



Original Article

Clinical safety evaluation of a tea containing *Cissampelos sympodialis* in healthy volunteers



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ABSTRACT

Cissampelos sympodialis Eichler, Menispermaceae, is widely used by Indian tribes and folk medicine to treat various inflammatory disorders, including asthma. Clinical toxicological trials were made with the tea of *C. sympodialis*, a medicinal plant. The study took place at Lauro Wanderley Hospital/UFPB-PB, where seventeen healthy volunteers were chosen, among those six men and eleven women who orally ingested, during four weeks uninterruptedly, 150 ml of the tea, once a day. Before the first ingestion and after the last one, the participants were subjected to clinical and laboratorial tests for their overall conditions in order to analyze the toxicity of the plant. The results demonstrated that the volunteers neither experience clinical nor laboratorial alterations, as well as no significant adverse effects, apart from little change detected in their hematological tests. Nevertheless, none demonstrated any pathological conditions, just alterations of the normal human being physiology. Therefore, it is concluded that these data complement that obtained during pre-clinical studies and confirm a low toxicity of this plant.

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Introduction

The herbal infusion is a drink made from leaves, flowers, seeds, fruit, stalks and some plant species roots (Zhao et al., 2013; Anvisa, 2010). The preparation consists of pouring boiling water over the herbal drug (Anvisa, 2010).

Herbal products are easily available and widely commercialized (Zhao et al., 2013; Owens et al., 2014), therefore being a way of complementing traditional medicine all over the world (Owens et al., 2014). However, those are not completely free of possible toxicity or other adverse effects (De Smet, 2004).

The infusion of *Cissampelos sympodialis* Eichler, Menispermaceae, species is widely used by Indian tribes and folk medicine to treat various inflammatory disorders, including asthma (Bezerra-Santos et al., 2004; Costa et al., 2008; Rocha et al., 2010; Marinho et al., 2012; Cavalcanti et al., 2013; Vieira et al., 2013). Asthma

is a medical condition which presents as main physiopathological characteristic bronchial inflammation, accompanied by lower airway hyper responsiveness and variable airflow limitation. This inflammation is associated with severe leukocyte recruitment and their activation at the site of lesion (Bezerra-Santos et al., 2012; Cavalcanti et al., 2013; Ribeiro-Filho et al., 2013).

The species is endemic in the Northeast and Southeast of Brazil, frequently occurring in open areas, as shrubs in sandy soil (Barbosa-Filho et al., 1997). The plant is popularly known as “milona”, “jarrinha”, “orelha-de-onça” and “abuteira” (Agra et al., 2007a,b).

Several studies were conducted with this plant, which have proved its therapeutic potential (Cavalcanti et al., 2013). Studies revealed anti-inflammatory activity and the potential for modulating the microbicide activity of macrophages by increasing the IL-10 production along with inhibition of NO synthesis. Furthermore, the findings proved the efficacy of *C. sympodialis* upon the regulation of B cell function and the immunoglobulin secretion in allergic diseases, as well as autoimmune disease synthesis (Cavalcanti et al., 2013; Vieira et al., 2013; Piuvezam et al., 2012).

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Vieira et al. (2013) demonstrated that the inhalation of *C. sympodialis* in animals with allergic inflammation of the airways is as effective as the oral treatment with dexamethasone for controlling the inflammatory response in the lungs and the production of IgE. Ultimately, the results suggest that the leaves of *C. sympodialis* may be material for a herbal medicine.

In pre-clinical toxicological assays with the AFL (alcoholic fraction of leaves) of *C. sympodialis* done in rats (male and female), it was investigated the sub-acute (four weeks) and chronic (thirteen weeks) toxicity of the popular administration (9 mg/kg/orally). These studies suggested lack of toxicity in these animals. Doses administrated 5–225 times higher than the ones ingested by men evidenced, in mice, inflammatory processes and an increase of hepatic enzymes, besides the hyperplasia of Kupffer cells, reversible thirty days after the administration of the extract was suspended (Diniz, 2000).

Data published in pre-clinical studies with *C. sympodialis* leaves enable clinical assays which may initially establish the safety and subsequently the effectiveness of *C. sympodialis* in humans. Thus, this study intended to ascertain the safety of this medicine in potential. Based on clinical phase 1 parameters in conjunction with the ones in the pre-clinical studies which have already been published, there is craving for registering with Anvisa (CNS, 1997; Anvisa, 2010).

Material and methods

Plant material

The leaves of *Cissampelos sympodialis* Eichler, Menispermaceae, were collected during the months of March and September of 2012 in the garden of medicinal plants at the Laboratory of Pharmaceutical Technology Prof. Delby Fernandes de Medeiros, Campus I at Federal University of Paraíba, where the plant has been cultivated. The identification and morphological description of the plant were made by Dra. Maria de Fátima Agra. A sample is located in the Lauro Pires Xavier herbarium, at UFPB, by the voucher specimen number Agra 1456 (JPB).

The acquisition of *C. sympodialis* sachets

The leaves of *C. sympodialis* were dehydrated in a greenhouse with air flowing at 38 °C for 72 h and ground in a Harley type grinder, having the average yields calculated. After the pounding, the dried leaves were submitted to a phytochemical/quality control triage, and then sent to the Aplaf Ltda, São Paulo-SP, being this company responsible for producing and ratifying the quality control of the *C. sympodialis* sachets. Each unit, produced with filter paper for specific use, contained 1 g of the powder.

Phytochemical

A phytochemical trial of *C. sympodialis* leaves infusion was conducted according to Matos (1997). During the trial, the presence of alkaloids, steroids, tannins, flavonoids and saponin were detected.

The classes of chemical substances present on the leaves of *C. sympodialis* were characterized before the sachets production, thus being considered an indispensable stage for their standardization.

Search of warifteine and methylwarifteine alkaloids in teas sachets of *C. sympodialis*

The teas were prepared under the same conditions of clinical studies (one sachet containing 1 g of the powder *C. sympodialis* was subjected to infusion for 15 min).

HPLC equipment and conditions

All solvents used were HPLC level. Deionized Milli Q water (Millipore, Bedford, MA) was used to prepare the mobile phase and diluents solutions. All chromatographic runs were carried out using a Sykam HPLC System, consisting of a S7131 pump. A S3240 photodiode-array detector (DAD) was used for detection. Full spectra were recorded in the range 200–400 nm. Equipment control, data acquisition and integration were performed with Clarity software. Chromatographic separations were achieved using methodologies based on Aragão (2002) and Marinho (2011). The mobile phase consisted of a mixture of methanol/CH₃CO₂H 0.1% (35:65, v/v). Flow-rate was set to 0.3 ml/min and the injection volume was 20 µl. All experiments were carried out at room temperature. The DAD detected the presence or not of alkaloids peaks in sachets of *C. sympodialis*. It was obtained spectrum UV of alkaloid templates of *C. sympodialis* (warifteine and methylwarifteine) within the same conditions proposed by the method and saved on a data base supported by the Clarity software. The substances were analyzed through HPLC/UV-DAD and by comparing the time retention in the extract peaks with the ones collected with authentic reference standards.

Research field

This research took place in Lauro Wanderley University Hospital at Federal University of Paraíba, where further trials were done in the clinical analysis laboratory, in the cardiology room as well as in the ambulatory at CRAS (Reference Server Care Center). Protocol clinic was conducted/defined according to the Brazilian resolutions n° 251/97 and 466/12 from the CNS, the international standards from the World Health Organization 2011 and good clinical practice (GCP) (CNS, 1997; CNS, 2012).

Volunteers

The clinical study was open and not randomized, performed with individuals participating voluntarily. The sample consisted of eighteen volunteers, six men and eleven women (one quitclaim) between 23 and 60 years old, were chosen after complete clinical and laboratorial trials which aimed to ascertain proper health conditions in order to participate in this research.

Experimental protocol

The volunteers had a 150 ml dose of the herbal product orally, once a day, using the sachets with leaves of *C. sympodialis* for four weeks. Thus, this study was conducted from May 2012 to June 2013. After receiving the sachets, the participants were weekly monitored (0-1-2-3-4 weeks), starting from day 0.

Before the first ingestion of the product and a day after the last one, the participants (men and women) were subjected to a clinical and laboratorial evaluation for their overall conditions. They did the following tests: glucose, creatinine phosphokinase (CPK), triacylglyceride, total cholesterol and fractions, lactate dehydrogenase (LDH), amylase, sodium, potassium, aspartate transaminase, alanine transaminase, total bilirubin and fractions, gamma GT, alkaline phosphatase, total protein and fractions, creatinine, uric acid, urea, complete blood count, platelet count and urinalysis I. A twelve-lead electrocardiogram was also done.

Throughout the course of the study, the volunteers were instructed to report to the researchers any signs or symptoms that might present adverse reaction and were also given a questionnaire to fill in, if at all.

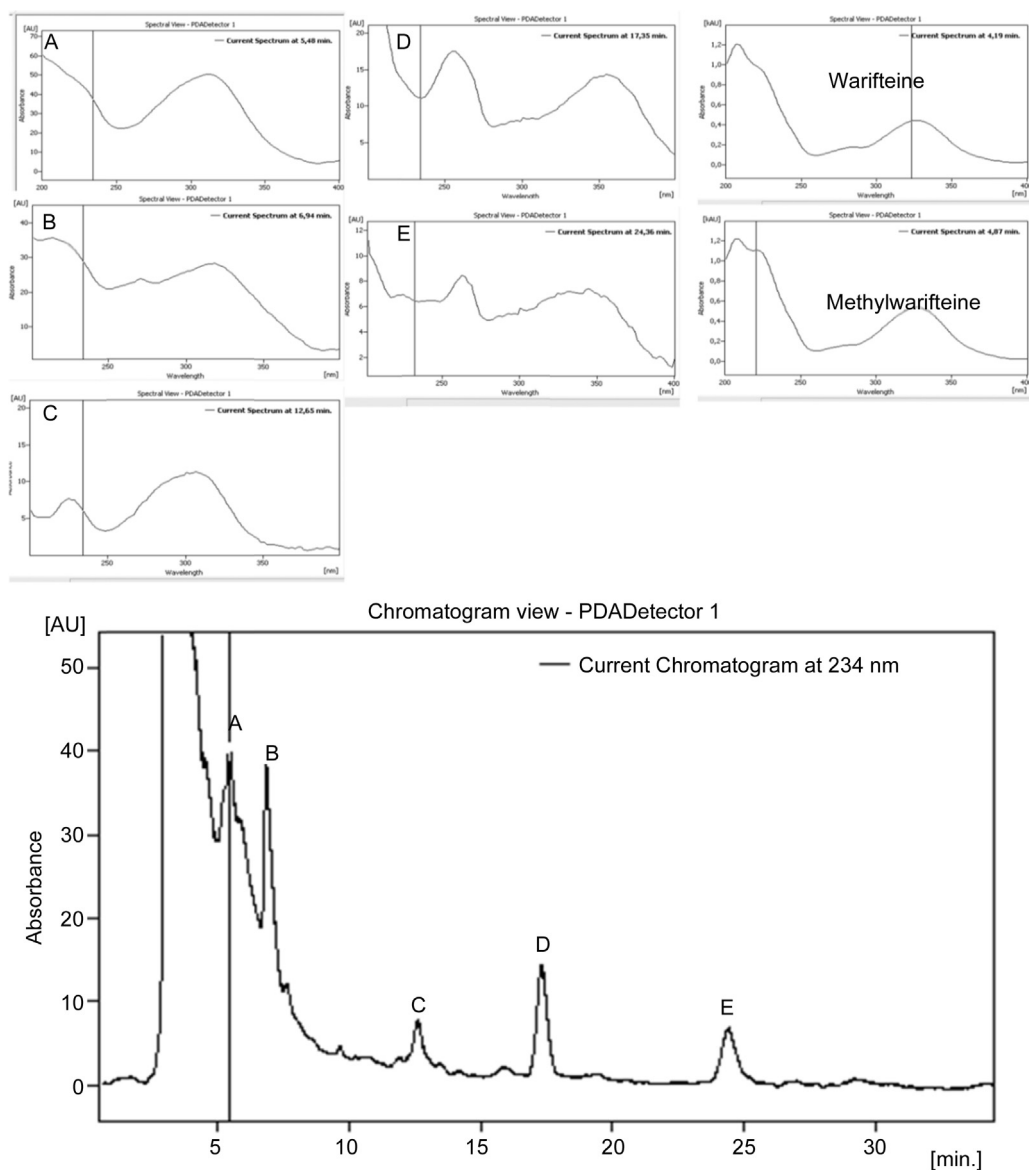


Fig. 1. The chromatogram and UV spectra of the separation of tea of *Cissampelos sympodialis*. Five unknown peaks are eluting: A, B, C, D and E.

Exclusion criteria

Individuals who had any clinical or laboratorial alterations – hepatic, renal or cardiac dysfunction; pregnancy; use of alcohol and/or any medicines – during the initial clinical tests were excluded from this study.

Ethical aspects

The research project, with the protocol and consent forms, was submitted and approved by the Committee of Ethics in Research with humans at Lauro Wanderley University Hospital – UFPB on 25/05/2010 – protocol no. 284/10.

All volunteers were informed about the nature and objectives of the study and those who agreed to participate gave their formal written consent after signing the Statement of Informed Consent.

Statistical analysis

The evaluation of the volunteers' hematological and biochemical parameters, which aimed to diagnose whether the participants met

the standards for an individual considered healthy, was performed by comparing the results obtained throughout the treatment and the ones acquired during the initial trial for each volunteer (basal time). The figures were expressed by mean \pm standard error of mean (SEM) of the seventeen participants, separating them by gender, according to the type of test and evaluation period. It was used the Student's "t" test for paired samples $*p < 0.05$ and One-way ANOVA/Tukey. $*p < 0.05$. All data were analyzed using the statistical program Graph Pad Prism $\text{\textcircled{R}}$ version 6.02.

Results and discussion

The search for warifteine and methylwarifteine in teas prepared with *C. sympodialis* sachets were performed through analysis by High Performance Liquid Chromatography coupled to UV detector with photodiode array (HPLC/DAD). The HPLC chromatogram, Fig. 1, in *C. sympodialis* tea showed five unknown peaks defined as: A, B, C, D, E and the absence of warifteine and methylwarifteine in measurable concentrations. This fact can be explained because previous studies used ethanolic extraction. This study used hot water extraction.

Table 1
Patients' biochemical parameters, either sex, from the Clinical Trial with infusion of the leaves of *Cissampelos sympodialis*. Values are expressed in mean \pm SEM.

Groups	Glucose (mg/dl)	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)	Indirect bilirubin (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)	Urea (mg/dl)
<i>Men</i>							
Reference	70–99	0.2–1.2	0.0–0.5	0.0–0.5	3.5–7.0	0.9–1.3	19.0–44.0
1° Appointment	89.67 \pm 1.76	0.74 \pm 0.23	0.21 \pm 0.06	0.54 \pm 0.17	6.05 \pm 0.43	1.03 \pm 0.07	29.50 \pm 3.15
30 Days	90.33 \pm 2.17	0.96 \pm 0.34	0.26 \pm 0.07	0.70 \pm 0.27	6.03 \pm 0.49	1.03 \pm 0.07	28.33 \pm 2.08
<i>Women</i>							
Reference	70–99	0.2–1.2	0.0–0.5	0.0–0.5	2.5–6.2	0.6–1.1	14.9–40
1° Appointment	87.55 \pm 1.97	0.63 \pm 0.15	0.16 \pm 0.03	0.46 \pm 0.12	4.23 \pm 0.34	0.73 \pm 0.03	27.20 \pm 2.39
30 Days	90.55 \pm 4.10	0.64 \pm 0.16	0.18 \pm 0.03	0.46 \pm 0.13	4.25 \pm 0.34	0.76 \pm 0.04	26.33 \pm 2.84

Student's "t" test * $p < 0.05$ probability.**Table 2**
Patients' biochemical parameters, either sex, from the Clinical Trial with infusion of the leaves of *Cissampelos sympodialis*.

Groups	AST (U/l)	ALT (U/l)	GGT (U/l)	Total phosphinic creatine (U/l)	LDH (U/l)	Amylase (U/l)
<i>Men</i>						
Reference	5–34	6–55	12–24	<190	125–243	25–125
1° Appointment	25.17 \pm 3.11	42.50 \pm 8.96	47.33 \pm 10.67	122.00 \pm 18.18	65.80 \pm 4.42	79.50 \pm 13.71
30 Days	25.17 \pm 3.03	47.33 \pm 12.65	47.33 \pm 14.78	120.00 \pm 24.45	186.00 \pm 18.51	78.33 \pm 13.07
<i>Women</i>						
Reference	5–34	6–55	9–36	<167	125–243	25–160
1° Appointment	21.40 \pm 4.10	25.70 \pm 6.55	23.90 \pm 5.65	97.50 \pm 15.99	153.80 \pm 4.35	67.30 \pm 3.17
30 Days	19.40 \pm 2.11	22.40 \pm 3.40	22.85 \pm 3.51	103.5 \pm 11.05	156.00 \pm 4.79	73.43 \pm 5.07

AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, Gamma-glutamyl transpeptidase; LDH, lactate dehydrogenase. Values are expressed in mean \pm SEM. Student's "t" test * $p < 0.05$ probability.**Table 3**
Patients' hematological parameters, either sex, from the Clinical Trial with infusion of the leaves of *Cissampelos sympodialis*.

Groups	RBC ($10^6/\text{mm}^3$)	Hemoglobin (g/dl)	Hematocrit (%)	MCV (fl^3)	MCH (pg)	MCHC (%)	RDW (%)
<i>Men</i>							
Reference	4.5–6.0	12.8–17.8	40–52	82–98	27–33	32–36	11.5–14.5
1° Appointment	5.33 \pm 0.10	15.72 \pm 0.46	45.90 \pm 0.99	86.13 \pm 1.44	29.51 \pm 0.91	34.24 \pm 0.52	10.63 \pm 0.33
30 Days	5.32 \pm 0.13	15.76 \pm 0.29	46.06 \pm 1.01	86.65 \pm 1.01	29.68 \pm 0.55	34.26 \pm 0.33	10.92 \pm 0.15
<i>Women</i>							
Reference	3.9–5.3	12–15.6	36–48	82–98	27–33	32–36	11.5–14.5
1° Appointment	4.42 \pm 0.09	13.33 \pm 0.23	39.02 \pm 0.68	88.31 \pm 1.19	30.20 \pm 0.52	34.17 \pm 0.22	11.22 \pm 0.34
30 Days	4.38 \pm 0.10	13.22 \pm 0.23	38.51 \pm 0.61	88.07 \pm 1.04	30.25 \pm 0.61	34.34 \pm 0.38	11.38 \pm 0.38

RBC, red blood cells; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red cell distribution width. Values are expressed in mean \pm SEM. Student's "t" test * $p < 0.05$ probability.

This study investigated the clinical toxicity in seventeen humans, in search of the safety of this medicine in potential. The clinical phase I concerns the first moment when the medicine is tested in a group of healthy volunteers. This phase seeks to establish the safety, pharmacokinetic profile and tolerability in a preliminary format of the substance in humans (CNS, 1997; Mesia et al., 2011).

Tests were carried out for urinalysis I, hematological and biochemical parameters of patients, which showed that there was just a significant change in statistics in four hematological parameters, in which it was observed a decline in leucocytes and neutrophils in men, an increase in eosinophils in women and in lymphocytes in men. Although these changes were statistically significant, they

do not represent a pathological condition, and perhaps are just an alteration of normal human physiology (Tables 1–4).

The leucocytes and lymphocytes are cells responsible for protecting the organism against infections. Lymphocytosis is caused by neoplasia, viral infections such as rubella, mononucleosis, and mumps; bacterial infections, protozoans, among others (Hoffbrand and Moss, 2013; Musso et al., 2014). The neutrophils are mainly responsible for the phagocytes is of cells and extraneous material. Neutropenia is mainly caused by dysplasia, infections, inflammation, intravascular destruction (immune), drugs and chemicals, among others (Musso et al., 2014; Spaan et al., 2013). On the other hand, the eosinophilia is related to allergic responses, parasitic

Table 4
Patients' hematological parameters, either sex, from the Clinical Trial with infusion of the leaves of *Cissampelos sympodialis*.

Groups	Leukocytes ($10^3/\text{mm}^3$)	Neutrophils (%)	Eosinophils (%)	Lymphocytes (%)	Monocytes (%)	Platelet ($1/\text{mm}^3$)
<i>Men</i>						
Reference	4.0–11.0	45–70	1–6	20–45	2.0–10.0	150,000–450,000
Basal	9265 \pm 718.40	58.83 \pm 3.08	2.83 \pm 0.60	30.83 \pm 3.12	7.5 \pm 0.68	207717 \pm 31716
30 Days	8293 \pm 636.10	53.67 \pm 2.33	3.00 \pm 0.37	35.67 \pm 2.14	7.5 \pm 0.56	237683 \pm 23855
<i>Women</i>						
Reference	4.0–11.0	45–70	1–6	20–45	2.0–10.0	150,000–450,000
Basal	6360 \pm 342.50	55.00 \pm 1.87	3.00 \pm 0.73	34.64 \pm 2.16	6.73 \pm 0.57	220391 \pm 19885
30 Days	6579 \pm 412.70	56.02 \pm 2.81	3.87 \pm 0.69	32.76 \pm 3.01	6.49 \pm 0.62	224491 \pm 15365

Values are expressed in mean \pm SEM. Student "t" test * $p < 0.05$ probability.

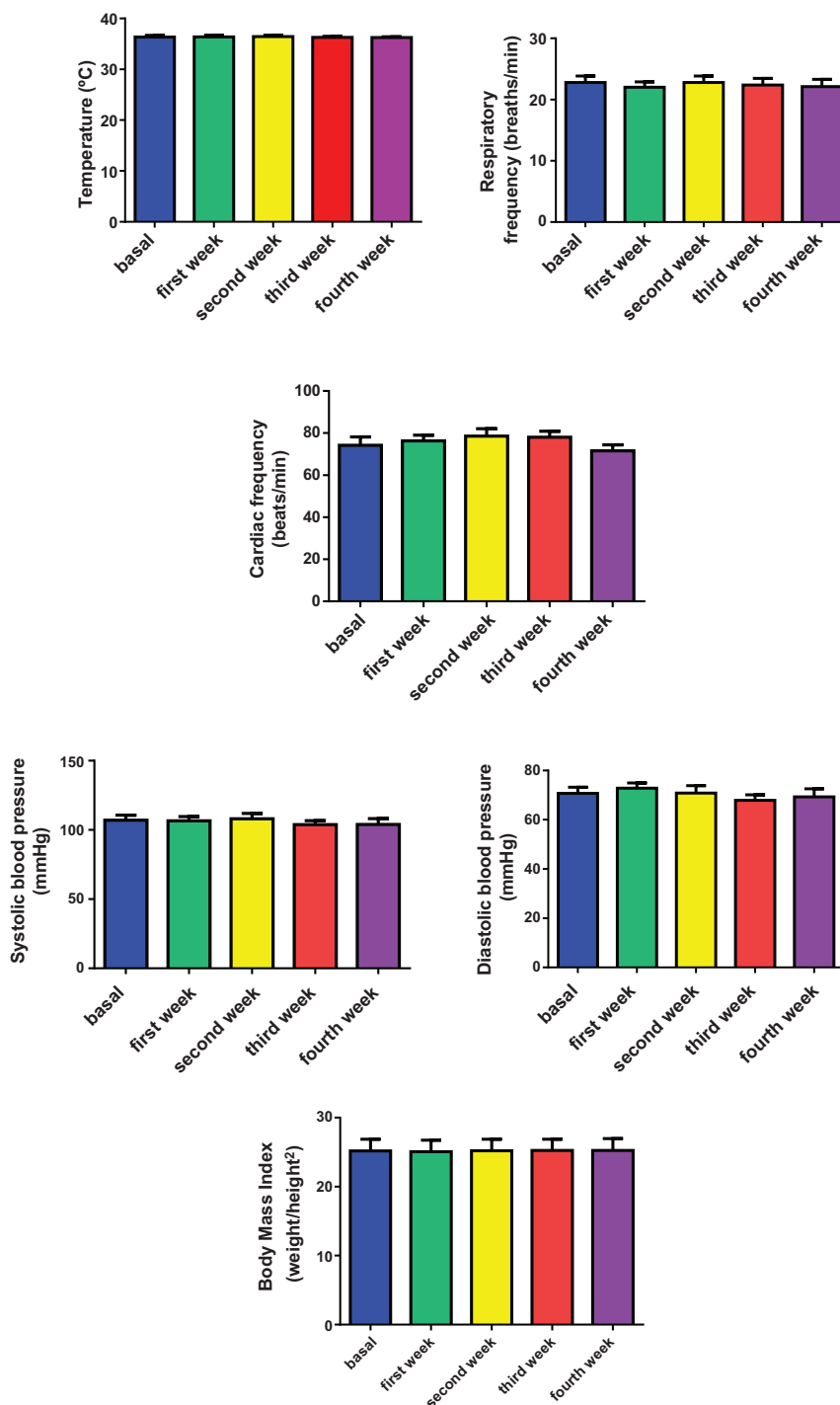


Fig. 2. Weekly evaluation of women according to the following parameters: temperature, respiratory and cardiac frequency, systolic and diastolic blood pressure and body mass index. Values are expressed in mean \pm SEM. One-way ANOVA/Tukey. * $p < 0.05$.

diseases, acute infection, certain skin diseases, drug sensitivity, among others (Chen et al., 2013; Dasgupta et al., 2013; Musso et al., 2014). These pathologies were not detected in the volunteers who participated in the study.

Within this context, the low toxicity of *C. sympodialis* was verified, since it was not revealed any significant statistical changes in the volunteers' biochemical tests, despite the fact that studies demonstrate hepatic alterations in humans throughout continuous use of medicinal plants (Paulo et al., 2009; Bunchorntavakul and Reddy, 2013). Furthermore, when comparing the results from

the pre-clinical trials to the clinical ones, we realized that there may be some statistical variation, yet only in overdose (Diniz, 2000).

Along the clinical tests, anamnesis, temperature measurement, blood pressure investigation, respiratory and cardiac frequency, body mass index and application of questionnaires related to possible side and adverse effects were conducted by the physician responsible for the research, who evaluated and diagnosed that the individuals involved in the study were in normal standards (Figs. 2 and 3). The electrocardiograms showed eleven normal ones before and after ingestion of the product; two conduction disorders

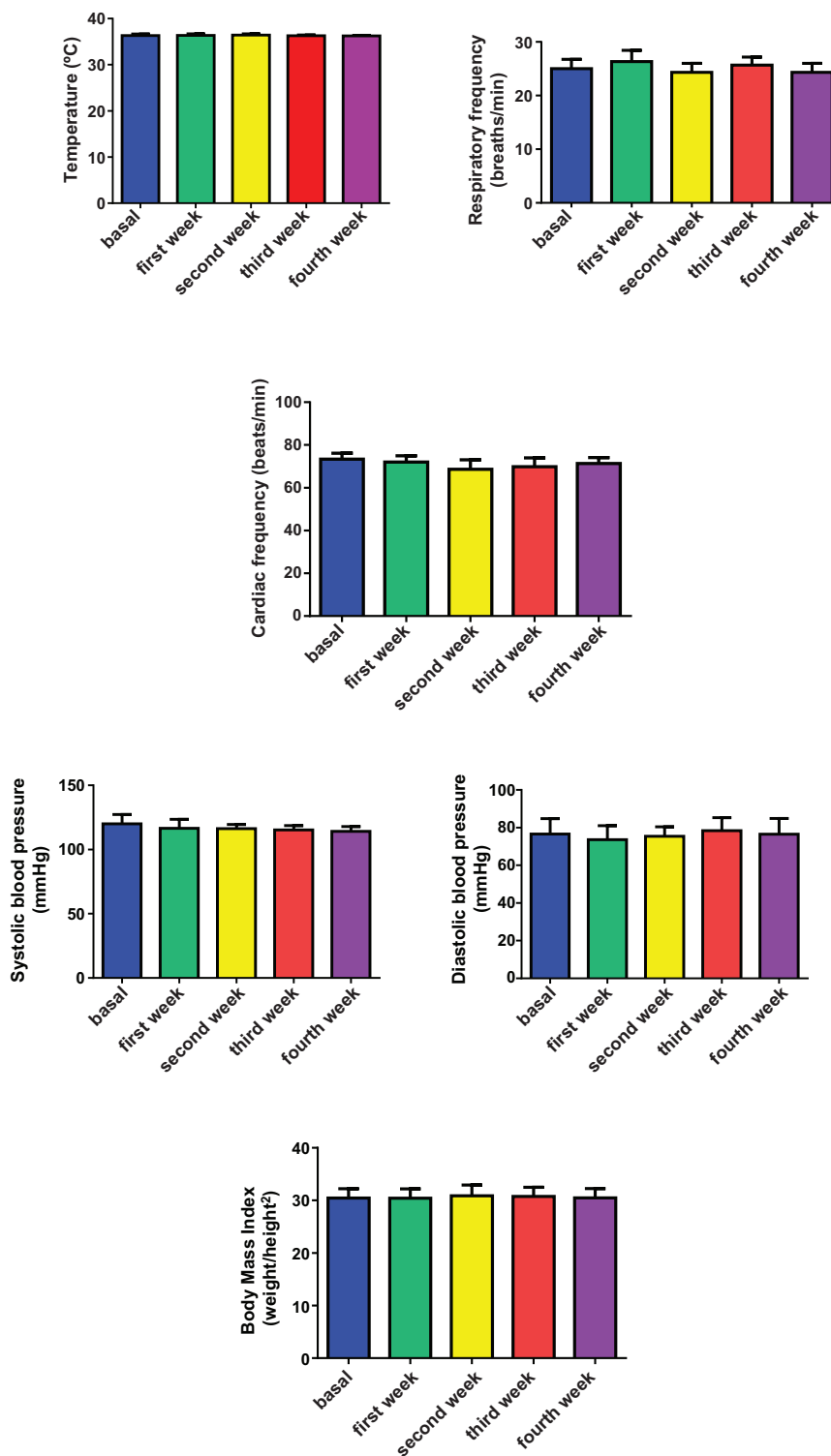


Fig. 3. Weekly evaluation of men according to the following parameters: temperature, respiratory and cardiac frequency, systolic and diastolic blood pressure and body mass index. Values are expressed in mean \pm SEM. One-way ANOVA/Tukey. * $p < 0.05$.

of the right bundle branch before and after; one short PR before and after; one bradycardic before and after; one normal before and one altered ventricular repolarization after; one bradycardic before and one altered ventricular repolarization after. Nevertheless, all electrocardiographic tests were as expected based on normal limits.

The electrocardiogram (ECG) is a simple and low cost test which provides data about the myocardium status, perhaps useful in

cardiovascular epidemiology, therefore supporting the diagnosis of the myocardial infarction, ischemia and cardiac hypertrophy, and yet detecting the risk of future cardiac events (Cardoso et al., 2002; Ribeiro et al., 2012).

However, the normal range of the electrocardiogram is controversial, hence it demands a thorough analysis of variations considered expected in the ECG of healthy people, and, only after, some reflection on the meaning of the electrocardiograms which

Table 5
Side effects and adverse reactions described throughout the clinical study.

Effects	Numbers	Frequency (%)
Fluid retention	1/17	5.88
Decreased muscle aches	2/17	11,76
Hydration of heels	1/17	5.88
Decreased appetite	5/17	29.41
Reduction in candida	1/17	5.88
Decrease tingling	1/17	5.88
Increased intestinal motility	2/17	11.76
Dizziness	1/17	5.88
Somnolence	1/17	5.88
Insomnia	1/17	5.88

are undoubtedly abnormal (Moffa and Sanches, 2001; Hampton, 2011).

Sinus rhythm is the only one sustained as normal. In youth, the space RR is reduced, in other words, the cardiac frequency is increased during inspiration and it is named respiratory sinus arrhythmia. When the sinus arrhythmia is intense, it may simulate an atrial one (Moffa and Sanches, 2001; Hampton, 2011).

There is no single definition for a normal cardiac frequency, and the terms bradycardia and tachycardia ought to be used cautiously. There is no point at which a high cardiac frequency in sinus rhythm should be called sinus tachycardia and no upper limit for sinus bradycardia. However, unexpected high or low rates should be investigated. The possible causes for a sinus rhythm of low cardiac frequency are: good physical condition, hypothyroidism, hypothermia, acute myocardial infarction, vasovagal attacks and use of beta-blockers. The most common causes described as sinus rhythm of high cardiac frequency is: pain, fear, obesity, acute myocardial infarction, pulmonary embolism, anemia, thyrotoxicosis, use of beta-adrenergic drugs (Moffa and Sanches, 2001; Hampton, 2011). These pathologies were not detected in the volunteers who participated in the study.

Studies showed that small changes in the ECG may be regarded as “predictive” for clinical signs of a coronary heart disease, which is related to cardiovascular mortality (Moffa and Sanches, 2001; Hampton, 2011). Some authors suggest that an abnormality in the ST-T segment is an independent indicator of morbidity and mortality from coronary atherosclerosis (Moffa and Sanches, 2001; Baranowski et al., 2012).

For this reason, the ECG has been widely used to identify individuals at risk for ischemic heart diseases, still during asymptomatic stage. This population, once subjected to preventive and quite aggressive strategies, may be benefited.

Throughout the clinical research, some side and adverse effects were observed, such as: decreased muscle aches; skin hydration; increased intestinal motility; decreased tingling sensation. Moreover, dizziness, insomnia, fluid retention, somnolence, reduction in candida and decreased appetite were also reported as side and adverse effects (Table 5).

These adverse reactions may be classified Type A, referred to as toxic or side effects, which can be explained by the action mechanism of drugs, being a common and expected reaction and of low mortality rate (Edwards and Aronson, 2000; Sobrafo, 2011).

The data collected from this study demonstrated that the administration of *C. sympodialis* leaves infusion in humans, ingested during thirty days, was well tolerated, indicating neither clinical nor laboratorial alterations, nor any significant adverse reactions. These results complement those obtained in the pre-clinical toxicological studies, suggesting low toxicity, in the dosage and route of administration tested. With this data, there is craving for registering this medicine in potential with Anvisa and proceed with the phase II studies.

Authors' contributions

LFBM (PhD student) main author, involved in the study design, conducted the interviews, field and laboratory work, literature review and general data collection, systematization and analysis, wrote the first draft this paper. LSNR and AIPAT, contributed to designing and following progress of the research and fieldwork. JAR and KMO, contributed to in achieving the laboratory test and data analyses. CFSa contributed to chromatographic analysis. VMVN contributed to cardiac monitoring and reports. ABL and CMBLL, contributed in writing the final version of this paper. MFFMD designed the study, supervised the laboratory work and contributed to critical reading the manuscript. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

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