Managing murine food allergy with *Cissampelos sympodialis* Eichl (Menispermaceae) and its alkaloids

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**A B S T R A C T**

Food allergy is a severe human disease with imminent risk of life. *Cissampelos sympodialis* (Menispermaceae) is a native Brazilian plant used in Brazilian folk medicine for the treatment of respiratory allergies. In this study the experimental model of food allergy induced by ovalbumin (OVA) was used to determine whether the alcoholic extract of the plant (AFL) and its alkaloids match a therapeutic approach for this disease. Animal weight, diarrhea, OVA-specific IgE levels, inflammatory cell and cytokine profiles, mucus production and proportion of T cells on the mesenteric lymph node (MLN) were evaluated. Warifteine (W) or methyl-warifteine (MW) alkaloids slightly improve diarrhea score independently of AFL and all treatments decreased the OVA-specific IgE levels. Stimulated mesenteric lymph node (MLN) cells in the presence of the alkaloids diminished the IL-12p70 levels independently of IFN-γ or IL-13 secretion. The alkaloids increased the number of Treg cells on MLN and reduced the number of eosinophils and mast cells as well as mucus production in the gut. Therefore, the alkaloids modulate the immune response in food allergy by increasing regulatory T cells in MLN independently of Th1 or Th2 profiles.

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1. Introduction

Food allergy is an adverse health effect with intense diarrhea and it is dependent on Th2 immune response. This disorder can occur repeatedly after an exposure to a given specific food allergen [1]. There is evidence of a high and increasing prevalence of food allergies all over the world [2–4].

Increased health cost in ambulatory and emergency services besides difficult symptom control is described as important limitations to food allergy treatments [5]. Besides, there is no effective therapy and its management consists of changing patients’ behavior, that is, by asking them to avoid foods which may contain a given group of allergens [1]. This radical change in the behavior of allergic patients, as well as in their family’s, may lead to a poor quality of life and may result in nutritional deficiency and psychosocial distress [6,7].

The pathophysiology of food allergies is largely studied in murine models, which present similarities with human food allergies [8]. In these animal models, enhanced immunoglobulin-E (IgE) levels, inflammatory cell recruitment and Th2 cytokine secretions can be observed [8–10]. The IL-4, IL-5, IL-9 and IL-13 productions represent the Th2 profile observed in allergies. These cytokines are involved in the specific IgE production, eosinophilia and mast cell activation. However, to modulate the process, the presence of regulatory T cells (Treg cells) into the mesenteric lymph nodes (MLN) has been described with the presence of regulatory cytokines IL-10 and TGF-β [11,12]. Another regulatory cytokine profile observed in this disorder is from Th1 cells, mainly with IFN-γ secretion.

Search for new therapies to treat food allergy has emerged and in this perspective the medicinal plant extracts and isolated compounds are being tested in food allergy models with promising results, ranging from an attenuation of food allergy to an inhibition of the development and progression of the disease [13,14].

*Cissampelos sympodialis* Eichl. (Menispermaceae) popularly named “milona” is a plant used in the Northeastern Brazil to treat several inflammatory diseases including allergic disorders. Studies from our laboratory showed that the hydroalcoholic fraction from the leaves (AFL) of the plant extract induced an anti-allergic cytokine profile with IL-10 and IFN-γ secretions by spleen cells [15–17]. In an experimental model of asthma, AFL reduced IgE serum levels and leukocyte recruitment to bronchoalveolar lavage [17–19]. Additionally, it was demonstrated that AFL inhibited ERK and NF-κB expression in B cell cultures [20].

In the perspective to describe which isolated compounds of AFL were implicated in the anti-allergic effect, our group demonstrated that warifteine, an alkaloid isolated from AFL, represents one of the bioactive molecules. In a previous report it was showed that warifteine inhibited IgE production, paw edema induced by ovalbumin (OVA),
scratching behavior induced by the compound 48/80 and a hyperalgesia reaction induced by IgE-anti-DNP-OVA in rats [22]. Indeed, warifteine inhibited histamine release of mast cells after antigen challenge indicating that the alkaloid is one of the compounds in the plant extract with an immunomodulatory effect [21].

Similarly to AFL, warifteine acts on B cell function by inhibiting cell proliferation and Ig secretion, modifying the phosphorylation pattern of mitogen-activated protein kinase (MAPK) ERK and the intracellular levels of transcription factor NF-κB. Warifteine also attenuated the intracellular calcium levels and increased the cAMP level in these cells [20].

Given the knowledge about the plant effects in pulmonary allergy, we postulate the hypothesis that the plant could also modulate food allergy — an important public health problem all over the world. Therefore, the aim of this study was to demonstrate the immunomodulatory effect of the C. sympodialis extract, warifteine and its natural methylated alkaloid, methyl-warifteine, in an experimental model of food allergy.

2. Material and methods

2.1. Animals

Female BALB/c mice (20–25 g) and Wistar rats (250–300 g) were used in the study. The animals were supplied by animal facility of Federal University of Paraíba (UFPB/PB/Brazil). Experimental animals were maintained with water and food ad libitum in a room temperature ranging from 22 to 24 °C and a 12 h light/dark cycle. Groups of 10 animals were used in each test. All experiments were carried out with strict adherence to ethical guidelines and approved by the Ethical Committee for Experimental Animal/UFPB (CEPA No. 0302/11).

2.2. Plant extract and alkaloids

Leaves from C. sympodialis were obtained from the Botanical Garden of the Laboratory of Pharmaceutical Technology, UFPB (voucher specimen Agra 1456). The leaves were dried at 50 °C and pulverized. The dried extract (AFL) was dissolved and filtered in water. Known volumes were dried to determine the final concentration of the water-soluble components (78% on average). The standardized AFL had a nominal concentration of 0.95% of warifteine [19–21]. Alkaloid solutions were prepared using 1 mg of the crystal in 50 μL of HCl 1 N and 800 μL of distilled water. The pH was adjusted to 7.0 with NaOH 1 N. The volume was completed to 1000 μL and the dilutions were prepared using 1 mg of aluminum potassium sulfate adjuvant (alum, Sigma-Aldrich, St. Louis, Missouri, USA) in the presence of 1 mg of aluminum potassium sulfate adjuvant (alum, Sigma-Aldrich) by intraperitoneal injection. Two weeks later, mice were held in the supine position and an OVA solution (50 mg/250 μL) was orally administered. Before each intragastric challenge and treatment, mice were deprived of food for 3 h, and 1 h before each OVA-challenged animal was treated with different drugs. Groups of animals (n = 10) were divided in: positive control (CTR +, saline), AFL200 (AFL at 200 mg/kg), W2 (warifteine at 2 mg/kg), MW2 (methyl-warifteine at 2 mg/kg) or CROMO 10 (sodium cromoglycate at 10 mg/kg) and the negative control (CTR −, non-sensitized and OVA-challenged). Besides the CTR −, the other groups were OVA-sensitized and OVA-challenged. Mice demonstrating profuse liquid stool (diarrhea) were visually recorded for 60 min after challenges [22,23].

2.4. Body weight and diarrhea evaluation

The body weight of each group of animals was obtained from the means of the individual values and expressed in grams (g). The gain of weight to each animal in group was determined as:

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\text{% of weight gain =} \left( \frac{\text{animal weight on 50th day} - \text{animal weight on 28th day}}{\text{animal weight on 28th day}} \right) \times 100
\]

and the percentage of body weight gain in each group was determined by the means ± S.E.M. of % of weight gain and compared between groups [24,25]. Diarrhea was defined as diarrhea scores according to feces appearance as follows: score 0 = no changes; score 1 = soft but well formed; score 2 = soft, non-formed; score 3 = one episode of liquid diarrhea; and score 4 = at least two episodes of liquid diarrhea. One hour after the last challenge mice were sacrificed by exsanguination under anesthesia and blood, mesenteric lymph nodes (MLN), and intestinal tissue were taken for further analysis.

2.5. Determination of OVA-specific IgE titer

Briefly, OVA-specific IgE titer was determined using the passive cutaneous anaphylaxis (PCA) reaction known as a reaction that is extremely precise to determine the antigen-specific IgE levels. Treated and non-treated OVA-sensitized and non-sensitized groups were bled and 50 μL of a serial dilution of each serum was inoculated intradermally on the shaved back of Wistar rats at marked sites. After 24 h, the rats were challenged intravenously with OVA (2.0 mg) in 1% Evans Blue dye. Thirty minutes later the rats were euthanized and OVA-specific IgE titers were measured. The highest serum dilution giving up to 5 mm diameter flare or bluing reaction was taken as the PCA titer [26].

2.6. Cell preparation

Mesenteric lymph node cell suspensions from each animal group were removed and processed in tissue grinder homogenizer. Cells were washed twice in RPMI 1640 medium by centrifugation at 1200 rpm for 10 min at 4 °C. The pellet was resuspended in RPMI

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![Fig. 1. Experimental design.](image-url)
medium supplemented with 2 mM L-glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, 5 μM l-mercaptoethanol and 10% fetal calf serum (FCS — Gibco, Paisley, UK). Viable cells were determined in a hemocytometer by the exclusion of the Trypan blue dye. Duplicate cultures were performed in 24-well flat-bottomed tissue culture plates (Costar, Cambridge, MA, USA) in a final volume of 1 mL/well containing 1 x 10^6 cells. Concanavalin-A (5 μg/mL) and warifteine or methyl-warifteine at 0.1 or 1.0 μM were added in the cell cultures. The plate was incubated for 12–48 h at 37 °C, 5% CO2. A volume of 900 μL of the cell culture was removed and frozen at −20 °C for further cytokine analysis [27].

2.7. Cytokine analysis

IL-12p70, IL-13 and IFN-γ in the supernatants of MLN cell cultures were measured by ELISA, using the recommended protocol from the antibodies' suppliers. Antibody pairs and standard recombinant cytokines for ELISA assay were purchased from ebiosciences.

2.8. Flow cytometric analysis of mesenteric lymph node cells

Two aliquots of 1.0 x 10^6 MLN cells/3 mL of PBS (4 °C) from each animal group were centrifuged (1200 rpm, 10 min, 4 °C) and the supernatant was discarded. The cell suspensions were kept in PBS with 2 μL of mouse serum (1:100) for 5 min. One aliquot was used to evaluate the amount of CD4+ and CD8+ T cells using monoclonal antibodies: anti-mouse CD4-PE conjugated and CD8-FITC conjugated purchased from ebiosciences. Another cell aliquot was evaluated for the regulatory T cells using a kit from ebiosciences: anti-mouse CD4-FITC conjugated, CD25-APC conjugated and FoxP3-PE conjugated. The regulatory T cells were identified with CD4+, CD25+ and FoxP3+ cell populations. Flow cytometry (BD Biosciences™, FACScalibur, San Jose, CA, USA) and Cell Quest™ for Macintosh software (BD Biosciences™) was used to analyze the data [28].

2.9. Gut histology

Gut segments (jejunum portion) were washed with cold saline, fixed with 4% buffered formalin and embedded in paraffin. 5-μm thick gut sections were stained with Hematoxylin & Eosin (eosinophil cells), Toluidine blue (mast cells) or periodic acid-Schiff (PAS) (mucus production) according to standard protocols and were examined under light microscopy. Analysis of tissue sections and captured images were obtained at 40 × (56 μm²/field) using a computer-assisted image analyzer (software AV soft Bio view (Spectra Module) version 4.0 for Windows, Silver Spring, MD, USA). One observer who was unaware of the experimental setting examined all tissue sections randomly. Digital photographs of at least 10 sections per tissue (cross-sectional diameters — 50 μm²) were obtained under light microscopy at ×400 magnifications. For quantifying eosinophil and mast cell infiltration of the gut tissue, the total numbers of the cells were counted in ten areas, and the mean was expressed as eosinophils or mast cells/high power field (HPF). Mucus secretion from goblet cells of the gut epithelium was quantified by the staining of sections with PAS. For the determination of goblet cell volume, all pixels with green hues were selected for the creation of a binary image and subsequent calculation of the total area, and the resulting data were reported as mm² PAS/field.

2.10. Statistical analyses

Data were analyzed by Student’s t-test, ANOVA followed by Tukey using software GraphPad Prism (GraphPad, San Diego, CA). Values given are means ± S.E.M. A value of P < 0.05 was considered significant. For flow cytometry data analysis we used Win MDI software (version 2.9, available at http://facs.scripps.edu/software.html).

3. Results

3.1. Effect of AFL or alkaloids on the body weight in murine food allergy

Treatment with the extract (AFL), warifteine (W), methyl-warifteine (MW) or sodium cromoglycate (CROMO) was evaluated based on the body weight loss caused by OVA challenges in mice. It was observed that the mean body weight of treated animals from each experimental group did not differ from one another during the OVA challenge period (data not shown).

3.2. Effect of AFL or alkaloids treatment on the allergic diarrhea

Treatments with alkaloids (W and MW) slightly improved the diarrhoea process similar to the pattern drug, sodium cromoglycate. However, animals treated with the extract (AFL) did not show improvement on the diarrhoea process (Fig. 2). These results showed that the anti-allergic properties of the alkaloids previously described in an asthma model also prevented the diarrhoea occurrence in this food allergic model.

3.3. Effect of AFL or alkaloids treatment on IgE production

We also investigated the ability of the treatments in modulating the production of OVA-specific IgE sera titer, a parameter related to severity of immediate anaphylaxis hypersensitivity. As observed in Fig. 3, the
treatments with plant extract (AFL), alkaloids (W and MW) or sodium cromoglycate (CROMO) were significantly (P < 0.01) effective in decreasing the amount of OVA-specific IgE serum levels (titers — 1:288 ± 52, 1:227 ± 117; 1:268 ± 78; 1:248 ± 65, respectively) as compared with the non-treated group (CTR+, titer — 1:632 ± 65). Additionally, non-sensitized mice did not present IgE into the plasma. These results indicate that the treatments are affecting the immune system response by modulating B cells.

3.4. Effect of the alkaloids on the cytokine production

To identify the mechanisms by which warifteine or methyl-warifteine treatment delayed diarrhea on the food allergy model, we measured in vitro cytokine production in MLN cells from ovalbumin sensitized mice. Con-A-stimulated cells produced, significantly (P < 0.05), IL-12p70, IFN-γ (Th1 profile), and IL-13 (Th2 profile) as compared to non-stimulated cells (Fig. 4). In vitro treatment with warifteine at 0.1 and 1 μM and methyl-warifteine at 1 μM inhibited (P < 0.05) IL-12p70 production induced by Con-A (Fig. 4A) independent of IFN-γ (Fig. 4B). However both alkaloids did not inhibit IL-13 production (Fig. 4C). The IL-12p70 modulation by the alkaloids indicates that they are interfering with cells from the innate immune system regardless if the cells from an adaptive immune system.

3.5. Effect of AFL or alkaloids on T cell proportion

In order to identify the T cell profile of MNL of OVA-sensitized and alkaloid-treated mice, flow cytometry analyses were carried out. The number of CD4+, CD8+ and CD25+Foxp3+ (regulatory T cells) after OVA challenges was measured. Fig. 5A shows that the process...
of ovalbumin-sensitization and -challenges (CTR+) significantly reduced (P < 0.01) the percentage of CD4+ T cells in MLN as compared with the non-sensitized group (CTR−) (38.9% vs 49.1% respectively). AFL, W (P < 0.01) or MW (P < 0.05) treatments restored and even increased the proportion of CD4+ T cells (48.2%; 51.1% and 43.8% respectively) as compared with CTR+ group (38.9%). The treatment with sodium cromoglycate decreased the amount of CD4+ T cells (39.6%) similar to the CTR+ group (38.9%).

Fig. 5A also showed that AFL, alkaloids (W, MW) or even sodium cromoglycate treatments significantly (P < 0.05, P < 0.01 and P < 0.001) increased and restored the proportion of CD4+ T cells (15.9%, 16.5%, 18.8 and 19.3%, respectively) as compared with CTR+ group (13.3%). The proportion of CD25+FOXp3+ T cells among MLN cells is represented in Fig. 6A. MLN cells in the presence of the alkaloids (W or MW) or extract (AFL) maintained and even increased the proportion of CD25+FOXp3+ T cells (12.4%, 12.4% and 14.6% respectively) as compared with the CTR+ group (11.8%). An interesting result observed in this study was that the pattern drug treatment promoted a profound reduction in this cell population (1.9%) as compared with CTR+ group (11.8%).

3.6. Effect of AFL and alkaloids in leukocyte influx and mucus production

Stained gut tissue sections were analyzed to evaluate the effect of AFL and alkaloid (W and MW) treatments on leukocyte recruitment and mucus production. AFL and the alkaloids inhibited eosinophil recruitment to the gut tissue compared to the CTR+ group (P ≤ 0.05) (Fig. 7A, B). Both alkaloids also inhibited mast cell recruitment to the gut tissue (P ≤ 0.05) (Fig. 8A, B) independent of the AFL. However, AFL and the alkaloid treatments were able to inhibit mucus production in the gut tissue (P ≤ 0.05) (Fig. 9A, B). Animals from the control group did not present leukocytes or mucus production. These data clearly demonstrated that the plant compounds are responsible for the anti-allergic property of the plant.

4. Discussion

Food allergies are pathological conditions common in children and adults justifying the development of therapeutic strategies to reverse these conditions. Induction of oral tolerance by oral or sublingual route, administration of allergenic molecules, clinically-tested drugs or medicinal plants used in folk medicine can interfere with food allergy symptoms such as diarrhea and loss of weight [8].

The oral antigen tolerance procedure can result in accidents with drastic consequences such as anaphylactic shock reactions [29]. To avoid severe side effects, a better regulation of the immune system using plant extracts or even their compounds should be analyzed.

Studies with medicinal plants used in folk medicine to avoid allergic symptoms have been addressed in different laboratories around the world [30]. This perspective directed us to study the C. sympodialis extract (AFL) and its alkaloids warifteine and methyl-warifteine in an experimental model of ovalbumin-induced food allergy [31]. These plant products have been systematically studied and it has been shown that the plant...
Fig. 6. Effect of the plant extract and alkaloids on regulatory T cells from mesenteric lymph node (MLN) of ovalbumin sensitized mice. The proportion of CD25, FoxP3 T cells on CD4 T cell population was analyzed by flow cytometry using monoclonal antibodies anti-CD25 APC and anti-FoxP3 FITC. A. Single cell suspensions of MLN stained for CD25+ and Foxp3 cells and analyzed by FACS. Numbers represent the proportion of CD25+FoxP3+ cells among the CD4+ population from animal groups: negative control (CTR−), positive control (CTR+), plant extract (AFL 200), warifteine (W2), methyl-warifteine (MW2) or sodium cromoglycate (CROMO10). B. Percentage of CD25+, FoxP3+, and CD4+ population into the MLN cells. Data were analyzed by Student’s t-test and results are expressed as means ± S.E.M. in two independent experiments. +, P < 0.05 significantly different compared with the CTR+ group.

Fig. 7. Effect of the plant extract and alkaloids on eosinophil recruitment to gut mucosa. Twenty-four hours after the last day of challenge, gut segments were fixed in 4% formalin, embedded in paraffin, and sectioned. Tissue section slides were stained with Hematoxylin & Eosin for eosinophil tissue evaluation. Different mice groups were analyzed: negative control (CTR−), positive control (CTR+), plant extract (AFL 200), warifteine (W2) and methyl-warifteine (MW2). (A) Microscopic analysis of stained tissue sections. (B) Quantitative analysis of tissue sections stained with Hematoxylin & Eosin. *P ≤ 0.05, significantly different from the CTR+ group. +P ≤ 0.05, significantly different from the OVA-challenged group. Representative of three independent experiments.
Fig. 8. Effect of the plant extract and alkaloids on mast cell recruitment to gut mucosa. Twenty-four hours after the last day of challenge, gut segments were fixed in 4% formalin, embedded in paraffin, and sectioned. Tissue section slides were stained with Toluidine blue for mast cell tissue evaluation. Different mice groups were analyzed: negative control (CTR−), positive control (CTR+), plant extract (AFL 200), warifteine (W2) and methyl-warifteine (MW2). (A) Microscopic analysis of stained tissue sections. (B) Quantitative analysis of tissue sections stained with Toluidine blue. *P ≤ 0.05, significantly different from the CTR+ group. +P ≤ 0.05, significantly different from the OVA-challenged group. Representative of three independent experiments.

Fig. 9. Effect of the plant extract and alkaloids on mucus production. Twenty-four hours after the last day of challenge, gut segments were fixed in 4% formalin, embedded in paraffin, and sectioned. Tissue section slides were stained with PAS for mucus production tissue evaluation. Different mice groups were analyzed: negative control (CTR−), positive control (CTR+), plant extract (AFL 200), warifteine (W2) and methyl-warifteine (MW2). (A) Microscopic analysis of stained tissue sections. (B) Quantitative analysis of tissue sections stained with PAS. *P ≤ 0.05, significantly different from the CTR+ group. +P ≤ 0.05, significantly different from the OVA-challenged group. Representative of three independent experiments.
can modulate the immune system towards a beneficial cytokine profile in an experimental model of asthma [15–21].

In this study AFL, warifteine or methyl-warifteine did not improve the weight gain during OVA challenge procedures. However, these treatments slightly decreased the allergic diarrhea process during the OVA challenges demonstrating that the natural methylation of warifteine did not improve its effect. This protective effect of the treatments, mainly by the alkaloids, can be associated with inhibition of mast and eosinophil cell activities. Indeed, a previous report demonstrated that warifteine inhibited the hyperalgesia and scratching behavior, which are both phenomena associated to mast cell degranulation [21]. Interestingly AFL oral treatment showed no effect on the allergic diarrhea process. However, all three treatments decreased the amount of mucus on the goblet cells of the intestinal tissue.

The absence of a strong anti-diarrhea effect induced by the alkaloids and the failure of the AFL treatment can be justified by a previous report on which the authors showed that AFL, warifteine or methyl-warifteine inhibited phosphodiesterase (PDE) activity in intestinal smooth muscle and consequently increased cAMP levels. cAMP is associated with an increased Ca2+ channel activity whose result is the influx of Na+ and H2O into the intestinal tract [32,33]. Therefore the slight anti-diarrhea effect of the compounds tested may be justified based on the anti-immunoglobulin effect.

Antigen-specific IgE levels are induced in allergic mice after challenge procedure similar to what happens to humans [34,35]. Of note, oral treatment with AFL or the alkaloids on this food allergy model, one hour before each ovalbumin challenge, reduced the IgE levels indicating a systemic protective effect. This phenomenon was also observed in an experimental model of asthma and in B cell activation studies [16–20].

Previously, warifteine inhibited the phosphorylation of mitogen activated protein kinase (MAPK) ERK1/2 and the intracellular levels of the transcription factor NF-kB (NFk-B) in activated B cells. In addition warifteine increased cAMP levels in these cells. This phenomenon inhibits the phosphorylation of k-B required for the migration of NF-kB to the cell nucleus and consequently inhibited B cell function [20,36]. Recently, we have been studying MAPK phosphorylation profile in mice peritoneal macrophages and, as preliminary results; we observed that the methylated warifteine decreased the JNK phosphorylation in LPS-activated macrophages indicating the inhibition of macrophage function (unpublished data).

Macrophages also have been studied as an immunomodulatory target to C. symposialis. Previous report showed that AFL treatment decreased NO production and increased IL10 levels in T. cruzi infected macrophages leading to proliferation of intracellular parasite [37]. These data indicated for the first time that the plant present compounds that modulate macrophage function. In order to study mechanisms of action of the alkaloids, Th1 and Th2 cytokine profiles in the mesenteric lymph node cells from food allergic mice were measured. In this study, both alkaloids inhibited the IL-12p70 secretion on MLN cells stimulated by Con-A independent of IFN-γ secretion.

According to data already published with the plant extract and its alkaloids we suggest that in the experimental model of food allergy used in this study, compounds of the plant are down regulating monocytes and/or macrophages which represent the main sources of IL-12p70 probably without modifying T cell cytokine secretion. Indeed the treatments did not alter IL-13 levels in Con-A stimulated MLN cell either.

To clarify this hypothesis we analyzed the proportion of CD4+, CD8+ and Treg cells in the MLN of OVA-sensitized, -challenged mice and treated them with alkaloids or the extract. The proportion of CD4+ T or CD8+ T cells in MLN was restored to normal levels by AFL or alkaloid treatments. Interestingly, we observed that the treatments increased the proportion of Treg cells in the CD4+ T cell pool in the MLN of mice. Treg cells are important in controlling a wide range of immune-mediated pathologies including autoimmunity and allergies [38,39]. The regulatory mechanism of Treg cells is related to the secretion of IL-10 and/or TGF-β [40]. Therefore, further studies will be done to explain this phenomenon.

Thus, we suggest that C. sympodialis (milona) used in the folk medicine in Northeast Brazil modulates cells from the innate immune mainly, but also T regulatory cells in the experimental model of food allergy. The data clearly showed that the treatments ameliorated the disease symptoms. So, we postulate that the alkaloids from the plant may be useful as adjuvant to improve treatments to food allergic diseases, however further studies should be addressed to demonstrate the binding of these alkaloids to molecular targets.

Abbreviations

AFL hydroalcoholic fraction from the leaves of plant extract
OVA ovalbumin
CROMO sodium cromoglycate
MLN mesenteric lymph node cell
HPF high power field
PAS periodic acid-Schiff

Safety

The authors declare that there were no issues related to human hazard during the term of this research work, neither when handling the chemicals nor while performing the protocols described herein.

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