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Memory retrieval improvement by *Ptychopetalum olacoides* in young and aging mice

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Abstract

Amazonian peoples use traditional remedies prepared with *Ptychopetalum olacoides* (PO) roots for treating various age-related conditions. This study shows that a single intraperitoneally (i.p.) administration of *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 (800 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.

Keywords: *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 *Gingko biloba* and *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, *Ptychopetalum olacoides*; POEE, *Ptychopetalum olacoides* ethanol extract; DMSO, dimethyl sulphoxide; ACTH, adrenocorticotrophic hormone

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Ptychopetalum olacoides (PO) Bentham (Olacaceae), known as marapuama, muirapuama or miriantã, 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 apormophine-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 modulation (acquisition, consolidation 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, 12 h-light/12 h-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 (12 L) 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 1000 mg/kg p.o.) was dissolved in a 20% DMSO solution (in water).

2.5. Step-down inhibitory avoidance

The test used was adapted from Netto and Izquierdo (1985) and from Maurice et al. (1994). Mice were habituated in a dimly-lit room for at least 30 min before the experiments. The inhibitory avoidance training apparatus was a plastic box (30 cm × 30 cm × 40 cm), with a platform (5 cm × 5 cm × 4 cm) fixed in the center of the grid floor. Each mouse was placed on the platform and the latency to step down (four paws on the grid), was automatically recorded in training and test sessions. In the training session, the mouse received a 0.3 mA scrambled foot shock for 15 s, upon stepping down. Animals exhibiting step-down latencies greater than 30 s in training were excluded from experiments; less than 5% of the animals met this exclusion criterion. The test session was performed 24 h later, with the same procedure except that no shock was administered after stepping down; an upper cut-off time of 300 s was set.

Drugs (saline, DMSO or POEE) were administered intraperitoneally (i.p.) as follows: 30 min before training, to evaluate effects on task acquisition (in this case, no exclusion criterion was applied); immediately after training, to evaluate effects on memory consolidation, and 30 min before testing, to assess memory retrieval.

In order to assess memory retrieval with orally (p.o.) administered treatments, the drugs (saline, DMSO or POEE) were administered 90 (adult mice) or 120 min (aging mice) before the test session.

2.6. Statistical analysis

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.

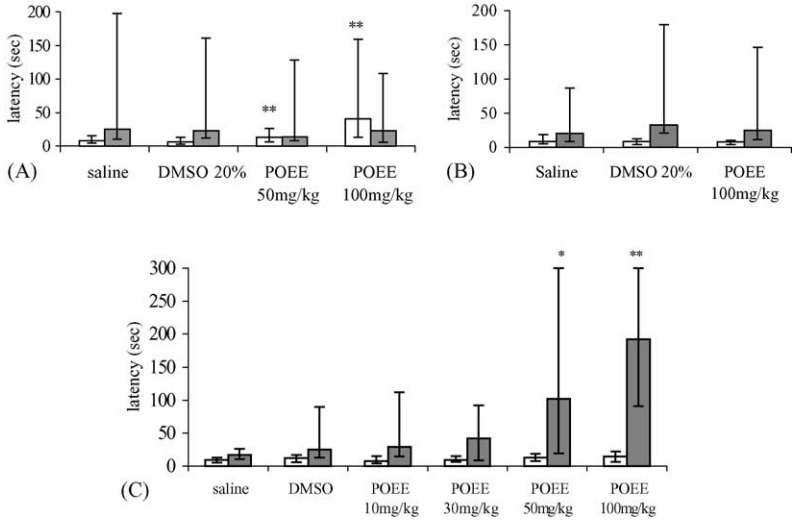

Fig. 1. Effect of *Ptychopetalum olacoides* ethanol extract (POEE) given i.p. 30 min prior to training (A), immediately post-training (B), and 30 min prior to testing (C) on retention test performance of adult mice trained in step-down inhibitory avoidance (0.3 mA footshock, 24 h training-test interval). 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 U 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 ($r = 1$, $P < 0.001$).

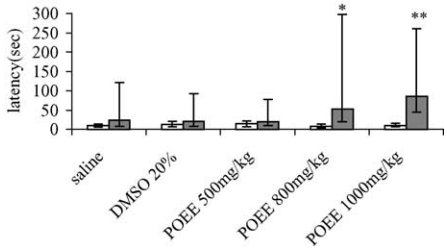

Fig. 2. Effect of *Ptychopetalum olacoides* ethanol extract (POEE) given p.o. 90 min prior to retention test performance of adult mice trained in step-down inhibitory avoidance (0.3 mA footshock, 24 h training-test interval). DMSO, dimethyl sulphoxide 20%. $N = 20$ per group. Each column represents the 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.,

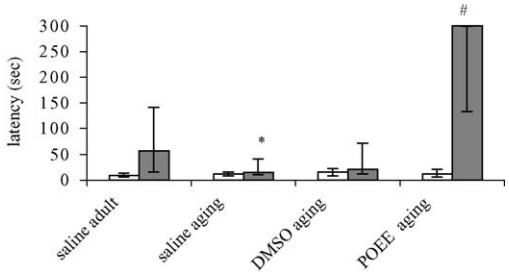

Fig. 3. Effect of *Ptychopetalum olacoides* ethanol extract (POEE) given i.p. 30 min prior to retention test performance of aging mice trained in step-down inhibitory avoidance (0.3 mA footshock, 24 h training-test interval). DMSO, dimethyl sulphoxide 20%; POEE, POEE 100 mg/kg. Each column represents the median and interquartile ranges of training (light columns) or test (dark columns) session latencies. $N = 20$ per group. $^{*}P < 0.05$, as compared to saline-treated adult mice, $^{\#}P < 0.01$ as compared to saline- and DMSO-treated aging and saline-treated adult mice, in Mann–Whitney following Kruskal–Wallis.

1996), although the step-down inhibitory avoidance test is more reliable than other methods for memory assessment, false positives related to diminished motor activity leading to increased test session latencies should, nevertheless, be considered. In this case, influence on motor activity by the extract can be ruled out since previous studies demonstrated that POEE (30–100 mg/kg) affected neither locomotor activity in the hole-board test nor motor coordination as evaluated in the rota-rod test (da Silva et al., 2001). However, POEE decreases exploratory behavior (head dipping) in the hole-board test, a result compatible with the profile of anxiogenic drugs (Takeda et al., 1998; da Silva et al., 2001).

The step-down inhibitory avoidance task is a classic paradigm to assess memory with a strong aversive component (Cahill et al., 1986). An anxiogenic effect could account for the increased latencies observed in training sessions when POEE was injected pre-training (with no significant effect in test session latencies with this particular drug administration paradigm). It has been reported that stress hormones, when administrated pre-test, may enhance memory retrieval (Izquierdo et al., 2002). Catecholamines and glucocorticoids have been reported to improve memory consolidation and impair memory retrieval (Cahill and McGaugh, 1998; Roozental, 2002). ACTH, adrenaline, vasopressin and β-endorphin enhanced step-down memory retrieval at low doses in 3 month-old rats (Izquierdo et al., 2002). Furthermore, low to moderate doses of neuromodulators and peripheral hormones can be associated with memory retrieval enhancement (Izquierdo et al., 2000). Further studies are, therefore, necessary to distinguish POEE effects on anxiety and memory retrieval. It is noteworthy that POEE did not present different effects depending on the route of administration, since administration by the intraperitoneal or oral routes improved memory retrieval dose-dependently in adequate dose ranges. In the aging mice oral gavage was given 120 min before session tests (instead of 90 min in adult mice) due to age-related changes in drug absorption, including increased gastric pH, reduced gastrointestinal motility and decreased absorption surface (Gareri et al., 2000).

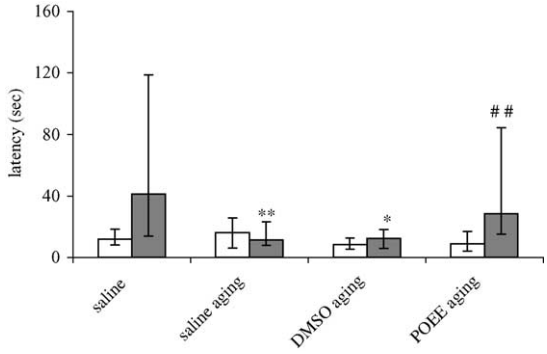

Fig. 4. Effect of *Ptychopetalum olacoides* ethanol extract (POEE) given p.o. 120 min prior to retention test performance of aging mice trained in step-down inhibitory avoidance (0.3 mA footshock, 24 h training-test interval). DMSO, dimethyl sulphoxide 20%; POEE, 800 mg/kg. Each column represents the median and interquartile ranges of training (light columns) or test (dark columns) session latencies. $N = 20$ per group. $^{*}P < 0.05$ as compared to saline adult mice, $^{**}P < 0.01$ as compared to saline adult mice, $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ as compared to saline aging mice, in Mann–Whitney following Kruskal–Wallis.

The molecular mechanism by which POEE facilitates memory retrieval remains to be elucidated. Its behavioral profile (Siqueira et al., 1998; da Silva et al., 2001) suggests interactions with a diversity of neurotransmitters (including noradrenaline, serotonin and dopamine), a pattern consistent with the current understanding of the modulation of memory processes (Izquierdo et al., 2000). Moreover, we have recently reported that POEE has the ability to inhibit AChE as evaluated by in vitro and ex vivo assays (Siqueira et al., 2003), indicating improvement of cholinergic function as a potential neurochemical correlate of POEE effects on memory retrieval deficit in aging mice.

It is estimated that within the next 50 years, approximately 30% of the world population will be aged 65 years or older (Youdim and Joseph, 2000); more importantly, by the year 2025 70% of world's older population will be living in developing countries (Kalache et al., 2002). According to Chaimowicz (1997), the Brazilian population has been aging quickly (aging population index of 6.4 in 1960 reaching 13.9 in 1991), an increase of more than 100% in only three decades. It is, therefore, crucial to develop appropriate health care means for age-related neurodegenerative disorders, including cognitive deficits. It is worth noting that the results of this study are remarkably in agreement with therapeutic claims by traditional communities for *Ptychopetalum olacoides*-based home-made remedies. Particularly because experimental results are consistent with anecdotal reports of therapeutic effects in humans taking such remedies orally, the data revealed in this study are of relevance for the development of drugs for managing memory deficits associated with age and with a number of neurological disorders.

Considering both the lack and the need of drugs proven to be effective in improving memory retrieval (Espinola et al.,

1997; Abe and Saito, 2000; Bhattacharya et al., 2000; Vohora et al., 2000), the specific facilitating effect on retrieval reported here is of particular interest and deserves further scrutiny, including POEE effects in memory paradigms devoid of aversive components.

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