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Evaluation of the anxiolytic-like effects of *Cecropia glazioui* Sneth in mice

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Abstract

Cecropia glazioui Sneth has been used in most Latin American countries as an antihypertensive, cardiotonic, and antiasthmatic folk medicine. In the cardiovascular studies to define its antihypertensive action it was noteworthy that animals treated with the aqueous extract (AE) of *C. glazioui* were much calmer than control animals. That observation prompted the present study, aimed at an investigation of the effects of AE and of two semipurified fractions on mouse behavior as evaluated in the elevated plus-maze test (EPM). Male adult Swiss mice were treated with AE (0.25–1 g/kg po) acutely (1 h) or repeatedly (24, 7, and 1.5 h before the test). After repeated administration of AE, the frequency of entries in the open arms of EPM was increased threefold. A similar profile of action was observed after treatment with the butanolic fraction (Fbut) but not with the aqueous fraction (Faq). These findings suggest that the AE of *C. glazioui* promotes an anxiolytic-like effect in mice. The active principles responsible for this action are present in the less polar fraction of the extract, the main constituents of which are flavonoids and terpenes, among other compounds. © 2002 Elsevier Science Inc. All rights reserved.

Keywords: Anxiety; Medicinal plant; Validation; Phytomedicine; Plus-maze

1. Introduction

Anxiety is a common psychopathology that in adulthood affects 1 in 5 Western women and 1 in 10 men, most of whom are prescribed benzodiazepines for treatment (Brawman-Mintzer and Lydiard, 1997). Benzodiazepines have been extensively used for the last 40 years to treat several forms of anxiety (Jordan et al., 1996; Rickels and Schweizer, 1997) and, although these compounds have well-known benefits, their side-effects are prominent, including sedation, muscle-relaxation, ethanol potentiation, anterograde amnesia, and pharmacological dependence (Jordan et al., 1996). In the search for an alternative, more specific, and perhaps cost-free therapy, research has been conducted to investigate natural anxiolytic drugs as well as new depressant principles (Luk et al., 1983; Nielsen et al., 1988; Medina et al., 1990, 1991; Picq et al., 1991; Marder et al., 1996; Haberlein et al., 1994; Viola et al., 1994, 1995; Wolfman et al., 1994; Okuyama et al., 1996). Plants have long been used to treat central nervous system (CNS) disorders. Folk medicine particularly values, for example, plants that "calm down," tranquilize, and raise mood, such as *Passiflora coerulea* (Medina et al., 1990), *Valeriana officinalis* (Santos et al., 1994a,b; Cavadas et al., 1995), *Matricaria recutita* (Viola et al., 1995), *Jatropa cilliata* (Okuyama et al., 1996), *Salvia guaranitica* (Marder et al., 1996), *Tilia tormentosa* (Viola et al., 1994), and *Tilia europeae* (Cavadas et al., 1997).

Cecropia glazioui Sneth (Moraceae) is popularly named "embaúba" in Brazil. There are ethno-pharmacological reports in tropical and subtropical Latin America of the plant being used as an antihypertensive, cardiotonic, and antiasthmatic remedy (Pio Correa, 1984; Simões et al., 1986; Di Stasi et al., 1989; Matos, 1991). These folk

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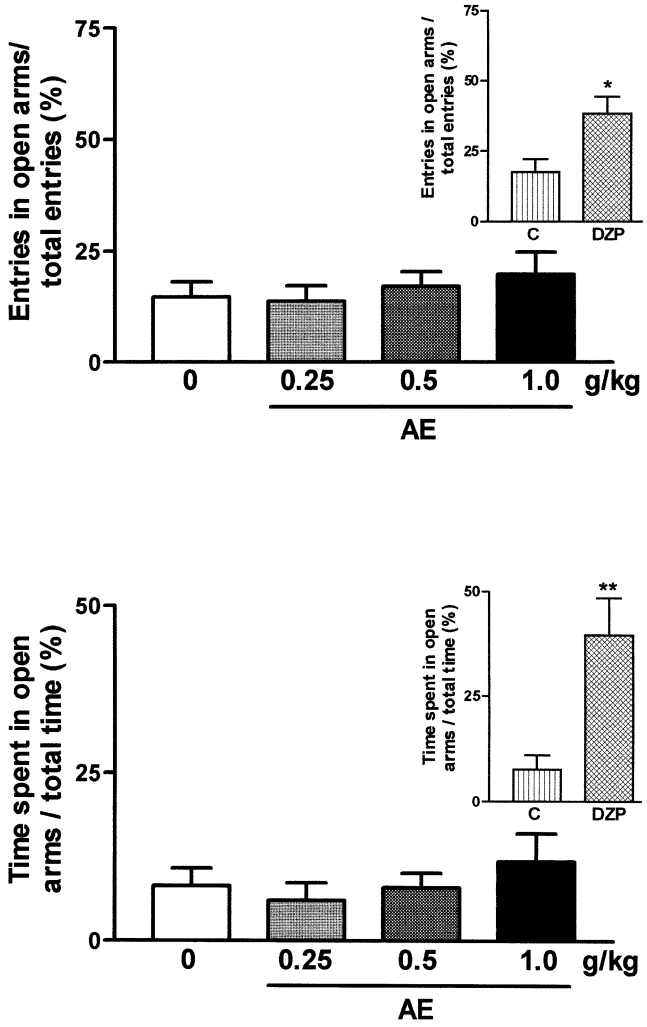

Fig. 1. Effect of a single-dose oral treatment with the AE of *C. glazioui* Sneth on plus-maze test performance in mice. Percentage of entries and time spent in open arms (open/total × 100) is shown. AE was administered per os. Diazepam 1 mg/kg (DZP) and its control solution were administered intraperitoneally. Bars and vertical lines represent the means and S.E.M. of each group. ** $P<.01$; * $P<.05$ compared to control groups (ANOVA followed by Bonferroni's test, except for diazepam, for which nonpaired Student's *t* test was used).

Table 2
Effects of a single-dose treatment with the reference drug diazepam (1.0 mg/kg ip) upon the behavioral parameters recorded in the plus-maze in mice

Plus-maze measures	Vehicle	Diazepam (1 mg/kg)	*t* (14)
Total arm entries	9.0 ± 1.74	20.0 ± 2.07**	4.065
Closed-arm entries	7.1 ± 1.20	11.9 ± 1.56*	2.397
Closed-arm time (s)	183.5 ± 11.68	108.5 ± 1.56**	4.564
Central time (s)	102.1 ± 8.48	102.2 ± 19.11	0.0059
HD	10.9 ± 1.61	33.8 ± 5.06**	4.309
Rearing	14.8 ± 1.98	13.1 ± 2.18	0.5514
SAP	20.0 ± 1.18	10.9 ± 2.26**	3.166
Grooming	0.9 ± 0.23	1.5 ± 0.38	1.418
Fecal boli	0.2 ± 0.25	0.4 ± 0.38	0.2774
Number of animals	8	8	–

Data are expressed as mean ± S.E.M.
* $P<.05$ compared to control group; nonpaired Student's *t* test.
** $P<.01$ compared to control group; nonpaired Student's *t* test.

indications have been scientifically investigated in the last 10 years, mainly with regard to the plant's cardiovascular actions (Vidrio et al., 1982; Nicolau et al., 1988; Borges, 1992; Borges et al., 1990; Cysneiros, 1996). It has been shown that the antihypertensive/hypotensive action may be related to blockade of voltage-gated calcium channels in vascular smooth muscle (Cysneiros, 1996; Cysneiros et al., 1994, 1995; Lapa et al., 1999), whereas the positive inotropic/chronotropic and broncho-relaxant effects seem to be produced by a β-adrenergic activity (Cysneiros et al., 1996).

During experiments in which the tail blood pressure was being recorded in rats chronically treated with *C. glazioui* it was noteworthy that the animals were much calmer than the control water-treated rats (Lapa et al., 1999). They were easy to handle although no hindrance of movements or depression could be detected. Anecdotal reports of a 'tranquilizer' effect of *C. glazioui* are uncommon, although no systematic studies of the central effects have been reported yet. These observations prompted us to study the effects of *C. glazioui* upon the CNS. The present study aimed to investigate the putative anxiolytic-like activity of the aque-

Table 1
Effects of a single-dose treatment with the AE of *C. glazioui* Sneth (0.25–1.0 g/kg po) upon the behavioral parameters recorded in the plus-maze in mice

Plus-maze measure	Vehicle	AE (0.25 g/kg)	AE (0.5 g/kg)	AE (1.0 g/kg)	*F*(3,59)
Total arm entries	7.1 ± 0.78	6.1 ± 0.82	7.5 ± 0.61	8.3 ± 0.83	1.442
Closed-arm entries	5.9 ± 0.64	5.06 ± 0.57	5.5 ± 0.36	5.9 ± 0.45	0.6434
Closed-arm time (s)	196 ± 13.04	194.7 ± 13.66	179.4 ± 11.69	177.9 ± 13.40	0.5549
Central time (s)	78.6 ± 10.07	91.4 ± 14.03	87.5 ± 13.04	96.1 ± 12.19	0.3533
HD	7.9 ± 1.20	10.1 ± 2.00	11.2 ± 1.69	12.3 ± 1.21	1.623
Rearing	12.7 ± 1.22	11.4 ± 0.95	11.8 ± 1.25	12.3 ± 1.21	0.2284
SAP	20.0 ± 2.0	18.0 ± 1.69	19.2 ± 2.04	17.9 ± 1.61	0.3032
Grooming	0.8 ± 0.17	1.2 ± 0.33	1.12 ± 0.29	0.8 ± 0.30	0.6453
Fecal boli	1.2 ± 0.55	0.7 ± 0.25	1.2 ± 0.46	0.8 ± 0.34	0.3897
Number of animals	16	16	16	15	–

Data are expressed as mean ± S.E.M.
All comparisons were made with ANOVA followed by Bonferroni's test.

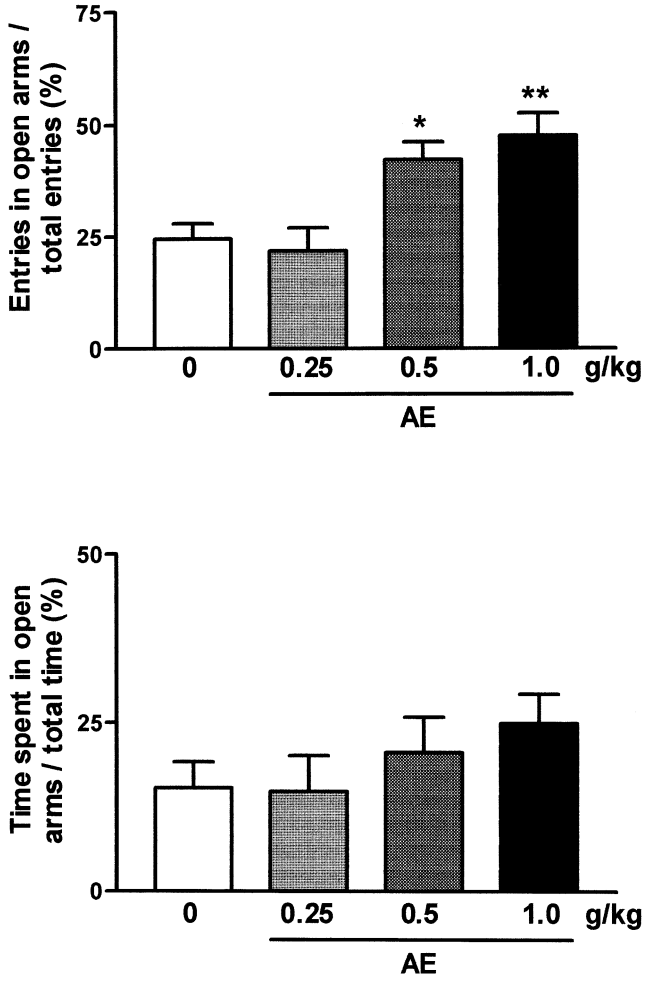

Fig. 2. Effect of the oral treatment with repeated doses (three doses in 24 h) of the AE of *C. glazioui* Sneth on plus-maze test performance in mice. Percentage of entries and time spent in the open arms (open/total × 100) is shown. AE was administered per os. Bars and vertical lines represent the means and S.E.M. of each group. ** $P < .01$; * $P < .05$ compared to control groups (ANOVA followed by Bonferroni's test).

ous extract (AE) and two semipurified fractions obtained from *C. glazioui*. The plus-maze test in mice was selected to evaluate the CNS effect.

2. Method

2.1. Animals

Male adult Swiss mice weighing 30–35 g were used in all experiments. Animals were maintained on a 12-h light–dark cycle (lights on at 7:00 a.m.) at constant room temperature (23 ± 2 °C). Mice were housed in groups (20 per cage) and had free access to food and water, except during the experiments. All animals were allowed to adapt to the laboratory conditions for at least 1 week before the beginning of the experiments. Each animal was used just once. All experiments were conducted in accordance with international standards of animal welfare recommended by the Brazilian Society of Neuroscience and Behavior (Act 1992) and approved by the University Committee for Animal Care in Research. The minimum number of animals and duration of observation required to obtain consistent data were employed.

2.2. Drugs

Diazepam (Dienpax, Sanofi-Winthrop Lab., Brazil) was used as a reference drug (positive control). It was dissolved in saline (0.9% NaCl) immediately before intraperitoneal (ip) injection.

2.3. Botanical material

The leaves of *C. glazioui* Sneth were obtained from a controlled plantation at the farm of CPQBA, an interdisciplinary research center at the University of Campinas (São Paulo, Brazil). The harvest was directed by Dr. P.M. Magalhães and Dr. I. Montanari who were also responsible for the plant identification and stabilization. A voucher specimen is deposited at that university.

2.4. Extraction and purification

The extract of *C. glazioui* was prepared as described by folk medicine. The ground dried leaves were extracted in hot water (2.5%, 72 °C) for 30 min (yield 10%). The AE

Table 3
Behavioral parameters recorded in the plus-maze from mice treated with repeated doses of the AE of *C. glazioui* Sneth (0.25–1.0 g/kg po)

Plus-maze measures	Vehicle	AE (0.25 g/kg)	AE (0.5 g/kg)	AE (1.0 g/kg)	$F(3,38)$
Total arm entries	9.3 ± 0.87	10.3 ± 1.15	9.6 ± 1.14	12.1 ± 1.76	0.9640
Closed-arm entries	5.9 ± 0.79	7.7 ± 0.60	5.2 ± 0.58	6.3 ± 1.03	1.893
Closed-arm time (s)	183.9 ± 17.42	178.1 ± 15.64	200.8 ± 14.69	168.3 ± 16.31	0.7728
Central time (s)	83.7 ± 9.79	91.7 ± 11.85	47.2 ± 5.39*	52.7 ± 7.40*	6.592
HD	11.7 ± 1.56	10.9 ± 1.99	13.7 ± 2.82	15.7 ± 2.65	0.7824
Rearing	11.9 ± 1.80	12.2 ± 1.16	12.2 ± 0.99	11.3 ± 1.34	0.09627
SAP	16.5 ± 2.36	17.5 ± 2.32	11.8 ± 1.14	9.8 ± 2.31	3.256
Grooming	1.1 ± 0.31	1.1 ± 0.35	1.91 ± 0.54	1.4 ± 0.30	0.9441
Fecal boli	0.5 ± 0.34	0.8 ± 0.47	0.4 ± 0.15	0.6 ± 0.30	0.2658
Number of animals	10	10	12	10	–

Data are expressed as mean ± S.E.M.

* $P < .05$ compared to control group; ANOVA followed by Bonferroni's test.

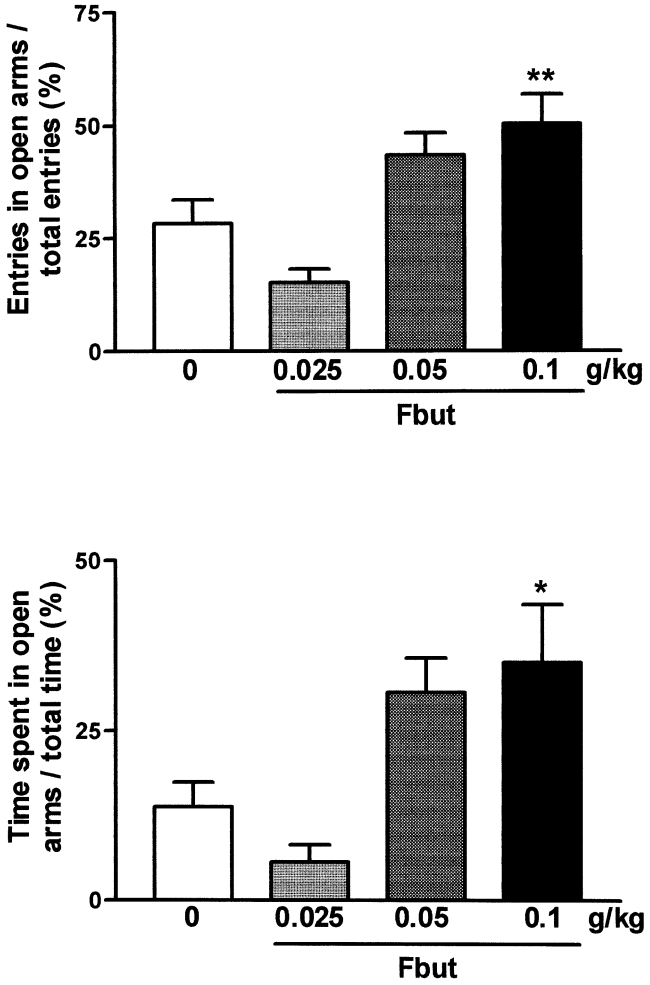

Fig. 3. Effect of the oral treatment with repeated doses (three doses over 24 h) of the Fbut obtained from the AE of *C. glazioui* Sneth on plus-maze test performance in mice. Percentage of entries and time spent in the open arms (open/total × 100) is shown. Fbut was administered per os. Bars and vertical lines represent the means and S.E.M. of each group. ** $P<.01$; * $P<.05$ compared to control groups (ANOVA followed by Bonferroni's test).

was concentrated under vacuum to one fifth of its original volume and freeze-dried. The concentrated AE (200 ml) was partitioned with *n*-butanol (200 ml) and the resulting butanolic (Fbut; yield 10%) and aqueous (Faq; yield 12%) fractions were concentrated under vacuum and freeze-dried. To control the quality of all plant preparations, each extract or fraction was analyzed by high-pressure liquid chromatography (HPLC) using known chemical constituents of the plant as chemical markers. All the extracts were freshly resuspended in tap water to be administered per os (po).

2.5. Behavioral testing

The elevated plus-maze (EPM) was slightly modified from that used by Lister (1987). It consisted of two open arms ($30 \times 5 \times 0.25$ cm) and two enclosed arms ($30 \times 5 \times 15$ cm), extending from a central platform (5×5 cm) and arranged such that two pairs of identical arms were opposite to each other. The apparatus was raised to a height of 50 cm above floor level. The maze floor was constructed from black Plexiglas and the walls from clear Plexiglas. At the beginning of the test, each mouse was placed on the central platform facing an enclosed arm. After the test (5 min), the maze was carefully cleaned with wet tissue paper (10% ethanol solution). Tests were carried out during the light period (1:00–5:00 p.m.). Mouse behavior was filmed under red light illumination (15 W) by using a video camera located 100 cm above the maze. The conventional spatial–temporal measures were the number of entries (all four paws on open or enclosed arms and expressed as percentage of total entries), the time spent on open or enclosed arms (expressed as percentage of closed + open arm time spent), and the time on the central platform. Ethologically derived measures were grooming, rearing, stretched attend postures (SAP), head dipping (HD), and defecation as an emotionally related parameter (Rodgers and Dalvi, 1997).

2.6. Experimental procedures

In Experiment 1, mice were treated with the AE of *C. glazioui* (0.25, 0.5, and 1.0 g/kg po) and 1 h afterwards they were submitted to the plus-maze test for 5 min. Mice treated with either diazepam (1 mg/kg ip) or water (0.5 ml po) were the positive and negative control, respectively.

Table 4
Behavioral parameters recorded in the plus-maze from mice tre1ated with repeated doses of the Fbut (25–100 mg/kg po) obtained from the AE of *C. glazioui* Sneth

Plus-maze measures	Vehicle	Fbut (25 mg/kg)	Fbut (50 mg/kg)	Fbut (100 mg/kg)	*F*(3,30)
Total arm entries	9.7 ± 1.11	9.9 ± 1.14	11.6 ± 1.50	13.9 ± 2.40	1.487
Closed-arm entries	7.1 ± 1.04	8.2 ± 0.88	6.6 ± 1.11	6.2 ± 0.80	0.4973
Closed-arm time (s)	190.8 ± 11.97	214.8 ± 10.71	169.6 ± 11.37	150.0 ± 24.51	2.887
Central time (s)	78.3 ± 8.91	72.8 ± 8.54	55.3 ± 8.15	46.3 ± 7.32*	3.355
HD	11.5 ± 1.52	6.8 ± 1.03	16.4 ± 3.34	20.8 ± 4.72	4.006
Rearing	12.3 ± 1.25	13.1 ± 2.03	13.9 ± 1.84	11.3 ± 1.96	0.3615
SAP	11.9 ± 1.78	8.8 ± 1.62	10.7 ± 2.98	10.4 ± 1.70	0.438
Grooming	1.0 ± 0.15	1.8 ± 0.59	0.7 ± 0.28	1.4 ± 0.38	1.413
Fecal boli	0.2 ± 0.13	0.1 ± 0.12	0.1 ± 0.14	0.4 ± 0.34	0.4875
Number of animals	10	8	7	9	–

Data are expressed as mean ± S.E.M.

* $P<.05$ compared to control group; ANOVA followed by Bonferroni's test.

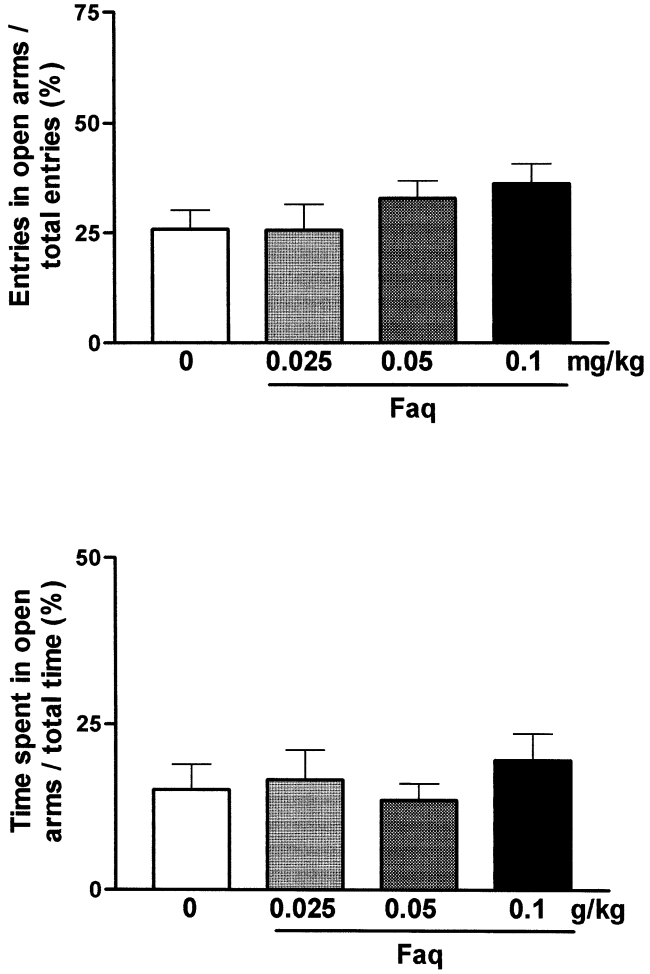

Fig. 4. Effect of the oral treatment with repeated doses (three doses over 24 h) of the Faq obtained from the AE of *C. glazioui* Sneth on plus-maze test performance in mice. Percentage of entries and time spent in the open arms (open/total × 100) is shown. Faq was administered per os. Bars and vertical lines represent the means and S.E.M. of each group. $P > .05$ compared to control groups (ANOVA followed by Bonferroni's test).

In Experiment 2, mice were treated three times with AE at 24, 7, and 1.5 h before the 5-min test, as proposed by Porsolt et al. (1977) to evaluate antidepressant drugs.

In the third experiment, mice were treated three times with either the Fbut or the Faq of the AE of *C. glazioui* (25, 50, and 100 mg/kg po), as previously described, and submitted to the plus-maze test for 5 min.

2.7. Statistics

Data were analyzed by Graphpad INSTAT version 2.05 software and they were presented as mean ± S.E.M. values. The statistical tests used were the nonpaired Student's *t* test (diazepam data) or one-way ANOVA followed by Bonferroni's test (AE, Fbut and Faq data). Differences between experimental groups were considered statistically significant when P was less than 0.05.

3. Results

3.1. Effect of acute oral treatment with the AE of C. glazioui

The acute treatment with AE did not alter the parameters evaluated in the plus-maze ($P > .05$). The frequency of open-arm entries and time spent on these arms are shown in Fig. 1, and the other parameters are shown in Table 1. Compared to the acute treatment group, the positive control (mice treated with diazepam) spent significantly more time on the open arms ($P < .01$) and presented an increased percentage of entries in these arms ($P < .05$), as depicted in Fig. 1. These animals also showed increases in the number of HD, the number of entries into the enclosed arms, and total entries, and a reduced number of SAP and time spent on the maze-enclosed arms, as shown in Table 2. The time spent in the central platform, grooming behavior, and defecation did not differ from the vehicle-treated group (Table 2).

3.2. Effect of repeated oral treatment with the AE of C. glazioui

The animals treated with AE (0.5 and 1.0 g/kg) three times over a 24-h period increased the number of open-arm entries ($P < .05$) although the time spent on these arms was

Table 5
Behavioral parameters recorded in the plus-maze from mice treated with repeated doses of the Faq (25 – 100 mg/kg po) obtained from the AE of *C. glazioui* Sneth

Plus-maze measures	Vehicle	Faq (25 mg/kg)	Faq (50 mg/kg)	Faq (100 mg/kg)	$F(3,36)$
Total arm entries	9.5 ± 1.46	11.0 ± 1.45	9.6 ± 1.16	8.6 ± 0.88	0.6098
Closed-arm entries	6.8 ± 1.10	7.5 ± 0.62	6.5 ± 0.93	5.2 ± 0.40	1.356
Closed-arm time (s)	196.9 ± 19.61	173.7 ± 11.36	205.6 ± 15.30	194.4 ± 15.4	0.8099
Central time (s)	72.1 ± 14.30	89.4 ± 10.85	70.5 ± 12.06	58.7 ± 10.53	1.119
HD	11.9 ± 3.20	10.6 ± 1.57	9.7 ± 1.48	15.8 ± 1.78	1.497
Rearing	11.6 ± 1.47	12.7 ± 1.44	13.5 ± 2.54	15.2 ± 1.59	0.6901
SAP	16.9 ± 2.48	16.4 ± 1.96	13.8 ± 1.87	12.6 ± 2.25	0.9244
Grooming	0.9 ± 0.18	0.9 ± 0.25	0.7 ± 0.21	1.9 ± 0.59	2.467
Fecal boli	0.2 ± 0.13	0.5 ± 0.39	0.7 ± 0.21	1.0 ± 0.5	0.9418
Number of animals	10	11	10	9	–

Data are expressed as mean ± S.E.M.
All comparisons were made with ANOVA followed by Bonferroni's test.

not changed ($P>.05$; Fig. 2). The treated mice also spent less time on the central platform ($P<.05$) but the other recorded parameters were not modified by the repeated treatment (Table 3). The lowest dose (AE 0.25 g/kg) did not modify any parameter evaluated in the EPM (Fig. 2 and Table 3).

3.3. Effect of repeated oral treatment with the semipurified fractions of C. glazioui

Mice treated three times with Fbut (25–100 mg/kg) increased the frequency of open-arm entries ($P<.05$) and spent more time on these arms ($P<.01$; Fig. 3). This dose also reduced the time spent in the central platform ($P<.05$; Table 4). Other behavioral parameters were not modified by the treatment, as shown in Table 4 ($P>.05$).

The treatment with Faq (25–100 mg/kg) did not alter any parameter evaluated in the EPM test. The entries into and time spent on open arms ($P>.05$) are shown in Fig. 4. The other behavioral parameters are presented in Table 5.

4. Discussion

In a previous publication on the central effects of *C. glazioui* we showed that long-term treatment with the AE induces anxiolytic and antidepressant effects in normotensive rats as well as in L-NAME hypertensive rats (Lapa et al., 1999). The aim of the current study was to further analyze the anxiolytic-like action of the AE of *C. glazioui* and its semipurified fractions. The activity of semipurified fractions of the active extract was studied in order to guide the future chemical identification of the active compounds. The behavioral data showed that the effect of *C. glazioui* was not quite evident after a single-dose treatment with AE but a significant anxiolytic-like activity was observed after the repeated treatment, a pattern already reported for the hypotensive action (Lapa et al., 1999). Furthermore, it was found that the anxiolytic-like activity was 5 to 10 times greater in the Fbut than in AE, indicating the purification of their active principles.

An anxiolytic-like effect was indicated by the increased frequency of entries into the open arms of the plus-maze. This primary index of anxiety is spatiotemporal in nature: it is reduced by anxiolytic drugs and can be increased by anxiogenic compounds (Rodgers and Dalvi, 1997). The decreased time spent on the central platform is another indication of a reduced 'decision-making' behavior. Both parameters are accepted as reliable indicators of anxiety and fearfulness (Ramos et al., 1997).

No treatment altered the other behavioral parameters registered in the plus-maze, including the number of entries into the enclosed arms, a well accepted measure of locomotor activity (Ramos et al., 1997; Rodgers and Dalvi, 1997; Rodgers et al., 1999). These observations also indicate that the anxiolytic-like effect of *C. glazioui* is quite selective, and not merely the result of either a general stimulation of locomotor activity or an exploratory behavior consequent to the exposure to a novel environment. Essentially, the same reasoning can be applied to the results obtained using the Fbut of *C. glazioui*, which also enhanced the frequency of entries into and the time spent on the open arms (both anxiolytic-like effects) without affecting the number of entries into the enclosed arms (locomotor activity effect). Such a view is reinforced by this compound's reported failure to modify motor coordination in the rotarod test (Baretta et al., 1998).

Cecropia is rich in flavonoids (Neidlein and Koch, 1980). Flavonoids with anxiolytic and/or antidepressant activity have also been described in many plant species used in folk medicine to depress the CNS. This effect has been ascribed to their affinity for the central benzodiazepine receptor (Medina et al., 1993, 1997; Griebel et al., 1999; Paladini et al., 1999). The two flavonoids orientin and iso-orientin, isolated from the active Fbut, could be responsible for the observed anxiolytic-like effect of *C. glazioui* (Dr. Luce M. Brandão Torres, personal communication). The mild sedative and anxiolytic effect produced by these compounds (Okuyama et al., 1996) might be regarded as additional evidence for the results herein described, although the low affinity of these flavonoids for the benzodiazepine receptor in vivo does not fully support the pharmacological action observed in our experiments. Nevertheless, alternative explanations can be raised to the underlying mechanisms of this effect. Since preliminary studies showed an increase in the hippocampal levels of 5-HT in rats treated with AE (Lapa et al., 1999), this biochemical effect could be a plausible explanation to its anxiolytic-like effect and this hypothesis is presently under investigation. 5-HT_{1A} receptor agonists, such as buspirone, are also used to treat anxiety (Lister, 1987; Jordan et al., 1996). Moreover, selective serotonin reuptake inhibitors, such as fluoxetine, are effective in treating a wide spectrum of mood disorders including depression, panic disorder, and anxiety (Kilts, 1994; Rodgers and Dalvi, 1997). Actually, the effects of serotonergic agents on different anxiety models are controversial (for review see Griebel, 1995).

Another possibility to explain the anxiolytic-like effect reported here is the action of some constituents of the AE from *C. glazioui* on the voltage-dependent calcium influx since Fbut and F4, one of its subfractions, blocked both the [^{45}Ca] influx in a rat uterus ring preparation and the Ca^{2+} currents in chromaffin cells from the PC12 cell line (Lapa et al., 1999). Some calcium channel blockers, such as nimodipine and nifedipine, can cross the blood–brain barrier and exert central actions including anxiolytic, anticonvulsant, antidepressant, among other central effects (Raeburn and Gonzalez, 1988; Soubrié, 1989; Pucilowski, 1992; De Vry et al., 1997). The exact site at which these compounds act to achieve their psychotropic and behavioral effects is yet unknown. However, it should be noted that the hippocampus has high levels of dihydropyridine receptors (Belleman et al.,

1983) that amplify the clinical potential of calcium channel antagonists at the CNS level. This hypothesis on the underlying mechanism of action is also under investigation, although we do not believe it is feasible since the doses of calcium channel blockers necessary to promote behavioral changes are generally higher than the doses that produce other pharmacodynamic effects (Soubrié, 1989).

In summary, the present results demonstrate an anxiolytic-like effect of the AE from leaves of *C. glazioui* Sneth. The purification process was effective in concentrating the active principle(s) responsible for the anxiolytic-like action of *C. glazioui* since its Fbut was 5 to 10 times more potent in promoting a similar activity. This anxiolytic-like effect may involve the serotonergic system or, alternatively, it may be due to a mechanism involving the blockade of calcium channels. The exact underlying mechanism of action remains to be elucidated but the present findings are important because they validate one of the folk uses of the *C. glazioui* as a medicinal plant in Latin America.

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References

Baretta IP, De Lima TCM, Cysneiros RM, Tanae MM, Lima-Landman AJ, Lapa AJ. Avaliação dos efeitos centrais da *Cecropia glazioui* Sneth em ratos normo e hipertensos. XV Simpósio de Plantas Medicinais do Brasil, Águas de Lindóia.1998;P01.189.

Belleman P, Schade A, Toward R. Dihydropyridine receptor in rat brain labeled with (^{3}H)nimodipine. Proc Natl Acad Sci USA 1983;80: 2356–60.

Borges ACR. Mecanismo de ação hipotensora da *Cecropia glazioui* Sneth. MSc dissertation, UNIFESP-EPM, 1992.

Borges ACR, Torres LMB, Souccar C, Lapa AJ. Atividade hipotensora do extrato aquoso e de frações semi-purificadas de *Cecropia glazioui* Sneth. XI Simpósio de Plantas Medicinais do Brasil, João Pessoa. 1990;P089.

Brawman-Mintzer O, Lydiard RB. Biological basis of generalized anxiety disorder. J Clin Psychiatry 1997;51:16–25.

Cavadas C, Araujo I, Cotrim MD, Amaral T, Cunha AP, Macedo T, Ribeiro CF. In vitro study on the interaction of *Valeriana officinalis* L. extracts and their amino acids on $GABA_A$ receptor in rat brain. Arzneim-Forsch 1995;45(7):753–5.

Cavadas C, Fontes-Ribeiro CA, Santos MS, Cunha AP, Macedo T, Caramona MM, Cotrim MD. In vitro study on the interaction of *Tilia europeae* L. aqueous extract with $GABA_A$ receptors in rat brain. Phytother Res 1997;11:17–21.

Cysneiros RM. Mecanismo de ação hipotensora do extrato aquoso e frações purificadas da *Cecropia glazioui* Sneth. PhD thesis, UNIFESP-EPM, 1996.

Cysneiros RM, Nasser ALM, Souccar C, Lapa AJ. Substância(s) moduladora(s) dos canais de cálcio voltagem dependentes presentes na *Cecropia glazioui* Sneth. XIII Simpósio de Plantas Medicinais do Brasil, Fortaleza.1994;P325.

Cysneiros RM, Nasser ALM, Lapa AJ. Purificação do composto responsável pelo bloqueio do influxo de cálcio presente na *Cecropia glazioui* Sneth. X Reunião Anual da Federação das Sociedades de Biologia Experimental, Serra Negra.1995;P16.97.

Cysneiros RM, Tanae MM, Lapa AJ. Ação β-adrenérgica da *Cecropia glazioui* Sneth. XI Reunião Anual da Federação das Sociedades de Biologia Experimental, Caxambu.1996;P0.071.

De Vry J, Fritze J, Post RM. The management of coexisting depression in patients with dementia: potential of calcium channel antagonists. Clin Neuropharmacol 1997;20(1):22–35.

Di Stasi LC, Santos EMG, Santos CM, Himura CA. Plantas medicinais da Amazônia. São Paulo: Editora UNESP, 1989. pp. 137–8.

Griebel G. 5-Hydroxytryptamine-interacting drugs in animal models of anxiety disorders: more than 30 years of research. Pharmacol Ther 1995;65:319–95.

Griebel G, Perrault G, Tan S, Schoemaker H, Sanger DJ. Pharmacological studies on synthetic flavonoids: comparison with diazepam. Neuropharmacology 1999;38(7):965–77.

Haberlein H, Tschiersch KP, Schafer HL. Flavonoids from *Leptospermum scoparium* with affinity to the benzodiazepine receptor characterized by structure activity relationships and in vivo studies of a plant extract. Pharmazie 1994;49(12):912–22.

Jordan AD, Kordik CP, Reitz AB, Sanfillipo PJ. Novel anxiolytics agents—1994 to present. Expert Opin Ther Pat 1996;6(10):1047–60.

Kilts CD. Recent pharmacological advances in antidepressant therapy. AJM 1994;97(Suppl. 6A):3S–12S.

Lapa AJ, Lima-Landman MTR, Cysneiros RM, Borges CR, Souccar C, Baretta IP, De Lima TCM. A topic on new antihypertensive drug research. In: Hostettsmann K, Gupta MP, Marston A, editors. Chemistry, biological and pharmacological properties of medicinal plants from the Americas. Amsterdam: Harwood Academic Publishers, 1999. pp. 185–96.

Lister RG. The use of a plus-maze to measure anxiety in the mouse. Psychopharmacology 1987;92(2):180–5.

Luk KC, Stern L, Weigele M, O'Brien RA, Spirt N. Isolation and identification of "diazepam-like" compounds from bovine urine. J Nat Prod 1983;46(6):852–61.

Marder M, Viola H, Wasowski C, Wolfman C, Waterman PG, Cassels BK, Medina JG, Paladini AC. 6-Bromoflavone, a high affinity ligand for the central benzodiazepine receptors is a member of a family of active flavonoids. Biochem Biophys Res Commun 1996; 223(2):384–9.

Matos FJA. Farmácias vivas. Fortaleza. EUFC, 1991, p. 75.

Medina JH, Paladini AC, Wolfman C, Levi de Stein M, Calvo D, Diaz LE, Pena C. Chrysin (5,7-di-OH-flavone), a naturally-occurring ligand for benzodiazepine receptors, with anticonvulsant properties. Biochem Pharmacol 1990;40(10):2227–31.

Medina JH, Danelon JL, Wasowski C, Levi de Stein M, Paladini AC. Production of benzodiazepine-like compounds in bovine rumen. Biochem Biophys Res Commun 1991;181(3):1048–55.

Medina JH, Paladini AC, Izquierdo I. Naturally-occurring benzodiazepines and benzodiazepines-like molecules in brain. Behav Brain Res 1993; 58:1–8.

Medina JH, Viola H, Wolfman C, Marder M, Wasowski C, Calvo D, Paladini AC. Overview—flavonoids: a new family of benzodiazepine receptor ligands. Neurochemistry 1997;22(4):419–25.

Neidlein R, Koch E. Isolation and structure of constituents from *Cecropia adenopudus*. Arch Pharm 1980;313(3):199–207.

Nicolau M, Antoniolli A, De Lima TCM, Rae GA, Yunes RA, Calixto JB. Efeito do extrato bruto (EB) e de frações semipurificadas de *Cecropia glazioui* sobre a musculatura lisa e cardíaca in vitro e o sistema cardiovascular. X Simpósio de Plantas Medicinais do Brasil, São Paulo, 1988;4.

Nielsen M, Frokjaer S, Braestrup C. High affinity of the naturally-occurring biflavonoid, amentoflavon, to brain benzodiazepine receptors in vitro. Biochem Pharmacol 1988;37(17):3285–7.

Okuyama E, Okamoto Y, Yamazaki M, Satake M. Pharmacologically active components of a Peruvian medicinal plant, Huanarpo (*Jatropha cilliata*, M. Arg.). Chem Pharm Bull (Jpn) 1996;44:333–6.

Paladini AC, Marder M, Viola H, Wolfman C, Wasowski C, Medina JH. Flavonoids and the central nervous system: from forgotten factors to potent anxiolytic compounds. J Pharm Pharmacol 1999;51(5): 519–26.

Picq M, Cheav SL, Prigent AF. Effect of two flavonoid compounds on central nervous system. Analgesic activity. Life Sci 1991;49(26):1979–88.

Pio Correa M. Dicionário das Plantas úteis do Brasil e das exóticas cultivadas. Inst. Bras. Desenv. Florestal, Rio de Janeiro, vol. IV, 1984;199–200.

Porsolt RD, Le Pichon M, Jalfre M. Depression: a new animal model sensitive to antidepressant treatments. Nature 1977;266:730–2.

Pucilowski O. Psychopharmacological properties of calcium channel inhibitors. Psychopharmacology 1992;109(1–2):12–29.

Raeburn D, Gonzales RA. CNS disorders and calcium antagonists. Trends Pharmacol Sci 1988;9(4):117–9.

Ramos A, Berton O, Mormede P, Chaouloff F. A multiple-test study of anxiety-related behaviours in six inbred rat strains. Behav Brain Res 1997;85(1):57–69.

Rickels K, Schweizer E. The clinical presentation of generalized anxiety in primary-care settings: practical concepts of classification and management. J Clin Psychiatry 1997;58:4–9.

Rodgers RJ, Dalvi A. Anxiety, defence and the elevated plus-maze. Neurosci Biobehav Rev 1997;21(6):801–10.

Rodgers RJ, Haller J, Holmes A, Halasz J, Walton TJ, Brain PF. Corticosterone response to the plus-maze: high correlation with risk assessment in rats and mice. Physiol Behav 1999;68(1–2):47–53.

Santos MS, Ferreira F, Faro C, Pires E, Carvalho AP, Cunha AP, Macedo T. The amount of GABA present in aqueous extracts of valerian is sufficient to account for [^{3}H]GABA release in synaptosomes. Planta Med 1994a;60(5):475–6.

Santos MS, Ferreira F, Faro C, Cunha AP, Carvalho AP, Ribeiro CF, Macedo T. Synaptosomal GABA release as influenced by valerian root extract — involvement of GABA carrier. Arch Int Pharmacodyn Ther 1994b;327(2):220–31.

Simões CMO, Mentz LA, Schenkel EP, Irgang BE, Stehmann JR. Plantas da medicina popular no Rio Grande do Sul, Porto Alegre. Ed. UFRGS, 1986, pp. 96–7.

Soubrié P. Neuropsychological profiles of calcium antagonists. Fundam Clin Pharmacol 1989;3(Suppl.):71s–8s.

Vidrio H, Garcia-Marquez F, Reyes J, Soto RM. Hypotensive activity of *Cecropia obtusifolia*. J Pharm Sci 1982;71(4):475–6.

Viola H, Wolfman C, Levi de Stein M, Wasowski C, Pena C, Medina JH, Paladini AC. Isolation of pharmacologically active benzodiazepine receptor ligands from *Tilia tormentosa* (Tiliaceae). J Ethnopharmacol 1994;44(1):47–53.

Viola H, Wasowski C, Levi de Stein M, Wolfman C, Silveira R, Dajas F, Medina JH, Paladini AC. Apigenin, a component of *Matricaria recutita* flowers, is a central benzodiazepine receptors-ligand with anxiolytic effects. Planta Med 1995;61(3):213–6.

Wolfman C, Viola H, Paladini AC, Dajas F, Medina JH. Possible anxiolytic effects of chrysin, a central benzodiazepine receptor ligand isolated from *Passiflora coerulea*. Pharmacol, Biochem Behav 1994;47(1):1–4.