Memory retrieval improvement by \textit{Ptychopetalum olacoides} in young and aging mice

Adriana L. da Silva\textsuperscript{a,b}, \textsuperscript{*}Ángelo L.S. Piat\textsuperscript{a}, Simone Bardini\textsuperscript{a}, Carlos A. Netto\textsuperscript{b}, Domingos S. Nunes\textsuperscript{c}, Elaine Elisabetsky\textsuperscript{a,b,}\textsuperscript{*}

\textsuperscript{a} Laboratório de Etnofarmacologia, ICBS, Universidade Federal do Rio Grande do Sul, Av. Sarmento Leite 500/202, 90046-900 Porto Alegre, RS, Brazil

\textsuperscript{b} PPG em Ciências Biológicas-Bioquímica, ICBS, Universidade Federal do Rio Grande do Sul, Av. Ramiro Barcelos 2600, 90035-003 Porto Alegre, RS, Brazil

\textsuperscript{c} Departamento de Química, Universidade Estadual de Ponta Grossa, Campus Uvaranas, Bloco M, CEP: 84030-350 Ponta Grossa, PR, Brasil

Received 15 October 2003; received in revised form 22 June 2004; accepted 1 July 2004

\textbf{Abstract}

Amazonian peoples use traditional remedies prepared with \textit{Ptychopetalum olacoides} (PO) roots for treating various age-related conditions. This study shows that a single intraperitoneally (i.p.) administration of \textit{Ptychopetalum olacoides} ethanol extract (POEE, 50 and 100 mg/kg) improved memory retrieval in step-down inhibitory avoidance ($P \leq 0.05$ and $P \leq 0.01$, test session latency 102 [19.38–300] and 192 [91.3–300] s, respectively versus control 24.7 [12.9–89.6]), without interfering with acquisition or consolidation in adult (2.5-month-old) mice. Comparable results were obtained with POEE given p.o. at 800 and 1000 mg/kg ($P \leq 0.05$ and $P \leq 0.01$, 52.7 [19.5–297.2] and 85.7 [44.4–260.4] versus control 20.5 [8–92.6]). Moreover, memory amelioration was also observed ($P \leq 0.01$) in aging (14 months) mice presenting memory deficit (14.95 [10.8–41]) as compared to adult (2.5 months) mice (57 [15.7–141.2]), with the extract given acutely i.p. 100 mg/kg (300 [133.1–300] versus control 14.95 [10.8–41]) or p.o. 800 mg/kg (28.4 [15.1–84.6] versus control 11.5 [7.8–23.3]). Indeed, aging mice treated with POEE (100 mg/kg, p.o.) performed as well as adult mice. Consistently with its traditional use, the data suggest that POEE facilitates memory retrieval. Although the antioxidant and acetylcholinesterase inhibitory properties previously described for this extract may be of relevance, the molecular mechanism(s) underlying the improvement in memory retrieval here reported merit further scrutiny.

\textcopyright 2004 Elsevier Ireland Ltd. All rights reserved.

Keywords: \textit{Ptychopetalum olacoides}; Marapuama; Memory retrieval; Step-down; Aging; Neurodegenerative disorders

1. Introduction

Cognitive deficits are often observed in old humans, as well as in various neurological conditions. It has been previously proposed (Kubanis and Zornetzer, 1981) that memory retrieval in the elderly appears to be more impaired than acquisition or storage. Moreover, the first symptoms in Alzheimer’s disease include impairment of new information storage or retrieval (Dringenberg, 2000). With the increase of life expectancy and the consequent increase in the number of patients suffering from brain degenerative disorders, the search for products able to reduce or minimize cognitive deficits associated with aging has become even more attractive. Plant species traditionally used in non-western medical systems for enhancing cerebral function, like \textit{Gingko biloba} and \textit{Panax ginseng}, have proven to be effective in animal memory tests and useful in cognitively impaired humans (LeBars et al., 1997; Yamaguchi et al., 1997; Curtis-Prior et al., 1999; Zhong et al., 2000).

Abbreviations: PO, \textit{Ptychopetalum olacoides}; POEE, \textit{Ptychopetalum olacoides} ethanol extract; DMSO, dimethyl sulphoxide; ACTH, adrenocorticotropic hormone

* Corresponding author. Tel.: + 55 51 3316 3560; fax: +55 51 3316 3121.

E-mail address: elisasky@ufrgs.br (E. Elisabetsky).

0378-8741/$ – see front matter © 2004 Elsevier Ireland Ltd. All rights reserved.
were collected in Pará State (Brazil) and identified by Nelson Rosa. Voucher specimens were deposited at the herbarium of the Goeldi Museum (MPEG 108.036).

Ptychopetalum olacoides (PO) Bentham (Olacaceae), known as marapuama, mairapuama or mirante, is traditionally used in the Brazilian Amazon as a "brain tonic", specially by those recovering from central nervous systems (CNS) illnesses, by the elderly, and in general to cope with stressful situations; the pharmacological meaning and specific properties of such tonics have yet to be elucidated (Elisabetsky et al., 1992; Elisabetsky and Siqueira, 1998a). Therapeutic outcomes expected from the use of "brain tonics" include facilitating the recovery of cognitive and motor deficits after brain injuries (such as stroke), as well as improvement of cognitive functions, like alertness and memory, in the elderly.

Ptychopetalum olacoides is currently found in dozens of herbal products and multivitamin supplements in several American and European countries (Elisabetsky, 1987; Elisabetsky and Siqueira, 1998b; Paiva et al., 1998), with a diverse range of alleged effects. Previous pharmacological studies demonstrated that an ethanol extract of PO roots potentiated yohimbine-induced lethality, reversed reserpine-induced ptosis and prevented apomorphine-induced stereotypy in mice (Siqueira et al., 1998), favorably influenced performance in the forced swimming test (Paiva et al., 1998) and acted as an anxiogenic in the hole board model (da Silva et al., 2001), supporting the hypothesis that Ptychopetalum olacoides has central nervous system effects likely to affect the dopaminergic and noradrenergic systems.

The purpose of this study was to investigate the effects of Ptychopetalum olacoides ethanol extract (POEE) on memory consolidation, acquisition and retrieval in adult (2.5-month-old) and aging mice (14-month-old), using the step-down inhibitory avoidance test.

2. Material and methods

2.1. Animals

Experiments were performed using male adult mice, CF1 strain, received from the Fundação Estadual de Produção e Pesquisa em Saúde (FEPPS) immediately after weaning (21 days) or at 14 months of age (35–50 g, aging mice). Animals were maintained in our own animal facility room under controlled environmental conditions (22 ± 1 °C, 12h-light/12h-dark cycle, free access to food [Nuvilab CR1] and water); animals were maintained up to 10 weeks of age (25–40 g, adult mice), or in the case of aging mice for at least two weeks before the experiments. All procedures were carried out in accordance with institutional policies on experimental animal handling.

2.2. Plant material

Roots of Ptychopetalum olacoides Bentham (Olacaceae) were collected in Pará State (Brazil) and identified by Nelson Rosa. Voucher specimens were deposited at the herbarium of the Goeldi Museum (MPEG 108.036).

2.3. Preparation of extract

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

2.4. Drugs

Saline (NaCl 0.9%) and dimethyl sulphoxide (DMSO) were acquired from Delaware. POEE (10, 30, 50 and 100 mg/kg i.p. and 500, 800, and 1000mg/kg p.o.) was dissolved in a 20% DMSO solution (in water).

2.5. Step-down inhibitory avoidance

The step-down latencies are expressed as medians (interquartile ranges). Data were analyzed by Kruskal–Wallis non-parametric analysis of variance; comparisons between groups were run using the Mann–Whitney U test (two-tailed).

The step-down latencies are expressed as medians (interquartile ranges). Data were analyzed by Kruskal–Wallis non-parametric analysis of variance; comparisons between groups were run using the Mann–Whitney U test (two-tailed). Comparisons between training and test sessions within each group were made by the Wilcoxon test. The Spearman-rank correlation coefficient was used to check dose-effect associations.
Fig. 1. Effect of *Ptychopetalum olacoides* ethanol extract (POEE) given i.p. 30 min prior to training (A), immediately post-training (B), and 30 min prior to testing (C) on retention test performance of adult mice trained in step-down inhibitory avoidance (0.3 mA footshock, 24 h training-test interval). DMSO, dimethyl sulphoxide 20%. *N* = 20 per group. Each column represents the median (interquartile ranges) of training (light columns) or test (dark columns) session latencies *P* < 0.05, **P** < 0.01 significant difference compared with controls (saline and DMSO) in Mann–Whitney test, following Kruskal–Wallis.

3. Results

Confirming that learning and memory took place with the training paradigm used in this study, there were significant and consistent differences (*P* < 0.05) between training and test session latencies, in both saline-treated and DMSO-treated adult (2.5 month-old) and aging (14-month-old) groups. No differences in latencies were found in the various groups of training sessions, except for POEE (50 and 100 mg/kg) injected pre-training, when significant (*P* < 0.01) increases in step-down latencies were observed. Due to the marked increase in training session, latency induced with POEE 100 mg/kg administered pre-training, this is the only group where there is no significant difference between the training and test sessions (Wilcoxon, *P* = 0.22), although its test latency does not differ from those observed in all of the other similarly treated groups (Kruskal–Wallis, *P* = 0.42).

The doses found to be effective in facilitating retrieval were tested for potential effects in memory acquisition and consolidation. When tested for effects in memory acquisition, it was found that the higher POEE dose (100 mg/kg) significantly increased the latency in the training session; therefore, only the lowest dose effective in facilitating retrieval (50 mg/kg) could be properly analyzed for its effects on memory acquisition. No differences in test session performance were found after either pre- (Fig. 1A) or post-training (Fig. 1B) DMSO and POEE administrations. Most importantly, POEE (50 and 100 mg/kg i.p. or 800 and 1000 mg/kg p.o.) significantly improved retrieval (*P* < 0.05 and *P* < 0.01, respectively) in adult mice that received pre-test injections (Figs. 1C and 2). Correlation analysis using the Spearman test showed that this effect is dose-dependent (*r* = 1, *P* < 0.001).

Fig. 2. Effect of *Ptychopetalum olacoides* ethanol extract (POEE) given p.o. 90 min prior to retention test performance of adult mice trained in step-down inhibitory avoidance (0.3 mA footshock, 24 h training-test interval). DMSO, dimethyl sulphoxide 20%. *N* = 20 per group. Each column represents the median (interquartile ranges) of training (light columns) or test (dark columns) session latencies *P* < 0.05, **P** < 0.01 compared with controls (saline and DMSO), in Mann–Whitney following Kruskal–Wallis.

With aging (14 months) saline-treated mice there were no significant latency differences between training and test sessions, evidencing learning and memory deficits for this task; these aging mice also presented a retrieval deficit (*P* < 0.05) when compared to adult (10-week-old) animals. POEE (100 mg/kg i.p. and 800 mg/kg p.o.) administered pre-test improved memory retrieval (Figs. 3 and 4) in aging mice when compared to their controls (*P* < 0.01).

4. Discussion

This study showed that *Ptychopetalum olacoides* ethanol extract (POEE) improves retrieval in the step-down inhibitory avoidance test in a dose-dependent way, affecting neither memory consolidation nor task acquisition. With reference to the influences of locomotor activity (Zarrindast et al.,...
Considering both the lack and the need of drugs proven to be effective in improving memory retrieval (Espinola et al.,...
References


