Antidepressant and anxiolytic effects of hydroalcoholic extract from *Salvia elegans*☆

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Abstract

Salvia elegans Vahl (Lamiaceae), popularly known as "mirto", is a shrub that has been widely used in Mexican traditional medicine for the treatment of different central nervous system (CNS) diseases, principally, anxiety. Nevertheless, the available scientific information about this species is scarce and there are no reports related to its possible effect on the CNS. In this work, the antidepressant and anxiolytic like effects of hydroalcoholic (60%) extract of *Salvia elegans* (leaves and flowers) were evaluated in mice. The extract, administered orally, was able to increase the percentage of time spent and the percentage of arm entries in the open arms of the elevated plus-maze, as well as to increase the time spent by mice in the illuminated side of the light–dark test, and to decrease the immobility time of mice subjected to the forced swimming test. The same extract was not able to modify the spontaneous locomotor activity measured in the open field test. These results provide support for the potential antidepressant and anxiolytic activity of *Salvia elegans*.

Keywords: Anxiety; Depression; *Salvia elegans*; Elevated plus-maze; Forced swimming test; Open field

1. Introduction

According to the World Health report (WHO, 2001), approximately 450 million people suffer from a mental or behavioral disorder, yet only a small minority of them receive even the most basic treatment. This amounts to 12.3% of the global burden of disease, and will rise to 15% by 2020 (Reynolds, 2003). In the search for new therapeutic products 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 animal models (Zhang, 2004).

In Mexican traditional medicine, an infusion is prepared from the leaves and flowers of *Salvia elegans* Vahl (Lamiaceae), popularly known as "mirto", and administered orally for treating CNS diseases. This plant constitutes one of the most used species in Mexico for the treatment of anxiety, and insomnia (Aguilar et al., 1994). It is important to mention that the common name of this plant is shared with other members of the genus (Martínez, 1979; Bello, 1993). Despite the widely popular use of the plant, it was not possible to find pharmacological data confirming some activity of this plant on the mentioned diseases. The only evidence of probable CNS activity was found in a report showing that the methanolic extract of *Salvia elegans* was able to displace [^{3}H]-(*N*)-scopolamine from muscarinic receptors in homogenates of human cerebral cortical cell membranes (Wake et al., 2000). Despite the scarce pharmacological scientific information about this species, there are other members of the family which possess demonstrated CNS activities. *Salvia divinorium* Epling and

Abbreviations: CNS, central nervous system; DZP, diazepam; EPM, elevated plus maze; FST, forced swimming test; IMI, imipramine; LDT, light–dark test; OFT, open field test; PTX, pycrotoxine

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Játiva has hallucinogenic (Siebert, 1994; Chavkin et al., 2004) and antidepressant properties (Hanes, 2001), and *Salvia officinalis* L. possesses metabolites with benzodiazepine-like effects (Kavvadias et al., 2003), *Salvia miltiorrhiza* f. *alba* Wu and Li, used in Chinese traditional medicine, has shown neuroprotective effect, due to the NMDA receptor antagonistic activity (Sun et al., 2003), and *Salvia reuterana* Boiss recently showed anxiolytic effect in mice (Rabbani et al., 2005).

Twenty-eight volatile constituents were identified in essential oil from *Salvia elegans*, mono- and sesqui- terpenoids such as *trans*-ocimene, linalool, β-caryophyllene, germacrene D and spathulenol, aliphatic alcohols such as 2-propanol and 3-octanol, and trans-3-hexenal (Makino et al., 1996).

These data confirm that different members of the Lamiaceae family are able to modulate the physiology of the CNS. On this basis, the objective of the present study was to evaluate the anxiolytic and antidepressant effect produced by the hydroalcoholic extract from *Salvia elegans* in mice. Experiments were conducted with the ICR mice strain, which permits the analysis of the effects produced by *Salvia elegans* in different mice models.

2. Methods

2.1. Plant material and extract preparation

Leaves and flowers of *Salvia elegans* were collected from the state of Puebla, south west of Mexico. Plant material was identified by Abigail Aguilar-Contreras, M.Sc., the IMSSM Herbarium Director (located in National Medical Center, Mexico City). Voucher specimens were stored at this site for future reference (IMSSM-14588). From the collected plant, the leaves and flowers were selected and dried under dark conditions at room temperature for 2 weeks. Dry material was milled in an electrical grinder (Pulvex) obtaining particles less than 4 mm. Milled material was extracted in 60% ethanol solution at 50 °C for 2 h. Afterwards, the extract was filtered through a Wattman #1 paper and extracted once again (under the same conditions) with a new solvent. The obtained extracts were reunited and the solvent was evaporated to dryness with a rotary evaporator under reduced pressure. The yield of the extract was quantified (16.48%) and the obtained material was protected from direct light and stored under 4 °C until its use.

2.2. Drugs

The hydroalcoholic extract from *Salvia elegans* was used as experimental extract (25–2000 mg/kg; dissolved in saline solution), and as positive controls were used: diazepam (DZP, 1.0 mg/kg, Sigma) as an anxiolytic drug; picrotoxin (PTX, 2.0 mg/kg, Sigma St. Louis, USA) as an anxiogenic drug; and imipramine hydrochloride (IMI, 15 mg/kg, Sigma St. Louis, USA) as an antidepressant drug.

2.3. Animals and treatments

All experiments were conducted in accordance with international standards of animal welfare recommended by the Society for Neuroscience (USA). The experimental protocol was approved by the Institutional Research Committee. The minimum number of animals and duration of observation required to obtain consistent data were employed.

Male ICR mice (32–38 g) purchased at Harlan Mexico were used. All animals were maintained under controlled conditions of temperature (22 ± 2 °C), and illumination (12 h light–dark cycle), with free access to food (Harlan rodent lab diet) and water. Groups of eight animals were organized and, in order to reduce the influence of day variation, all assays were conducted from 8 to 13 h, in a special noise-free room with controlled illumination.

Mice were treated orally with different doses of the hydroalcoholic extract of *Salvia elegans* (125, 250, 500, 1000 and 2000 mg/kg). A negative control group was included which received physiological saline solution (p.o.). Positive control groups were administered with 15 mg/kg of IMI i.p. (for forced swimming test) or with 1.0 mg/kg of DZP i.p. (for the other tests) and an anxiogenic group was treated with 2.0 mg/kg of PTX i.p. (for elevated plus-maze, light–dark test and open field test). All treatments were administered 1 h before the test, with the exception of forced swimming test, in which IMI and the plant extract were administered 24, 18 and 1 h before the test.

2.4. Elevated plus-maze (EPM)

This test has been widely validated to measure anxiety in rodents (Pellow et al., 1985; Lister, 1987). This apparatus was made of Plexiglas and consisted of two open arms (30 cm × 5 cm) and two closed arms (30 cm × 5 cm) with 25 cm walls. The arms extended from a central platform (5 cm × 5 cm). The maze was elevated 38.5 cm from the room floor.

Each animal was placed at the center of the maze, facing one of the enclosed arms. Number of entries and the time spent in enclosed and open arms was recorded for 5 min test. Entry into an arm was defined as the animal placing all four paws onto the arm. All tests were taped by using a video camera. After each test, the maze was carefully cleaned up with a wet tissue paper (10% ethanol solution).

2.5. Forced swimming test (FST)

The FST is the most widely used pharmacological in vivo model for assessing antidepressant activity (Porsolt et al., 1977). The development of immobility when the mice are placed in an inescapable cylinder filled with water, reflects the cessation of persistent escape-directed behavior (Lucki, 1997). The apparatus consisted of a clear plexiglas cylinder (20 cm high × 12 cm diameter) filled to a 15 cm depth with water (24 ± 1 °C). In the pre-test session, every animal was placed individually into the cylinder for 15 min, 24 h prior to the 5 min swimming test. *Salvia elegans* extract, imipramine and distilled water were administered three times: immediately after the initial 15-min pre-test, 18 and 1 h prior to the swimming test. During the test session a trained observer registered the immobility time, considered to be

when the mouse made no further attempts to escape, apart from the movements necessary to keep its head above the water. It was suggested (Porsolt et al., 1977) that the immobility reflected a state of lowered mood in which the animals had given up hope of finding an exit and had resigned themselves to the experimental situation.

2.6. Light–dark test (LDT)

The apparatus consisted of a Plexiglas box with two compartments (20 cm × 20 cm each), one of which was illuminated with a white light while the other remained dark. Each animal was placed at the center of the illuminated compartment, facing one of the dark areas. The time spent in illuminated and dark places, as well as the number of entries in each space, was recorded for 5 min (Crawley and Goodwin, 1980).

2.7. Open field test (OFT)

The open-field area was made of acrylic transparent walls and black floor (30 cm × 30 cm × 15 cm) divided into nine squares of equal area. The open field was used to evaluate the exploratory activity of the animal (Archer, 1973). The observed parameters were the number of squares crossed (with the four paws) and number of rearings.

2.8. Statistical analysis

The statistical analysis of the results was carried out with a SPSS 11.0 program and based on an analysis of variance (ANOVA) followed by the Dunnet's test, in which a significant difference was established among groups when the p value was lower than 0.05.

3. Results

3.1. Elevated plus-maze

The oral administration of different doses of *Salvia elegans* extract in ICR mice exposed to the EPM test significantly [Veh 101.6 ± 31.0; 125 mg/kg, 137 ± 39.0; 250 mg/kg, 118.53 ± 30.21; 2000 mg/kg, 153.0 ± 17.4; $F(5.59) = 18.101$; $p < 0.001$] increased the time that mice spend in open arms comparing with the negative control (Fig. 1a). Furthermore, these results were not significantly different from the group treated with diazepam [Veh, 101.6 ± 31.0; DZP 1.0 mg/kg, 178.0 ± 50; $F(5.59) = 18.101$; $p < 0.05$]. In this model, the animals that received PTX [2.0 mg/kg, 11.9 ± 40; $F(5.59) = 18.101$; $p < 0.05$] showed a diminution of the activity and the time spent in open arms. Compared with the negative control group, the percentage of entries [Veh, 35.4 ± 7.1; 125 mg/kg, 46.3 ± 14.5; 250 mg/kg, 46.7 ± 20.6; 500 mg/kg, 46.0 ± 7; 1000 mg/kg, 49 ± 11.6; 2000 mg/kg, 55.8 ± 10; $F(5.59) = 16.506$; $p < 0.05$; DZP 1.0 mg/kg, 59.5 ± 12.7; $F(5.59) = 16.506$; $p < 0.001$] and the percentage of time spent into open arms were higher in mice treated with the extract and DZP [Veh, 39.6 ± 10.6; 125 mg/kg, 46.6 ± 20.3; 250 mg/kg, 48.47 ± 25.5; 500 mg/kg, 46.3 ± 13; 1000 mg/kg, 40.2 ±15.6; 2000 mg/kg, 50.8 ± 6.5; $F(5.59) = 14.434$; $p < 0.05$; DZP 1.0 mg/kg, 53.7 ± 19; $F(5.59) = 14.434$; $p < 0.001$] (Fig. 1b).

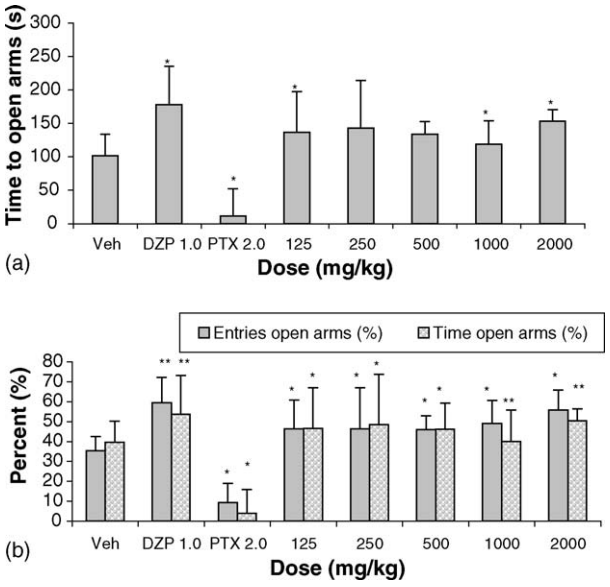

Fig. 1. Effect produced by the oral administration of different doses of the hydroalcoholic extract from *Salvia elegans* on the time spend into the open arms (a), as well as, on the percentage of time spend and percentage of entries into the open arms (b) by mice exposed to the EPM test. $^{*}p < 0.05$, $^{**}p < 0.001$ on the ANOVA followed by post hoc Dunnet test (mean ± S.D.). DZP = diazepam, PTX = picrotoxina, Veh = vehicle.

3.2. Light–dark test

The administration of different doses of *Salvia elegans* extract in ICR mice induced a significant increment of the time spent by mice on the illuminated side of the light–dark apparatus [Veh, 136 ± 15; 125 mg/kg, 157 ± 28; 250 mg/kg, 166 ± 5; 500 mg/kg, 180 ± 21; 1000 mg/kg, 167 ± 30; 2000 mg/kg, 173 ± 9; $F(5.99) = 5.374$; $p < 0.05$]; similar to the effect observed in animals treated with DZP [Veh, 136 ± 15; DZP 1.0 mg/kg, 195 ± 26; $F(5.99) = 5.374$; $p < 0.001$] (Fig. 2).

Fig. 2. Effect produced by the oral administration of different doses of the hydroalcoholic extract from *Salvia elegans* on the time spent by mice into the illuminated compartment on the light–dark test. $^{*}p < 0.05$ with ANOVA followed by post hoc Dunnet test (mean ± S.D.). DZP = diazepam, Veh = vehicle.

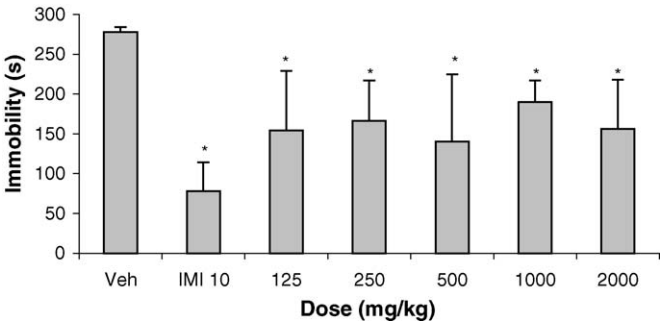

Fig. 3. Effect produced by the oral administration of different doses of the hydroalcoholic extract from *Salvia elegans* on the immobility time of ICR mice exposed to the the swimming forced paradigm. $^{*}p<0.05$, $^{**}p<0.01$ with ANOVA followed by post hoc Dunnet test (mean ± S.D.). IMI = Imipramine, Veh = vehicle.

3.3. Forced swimming test

All doses of *Salvia elegans* extract administered in mice provoked a significant [Veh 278 ± 6; 125 mg/kg, 154 ± 75; 250 mg/kg, 166 ± 51; 500 mg/kg, 140 ± 80; 1000 mg/kg, 190 ± 27; 2000 mg/kg, 156 ± 60; $F(5.99) = 6.145$; $p < 0.05$]; diminution of immobility time when the animals were exposed to the FST. It is important to mention that IMI, the antidepressant used in the positive control group, induced a similar modification to that observed with the plant extract [IMI 10 mg/kg, 80 ± 36; $F(5.99) = 6.145$, $p < 0.05$] (Fig. 3).

3.4. Open field test

The OFT was done in order to determine the effect of the administration of the plant extract upon spontaneous motor activity. The total number of entries into open field was statistically similar in groups treated with the vehicle and different doses of *Salvia elegans* extract [Veh 128 ± 46; 125 mg/kg, 145 ± 46; 250 mg/kg, 107 ± 17; 500 mg/kg, 127 ± 55; 1000 mg/kg, 153 ± 55; 2000 mg/kg, 121 ± 62; $F(5.99) = 1.165$; $p > 0.05$]. Animals treated with DZP showed a light decrease on the number of entries; nevertheless, it did not reach statistical significance [Veh 128 ± 46; DZP 1.0 mg/kg, 92 ± 32; $F(5.99) = 1.165$; $p > 0.05$] (Fig. 4). The number or rearings [Veh 54 ± 13; 125 mg/kg, 47 ± 13; 250 mg/kg, 43 ± 13; 500 mg/kg, 46 ± 15; 1000 mg/kg, 48 ± 10; 2000 mg/kg, 45 ± 19; $F(5.99) = 3.634$; $p > 0.05$] was only modified in mice that received DZP which presented a significant diminution of this parameter with respect to the control [Veh 54 ± 13; DZP 1.0 mg/kg, 21.50 ± 13; $F(5.99) = 3.634$; $p > 0.05$].

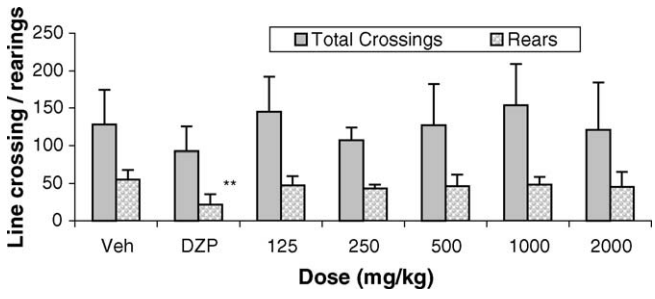

Fig. 4. Effect produced by the oral administration of different doses of hydroalcoholic extract from *Salvia elegans* on the total crossings and rearings number of ICR mice exposed to the open field paradigm. $^{*}p<0.05$, $^{**}p<0.01$ with ANOVA followed by post hoc Dunnet test (mean ± S.D.). DZP = diazepam, Veh = vehicle.

4. Discussion

Despite the widely popular use of *Salvia elegans* for treating nervous disorders, there is an absence of scientific reports about the evaluation of its pharmacological effects. In this work, it was demonstrated that the administration of different doses of the hydroalcoholic extract of *Salvia elegans* in mice was able to induce anxiolytic effects, without modifying significantly the spontaneous motor activity. When the animals were exposed to the EPM test, all mice were sensitive to the extract and showed a similar behavior to that of mice treated with DZP (there were no statistical differences between these two groups). This animal model is considered one of the most widely validated tests for assaying sedative and anxiolytic substances such as the benzodiazepines (Pellow et al., 1985). The plant extract induced anxiolytic effect beginning at the lower doses employed. An increase of the most important variables of the EPM test was found, as follows: the percentage of time that mice spend in the open arms as well as the percentage of entries into these arms. The anxiolytic effect was also evidenced through the light–dark test. As with the EPM test, this model is useful for modeling anxiety, and it has been developed for predicting the potency of clinically used compounds for treating this disease. It has been assumed that the time mice spend in the illuminated side of the box is the most useful and consistent parameter of anxiety (Young and Johnson, 1991). The lack of dose-dependent effect could be attributed to the biological variability, as well as to the chemical complexity of the crude extract. These factors, in statistical terms, turn out more dispersion of results.

Regarding the medical treatment of psychiatric disorders, the results obtained in this work became important because not only anxiolytic effects were observed; antidepressant activity was also shown. Results showed that the administration of the *Salvia elegans* extract produced a diminution of immobility time of mice exposed to the forced swimming test. These behavioral effects were similar to that found by other authors after treating mice with classical antidepressant drugs as IMI (Porsolt et al., 1977; Borsini and Meli, 1988).

The effects produced by *Salvia elegans* and DZP (1.0 mg/kg) upon the open field test demonstrated that these products do not modify the spontaneous locomotor activity of mice, which indicates that the plant extract exerts antidepressant and anxiolytic effects at different doses, without modifying significantly this parameter. Therefore, it is probable that these effects are not related to the stimulation of general motor activity (Novas et al., 1988). Other authors have also reported that 1.0 mg/kg of DZP did not modify the spontaneous locomotor activity, nevertheless, higher doses decreased this parameter (Sousa et al., 2004; Klodzinska et al., 2004).

With the experimental test used in this work (which gives us information about motor activity, anxiety and depression) it is not possible to elucidate the action mechanism through which *Salvia elegans* exerts both effects. Therefore, it is necessary to develop biochemical and pharmacological studies that allow us to establish if the effects here reported are a consequence of the separate activation of nervous structures by one chemical compound by itself, or if the biological activities are produced by different secondary metabolites of the plant.

Pharmacological studies have demonstrated that plants of the *Salvia* genus and some of their chemical constituents, including essential oils and flavonoids, display nervous system depressor activities (Lu and Foo, 2002). For example, the flavonoid apigenin (Kavvadias et al., 2003), which selectively binds with high affinity to the central benzodiazepine receptor, possesses important anxiolytic (Salgueiro et al., 1997; Paladini et al., 1999) and antidepressant activities (Nakazawa et al., 2003). *Salvia elegans* contains linalool, an essential oil that exerts sedative effects in humans (Kuroda et al., 2005). This species probably include other compounds like apigenin, or others flavonoids with depressor nervous system activities.

Until now, there was just one pharmacological report about the biological effects produced by *Salvia elegans*. That report demonstrated the ability of the methanolic extract to bind with cholinergic receptors on CNS of humans. Particularly, the extract was able to displace [^{3}H]-(*N*)-scopolamine from muscarinic receptors, which could indicate the presence of some chemical compound with cholinergic activity (Wake et al., 2000). Despite the fact that the substance with binding abilities is still unknown, leaving undefined whether there is an agonist or antagonist relation upon the receptors, the information is important because the cholinergic neurons located in the septum participate in mood states such as: waking up, the motivation and vegetative functions, through nervous pathways that receive innervation from mesencephalyc structures and from the brain stem. These neurons integrate the information for the hippocampus, using the septo-hypocampal cholinergic pathway, allowing a modulation of the response of hippocampus directed to the brain cortex. This last group of pathways participates in such different behavioral processes as learning, motivation, exploration and anxiety (Dutar et al., 1995); which could be part of the fundamental principle of findings obtained in the behavioral models here studied with *Salvia elegans*.

Data here obtained allows us to propose this plant species as an excellent candidate for isolating new substances with potential anxiolytic and antidepressant activity.

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