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The antidepressant-like effects of *Aloysia polystachya* (Griseb.) Moldenke (Verbenaceae) in mice

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Abstract

The aim of the present work is to evaluate the putative antidepressant-like effects of a hydro-ethanolic extract (CEAp) and their fractions from the aerial parts of *Aloysia polystachya* (Griseb.) Moldenke (Verbenaceae) on the performance of male mice in the forced swimming test (FST). A single dose (100.0 mg/kg p.o.) of CEAp, in male mice provoked a significant reduction of the immobility time ($p<0.01$). Such effect was also observed with short-term treatment (7 days) with single doses of 1.0 ($p<0.01$), 10.0 ($p<0.05$) and 100.0 ($p<0.05$) mg/kg/day of CEAp. Additionally, in a different set of experiments, repeated administration in a 24-h period (24, 18 and 1 h before swimming test) with doses of 1.0 ($p<0.05$) and 10.0 ($p<0.05$) mg/kg p.o., of CEAp and 10.0 mg/kg p.o., ($p<0.05$) of ethyl acetate fraction, provoked significant reduction of the immobility time of male mice in the FST. Moreover, it was noted important differences in the onset of the antidepressant-like effect in the FST, depending on the modality of treatment with CEAp (acute, short-term or repeated). Both, efficacy and potency were higher when repeated administration of CEAp was used, and surprisingly the dose of 10 mg/kg (24, 18 and 1 h before swimming test) was more effective than imipramine. In the same way, the short term administration (7 days) improved significantly efficacy and potency of the CEAp in comparison to a single dose treatment. The ethyl acetate fraction submitted to TLC demonstrated that main and minor components are phenolics and terpenes, respectively. In addition, this fraction gives a negative Shinoda's test for flavonoids. These results indicate an antidepressant-like profile of action for the hydro-ethanolic extract and the component(s) of the ethyl acetate fraction obtained from *A. polystachya*, which deserve further investigation.

Keywords: *Aloysia polystachya*; Verbenaceae; Antidepressant effect; Forced swimming test; Immobility

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Introduction

Depressive disorders are among the most common diseases in humans, with approximately 11.3% of all adults afflicted during any 1 year (Judd, 1995). Roughly, 90% of patients with depressive symptoms suffer from mild to moderate depression, while only 10% are severely depressed. In mild to moderate depression, in particular, some practitioners and patients are reluctant to use standard antidepressants like tricyclic antidepressants or specific serotonin reuptake inhibitors (SSRIs), because of their side effects. Therefore, additional treatment strategies with favourable side effect profile, credible benefits and moderate costs are of particular interest (Ernst, 1995; Laakmann et al., 1998).

A half of a century ago, antidepressants was discovered by serendipity. There are a few reliable animal models which both, resemble the clinical phenomenon of depressive illness and are selectively sensitive to treatments known to be effective for its management (Gluckman and Baum 1969; Van Riezen, 1972). In a series of publications, Porsolt et al. (1977, 2001) and Cryan et al. (2002), have reported a new behavioural test in rodents, developed as a primary screening procedure, to detect the efficacy of antidepressants. In addition, the efficacy of the forced swimming test (FST) with mice was reviewed by Petit-Demouliere et al., (2005) and concluded that the assay has good reliability and predictive validity, being the use of Swiss strain mice one of the most important factors for evaluating antidepressant activity. Moreover, at the present time, the FST is the most widely used model for assessing potential antidepressant activity in rodents, following acute and short-term treatment (Cryan et al., 2005).

In the search for new molecules useful for the treatment of neurological disorders, medicinal plant research, worldwide, has progressed constantly, demonstrating the pharmacological effectiveness of different plant species in a variety of animals models (Zhang, 2004). The pharmacological approach to the treatment of depression includes long-term employment of antidepressant, either in the form of monotherapy or as a combination of several antidepressants with various mechanisms of action. Currently, *Hypericum perforatum* L. (St. John's wort) is probably one of the most commonly used natural antidepressant. Several constituents of the extract of *H. perforatum*, such as hypericin and hyperforin, seem to be important for this effect. *H. perforatum* is considered to be an effective alternative to conventional therapeutic agents in the treatment of mild to moderate depression (Bach-Rojecky et al., 2004).

Aloysia polystachya (Griseb.) Moldenke, is an aromatic native plant of Verbenaceae family which is widely distributed in subtropical regions of South America, mainly Paraguay and the North of Argentine. In Paraguayan folk medicine, is a well-known medicinal plant, popularly named "burrito", which has been used for a wide variety of indications, including digestive and respiratory tract disorders (González Torres, 1996). In Luque District, Paraguay, it was mentioned its use as sedative and as "tonic for the nerves", similarly as reported by Del Vitto et al. (1997) in Argentine. Leaves of *A. polystachya* are used, in Argentine, for respiratory diseases (colds and cough), gastrointestinal pain (Filipoy, 1994), as antiemetic (Martinez Crovetto, 1981) and as a sedative remedy (Del Vitto et al., 1997). Therapeutic uses of other species of *Aloysia* (i.e., *A. triphilla*) include febrifuge, sedative, stomachic, diuretic, and antispasmodic activities (Oliveira de Figueiredo et al., 2002). Concerning its chemical composition, the presence of monoterpenes (carvacrol, carvone, eucarvone, limonene, (−) limonene, α-pinene, sabinene, (+) sabinene, (−) thujone, α-thujone, α-(−) thujone, β-thujone, isothujone, (+) isothujone), in leaf essential oil, was revealed by several studies (Fester et al., 1956; Huergo and Retamar, 1973; Gatto et al., 1981).

Recently, we have found that crude extract of *A. polystachya* (CEAp) administered orally to mice, exhibits anxiolytic-like effect, devoid of any hypnosedative activity. We also demonstrated that CEAp has low toxicity and no lethality, being well tolerated by mice. Moreover, hydroalcoholic extract from *A. polystachya* also showed anxiolytic and antidepressant-like effects in rats (Mora et al., 2005).

The present study was undertaken to study in mice the influence of CEAp on the duration of immobility in the FST, after acute administration, and after short-term treatment (7 days) and, to determine the influence of several fractions of CEAp in the FST after repeated administration in a 24-h period (24, 18 and 1 h before swimming test).

Materials and methods

Extraction and fractionation

Aerial parts of cultivated *Aloysia polystachya*, in blossom, were collected from the Botanical Garden for Medicinal Plants of the Chemical Science Faculty, San Lorenzo, Paraguay, on December 2001. A voucher herbarium specimen has been deposited at the Department of Botany under the number Ortiz 1498. Fresh aerial parts were air-dried in the shadow and grounded, yielding 1474 g of powder which was extracted with a mixture of ethanol:water (60:40) at 50 °C for 1 h in a bathing apparatus. The extraction was repeated twice and the filtered hydro-ethanolic extract were mixed and evaporated under reduced pressure. The concentrated

extract (CEAp) was frozen and finally freeze-dried to yield 208.7 g (14.48%) of dry extract which was used for the biological tests. A sample of about 1.1 g of CEAp was suspended in 500 mL of deionised water and successively extracted with hexane, dichloromethane and ethyl acetate, using 500 mL of each solvent, three times (Fig. 1). Hexane and dichloromethane extracts were dried with anhydrous sodium sulphate, filtered and evaporated under reduced pressure. Ethyl acetate extract was concentrated under reduced pressure and the water fraction was freeze-dried. In order to determine what kind of the constituents are present, the ethyl acetate fraction was submitted to analytical TLC on a silica gel-precoated plastic plate (Wagner and Bladt, 1996). With the purpose to detect naturally occurring phenolics, a mixture of ethyl acetate–formic acid–glacial acetic-acid–water (100:11:11:26) was used as mobile phase and UV light detection at 254 and 365 nm was used as suitable screening system. Fast Blue B was used as spraying reagent. When occurring phenolics compounds, blue or blue–violet (vis) spots are developed. The color is intensified by further spraying with 10% aqueous sodium hydroxide solution. To terpenoids detection TLC was performed using toluene–dichloromethane–ethanol (40:40:10) as a solvent system and further developed by spraying with a mixture of anisaldehyde–sulphuric acid. This system, in the presence of terpenes, yields mainly blue, blue–violet and sometimes red or yellow–brown spots (Fig. 1).

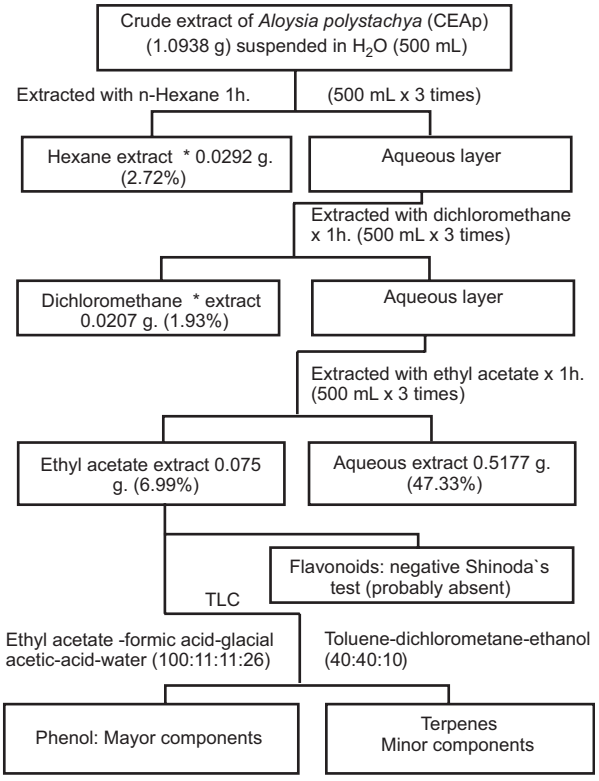

Fig. 1. Flow diagram of fractionation of the CEAp of *Aloysia polystachya*. Number in parenthesis indicate percent yields of each fraction (*dried with sodium sulphate anhydrous).

Animals

Swiss albino male mice, weighing between 20 and 30 g, kept under controlled conditions (12 h dark–light cycle, 23–25 °C temperature and 50–60% humidity) were used. All experiments were conducted in accordance with international standards of animal welfare and the experimental protocols were approved by the local Animal Care and Use Committee (FCQ-2006/04). The minimum number of animals and duration of observation required to obtain consistent data were used. Behavioural experiments were conducted from 10:00 AM to 2:00 PM. The animals received a standard food pellet and before the experiments they were fasted overnight with water *ad libitum*.

Drugs

Imipramine hydrochloride was obtained from Wako Pure Chemical Industries Ltd. (Japan), dichloromethane, ethanol, ethyl acetate, hexane, Tween 80 and propylenoglycol for pharmaceutical use were locally purchased.

Behavioural despair (forced swimming test) in mice

This test was performed according to the procedure described by Porsolt et al (1977, 2001), with slight modifications. Briefly, 1 h after dosing, the animals were individually forced to swim in a transparent glass vessel (25 cm high, 15 cm in diameter) filled with (12.5 cm high) water at 21–24 °C. The total duration of immobility (in seconds) was measured during the last 4 min of a single 6 min test session. Groups of 10 mice were treated acutely with single dose of vehicle, CEAp (1.0, 10.0, 100.0 and 1000.0 mg/kg) p.o., and 1 h later mice were individually forced to swim in the glass vessel. Other groups of 8–9 mice were short-term treated (7 days) with a single daily dose of vehicle, CEAp (1.0, 10.0, 100.0 and 1000.0 mg/kg/day) p.o., or imipramine i.p. (32 mg/kg), and 1 h after the last administration they were individually forced to swim in same conditions. In a different set of experiments, groups of 10 animals were subjected to repeated administration of three doses of vehicle, CEAp and their fractions (hexane, dichloromethane, ethyl acetate and aqueous), using 1.0 and 10.0 mg/kg p.o., 24, 18 and 1 h prior to the swimming test. Behaviour was monitored from the frontal side by a video camera for subsequent analysis. Mice were considered immobile when they made no further attempts to escape except the movements necessary to keep their heads above the water.

Statistical analysis

The results are expressed as mean ±S.D, and statistical analysis of the data was performed by the Dunn's Multiple Comparison test after Kruskal–Wallis non-parametric ANOVA. Probability level less than 0.05 was considered as statistically significant.

Results

Effects of CEAp on the immobility time

A significant shortening of the immobility time was observed ($p<0.01$) when an acute dose of 100.0 mg/kg p.o., of CEAp was administered to mice. Imipramine (32.0 mg/kg i.p.) also showed a significant reduction of immobility time ($p<0.001$), compared to vehicle treated animals (Fig. 2). Short-term treatment (7 days) induced a decrease of the immobility time when the animals were exposed to the FST after dosing orally, once a day, with 1.0 ($p<0.01$), 10.0 ($p<0.05$) and 100.0 mg/kg ($p<0.05$) of CEAp (Fig. 3). In addition, repeated administration in a 24 h period, showed a significant reduction of the immobility time with 1.0 ($p<0.05$) and 10.0 mg/kg ($p<0.01$) of CEAp in a different groups of mice submitted to FST in comparison to control group (Fig. 4).

Effect of fractions of *A. polystachya* on the immobility time

Repeated oral administration (three doses in a 24 h period) of 1.0 and 10.0 mg/kg of hexanic, dichloromethane

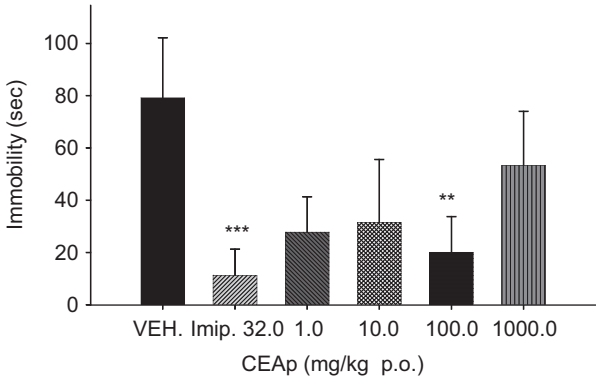

Fig. 2. Effect of the vehicle (0.1 mL/10 g body weight) (VEH), imipramine (imip, 32.0 mg/kg, i.p.) and increasing single doses of CEAp (1.0, 10.0, 100.0 and 1000.0 mg/kg p.o) of *Aloysia polystachya* on the forced swimming test (FTS) in mice. Each bar represents the mean±SD of 10 animals. ***$p<0.001$; **$p<0.01$, significantly different from vehicle, Dunn's multiple comparison test after Kruskal–Wallis non-parametric ANOVA.

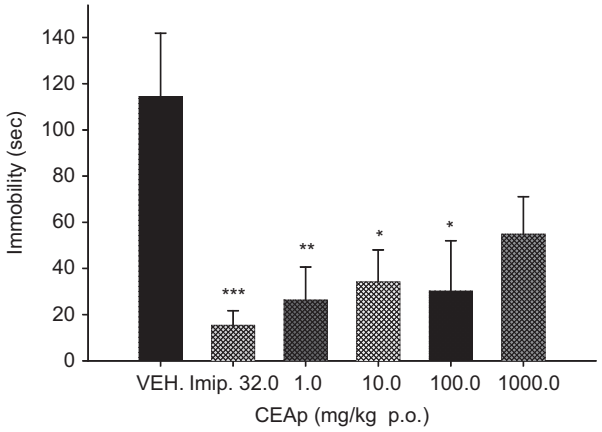

Fig. 3. Effect of the vehicle (0.15 mL/30 g body weight) (VEH), imipramine (imip, 32.0 mg/kg, i.p.) and increasing doses during 7 days of CEAp (1.0, 10.0, 100.0, and 1000.0 mg/kg/day, p.o.) of *Aloysia polystachya* on the forced swimming test (FTS) in mice. Each bar represents the mean±SD of 8 animals. ***$p<0.001$; **$p<0.01$; *$p<0.05$, significantly different from vehicle, Dunn's multiple comparison test after Kruskal–Wallis non-parametric ANOVA.

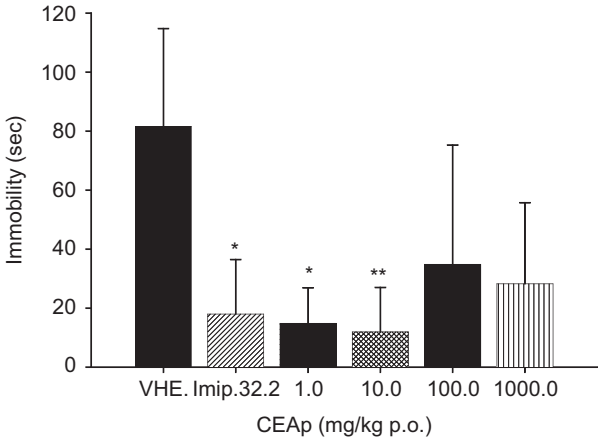

Fig. 4. Effect of repeated administration of three doses in 24 h of vehicle (0.1 mL/g de peso corporal) (VEH), imipramine (32.0 mg/kg i.p) and increasing doses of CEAp (1.0, 10.0, 100.0, and 1000.0 mg/kg p.o) obtained from *Aloysia polystachya* on the forced swimming test (FTS) in mice. Each bar represent the mean±SD of 10 animals. **$p<0.01$; *$p<0.05$, significantly different from vehicle, Dunn's multiple comparison test after Kruskal–Wallis non-parametric ANOVA.

and aqueous fractions of *A. polystachya*, did not produce any significant change on the immobility time in mice, when exposed respectively, to the FST. However, repeated treatment with dose of 10.0 mg/kg of ethyl acetate fraction of *A. polystachya*, reduced significantly the immobility time ($p<0.05$), in comparison to control group receiving the vehicle and submitted to FST. Imipramine (32.0 mg/kg i.p.) showed a significant reduction of immobility time ($p<0.01$), compared to vehicle treated animals (Fig. 5). The ethyl

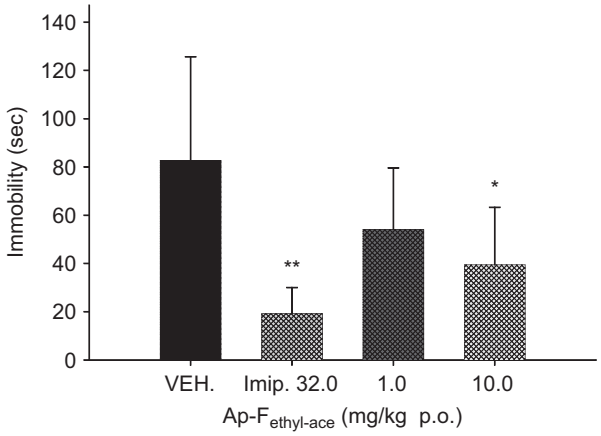

Fig. 5. Effect of repeated administration of three doses in 24 h of vehicle (0.1 mL/g de peso corporal) (VEH), imipramine (32.0 mg/kg i.p) and increasing doses of Ap-$F_{ethyl-ace}$ (1.0, 10.0 mg/kg p.o) obtained from *Aloysia polystachya* on the forced swimming test (FTS) in mice. Each bar represent the mean ± SD of 14 animals. $^{**}p<0.01$; $^{*}p<0.05$, significantly different from vehicle, Dunn's multiple comparison test after Kruskal–Wallis non-parametric ANOVA.

acetate fraction submitted to TLC demonstrated that main components are phenolic compounds. In addition, terpenes are also present but as minor constituents. The ethyl acetate fraction gave a negative Shinoda's test for flavonoids.

Discussion

The present study characterized the effects of the administration of crude hydroalcoholic extract (CEAp) and fractions obtained from *A. polystachya*, on mice performance in forced swimming test (FST) following acute, short-term and repeated treatment with extracts and fractions obtained from a plant popularly used to treat "nervous diseases" in Paraguayan traditional medicine. In two previous papers we have reported the anxiolytic and antidepressant-like properties in rats (Mora et al., 2005) and the anxiolytic-like effect in mice of CEAp, obtained from the aerial parts of *A. polystachya*. Besides that, we demonstrated that CEAp exhibits low toxicity, no lethality, was well tolerated, did not induce significant changes in several behavioural and physiological parameters and is devoid of any hypnosedative activity, when administered to mice (Hellion-Ibarrola et al., 2006).

Because the pharmacotherapy of depression typically requires chronic drug treatment to obtain a full response in terms of antidepressant effect, it is critical to perform, not only acute, but also short-term, chronic and repeated treatments in the FST mice model. Regarding the medical treatment of psychiatric disorders, the results obtained in this work became important, because not only anxiolytic effects were observed with CEAp, but also the antidepressant properties in mice were established. Certainly, both the administration of CEAp and its ethyl acetate fraction produced a diminution of immobility time of mice exposed to the FST under acute, short-term or repeated modalities. Indeed, single dose of 100.0 mg/kg p.o., of CEAp provoked a highly significant reduction of immobility time ($p<0.01$). Similar results were also observed with short-term treatment (7 days) with single dose of 1.0 ($p<0.01$), 10.0 ($p<0.05$) and 100.0 ($p<0.05$) mg/kg/day of CEAp. Additionally, in a different set of experiments, repeated administration in a 24-h period (24, 18 and 1 h before swimming) doses of 1.0 ($p<0.05$) and 10.0 ($p<0.05$) mg/kg p.o., of CEAp and 10.0 mg/kg p.o., ($p<0.05$) of ethyl acetate fraction, provoke significant reduction of the immobility time of male mice subjected to the FST, when compared to control group (vehicle). Moreover, depending on the modality of treatment with CEAp (acute, short-term or repeated) in the FST, it was observed important differences in the onset of the antidepressant-like effect. In fact, both efficacy and potency of CEAp as antidepressant were higher when repeated administration was used, and interestingly 10 mg/kg (24, 18 and 1 h before swimming) was more effective than imipramine (32.0 mg kg i.p.) used as positive control. In the same way, the short term administration (7 days), improves significantly the efficacy and potency of the CEAp, in comparison to single dose treatments. These behavioural effects were similar to those seen in rats treated with *A. polystachya* (Mora et al., 2005) and those found in mice treated with conventional antidepressant drugs, such as tricyclic, monoamine oxidase inhibitors and selective serotonine re-uptake inhibitors agents (Porsolt et al., 1977; Borsini and Meli, 1988; Petit-Demouliere et al., 2005; Cryan et al., 2005).

The preliminary standardization of the active ethyl acetate fraction, using TLC, let recognize phenolics as the main components and terpenes as the minor. Flavonoids are probably absent in this fraction, taking into account the negative result in the Shinoda's test. Therefore, according to the TLC fingerprinting assay, the active antidepressant principles are unknown and may correspond to one or more component of this fraction.

In conclusion, the results of this preclinical study provide evidence about the antidepressant effects of the hydroalcoholic extract of *Aloysia polystachya* and its ethyl acetate fraction, administered either acute or repeatedly in mice. Additionally, it provides the TLC fingerprinting for standardization of the active ethyl acetate fraction. These results contribute to the scientific validation of the indications of this plant in Paraguayan folk medicine. However, further experiments are needed

to identify its active compounds and the corresponding mechanisms of action. Our results encourage us to pursue the identification of the molecules associated to the effect observed in CEAp.

Acknowledgements

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