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Anxiogenic Properties of *Ptychopetalum olacoides* Benth. (Marapuama)

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Alcohol infusions of roots of *Ptychopetalum olacoides* Benth. (PO), known as Marapuama or Muirapuama, are used in the Brazilian Amazon as a 'nerve tonic'. Over the years PO has been found increasingly in phytoformulations and regarded as a stimulant, claimed to enhance physical and mental performances. This study determined that a *P. olacoides* ethanol extract (30, 100 and 300mg/kg) decreased exploratory behaviour in the hole-board test, without interfering with locomotion or motor coordination (rota-rod test). The data are comparable to that obtained with pentylenetetrazol (40 mg/kg), suggesting an anxiogenic effect of *P. olacoides*. Copyright © 2002 John Wiley & Sons, Ltd.

Keywords: Ptychopetalum olacoides; Marapuama; Muirapuma; anxiogenic; hole-board; rota-rod.

Introduction

Ethnopharmacological studies indicate that Amazonian 'caboclos' (Amazonian native rural inhabitants) use an alcohol infusion of the roots of *Ptychopetalum olacoides* Benth. (PO) (Olacacea), known as Marapuama, as a 'nerve tonic', aphrodisiac, appetite modulator and as an anti-tremor agent (Elisabetsky, 1987; Grenand *et al.*, 1987). The concept of a 'nerve tonic' has been discussed in detail elsewhere (Elisabetsky *et al.*, 1992); relevant to this study is to mention that the use of a 'nerve tonic' includes recovery of cognitive and motor functions after brain injuries (such as stroke), and cognition improvement (including alertness and memory) in the elderly. *P. olacoides* is currently present in herbal products in several American and European countries (Table 1).

Although little is known about the chemistry or pharmacology of this species (Paiva *et al.*, 1998; Uber Bucek *et al.*, 1987), we previously reported that a PO ethanol extract (POEE) potentiated yohimbine-induced lethality, reversed reserpine-induced ptosis, and prevented apomorphine-induced stereotypy in mice (Siqueira *et al.*, 1998). These data support the hypothesis of a PO modulatory effect on brain functions, suggesting the involvement of cateholaminergic transmission.

Considering the therapeutic claims of the marketed *P. olacoides* formulations, the purpose of this paper was to investigate the effects of *P. olacoides* ethanol extract in the hole-board model (exploratory behaviour) and the rota-rod test (motor coordination).

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Material and Methods

Plant material. Roots of *Ptychopetalum olacoides* Benth. (Olacaceae) were collected in the State of Pará, Brazil, and identified by Nelson Rosa. Voucher specimens were deposited at the herbarium of the Museu Paraense Emilio Goeldi (MPEG 108.036). The collection was authorized by CNPq and is in line with the Brazilian policy regarding access to genetic resources.

Preparation of extract. *Ptychopetalum olacoides* ethanol extract (POEE) was prepared as detailed elsewhere (Elisabetsky and Siqueira, 1998). Briefly, the dried roots (2.5 kg) were peeled, ground and extracted with ethanol (12 L), using a Soxhlet apparatus (40 h). The extract was evaporated under reduced pressure resulting in the POEE (150 g; 6% yield).

Animals. Experiments were performed with male adult mice, CF1 strain, received from Fundação Estadual de Experimentação e Produção da Saúde (FEEPS) immediately after weaning (21 days). Animals were maintained in our own animal facilities under a controlled environment (22 °±1 °C, 12 h light/dark cycle, free access to food [Nuvilab CR1] and water) up to 10 weeks old (25–40 g). All procedures were carried out according to institutional policies on experimental animal handling.

Drugs. Diazepam and propylene glycol (PPG) were acquired from Sigma; dimethyl sulphoxide (DMSO) from Delaware and pentylenetetrazol (PTZ) from Knoll A.G-Ludwingshafen/Rheno. Diazepam (0.5 mg/kg) was suspended in propylene glycol 10% (v/v). POEE (30, 100 and 300mg/kg) was dissolved in DMSO 20%(v/v). PTZ was dissolved in saline.

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Table 1. Some herbal products containing *Ptychopetalum olacoides*

Product	Description of Contents	Country	Reference
X-Action Pack or Men X Action	*Ptychopetalum olacoides* (Muira puama) *Pausinystalia yohimbe* (yohimbe bark) Arginine Damiana leaves Oat straw leaves concentrate Saw palmetto berries	USA	http://www.herbs-are-us.net/xaction.html
Touchfire HIS	*Ptychopetalum olacoides* (Potency Wood) *Juniperis brasiliensis* (Catuaba) *Turnera aphrodisiaca* (Damiana) *Smilax papyracea* (salsaparilla) *Urtica dioica* (Nettle Root)	USA Canada	http://www.naturesvitamins.com/hisfire.html
Doctor's Choice for Men	Essential vitamins and minerals *Zingiber officinale* (Ginger Root extract) *Camellia sinensis* (Green Tea Extract) *Ptychopetalum olacoides* (Muira puama extract) *Serenoa repens* (Saw Palmeto extract) *Panax Ginseng* (Korean Ginseng Root Extract)	USA	http://www.enzy.com/products/individual/eprod004.html
Masculex	Vitamin E *Ptychopetalum olacoides* (Muira puama extract) *Turnera diffusa* (Mexican Damiana leaves extract) *Serenoa repens* (Saw Palmeto extract) *Cola nitida* (Cola Nut extract) *Panax Ginseng* (Korean Ginseng Root Extract) *Ginkgo biloba* Extract	USA	http://www.enzy.com/products/individual/eprod149.html
Potency Wood	*Ptychopetalum olacoides* (Muira puama extract)	USA	http://www.rain-tree.com/muiraprod.html
Marapuanatus	*Ptychopetalum olacoides* (Marapuama extract)	Brazil	http://www.pronatus.com.br/productos_capsulados.htm
Guaracola	*Paullinia cupana* *Sterculia acuminata* *Anemopaegma mirandum* *Ptychopetalum olacoides*	Brazil	http://www.pharmaciacordeiro.com.br/

Hole-board. The hole-board apparatus (Ugo Basile, Italy) consisted of a grey Perspex panel ($40 \times 40 \times 40$ cm, 2.2 cm thick) with 16 equidistant holes (3 cm in diameter) in the floor. Photocells below the surface of the holes provided measures of the number of head-dips. The board was positioned 15 cm above the table and divided (with black water-resistant marker) in 9 squares of 10×10 cm. The method was adapted from Takeda *et al.* (1998). Mice were transported to the dimly lit laboratory at least 1 h prior to testing. Each animal was individually placed in the centre of the board (facing away from the observer) and the following parameters were noted for 5 min: the latency to the first head-dip, measured using a stopwatch; the number of rearings and spontaneous movements (number of squares crossed with all four paws). The animals were divided into eight groups (15–30 animals) and treatments (saline, PPG, DMSO, diazepam, POEE and PTZ) administered intraperitoneally (i.p, 10 mL/kg) 30 min prior to the testing.

Rota-rod. The rota-rod apparatus (Ugo Basile, Italy) consisted of a rotating bar which is suitably machined to provide grip. Six flanges divide the bar, enabling five mice to be at the treadmill simultaneously. Latency to fall from the bar is automatically recorded in seconds. The method was adapted from Dalmmeier and Carlini (1981). Mice were initially selected for their ability to remain in the rota-rod (18 rpm) for at least two of three consecutive 90 s trials. On the test day (24 h after selection), the latency to fall from the rota-rod (one trial of 60 s) was determined 30 and 60 min after treatments.

Statistical analysis. The results are expressed as mean $\pm$ SEM, and were analysed by ANOVA followed by Student–Newman–Keuls test.

Results

The POEE (30,100 and 300 mg/kg) significantly reduced the number of head-dips, as did PTZ (40 mg/kg) (Fig. 1A). POEE increased the latency to the first head-dip at 100 and 300 mg/kg (Fig. 1B), while reducing locomotion only at 300 mg/kg (Fig. 1C). POEE significantly reduced the number of rearings at 100 and 300 mg/kg (Fig. 1D). Diazepam 0.5 mg/kg increased the number of head-dips (Fig. 1A) and locomotion (Fig. 1C).

There were no deficits nor differences in the rota-rod performance with any POEE doses, nor with DMSO compared with saline (data not shown).

Discussion

Anxiety, a symptom accompanying various central nervous system disorders and a disorder by itself, is characterized in humans by a tense and physically

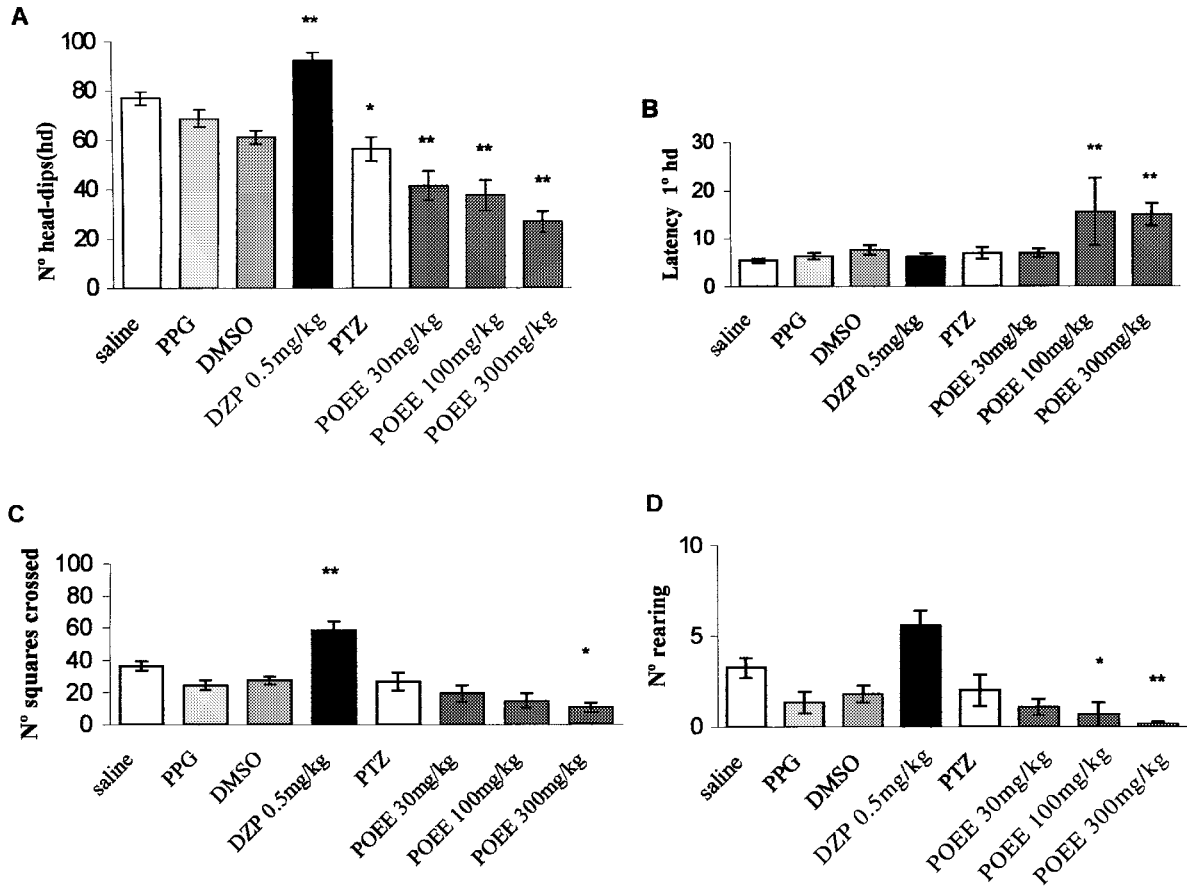

Figure 1. Effects of *Ptychopetalum olacoides* ethanol extract (POEE) in the hole-board test. Number of head-dips (A), latency for the first head-dip (B), number of squares crossed (C) and number of rearings (D). PTZ, pentylenetetrazol; PPG, Propylene glycol 10%; DZP, diazepam 0.5 mg/kg; DMSO, Dimethyl sulphoxide 20%. Each column represents the mean ± SEM. ANOVA * = $p < 0.05$ and ** $p < 0.01$ compared with saline.

exhaustive alertness (Treit, 1985). Other animal species display a variety of defensive reactions in response to predators, some understood as animal correlates of anxiety states (Rodgers *et al.*, 1995). Rodents demonstrate anxiety, fear and curiosity when placed in a new environment, and an overall assessment of behaviour can be determined by observing freezing, grooming (fear) or rearing, head-dips (curiosity) and the number of faecal pellets (stress) (File, 1987a; Kennedy, 1978; Dalvi and Rodgers, 1999).

The so-called hole-board model has become increasingly the most frequently used test to detect and evaluate the anxiolytic/anxiogenic properties of drugs (File and Wardill, 1975; Durcan and Lister, 1988; Moreira *et al.*, 1996; Takeda *et al.*, 1998). The present study demonstrated that the *P. olacoides* ethanol extract reduced the number of head-dips and rearings, and increased the latency to the first head-dip; these behaviour alterations are compatible with the profile of anxiogenic drugs (Moreira *et al.*, 1996; Takeda *et al.*, 1998). Accordingly, pentylenetetrazol, a known anxiogenic agent, reduced the number of head-dips.

A decrease in locomotion was observed only with the highest POEE dose studied, and the extract did not induce motor deficits in the rota-rod.

Changes in emotional states of animals are associated with benzodiazepine and non-benzodiazepine associated mechanisms. Yohimbine, an α_2-adrenergic antagonist, causes anxiety in humans, and in animal models (File, 1987b). Buspirona, a 5-HT_{1A} agonist, and ritanserine, a 5-HT_2 antagonist, possess anxiolytic proprieties in the human as well as animal models. Adrenocorticotrophic hormone (ACTH) has anxiogenic effects in the social interaction test, being antagonized by chlordiazepoxide (File, 1987b).

In this study, diazepam (0.5 mg/kg) increased the number of head-dips and locomotion, without interfering with rearing. Takeda *et al.* (1998) reported that diazepam increases head-dipping behaviour at doses similar to those used in this study, but did not observe modifications in locomotion or rearings. Nevertheless, several studies reported that diazepam increased locomotor activity in the open field (Bhattacharya and Mitra, 1991; Wieland *et al.*, 1991; Ramanathan *et al.*, 1998). Moreover, increases in the number of squares crossed in the open field and in head-dips in the hole-board were obtained with desmethyldiazepam and chlordesmethyldiazepam (De Angelis *et al.*, 1982). Our results indicate that a non-sedative but anxiolytic dose of diazepam facilitates exploratory behaviour, expressed in increased head-dips and locomotion.

The present results add to previously reported data obtained with POEE (Elisabetsky and Siqueira, 1998) indicating a central stimulant profile. An anxiogenic effect is in line with traditional therapeutic claims for *Ptychopetalum olacoides*, since moderate anxiogenesis is associated with alertness and increased physical and

psychological endurance (Jaffe, 1990). Further studies are necessary to complete the psychopharmacological profile of PO and unveil the mechanism(s) that underlie the central effects of POEE.

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References

Bhattacharya SK, Mitra SK. 1991. Anxiolytic activity of *Panax ginseng* roots: an experimental study. *J Ethnopharm* **34**: 87–92.

Dalmmeier K, Carlini EA. 1981. Anesthetic, hypothermic, myorelaxant and anticonvulsant effects of synthetic eugenol derivatives and natural analogues. *Pharmacology* **22**: 113–127.

Dalvi A, Rodgers RJ. 1999. Behavioral effects of diazepam in the murine plus-maze: flumazenil antagonism of enhanced head dipping but not the disinhibition of open-arm avoidance. *Pharm Biochem Behav* **62** (4): 727–734.

De Angelis L, Bertolissi M, Nardini G, Traversa U, Vertua R. 1982. Interaction of caffeine with benzodiazepines: behavior effects in mice. *Arch Int Pharmacodyn* **255**: 89–102.

Durcan MJ, Lister RG. 1988. Time course of ethanol's effects on locomotor activity, exploration and anxiety in mice. *Psychopharmacology* **96**: 67–72.

Elisabetsky E. 1987. From indigenous disease concepts to laboratory work hypothesis: the case of 'nerve tonics' from the Brazilian Amazon. *International Foundation for Science, Provisional Report Series*, 84–100.

Elisabetsky E, Figueiredo W, Oliveira G. 1992. Traditional Amazonian nerve tonics as antidepressant agents: *Chaunochiton kappleri:* a case study. *J Herbs Spices Med Plants* **1**: 125–161.

Elisabetsky E, Siqueira IR. 1998. Is there a psychopharmacological meaning for traditional tonics? In *Plants for Food and Medicine*, Prendergast HDV, Etkin NL, Harris DR, Houghton PJ (eds). Royal Botanic Gardens: Kew; 372–385.

File SE. 1987a. The contribution of behavioural studies to the neuropharmacology of anxiety. *Neuropharmacology* **26** (7B): 877–886.

File SE. 1987b. Beyond the benzodiazepines: search for new anxiolytics. *Human Psychopharmacol* **2**: 151–158.

File SE, Wardill GA. 1975. Validity of head-dipping as a measure of exploration in a modified hole-board. *Psychopharmacologia (Berl.)* **44**: 53–59.

Jaffe JH. 1990. Drug addition and drug abuse. In *The Pharmacological Basis of Therapeutics*, Gilman AG, Rall TW, Nies AS, Taylor P (eds). Pergamon Press: Oxford; 522–573.

Grenand P, Moretti C, Jacquemin H. 1987. *Pharmacopées Traditiomelles en Guyane*, 1st edn. Editions de L'Or stom: France; 326–328.

Kennedy B. 1978. Experimental approaches to the detection of anxiolytic activity in the rat. *Irish J Med Sci* **147** (1): 38–42.

Moreira EG, Nascimento N, Rosa GJM, Rogero JR, Vassilieff VS. 1996. Crotoxin-induced behavioral effects in rats. *Braz J Med Biol Res* **29**: 629–632.

Paiva LAF, Rao VSN, Silveira ER. 1998. Effects of *Ptychopetalum olacoides* extract on mouse behavior in forced swimming and open field test. *Phytother Res* **12**: 294–296.

Ramanathan M, Jaiswal AK, Bhattacharya SK. 1998. Differential effects of diazepam on anxiety in streptozotocin induced diabetic and non-diabetic rats. *Psychopharmacology* **135**: 361–367.

Rodgers RJ, Cole JC, Aboualfa K, Stephenson LH. 1995. Ethopharmacological analysis of the effects of putative 'anxiogenic' agents in the mouse elevated plus-maze. *Pharmacol Biochem Behav* **52**: 805–813.

Siqueira IR, Lara DR, Gaieski FS, Nunes, DS, Elisabetsky E. 1998. Psychopharmacology proprieties of *Ptychopetalum olacoides* Bentham (Olacaceae). *Pharm Biol* **36**: 327–334.

Takeda H, Tsuji M, Matsumiya T. 1998. Changes in head-dipping behavior in the hole-board test reflect the anxiogenic and/or anxiolytic state in mice. *Eur J Pharmacol* **350**: 21–29.

Treit D. 1985. Animal models for the study of anti-anxiety agents: a review. *Neurosci Biobehav Rev* **9**: 203–222.

Uber Bucek E, Fournier G, Dadoun H. 1987. Volatile constituents of *Ptychopetalum olacoides* root oil. *Planta Med* **53**: 231.

Wieland S, Lan NC, Mirasedeghi S, Gee KW. 1991. Anxiolytic activity of the progesterone metabolite 5α-pregnan-3α-ol-20-one. *Brain Res* **565**: 263–268.

