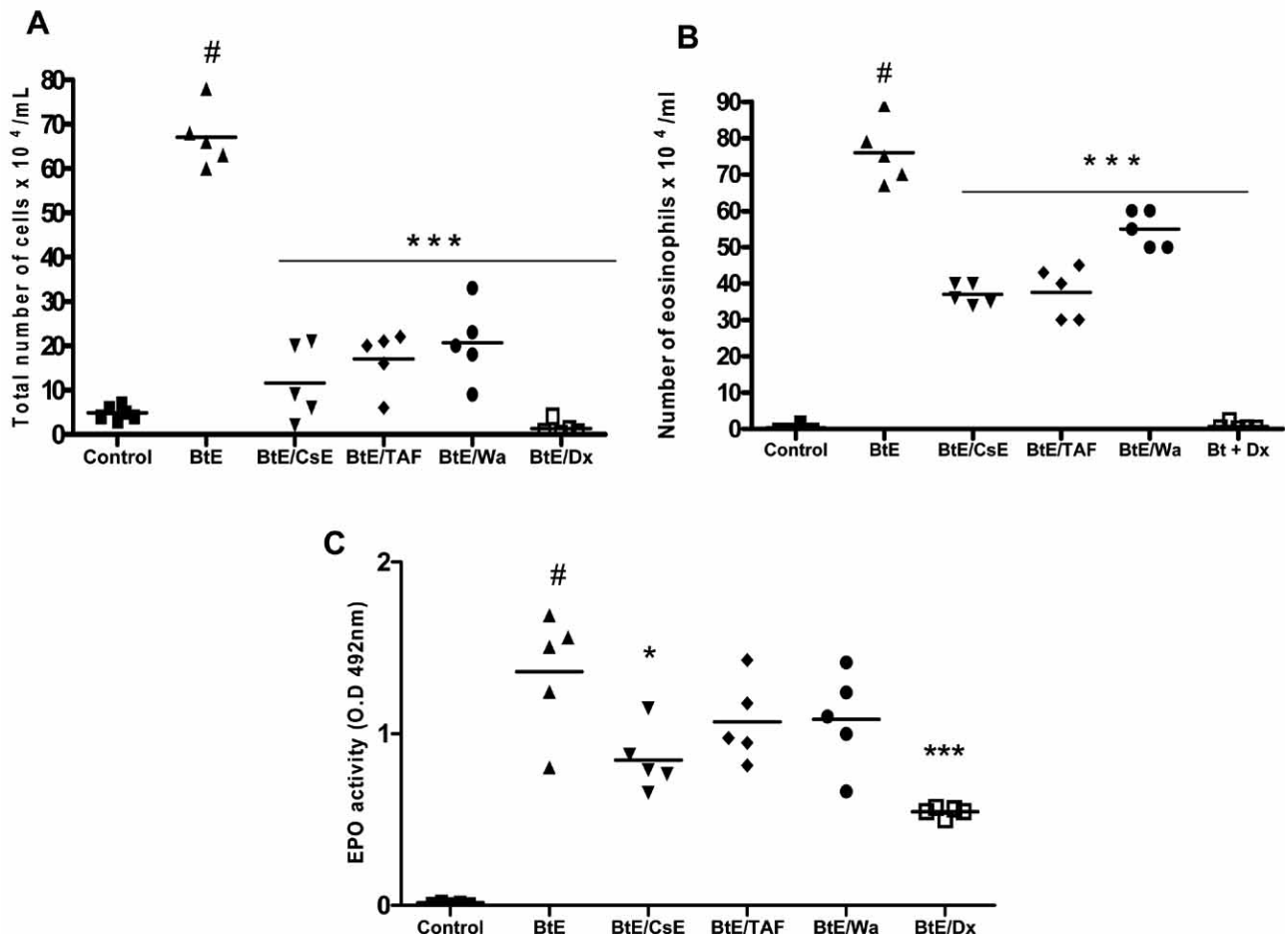


Fig. (2). Effect of oral treatment of mice experimentally sensitized to *Blomia tropicalis* (BtE) with hydroalcoholic extract of *Cissampelos sympodialis* (CsE), total alkaloids (TAF) or Warifteine (Wa) in total (**A**) and differential (**B**) cell counts and in eosinophil peroxidase EPO activity (**C**). # P< 0.001 in relation to control; * P<0.05 in relation to BtE sensitized group, ***p<0.001 in relation to BtE sensitized group, ANOVA-Tukey.

3.2. Effect of *C. sympodialis* Extract on Total and Differential Bronchoalveolar Lavage Fluid Cell Counts

As expected, the sensitization with *Blomia tropicalis* led to an increase in total cell counts in the BAF (Fig. **2a**, P<0.001). On the other hand, when the BtE-sensitized mice were treated with CsE, the total cell count significantly decreased (Fig. **2a**, P<0.001). The number of cells in the BAF of animals treated with TAF and Wa was also reduced (Fig. **2a**, P<0.001) as well as in dexametasone (Dx) treated animals (Fig. **2a**, P<0.001). Regarding the differential cell counts in the BAF, no difference was found in macrophages, neutrophils and lymphocytes counts among treated and untreated groups. However, a significant increase in eosinophil numbers was found in BtE-sensitized mice (Fig. **2b**, P<0.001). The CsE, TAF and Wa treatments led to a significant decrease in eosinophils counts in the BAF in relation to untreated BtE-sensitized animals (Fig. **2b**, P<0.001).

3.3. Eosinophil Peroxidase Levels in Bronchoalveolar Lavage Fluid

The presence of EPO activity is indicative of eosinophil degranulation. As can be seen in Fig. (**2c**), the BtE sensitization produced a significant augmentation of EPO activity in BAF (P<0.001), which is not surprisingly, considering the increased presence of eosinophils to the lungs (Fig. **2b**). The CsE (Fig. **2c**, P<0.05) and Dx (Fig. **2c**, P<0.001) treatment reduced the EPO activity found in the BAF of BtE-sensitized mice, a fact that may reflect the inhibition of eosinophil migration to the lung. The same phenomenon was observed in animals treated with TAF or WA (Fig. **2c**), although it was not statistically significant.

3.4. Lung Histopathological Changes in *B. tropicalis*-Sensitized *C. sympodialis* Treated Mice

The effect of CsE, TAF and Wa treatments on the lung architecture of BtE-sensitized animals can be seen in Fig. (**3a-e**). In Fig. (**3b**) we can observe an marked cell infiltration in animals sensitized with BtE in comparison to normal control mice (Fig. **3a**). The treatment with CsE (Fig. **3c**) led to a significant decrease in cell infiltration in the lungs. However, the reduction of inflammatory cells in the lungs of animals treated with TAF or Wa was lower than in the lungs of CsE-treated animals (Fig. **3d** and **3e**). The reduction of inflammation can be confirmed by quanti-

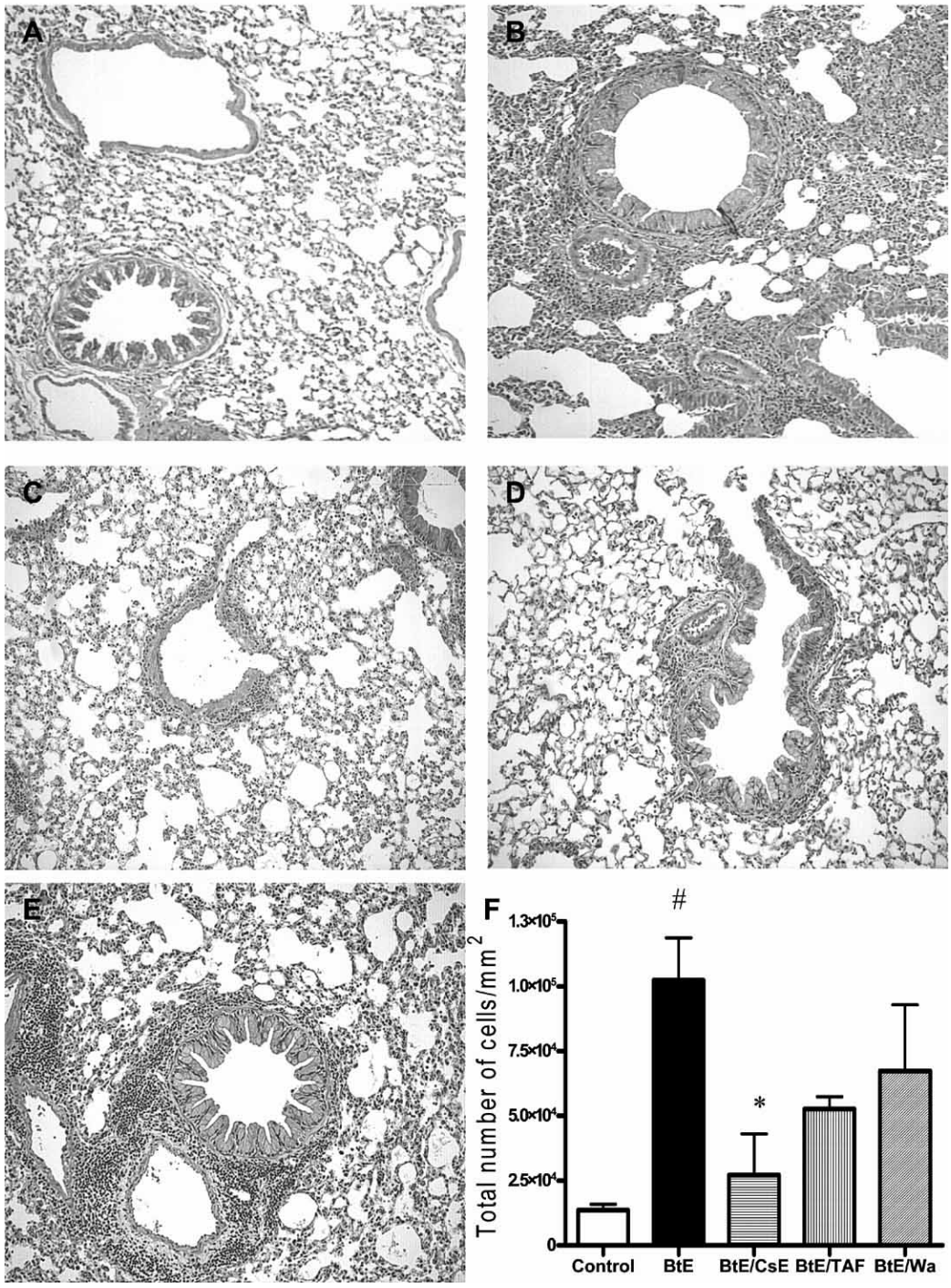


Fig. (3). Effect of oral treatment of mice experimentally sensitized to *Blomia tropicalis* (BtE) with hydroalcoholic extract of *Cissampelos sympodialis* (CsE), total alkaloids (TAF) or Warifteine (Wa) in lungs histopathology (HE staining and 20X magnification, HE) (Fig. **3a-e**) and in the quantification of the area of inflammation (Fig. **3f**). (**A**) control untreated, non-sensitized animal; (**B**) BtE-sensitized animal; (**C**) BtE-sensitized, CsE-treated animal; (**D**) BtE-sensitized, TAF-treated animal; (**E**) BtE-sensitized, Wa-treated animal. The images are representatives of each group. # $P < 0.05$ in relation to control; * $P<0.05$ in relation to BtE sensitized group, ANOVA-Tukey.

fication of areas of inflammation (Fig. **3f**). These results show that, in addition to decreasing eosinophil migration and degranulation, the treatment of BtE-sensitized mice with CsE and its alkaloids reduced lung tissue inflammation (only statistically significant for CsE treatment).

3.5. Effect of CsE on Anti-*B. tropicalis* IgE Antibody Titers

Fig. (**4**) presents the effect of CsE treatment on anti-BtE IgE antibody titers in BtE-sensitized mice. The BtE-sensi-

tized animals produced higher titers of anti-BtE IgE antibodies than the control, non-sensitized animals (P<0.05). The treatment of the BtE-sensitized mice with CsE decreased the IgE titers, although the decrease was not statistically significant (P>0.05).

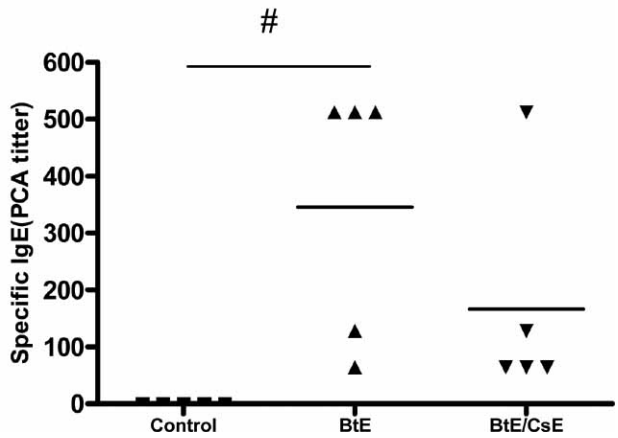


Fig. (4). Effect of oral treatment of mice experimentally sensitized to *Blomia tropicalis* (BtE) with hydroalcoholic extract of *Cissampelos sympodialis* (CsE) in anti-BtE IgE antibody titers as determined by PCA reaction. # P< 0.05 in relation to control, ANOVA-Tukey.

3.6. Cytokine Profile in the Bronchoalveolar Lavage Fluid of Treated Animals

In order to study the mechanisms underlying the protective effect of *Cissampelos sympodialis* extract (CsE), total alkaloid fraction (TAF) and warifteine (Wa) on the migration of leukocytes, mainly eosinophils, to the lungs, the cytokine profile in the BAF of treated mice was determined. The levels of Th1 (IFN-γ), Th2 (IL-5, IL-13) and Treg (IL-10)-associated cytokines was investigated. BtE-sensitized animals had higher levels of IL-5 and IL-13 in the BAF than the control non-sensitized mice (Fig. **5a** and **b**, P<0.001). When the sensitized animals were treated with CsE, but not when they were treated with TAF or Wa, the production of IL-5 was significantly decreased. The decrease in level of the other cytokine investigated in this work, IL-13, observed in animals treated with CsE, TAF or Wa, was not statistically significant. In addition, the CsE, but not TAF or Wa promoted a significant increase in the production of the regulatory cytokine IL-10 (P<0.05, Fig. **5c**). The Th1-type cytokine IFN-γ could not be detected in the BAF of untreated and CsE, TAF or Wa-treated, BtE-sensitized mice (data not shown).

4. DISCUSSION

We report here for the first time that *Cissampelos sympodialis* extract (CsE) effectively reduced immunolo-

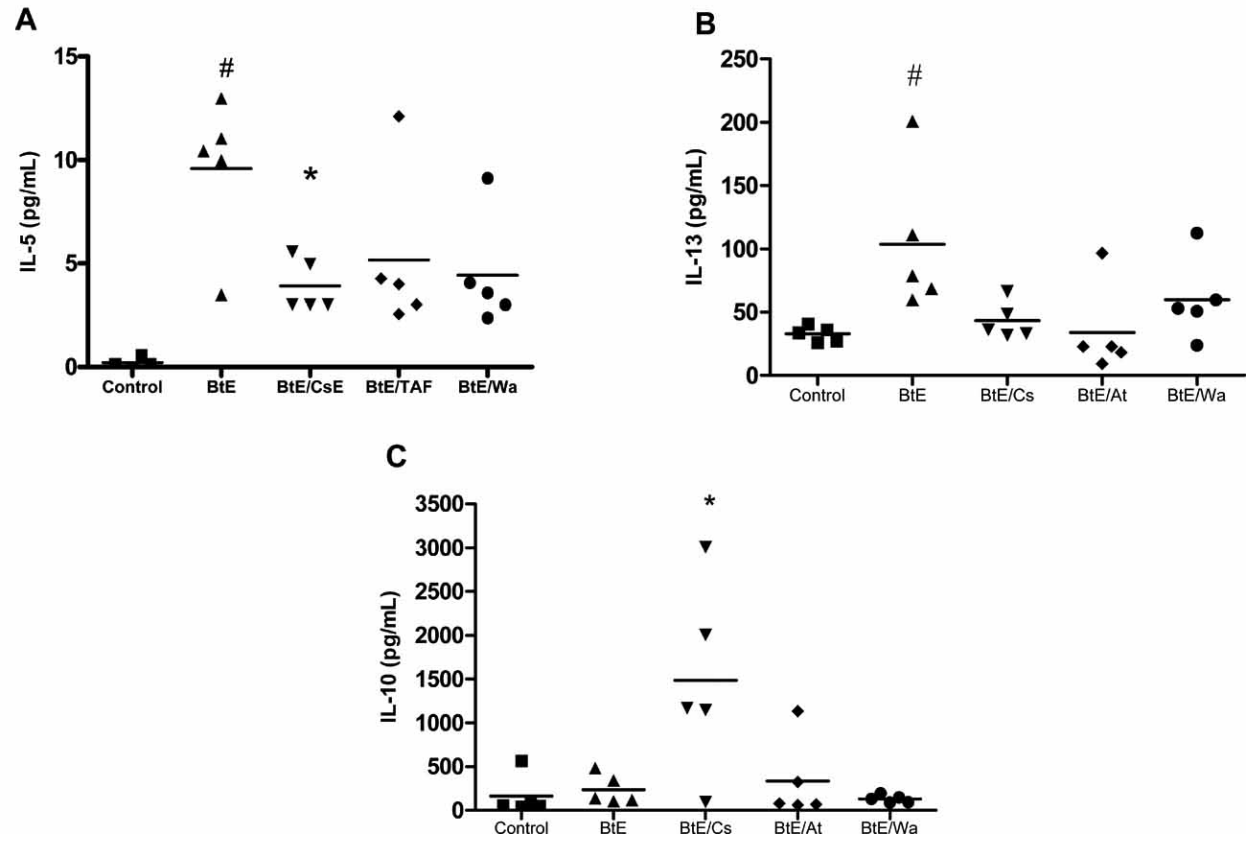


Fig. (5). Effect of oral treatment of mice experimentally sensitized to *Blomia tropicalis* (BtE) with hydroalcoholic extract of *Cissampelos sympodialis* (CsE), total alkaloids (TAF) or Warifteine (Wa) in cytokines production in BAF. IL-5 (**A**), IL-13 (**B**) and IL-10 (**C**). # P< 0.001 in relation to control; * P<0.05 in relation to BtE sensitized group, ANOVA-Tukey.

gical parameters in a murine model of *Blomia tropicalis*-induced allergy which is an model that mimics human allergy. The parameters analyzed were specific IgE production, cell migration and histopathological alterations in the lungs, peroxidase (EPO) production in bronchoalveolar lavage fluid (BAF) and cytokine production. These activities are correlated to the immunomodulatory and anti-inflammatory properties of the alkaloid warifteine but showing an addictive effect of other components within the extract to obtain an optimal activity.

Eosinophils are extremely important for the pathophysiology of asthma. They constitute a fundamental trait of allergic asthma: the presence of these cells in the lung indicates the late phase of asthma and is also associated with disease severity [19]. The traffic of eosinophils to the sites of allergic reactions is activated by cytokines, chemokines and chemoattractants [20].

The resolution of symptoms of asthma has been associated with reduction of lung eosinophil infiltration [21]. We and others have previous described the presence of eosinophils in inflammatory infiltrates in the lungs of mice with BtE-induced respiratory allergy model [22, 23]. Drugs modulating eosinophil recruitment and/or activation may therefore be important to reduce lung inflammation in mite antigenelicited asthma.

The reduction of eosinophil infiltration to the lungs has been shown in several other studies in which evaluated the effect of plants on eosinophils. Sohn *et al.* (2009) [24] found in cultured cells that *Vitex rotundifolia* a plant popularly used for the treatment of asthma reduced the production of substances chemoattractant for eosinophils.

Our results corroborate the anti-allergic potential of CsE and Wa observed by Bezerra-Santos and collaborators (2006) [6] and Costa and collaborators (2008) [11] where CsE and its alkaloids reduce the numbers of eosinophils in the BAF, IgE production, leukocyte activation, thermal hyperalgesia, mast cell degranulation and scratching behavior in OVA-sensitized mice.

Previous reports have also described the inhibitory effect of natural compounds, such as D-pinitol [25] and bee pollen phenolic extract [26], on eosinophil degranulation, as disclosed by peroxidase activity measurements. Unlike the other peroxidases, the EPO has a high cationic charge, with an isoelectric point of 10.8, and binds strongly to the extracellular matrix [27], causing damage to the tissue. The reduction of EPO release by CsE treatment may therefore be of relevance in ameliorating inflammation and/or tissue remodeling in allergic asthma.

IgE is the main immunoglobulin class associated with allergic diseases, and the role it plays as effector of activation events related to eosinophil and mast cell degranulation is well known [28]. The production of IgE depends on Th2-type cytokines, such as IL-4. Maturation, migration and activation of eosinophils are stimulated by IL-5 [29]. The down-modulation of IgE production constitutes an important strategy for treating allergic diseases, as demonstrated by the existence of relatively efficacious drugs, such as Omalizumab [30] and the anti-IL-4 monoclonal antibody Pascolizumab [31], which have been designed with this aim.

The protective effect of CsE on IgE titers observed in this study was not statistically significant and this could be due to the lack of efficacy on modulating IL-13 which in addition to IL-4 are important regulators of IgE production. IL-13 is a cytokine produced by T cells, mast cells, dendritic cells and many other cell types [32]. It is especially related to mucus production by goblet cells in airways epithelium and, in association with IL-4 by interaction with IL-4Rα chain and activation of STAT 6 transcriptional factor, induces IgE class switching [33]. However, recently studies have shown that in parallel of this classical IL-4Rα chain activation, a novel pathway for IL-13 signaling independent of STAT 6 has been suggested [34]. In humans, levels of IL-13 are systematically increased in the lung during asthmatic attacks [35]. Although IL-13 levels were elevated in BtE-sensitized mice, its levels were not significantly reduced by CsE, TAF and Wa treatments, a fact that may explain the non-significant effect of the treatments in the IgE antibody titers as mentioned previously.

On the other hand, the role played by IL-5 in the pathogenesis of asthma is well described [25] and several studies demonstrated the role of this cytokine in the development, activation and migration of eosinophils [36, 37]. The down-modulation of IL-5 production by the CsE could explain the impact of the CsE treatment on the infiltration of leukocytes, especially eosinophil, in the lungs. IL-5, therefore, would be the main cytokine involved in the pathogenesis in this respiratory allergy model. The mechanism by which CsE would affect IL-5 levels remains unknown, but one possibility is that it is modulating the transcription factor related to IL-5 production. The absence of a statistically significant effect of TAF and Wa in IL-5 levels (Fig. **5a**) could be ascribed to an additive or synergistic effect of the various alkaloids that are present in the CsE and other yet undescribed antiinflammatory substance. As described by Chen and collaborators (2007) [38], when OVA-sensitized animals were treated with budesonide, a glucocorticoid widely used for the treatment of asthma, a reduction of the expression of a transcription factor related to IL-5 was observed. The fact that IL-5 but not IL-13 was significantly reduced by CsE could be due to inhibition of STAT 6 transcription factor that regulates IL-5 production but is not the only factor associaded to IL-13 up-regulation.

In addition, the CsE, but not TAF and Wa promoted a significant increase in the production of IL-10. Previous reports described the ability of CsE in up-regulate IL-10 production *in vitro* and *in vivo* [6, 8]. The up-regulation of regulatory cytokine production is an important mechanism for inhibiting the development of asthma by means of controlling the excessive inflammation caused by massive eosinophil influx and the IgE-dependent immediate hypersensitivity reaction, which involves mast cell degranulation and markedly contributes to allergic asthma pathogenesis [39].

The principal function of IL-10 appears to be to limit and ultimately terminate inflammatory responses. In addition to these activities, IL-10 regulates growth and/or differentiation of B cells, NK cells, cytotoxic and helper T cells, mast cells, granulocytes, dendritic cells, keratinocytes, and endothelial cells. IL-10 plays a key role in the differentiation and function of a newly identified type of T cell, the regulatory T

cell, which is an important cell type in the control of immune responses and maintenance of immunological tolerance *in vivo* [40].

The immunodulatory action of IL-10 results from the inhibition of the synthesis of proinflammatory cytokines, such as TNF-α, from decreasing the activity of neutrophils and macrophages, and from reducing the expression of major histocompatibility complex (MHC) proteins class II. Moreover, IL-10 also inhibits the production of Th2 cytokines, such as IL-5 and IL-13, involved in asthma pathogenesis, inhibits histamine release by mast cells and basophils, and stimulates the production of blocking IgG4 antibodies [41, 42]. It is possible, therefore, that the decreased levels of IL-5, and consequent reduction in the number of eosinophils in BAF on CsE-treated animals could be due to this IL-10 up-regulation.

The molecular mechanism by which CsE up-regulates IL-10 remain unknown but the immunossupression exerted by this cytokine on Th2 immune response can be explained, at least in part, by inhibiting transcriptional factors related to Th2 cytokines such as the STAT6 (transcription factor). In addition, IL-10 also suppresses the NF-kB pathway at least by two different ways: by inhibiting activation of IkB kinase–similar to salicylate –and by inhibiting NFkB DNA binding [40]. One study conducted by Lentsch and collaborators (1997) [43] has demonstrated that IL-10 inhibits the expression of NF-kB and this event was associated with reduced inflammation in the lung and that inhibition of NF-kB was achieved by inhibiting the degradation of IkBa. The same group showed that administration of oral IL-10 in mice inhibited the lung injury by IgG immune complex in 95% and reduced levels of TNF-alpha in the BAL of these animals [44]. In this regard, the ability of CsE to restore physiological balance by IL-10 up-regulation with apparent lack of toxicity and low cost might open a field of interest concerning its possible use in clinical applications such as asthma and other immunomediated diseases.

5. CONCLUSION

CsE showed interesting effects on BtE-induced experimental respiratory allergic disease. The induction of IL-10 by CsE could be the major effect mediating the anti-allergic activity, by modulating cell influx and degranulation. The CsE-induced IL-10 could be acting through the suppression of IL-5 production. Intriguingly, the hydroalcoholic extract of CsE showed better results in all parameters analyzed in comparison to the total alkaloids fraction (TAF) or bisbenzylisoquinoline alkaloid warifteine (Wa), what could be explained by the additive or synergistic effect of different compounds within the extract. These results also suggest the recent concept of purity-activity relationships of natural products [45] to improve drug discovery and synergy research of these compounds.

6. ABBREVIATIONS

BAF = Bronchoalveolar fluid

BtE = *Blomia tropicalis*

CsE = *Cissampelos sympodialis* extract

EPO = Eosinophil peroxidase

HBSS = Hanks' balanced salt solution

HCl = Chloride acid

i.n. = Intranasal route

IFN-γ = Interferon-gamma

IkBa = Nnuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha

IL = Interleukin

MeOH = Methanol

NaOH = Sodium hydroxide

NF-kB = Nuclear factor kappa-light-chain-enhancer of activated B cells

NMR = Nuclear magnetic resonance

PCA = Passive cutaneous anaphylaxis

RPM = Rotations per minute

s.c. = Subcutaneous route

STAT6 = Signal transducer and activator of transcription 6

TAF = Total alkaloid fraction

TGF-beta= Transforming growth factor beta

v.o. = Oral route

Wa = Warifteine

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