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# *Cissampelos sympodialis* (Menispermaceae): A Novel Phytotherapic Weapon Against Allergic Diseases?

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## 1. Introduction

Allergic diseases affect millions of people around the world. Enhanced prevalence and the chronic characteristics of these illnesses represent an important public health problem. Most prevalent allergic diseases are classified as immediate-type reactions such as urticaria, allergic conjuctivitis, food allergy, allergic rhinitis, anaphylaxis and asthma (Sicherer & Leung, 2004; Fonacier et al., 2010; Sicherer, 2011). The immediate-type reaction terminology is applied to allergic reactions because the symptoms develop few minutes after allergen contact. The immune mechanisms responsible for the initiation of these reactions depend on the production of immunoglobulins (IgG1 and/or IgE) that activate cells such as mast cells, eosinophils and basophils. Once activated, these cells are responsible for release of inflammatory mediators, contributing to the exacerbation and maintenance of the allergic processes (Lampinen et al., 2004). Urticaria is characterized by pruritic, edematous and erythematous lesions that affect 15% to 25% of individuals during their lives. Most of the cases are acute but about 30% of patients present symptoms for more than six weeks and are considered as having chronic disease. Women are more susceptible (75%) than men and only 1% to 5% of the cases are related to IgE-dependent reaction while most of the cases are considered to be induced by physical stimuli or of idiopathic nature, including autoimmune urticaria (Antunez et al., 2006). Allergic urticaria depends on skin mast cell activation which delivers preformed mediators, mainly histamine, few minutes after allergen exposure. On the other hand activated mast cells also produce and deliver neo-formed mediators, i.e., prostaglandin D2 ($PGD_2$) and cisleukotrienes (*cis*LT) that stimulate inflammatory responses mediated by neutrophils, basophils, eosinophils and T lymphocytes (Funk, 2001; Harizi et al., 2008; Kambe et al., 2010). Another allergic disease of great importance in public health is allergic conjunctivitis. Allergic conjuctivities prevalence in the United States was estimated to affects about 40 million people. Allergic conjunctivitis is marked by the presence of eosinophil cells in conjunctiva mucosa (Bezerra & Santos, 2010). Food allergy, another allergic disorder, is related to the genetic susceptibility of individuals to eggs, peanuts, seafood (e.g. shrimp, lobsters, crabs, squids and mussels milk and others). Oral sensitization

with shrimp tropomyosin induces in mice allergen-specific IgE, T cell response and systemic anaphylactic reactions (Capobianco et al, 2008). Food allergy has been reported in some cohort studies which describe variable rates of food allergy prevalence in the United States, Canada, the United Kindom, Singapore and the Philippines (Berin & Mayer, 2009). Approximately 1% of food allergic patients develop signs and symptoms characterized by intense diarrhea, urticaria and anaphylaxis. There are divergences about the period of exposition to food allergens and development of disease symptoms. Studies showed that allergenic food ingested during pregnancy increases the risk of higher prevalence of allergic response in infants (Sausenthaler et al., 2011). In contrary to the aforementioned, other studies suggested that earlier exposure to food allergens may promote a protective response to allergic conditions. Serologic diagnosis of patients with food allergies have demonstrated high levels of specific IgE. The pathological immune response observed in these patients depended on: (i) the presence of an adjuvant responsible for stimulating inflammatory response which is considered one necessary step to initiate lymphocyte responses, (ii) allergen doses which can induce classical or alternative mechanisms of allergic process in response to food as seen in cutaneous sensitization to food allergens and (iii) the types of mediators delivered which can lead to systemic allergic reactions such as anaphilaxis (Berin & Mayer, 2009; Sicherer & Leung, 2011). In addition, asthma, which probably may be the most important allergic disorder, affects about 300 million people in the world and causes an estimated 250,000 deaths annually. This illness is characterized by a reversible lower airway inflammation, airway hyperresponsivenss, mucus hypersecretion, leukocyte recruitment to lung tissue and airway remodeling that might cause respiratory deficits. Increased prevalence and difficulties in asthma control are responsible for the elevated costs to health systems around the world (Busse & Lemanski, 2001; Mayr et al., 2003; Bateman et al., 2008).

During asthmatic crises, patients develop an intense breathing difficulty called airway hyperreactivity (AHR). This response occurs as a consequence of the exposure of the inhaled route to the environmental allergen, thereby increasing the respiratory pause. An array of inflammatory mediators such as histamine, *cis*LT, PGs, cytokines, chemokines and others present in the lung tissue elicit smooth muscle cell contraction, mucus production, lung inflammation and airway remodeling. Mucus hypersecretion and bronchiole obstructions are important features of asthmatic patients. These effects of the inflammatory mediators may worsen the respiratory functions. In the last decade studies have shown the involvement of some mediators in stimulating the mucus production by cells named globlet cells. Concomitant to lung enhanced respiratory pause and lung obstruction, recruitment of inflammatory leukocytes to bronchoalveolar space initiates a cellular response that might become a destructive response to the lung tissue architecture in a chronic phase of the disease. Different leukocytes participate in the inflammatory process in the lung, i.e., neutrophils, mononuclear cells and mainly eosinophil cells (Cowden et al., 2010). Similar cellular and molecular immunological mechanisms related to asthma are described in allergic rhinitis demonstrating a strong correlation between these two allergic disorders with the same etiology. Rhinitis is an upper airway allergic inflammation and is considered co-morbidity to asthma as several studies have suggested that upper and lower airway inflammations are a unique entity. The prevalence of this disease has been increasing in many countries and an association between asthma and allergic rhinitis has been shown. Although asthma and allergic rhinitis show certain particularities, both present similar pathophysiology with IgE-dependent allergic reactions. Another severe IgE-dependent

allergic reaction is the anaphylactic shock triggered by allergens such as bee venom, domiciliary dust, cockroaches, food, pollen, and/or medicines after mast cell sensitization and activation (Bateman et al., 2008). The term 'anaphylaxis' was used for the first time by Richet and Portier (1902) to describe a potentially fatal reaction that may affect different organs and systems and the process by which all the symptoms derived from pharmacologic mediators, i.e. histamine, are released by blood leukocytes as eosinophils and basophils. Mortality rate of anaphylaxis have been increasing around the world in the last decades and at least twenty people die every year in UK due to anaphylactic reactions representing one death in three million habitants a year. Although the majority of the cases of anaphylactic reactions are related to high levels of IgE and histamine, some patients do not present these serum biologic markers, suggesting in these cases an IgE-independent mechanisms (Pumphrey, 2004; Moneret-Vautrin & Mertes, 2010; Seidel et al., 2010).

## 2. Immune mechanisms for the initiation of allergic reactions

The allergen sensitization phase is related to multiple factors including gene polymorphisms of HLA, FcεRI-β and IL-4 family, environmental factors like vaccination for prevention of diseases, pollutants present indoors and outdoors and viral infections. IgE is a critical participant in the onset of the effector phase of allergies due to its affinity to receptors (FcεRI) present on the surface of mast cells, basophils and/or eosinophils. The cross-linking between two IgE molecules and the allergen is responsible for cellular activation and subsequent release of preformed mediators, i.e., histamine from the cytoplasmic granules as well as neo-formed mediators such as eicosanoids (leukotrienes, prostaglandins and thromboxane) (Maddox & Schwartz, 2002). These mediators increase vascular permeability, induce smooth muscle contraction causing difficulty in breathing as well as the proliferation of fibroblasts and smooth muscle cells (Kanaoka & Boyce, 2004). The IgE production by allergen-specific B cells is associated with Th2 cell profile with IL-4, IL-5 and IL-13 productions. The IL-4 and IL-13 induce in B cells the production of allegen-specific- IgE (Munitz et al., 2008) and IL-5 induces the production, activation and differentiation of bone marrow-derived eosinophil (Takatsu & Nakajima, 2008). Among the cell types mentioned above, mast cells are of fundamental importance in the first phase of allergic disease due to their wide distribution throughout the body including skin, lungs and gastrointestinal tract (Maurer & Metz, 2005). Recently, some scientific work showed that mast cells are able to migrate to the smooth muscles of the airways of asthma patients, corroborating the interaction between mediators released by mast cells and smooth muscle response in the asthmatic lung (Brightling et al., 2003).

Another important cell population in the pathophysiology of asthma is the eosinophil. This cell participates in the late phase of the inflammatory response and it was initially described as a component of defense against intestinal parasites (Gleich et al., 1993; Weller, 1997; Rothenberg, 1998). However, there are several lines of evidence that contradicts this view, and demonstrate the eosinophils as multifunctional cells involved in the initial processes and propagation of various inflammatory diseases. They are also involved in the regulation of innate and adaptive immune responses (Rothenberger & Hogan, 2006). Eosinophils can respond to different stimuli as nonspecific tissue injury, viral infections, allograft, allergens, and tumors. Additionally, these cells release cationic proteins stored in granules as eosinophil peroxidase (EPO), major basic protein (MBP), eosinophil cationic protein (ECP) and eosinophil derived neurotoxin (EDN). Eosinophils also release a range of cytokines including the Th2

profile as well as chemokines RANTES, eotaxin 1 and MIP-1α (Rothenberger & Hogan, 2006). Eosinophils participate in many pathological processes such as parasite infections, gastrointestinal disorders and allergic processes such as asthma. Several studies have revealed the presence of high levels of MBP in bronchoalveolar lavage (BAL) of asthmatic patients that induces cytotoxicity to various body tissues, especially the airway epithelium (Rothenberg, 1998). Additionally, MBP increases the reactivity of airway smooth muscle to cause dysfunction of the muscarinic M2 vagal nerve, known to contribute to the development of airway hyperreactivity, a key feature of asthma (Jacoby et al., 1993). Several studies have demonstrated that eosinophil activation in inflammatory reactions is associated with increasing number of lipid bodies (LBs) (Bozza et al., 2011). The LBs are defined as cytoplasmatic organelles rich in lipids, surrounded by a phospholipid monolayer, posses high amounts of enzymes that produce eicosanoids such as PLA2, 5-LO, 15-LO, COX, $LTC_4$ and PGE synthases and also cytokines, chemokines and several kinases related with signal transductions. However LBs are found in small quantities in non-activated cells, they are associated with a wild range of pathological conditions such as cancers, infectious and inflammatory diseases like asthma (Bozza et al., 2009). Several inflammatory mediators are able to induce the leukocyte LB formation as platelet activator factor (PAF) (De Assis et al., 2003). In eosinophils other stimuli such as prostaglandin D2 ($PGD_2$) (Mesquita-Santos et al., 2006), IL-5 (Bozza et al., 1998), RANTES and eotaxin also induced the LB formation (Vieira-de-Abreu et al., 2005). In addition, in the allergic inflammation, the new LBs are observed and this process is mediated mainly by a *cross-talk* between eotaxin/RANTES via chemokine receptors (CCR3) with MAPK, PI3K and tyrosine kinases activation and PGD2 via an unknown receptor. The main site of *cis*LT generation in eosinophils is the LBs in the pulmonary allergic inflammation (Bozza et al., 2009). Moreover, the regulation of allergic reactions is carried out by T cells called Th1 cells (Teixeira et al., 2005), which secret cytokines such as IL-2 and INF-γ, and by the Th17 cells that produce IL-17. Both profiles can reduce the eosinophil onset in the lung and the bronchial hyperreactivity (Schnyder-Candrian, et al. 2006) (Figure 1).

## 3. Conventional treatment of allergic diseases

A wide variety of medicines are used to treat allergic diseases. The β2-adrenergic agonist therapy is widely used as first choice for addressing the crisis of asthma (O'Byrne, 2009). Phenoterol and salbutamol are members of this group and are largely used to reverse bronchoconstriction by binding directly to β2-receptors of lung smooth muscle cells and inducing breathing relieve due to bronchodilatation. *In vitro* studies showed these drugs are responsible for increasing the levels of cAMP described as a regulatory second messenger of intracellular calcium-dependent mechanisms. Calcium is one of several molecules responsible for the smooth muscle contraction during acute phase of asthma crises (Mahn et al., 2010). Of note, side effects are observed in patients under β2-agonists therapy, mainly cardiac frequency increases in response to activation of cardiac β1-receptors. Potent anti-inflammatory steroid therapy is also used to control asthma manifestations. These medicines are indicated to block lung inflammation mediated by inflammatory leukocytes such as neutrophils, eosinophils, basophils and lymphocytes that contribute to exacerbation of inflammatory response (Jarjour et al., 2006). As consequence of inflammation, lung tissue might present a cell phenotype change, referred to as 'remodeling' which impairs the physiological lung function causing respiratory deficiency and death in some of asthmatic patients.

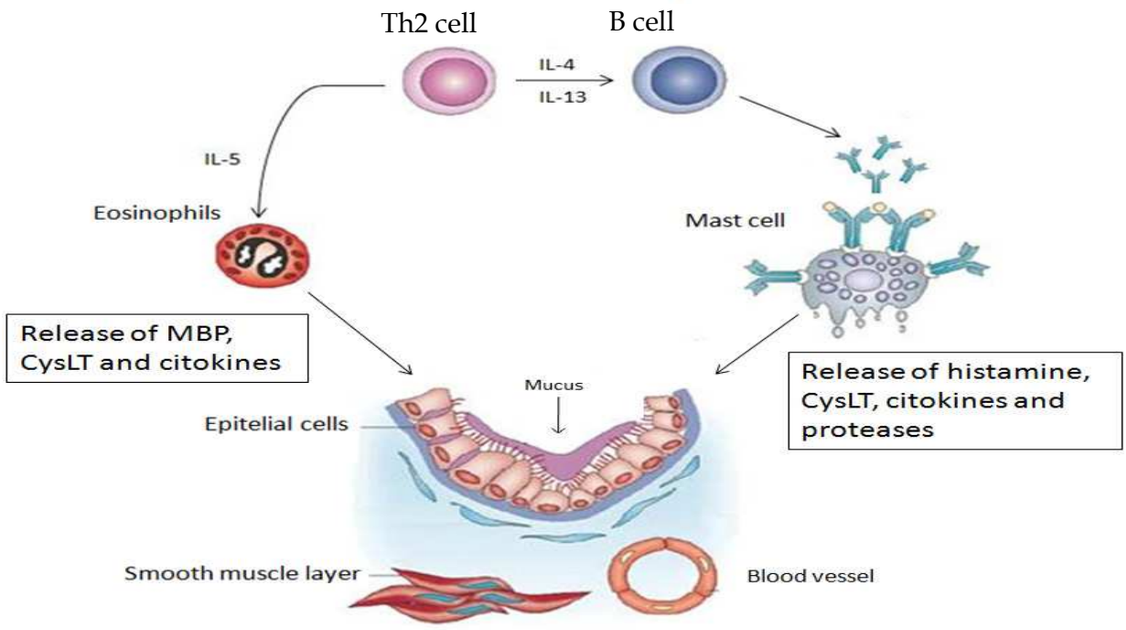



Fig. 1. Mechanism of immediate allergic reactions. Immediate allergic reactions are orchestrated by Th2 lymphocytes and its major cytokines (IL-4, IL-5 and IL-13) responsible for inducing B cell activation and IgE secretion. Mast cell sensitization depends on IgE cross-linking and binding with FcεRI, subsequent cellular activation, histamine and leukotrienes releases that are crucial to bronchospasm induction. Concomitantly, eosinophils migrate to bronchoalveolar cavity by an IL-5 (and others) dependent mechanism. Lung damage and airway remodeling are caused by cationic proteins delivered by eosinophils and matrix extracellular protein deposition.

Steroidal anti-inflammatory drugs are able to control these pathologic responses in airways by inhibition of lymphocyte functions and mainly inducing apoptosis in eosinophils, which is considered the major component of lung tissue damage. Steroids also can induce a variety of side effects like endocrine alterations, cardiovascular disturbances, psychotic crises and cancers (Belvisi, 2004). Combinations of β2-agonists and steroids are commonly used to control asthma. Another class of antiasthmatic drug that was developed about 10 years ago, the antileukotrienes (Montelukast® and derivatives). These drugs present both bronchodilator and anti-inflammatory properties. *cis*LT are known pro-inflammatory mediators and they induce vascular permeability, lung smooth muscle cell contraction/bronchospasm and leukocyte activation and chemotaxis (Funk, 2001). Previous studies reported *cis*-LT as the major bronchoconstrictor mediators in an asthmatic lung, causing sustained smooth muscle contraction. The anti-leukotriene drugs block leukotriene receptors in lung tissue, reversing bronchospasm in asthmatic patients. Additionally, leukotriene modifiers like zileutron act by blocking the 5-lipooxygenase enzyme (5-LO), thus inhibiting the leukotriene generation. Therefore, anti-leukotriene therapies strongly impair airway inflammatory response and ameliorate respiratory function (Terashima, et al. 2002; Angelova-Fischer & Tsankov, 2005). Antagonists of the enzyme phosphodiesterase, aminophylline and theophylline as well as muscarinic blockers also occupy space in the therapeutic arsenal.

### 3.1 Immunotherapy of asthma

Immunotherapy with anti-IgE has also contributed to the treatment of asthma patients who do not have a good response to conventional therapies (Lazaar & Panettieri, 2004; Foster et al., 2011). Despite anti-IgE therapy, which represents a major breakthrough in the treatment of asthma, the high cost of this therapy remains the major obstacle.

## 4. Botanical and pharmacological study with *Cissampelos sympodialis*

In northeastern Brazil, diseases such as asthma, influenza, bronchitis and rheumatism are traditionally treated with infusions of the root bark of *Cissampelos sympodialis* Eichl (Menispemaceae), popularly known in the region as milona, abuteira or orelha de onça (Correa, 1984). The Menispermaceae family was described by AL Jussieu (1789). This term is an allusion to the morphology of the seed that looks like the fourth form of the moon. This species belongs to the order Ranunculales, subdivided by Diels (1910) into eight tribes, three subtribes, 72 genera and approximately 400 species. These species are found on all continents, especially in tropical and subtropical regions. In Brazil, the Menispermaceae family is represented by 12 genera and 106 species distributed mostly in the Amazon forest (Barroso, 2004).

The genus *Cissampelos* belongs to the tribe Cocculeae, and subtribe Cissampelinae and comprises 19 species of which nine occur in Brazil (Rhodes, 1975). This genus is one of the few among the angiosperms that shows diversity and uniformity. The diversity can be seen in vegetative habitat and leaves. The uniformity is found in the sexual expression of simple flowers, pistils and small flowers. In the state of Paraiba the genus *Cissampelos* is represented by three species: *Cissampelos ovalifolia* DC, *Cissampelos glaberrima* St. Hill and *Cissampelos sympodialis* Eichl. These species are found in different types of habitat, soil and vegetation, occurring mainly in rainforests on the Atlantic coast and hills (Barbosa-Filho et al., 1997). The species *Cissampelos sympodialis* is endemic in Brazil and is found in the Northeast and Southeast, from Ceara to Minas Gerais states. This species often occurs in open areas as shrubs in sandy soil and can be distinguished mainly by the shape of the deltoid leaves (Barbosa-Filho et al., 1997). The roots of *Cissampelos sympodialis* are widely used by Indian tribes and in folk medicine to treat various diseases such as diarrhea, diseases of the genitourinary tract and especially in respiratory tract diseases such as asthma (Corrêa, 1984). Both alcoholic fraction of roots (AFR) and of leaves (AFL) and some of the chemical components (bisbenzylisoquinolinic type alkaloids) isolated from these extracts have been studied. These alkaloids have been shown to have paralytic effect, cytotoxic activity (Kupchan et al., 1965), to stimulate the central nervous system (Sur & Pradhan, 1964), to prevent hypersecretion of reactive products from neutrophils and macrophages (Castranova et al., 1991), to inhibit the inflammatory cytokine production by peripheral blood mononuclear cells (Onai et al. , 1995) and bronchodilator activity (Thomas et al., 1995). Thomas et al (1995) showed that the AFR had a relaxing effect on smooth muscle of trachea and increased the cyclic adenosine monophosphate (cAMP) levels from alveolar leukocytes in guinea pigs in a manner similar to aminophylline which antagonizes bronchial muscle contractions. Similarly, studies of AFL showed inhibition of histamine and ovalbumin (OVA)-induced bronchospasm in guinea pigs (Thomas et al., 1997a), synthesis of phosphodiesterase (PDE) IV and V in the lungs of mice and induced increased levels of cAMP in guinea pig trachea muscle cells (Thomas et al. 1997b). Also AFL had an antidepressant effect probably associated with the phosphodiesterases inhibition in rat brain

(Almeida et al., 1998), inhibited human neutrophils degranulation (Thomas et al., 1999) and induced contraction of vascular smooth muscle (Freitas et al., 2000).

## 5. Phytochemical study of *Cissampelos sympodialis*

Chemical studies of *Cissampelos sympodialis* led to the isolation of different alkaloids (Barbosa-Filho et al., 1997) such as bisbenzylisoquinolinic (warifteine, methilwarifteine, roraimine and simpodialine); morfinic (milonin); aporfinic (laurifolin) and oxoaporfinic (liriodenine) which have allowed for a more accurate immunopharmacological studies (Freitas et al., 1996, De Lira et al., 2002) (Table 1). Analysis of quality control of *Cissampelos sympodialis* extracts by thermogravimetry test showed that both AFL and AFR present alkaloids as major compounds and also both extracts showed the same kinetic behavior of bisbenzylisoquinolinic alkaloids (Aragão et al., 2002). Among these alkaloids warifteine showed spasmolytic activity by modifying various regulatory processes involving intracellular calcium channels and cAMP levels, which are essential for muscle contraction (Somlyo & Somlyo, 1994; Freitas et al., 1996). Therefore the purpose of our scientific study has been to develop a herbal medicine from the leaf extract of *Cissampelos sympodialis* to treat asthma as an alternative therapy.

## 6. Current stage of knowledge of *Cissampelos sympodialis*

### 6.1 Immunological study of *Cissampelos sympodialis*

Since the relaxant effect of *Cissampelos sympodialis* extracts (roots and leaves) on bronchial smooth muscle cells (Thomas et al. 1995), inhibition of phosphodiesterases (PDE) IV and V in the lung with increased levels of cAMP in muscle cells of the trachea (Thomas et al. 1997b), biological effects that corroborate with the anti-asthmatic activity of the plant were demonstrated, we began the immunological studies. Our research group, with laboratory complex located in the Laboratory of Pharmaceutical Technology (LTF), Federal University of Paraíba (UFPB), and in collaboration with Federal University of Rio de Janeiro and the Oswaldo Cruz Foundation/Rio, Brazil, has systematically studied the immunomodulatory effect of *Cissampelos sympodialis* since 1997. *In vivo* and *in vitro* tests have been conducted to understand the mechanisms of action of AFL as well as the isolated alkaloid warifteine in experimental models of allergy and inflammation.

### 6.2 Toxicological study of *Cissampelos sympodialis*

Several parameters can be analyzed to demonstrate the toxic potential of a plant (extracts or compounds) such as loss of weight, death, anorexia, and change of behavior. Therefore toxicological studies showed that the use of AFL in acute treatment was considered nontoxic with no deaths among rats after administration at dose of 5 g/kg orally (po) or of 2 g/kg intraperitoneally (ip) (Diniz et al., 2004). However AFL chronic treatment caused an anorexic effect in female rats and behavioral changes (Almeida et al., 2005).

The alkaloids warifteine and milonine isolated from *C. sympodialis* showed cytotoxicity in fibroblast cell line (V79) derived from hamster and in hepatocytes of Wistar rats (Melo et al., 2003). Given the mixed results of acute and chronic treatments in rats, our research group began studying the effect of chronic oral treatment (more than 15 days) with AFL into inbred BALB/c mice. We observed that this treatment induced weight gain throughout the treatment, suggesting lack of toxicity in these experimental animals (Bezerra-Santos et al., 2004).

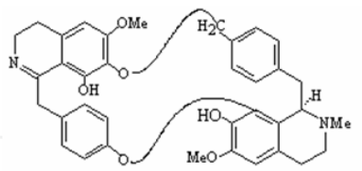
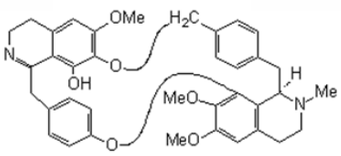
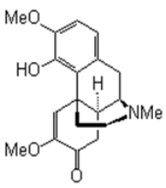
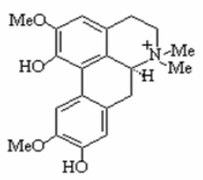
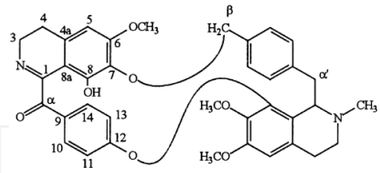
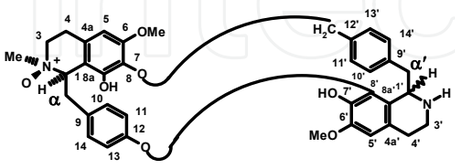
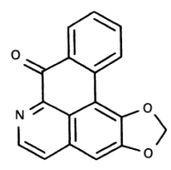
| Structure | Compound name | References |
|---|---|---|
|  | Warifteine | Barbosa et al., 1997 |
|  | Methilwarifteine | Barbosa et al., 1997 |
|  | Milonine | Barbosa et al., 1997 |
|  | Laurifoline | Alencar, 1994 |
|  | Roraimine | De Lira et al., 2002 |
|  | Simpodialine β-*N*-oxide | Alencar, 1994 |
|  | Liriodenine | De Lira et al., 2001 |

Table 1. Alkaloids of *Cissampelos sympodialis* Eichl.

### 6.3 Anti-inflammatory activity of *Cissampelos sympodialis*

The inflammatory process is a complex program of intracellular signal transduction and transcription events driven by multiple pro-inflammatory mediators and cytokines (Sherwood & Toliver-Kinsky, 2004). The acute inflammation is characterized by exudation of protein-rich fluid, edema, vasodilation and cell migration, primarily of neutrophils, into the site of injury (Sherwood & Toliver-Kinsky, 2004). Investigations on the anti-inflammatory activity of AFL were performed in experimental models of acute inflammation using phlogistic agents in Swiss mice or rats. Prophylactic treatments (before the phlogistic administration) demonstrated an AFL inhibitory effect on the ear edema formation induced by either TPA (12-O-tetradecanoyl phorbol-13-acetate) or capsaicin in Swiss mice (Batista-Lima et al., 2001). The experimental model of edema induced by TPA involves the activation of phospholipase A2 and production of prostaglandins and leukotrienes while the edema induced by capsaicin involves the release of substance P, histamine and eicosanoids such as serotonin and prostaglandins. These mediators are produced and released mainly by inflammatory cells such as mast cells, basophils, eosinophils and macrophages (Funk, 2001). Based on the anti-inflammatory effect of AFL, we inferred that the plant acts on the inflammatory cells by modulating the production of mediators. Corroborating this hypothesis was the observation that prophylactic treatment of experimental animals (rats) with AFL also showed inhibition of neutrophil migration into the intraperitoneal cavity induced by carrageenan (Batista-Lima et al., 2001). The migration of neutrophils into the peritoneal cavity of rats induced by carrageenan is dependent on the release of eicosanoids and chemotactic agents such as leukotriene B4 and/or IL-8, respectively, produced by mast cells and/or resident macrophages (Lefebvre et al., 2010, Nakagome & Nagata, 2011). Taken together the results support the hypothesis that AFL treatment is modulating cytokines as well as antiinflammatory mediator effects.

## 7. Immunomodulatory activity of *Cissampelos sympodialis*

### 7.1 Effect of *Cissampelos sympodialis* on IL-10 and NO production

Although eicosanoids and chemotactic agents produced by inflammatory cells are responsible for triggering the inflammatory process, these cells are also responsible for producing cytokines which control inflammation. IL-10 produced by mononuclear cells has been described as a potent regulatory molecule in the inflammatory process (Moore et al., 2001). Surprisingly, *in vitro* studies showed, for the first time, that the inhibitory effect of AFL on the proliferative response of BALB/c mice spleen cells stimulated with the mitogen concanavalin A was associated with the production of IL-4 and IL-10 by these cells (Piuvezam et al., 1999). Macrophages are cells that produce IL-10 and from this perspective, we investigated the effect of AFL on murine resident and elicited (sodium thioglycollate) macrophages. The experimental model used for this purpose was the infection of macrophages with trypomastigote form of *Trypanosoma cruzi*. The AFL treatment induced an increase in the release of trypomastigote forms by the cells with increase in IL-10 production. This phenomenon was shown in both types of macrophages (resident or elicited). The AFL was also able to increase the production of IL-10 even in the absence of the parasite. In addition, AFL inhibited the NO synthesis induced by interferon-gamma (IFN-γ) and lipopolysaccharide (Ding, et al., 1988; Alexandre-Moreira et al., 2003). Therefore these results confirm the effect of AFL in modulating the microbicidal activity of macrophages by increasing the IL-10 production as well as inhibition of NO synthesis.

### 7.2 Effect of *Cissampelos sympodialis* on the immunoglobulin production

B cells are responsible for the production of immunoglobulins (Ig) after antigen recognition and activation (Snapper & Paul, 1987; Wong & Koh, 2000). Asthma reaction is an immediate-type reaction mediated mainly by IgE (Busse & Lemanski, 2001; Mayr, et al., 2003). The release of mediators associated with the inflammatory cells to the reaction site induces the clinical symptoms of asthma (Maddox & Schwartz, 2002) such as bronchocontraction, mucus production and the strangling sensation (Funk, 2001). Based on the asthma symptoms and the fact that Brazilian folk medicine has systematically used *Cissampelos sympodialis* to prevent asthma symptoms, several studies have been conducted using the experimental model of asthma to demonstrate the AFL effect. The strain of inbred BALB/c mice is hypersensitive to ovalbumin (OVA) with the production of OVA-specific IgE, pulmonary hyperactivity and mucus production after sensitization and challenge with OVA. The chronic oral treatment (15 days before OVA sensitization) with AFL inhibited the total OVA-specific IgE production and increased the production of IFN-γ by spleen cells of these mice (Bezerra-Santos et al., 2004). Alexandre-Moreira and co-workers (2003) demonstrated that AFL inhibited activated B cell function through an increase in intracellular cAMP levels. Several studies have identified cAMP as an antagonist of B cell proliferation induced by mitogens (Cohen & Rothstein, 1989). Also it was demonstrated that cAMP is a second messenger that plays an important role in the regulation of B cell apoptosis (Myklebust et al., 1999). In general, an increase in cAMP levels is associated with anti-inflammatory and immunosuppressive effects (Cohen & Rothstein, 1989; Wong & Koh, 2000; Torgersen et al., 2002). Finally, the finding that AFL inhibited immunoglobulin secretion suggests a therapeutic use for the *Cissampelos sympodialis* extract in conditions associated with up regulation of B cell function and enhanced immunoglobulin secretion such as allergic diseases as well as autoimmune disease.

### 7.3 Activity of *Cissampelos sympodialis* in anaphylactic shock reaction

Anaphylaxis is a severe allergic reaction and is often fatal. It is mediated by IgE antibodies, mast cells and their mediators such as histamine. Medicines, insect bites and certain foods can trigger anaphylactic shock in genetically predisposed individuals (Teo et al., 2009; Dybendal et al., 2003). To have a better understanding of the effect of AFL treatment in allergic reactions, we evaluated the therapeutic potential of the acute treatment (five days before sensitization) in experimental model of anaphylactic shock using ovalbumin (OVA) challenge. The results demonstrated that AFL treatment was able to inhibit up to 70% death of OVA-sensitized mice after 1 hour of the OVA challenge. However, the same treatment was not able to inhibit the anaphylactic shock induced by compound 48/80. These data show that the effect of the extract is dependent on mechanisms involving IgE production (Bezerra-Santos et al., 2005).

### 7.4 Eosinophil lipid body inhibition by *Cissampelos sympodialis*

LBs are specialized organelles in the synthesis and storage of arachidonic acid derivatives such as prostaglandins and leukotrienes, and are present in the cytoplasm of various leukocytes and activated eosinophils (Bozza & Viola, 2010). A single treatment with AFL inhibited the formation of lipid bodies in eosinophils from mice sensitized and challenged with OVA. These results suggest that the extract is capable of modulating the synthesis of

inflammatory mediators important in the chemotaxis of inflammatory cells to the lungs during asthma attacks, as well as contraction mediators that cause bronchospasm.

## 8. Warifteine, a bisbenzylisoquinoline alkaloid from *Cissampelos sympodialis*

Warifteine is a major bisbenzylisoquinolinic alkaloid found in AFR as well as AFL. The isolated compound is an amorphous yellow crystal and the chemical name is (R)-2,8,13,13a,14,15,16,25-Octahydro- 18,30- dimethoxy-14 -methyl -4,6:9,12:21,24 -trietheno-3H pyrido (3',2':14,15) (1,11) dioxacycloeicosino (2,3,4- ij) isoquinoline-5,19-diol with molecular weight of 592.68084 g/mol. Warifteine is insoluble in polar solvents but in acidic conditions becomes a water-soluble salt, allowing its *in vivo* and *in vitro* analysis without addition of other toxic solvents. Warifteine becomes an important compound marker for the extract standardization of the plant as well as a candidate for a phytomedicine (Cerqueira-Lima et al., 2010).

### 8.1 Warifteine inhibits the histamine release

Allergic reactions trigger organic changes according to body region affected as atopic dermatitis (skin), hay fever or rhinitis (upper respiratory tract), asthma (lower respiratory tract), food allergy (digestive tract), anaphylactic shock (systemic reaction) (Cavalher-Machado et al., 2004; Sicherer & Leung, 2011). All of these conditions are consequence of sensitized mast cell degranulation which releases several mediators (histamine, CisLT or prostaglandins) that cause smooth muscle contraction. Histamine is also of fundamental importance in triggering the allergic symptoms such as swelling (Baroody & Naclerio, 2000), itching (Davidson & Giesler, 2010), bronchospasm (Larsen, 2001) and anaphylactic shock (Valent et al., 2011). Warifteine effect in mast cell degranulation was then investigated. Initial findings came from *in vitro* assays which showed that warifteine was able to relax smooth muscle independently of endothelium, i.e., it did not only control the tone muscle in vessels but also relaxed the bronchioles muscles (Freitas et al., 1996). Warifteine then becomes an important tool in attempting to prevent or reverse the respiratory distress occurring during asthmatic attacks (Priel et al., 1994). To evaluate the alkaloid activity on mast cell degranulation we used several experimental models. At first OVA-sensitized mice were orally treated with warifteine then OVA-challenged in their paws. An inhibition of edema formation was observed (Costa et al., 2008). Passively IgE anti-DNP/BSA-sensitized-paw of rats were treated with warifteine and challenged with DNP/BSA and the results demonstrated that the treatment inhibited the hyperalgesia reaction, showing modulation among mast cells, vessels and nerves. Mimicking a local allergic reaction like a bee sting, the intra dermal administration of the secretagogue compound 48/80 in mice induces mast cell degranulation with histamine release and consequently induction of scratching behavior (Inagaki et al., 2002). We demonstrated that warifteine treatment inhibited the itching, indicating a direct effect in mast cell degranulation (Costa et al., 2008). Mast cells from dorsal subcutaneous tissue and peritonea from OVA sensitized rats were cultured with warifteine and after OVA challenge the histamine release was measured. The warifteine inhibited significantly the histamine release from tissue and peritoneal mast cells in a similar manner to sodium cromoglycate (CGS) (Costa et al., 2008). These data indicate that warifteine is inhibiting the mast cell degranulation and histamine release.

### 8.2 Warifteine inhibits the B cell functions

Several models have been employed for analyzing B cell response *in vitro*. Anti-IgM antibodies (Ab) have been used as a model for studying signals induced by binding to B cell surface Ig (Mond et al., 1995) and also T-independent type 2 antigens (TI-2), which activate B cells through a broad cross-linking of their Toll-like receptors (TLR) (Vos et al., 2000; Peng, 2005). Warifteine was then analyzed on B cells. It was observed that warifteine inhibited both B cell proliferation and Ig secretion induced by TLR ligands (LPS, Pam3Cys and CpG oligodeoxynucleotide) or anti-IgM Ab. These effects were not due to a toxicity since warifteine neither induced alteration in propidium iodide labeling of fresh spleen B cells or modified XTT metabolization by the B cell line A20. Also the inhibitory effect of B cell activated with TLR activators or anti-IgM Ab did not modify the total protein phosphorylation pattern, however it attenuated the rise in intracellular calcium levels, the phosphorylation of mitogen-activated protein kinase (MAPK) ERK and the intracellular levels of transcription factor NFκB. Warifteine also increased the cAMP level. *In vivo* study showed that pre-treatment with warifteine inhibited the anti-TNP-ficoll titles in BALB/c mice immunized with TI-2 antigen TNP-ficoll (Rocha et al., 2010). Taken together, the data showed that the alkaloid present in the AFL of *Cissampelos sympodialis* is one of the compounds responsible for the B cell modulatory effect.

### 8.3 Warifteine inhibits the eosinophil activity

A characteristic feature of asthma is a chronic inflammation with degeneration of bronchial epithelium in an eosinophil-dependent mechanism. Eosinophils release cationic proteins, chemotactic agents (eotaxin) and eicosanoids (*cis*-LT) (Ono et al., 2008). The treatments with warifteine or AFL inhibited eosinophil migration into the pleural and bronchoalveolar cavities of OVA sensitized BALB/c mice. Both warifteine and AFL were also capable of inhibiting the secretion of *cis*-LT and eotaxin, suggesting a role for AFL and its alkaloid in controlling the inflammatory process, thus corroborating the belief of an alternative treatment for diseases associated with eosinophil activity.

## 9. Cellular and molecular therapeutic targets for *Cissampelos sympodialis*

Studies performed for 15 years have contributed to the unraveling of part of the immunopharmacological mechanisms involved in *Cissampelos sympodialis* and warifteine effects. Figure 2 presents different cellular and molecular therapeutic targets for the plant extract and its alkaloid.

## 10. Relevance of the proposal for new herbal medicine

Some allergic diseases of major public health concerns are classified as immediate-type hypersensitivity, atopic dermatitis, food allergy, rhinitis, allergic asthma and anaphylactic shock. The incidence of allergic asthma is increasing at an alarming rate in developing countries like Brazil where around 35% of the population experience allergic diseases including asthma (Brazilian Association of Allergy and Immunopathology. 2007). Asthma is considered a public health problem. A significant variety of medicines such as bronchodilators and potent anti-inflammatory drugs that mitigate the crisis is used to treat asthma but with undesirable side effects. Our research group, with multidisciplinary profile, has been studying in a systematic way, the plant extracts of *Cissampelos sympodialis* and its components on

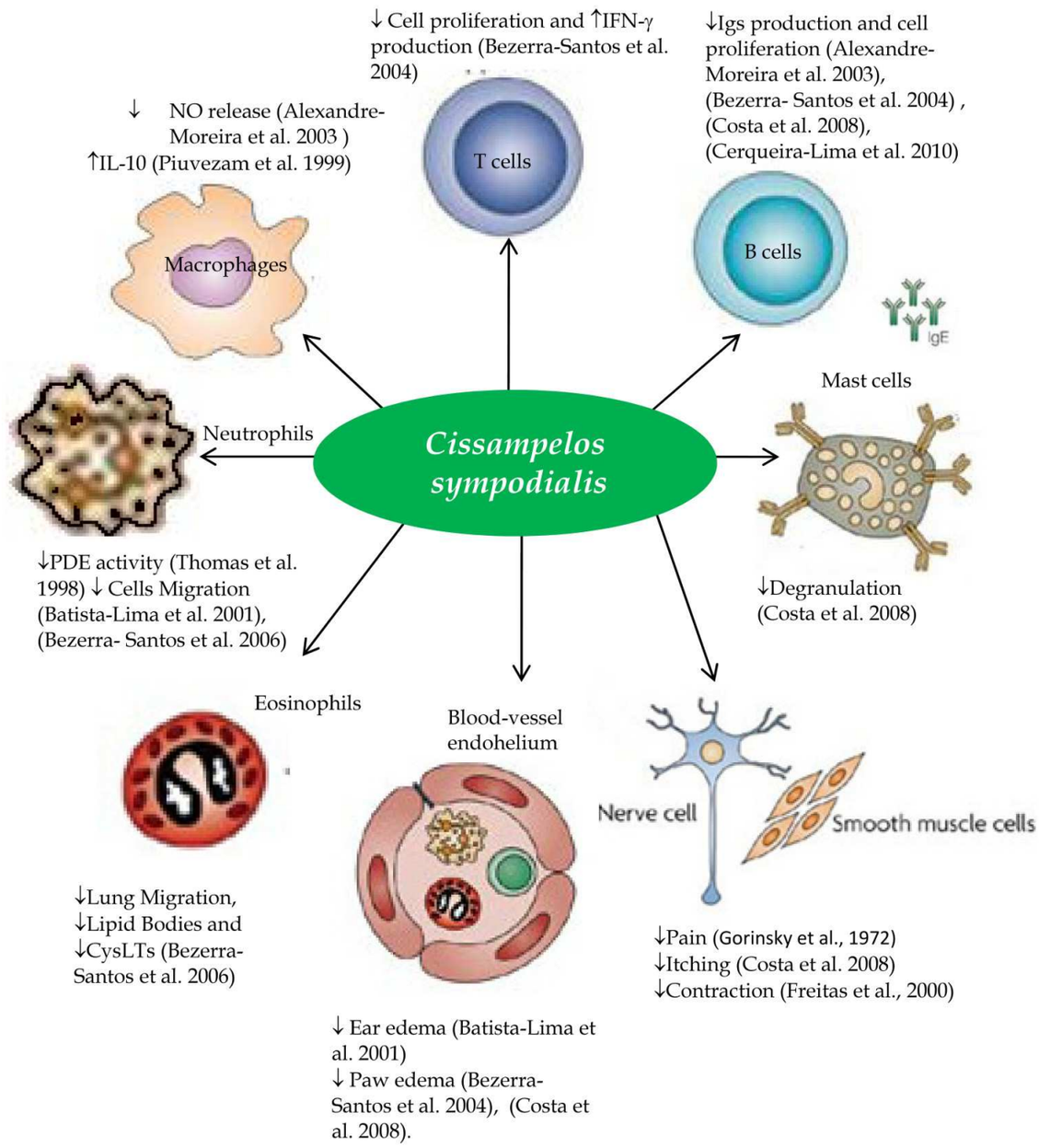



Fig. 2. Therapeutic targets for *Cissampelos sympodialis*. *In vitro* and *in vivo* studies showed that *C. sympodialis* induces IL-10 production by macrophages and IFN-γ production by splenocytes from OVA-sensitized mice. Oral treatment with AFL and warifteine inhibited OVA-specific IgE serum titer and mononuclear cell proliferation. Also both AFL as well as warifteine inhibited neutrophil and eosinophil migration and activation (PDE activity, leukotriene generation and lipid body formation) to the pleura and bronchoalveolar cavity induced by flogistic stimulus or allergens. Additionally, warifteine inhibited histamine delivery by mast cell and attenuated hyperalgesic reaction in rats.

experimental models of inflammation and allergy. The accumulated data showed that the extracts and its major alkaloid, warifteine, present potent anti-inflammatory effects, prolong the time of onset of anaphylactic shock reaction with reduction in allergen-specific IgE production, inhibit the inflammatory cell recruitment to the airways, relax airway smooth muscle in guinea pigs as well as modulate the production and release of inflammatory mediators such as histamine and cytokines. In addition, the great similarity in chemical structure among the alkaloids warifteine and milonine of *Cissampelos sympodialis* with drugs traditionally used in therapy: tubocurarine (potent muscle relaxant) and codeine (analgesic, antitussive and narcotic) respectively, (Figure 3), justified the popular use of the plant to treat respiratory diseases and the effort to produce an herbal medicine from this Brazilian plant.

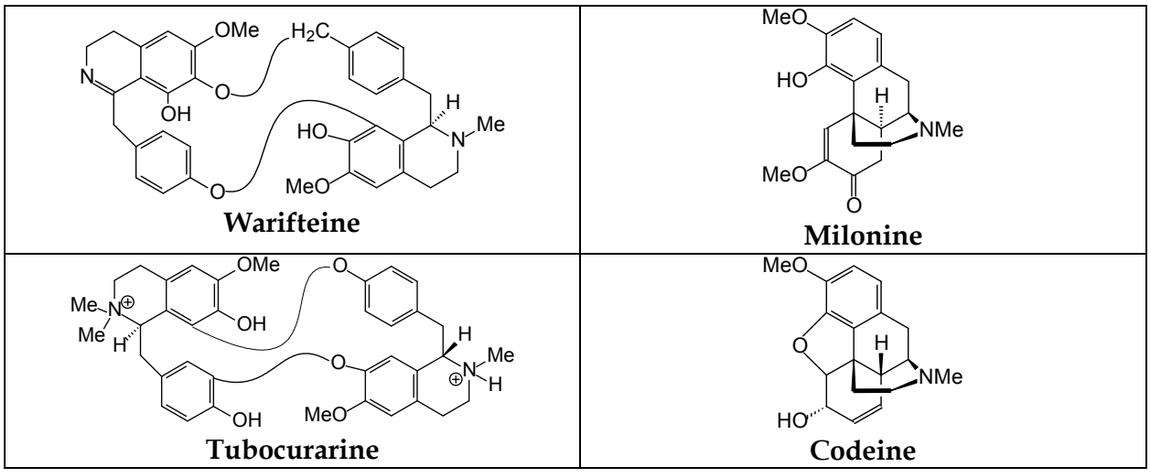



Fig. 3. Warifteine and tubocurarine are alkaloids that have the same chemical skeleton and belong to the class of bisbenzylisoquinoline. Milonine and codeine are alkaloids that have the same chemical skeleton and belong to the class of Morphinans.

## 11. Why *Cissampelos sympodialis* has potential as a herbal medicine?

1. *Cissampelos sympodialis* is used in folk medicine and by Indian tribes, in Northeast Brazil, for the treatment of disorders of airways such as asthma and rhinitis.
2. The preclinical data showed low or no toxicity on oral administration of the extract depending on the animal model used.
3. Studies of mechanisms of action of the extract have demonstrated efficacy in reduction of pathophysiological characteristics of allergic diseases associated with chronic inflammations such as asthma.
4. The leaf extract of the plant presents milonine, which is a morphinic alkaloid with a codeine-like chemical structure. Codeine is a classic drug with antitussive and analgesic properties.
5. Warifteine, one of the major alkaloids of the plant, presented similar effect of the extract in reducing asthma pathological profile.
6. Chemical structure of warifteine is similar to the tubocurarine chemical structure. Tubocurarine is a classic drug with muscle relaxant property.
7. Warifteine can be used as a molecular marker for the standardization of herbal medicine.

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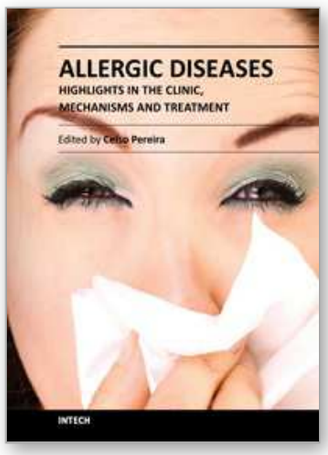


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The present Edition "Allergic diseases - highlights in the clinic, mechanisms and treatment" aims to present some recent aspects related to one of the most prevalent daily clinical expression disease. The effort of a group of outstanding experts from many countries reflects a set of scientific studies very promising for a better clinical care and also to the treatment and control of the allergy. This book provides a valuable reference text in several topics of the clinical allergy and basic issues related to the immune system response. The inflammatory reaction understanding in allergic disease is clearly evidenced, as well as new strategies for further researches.

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