Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Ethnopharmacology 107 (2006) 19–24
Journal of ETHNO-PHARMACOLOGY
www.elsevier.com/locate/jethpharm

Pharmacological activities investigation of crude extracts and fractions from *Qualea grandiflora* Mart.

F.O.G. Gaspi [a,*], M.A. Foglio [b], J.E. Carvalho [b], R.A. Moreno [c]

[a] *Faculdade de Farmácia do Centro Universitário Hermínio Ometto (UNIARARAS), Av. Dr. Maximiliano Baruto, 500, Jardim Universitário, 13607-339 Araras, SP, Brazil*
[b] *Centro Pluridisciplinar de Pesquisas Químicas, Biológicas e Agrícolas (CPQBA-UNICAMP), Campinas, SP, Brazil*
[c] *Faculdade de Ciências Médicas-Sub-Comissão de Pós-Graduação em Farmacologia (UNICAMP), Campinas, SP, Brazil*

Received 10 May 2005; received in revised form 18 January 2006; accepted 28 January 2006
Available online 29 March 2006

Abstract

This study investigates pharmacological activities of crude hydroalcoholic extract and fractions of *Qualea grandiflora* Mart. leaves employing different experimental models using mice. The treatment with crude hydroalcoholic extract (EH) in a dose of 500 mg/kg, i.p. caused: signs of central nervous system depressant action in the Hippocratic screening test, confirmed by the potentiation of sodium pentobarbital sleeping time. Increasing in the latency time of hot plate assay that indicate an analgesic effect; significantly delaying of the onset of clonic PTZ convulsions, increasing in the time for death, suppressing of the tonic PTZ convulsion, and decreasing of severity and number of convulsions. The median lethal dose of EH was 1.321 mg/kg. The convulsions induced by PTZ, ethyl ether fraction (300 mg/kg, i.p.) was more active in increasing the latency time for first convulsion, moreover, the hexane fraction, at the same dose, was more active in increasing the time for death and/or avoiding the death. Both did not cause disturbance in motor coordination at the dose of 500 mg/kg, assessed by rotarod test. These results suggest that the crude extract of leaves of *Qualea grandiflora* Mart. has a central nervous system depressant action, an analgesic effect and behave as a potential anticonvulsant.

Keywords: *Qualea grandiflora*; Vochysiaceae; Anticonvulsants; Medicinal plant

1. Introduction

Qualea grandiflora Mart. (Vochysiaceae) is a tree, symbol of Brazilian Cerrado, widely found in these regions (Silva et al., 2002). This specie have many popular names such as pau-terra-de-folha-larga, pau-terra-do-campo, pau-terra-do-cerrado and is about 5 m tall, yellow flowers and triangular fruits (Correa, 1978; Joly, 1993). The Cerrado, known as Brazilian savanna, comprises a very rich and characteristic flora that covers more than 2 million square kilometers of Brazilian inland. Many of these plants are used in folk medicine to treat several tropical diseases (Alves et al., 2000). The infusion or decoction of the *Qualea grandiflora* leaves have been usually to treat the diarrhea with blood, the intestinal colic and against amoeba (Rodrigues and Carvalho, 2001). The methanolic extract of the bark of this plant was studied (Alves et al., 2000) and possessed antibacterial activity against *Staphylococcus aureus*, *Bacillus cereus* and *Pseudomonas aeruginosa*. Despite the pharmacological effects of the extracts of their leaves have never been studied. Therefore, the present work was undertaken to evaluate the effects of the crude hydroalcoholic extract and fractions of this important Brazilian plant species on different experimental animal models using mice.

2. Material and methods

2.1. Plant material and crude extract preparation

The leaves of *Qualea grandiflora* were collected at "Reserva Biológica e Estação Experimental de Mogi Guaçu" in São Paulo-Brazil by both botanist Prof[a]. Dr.[a] M. Carmo E. do Amaral

Abbreviations: CNS, central nervous system; EH, crude hydroalcoholic extract; FE, ethyl ether fraction; FH, hexane fraction; FA, aqueous fraction; GABA, gamma-aminobutyric acid; i.p., intraperitoneal; Mart., Carl Friedrich Philipp von Martius; PTZ, pentylenetetrazol

* Corresponding author. Tel.: +55 19 3541 7950; fax: +55 19 3541 7950.
E-mail addresses: fernandagaspi@uniararas.br, fergaspi@ig.com.br (F.O.G. Gaspi).

0378-8741/$ – see front matter © 2006 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.jep.2006.01.023

and Prof. Dr. Volker Bittrich. Voucher specimen is deposited at Instituto de Biologia/UNICAMP under registration number UEC-264.

The leaves were allowed to dry under air circulation (40 °C). The material was grinded to a fine power prior to use. The powder was submitted to dynamic maceration with aqueous ethanol (7:3, v/v) at room temperature during 4 h. This procedure was repeated three times with the same powder. After filtration, the solvent was evaporated completely under vacuum at 40 °C resulting in the crude hydroalcoholic extract (EH). The yield of the lyophilized extract was 23%. The EH was partitioned by two organic solvents, hexane and ethyl ether. This procedure was repeated three times with each solvent. The concentration of each fraction under reduced pressure yielded the hexane (FH), ethyl ether (FE) and aqueous fractions (FA).

The EH was pre-purified by dry column chromatography on silica gel 60 (Merck 7734) with chloroform/methanol 5% providing each different fractions.

From the hexane extract the fractions obtained were 2H, 6H and 8H. From the ethyl ether extract resulted the fractions were CL., 1E, 2E, 3E and 4E.

The extracts and all fractions were monitored by thin layer chromatography using chloroform/methanol (90:10) as eluent and anisaldehyde reagent for detection.

After evaporation under vacuum of the solvents, the EH and fractions were lyophilized for withdraw any humidity and were calculated the respective yieldings. They were freshly prepared in saline solution 0.9%, before each pharmacological test. They were administered in a volume not higher than 0.1 ml/10 g of body weight for mice.

2.2. Animals

Male Swiss mice, weighting 25–30 g, purchased from Animal Experimental Care Center of Campinas State University, were employed in all pharmacological experiments. These animals were maintained under standard conditions of light cycle (12 h light, 12 h dark) and temperature (23 ± 2 °C), for at least 7 days before the experiments, with free access to tap water and food. The animals fasted for 24 h before each experimentation.

The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 82-23, revised 1996) and International Guiding Principles for Biomedical Research Involving Animals (CIOMS)—Genebra, 1985.

2.3. Drugs

The drugs used in pharmacological experiments were dissolved in saline solution and were prepared freshly each time and administered in a volume of 0.1 ml/10 g body weight. Control animals received the same volume of vehicle (saline solution).

2.4. Hippocratic screening and toxicity assessment

Groups of mice were treated with intraperitoneal injections of EH (300, 500, 1000 and 1500 mg/kg) or vehicle and the reactions were continuously observed for 4 h and at 24 h time interval to detect and noted any reaction (Malone, 1977; Kanjanapothi et al., 2004). The incidence of mortality was noted up to 24 h after injection. The median lethal dose (DL50) of EH was determined (Lichfield and Wilcoxon, 1949).

The same procedure was carried out for aqueous, ethyl ether and hexane fractions, they were administered intraperitoneally to mice in a dose of 100, 300 and 500 mg/kg.

2.5. Potentiation of sodium pentobarbital sleeping time

Groups of mice were treated with intraperitoneal injections of EH (500 or 1000 mg/kg) or vehicle. After 30 min all animals received sodium pentobarbital (40 mg/kg, i.p.). Each animal was placed gently on its back. If the animal remained on its back 30 s, loss of the righting reflex was considered to occur. The sleep latency and the sleeping time were recorded. The sleeping time was measured as the interval between the loss and the recovery of the righting reflex (Carlini and Burgos, 1979).

2.6. Hot plate test

Groups of mice were treated with intraperitoneal injections of EH (500 or 1000 mg/kg). After 60 min the animals were placed on the hot plate one at a time, which is surrounded by a clear acrylic cage and with constant temperature of 56.5 °C. The latency of the animals presented the painful reflex (lift and lick their front and/or back paws) was observed and recorded for each animal. After reaction or no response within 30 s, the mouse was immediately removed from the hot plate and returned to its home cage. Each animal was tested only once. A control group was used (Mazella et al., 1991; Malmberg and Bannon, 1999).

2.7. Motor impairment assessment

The rotarod test was used to determine the effect of the extract on motor coordination. This test used a custom built apparatus; which consisted of an elevated rod (diameter of 2.5 cm and height of 25 cm) that rotated at a constant speed (16 rpm). Mice were trained to walk continuously on the rod for a period of 120 s. The animals were then evaluated for motor coordination at 30 min after intraperitoneal administration of the ethyl ether and hexane fractions (500 mg/kg). The time each animal falls off from the rod was noted. A control group was also used (Duham and Myia, 1957).

2.8. PTZ-induced seizure

Pentylenetetrazol at the dose of 85 mg/kg was injected subcutaneous to induce clonic–tonic convulsions in mice. Doses of 500 mg/kg of the extracts or 300 mg/kg of the fractions were administered intraperitoneally 30 min before PTZ injection. A control group was used and phenobarbital (5 mg/kg, i.p.) was administered to a group of animals as positive control. After injection of PTZ the animals were individually placed in plastic boxes and observed for at least 30 min to detect an occurrence of

the first episode of clonic or tonic seizures. The latency period for any seizure and as well as death was recorded for each animal. If none of these episodes occurred during the time limit (1 h), the animal was considered protected (Krall et al., 1978; Swinyard et al., 1989; Wang et al., 2000). Due to seasonal or circannual variations observed in laboratory animals despite constant environmental conditions and the most marked loss of anticonvulsant activity was in late winter and early spring (Löscher and Fiedler, 2000), all experiments was conducted out of this period.

2.9. Statistical analysis

The results were expressed as mean ± S.E.M. The data obtained from potentiation of sodium pentobarbital sleeping time were submitted to one way analysis of variance with critical range at $p < 0.05$ and afterwards to Duncan's test.

The results obtained from PTZ-induced test were statistically evaluated using survival analysis techniques and log-rank test. A value of $p < 0.05$ was considered significant (Kalbfleisch and Prentice, 1980).

The median lethal dose (DL50) of the EH was determined in mice along with their respective 95% confidence limits (Miller and Tainter, 1944; Berkson, 1951).

3. Results

3.1. Hippocratic screening and acute toxicity

After the intraperitoneal administration of crude hydroalcoholic extract (EH), ethyl ether fraction or hexane fraction, the animals were observed during 4 h and after 24 h. No abnormal behaviours were observed in mice using 300 mg/kg of EH. With the dose were 500 mg/kg the animals became hyporeactive to external stimuli like touch and tail press. Their exploratory activity was decreased 30 min after the injection and after 1 h presented piloerection. With the dose was 1000 mg/kg, the animals presented all the signs mentioned above and after 1 h diminished noise response. Moreover, these behaviours, using a dose of 1500 mg/kg after 30 min the mice presented decreased grip strength, and after 1 h the animals presented abdominal contortion. Tremors, loss of righting reflex and lacrimation were not observed. A dose of 1321 mg/kg of the EH was the median lethal dose, whereas 1500 mg/kg produced death in all animals. The signs were observed as shown in Table 1.

The mice that received the ethyl ether fraction (100 mg/kg) did not presented abnormal signs. While at the dose of 300 mg/kg presented decreased touch and the tail press response, after 30 min and after 1 h presented piloerection, decreased the noise response and the exploratory activity (Table 1). However, using a dose of 500 mg/kg the animals presented the mentioned above signs and abdominal edema and contortion, beyond lacrimation and agressivity after 12 h. This dose was lethal to animals after 24 h. The hexane fraction (300 mg/kg) just presented decreased the touch response using a dose of 500 mg/kg (Table 1). It is important to note that the piloerection remained until 24 h after the injection, except using hexane fraction. The animals that received the aqueous fraction did not have any different sign comparing to control group.

3.2. Potentiation of sodium pentobarbital sleeping time

The pre-treatment with the EH prolonged pentobarbital-induced sleeping time. The extract increased sleeping time from 39.6 ± 15.6 to 77.6 ± 29.8 ($p < 0.05$) in a dose of 500 mg/kg. In a dose of 1000 mg/kg the results did not produce a significant alteration.

3.3. Hot plate test

The EH increased the latency time significantly that indicates an analgesic effect. Using a dose of 500 mg/kg increased by 120% and 251.7% in a dose of 1000 mg/kg.

Table 1
Effects of crude hydroalcoholic extract (EH), ethyl ether (FE) and hexane fractions (F H) of *Qualea grandiflora* on Hippocratic screening and acute toxicity

Signs	EH (mg/kg)				FE (mg/kg)			FH (mg/kg)		
	300	500	1000	1500	100	300	500	100	300	500
Touch response (↓)	–	30 min (5/5)	30 min (5/5)	30 min (5/5)	–	30 min (5/5)	30 min (5/5)	–	–	30 min (5/5)
Tail press response (↓) (↓)	–	30 min (5/5)	30 min (4/5)	30 min (5/5)	–	30 min (2/5)	30 min (3/5)	–	–	–
Grip strength	–	–	–	30 min (4/5)	–	–	–	–	–	–
Exploratory activity (↓)	–	30 min (4/5)	30 min (4/5)	30 min (5/5)	–	1 h (4/5)	30 min (4/5)	–	–	–
Piloerection	–	1 h (5/5)	30 min (4/5)	30 min (5/5)	–	30 min (3/5)	30 min (5/5)	–	–	–
Noise response (↓)	–		1 h (4/5)	30 min (5/5)	–	1 h (4/5)	30 min (5/5)	–	–	–
Abdominal contortation (↓)	–	–	–	1 h (4/5)	–	–	30 min (3/5)	–	–	–
Tremors	–	–	–	–	–	–	–	–	–	–
Loss of righting reflex	–	–	–	–	–	–	–	–	–	–
Lacrimation	–	–	–	–	–	–	30 min (4/5)	–	–	–
Agressivity	–	–	–	–	–		30 min (3/5)	–	–	–
Death	–	–	20 h (1/5)	20 h (5/5)	–		20 h (5/5)	–	–	–
DL50	1321 mg/kg									

The data showed the time and the number of mice that had abnormal signs compared to the control mice after administration of EH and fractions via i.p., $n = 6$. (↓) Indicated decreased the normal sign.

Table 2
Motor impairment assessment—hexane and ethyl ether fractions

Time after administration (min)	Time for fall off (s)		
	Saline	Hexane fraction 500 mg/kg	Ethyl ether fraction 500 mg/kg
30	68 ± 15.0*	68 ± 8.26[a]	67 ± 6.50[a]

Time for fall off in seconds.
[a] Median ± S.E.M.

3.4. Motor impairment assessment

The fall-off time of mice treated with hexane and ethyl ether fractions did not differ from saline solution, therefore the fractions did not induce disturbance in motor coordination (Table 2).

3.5. PTZ-induced seizures

The EH (500 mg/kg) significantly delayed the onset of clonic PTZ convulsion, increased the time for death and suppressed tonic PTZ convulsion (Fig. 1). Using a dose of 1000 mg/kg the results had no significant variation. The ethyl ether fraction was more active in delaying the onset of clonic PTZ convulsion and decreasing the severity and the number of convulsions, however the hexane fraction was more active in delaying the time for death and/or avoiding the death (Fig. 1). Those fractions that resulted of the hexane fraction the most active in death was the 2H and those that resulted of the ethyl ether fraction the 2E was more active in delaying the onset of clonic PTZ convulsion (Fig. 2). In those animals that were no protected, the onset of convulsion was remarkably in delaying, while the duration of convulsion was reduced.

4. Discussion

In this work, the effects of crude hydroalcoholic extract (EH) and their fractions were studied in several pharmacological experiments.

The findings observed when the EH and fractions were tested by Hippocratic screening test, demonstrated signs that indicated central nervous system depression (CNS) effect carrying out a diminished exploratory activity and a decreased response to touch, tail press and noise. This CNS depressant effect was assessed by potentiation of sodium pentobarbital sleep and by PTZ-induced convulsions test for the intraperitoneal route. In the potentiation of sodium pentobarbital sleep assay, the EH presented increase in sleeping time and this is classically related to the CNS depressant drugs (Willianson et al., 1996). Despite this test is not specific because compounds that interfere with biotransformation of pentobarbital by cytochrome P450 complex can show the same effects of CNS depressant drugs (Goloubkova et al., 1998). The mice treated with EH were partially protected from convulsions induced by PTZ, which can be indicative of a more specific effect due to action on GABA system (Löscher, 1998).

The PTZ test was used because this is one of the first assays developed to conventionally accepted anticonvulsant screening procedure. It is used to identify chemical substances that alter seizure threshold (Löscher and Schmidt, 1988; Swinyard and Kupferberg, 1988).

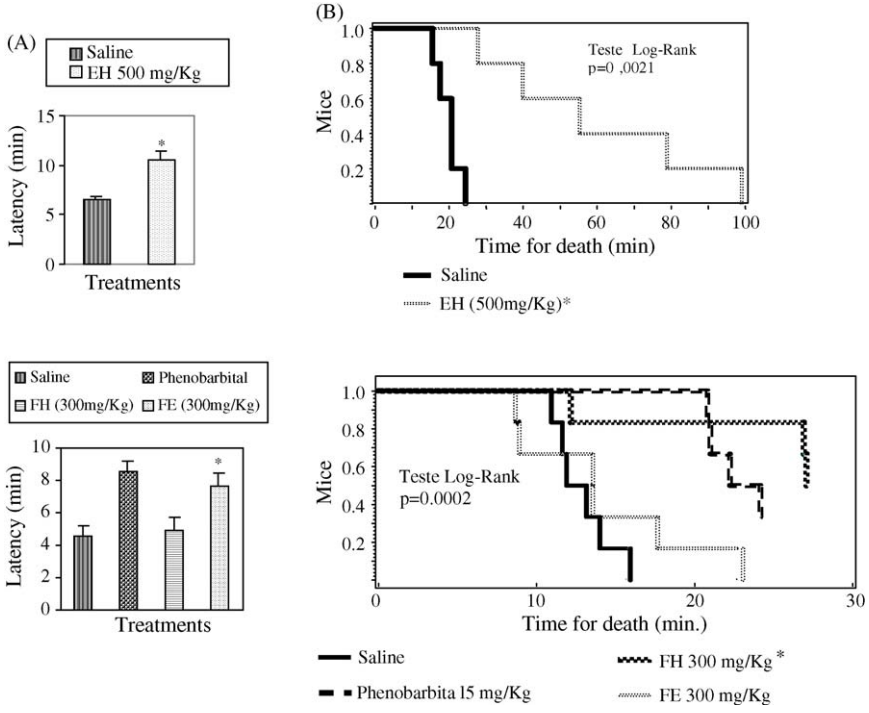

Fig. 1. Effect of crude hydroalcoholic extract (EH) of *Qualea grandiflora*, hexane (FH) and ethyl ether (FE) fractions on PTZ-induced seizures in mice. The EH and fractions were administrated via i.p. 30 min before the administration via s.c. of PTZ ($n = 6$). Graphics represent mean ± S.E.M. ($^{*}p < 0.05$) of the time for latency of first convulsion (A) and time for death for each animal (B).

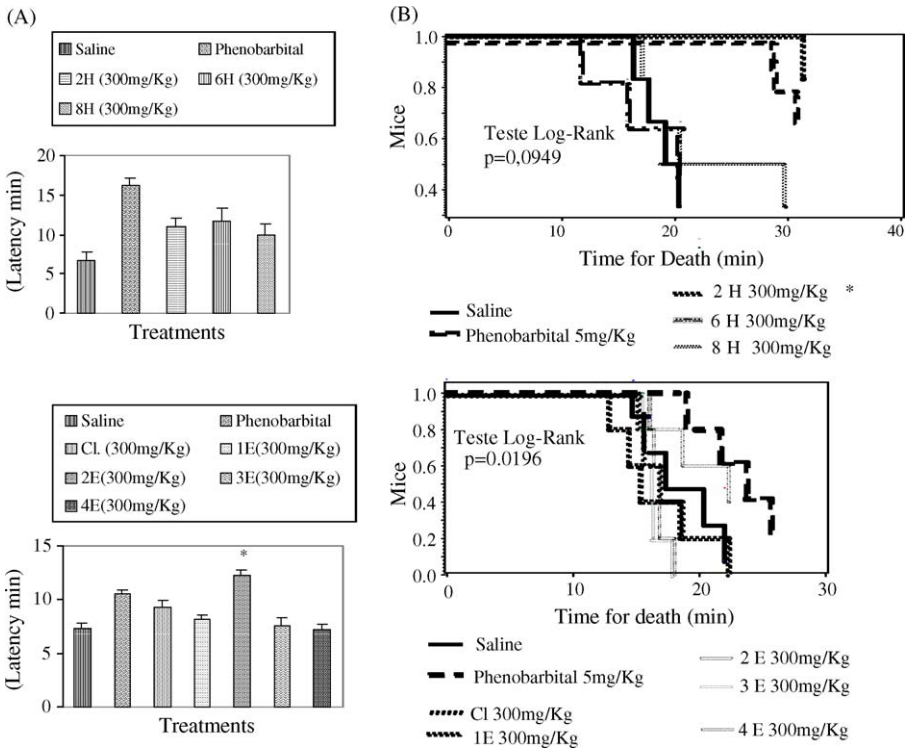

Fig. 2. Effect of 2H, 6H e 8H fractions obtained from hexane fraction (FH) and Cl., 1E, 2E, 3E and 4E fractions obtained from ethyl ether fraction of *Qualea grandiflora* on PTZ-induced seizures in mice. The fractions were administrated via i.p. 30 min before the administration via s.c. of PTZ ($n = 6$). Graphics represent mean ± S.E.M. ($^{*}p < 0.05$) of the time for latency of first convulsion (A) and time for death for each animal (B).

In addition, the results showed that the extract did not induces disturbance of motor coordination at the dose in which the anticonvulsant activity was observed.

We conclude that the extract of *Qualea grandiflora* possesses anticonvulsant activity against PTZ-induced convulsions. The mechanisms and the active compound(s) involved in these pharmacological effects are unknown and need to be elucidated in further studies.

Acknowledgements

We thank Prof[a]. Dr.[a] M. Carmo E. do Amaral and Prof. Dr. Volker Bittrich for plant collection and identification. We are grateful to Dr. Antonio Amarante for the excellent assistance in statistical analysis. This research was supported by Fapesp.

References

Alves, T.M.A., Silva, A.F., Brandão, M., Grandi, T.S.M., Smânia, E.F.A., Smânia Jr., A., Zani, C.L., 2000. Biological screening of Brazilian medicinal plants. Memórias do Instituto Oswaldo Cruz 95, 367–373.

Berkson, J., 1951. Why I prefer logists to probits. Biometrics 7, 327–339.

Carlini, E.A., Burgos, V., 1979. Screening farmacológico de ansiolíticos: metodologia laboratorial e comparação entre o diazepam e clorobenzapam. Revista da Associação Brasileira de Psiquiatria de São Paulo 1, 25–31.

Correa, M., 1978. PIO—Dicionário de Plantas Úteis do Brasil, Volume V. Imprensa Nacional, Rio de Janeiro.

Duham, N.W., Myia, T.S., 1957. A note on a simple apparatus for detecting neurological deficit in rats and mice. Journal of the American Pharmacists Association 46, 208–209.

Goloubkova, T.D., Heckler, E., Rates, S.M.K., Henriques, J.A.P., Henriques, A.T., 1998. Inhibition of cytochrome P450-dependent monooxygenases by an alkaloid fraction from Helietta apiculata markedly potentiate the hypnotic action of pentobarbital. Journal of Ethnopharmacology 60, 141–148.

Joly, A.B., 1993. Botânica—Introdução à Taxonomia Vegetal, 11[a] ed. Companhia Editora Nacional, São Paulo.

Kalbfleisch, J.D., Prentice, R.L., 1980. The Statistical Analysis of Failure Time Data. John Wiley and Sons, New York.

Kanjanapothi, D., Panthong, A., Letiprasertsuke, N., Taesotikul, T., Rujjanawate, C., Kaewpinit, D., Sudthayakorn, R., Choochote, W., Chaithong, U., Jitpakdi, A., Pitasawat, B., 2004. Toxicity of crude extract of *Kaempferia galanga* L. (Proh Hom). Journal of Ethnopharmacology 90, 359–365.

Krall, R.L., Penry, J.K., White, B.G., Kupferberg, H.J., Swinyard, E.A., 1978. Antiepileptic drug development: anticonvulsants drug screening. Epilepsia 19, 409–428.

Lichfield Jr., J.T., Wilcoxon, F., 1949. A simplied method of evaluating dose effect experiments. Journal of Pharmacology and Experimental Therapeutics 96, 99–113.

Löscher, W., Schmidt, D., 1988. Which animal models should be used in the search for new antiepileptic drugs? A proposal based on experimental and clinical considerations. Epilepsy Research 2, 145–181.

Löscher, W., 1998. Review: new visions in the pharmacology of anticonvulsant. European Journal Pharmacology 342, 1–13.

Löscher, W., Fiedler, M., 2000. The role of technical, biological, and pharmacological factors in the laboratory evaluation of anticonvulsant drugs VII. Seasonal influences on anticonvulsant drug actions in mouse models of generalized seizures. Epilepsy Research 38, 231–248.

Malmberg, A.B., Bannon, A.W., 1999. Models of nociception: hot-plate, tail-flick, and formalin tests inrodents. Current Protocols in Neuroscience, 8.9.1–8.9.15.

Malone, M.H., 1977. Pharmacological approaches to natural product, screening and evaluation. New natural products and plant drugs with pharmacological biological or therapeutical activity. Springer-Verlag, Berlin, pp. 24–53.

Mazella, A.G., Oliveira, A.A.M., Silva, F.B., Caldeira, T.T.O, Sertie, J.A.A., 1991. Atividade antinflamatória tópica de Cordia Verbenacea e sua toxicidade. Anais da VI Reunião anual da Federação de Sociedades de Biologia Experimental, Resumo 06.33, p. 284.

Miller, L.C., Tainter, M.L., 1944. Estimation of ED50 and its error by means of log probit graph paper. Proceedings of the Society For Experimental Biology and Medicine 57, 261–264.

Rodrigues, V.E.G., Carvalho, D.A., 2001. Levantamento Etnobotânico de Plantas Medicinais no Domínio do Cerrado na Região do Alto Rio Grande—MG. Ciências Agrotecnológicas 25, 102–123.

Silva, L.O., Costa, D.A., Santo Filho, K.E., Ferreira, H.D., Brandão, D., 2002. Levantamento florístico efitossociológico em duas áreas de cerrado. Acta Botânica Brasílica 16, 43–53.

Swinyard, E.A., Kupferberg, H.J., 1988. Antiepileptic drugs: detection, quantification and evaluation. Federation Proceedings 44, 2629–2633.

Swinyard, E.A., Woodhad, J.H., White, H.S., Franklin, M.R., 1989. Experimental selection, quantification and evaluation of anticonvulsants. In: levy, R., Mattson, R., Meldrum, B., Dreifuss, J.K. (Eds.), Antiepileptic Drugs, 5, 3rd ed. Raven Press Ltda, New York, cap., pp. 85–102.

Wang, H.H., Liao, J.F., Chen, C.F., 2000. Anticonvulsant effect of water extract of Scutellariae radix in mice. Journal of Ethnopharmacology 73, 185–190.

Willianson, E., Okpako, D., Evans, F.J., 1996. Selection Preparation and Pharmacological Evaluation of Plant Material. John Wiley and Sons, Chichester.