



Effectiveness of *Cissampelos sympodialis* and its isolated alkaloid warifteine in airway hyperreactivity and lung remodeling in a mouse model of asthma

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ABSTRACT

Background: *Cissampelos sympodialis* Eichl. (Menispermaceae) is a plant found in Northeastern and Southeast of Brazil and hot water infusion of *C. sympodialis* root bark is largely used in the indigenous and folk medicine to treat several inflammatory disorders, including asthma. Asthma is a chronic inflammatory allergic disease characterized by airway hyperreactivity (AHR), eosinophil tissue infiltration and lung remodeling. The aim of this study was to evaluate the therapeutic effect of *C. sympodialis* and its isolated alkaloid warifteine on allergen triggered airway hyperreactivity (AHR) and lung remodeling in murine model of asthma.

Methodology/principal findings: The oral pre-treatment with *C. sympodialis* or warifteine inhibited allergen-induced AHR to inhaled methacholine and IL-13 levels in the bronchoalveolar lavage (BAL). In order to investigate the therapeutic potential of *C. sympodialis* and warifteine, animals were treated 1 h after the last ovalbumin (OVA) challenge in sensitized animals. Similarly to the pre-treatment, post-treatment with warifteine was effective to inhibit significantly AHR to inhaled methacholine and to reduce IL-13 levels in the BAL. In addition, oral pre- or post-treatments with *C. sympodialis* or warifteine reduced OVA-induced eosinophil tissue infiltration, mucus production and subepithelial fibrosis to values similar to nonallergic controls.

Conclusions: Our data show the anti-allergic and immunoregulatory properties of *C. sympodialis*, acting mostly through the active compound warifteine, to inhibit the airway hyperreactivity and lung remodeling through a mechanism at least partially dependent of IL-13 and eosinophil inhibition. Therefore placing warifteine as an interesting therapeutic candidate in allergic inflammation and corroborating the folk medicine use of *C. sympodialis* as anti-allergic plant.

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1. Introduction

Asthma is a chronic inflammatory disease characterized by eosinophil tissue infiltration, airway hyperreactivity (AHR) and lung remodeling [1]. Anti-inflammatory treatment with inhaled glucocorticoid alone or combined therapy of corticosteroids with long-acting β_2 -agonist bronchodilators is the current preconized therapy for asthma. Although, most asthmatics respond to these treatments, 5–10% of patients are entirely insensitive to corticosteroids. In addition, some patients require additional oral glucocorticoids, and long-term use of corticosteroids has been strongly associated with a number of

adverse effects [2,3]. Thus, there is a need for new anti-asthmatic drug development.

Medicinal plants and their bioactive molecules are alternative options to conventional therapies for many diseases. *Cissampelos sympodialis* Eichl. (Menispermaceae) is a plant species found in Northeastern 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]. Phytochemical analysis of *C. sympodialis* root extracts leads to isolation of several alkaloids, of which warifteine has shown pharmacological effects [5–7]. In order to investigate the effect of *C. sympodialis* in immunological responses, previous studies showed that this plant enhanced IL-10 and IFN- γ levels in spleen cells from allergic mice and IL-10 levels in macrophage cultures [8,9]. This modulatory effect of *C. sympodialis* was correlated with decreased Ig-E production.

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Pertinent to the potential anti-asthmatic effects of *C. sympodialis*, recent published data showed that *C. sympodialis* and warifteine strongly reduced the eosinophilic inflammation into the bronchoalveolar lavage and pleural cavities in ovalbumin (OVA) sensitized mice [10]. This effect was dependent of cysteinyl leukotrienes generation and lipid body formation observed in activated eosinophils. *C. sympodialis* and warifteine were also capable of inhibiting the generation of the eosinophil chemoattractant factor, eotaxin. In addition, recent study showed in an experimental model of respiratory allergy to *Blomia tropicalis* which is the most important indoor allergen associated with asthma and rhinitis that the hydroalcoholic extract of *C. sympodialis* leaves and warifteine significantly reduced eosinophil migration and modulated Th2 cytokine production inducing IL-5 levels reduction in the bronchoalveolar lavage, while presenting high levels of the anti-inflammatory cytokine IL-10 [11].

Among the effects observed on inflammatory leukocytes, mast cell activation was also modified by warifteine. *In vitro* studies showed that warifteine inhibited mast cell degranulation induced by immunological or pharmacological challenge [12]. In these previous studies, it was also observed that warifteine decreased the immediate allergic reactions such as anaphylactic shock by IgE dependent mechanisms and thermal hyperalgesia.

The effects of *C. sympodialis* and warifteine on airway hyperreactivity and lung tissue pathological changes in OVA-induced allergic airway disease have not been addressed. The aim of this study was to evaluate the effectiveness of *C. sympodialis* on airway hyperactivity and collagen fibers and mucus production. For this purpose we used either the standardized hydroalcoholic extract of the *C. sympodialis* leaves, as well as, the *C. sympodialis*-derived alkaloid warifteine in OVA-induced lung inflammation in actively sensitized mice.

2. Materials and methods

2.1. Preparation of *C. sympodialis* extract and warifteine purification

Leaves from *C. sympodialis* were obtained from the Botanical Garden of the Laboratório de Tecnologia Farmacêutica/Universidade Federal da Paraíba (voucher specimen Agra 1456). The leaves were dried at 50 °C in an oven and pulverized. The powder was extracted with 70% ethanol in water at 70 °C for 5 days. The dried extract was dissolved in water; filtered and known volumes were dried to determine the final concentration of the water-soluble components. All doses are expressed in terms of the concentration of the soluble components (mg/kg of body weight). The yield was 22% on average, based on solid residues present [13]. *C. sympodialis* extract was dissolved in sterile water immediately before use. The extract used in all experiments described in this paper had a nominal concentration of 0.95% of warifteine [11].

The extract of *C. sympodialis* was submitted to procedures aimed to isolate the alkaloids, using column and thin layer chromatography (TLC). Briefly, the extract was dissolved in 3% HCl and extracted several times with CHCl₃. The aqueous fraction was basified with NH₄OH to pH 9 and again extracted with CHCl₃. The CHCl₃ extract was washed with H₂O, dried (MgSO₄) and the solvent evaporated to afford the total tertiary alkaloid fraction (TTA). The TTA was subjected to column over alumina, eluting with hexane containing increasing amounts of CHCl₃, CHCl₃ with increasing amounts of MeOH and finally with MeOH. The fraction eluted with CHCl₃–MeOH (49:1), after further purification by TLC (1.0 mm layer), yielded the isolation of the bisbenzylisoquinoline alkaloid warifteine (0.031%). The identification of the warifteine was performed by analyzing ¹H and ¹³C NMR spectral data compared with those published in the literature (Fig. 1) [11]. Warifteine was quantified in the leaf extract (cSE) by means of High Performance Liquid Chromatography (HPLC) with ultraviolet detection and it was calculated at 96.4% pure.

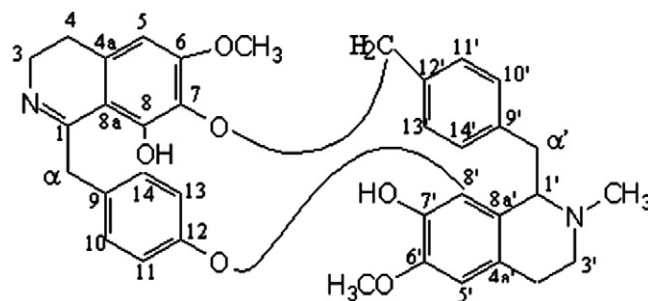


Fig. 1. Structure of warifteine.

2.2. Animals

Female BALB/c mice weighing 16–20 g were used. They were obtained from Oswaldo Cruz Foundation breeding unit. Animals were maintained with food and water *ad libitum* in a room with temperature ranging from 22 to 24 °C and a 12 h light/dark cycle. This study was carried out in accordance with the recommendations of the Brazilian National Council of Control of Animal Experimentation (CONCEA). The protocols were approved by the Animal Welfare Committee of the Oswaldo Cruz Foundation (CEUA/FIOCRUZ protocol # L-002/08).

2.3. Asthma model in actively sensitized mice

OVA-induced allergic airway disease was generated in mice as described previously [14]. Briefly, mice (n = 8) were sensitized with intraperitoneal injection of OVA (10 µg/mouse) and Al(OH)₃ (10 mg/mL) in 0.9% NaCl solution (saline; 0.2 mL) on days 1 and 10. From day 19 to day 24 after sensitization, mice were challenged daily for 20 min with OVA (5%) in phosphate buffered saline (PBS) by aerosol. Aerosolized PBS was administered to sensitized mice as a negative control. These procedures were performed in a 30 × 20 × 10 cm acrylic chamber and the aerosol was generated with an ultrasonic nebulizer.

2.4. Treatments

To evaluate the effect of *C. sympodialis* and warifteine in airway hyperreactivity and lung histopathological changes, mice were treated 1 h before (pre-treatment) or 1 h after (post-treatment) the last aerosol OVA challenge. *C. sympodialis* extract (40 mg/kg), warifteine (2 mg/kg) or dexamethasone (2 mg/kg) was administered via oral route (p.o). Of note, the *C. sympodialis* extract and warifteine doses were used according to previous reports [9–11].

2.5. Airway hyperreactivity analysis

Airway hyperreactivity (AHR) was analyzed in mice using non-invasive whole-body plethysmography (Buxco, Sharon, CT) one day after the last OVA challenge. AHR was measured following aerosolization of PBS followed by increasing concentrations of methacholine (0, 6, 25 mg/mL; Sigma-Aldrich) for 2 min into the chamber. AHR was expressed as an average enhanced pause (Penh). There was an interval of 10 min between each aerosol exposure and within this period of time the Penh values had returned to baseline.

2.6. Bronchoalveolar lavage (BAL) and quantification of IL-13

Animals were euthanized by CO₂ and the trachea was surgically exposed and cannulated at 6 or 24 h after the last allergen challenge. BAL was collected from the mice by washing the lungs with 1 mL PBS. Samples of BAL were centrifuged at 500 g for 8 min at 4 °C to obtain the supernatants. Then levels of IL-13 were measured according to

the manufacturer's instructions by the mouse IL-13 DuoSet kit (R&D Systems).

2.7. Lung histology

Lungs were inflated by injecting 1.0 mL of 4% buffered formalin through the catheter used to perform BAL. Lungs were then removed, fixed in the same solution, and embedded in paraffin. Lung sections of 5- μ m thickness were stained with Sirius red, Mason's trichrome, and periodic acid-Schiff (PAS) according to standard protocols and examined under light microscopy. Analysis of tissue sections and captured images was performed by using a computer-assisted image analyzer (Image-Pro Plus Version 4.1 for Windows, Media Cybernetics, LP, Silver Spring, MD, USA). One observer who was unaware of the experimental setting examined all tissue sections randomly. Digital photographs of at least 10 bronchovascular bundles per tissue section (with bronchioles cross-sectional diameters ranging from 120 to 250 μ m) were obtained under light microscopy at $\times 400$ magnifications. For quantifying eosinophil infiltration of the pulmonary tissue, the total number of eosinophils was counted in the ten areas, and the mean was expressed as eosinophils/high power field (HPF). Mucus secretion from goblet cells of the bronchial epithelium was quantified by the staining of sections with PAS. Goblet cells were recognized by the intense dark red staining of their mucus content with PAS, and their characteristic distended lateral border and basal nucleus. The number of goblet cells was expressed as the percentage of positive cells in at least 500 cells of the bronchial epithelium. The Mason's trichrome dye was used to stain collagen fibers in tissue. Density of collagen fibers was defined by the positively stained area in relation to total tissue in the area of the bronchovascular bundles per millimeter squared, at a magnification of $\times 100$.

2.8. Statistical analysis

Statistical analysis was carried out using the statistical software SPSS for Windows (Version 10.0.1, SPSS Inc., 1989–1999, USA) or GraphPad Prism statistical analysis and graphing software (GraphPad, San Diego, CA). Data were analyzed by ANOVA followed by Newman Keuls *t* test or Dunnett's test. Correlations between the densities of positive cells and the collagen fibers were assessed using the Spearman rank correlation coefficient. Values are expressed as means $\pm$ SEM. The level of significance was set at $P < 0.05$.

3. Results

3.1. *C. sympodialis* and its alkaloid warifteine inhibit AHR and IL-13 production

OVA sensitization and airway challenge led to the development of AHR in mice illustrated by significant increases in Penh values compared with PBS-challenged control mice. As shown in Fig. 2A, the pre-treatment with *C. sympodialis* reduced AHR to inhaled methacholine (25 mg/mL) observed 24 h after allergen challenge. This effect was similar to the glucocorticoid dexamethasone a potent anti-inflammatory drug. The pre-treatment with *C. sympodialis* derived-alkaloid warifteine was also effective in blocking AHR (Fig. 3A).

The involvement of IL-13 in AHR development is well described. OVA-induced allergic airway inflammation leads to elevated levels of IL-13 when compared to PBS challenged animals (Figs. 2B and 3B). As illustrated in the same figures, OVA-sensitized and challenged mice pre-treated with either *C. sympodialis* or warifteine showed a significant reduction in IL-13 levels, comparable to dexamethasone treated animals.

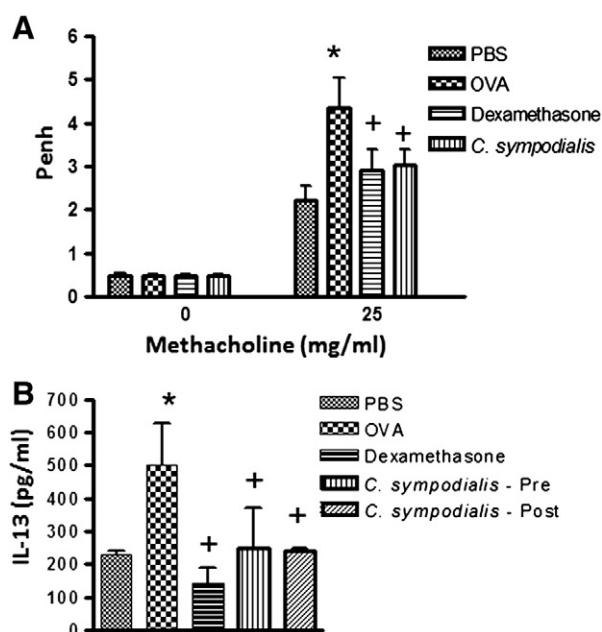


Fig. 2. Effect of *C. sympodialis* treatment on airway hyperreactivity (AHR) development and IL-13 production. OVA-sensitized mice were treated with a single oral dose of dexamethasone (2 mg/kg) 1 h before, or *C. sympodialis* extract (40 mg/kg) 1 h before (pre-) or 1 h after (post-) the last OVA challenge. PBS aerosolized mice were used as control. (A) AHR was measured by aerosolization of PBS followed by increasing concentrations of methacholine. AHR was analyzed 24 h after the last allergic challenge and expressed as an average enhanced pause (Penh). (B) IL-13 production was measured in BAL 24 h after the last allergic challenge. Results are expressed as the mean $\pm$ SEM from at least six animals. *, significantly different from PBS-challenged group ($P \leq 0.05$). +, significantly different from allergen-challenged group ($P \leq 0.05$).

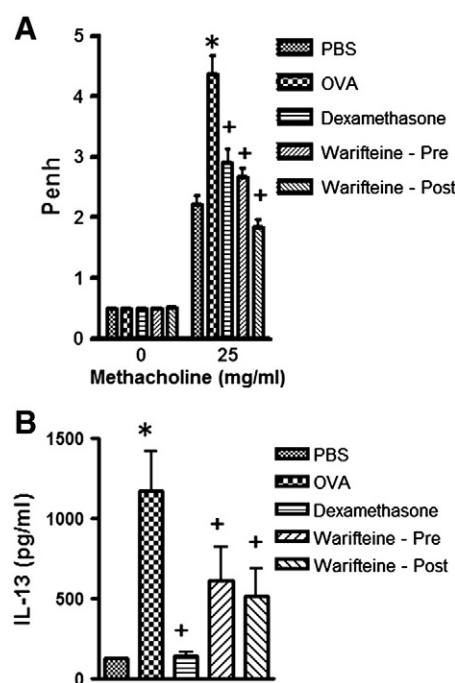


Fig. 3. Effect of warifteine treatment on airway hyperreactivity (AHR) and IL-13 production. OVA-sensitized mice were treated with a single oral dose of dexamethasone (2 mg/kg) 1 h before, or warifteine (2 mg/kg) 1 h before (pre-) or 1 h after (post-) the last OVA challenge. (A) AHR was measured by aerosolization of PBS followed by increasing concentrations of methacholine. AHR was analyzed 24 h after the last allergic challenge and expressed as an average enhanced pause (Penh). (B) IL-13 production was measured in BAL 6 h after the last allergic challenge. Results are expressed as the mean $\pm$ SEM from at least six animals. *, significantly different from PBS-challenged group ($P \leq 0.05$). +, significantly different from allergen-challenged group ($P \leq 0.05$).

In order to investigate the potential therapeutic effect of *C. sympodialis* and its active derivative warifteine, animals were treated 1 h after the last OVA challenge in sensitized animals. Similarly to the pre-treatment, oral post-treatment with warifteine but not with *C. sympodialis* extract (data not shown) was able to inhibit AHR in a significant manner. In addition, post-treatment with warifteine reduced IL-13 BAL levels detected 6 h after the last OVA challenge in the asthma model (Fig. 3B). These data indicate that *C. sympodialis*, mostly through the active compound warifteine, is capable to ameliorate an established inflammatory allergic reaction.

3.2. Effects of *C. sympodialis* and warifteine in mucus accumulation

The evaluation of airway mucins assessed by PAS staining is shown in Fig. 4A. The analysis of airway mucins demonstrated a significant metaplasia of goblet cells and mucus accumulation 24 h after the last OVA challenge in actively sensitized mice. Oral pre- or post-treatment with *C. sympodialis* or pre-treatment with warifteine reduced OVA-induced mucus accumulation and the percentage of mucus producing cells to values similar to nonallergic controls (Fig. 4B). Pre-treatment with dexamethasone showed decreased mucus production as expected.

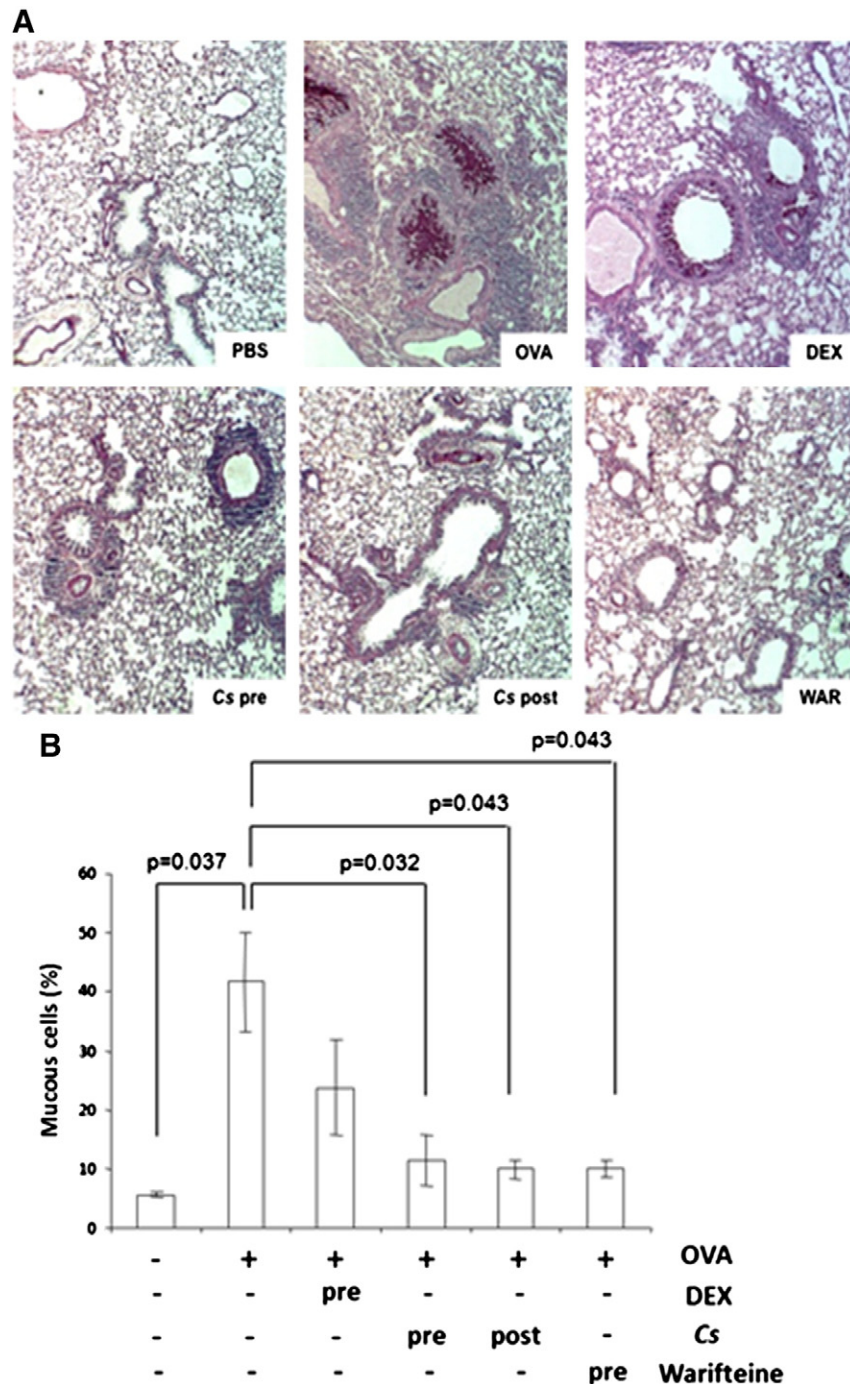


Fig. 4. Effects of *C. sympodialis* and warifteine treatments in airway mucus deposition. Twenty-four hours after the last day of challenge, lungs were inflation-fixed in 4% formalin, embedded in paraffin, and sectioned. The tissue section slides were stained with periodic acid-Schiff (PAS) for evaluation of airway mucins and number of mucus-producing cells. OVA-sensitized mice were treated with a single oral dose of dexamethasone or warifteine (2 mg/kg) 1 h before, or *C. sympodialis* extract (40 mg/kg) 1 h before (pre-) or 1 h after (post-) the last OVA challenge. PBS aerosolized mice were used as control. (A) Microscopic analysis of stained tissue sections. (B) Quantitative analysis of tissue sections stained with PAS. Data are representative of two independent experiments ($n=6$). *, significantly different from PBS-challenged group ($P \leq 0.05$). +, significantly different from allergen-challenged group ($P \leq 0.05$).

3.3. Treatment with *C. sympodialis* or warifteine inhibits tissue eosinophil infiltration in OVA-induced allergic airway inflammation

Eosinophil activation and recruitment are hallmarks of allergic disease and tissue eosinophil infiltration has been associated with collagen deposition and lung remodeling. The effect of *C. sympodialis* or warifteine oral treatment on OVA-induced eosinophil tissue infiltration was investigated. As shown in Fig. 5A, Sirius red stained lung sections revealed an increased leukocyte infiltration comprised mostly by a marked perivascular eosinophilia in OVA-challenged allergic mice when compared to nonallergic mice. Oral pre-treatment with *C. sympodialis* or warifteine significantly inhibited

OVA-induced eosinophil tissue infiltration similar to pre-treatment with dexamethasone (Fig. 5B). Post-treatment with *C. sympodialis* extract was also able to significantly inhibit eosinophil tissue infiltration observed after 24 h (Fig. 5A and B).

3.4. Inhibition of collagen fiber deposition by *C. sympodialis* and warifteine

Airway remodeling is associated with clinical outcomes in asthmatic patients and contributes to thickening of airway walls leading to hyperreactivity and narrowing of airways. As shown in Fig. 6A, OVA induced significant subepithelial fibrosis with collagen fiber

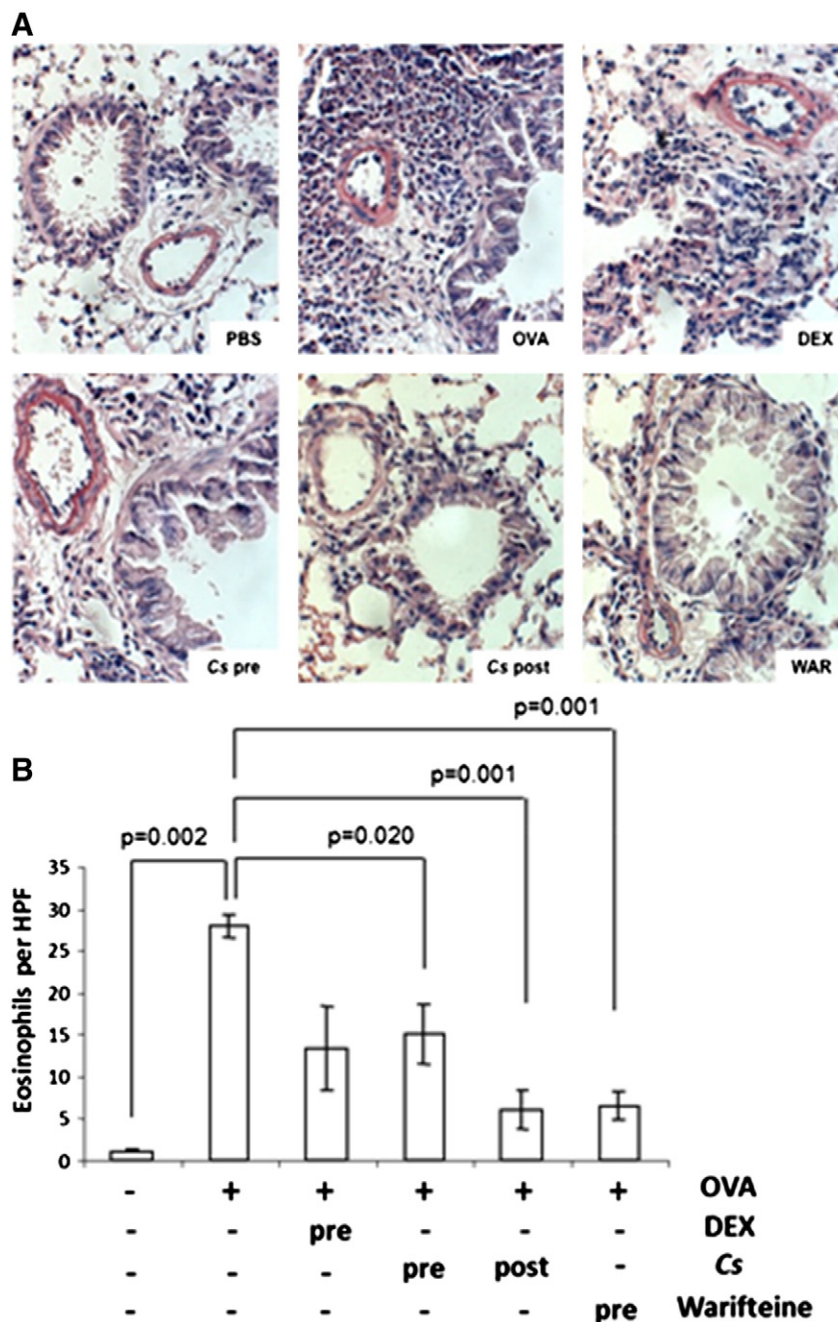


Fig. 5. Effect of *C. sympodialis* and warifteine treatment in eosinophil tissue infiltration following OVA-induced allergic airway disease. Twenty-four hours after the last day of challenge, lungs were inflation-fixed in 4% formalin, embedded in paraffin, and sectioned. The tissue section slides were stained with Sirius red for eosinophil tissue evaluation. OVA-sensitized mice were treated with a single oral dose of dexamethasone or warifteine (2 mg/kg) 1 h before, or *C. sympodialis* extract (40 mg/kg) 1 h before (pre-) or 1 h after (post-) the last OVA challenge. PBS aerosolized mice were used as control. (A) Microscopic analysis of stained tissue sections. (B) Quantitative analysis of tissue sections stained with Sirius red. *, significantly different from PBS-challenged group ($P \leq 0.05$). +, significantly different from allergen-challenged group ($P \leq 0.05$).

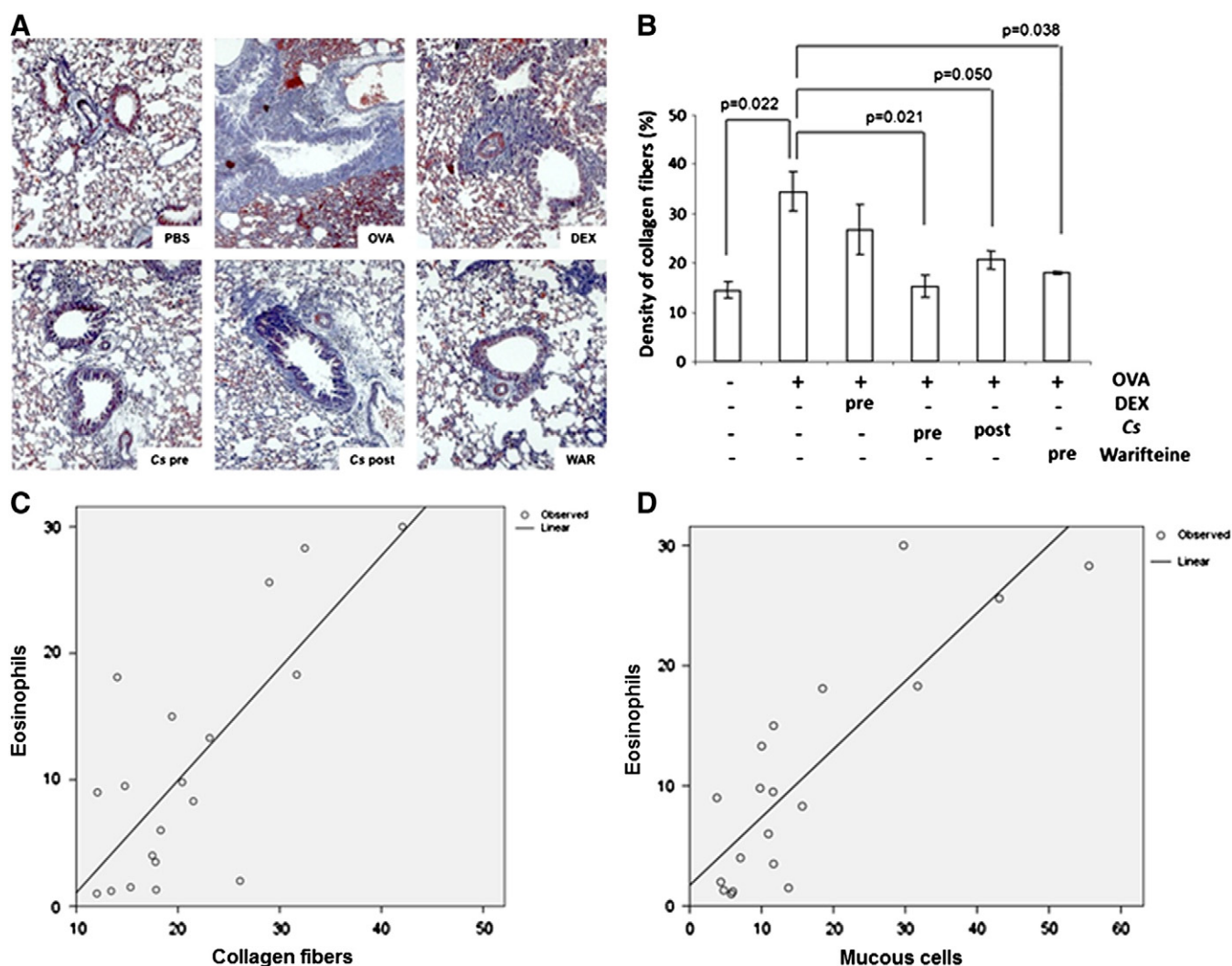


Fig. 6. Effects of *C. sympodialis* and warifteine treatments in collagen fiber deposition. (A) Lung serial sections were embedded in paraffin by routine methods and stained with Mason's trichrome stain to collagen fiber observations. OVA-sensitized mice were treated with a single oral dose of dexamethasone or warifteine (2 mg/kg) 1 h before or *C. sympodialis* extract (40 mg/kg) 1 h before (pre-) or 1 h after (post-) the last OVA challenge. PBS aerosolized mice were used as control. (A) Microscopic analysis of stained tissue sections. (B) Quantitative analysis of collagen fiber density was expressed in percentage. (C) Correlation between eosinophil tissue infiltration and collagen fiber deposition and (D) correlation between eosinophil tissue infiltration and mucus-producing cells. *, significantly different from PBS-challenged group ($P \leq 0.05$). +, significantly different from allergen-challenged group ($P \leq 0.05$).

deposition assessed by Gomoris staining in allergic animals when compared to nonallergic controls. As observed in Fig. 6A and quantified in Fig. 6B, oral treatment with *C. sympodialis* (pre or post) or warifteine (pre) reduced OVA-induced collagen deposition to values similar to nonallergic controls (Fig. 6A, B). In accordance to a role for tissue eosinophils in collagen and mucus deposition and lung remodeling, there is a strong correlation between eosinophil infiltration and collagen deposition (correlation coefficient of 0.651, $P < 0.003$) and mucus (correlation coefficient of 0.723, $P < 0.001$) in the lungs (Fig. 6C, D).

4. Discussion

In this study we evaluate the effect of *Cissampelos sympodialis* and its isolated alkaloid warifteine in a murine asthma model. We provide novel evidence related to the anti-allergic and immunoregulatory properties of *C. sympodialis*, acting mostly through the active compound warifteine, to inhibit the airway hyperreactivity and lung remodeling via a mechanism at least partially dependent of IL-13 and eosinophil inhibition.

In order to test the hypothesis that *C. sympodialis* has anti-allergic properties and potential for anti-asthmatic use, the effect of ethanolic extract of *C. sympodialis* leaves and its derived-alkaloid warifteine to prevent or ameliorate AHR and lung remodeling were investigated. Oral treatment with *C. sympodialis* 1 h before the last ovalbumin challenge significantly reduced AHR. Development of AHR depends on airway inflammation and smooth muscle lung contraction induced by different stimuli including IL-13, IL-4 and cysteinyl leukotrienes [15–17]. Accumulating evidence has placed IL-13 as a key mediator of AHR due to the protective effect demonstrated by different strategies of disruption of IL-13 production and/or effects [16]. To gain insights on the mechanism of action of *C. sympodialis* its role in IL-13 production was analyzed. In parallel to the AHR inhibition, we observed a reduced level of IL-13 in bronchoalveolar lavage of orally treated with *C. sympodialis* and challenged mice. Thus suggesting that, at least in part, the mechanism involved in *C. sympodialis* inhibitory effect on OVA-induced AHR may occur through inhibition of IL-13 production. In order to investigate candidate compounds to anti-asthmatic effect from *C. sympodialis* the isolation of one bisbenzylisoquinoline alkaloid warifteine was performed [6]. Similar to the one observed with *C. sympodialis*, oral treatment with warifteine inhibited

allergen-induced AHR and IL-13 production, suggesting that warifteine represents the main active component responsible for the effects of *C. sympodialis*. However, other constituents mainly alkaloids such as methylwarifteine, milonine and roraimine may also contribute to the anti-allergic effects of *C. sympodialis* extract.

Of note, warifteine also has an additional property that may explain its inhibitory effects on AHR. Previous *in vitro* and *in vivo* studies demonstrated direct spasmolytic effects and tracheal muscle contraction inhibition of warifteine [6,7,13,15]. In addition, warifteine treatment was very effective impairing *in vivo* lipid body biogenesis and BAL levels of LTC₄ [10] which is produced within eosinophil lipid bodies recruited to BAL in the asthma model [18].

As key components in asthma, lung eosinophils influx during inflammatory response has been demonstrated as important players of progressive tissue damage and lung remodeling [19–21]. Eosinophils are multifunctional leukocytes capable of producing immunoregulatory cytokines and lipid mediators and are actively involved in allergic disease through regulation of Th2-type immune responses [22]. *C. sympodialis* and warifteine also significantly reduced the eosinophil influx into the lung tissue of OVA-induced allergic inflammatory disease in mice. Accordingly, we have showed that *C. sympodialis* and its isolated alkaloid warifteine reduced eosinophil recruitment and activation in bronchoalveolar lavage as well as into pleural cavity of OVA sensitized mice in part through mechanisms dependent of eotaxin [10].

Allergic lung inflammation also induces changes in tissue phenotype named hyperplasia of goblet cells leading to excessive mucus production causing airway obstruction [23] and collagen deposition leading to remodeling of the airways. An array of molecules might trigger mucus production but IL-13 has been mentioned as a pivotal stimulus [24]. Mice orally treated with *C. sympodialis* and warifteine showed a diminished mucus production in lung tissue. Airway remodeling is characterized by altered composition, content, and organization of cellular and molecular constituents of the airway wall [1]. Oral treatment with *C. sympodialis* also preserved structural components of lung tissue such as airway smooth muscle (ASM) mass, distance between epithelium and ASM cells, proliferation of blood vessels, airway edema, goblet cell numbers, collagen fiber deposition and mucus production. Deposition of collagen fibers and mucus has been described as airway change able to cause narrowing of airway, rigidity, loss of elastic recoil and reversible obstruction [1]. *C. sympodialis* and warifteine reduced collagen fiber deposition and mucus in lung tissue. These data correlated to decrease of eosinophil numbers in the tissue. Previous studies related that different stimuli can initiate airway remodeling before the appearance of asthma symptoms suggesting remodeling as contributive phenomena to lung inflammation and AHR development, and there is compelling evidence that tissue eosinophil accumulation contributes to lung remodeling in OVA-induced airway allergic disease since animals genetically ablated in eosinophils were significantly protected from peribronchiolar collagen deposition and increases in airway smooth muscle [25].

In this work we demonstrated for the first time that *C. sympodialis* and its isolated alkaloid warifteine inhibits airway hyperreactivity and lung remodeling known as the main asthma features and we provide evidence that the protective effect occurs partially by an IL-13 dependent mechanism. These findings, together with previous reports of this plant and warifteine inhibiting anaphylactic shock reaction in OVA sensitized mice, allergic pleurisy and pulmonary inflammation; suggest their therapeutic potential as anti-allergic and anti-asthmatic [9,26,10]. This alkaloid also reduced mast cell degranulation and hyperalgesia response in rats [12]. Of interest for potential therapeutic applications, both *C. sympodialis* extract and purified warifteine showed unique post-treatment properties via oral administration maintaining the anti-allergic inhibition of AHR, IL-13 production and lung eosinophil infiltration even when administered after the installed allergic process.

5. Conclusion

Indigenous and other people living in non-developed countries use *C. sympodialis* as a preventive method to asthma condition. Our data corroborate the folk anti-allergic medicine use of *C. sympodialis*. In addition, our findings indicate that the anti-allergic and immunoregulatory properties of *C. sympodialis* occur mostly through the active compound warifteine, to inhibit the airway hyperreactivity and lung remodeling through a mechanism at least partially dependent of IL-13 and eosinophil inhibition. Therefore, warifteine is suggested as an interesting therapeutic candidate in allergic inflammation.

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Glossary

CS: *Cissampelos sympodialis*

DEX: Dexamethasone

WAR: Warifteine

OVA: Ovalbumin

AHR: Airway hyperreactivity

IL-13: Interleukin-13

Penh: Enhanced pause

BAL: Bronchoalveolar lavage

PAS: Periodic acid-Schiff

PBS: Phosphate buffered saline

Pre/Post: Pre-treatment/Post-treatment