Research Article
Anxiolytic and Anticonvulsant Effects on Mice of Flavonoids, Linalool, and α-Tocopherol Presents in the Extract of Leaves of Cissus sicyoides L. (Vitaceae)

Edvaldo Rodrigues de Almeida,1 Krissia Rayane de Oliveira Rafael,1
Geraldo Bosco Lindoso Couto,2 and Ana Beatriz Matos Ishigami2

1 Evaluation Laboratory of Psychobioactive and Toxicology, Department of Antibiotics, Federal University of Pernambuco, 50670-901 Recife, PE, Brazil
2 Department of Odontology, Federal University of Pernambuco, 50670-901 Recife, PE, Brazil

Correspondence should be addressed to Edvaldo Rodrigues de Almeida, edvaldo.ra@gmail.com

Received 6 September 2008; Revised 7 November 2008; Accepted 15 December 2008

Recommended by Omar Benzakour

The aim of the present study is to demonstrate the anxiolytic and anticonvulsant effects of a hydroalcoholic extract obtained from the aerial parts of Cissus sicyoides L. (CS) (Vitaceae) on male and female mice using several behavioral assays. Groups of males and females treated via intraperitoneal (IP) with doses of 300, 600, and 1000 mg/kg of the extract showed significant action in the elevated plus-maze (EPM), time spent in the open arms, and number of entries in the open arms. The board-hole test also showed a significant increase in the time spent in head-dipping and in marble-burying test of the number of marbles buried. The same treatment increased the duration of sleeping time induced by sodium pentobarbital and also showed a significant increase in protection against pentylenetetrazole-induced convulsions. These results indicate an anxiolytic and anticonvulsant-like action from C. sicyoides L. extract on mice, probably due to the action of flavonoid(s), Linalool, and α-tocopherol present in the C. sicyoides leaves.

Copyright © 2009 Edvaldo Rodrigues de Almeida et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

1. Introduction

Cissus sicyoides (CS) belonging to the Vitaceae family comprises of about 165 genus and 1370 species, which are distributed throughout the tropics, mainly in Brazil and the Caribbean. It is popularly known as “insulinas, cipó-pucá, bejucu de porra, bejucu caro, puci, and anil trepador” [1]. Originally from the Dominican Republic [2], it is used in popular medicine as a diuretic, anti-inflammatory, and antidiabetic [3, 4]. It has also demonstrated a vasoconstrictor effect on guinea-pig aorta rings [5] and an antibacterial activity [6]. In Brazil, CS was evaluated for its anticonvulsant property, where it is used against epilepsy and cytotoxic activities [7–9]. The fact that treatment with tea induced an increase in the amount of chromosomal damage in bone marrow cells without altering the cell division cycle was also demonstrated. This plant also presents antibacterial and oxytocic activities [10], and CS contains significant amounts of α-tocopherol, a compound proved to be a useful adjunct to anticonvulsants in clinical medicine [11]. Alpha-tocopherol protects against pentylenetetrazol and methylmalonate-induced convulsions [12] and prevents the occurrence of epileptic foci in a rat model of posttraumatic epilepsy [13]. The central antinociceptive effect of C. sicyoides on mice as well as the action of dry leaves extract in pregnant rats and offspring postal development was also demonstrated. [14–16]. Phytochemistry studies identified and isolated from the aerial parts of CS a new coumarin glycoside 5,6,7,8-tetrahydroxycoumarin-5β-xylorafanoside which was obtained together with known coumarin sabandin, two flavonoids kaempferol 3-rhamnoside and quercetin 3-rhamnoside, and two steroids, sitosterol and 3β-O-β-d-glucopyranosylsitosterol [17] (see Figure 1). Leaves of the genus Cissus contain sterols, quinones, and phenolic compounds. Anthocyanins, saponins, and flavonoids are also found in the plants leaves and fruits [3]. The effect of
linalool present in the leaves of the CS was demonstrated in the protection against seizures induced in mice [7, 8, 18] (see Figure 2). However, we found no reference on its activity on the central nervous system (CNS) relating to anxiety as well as information on its acute toxicity. Benzodiazepines (BDZs) are considered safe drugs and are widely prescribed for their anxiolytic and anticonvulsant actions [19–21]. However, they may produce side effects, such as sedation and myorelaxation that are considered as unwanted effects in an anxiolytic drugs [20]. On the other hand, the existence of natural flavonoids that possess anxiolytic effect not associated with myorelaxant, amnesic, or sedative actions has been demonstrated [22]. Although alternative treatments with herbs are increasingly used by the population to alleviate affective disorders, there is a strong rejection among doctors as the use of herbs for treatment of various diseases is still scarce [23]. The antidiabetic action of the CS is in the making of clinical trials (phase II), the results obtained by the authors are promising for the future use in medical clinics [24]. This study also aims to assess the possible effects of flavonoids in the hydroalcoholic extract of the CS leaves in several behavioral tests related to anxiety in mice. The presence of α-Tocopherol has been identified in the leaves of C. sicyoides, used in clinical practice as an adjunct in the treatment of seizures [11] (see Figure 3). Our result indicates a new action for use of the C. sicyoides which can be related to the presence of the α-tocopherol as an adjuvant of the effect of sedatives together with the linalool and flavonoids present in this plant.

2. Materials and Methods

2.1. Plant Material and Extract Preparation. Aerial parts of CS were collected in the vicinity of the campus of the Federal University of Pernambuco—State of Pernambuco—Brazil in January 2005. The plant was identified by University Prof. Marlene Carvalho de Alencar Barbosa, and a voucher for specimen was deposited in the Geraldo Mariz Herbarium (UFPE) under Botanical Department N° 29040. The collected leaves were washed, dried at room temperature (28°C) in the laboratory for 25 days and ground in a mill to a grain size of <1 mm. Then, 360 grams of the powdered plant material were added to 1000 mL of alcohol and water (70:30, v/v). The dry powder yielded 30% of extract. For pharmacological testing, the extract was dissolved in saline plus Tween 80 (0.025%) solution.

2.2. Animals. Male and female two-month-old Swiss albino mice, weighing 20–30 g, were used in this experiment. The animals were housed in groups of ten per cage, with light/dark periods of 12 hours. They were fed and watered ad libitum. All experiments were conducted between 10:00 am and 4:00 pm. Female mice were tested without monitoring the oestrus cycle. All the animals were carefully monitored and maintained in accordance with the ethical recommendation of the Brazilian College of Animal Experimentation (COBEA) and the National Institute of Health Guide for Care and use of Laboratory Animals and approved by the Ethical Committee of the Federal University of Pernambuco (UFPE) protocol number 008196/2005-29.

2.3. Drugs. Diazepam (DPZ, 2.5 mg/kg, IP) was used as the standard anxiolytic drug. Pentobarbital sodium (PBS, 55 mg/kg, IP) was used as a hypnotic drug and pentylentetrazole (PTZ, 55 mg/kg, IP) as a convulsant. All drugs were obtained from Sigma Aldrich, Mo, USA, and Tween 80 was locally purchased.

2.4. Sodium Pentobarbital-Induced Sleeping Time. The mice were divided into four groups (10 animals/group). Three groups received three doses of extract (300, 600, and 1000 mg/kg IP). After 1 hour, all four groups received 55 mg/kg IP of sodium pentobarbital (PBS). The time that elapsed between the loss and recovery of the righting reflex was recorded, for control and drug pretreated animals [25].

2.5. Pentylentetrazole-Induced Convulsion. The mice were divided into four groups (10 animals/group). The first group received the pentylentetrazole (PTZ) (55 mg/kg IP)
were used in each group. The number of mice which exhibited convulsions, the lethal time, and the latency to first convulsion was recorded [25].

2.7. Board-Hole Test. To examine this premise, we tested groups received the CS extract at doses of 300, 600, and 1000 mg/kg (IP). After 1 hour, PTZ (55 mg/kg, IP) was administered to the animals in each group. The number of mice which exhibited convulsions, the lethal time, and the latency to first convulsion was recorded [25].

2.8. Elevated Plus-Maze Test. The elevated plus-maze (EPM) test consisted of two open arms (30 × 5 × 0.25 cm) and two closed arms (30 × 5 × 15 cm) emanating from a common central platform (5 × 5 cm). Two pairs of identical arms were opposite to each other. The entire apparatus was elevated to a height of 40 cm above floor level. At the beginning of the session, a mouse was placed at the centre of the maze, its head facing an open arm and allowed to explore the maze for 5 minutes, and the following parameters were scored: the time spent and number of entries in each type of arms. The plus maze was carefully cleaned with a wet towel after each animal test. The mice were divided into four groups (10 animals/group). DPZ (2.5 mg/kg, IP) was used as the positive control and CS extract at doses of 300, 600, and 1000 mg/kg, IP, in the three remaining groups. All experiments were carried out between 10:00 am and 4:00 pm. After each trial, the EPM apparatus was wiped clean with alcohol (70%) solution [29].

2.9. Statistical Analysis. Statistical analysis was performed using one-way ANOVA with post hoc Duncan’s test. P < .05 was considered significant. All data are expressed as mean ± S.D.

3. Results

3.1. Sodium Pentobarbital-Induced Sleeping Time. The effect of pentobarbital sodium-induced sleep is shown in Figure 1. The values, up to 1000 mg/kg of CS, were significantly different from the control group * [F (1.7) = 5.5; P < .01].

3.2. Effect of CS Pentylenetetrazole (PTZ-) Induced Convulsion. The CS inhibited generalized clonic-tonic convulsions induced by PTZ (55 mg/kg, IP) at doses of 600 and 1000 mg/kg (Figure 5), as in accordance with statistical analysis * [F (1.6) = 5.7; P < .01], using analysis of variance one way (ANOVA) and followed by a post hoc Duncan’s test.

3.3. Marble-Burying Test. To examine this premise, we studied the effect of the representative of CS on burying behavior. As expected, control exhibited significant decrease in the marble burying behavior. However, CS prompted an increase in marble burying (300, 600, and 100 mg. kg1, IP). These data were evaluated using the analysis of variance one way (ANOVA) followed by a post hoc Duncan’s tests. * [F (1.14) = 5.8; P < .01], and ** [F (1.12) = 5.7; P < .05] (Figure 6).

3.4. Effect of Board-Hole Test. The effect of CS on the board-hole test is shown in Figure 7. At the doses of 300, 600, and 100 mg/kg, IP, a significant increase in the amount of head-dipping behavior was shown * [F (1.13) = 5.7; P < .001].

3.5. Effect of CS on the Elevated Plus-Maze (EPM). CS in all the doses (300, 600, and 1000 mg/kg, IP) produced anxiolytic-like effects as determined by the increase in the percentage of open arm entries * [F (1.14) = 5.6; P < .01]. Conversely, the number of entries and the time spent in the
4 Journal of Biomedicine and Biotechnology

4. Discussion

C. sicyoides is a plant originating from the Dominican Republic [2]. It is popularly known as “insulina, cipo-pucá, bejuco de porra, bejuco caro, puci, and anil trepador” [1]. It is used in popular medicine as a diuretic, anti-inflammatory [4], and antidiabetic [5].

The aim of this study was to analyse the behavioral effects of the crude hydroalcoholic extract of the aerial parts of CS. The results presented here show that CS did not exhibit toxicity in mice and did not induce any significant changes in several behavioral and physiological parameters, and showed a slight decrease in spontaneous locomotor activity and an increase in breathing frequency (data not shown).

Treatment with CS reduced the latency of induction and increased the duration of the barbiturate-induced sleep (see Figure 3) indicating CNS depressant activity, since closed arms were reduced by CS treatment \( [F (1.13) = 5.5; P < .01] \) (Figures 8 and 9).

4. Discussion

C. sicyoides is a plant originating from the Dominican Republic [2]. It is popularly known as “insulina, cipo-pucá, bejuco de porra, bejuco caro, puci, and anil trepador” [1]. It is used in popular medicine as a diuretic, anti-inflammatory [4], and antidiabetic [5].

The aim of this study was to analyse the behavioral effects of the crude hydroalcoholic extract of the aerial parts of CS. The results presented here show that CS did not exhibit toxicity in mice and did not induce any significant changes in several behavioral and physiological parameters, and showed a slight decrease in spontaneous locomotor activity and an increase in breathing frequency (data not shown).

Treatment with CS reduced the latency of induction and increased the duration of the barbiturate-induced sleep (see Figure 3) indicating CNS depressant activity, since
sleeping time induced by PBS is related to its central depressant properties. These findings suggest that C. sicyoides, administered by the intraperitoneal route, has hypnotic activity due to the potential of barbiturate-induced sleep and might probably be due to pharmacokinetic interactions between CS and PBS through the presence of flavonoids, and activity due to the potential of barbiturate-induced sleep administered by the intraperitoneal route, has hypnosedative effects observed in the time spent in open arms and in relation to the vehicle, and similar to diazepam ∗ [F (1.13) = 5.5; P < .01] and ∗∗ [F (1.11) = 5.5; P < .05] (see Figure 8). The EPM test is designed to evaluate drugs with anxiolytic-like nonspecific action. Extract or drugs that increase the time spent in open arms are considered anxiolytic by withdrawal of fear in the animals. The same happens with time spent in the closed arms, which are considered to produce fear or anxiety [29].

In this study, diazepam was used as a positive control, and, as expected, it increased the activity in the open arms of the EPM apparatus, confirming anxiolytic-like actions. The presence of flavonoids, linalool, and α-tocopherol in C. sicyoides leaves reinforces the anxiolytic-like and anticonvulsant-like effects of this plant found by us in this study.

Acknowledgments

The authors wish to express their gratitude to National Council for Research (CNPq) and to the Federal University of Pernambuco for their support.

References

[1] F. L. Beltrame, J. L. Sartoretto, R. B. Bazotte, R. N. Cuman, and D. A. G. Cortez, “Estudo fitoquímico e avaliação do potencial antidiabético do Cissus sicyoides L. (Vitaceae),” Química Nova, vol. 24, no. 6, pp. 783–785, 2001.
[2] J. H. Cano and G. Volpato, “Herbal mixtures in the traditional medicine of Eastern Cuba,” Journal of Ethnopharmacology, vol. 90, no. 2-3, pp. 293–316, 2004.
[3] M. C. F. Toledo, F. G. R. Reyes, M. Iaderoza, F. J. Fancis, and I. S. Draetta, “Anthocyanins from anil trepador (Cissus sicyoides, Linn.),” Journal of Food Science, vol. 48, no. 4, pp. 1368–1369, 1983.
[4] M. D. García, A. M. Quílez, M. T. Sáenz, M. E. Martinez-Dominguez, and R. de la Puerta, “Anti-inflammatory activity of Agave intermixta Trel. and Cissus sicyoides L., species used in the Caribbean traditional medicine,” Journal of Ethnopharmacology, vol. 71, no. 3, pp. 395–400, 2000.
[5] M. T. Pepato, A. M. Baviera, R. C. Vendramini, M. D. P. M. da Silva Perez, I. do Carmo Kettelhut, and I. L. Brunetti, “Cissus sicyoides (princess vine) in the long-term treatment of streptozotocin-diabetic rats,” Biotechnology & Applied Biochemistry, vol. 37, no. 1, pp. 15–20, 2003.
[6] M. D. García, M. T. Sáenz, R. Puerta, A. Quílez, and M. A. Fernandez, “Antibacterial activity of Agave intermixta and Cissus sicyoides,” Fitoterapia, vol. 70, no. 1, pp. 71–73, 1999.
[7] E. Elisabetsky, I. Marschner, and D. O. Souza, “Effects of linalool on glutamatergic system in the rat cerebral cortex,” Neurochemical Research, vol. 20, no. 4, pp. 461–465, 1995.
[8] E. Elisabetsky, G. P. Coelho de Souza, M. A. C. dos Santos, I. R. Siqueira, T. A. Amador, and D. S. Nunes, “Sedative properties of linalool,” Fitoterapia, vol. 66, no. 5, pp. 407–414, 1995.
[9] M. T. Sáenz, M. D. Garcia, A. Quílez, and M. C. Ahumada, “Cytotoxic activity of Agave intermixta L. (Agavaceae) and Cissus sicyoides L. (Vitaceae),” Phytotherapy Research, vol. 14, no. 7, pp. 552–554, 2000.
[10] V. E. P. Vicentini, M. L. Camparoto, R. O. Teixeira, and M. S. Mantovani, “Averrhoa carabambula L., Syzygium cumini (L.)
Skeels and Cissus sicyoides L.: medicinal herbal tea effects on vegetal and animal test systems,” Acta Scientarium, vol. 23, no. 2, pp. 593–598, 2001.

[11] W. L. R. Barbosa, Untersuchung der brasilianischen Arzneipflanze Cissus sicyoides, Ph.D dissertation, Bonn University, Bonn, Germany, 1994.

[12] M. C. P. Ribeiro, D. Silva de Avila, C. Y. M. Schneider, et al., “α-tocopherol protects against pentyleneetetrazol- and methylmalonate-induced convulsions,” Epilepsy Research, vol. 66, no. 1–3, pp. 185–194, 2005.

[13] N. Yamamoto, H. Kabuto, S. Matsumoto, N. Ogawa, and I. Yokoi, “α-tocopheryl-β-asorbate-2-O-phosphate diester, a hydroxyl radical scavenger, prevents the occurrence of epileptic foci in a rat model of post-traumatic epilepsy,” Pathophysiology, vol. 8, no. 3, pp. 205–214, 2002.

[14] E. R. de Almeida, R. P. F. Soares, F. F. R. Lucena, J. R. G. de Oliveira, J. F. C. Albuquerque, and G. B. L. Couto, “Central antinociceptive effects of Cissus sicyoides on mice,” Pharmaceutical Biology, vol. 44, no. 4, pp. 304–308, 2006.

[15] E. R. de Almeida, J. R. G. de Oliveira, F. F. R. Lucena, R. P. de Freitas Soares, and G. B. L. Couto, “The action of extract of the dry leaves of Cissus sicyoides L. in pregnant rats,” Acta Pharmaceutica Bonaerense, vol. 25, no. 3, pp. 421–424, 2006.

[16] E. R. de Almeida, J. R. G. Oliveira, F. R. S. Lucena, et al., “Embriofetotoxic effect and offspring postnatal development exposed to hydroalcoholic fraction extract of Cissus sicyoides L. during wistar rats pregnancy,” Journal of Medicinal Plants Research, vol. 1, no. 5, pp. 109–112, 2007.

[17] F. Beltrame, A. Ferreira, and D. Cortez, “Gomarin glycoside from Cissus sicyoides,” Natural Product Letters, vol. 16, no. 4, pp. 213–216, 2002.

[18] E. Elisabetsky, L. F. Brun, and D. O. Souza, “Anticonvulsant properties of linalool in glutamate-related seizure models,” Phytomedicine, vol. 6, no. 2, pp. 107–113, 1999.

[19] E. A. Carlini, “Plants and the central nervous system,” Pharmacology Biochemistry and Behavior, vol. 75, no. 3, pp. 501–512, 2003.

[20] J. H. Woods, J. L. Katz, and G. Winger, “Benzodiazepines: use, abuse, and consequences,” Pharmacological Reviews, vol. 44, no. 2, pp. 151–347, 1992.

[21] D. W. Gallager and R. J. Primus, “Benzodiazepine tolerance and dependence: GABA receptor complex locus of change,” Biochemical Society Symposium, vol. 59, pp. 135–151, 1993.

[22] M. Marder and A. C. Paladini, “GABA-A receptor ligands of flavonoid structure,” Current Topics in Medicinal Chemistry, vol. 2, no. 8, pp. 853–867, 2002.

[23] E. Ernst, “Herb-drug interactions: potentially important but woefully under-researched,” European Journal of Clinical Pharmacology, vol. 56, no. 8, pp. 523–524, 2000.

[24] H. B. Santos, J. Modesto-Filho, M. D. F. F. M. Diniz, et al., “Avaliação do efeito hipoglicemante de Cissus sicyoides em estudos clínicos fase II,” Revista Brasileira de Farmacognosia, vol. 18, no. 1, pp. 70–76, 2008.

[25] E. Speroni and A. Minghetti, “Neuropharmacological activity of extracts from Passiflora incarnata,” Planta Medica, vol. 54, no. 6, pp. 488–491, 1988.

[26] C. L. Broekkamp, H. W. Rijk, D. Joly-Gelouin, and K. L. Lloyd, “Major tranquillizers can be distinguished from minor tranquillizers on the basis of effects on marble burying and swim-induced grooming in mice,” European Journal of Pharmacology, vol. 126, no. 3, pp. 223–229, 1986.

[27] K. Njung’e and S. L. Handley, “Evaluation of marble-burying behavior as a model of anxiety,” Pharmacology Biochemistry and Behavior, vol. 38, no. 1, pp. 63–67, 1991.