

Cissampelos sympodialis Eichl. (Menispermaceae): oral treatment decreases IgE levels and induces a Th1-skewed cytokine production in ovalbumin-sensitized mice

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Abstract

The murine model of ovalbumin (OVA)-induced allergy was used to evaluate the effectiveness of oral treatment with the leaf extract of *Cissampelos sympodialis* Eichl. (Menispermaceae) (CS) in the modulation of immunoglobulin E (IgE) production and T cell activation. CS treatment with doses ranging from 200 to 600 mg/Kg/day for 15 days before and during OVA-sensitization promoted reduction in total and OVA-specific serum IgE. CS at 400 or 600 mg/Kg/day also reduced paw edema induced by local OVA challenge. Daily intake of up to 600 mg/Kg of oral CS by BALB/c mice did not reduce weight gain, which is indicative of a lack of systemic toxicity. To assess the effect of CS treatment on T cell proliferative response to stimuli in vitro, the mitogenic response of spleen cells of treated and control animals were evaluated. Cells from CS-treated animals showed an elevated background proliferative response to concanavalin-A (Con-A) when compared to those from control animals. Oral intake of CS increased the in vitro production of IFN- γ and IL-10 by Con-A stimulated cells. Mice treated with 200 mg/Kg/day CS showed increasing levels of IFN- γ . These results show that oral treatment with *Cissampelos sympodialis* extract has an immunomodulatory effect, reducing allergy-associated responses possibly by a preferential activation of Th1-type cytokines.

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1. Introduction

The plant genus *Cissampelos* (Menispermaceae) comprises 19 species, 9 of which can be found in Brazil (Rodhes,

1975). They are largely used in the indigenous and popular medicine to treat many illnesses including heart diseases, diarrhoea, genital and urinary diseases, colds and asthma. *Cissampelos sympodialis* Eichl., popularly known in Brazil as 'jarrinha', 'orelha de onça', 'abuteira' and 'milona' is used to treat asthma, bronchitis, influenza and rheumatism (Correa, 1984). It was shown to contain the tertiary alkaloids warifteine and methylwarifteine and the quaternary alkaloid laurifoline. The presence of alkaloids with a morphinan skeleton, such as milonie, roraimine (Barbosa-Filho et al., 1997) and warifteine have also been described, the later

Abbreviations: CS, *Cissampelos sympodialis*; OVA, ovalbumin; IgE, immunoglobulin E; Alum, aluminum hydroxide; p.o., oral treatment; i.p., intraperitoneal; Con-A, concanavalin-A; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide

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with spasmolytic action (Cortes et al., 1995; Freitas et al., 1996).

Other pharmacological studies on *Cissampelos sympodialis* have shown that the aqueous fraction of leaf and root bark ethanolic extracts promoted guinea-pig tracheal smooth muscle relaxation and increased intracellular cyclic adenosine monophosphate (cAMP) levels of cultured tracheal smooth muscle cells. In addition, the aqueous fraction of the ethanolic extract of the leaves of *Cissampelos sympodialis* (CS) inhibited both histamine-induced bronchospasm responses in ovalbumin (OVA)-sensitized guinea pigs and the activity of phosphodiesterases (PDE) IV and V (Thomas et al., 1997a,b). Studies using human peripheral blood neutrophils demonstrated that the CS inhibited degranulation of cells stimulated by formyl-Met.Phe.Pro, increased the levels of cAMP, and the activity of cAMP-dependent protein kinase A (PKA) (Thomas et al., 1999).

In vitro immunological studies showed that the CS increased the production of interleukin-10 (IL-10) and inhibited T cell proliferative response by mouse spleen cells stimulated with concanavalin-A (Piuvezam et al., 1999) and induced an IL-10-dependent inhibition of *Trypanosoma cruzi* killing by macrophages (Alexandre-Moreira et al., 2003). In vivo studies have demonstrated that intraperitoneal injection of the CS has an anti-inflammatory activity in the TPA (12-*O*-tetradecanoyl phorbol-13-acetate)-elicited mouse ear edema (Batista-Lima et al., 2001).

Taking together both the in vitro and the in vivo studies discussed above, it can be suggested that *Cissampelos sympodialis* has an interesting immunopharmacological activity. The above described data suggested that the plant produces substances that can modify the course of inflammatory diseases. However, it was not known yet whether the CS would be effective by the oral route, which is the form used in popular medicine. Also, we did not clarify whether CS could inhibit a chronic inflammatory response like asthma, which is the principal reason for the popular use of the extract. Asthma is now considered to be an airway disorder associated with mucosal inflammation (Hegele, 2000). Increased migration and activation of cells such as mast cells, eosinophils, T-lymphocytes and macrophages during this disease has been reported (Wills-Karp, 2000). IgE and cytokines such as IL-4, IL-5 and IL-13, are described to be responsible for the persistent chronic inflammation of the airways (Hogan et al., 1998; Wills-Karp et al., 1998). On the other hand, the up-regulation of IL-10 during allergic responses may help to control tissue inflammation that is characterized by leukocyte infiltration (Jae-Won et al., 2002; Asadullah et al., 2003).

To amplify our knowledge on the suitability of the use of *Cissampelos sympodialis* extract for the treatment of an allergic disease, we evaluated in this study the effect of oral treatment (p.o.) with CS in ovalbumin-sensitized mice. The immunological aspects investigated here were the production of ovalbumin-specific-antibody and the T cell cytokine secretion profile.

2. Materials and methods

2.1. Plant material and extract preparation

Leaves of *Cissampelos sympodialis* Eichl. (Menispermaceae) (a renewable source of the plant) 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, herein named CS, 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 average w/w yield was 22%, based on the solid residues present (Thomas et al., 1997b).

2.2. Animals

Male or female BALB/c mice (25–30 g) and Wistar rats (250–300 g) were used throughout the study. The animals were supplied by the Animal Facility of the Laboratório de Tecnologia Farmacêutica (Universidade Federal da Paraíba). Experimental animals were maintained with free access to water and food. Groups of 5–10 animals were used in each test. All experiments were carried out with strict adherence to ethical guidelines (Zimmerman, 1983).

2.3. Oral treatment with CS and sensitization with ovalbumin

Mice were allowed to drink water supplemented with CS to allow an estimated daily intake of 200, 400 or 600 mg/Kg for 40 days. The volume of CS solution consumed per animal per day calculated the CS doses. On days 0 and 14, the treated mice ($n = 10$) were actively immunized with OVA (grade II Sigma Chemical, St. Louis, MO) by intraperitoneal (i.p.) injection of 10 µg OVA adsorbed on 2.25 mg of aluminium hydroxide (alum). The control group (OVA) received only water during the immunization process (Oshiba et al., 1996). Three weeks after the first OVA injection, animals were exposed for 30 min to a 1% (w/v) aerosol of OVA in saline (Randolph et al., 1999).

2.4. Footpad swelling induced by OVA

Twenty-one days after initiation of OVA sensitization, CS-treated and control groups (OVA) of mice were challenged with 400 µg of OVA in 20 µl saline injected into the footpad. The measurement of the edema was performed with the aid of a gauge caliper (Mitutoyo) before and after (30 and 60 min) the OVA challenge (Facincone et al., 1997).

2.5. Cell preparation and proliferation assay

Spleen cell suspensions from each animal group were prepared and depleted of red blood cells by incubation in Boyle's solution (0.17 M Tris, 0.16 M ammonium chloride, BDH, UK). Cells were then washed twice in RPMI medium by centrifugation at $200 \times g$ for 10 min. The obtained pellet was resuspended in RPMI medium without phenol red and supplemented with 200 mM L-glutamine, 100 units/ml penicillin, 100 µg/ml streptomycin, 5 (M β-mercaptoethanol and 10% fetal calf serum (Gibco, Paisley, UK). Viable cell number was determined in a hemocytometer by exclusion of trypan blue. Triplicate cultures were performed in 96-well flat-bottomed tissue culture plates (Costar, Cambridge, MA, USA) in a final volume of 200 µl per well containing $4\text{--}5 \times 10^5$ cells. Concanavalin-A (Con-A) (Pharmacia, Uppsala, Sweden) was added at 2.5 µg/ml either in the absence or in the presence of 50 or 100 µg/ml of CS. Cultures were then incubated for 3 days at 37 °C in an atmosphere with 5% CO₂. A volume of 150 µl of the cell culture supernatants was removed and stored at –70 °C for cytokine measurement. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; Sigma Chem. Co., St. Louis, MO) was added and the cells were further incubated for 4 h at 37 °C, 5% CO₂ (Mosmann, 1983). After this time, a solution of SDS 10% in HCl 0.01N was added in each well and the plates were then incubated for 17 h at 37 °C, 5% CO₂ and read at 570 nm on a Titerk Multiskan plate reader.

2.6. Determination of OVA-specific IgE antibody by the PCA test

IgE antibody titers were determined using the passive cutaneous anaphylaxis (PCA) reaction. CS-treated (200, 400 or 600 mg/kg/day) and OVA group of mice were bled and the serum samples were diluted in PBS. Volumes of 50 µl were inoculated intradermally on the shaved backs of Wistar rats at marked sites. After 24 h, the rats were injected intravenously with OVA (2 mg/rat) in 0.5 ml of 1% Evans Blue (in PBS). Thirty minutes later, the rats were sacrificed, and OVA-specific IgE titers measured. The highest serum dilution giving a 5 mm diameter flare or bluing reaction was taken as the PCA titer (Holt et al., 1981).

2.7. Measurement of OVA-specific and total antibody IgE by ELISA

The serum samples from each animal group were used to determine the OVA-specific titers and total IgE levels by sandwich ELISA (Snaper and Paul, 1987; Kim et al., 1999). Plates (Corning, NY, EUA) were coated with 100 µg/ml of OVA diluted in PBS or 2 µg/ml of anti-IgE monoclonal antibody in coating buffer, respectively. Following overnight incubation at 4 °C, the plates were washed three times and blocked with 1% bovine serum albumin (BSA; Sigma Chem. Co., St. Louis, MO) for 2 h at 37 °C. Appropriate standards

and serum samples were serially diluted in PBS and the plates were washed and incubated with biotinylated anti-IgE monoclonal antibodies (Pharmigen, San Diego, CA) in PBS. After washing, wells were incubated with 1mg/ml of alkaline phosphatase-labeled avidin at room temperature for 2 h and washed three times. Then a solution of substrate (*p*-nitrophenylphosphate; Sigma Chem. Co., St. Louis, MO) was added to each well and incubated at room temperature. The plates were read at 405 nm. The calibration curve was obtained using purified IgE monoclonal antibody (obtained from Pharmigen, San Diego, CA).

2.8. Measurement of IL-10 and IFN-γ by ELISA

Spleen cells from each group of animals were plated at 5×10^5 cells per well. Con-A was added at 2.5 µg/ml. A volume of 150 µl of cell culture supernatant was removed after 3 days of culture and stored at –70 °C for cytokine assays. Cytokines (IL-10 and IFN-γ) were detected by capture ELISA (Schumacher et al., 1988). Flat-bottomed polystyrene plates (Dynatech, Alexandria, VA) were coated overnight at 4 °C with 50 µl of monoclonal anti-murine cytokine antibodies at previously determined optimal concentrations. The plates were then washed three times with PBS/Tween and were blocked for 60 min at 37 °C with a 10% FCS solution diluted in PBS. Culture supernatant (100 µl) and standards were added in duplicate to wells and incubated at 37 °C for 2 h. After washing three times, 100 µl of biotinylated anti-mouse cytokine monoclonal antibody was added to each well and incubated for 45 min at 37 °C. After washing as above, alkaline phosphatase–streptavidin conjugate (Pharmingen, San Diego, CA) diluted 1/2000 was added to each well and incubated for 30 min at 37 °C. Plates were washed three times as above and 100 µl of *p*-nitrophenylphosphate (1 mg/ml; Sigma, UK) in glycine buffer (0.1 M; pH 10.4) was added. Readings were performed at 405 nm on a Titerk Multiskan plate reader. Cytokine concentrations in the culture supernatants were determined from a standard curve using recombinant cytokines (regression coefficient, $r \geq 0.980$).

2.9. Statistical analyses

Data were analyzed statistically using a microcomputer program and the Student's *t*-test. Data were expressed as mean ± S.E.M. Results with $p < 0.05$ were considered statistically significant.

3. Results

3.1. Effect of CS oral treatment on body weight

Animals treated with either 200 or 400 mg/kg/day of the CS showed a significant increase in body weight during the experimental period, when compared to control mice ($p < 0.001$) (Fig. 1). A separate experiment showed that ani-

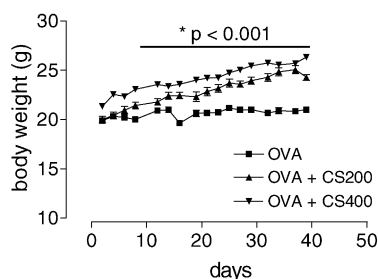


Fig. 1. Effects of *Cissampelos sympodialis* extract oral treatment on weight gain. OVA-sensitized mice ($n=10$) ingested CS at 200 mg/kg/day ($\blacktriangle$), 400 mg/kg/day ($\blacktriangledown$) or water alone ($\blacksquare$) for the periods of time indicated. Mean values $\pm$ S.E.M. * $p < 0.001$ as compared with time zero.

mals treated with 600 mg/kg/day showed weight gain similar to the control group (OVA) (data not shown). These data suggest that CS ingestion did not have a toxic effect.

3.2. Effect of CS on the footpad swelling induced by OVA injection

The effect of CS on active cutaneous anaphylaxis was tested. OVA-sensitized mice were treated with CS (200, 400, 600 mg/kg/day) and challenged with OVA in the footpad. Ingestion of CS at the doses of 400 and 600 mg/kg/day inhibited the induction of edema ($p < 0.001$) after 30 or 60 min of antigen challenge when compared with the edema developed in the OVA group (Table 1). Non-sensitized mice were used as controls and they did not present footpad edema on antigen challenge (data not shown).

3.3. Effect of CS on OVA-specific-IgE production

We next investigated the ability of CS to modify antigen-induced serum IgE production. Table 2 shows the CS-inhibitory effect on antigen-induced IgE production. OVA sensitization induced an OVA-specific-IgE titer of 1:1339 (100%), whereas oral administration of CS at 200, 400 or 600 mg/kg/day during the OVA sensitization period reduced the IgE production by 18%, 42% and 59%, respectively

Table 1

Measurement of footpad swelling (induced by OVA) in CS-treated and OVA-sensitized BALB/c mice

Period of time (min)	Δ Footpad diameter (mm)			
	Treatment			
	OVA	OVA + CS (mg/kg)		
		200	400	600
30	26	28	15*	1*
60	24	18	13*	8*

The footpad swelling is measured 30 and 60 min after the OVA challenge. Δ Footpad diameter values are calculated by subtracting normal control values from experimental group footpad diameter. The footpad diameter S.E.M. values are below 5%. Control (saline-treated group) values are as follows: 30 min—206 mm; 60 min—199 mm.

* $p < 0.001$ as compared with the saline treatment.

(Table 2). Similar but less striking results were obtained using the PCA assay (Table 2). We also observed a significant decrease in total IgE levels in the sera of all CS-treated mice (Table 2).

3.4. Effect of CS on T cell proliferative response

To investigate whether the prolonged CS treatment would modify the in vitro Con-A-induced T cell proliferation, mice were treated orally with the indicated doses of CS for 40 days and their spleen cells were tested in vitro for the induction of T cell proliferation. We observed that mice treated with doses of CS ranging from 200 to 600 mg/kg/day showed an increased background cell proliferative response that was not modified by incubation with Con-A (Table 3). The in vitro addition of CS to the cultures did not modify the background response of the cells.

3.5. Effect of CS on cytokine production

To demonstrate whether the CS treatment could modulate the pattern of cytokine production during OVA sensitization, ELISA assay analyzed supernatants from spleen cell cultures from each experimental group of mice. It was observed that Con A-stimulated spleen cells from OVA-sensitized animals treated with CS (200 or 600 mg/kg/day) secreted higher levels of IFN- γ when compared with the control group (OVA). The production of IL-10 was also increased, but it was not as high as the production of INF- γ (Table 4).

4. Discussion and conclusion

Earlier immunopharmacological studies demonstrated that the hydroalcoholic extracts from both roots and leaves inhibited the spontaneous tonus of the guinea pig tracheal smooth muscle by stimulating β -adrenoceptors (Thomas et al., 1995), histamine-induced bronchospasm in normal and antigen-induced anaphylactic responses in OVA-sensitized guinea pig (Thomas et al., 1997a) and phosphodiesterases (PDE) types IV and V from guinea pig lungs (Thomas et al., 1997b).

Since large-scale extraction of root components destroy the trees, extraction of the leaves alone would be preferable. Previous phytochemical studies of *Cissampelos sympodialis* showed similar chemical composition between root and leaf extracts (Thomas et al., 1995, 1997a,b). The extract of leaves alone (CS) showed strong immunomodulatory activity, inhibiting the spleen T cell proliferative response and inducing an increase in IL-10 production (Piuvezam et al., 1999). The CS was also shown to have anti-inflammatory activity when administered by the i.p. route, since it inhibited the in vivo induced inflammatory response (Batista-Lima et al., 2001).

All the studies described above were carried out in vitro or used an administration route different from that used popularly. Infusion of *Cissampelos sympodialis* leaves is nor-

Table 2

Antigen-specific and total IgE levels in *Cissampelos sympodialis* extract-treated OVA-sensitized mice

Treatments	Total serum IgE concentration ($\mu\text{g/ml}$)	OVA-specific IgE serum titer (%)	OVA-specific Ig serum titer (PCA) ^a
None	4.2	–	–
OVA	212	1:1339 (100)	1:256
OVA + CS 200	5.9*	1:1096* (18)	1:64*
OVA + CS 400	47	1:773* (42)	1:64*
OVA + CS 600	50*	1:545* (59)	1:64*

Mice ($n = 10$) are treated with different doses of CS (OVA + CS in mg/Kg/day) or water (OVA) and then sensitized with OVA. The control group is left untreated and unsensitized. Total serum IgE concentration and OVA-specific IgE titer are determined by ELISA assay. Data are representative of three independent experiments. (–): undetectable immunoglobulin.

^a PCA: passive cutaneous anaphylaxis.

* $p < 0.001$, as compared with the OVA group.

Table 3

Effect of *Cissampelos sympodialis* extract on the T cell proliferative response

Treatment	Medium	Con-A	Con-A + CS 50 $\mu\text{g/ml}$	Con-A + CS 100 $\mu\text{g/ml}$
OVA	0.152 $\pm$ 0.01	0.212 $\pm$ 0.04	0.162 $\pm$ 0.01	0.152 $\pm$ 0.00
OVA + CS 200	0.313 $\pm$ 0.01*	0.382 $\pm$ 0.01*	0.311 $\pm$ 0.01*	0.299 $\pm$ 0.00*
OVA + CS 400	0.261 $\pm$ 0.01*	0.248 $\pm$ 0.02 [#]	0.268 $\pm$ 0.02*	0.267 $\pm$ 0.01*
OVA + CS 600	0.148 $\pm$ 0.01	0.208 $\pm$ 0.02	0.223 $\pm$ 0.01*	0.212 $\pm$ 0.02*

OVA-sensitized mice are treated with the indicated doses of CS (mg/Kg/day). Their spleen cells are cultured in medium or stimulated with Con-A (2.5 $\mu\text{g/ml}$) alone or together with CS (50 or 100 $\mu\text{g/ml}$). The cell proliferation is measured after 3 days by the MTT test. Mean values of O.D. $\pm$ S.E.M.; ($n = 3$).

* $p < 0.001$, as compared with OVA group.

[#] $p < 0.01$, as compared with OVA group.

mally consumed in large oral doses and, a daily intake of about 15 leaves during chronic use (Melo-Diniz, 2000). To mimic the way the plant is popularly employed (Correa, 1984), we treated OVA-sensitized mice with the plant leaf extract by the oral route. We showed that orally treated animals gained weight, suggesting that the extract did not interfere with the normal growth of the animals. Pre-clinical toxicological studies (acute toxicity tests) showed that *Cissampelos sympodialis* extract ingestion at doses equivalent to those used in folk medicine did not produce any toxic effect (Melo-Diniz, 2000).

Our results showed that oral treatment with CS inhibited both total IgE and OVA-specific IgE production in sera from OVA-sensitized mice. Elevated levels of IgE are associated with immediate-type allergic reactions. In experimental models, the IgE production is dependent on the adjuvant used for immunization. It has been demonstrated that aluminium hydroxide is extremely effective in eliciting IgE antibody production in several animal species (Revoltella and Ovary, 1969). Our results suggest that oral CS administration may have anti-allergic activity through the decrease of IgE production. Our results validate the popular use of the CS for the treatment of asthma and indicate that a possible mechanism of its beneficial action is associated with a decrease in IgE production.

The role of IL-4 in regulating IgE production is well established, and Th2 cells, mast cells and NK 1.1 T cells have been described as the sources of IL-4 (Nakao, 2001; Alenius et al., 2002; Hameg et al., 1999). The inhibition of IgE production observed here could be, for instance, the result of an effect of the CS at the level of cytokine production. In the present study, we demonstrated that spleen cells from OVA-

sensitized mice also produced increased IFN- γ when treated with CS. Our previous results showed that spleen cells from normal BALB/c mice stimulated in vitro with CS and Con-A produced high levels of IL-10 (Piuevezam et al., 1999). Enhanced IL-10 production in acute inflammatory diseases represents a favorable prognosis due to its anti-inflammatory effect (Jae-Won et al., 2002), down-regulating the production of pro-inflammatory cytokines (i.e. TNF, IL-1, IL-6, IL-8) by macrophages/monocytes (De Wall Malefyt et al., 1991; Wang et al., 1994).

The increased IFN- γ in this experimental model may be associated with the lower IgE levels due to its role in down regulating the activation of Th2-type cells. It was previously shown that the induction of a Th2 T cell response depends on the balance between both IL-4 and INF- γ (Victoratos et al., 1997). We observed here that despite the moderate increase in IL-10 secretion, the increase in levels of IFN- γ is more pronounced, indicating a balance favoring a Th1-type response. It can be suggested that increased IFN- γ secretion would in-

Table 4

Effect of *Cissampelos sympodialis* extract on cytokine production

Treatments	Cytokines	
	INF- γ (ng/ml)	IL-10 (ng/ml)
OVA	0.30 $\pm$ 0.03	0.09 $\pm$ 0.01
OVA + CS 200	1.30 $\pm$ 0.35*	0.32 $\pm$ 0.08*
OVA + CS 600	1.00 $\pm$ 0.01*	0.26 $\pm$ 0.10 ^{&}

Mice are treated with CS and sensitized with OVA as for Table 3. Their spleen cells are then cultured with Con-A in vitro and the indicated cytokines quantified by ELISA assay. Mean values $\pm$ S.E.M. ($n = 3$).

* $p < 0.001$, as compared with the OVA group.

[&] $p < 0.01$, as compared with the OVA group.

terfere with the differentiation of Th2 cells, decreasing the production of IgE and hence the allergic reaction. IFN- γ also has an inhibitory effect on IgE secretion by B cells (Lack et al., 1996) and this may also be a mechanism to explain the decrease in IgE production.

The results presented here indicate for the first time that oral administration of the *Cissampelos sympodialis* extract is effective and safe in vivo and has immunomodulatory properties in actively sensitized mice. Oral CS treatment promoted decreased OVA-induced paw edema and IgE production, and it also increased the production of IFN- γ . These data highlight the important role of this plant in regulating murine IgE production and suggest its regulatory role in cytokine production in an IgE-dependent hypersensitivity model.

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References

- Alenius, H., Laouini, D., Woodward, A., Mizoguchi, E., Bhan, K., Castigli, E., Oettgen, H.C., Geha, R.S., 2002. Mast cells regulate IFN- γ expression in the skin and circulating IgE levels in allergen-induced skin inflammation. *Journal of Allergy and Clinical Immunology* 109, 106–113.
- Alexandre-Moreira, M.S., Freire de Lima, C.G., Trindade, M.N., Castro-Faria-Neto, H.C., Piuvezam, M.R., Peçanha, L.M.T., 2003. *Cissampelos sympodialis* Eichl. (Menispermaceae) leaf extract induces interleukin-10-dependent inhibition of *Trypanosoma cruzi* killing by macrophages. *Brazilian Journal of Medical and Biological Research* 36, 199–205.
- Asadullah, K., Sterry, W., Volk, H.D., 2003. Interleukin-10 therapy—review of a new approach. *Pharmacological Reviews* 55, 241–269.
- Barbosa-Filho, J.M., Agra, M.F., Thomas, G., 1997. Botanical, chemical and pharmacological investigation on *Cissampelos* species from Paraíba (Brazil). *Ciência e Cultura Journal* 49, 386–394.
- Batista-Lima, K.V., Ribeiro, R.A., Balestieri, F.M.P., Thomas, G., Piuvezam, M.R., 2001. Anti-inflammatory activity of *Cissampelos sympodialis* Eichl. (Menispermaceae) leaf extract. *Acta Farmaceutica Bonaerense* 20, 275–279.
- Correa, M.P., 1984. *Dicionário de Plantas Úteis do Brasil e Exóticas Cultivadas*. Ministério da Agricultura, Rio de Janeiro.
- Cortes, S.F., Alencar, J.L., Thomas, G., Barbosa-Filho, J.M., 1995. Spasmolytic actions of warifteine, a bisbenzylisoquinoline alkaloid isolated from the root bark of *Cissampelos sympodialis* Eichl. (Menispermaceae). *Phytoterapy Research* 9, 579–583.
- De Wall Malefyt, R., Abrams, J., Bennett, B., Figdor, C.G., De Vries, J.E., 1991. Interleukin-10 (IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 production by monocytes. *Journal of Experimental Medicine* 174, 1209–1220.
- Facincone, S., Pereira de Siquiera, A.L., Jancar, S., Russo, M., Marzagão Barbuto, M., Mariano, M., 1997. A novel murine model of late-phase reaction of immediate hypersensitivity. *Mediators of Inflammation* 6, 127–133.
- Freitas, M.R., Cortes, S.F., Thomas, G., Barbosa-Filho, J.M., 1996. Modification of calcium metabolism in rabbit aorta as a mechanism of spasmolytic action of warifteine, a bisbenzylisoquinoline alkaloid isolated from the leaves of *Cissampelos sympodialis* Eichl. *Journal of Pharmacy and Pharmacology* 48, 335–338.
- Hameg, A., Gouarin, C., Goubert, J., Hong, S., Van Kaer, L., Bach, J.F., Herbelin, A., 1999. IL-7 up-regulates IL-4 production by splenic NK1.1 and NK1.2 MHC class I-like/CD1-dependent CD41 T cells. *The Journal of Immunology* 162, 7067–7074.
- Hegele, R.G., 2000. The pathology of asthma: brief review. *Immunopharmacology* 48, 257–262.
- Hogan, S.P., Klaus, I., Young, M.J.M., Young, A.I.G., Foster, P.S., 1998. A novel T cell-regulated mechanism modulating allergen-induced airways hypersensitivity in BALB/c mice independently of IL-4 and IL-5. *The Journal of Immunology* 161, 1501–1509.
- Holt, P.G., Rose, A.H., Batty, J.E., Turner, K.J., 1981. Induction of adjuvant-independent IgE responses in inbred mice: primary, secondary and persistent IgE responses to ovalbumin and ovomucoid. *International Archives of Allergy and Applied Immunology* 65, 42–50.
- Jae-Won, Oh., Seroogy, C.M., Meyer, E.H., Akbari, O., Berry, G., Fathman, G., DeKruyff, H.R., Umetsu, D.T., 2002. CD4 T-helper cells engineered to produce IL-10 prevent allergen-induced airway hyper-reactivity and inflammation. *Journal of Allergy and Clinical Immunology* 110, 460–468.
- Kim, H.M., Kim, H.J., Park, S.T., 1999. Inhibition of immunoglobulin E production by *Poncirus trifoliata* fruit extract. *Journal of Ethnopharmacology* 66, 283–288.
- Lack, G., Bradley, K.L., Hamelmann, E., Renz, H., Loader, J., Leung, D.Y.M., Larsen, G., Gelfand, E.W., 1996. Nebulized IFN- γ inhibits the development of secondary allergic responses in mice. *The Journal of Immunology* 157, 1432–1439.
- Melo-Diniz, M.F.F., 2000. *Ensaios Toxicológicos Pré-Clínicos Com as Folhas de Cissampelos sympodialis Eichl.*, Thesis. Universidade Federal da Paraíba, Brazil.
- Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxic assays. *Journal of Immunological Methods* 65, 55–63.
- Nakao, A., 2001. Is TGF- β -1 the key to suppression of human asthma? *Trends in Immunology* 22, 115–118.
- Oshiba, A., Hamelmann, E., Takeda, K., Bradley, K.L., Loader, J.E., Larsen, G.L., Gelfand, E.W., 1996. Passive transfer of immediate hypersensitivity and airway hyperresponsiveness by allergen-specific immunoglobulin (Ig) E and IgG1 in mice. *Journal of Clinical Investigation* 97, 1398–1408.
- Piuvezam, M.R., Peçanha, L.M.T., Alexander, J., Thomas, G., 1999. *Cissampelos sympodialis* Eichl. leaf extract increases the production of IL-10 by concanavalin-A treated BALB/c spleen cells. *Journal of Ethnopharmacology* 67, 93–101.
- Randolph, D.A., Carruthers, C.J.L., Szabo, S.J., Murphy, K.M., Chaplin, D.D., 1999. Modulation of airway inflammation by passive transfer of allergen-specific Th1 and Th2 cells in a model of asthma. *The Journal of Immunology* 162, 2375–2383.
- Revolvella, R., Ovary, I., 1969. Reaginic antibody production in different mouse strains. *Immunology* 17, 45–54.
- Rodhes, D.G., 1975. A revision of the genus *Cissampelos*. *Phytologia* 30, 415–485.
- Schumacher, J.H., O'Garra, A., Schrader, B., 1988. The characterization of four monoclonal antibodies specific for mouse IL-5 and development of mouse and human enzyme-linked immunosorbent. *The Journal of Immunology* 141, 1576–1581.
- Snaper, C.M., Paul, W.E., 1987. B cell stimulatory factor-1 (interleukin-4) prepares resting murine B cells to secrete IgG1 upon subsequent stimulation with bacterial lipopolysaccharide. *The Journal of Immunology* 139, 10–17.
- Thomas, G., Araújo, C.C., Agra, M.F., Diniz, M.F., Bachelet, M., Vargaftig, B.B., 1995. Preliminary studies on the hydroalcoholic extract of the root of *Cissampelos sympodialis* Eichl. in guinea-pig tra-

- cheal strips and bronchoalveolar leucocytes. *Phytotherapy Research* 9, 473–477.
- Thomas, G., Araújo, C.C., Duarte, J.C., De Souza, D.P., 1997a. Bronchodilatory activity of an aqueous fraction of the ethanolic extract of the leaves of *Cissampelos sympodialis* Eichl. (Menispermaceae) in guinea-pig. *Phytomedicine* 4, 233–238.
- Thomas, G., Burnes, F., Pyne, S., Pyne, N.J., 1997b. Characterization of the extract from the leaves of *Cissampelos sympodialis* Eichl. (Menispermaceae) on spontaneous tone of isolated trachea. *Phytotherapy Research* 11, 496–499.
- Thomas, G., Selak, M., Henson, P.M., 1999. Effects of the aqueous fraction of the ethanol extract of the leaves of *Cissampelos sympodialis* Eichl. in human neutrophils. *Phytotherapy Research* 13, 9–13.
- Victoratos, P., Yiangou, M., Avramidis, N., Hadjipetrou, L., 1997. Regulation of cytokine gene expression by adjuvants in vivo. *Clinical and Experimental Immunology* 109, 569–578.
- Wang, P., Wu, P., Sielgel, M.I., Egan, R.W., Billah, M.M., 1994. IL-10 inhibits transcription of cytokine genes in human peripheral blood mononuclear cells. *The Journal of Immunology* 153, 811–816.
- Wills-Karp, M., Luyimbazi, J., Xu, X., Schofield, B., Neben, T.Y., Karp, C.L., Donaldson, D.D., 1998. Interleukin-13: central mediator of allergic asthma. *Science* 282, 2258–2261.
- Wills-Karp, M., 2000. Murine models of asthma in understanding immune dysregulation in human asthma. *Immunopharmacology* 48, 263–268.
- Zimmerman, M., 1983. Ethical guidelines for investigations of experimental pain in conscious animals. *Pain* 16, 109–110.