Acta Scientiarum. Health Sciences
ISSN: 1679-9291
eduem@uem.br
Universidade Estadual de Maringá
Brasil

Jacques Otobone, Fernanda; Colombo Martins, Juliana Vanessa; Trombelli, Marcos Alberto; Andreatini, Roberto; Audi, Elisabeth Aparecida
Anxiolytic and sedative effects of a combined extract of Passiflora alata Dryander and Valeriana officinalis L. in rats
Acta Scientiarum. Health Sciences, vol. 27, núm. 2, 2005, pp. 145-150
Universidade Estadual de Maringá
Maringá, Brasil

Anxiolytic and sedative effects of a combined extract of *Passiflora alata* Dryander and *Valeriana officinalis* L. in rats

Fernanda Jacques Otobone[1], Juliana Vanessa Colombo Martins[1], Marcos Alberto Trombelli[1], Roberto Andreatini[2] and Elisabeth Aparecida Audi[1*]

*[1]Departamento de Farmácia e Farmacologia, Laboratório de Neuropsicofarmacologia, Centro de Ciências da Saúde, Universidade Estadual de Maringá, Av. Colombo, 5790, 87020-900, Maringá, Paraná, Brasil. [2]Departamento de Farmacologia, Laboratório de Fisiologia e Farmacologia do Sistema Nervoso Central, Setor de Ciências Biológicas, Centro Politécnico, Universidade Federal do Paraná, Curitiba, Paraná, Brasil. *Author for correspondence. e-mail: eaaudi@uem.br*

ABSTRACT. This work investigated the effects of a combined extract of *Passiflora alata* Dryander and *Valeriana officinalis* L. (EPV) in rats under going elevated plus maze (EPM) and open-field test (OFT). No effects were detected after acute or repeated (3 or 7-days) treatment with EPV (5, 10 or 20 mg/kg, by gavage), on the EPM or the OFT. However, rats treated for 15 day (20 mg/kg) with EPV showed increased percentage of entries and time spent in the open arms on the EPM without alter locomotor activity in the OFT compared to control group. Acute or a 15 day administration of diazepam (2 mg/kg, i.p.), increased the same parameters on the EPM and OFT. Acute treatment with 300 or 600 mg/kg of EPV, decreased the locomotor activity in the OFT. Results suggest anxiolytic and sedative effects for the EPV and reveal a wide dose range for the anxiolytic effect.

Key words: anxiolytic effect, sedative effect, *Passiflora alata* Dryander, *Valeriana officialis* L.

RESUMO. Efeito ansiolítico e sedativo do extrato combinado de *Passiflora alata* Dryander e *Valeriana officinalis* L. em ratos. Este trabalho investigou o efeito do extrato combinado de *Passiflora alata* Dryander e *Valeriana officinalis* L. (EPV) em ratos submetidos aos testes do labirinto em cruz elevado (LCE) e campo aberto (TCA). Nenhum efeito foi detectado após o tratamento agudo ou repetido por 3 ou 7 dias com EPV (5, 10 or 20 mg/kg, gavagem) no LCE e TCA. Entretanto, ratos tratados por 15 dias com EPV (20 mg/kg) mostraram aumento na porcentagem de entradas e tempo gasto nos braços abertos no LCE, sem alterar a atividade locomotora no TCA, comparado ao controle. Diazepan (droga de referência, i.p.), aumentou os mesmos parâmetros analisados no LCE e OFT após o tratamento agudo ou por 15 dias. O tratamento agudo com 300 ou 600 mg/kg do EPV diminuiu significativamente a atividade locomotora no TCA. Estes resultados mostram que EPV produz efeito ansiolítico e sedativo, com ampla margem de segurança para o efeito ansiolítico.

Palavras chave: efeito ansiolítico, efeito sedativo, *Passiflora alata* Dryander, *Valeriana officialis* L.

Introduction

Pathological anxiety is one of most common mental disorders manifested by humans.

Anxiolytic substances, mostly belonging to the benzodiazepine group, occupy a prominent position in the ranking of drugs most utilized by man to minimize anxiety. Benzodiazepines enhance the activity of gama-aminobutyric acid (GABA), the main inhibitory neurotransmitter in the central nervous system (CNS), which results in its clinical effects, including anxiolysis, muscle relaxation, sedation and hypnosis (Kan *et al*., 1997; Uhlenhuth *et al.,* 1999). However, sedative and psychomotor deficits produced by benzodiazepine exposure are associated with an increased risk of motor vehicle accidents and hip fracture rates in the elderly, especially with longer acting compounds (Ray *et al*., 1989; Cummings and Klineberg, 1993; Hemmengarn *et al*., 1997; Barbone *et al*., 1998).

Owing to the unfavorable risks produced by classical anxiolytics, the development of new effective but less potent to induce adverse reactions is necessary. Thus, considerable attention has been given to the plant-derived therapeutics by the scientific community and the pharmaceutical industry.

Crude extracts of *Valeriana officialis* L. and *Passiflora alata* Dryander have been widely used since ancient times in many countries owing to their hypnotic, sedative and anxiolytic properties (Schultes, 1990; Carlini, 2003).

Clinical and pre-clinical evidences have confirmed these therapeutic properties of the crude extracts of different species of the genus *Valeriana* and *Passiflora*, including *Valeriana officialis* L. and *Passiflora alata* Dryander (Leathwood *et al*., 1982; Andreatini and Leite, 1994; Houghton, 1999; Herrera-Arelano *et al*., 2001; Dhawan *et al*., 2001; Petry *et al*., 2001; De Paris *et al*., 2002; Andreatini *et al*., 2002). *Passiflora alata* Dryander is known as *maracuja-doce* in Brazil and is the only species of the genus acknowledged, by the Brazilian Pharmacopeia (Vasconcelos and Cereda, 1994; Oliveira *et al*., 1994).

The aim of the present study was to examine the anxiolytic and sedative effects of acute and repeated treatments employing a combined extract of *Passiflora alata* and *Valeriana officinalis* L. (EPV) in rats submitted to elevated plus-maze (EPM), a behavioral test for anxiolytic drugs. We also profiled the secondary pharmacologic activity for the combined extract of EPV on spontaneous locomotion in the open-field test, a behavioral model for sedative/stimulant drug (Royce, 1977).

Material and methods

Animals

Wistar male rats (50-55 days old, 220-250 g), 5 per cage, at constant room temperature (22-23°C), under a 12h light-dark cycle, with free access to food and water, were deployed. Procedures adopted were approved by the UEM Ethics Committee, and followed the norms recommended by international guiding principles for Biomedical Research Involving Animals (CIMS, Geneva, 1985).

Preparation of the plant material

Each extract (*Passiflora alata* Dryander or Valeriana *officialis* L.) was produced separately and, after removal of the organic solvent, the remaining solid material was lyophilized. The phytotherapeutic compound (EPV) consisted of a combination of aqueous-ethanolic extracts from *Passiflora alata* Dryander leaves and *Valeriana officinalis* L. roots in a final composition of 65% ethanol with 9.3% dry residue. This combination standardizaton resulted in 0.02% valerenic acid and 0.13% flavonoids (apigenine).

Treatments

Treatment was performed only after the rats had been acclimatized to the above environment for at least 3 days. All experiments were carried out between 8:00 and 12:00 am. The dry residue (Herbarium Laboratório Botânico), was re-suspended into sterile saline (0.9% NaCl) immediately before use. EPV and saline, as control, were administered orally by gavage. The acute and the repeated treatments were performed once daily, with sterile saline (control) or EPV solution containing doses of 5, 10 or 20 mg/kg, or acutely with EPV solution in doses of 150, 300 or 600 mg/kg, in a final volume of 2 mL/kg body weight. Diazepan (2 mg/kg), administered by intraperitoneal route (i.p.), was used as positive control to acute or a 15 day treatment. Diazepam was used directy from the ampoule in solution (União Química). Each rat was used once in EPM and 24-hours later in the OFT Experimental sessions were conducted 30min after acute or final treatment.

Elevated plus-maze test (EPM)

The elevated plus-maze test procedure was performed according to original method (Pellow *et al.,* 1986). Number of entries and time spent in the open and closed arms of the maze were recorded for 5min. The percentage of open arm entries (100 x open/open + closed arm entries) and time spent in the open arms (100 x time in open/time in open + time in closed arms) were calculated for each rat.

Open-field test (OFT)

The animals submitted to the same experimental conditions were placed in the OFT. During a 5min period, the number of squares visited (four feet placed in the same square) and rearing behavior were registered using Royce's validation criteria (Royce, 1977).

Data analysis

Data were expressed as mean ± S.E.M. for each group. A one-way analysis of variance (ANOVA), followed by Dunnett`s test for multiple comparisons, was used. Effects and differences were considered significant at $p \leq 0.05$.

Results and discussion

In order to determine the effective dose of the combined extract of EPV on EPM in rats, increasing doses of the extract were tested after acute or repeated treatment (3, 7 or 15-days) by gavage. The combined extract of EPV at a dose of 20 mg/kg increased the percentage entries and time spent in

open arms in EPM, indicating anxiolytic-like effect only after a 15 day treatment. Only after acute administration the higher doses (300 or 600 mg/kg) of the combined extract of EPV produced a significant decrease in locomotor activity in the OFT, revealing sedative effect.

Figure 1 shows that the acute treatment with plant extract at any doses had no significant effect on any of the parameters that were measured on the EPM. Significant treatment effect was detected in percentage entries ($F_{4,30} = 9.566$, $P < 0.0001$) (upper panel) or in time spent (lower panel) in ($F_{4,30} = 5.517$, $p = 0.019$) open arms. Dunnett`s test revealed that only diazepan (2.0 mg/kg, i.p.), ($p < 0.01$) significantly increased percentage entry (upper panel) and time spent ($p < 0.01$) (lower panel) in open arms, confirming its anxiolytic effect after acute administration. Data with repeated treatment for 3 or 7 days were not shown.

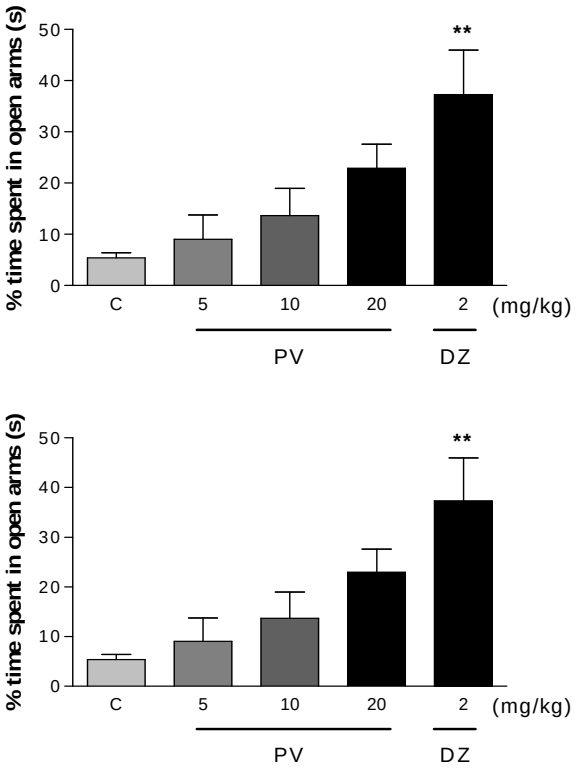

Figure 1. Mean (± SEM) of % entries (upper panel) and time spent (lower panel) in open arms in the EPM (n = 7) after acute treatment in control rats (C, sterile saline), combined extract of *Passiflora alata* Dryander and *Valeriana officinalis* L (EPV, 5, 10 or 20 mg/kg) by gavage or diazepan (DZ, 2 mg/kg, i.p.). Statistically significant from the control group at $^{**}p < 0.01$.

Figure 2 shows the effects of a 15 day treatment with EPV (5, 10 or 20 mg/kg, v.o.) or diazepan (2 mg/kg, i.p.), compared to the control group in rats submitted to the EPM. Significant treatment effect was detected in percentage entries ($F_{4,29} = 5.514$, $P = 0.0020$) (upper panel) and time spent (lower panel) in ($F_{4,29} = 7.688$, $P = 0.019$) open arms. Dunnett`s test revealed that diazepan ($p < 0.01$) and 20 mg EPV/kg ($p < 0.05$) significantly increased percentage entry (upper panel) and time spent (lower panel) in open arms, producing an anxiolytic effect.

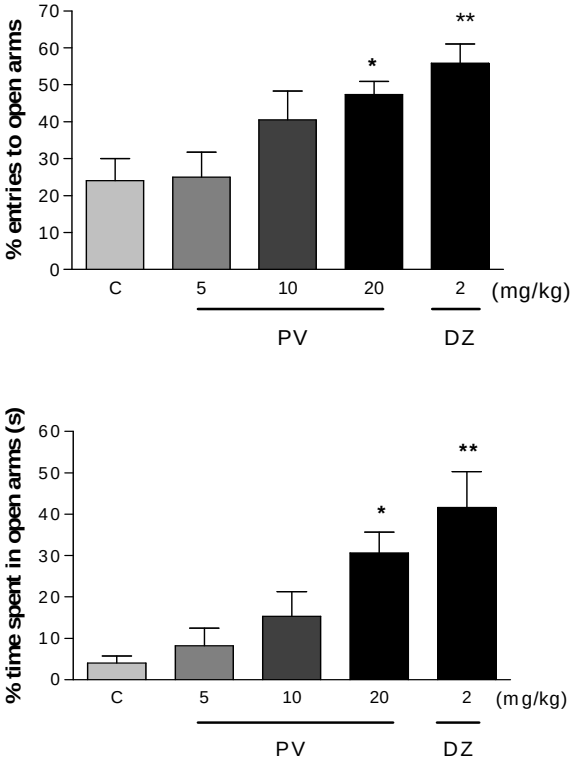

Figure 2. Mean (± SEM) % entries (upper panel) and time spent (lower panel) in open arms in the EPM (n = 6-8) after a 15 day treatment in control rats (C, sterile saline, v.o.), combined extract of *Passiflora alata* Dryander and *Valeriana officinalis* L (EPV, 5, 10 or 20 mg/kg, v.o.) or diazepan (DZ, 2 mg/kg, i.p.). Statistically significant from the control group at $^{*}P < 0.05$ and $^{**}p < 0.01$.

Figure 3 shows the effects of acute EPV treatment at higher doses (150, 300 or 600 mg/kg, v.o.) and diazepan (2 mg/kg, i.p.) in the EPM compared to rats receiving sterile saline (control, v.o.). Statistically significant treatment effect in percentage entries (upper panel) ($F_{4,35} = 9.706$, $p < 0.0001$) and time spent (lower panel) ($F_{4,35} = 22.11$, $p < 0.0001$) in open arms was observed. Dunnett's test revealed that only diazepam significantly increased the percentage entries ($p < 0.01$) and time spent ($p < 0.01$) in open arms in the EPM when compared to the treated control group.

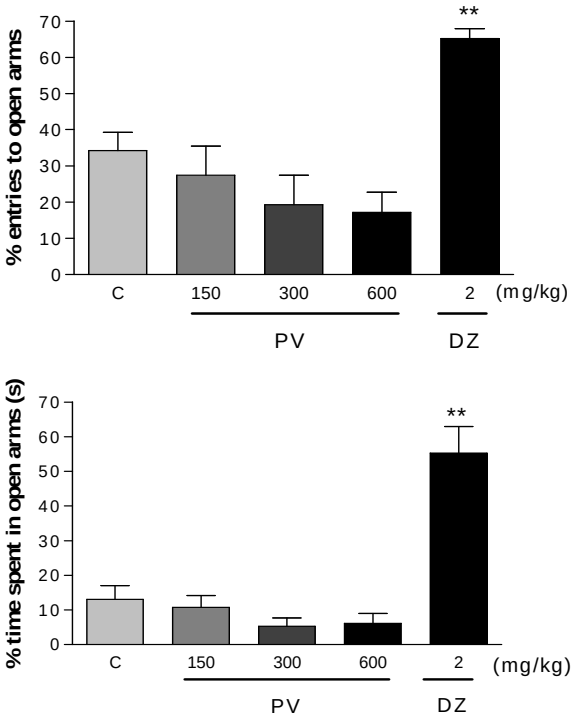

Figure 3. Mean (± SEM) percentage entries (upper panel) and time spent (lower panel) in open arms in the EPM (n =8) after a 15 day treatment in control group (C, sterile saline, v.o.), combined extract of *Passiflora alata* Dryander and *Valeriana officinalis* L (EPV, 150, 300 or 600 mg/kg, v.o.) or diazepan (Dz, 2 mg/kg, i.p.). ★★$p < 0.01$ significant difference of diazepan to C.

Table 1 shows that locomotor activity, analyzed by the number of crossings in the OFT was not statistically affected by EPV at doses 5, 10 or 20 mg/kg or diazepam, after acute (F4,29 = 1.066, p = 0.3910) or 15 day repeated (F4,30 = 1.842, p = 0.1468) treatment. However: ambulation was decreased after acute treatment with doses of 300.0 ($p < 0.05$), and 600.0 mgPV/kg ($p < 0.01$) treatment, indicating a significant sedative effect (F4,30 = 12.33, p = 0.0001) compared to the respective control groups.

Results show that a 15 day repeated treatment with 20.0 mg/kg combined EPV extract produced anxiolytic effect, as measure in the EPM. The same EPV dose 15 administered during 15 days did not alter locomotor activity in OFT, indicating a lack of sedative or stimulant effect.

Behavior in EPM is apparently driven by the conflict between fear (open space and novelty) and exploration (novelty). As a consequence, rats in EPM tend to avoid the open arms and remain longer in the closed arms. The percentage of open to total arm entries and time spent in the open arms have been used as indexes of anxiety. Anxiolytic drugs increase the number of entries into and/or time spent in open arms, while anxiogenic drugs promote the opposite effect (Pellow *et al*., 1986).

Table 1. Effect of EPV or diazepan after acute or 15 day treatment in OFT.

Crossing numbers		Treatment	
Drug	Doses (mg/kg)	1 day	15 day
Control		77.8±4.3	72.4±4.7
EPV	5	108.0±19.5	93±15.6
	10	86.7±10.5	77.0±6.2
	20	103.4±9.7	93.1±8.9
	150	64.7±5.2	–
	300	53.0±6.3★	–
	600	42.4±6.8★★	–
DZ	2	102.7±9.9	64.9±6.1

Mean (± SEM) number of crossings in rats receiving EPV extract (5, 10 or 20 mg/kg, v.o.) or diazepan (DZ, 2 mg/kg, i.p.) after acute or 15 day treatment or EPV extract (150, 300 or 600 mg/kg, v.o) after acute treatment compared to respective control rats (C, saline, v.o.) in the OFT (n = 6-8). Statistically significant from the respective control group at $p < 0.05$★ and $p < 0.01$★★.

Due to changes in locomotor activity may also influence exploratory behavior in EPM, ambulatory behavior, evaluated by the number of crossings of squares in OFT, has been used to quantify this factor. OFT is an experimental rodent model specifically employed for the study of locomotor activity (Wash and Cummins, 1976; Royce, 1977).

The effects of benzodiazepine drugs, which include clinical effects, like anxiolysis, have been detected after acute administration, and involve enhancement of GABA activity. Although relatively safe, these drugs can produce adverse effects, including decreased psychomotor activity and memory impairment (Longo and Johnson, 2000).

In the present study we demonstrated that the EPV extract affects locomotor activity only when administered at doses much higher than those eliciting anxiolytic effects. Thus, the acute administration of higher EPV doses produced no significant, dose-dependent decrease in the parameters analyzed in the EPM that might reflect tendency to anxiogenic or sedative effects. The significant decrease of the crossings numbers detected using 300 or 600 mg EPV/kg in the OFT, indicates sedative effect.

Clinical studies show a significant improvement in sleep quality in patients using *Valeriana officialis* extracts (Leathwood *et al*., 1982; Leathwood and Chauffard, 1985;). Different constituents of *Valeriana officialis* may interact with the GABA system in the brain, which may explain its anxiolytic and sedative/hypnotic effects (Morazzoni and Bombardelli, 1995).

Other studies have reported anxiolytic effect for an aqueous extract of *Passiflora alata* Dryander, but the active principles responsible for these effects have not yet been identified (Petry *et al*., 2001; De Paris *et al*., 2002; Carlini, 2003).

The benzodiazepine receptor antagonist flumazenil failed to block the anxiolytic/sedative activities and nootropic effect of a standardized extract of *Passiflora incarnata*, suggesting that these effects are not mediated by action on benzodiazepine/GABA receptors (Soleimani *et al*., 1997, Slomp Jr, 2004).

In our study the anxiolytic effect was detected after repeated, but not after acute PV administration. This suggests that combined extract may act through a different mechanism Than that of benzodiazepine drugs.

Some antidepressant drugs which act on serotoninergic transmission are also effective anxiolytic agents (Ballenger, 1993; Argyropoulos *et al*., 2000). However, the effectiveness in treating anxiety disorders of the different anti-depressant drugs has been observed only after chronic administration (Teixeira *et al*., 2000; Poltronieri *et al*., 2003).

Current results demonstrate anxiolytic and sedative effects of the combined extracts of *Passiflora alata* Dryander and *Valeriana officinalis* L., which may be of relevance to the treatment of anxiety and insomnia. The anxiolytic effect was detected in doses 15 times less than that required to produce a sedative effect, revealing a wide dose range for the anxiolytic effect of the compound, and a lower potential to induce adverse sedative effects than benzodiazepines.

Acknowledgemens

The combined extract of *Passiflora-Valeriana* was a donation from the Herbarium Laboratorio Curitiba, Brazil. The authors world like to thank Soledalva de Oliveira for technical assistance.

References

ANDREATINI, R.; LEITE, J.R. Effect of valeprotriates on the behavior of rats in the elevated plus-maze during diazepam withdrawal. *Eur. J. Pharmacol.,* Amsterdam, v. 260, p. 233-235, 1994.

ANDREATINI, R. *et al.* Effect of valeprotriates (Valeriana extract) in generalized anxiety disorder: a randomized placebo-controlled pilot study. *Phytother Res.*, London, v. 16, n. 7, p.650-654, 2002.

ARGYROPOULOS, S.V. *et al.* The psychobiology of anxiolytic drugs. Part 2: pharmacological treatments of anxiety. *Pharmacol. Ther.,* Oxford, v. 88, p. 213-227, 2000.

BALLENGER, J.C. Panic disorder: Eficacy of current treatments. *Psychol. Bull.*, Washington, D.C., v. 29, p. 477-486, 1993.

BARBONE, F. *et al.* Association of road-traffic accidents with benzodiazepine use. *Lancet*, London, v. 352, p. 1331-1336, 1998.

CARLINI, E.A. Plants and the central nervous system. *Pharmac Biochem Behav,* New York, v. 75, p. 501-512, 2003.

CUMMINGS, R.G.; KLINEBERG, R.J. Psychotropics, thiazide diuretcs and hip fractures in the elderly. *Med. J. Aust.*, North Sydney, v. 158, p. 414-417, 1993.

DE-PARIS, F. *et al.* Pharmacochemical study of aqueous extracts of *Passiflora alata* Dryander and P*assiflora edulis* Sims. *Acta Farm. Bonaer*, La Plata, v. 21, n. 1, p. 5-8, 2002.

DHAWAN, K. *et al.* Anti-anxiety studies on extracts of *Passiflora incarnata* Linneaus. *J Ethnopharmacol.,* Amsterdam, v. 78, n. 2-3, p. 165-170, 2001.

HEMMENGARN, B. *et al.* Benzodiazepine use and the risk of motor vehicle crash in the elderly. *JAMA*, Chicago, v. 278, p. 27-31, 1997.

HERRERA-ARELANO, A. *et al.* Polysomnographic evaluation of the hypnotic effect of Valeriana edulis standardized extract in patients suffering from insomnia. *Planta Med.,* Stuttgart, v. 25, p. 451-471, 2001.

HOUGHTON, P.J. The scientific basis for the reputed activity of valerian. *J. Pharm. Pharmacol.*, London, v. 51, p. 505-512, 1999.

KAN, N.C.C. *et al.* High prevalence of benzodiazepine dependence in out-patient users, based on the DSM-III-R and ICD-10 criteria. *Acta Psychiat Scand.,* Copenhagen, v. 96, p. 85-93, 1997.

LEATHWOOD, P.D. *et al.* Aqueous extract of valerian root (Valeriana officialis L.) improves sleep quality in man. *Pharmacol. Biochem. Behav.*, New York**, v.** 17, p. 65-71, 1982.

LEATHWOOD, P. D.; CHAUFFARD, F. Aqueou extract of valerian reduces latency to fall asleep in man. *Planta Med.,* Stuttgart, v. 51, p. 144-148, 1985.

LONGO, L.P.; JOHNSON B. Addition: Part 1. Benzodiazepines- side effects, abuse risk and alternatives. *Am Farm Physician*, Leawood, KS, v. 61, n. 7, p. 2121-2128, 2000.

MORAZZONI, P.; BOMBARDELLI E. Valeriana officialis: traditional use and recent evaluation of activity. *Fitoter*, Milano, v. 66, p. 99-112, 1995.

OLIVEIRA, J.C. *et al.* Aspectos gerais do melhoramento do maracujazeiro. *In:* AR São José (Ed.). *Maracujá*: produção e mercado. Vitória da Conquista: UESB-DFZ, 1994, p. 27-28.

PELLOW, S. *et al.* Validation of open: enclosed arm entries in elevated plus-maze as a measure of anxiety in rat. *J. Neurosc. Meth.*, Amsterdam, v.14, p. 149-167, 1986.

PETRY, R. D. *et al.* Comparative pharmacological study of hydroethanol extracts of *Passiflora alata* and *Passiflora edulis* leaves. *Phytother. Res.*, London, v. 15, p. 162-164, 2001.

POLTRONIERI, S.C. *et al.* Anti-panic-like effect of serotonin reuptake inhibitors in the elevated T-maze. *Behav. Brain Res.*, Shannon, v. 147, p. 185-192, 2003.

RAY, W.A. *et al.* Benzodiazepines of long and short elimination half-life and the risk of hip fracture. *JAMA*, Chicago, v. 262, p. 3303-3307, 1989.

ROYCE JR., On the construct validity of Open-Field

measures. *Psychol. Bull.*, Washington, D.C., v. 84, n. 6, p. 1098-1106, 1977.

SCHULTES, R.E. The virgin field in psychoactive plant research. *Bol. Mus. Para. Emílio Goeldi, Bot.*, Belém, v. 906, p. 6-83, 1990.

SLOMP JR., H. *Ausência de efeito amnésico de um composto de Passiflora alata Dryander*

Passifloraceae e Valeriana offinalis L. Valerianaceae em um protocolo de habituação e esquiva inibitória. 2004. Dissertação (Mestrado em Farmacologia)-Universidade Federal do Paraná, Curitiba, 2004.

SOLEIMANI, R. *et al.* Behavioral effects of *Passiflora incarnata* L. and its indole alkaloid and flavonoid derivatives and maltol in the mouse. *J. Ethnopharmacol.,* Amsterdam, v. 57, p. 11-20, 1997.

TEIXEIRA, R.C. *et al.* Behavioral effects of acute and chronic imipramine in elevated T-maze,model of anxiety. *Pharmacol. Biochem. Behav.,* New York, v. 65, p. 571-576, 2000.

UHLENHUTH, E.H. *et al.* Trends in recommendations for the pharmacotherapy of anxiety disorders by an international expert panel, 1992-1997. *Eur. Neuropsychopharmacol.*, Amsterdam, v. 9 (Supll 6), p. S393-398, 1999.

VASCONCELOS, M.A.; CEREDA, E. O cultivo de maracujá-doce. *In:* AR São José, *Maracujá:* produção e mercado. Vitória da Conquista:UESB-DFZ, 1994, p. 71-81.

WASH, R.N.; CUMMINS, R.A. The open-field test: a critical review. *Psychol. Bull*., Washington, D.C., v. 86, p. 482-504, 1976.

Received on May 09, 2005.
Accepted on November 21, 2005.