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Effects of the Single Supplementation and Multiple Doses of *Passiflora Incarnata* L. on Human Anxiety: A Clinical Trial, Double-blind, Placebo-Controlled, Randomized

ORIGINAL

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Abstract

Object: This study aimed to investigate the effects of *Passiflora incarnata* L. on anxiety in humans.

Method: The individuals were randomly assigned to one of the following groups: Placebo or experimental, n =30, single dose, as well as Placebo or Experimental, n =15, multiple dose. The experimental human anxiety was induced by simulated public speaking test in the following phases: Basal (B), stressful (A), speech 1 (S1), speech 2 (S2) and Final (F). We evaluated the Systolic Blood Pressure (SBP), and Diastolic (DBP), Heart Rate (HR), Electrical Conductance of Skin and extremities temperature (ET) and filled The state-trait anxiety inventory (STAI S e T).

Results: During the single dose, the HR was reduced at the end of the speech (86±2.0 to 74±3.0 bpm) the experimental group compared to the placebo group ($p<0.05$); the STAI-S did not change. In the delineation of multiple doses, SBP was reduced, in mmHg, in the experimental group compared to the placebo group during all phases. Experimental: 106±1.0 (B), 111±1.0 (A), 121±2.0 (S1), 115±3 (S2), 104±2.0 (F) e Placebo: 121±3.0; 127±3.0; 130±3.0; 130±4.5; 117±3.0 ($p<0.05$).

Conclusions: It is suggested that supplementation using *Passiflora incarnata* L. Capsules (500 mg) decreased cardiovascular signals both in single and in multiple doses, associated with the stress of public speaking.

Keywords
Anxiety; *Passiflora Incarnata* L; Anxiolytic; Nutraceutical.

Introduction

Anxiety disorders reach over 25% prevalence, including the fear of public speaking [1]. Anxiety comprises emotional and behavioral factors through fear manifestation, insecurity, apprehensive anticipation, catastrophic thinking, increase in wakefulness or alert period and physiological, as activation of the hypothalamic-pituitary-adrenal (HPA) axis, leading neurovegetative symptoms such as insomnia, tachycardia, pallor, increased perspiration, muscle tension, trembling, dizziness, intestinal disorders, among others [2].

The impact of anxiety on the individuals's health covers changes in blood pressure, heart rate and an increased risk of cardiovascular diseases such as coronary heart disease (risk tripled) and cerebrovascular [3].

Therefore, researchers have invested in as natural forms taken to treat or ameliorate the symptoms of anxiety, such as the use of herbs. Plants that are most studied with anxiolytic potential are Kawa, the St. John's Wort and Passiflora spp. [4-7]. The Passiflora extract has been used in many researches, presenting in their chemical constitution polyphenols, polyunsaturated fatty acids, fiber and other substances, such as passiflorin, one indole alkaloid, being the last one associated with anxiolytic effects [3].

The effects of *Passiflora incarnata* L on anxiety was previously assessed in animals [8, 4] and in humans [9-10] with satisfactory results [7]. Epidemiological studies have shown that the fear of public speaking is one of the biggest phobias that affect people, being highly prevalent among students [11].

The study proposal was to evaluate symptoms of anxiety of public speaking, under a single dose and multiple doses through the simulated public speaking (SPS) test through the intervention with the encapsulated dry extract of the whole plant *Passiflora incarnata* L.

Materials and Methods

Subjects A study population consisted of university students, adults, healthy, between 24 and 31 years, of both sexes, were excluded individuals who had any somatic or mental disorder diagnosed; pregnant women; individuals with chronic use of drugs.

The study, described as experimental (trial), randomized, double-blind, placebo-controlled, was approved by the Health Sciences Centre Research Ethics Committee of the Federal University of Paraíba (Protocol number 070/14) .All participants were informed about the specifications of the study and signed a Informed Consent Agreement which was drafted as the guidelines for research involving human beings (Resolution 466/12 of the national health council/ Ministry of Health).

Sample A sample was constituted by 90 individuals recruited by filling out the form for participation in the survey, sent via social network (*Facebook*) and emails. Of the 110 invitations sent, only 20 refused to participate or were withdrawn from the study due the exclusion criteria.

Of the total, the subjects were randomly assigned to one of the following groups: Placebo or Experimental, n = 30, single dose and placebo or experimental, n = 15, multiple doses, resulting in all four

groups. The sampling was based on previous studies using similar sample size and obtained positive results [14, 10].

Induction of experimental human anxiety The experimental human anxiety was induced by simulated public speaking test (SPS) [12, 13].This model causes anxiety for placing the volunteer to speak in front of a video camera . The induced anxiety and the effect produced by the substances used in the experiment can then be assessed using psychometric scales and physiological measurements [15, 16]. The **Table 1** shows the result of the experimental session.

Table 1. Sequence of the experimental session.

Session (min)	Phase	Procedures
-0:30		Adaptation to the laboratory; instructions on interventions and measurements
-0:15	Basal (B)	STAI-T, STAI-E, SBP, DBP, HR, ECS, ET
0		Ingestion of the capsule: placebo or experimental
+1:30	Stressful (A)	Instructions about SPS
+1:32		Discourse preparation, SBP, DBP, HR, ECS, ET, STAI-E
+1:34	Speech 1(S1)	Beginning of the speech
+1:36		Interruption in order to fill the STAI-E
+1:38	Speech 2 (S2)	Continuation of speech; DBP, SBP, HR, ECS, ET, STAI-E
+1:40		End of speech
+1:55	Final (F)	DBP, SBP, HR, ECS, ET, STAI-E

STAI-T: Trait anxiety inventory; STAI-E: State anxiety inventory; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; HR: Heart rate; ET: extremities temperature; ECS: Electrical Conductance of Skin; SPS: Simulated Public Speaking

Measurements The physiological measures include systolic blood pressure, diastolic blood pressure, heart rate, extremities temperature, electrical Conductance of skin. Together, these measures allow to investigate the occurrence of possible hemodynamic, thermal and perspiration of the extremities changes. The measurement of physiological parameters (ECS and ET) was performed using a suitable device, the physiograph I-330-C2 Plus Clinical System®. The SBP, DBP and HR were measured by a digital sphygmomanometer (Omrom, Brazil) attached to the voluntary's left wrist.

The psychological measurements were performed through the application of do state-trait anxiety inventory (STAI), produced by Spielberg, Gorsuch and Lushene (1970) [17], translated and validated to Portuguese by Biaggio and Natalício (1979) [18]. STAI is a test compound by two self-assessment questionnaires trait anxiety inventory and the state anxiety inventory.

Supplementation protocol The dry extract of *Passiflora incarnata* L., arising out of the whole plant, was standardized to a content of 0.29% of vitexin, purchased from a druggist in the city of João Pessoa, PB. The extract and the placebo capsules composed of 500 mg of identical appearance. Placebo capsules contained inactive substance (starch).

After baseline measurements were provided to each participant of the single administration groups one capsule. They waited 1 hour and a half and continued in the other experimental steps, while those from groups of multiple doses, after the baseline measurements, received 6 capsules properly labeled with all necessary information for 6 days ingestion. The subjects were instructed to eat them with water as a way to standardize the ingestion and maintain their lifestyle during the trial period. Regarding the timing of ingestion, the moment of ingestion of a single dose groups depended on the appointment with the participant, while the multiple doses groups were instructed to take after dinner. They had to take one capsule per day for 6 days. The experiments were performed in the morning shift.

A specific questionnaire on the appearance and the possible side effects characteristics was applied after the end of the experiment, being answered by the volunteer himself.

Statistical Analysis Statistical analysis was performed using the statistical program GraphPad Prism (Version 6.00, GraphPad Software Inc., San

Diego, CA, USA). Normality was evaluated by the test D´Agostino-Pearson omnibus. In the design of single administration, the physiological parameters SBP, DBP, HR, ET, ECS and psychological by STAI-T e E were compared between the placebo and experimental groups by the test *t* (Student) or nonparametric corresponding (U Mann-Whitney). To check the side effects it was used the Fisher's exact test. Data were analyzed with a significance level of 5% (P <0.05).

Results

Single Administration

The analysis of psychological and physiological parameters **(Table 2)** shows that the population had a uniformity during the initial evaluation, with no statistical difference between the groups (p> 0.05), except for the SBP.

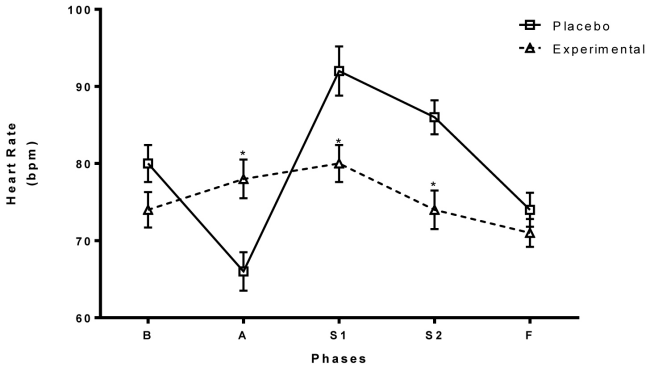
Table 2. Sample characterization of the groups at baseline moment, during the single administration.

Variables	Placebo	Experimental	P
Subject	30	30	-
Male	15	15	-
Female	15	15	-
Age[1](years)	24.9±3.4	24.9±3.4	0.90
STAI-T[2] (points)	46 (43-49)	46 (42-49)	0.80
STAI-E[2] (points)	42 (40-45)	43 (39-47)	0.90
SBP[3] (mmHg)	110±2.0	118±2.0	0.02*
DBP[3] (mmHg)	67±1.0	67±1.0	0.65
HR[3] (BPM)	80±2.0	74±2.0	0.09
ET[3] (°C)	30.15±0.5	30.13±0.5	0.80
ECS[3] (µS)	4±0.5	3.8±0.3	0.40

[1]:Mean ± standard deviation. [2]: Median (percentile 25 e 75). [3]: Mean ± standard error mean. STAI-T: Trait anxiety inventory; STAI-E: State anxiety inventory; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; HR: Heart rate; ET: extremities temperature; ECS: Electrical Conductance of Skin, mmHg: millimeters of mercury, bpm beats per minute. °C: degree Celsius. µS: microsiemens. *: Significant compared to placebo (p<0.05). Estatistic test: unpaired t test.

Comparing the SBP groups showed an increase in the experimental group (118 ± 2.0 mmHg) compared to the placebo group (110 ± 2.0 mmHg) at baseline phase (p <0.05). DBP increased in the experimental group (74 ± 1.7 mm Hg) compared to the placebo group (62 ± 1.6 mmHg) at phase S1, a statistical variation (p <0.05). HR increased in stressful phase (78 ± 3.0 bpm) in the experimental group compared to the placebo group (68 ± 3.0 bpm) (p <0.05), however declined throughout the speech (92 ± 3.0 to 80 ± 2.0 bpm and 86 ± 2.0 to 74 ± 3.0 bpm, S1 and S2, respectively) in the experimental group compared to the placebo group (p <0.05) **(Figure 1)**. Considering all stages of SPS analysis between the groups showed no statistical differences of the ECS and ET (p> 0.05).

Figure 1: *Passiflora incarnata* L. effect on the Heart rate of groups submitted to Simulated Public Speaking test.



*: Significant compared to the placebo group (p<0.05). Values are presented as mean ± standard error. Estatistic test: unpaired t test. B (Basal); A (Stressful); S1 (Speach 1); S2 (Speach 2) e F (Final).

The STAI-T experiment was evaluated only in the initial moment in each of the experimental groups, the analysis of the data shows that the same participants began the tests with moderate levels of anxiety (33-49 points) [17] **(Table 2)**. The comparisons between the groups showed no statistical differences (p> 0.05) compared to the scores of the STAI-E.

Multiple doses

The analysis of psychological and physiological parameters **(Table 3)** shows that the population had a uniformity during the initial evaluation, with no statistical difference between the groups. The exception was the DBP that showed a reduction in the experimental group ($p < 0.05$) compared to placebo.

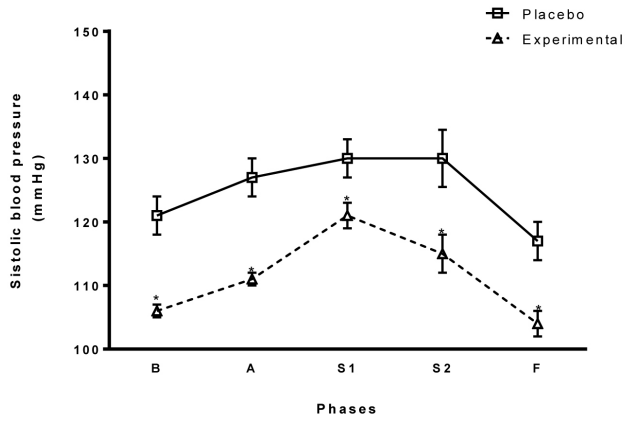
Table 3. Characteristics of the sample at baseline moment, during repeated doses.

Variables	Placebo	Experimental	P
Variables	Placebo	Experimental	P
Subject	15	15	-
Age[1](years)	24.7±3.5	26.4±3.0	0.16
STAI-T[2] (points)	44 (42-47)	46 (43-48)	0.40
STAI-E[2] (points)	42 (38-45)	45 (42-48)	0.07
SBP[3] (mmHg)	121±3.0	106±1.0	0.0001*
DBP[3] (mmHg)	66±3.0	67±1.0	0.70
HR[3] (bpm)	70±4.0	79±3.0	0.08
ET[3] (°C)	30.8±0.5	31±0.5	0.70
ECS[3] (μS)	3.5±0.5	3±0.3	0.40

[1]:Mean ± standard deviation. [2]: Median (percentile 25 e 75). [3]: Mean ± standard error mean. STAI-T: Trait anxiety inventory; STAI-E: State anxiety inventory; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; HR: Heart rate; ET: extremities temperature; ECS: Electrical Conductance of Skin, mmHg: millimeters of mercury, bpm beats per minute. °C: degree Celsius. μS: microsiemens. *: Significant compared to placebo ($p<0.05$). Estatistic test: unpaired t test.

The comparison between SBP groups was reduced, in mmHg, in the experimental group compared to the placebo group during all phases. Experimental: 106 ± 1.0 (B) 111 ± 1.0 (A) 121 ± 2.0 (S1) 115 ± 3 (S2) 104 ± 2.0 (F) and Placebo: 121 ± 3.0; 127 ± 3.0; 130 ± 3.0; 130 ± 4.5; 117 ± 3.0 ($p < 0.05$) **(Figure 2)**. There were no significant changes as the DBP in any of the five phases evaluated ($p > 0.05$). HR increased in stressful phase (86 ± 3.0 bpm) in the experimental group compared to the placebo group (77 ± 4.0 bpm), as well as during the speech 1 (91 ± 3.0 bpm) in the experimental group compared to the placebo group (3.0 ± 81 bpm) ($p < 0.05$). Considering all stages of SPS the analysis between the groups showed no significant differences of the ECS and ET ($p > 0.05$). **(Figure 2)**

Figure 2: *Passiflora incarnata* L. effect on the systolic blood pressure of groups submitted to Simulated Public Speaking test.



*: Significant compared to the placebo group ($p<0.05$). Values are presented as mean ± standard error. Estatistic test: unpaired t test. B (Basal); A (Stressful); S1 (Speach 1); S2 (Speach 2) e F (Final).

The STAI-T was evaluated only in the initial moment in each of the experimental groups, the analysis of the data shows that the same participants began the test with mild anxiety levels (40-60 points) [17]. There was no difference between groups ($p > 0.05$). There was an increase ($p < 0.05$) of the STAI-E scores in the experimental group in stressful phase (44 (41-46)) compared to the placebo group (41 (38-44 points)). This study showed no statistical difference between the groups in the side effects ($p > 0.05$) in any of the designs.

Discussion

Studies conducted investigating the Passiflora spp. effects on humans about anxiety are scarce, especially using SPS test, making it unprecedented in this respect.

The SPS test shows to be valid for the assessment of anxiety in healthy volunteers independent of the individual's anxiety-trait, unlike other methods, such

as the Stroop Color-Word Test, which is prerequisite high levels of trait anxiety [15]. In the present study there were no significant differences in anxiety-Trait Anxiety nor the State when the groups were compared. A likely explanation is the fact that of the little sensitivity by the scale effects of anxiolytic drugs [16].

In this study, using the *Passiflora incarnata* L., there was a reduction in heart rate (HR) during the whole speech in the experimental group with single administration, but not in multiple doses. In previous research with human beings, of cannabidiol (600mg) (single dose) did not alter the FC [19].

Preclinical study, acute and chronic, previously performed in spontaneously hypertensive rats (SHR) demonstrated satisfactory results in CF after the shell of the fruit of Passiflora edulis administration differing from the present study that used the dry extract of the whole plant from another species in healthy subjects. The effects were coming from the edulílico acid and anthocyanin fraction [20].

Regarding systolic blood pressure (SBP) there was a reduction in all phases of speech in experimental group compared to the placebo group in the design of repeated doses. The antihypertensive properties of passion fruit were previously reported in preclinical studies performed in SHR rats. A study supplemented the rats with the passion fruit flesh for five days to give hypotensive effect, and the effect attributed to the increased antioxidant activity in animals, since the pulp of the fruit is rich in phenolic compounds, ascorbic acid, carotenoids and flavonoids [21, 22].

In humans, the use of natural products such as Erytrina mulungu (500 mg) acute form Matricaria recutita (chamomile; 220 mg) for 8 weeks and oficinallis L. Melissa (lemon balm, 600 mg) for two weeks demonstrated anxiolytic effects by Corah scales modified, Hamilton Anxiety Scale and the Free Rating Scale for Anxiety, respectively [23, 24, 25].

The fermented ginseng was used in a clinical study of character and preclinical and demonstrated anxiolytic effect in the test light-dark transition in animals and Trait Anxiety Inventory-State, reducing the anxiety levels in humans. This study applied the STAI before and after within the same group evaluated (placebo or experimental). RNAm gene expression of enzymes related to the production of GABA in the hippocampus increased, although salivary markers such as cortisol and urinary such as 8-hidroxideoxiganosina stress did not change [26].

The exact mechanism of action of Passiflora spp. anxiety is unknown, but inhibition of monoamine oxidase (MAO) and activating receptors of the gamma-aminobutyric acid (GABA) may be involved. GABA is the major inhibitory neurotransmitter and orchestrates neuronal excitability. During stressful times it acts in the discontinuance of neuronal circuits. The low GABA levels are correlated with anxiety. In addition, GABA has hypotensive paper and is naturally available plants, herbs [27] and in some foods such as gabaron tea and fermented foods such as yogurt, milk and cheese, among others [22, 28]. GABA has been classified as bioactive compound in food and drugs. [29]

Anxiety has been widely studied, but few studies have evaluated the effects of food. There is an association between the consumption of milk with full fat such as ice creams and creams with higher levels of self-reported anxiety, stress, depression of mood and memory reduction [30]. Dietary interventions during pregnancy can reduce the high levels of anxiety during this period and promote a better reflection for birth and child development [31].

Studies of nutrients such as magnesium combined with other herbs [32, 33, 34] lysine with arginine [35, 36] have demonstrated anxiolytic effects. In addition, n-3 fatty acids (mood regulation), selenium, L-tryptophan amino acid (serotonin production), L-phenylalanine and L-tyrosine (production of dopamine and noradrenaline), vitamin E, vitamin C and vitamin D appear to have functional effects, further supporting the role of nutrition in alleviating the symptoms of anxiety [27].

The electrical conductance skin (ECS) did not change in any of the tests, even though the changes in the emotional state causes neurovegetative changes, for example, increase the activity of the sweat glands innervated by cholinergic fibers of the Central Nervous System (CNS) [16]. Likewise the temperature of the extremities has not changed. No studies were found that evaluated this variable to compare the results of the current study.

A study with Disorder Social Anxiety (SAD) patients with cannabidiol (600 mg) showed no change in physiological measures (SBP, DBP, HR and ECS) in either group (healthy, with and without SAD) and evaluated phases. The Group with SAD who received cannabidiol reduced the anxiety levels compared to Healthy Group in the phases of anticipation, speech and completion. The placebo group SAD reached high scores of anxiety about the healthy group during the phase of speech [19]. There was no change of these measures in this study.

One of the main investigations of the effects of P. incarnata on anxiety was a clinical, randomized and controlled in that evaluated the use of P. incarnata extract in the treatment of generalized anxiety disorder (n = 32). Similar results were obtained between the groups treated with oxazepam (30 mg/day) and with the product P. incarnata extract base (45 drops/day) for four weeks. The latter group showed better performance at work. The group that received P. incarnata had fewer adverse effects than synthetic drugs. The evaluator anxiety instrument was the Hamilton Anxiety Scale [37].

Conclusions

Supplementation using *Passiflora incarnata* L. capsules (500 mg) decreased cardiovascular signs, both in single and multiple doses, associated with the stress of public speaking. More research is needed to determine the exact mechanisms of action of *Passiflora incarnata* L., and and also its application in different anxiety disorders.

Conflict of Interest

The authors declare no conflict of interest.

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