The hydroalcoholic extract of *Salvia elegans* induces anxiolytic- and antidepressant-like effects in rats

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Abstract

Behavioral effects of a hydroalcoholic (60% ethanol) extract from the leaves of *Salvia elegans* Vahl (Lamiaceae) were studied in male Sprague–Dawley rats. The extract was administered intraperitoneally and its effects on spontaneous motor activity (total motility, locomotion, rearing and grooming behavior) were monitored. Putative anxiolytic and antidepressant properties of *Salvia elegans* were studied in the elevated plus-maze test (EPM) and in the forced swimming test (FST), respectively. Deleterious effects of *Salvia elegans* on learning and memory were also studied by using active and passive avoidance paradigms. The results revealed that all doses (3.12, 12.5, 25 and 50 mg/kg) of the extract caused a significant decrease in total motility, locomotion, rearing and grooming behavior. Only the dose of 12.5 mg/kg increased the exploration of the EPM open arms in a similar way to that of diazepam (1 mg/kg). In the FST, all doses of the extract induced a reduction of immobility, in a similar way to that of fluoxetine (10 mg/kg) and imipramine (12.5 mg/kg), along with a significant increase in the time spent in swimming behavior. Acquisition of active avoidance responses was disrupted by pre-treatment with the extract, but retention of a passive avoidance response was not significantly modified. These results suggest that some of the components of the hydroalcoholic extract of *Salvia elegans* have psychotropic properties, which deserve further investigation.

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

1. Introduction

Depression and anxiety are the most frequent mental disorders. More than 20% of the adult population suffer from these conditions at some time during their life (Buller and Legrand, 2001). The World Health Organization (WHO, 1999) predicts that depression will become the second leading cause of premature death or disability worldwide by the year 2020. Approximately two-thirds of the anxious or depressed patients respond to the currently available treatments but the magnitude of improvement is still disappointing. Then, the medical need for newer, better-tolerated and more efficacious treatments remains high.

People from different regions of the world have used herbal medicines to alleviate affective disorders for many years. In addition, the search for novel pharmacotherapy from medicinal plants for psychiatric illnesses has progressed significantly in the past decade (Zhang, 2004). An increasing number of herbal products have been introduced into psychiatric practice, as alternative or complementary medicines, and also there is a large number of herbal medicines whose therapeutic potential has been assessed in a variety of animal models (Zhang, 2004). In fact, these models have contributed to the screening of new psychopharmacological tools and to the understanding of their biological activity (Buller and Legrand, 2001).

Among the most commonly used herbal medications for the prevention or treatment of anxiety and depression we must mention kava (*Piper methysticum*) and St. John's wort (*Hypericum perforatum*), respectively. They have demonstrated efficacy and

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safety not only in animal models but also in clinical trials (Wong et al., 1998). In Mexico, several medicinal plants are used to alleviate insomnia, anxiety and depressed mood (Zolla, 1980). For instance, recently it has been reported that aqueous and hydroalcoholic extracts of *Casimiroa edulis* (Rutaceae), a plant commonly known as "white sapote" and widely used for the treatment of anxiety and insomnia, are able to induce anxiolytic- and antidepressant-like effects in rats (Molina-Hernández et al., 2004; Mora et al., 2005). *Salvia* (Lamiaceae family) is a large genus containing about 500 species of sage plants throughout the world. Only a few of these, however, have commercial, medicinal, or culinary value. *Salvia elegans* Vahl is a perennial shrub native to Mexico, commonly known as pineapple sage and pineapple-scented sage, in English, and "mirto", "flor del cerro", "limoncillo" and "perritos rojos", in Spanish. It is widely used in Mexican traditional medicine for alleviate central nervous system ailments (Aguilar et al., 1994). It is claimed as a "nervous system tonic, both soothing and stimulating, that releases stress, negativity and tension in heightened activity". Nevertheless there is no scientific evidence about potential effects of *Salvia elegans* in animal models of psychiatric diseases. That this plant could induce neurological effects is suggested by a report which demonstrated that the ethanolic extract of *Salvia elegans* is able to displace [3H]-(*N*)-scopolamine from muscarinic receptors in homogenates of human cerebral cortical cell membranes (Wake et al., 2000).

High-resolution gas chromatography/mass spectrometry analysis of the essential oil from *Salvia elegans* has led to the identification of twenty-eight volatile constituents (Makino et al., 1996). Among them, 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 were predominant components.

The present study was undertaken to investigate whether the hydroalcoholic extract of the leaves from *Salvia elegans* has potential activities on the central nervous system (CNS) and if it is able to induce behavioral modifications in rats. Spontaneous motor responses were monitored and the anxiolytic- and antidepressant-like effects were assessed in the plus-maze test and in the forced swimming test, respectively. Deleterious effects on learning and memory functions were evaluated through the acquisition and retention of active and passive avoidance conditioning, respectively.

2. Materials and methods

2.1. Plant material and preparation of the extract

With the support of professional collectors, aerial parts of *Salvia elegans* Vahl (Lamiaceae) were collected from Puebla, Mexico. Plant material was authenticated by Dr. Abigail Aguilar-Contreras, IMSSM Herbarium (located in Mexico City), where the voucher samples, with the record 14,688, were stored for future reference. Plant was dried under dark conditions at room temperature by 2 weeks and then ground in an electric mill until to obtain particles lesser than 4 mm. This material (2.6 kg) was extracted by maceration in 60% ethanol solution at 50 °C during 2 h. The extraction procedure was repeated once again under the same conditions with a new solvent. Extracts were filtered through a Wattman #1 paper and evaporated to dryness in a rotary evaporator under reduced pressure. The yield of the extract was quantified (16.46%), while the dried material was stored under refrigeration at 4–8 °C until its use.

2.2. Animals

Male Sprague–Dawley rats (200–250 g) from the breeding stock were housed in groups of six per cage, for a minimum of 5 days prior to the pharmacological experiments, with free access to standard rodent pellet diet and tap water, and maintained on a 12/12 h light-dark cycle. Each experimental group consisted of at least 10 animals. The room temperature was 22 ± 1 °C. Behavioral observations took place in soundproof rooms at the same period of the day to reduce the confounding influence of diurnal variation in spontaneous behavior. Each animal was tested only once.

All experiments were conducted in accordance with international standards of animal welfare recommended by the Society for Neuroscience (Handbook for the Use of Animals in Neuroscience Research, 1997). The experimental protocol was approved by the Bioethical Committee on Animal Research, Faculty of Medicine, University of Chile, under the code CBA#056 FMUCH. The minimum number of animals and duration of observations required to obtain consistent data were employed.

2.3. Drugs

Diazepam (F. Hoffmann-La Roche, Basel, Switzerland) was used as reference drug (positive control) for anxiolytic and sedative activities. Fluoxetine (Ely-Lilly Co., Indianapolis, USA) and imipramine (Novartis, Chile, S.A.) were used as standard drugs (positive controls) for antidepressant effect.

2.4. Treatment

The extract of *Salvia elegans* was freshly dissolved in distilled water to be acutely administered to the rats. Doses of the extract and the time intervals were determined in preliminary tests. Diazepam (1 mg/kg) was dissolved in 40% propylene glycol. Both fluoxetine (10 mg/kg) and imipramine (12.5 mg/kg) were dissolved in distilled water. Negative control groups received only distilled water. All administrations were performed intraperitoneally (i.p.) in a dose volume of 1 ml/kg body weight. Thirty minutes after i.p. treatment, the animals were submitted to a battery of behavioral tests.

2.5. Behavioral evaluation

2.5.1. Spontaneous motor activity

Thirty minutes after the treatment with the extract (3.12, 12.5, 25 and 50 mg/kg, i.p.), rats were individually placed in a Plexiglas cage (30 cm × 30 cm × 30 cm), located inside a soundproof chamber. The floor of the cage was an activity platform

connected to an amplifier and an electromechanical counter to monitor total motility (Lafayette Instrument Co., USA). Locomotor activity was also recorded with an infrared photocell activity monitor (Columbus Instruments, USA), provided with one array of 15 infrared photocells spaced 1″ (2.54 cm) apart. Spontaneous motor activity was monitored during 30 min and, simultaneously, the number of times each animal reared and the time (in s) spent in grooming behavior were recorded. Each animal was observed continuously via a video camera connected to a VHS tape-recorder.

2.5.2. Elevated plus-maze test (EPM)

This test has been widely validated to measure anxiety in rodents (File and Pellow, 1985; Lister, 1987). The apparatus, constructed from black Plexiglas, consisted of two open arms (50 cm × 10 cm each), two enclosed arms (50 cm × 10 cm × 40 cm each) and a central platform (10 cm × 10 cm), arranged in such a way that the two arms of each type were opposite to each other. The maze was elevated 70 cm above the floor. Sixty minutes after the i.p. treatment with *Salvia elegans* (3.12, 12.5, 25 and 50 mg/kg) or diazepam (1 mg/kg) or solvent, namely immediately after spontaneous motor activity recording, each animal was placed at the center of the maze, facing one of the enclosed arms. During the 5-min test period, the number of open and enclosed arms entries, plus the time spent in open and enclosed arms, was recorded (Pellow and File, 1986). Entry into an arm was defined as the point when the animal places all four paws onto the arm. Animal behavior was taped by using a video camera located above the maze. After the test, the maze was carefully cleaned with 10% ethanol solution.

2.5.3. Forced swimming test (FST)

The FST is the most widely used pharmacological model for assessing antidepressant activity (Cryan et al., 2002). The development of immobility when the rodents are placed in an inescapable cylinder of water reflects the cessation of persistent escape-directed behavior (Lucki, 1997). The test was performed according to a modification suggested by Lucki (1997) of the traditional method described by Porsolt et al. (1977). The apparatus consisted of a transparent Plexiglas cylinder (50 cm high × 20 cm wide) filled to a 30 cm depth with water at room temperature. In the pre-test, rats were placed in the cylinder for 15 min, 24 h prior to the 5-min swimming test. *Salvia elegans* extract (3.12, 12.5, 25 and 50 mg/kg), fluoxetine (10 mg/kg), imipramine (12.5 mg/kg) or distilled water were administered i.p. three times: immediately after the initial 15-min pre-test, 6 and 0.5 h prior to the FST. During the 5-min test, the following behavioral responses were recorded by a trained observer: *climbing behavior*, which is defined as upward-directed movements of the forepaws along the side of the swim chamber; *swimming behavior*, defined as movement throughout the swim chamber, which included crossing into another quadrant; and *immobility* was considered when the rat made no further attempts to escape except the movements necessary to keep its head above the water. Increases in active responses, such as climbing or swimming, and reduction in immobility, are considered as behavioral profiles consistent with an antidepressant-like action (Cryan et al., 2002).

2.5.4. Acquisition of active avoidance conditioning

The conditioning experiments were carried out with a two-way shuttle box (Lafayette Instrument Co.) composed of two stainless steel modular testing units, which were equipped with a 18-bar insulated shock grid floor, two 28-V DC lights and a tone generator (Mallory Sonalert 2800 Hz). Electric shocks were provided to the grid floor by a master shock supply (Lafayette Instrument Co.). Immediately after the EPM test, the rats were individually placed in the shuttle box and were trained in a one single session of 50 trials. Each trial consisted of the presentation of a tone that after 5 s was overlapped with a 0.20-mA foot shock until the animal escaped to the opposite chamber, with maximum shock duration of 10 s. A conditioned avoidance response was defined as a crossing within the first 5 s (tone alone).

2.5.5. Retention of passive avoidance conditioning

This test was carried out in the same apparatus described above with the difference that one of chamber remained illuminated and the other was darkened. A guillotine door was placed between the chambers. On Day 1 of testing, animals were habituated to the apparatus. Each rat received three trials with intervals of 4 h between them. On each trial, the rat was placed into the illuminated chamber facing away from the guillotine door. When the animal entered to the darkened chamber, the guillotine door was lowered noiselessly and the animal was removed from the apparatus 60 s later. The latency to enter was recorded. On Day 2, the animal was placed into the illuminated chamber and permitted to enter the darkened chamber. Upon entry the darkened chamber, the guillotine door was lowered and a 0.80 mA foot shock was applied for 3 s through the grid floor. Immediately after this training the rat was removed from the apparatus, injected with the *Salvia elegans* extract (12.5, 25 or 50 mg/kg i.p.), or distilled water, and returned to its cage. The retention test was given on Day 3 and consisted of a single trial without foot shock, in which each animal was placed into the illuminated chamber and the latency to enter the darkened chamber was recorded to an arbitrary maximum of 300 s.

2.6. Statistical analysis

Data were analyzed by Prism Graph Pad software and presented as mean ± S.E.M. values. The statistical tests used were one-way analysis of variance (ANOVA) followed by Newman–Keuls's multiple comparisons test. A probability level of 0.05 or less was considered statistically significant.

3. Results

3.1. Spontaneous motor activity

The overall effects of the i.p. administration of the extract on rat spontaneous motor activity are summarized in Table 1. One-way ANOVA revealed significant effects of the treatment on total activity, $F(4, 47) = 11, 84$; $p < 0.0001$, locomotion, $F(4,$

Table 1
Effect of *Salvia elegans* hydroalcoholic extract on spontaneous motor activity in rats[a]

Dose (mg/kg i.p.)	*n*	Total activity (counts)	Locomotor activity (counts)	Rearing (number)	Grooming (s)
Control	18	1057.9 ± 83.9	1147.6 ± 96.7	53.1 ± 5.2	309.5 ± 25.9
3.12	10	819.3 ± 170.4	791.4 ± 106.0*	25.2 ± 7.4*	127.0 ± 34.1*
12.5	10	563.2 ± 75.1*	859.3 ± 84.5*	25.4 ± 4.5*	110.5 ± 16.0*
25.0	10	315.3 ± 58.7*	653.6 ± 85.2*	26.3 ± 6.8*	73.4 ± 15.6*
50.0	10	272.3 ± 46.1*	436.9 ± 109.5*	14.1 ± 2.5*	74.7 ± 20.5*

[a] Data are means ± S.E.M.; *n* = number of animals.
* $p < 0.05$ compared with control animals (ANOVA followed by Newman–Keuls's test).

47) = 7, 91; $p < 0.0001$, rearing, $F(4, 47) = 8, 36$; $p < 0.0001$, and grooming behavior, $F(4, 47) = 6, 55$; $p < 0.001$. Subsequent Newman–Keuls's test demonstrated that the extract induced a significant and dose-dependent decrease in total motor activity, locomotor activity, rearing and grooming behavior.

3.2. Elevated plus-maze

The ANOVA revealed significant effects of the *Salvia elegans* extract treatment on the percentage of entries into the open arms, $F(5, 53) = 5, 47$; $p < 0.001$, and on the percentage of time spent in the open arms of the elevated plus-maze, $F(5, 53) = 6, 32$; $p < 0.0001$ (Fig. 1). Newman–Keuls's post hoc analysis showed a significant increase of the percentage of entries and in the percentage of time spent in the open arms after the dose of 12.5 mg/kg. The effect of diazepam (1 mg/kg) on the open arms exploration was not significantly different from that observed after *Salvia elegans* 12.5 mg/kg.

3.3. Forced swimming test

The effects of the *Salvia elegans* extract, fluoxetine and imipramine on active behaviors in the FST are shown in Fig. 2. The ANOVA revealed significant effects of treatment on immobility, $F(6, 84) = 7, 95$; $p < 0.0001$, swimming behavior, $F(6, 84) = 7, 34$; $p < 0.0001$, and climbing behavior, $F(6, 84) = 3, 30$; $p < 0.01$. Newman–Keuls's post hoc analysis demonstrated that all the four doses (3.12, 12.5, 25 and 50 mg/kg) of *Salvia elegans* injected significantly shortened the immobility time in comparison to negative control values. This effect was accompanied by a significant increase in swimming behavior, without modifying climbing behavior. As expected, both fluoxetine and imipramine significantly decreased the immobility time during the 5-min test session while inducing corresponding increase in swimming and climbing behaviors, respectively. There was no significant difference between the effect of the extract and that observed after fluoxetine and imipramine on the immobility time.

3.4. Active avoidance conditioning

The results of the active avoidance conditioning are showed in Fig. 3. ANOVA revealed a significant effect of the injection of *Salvia elegans* extract on the acquisition of the active avoidance response, $F(3, 36) = 4, 71$; $p < 0.01$. Post hoc Newman–Keuls's test indicated that the conditioning performance was seriously impaired after all doses of the extract.

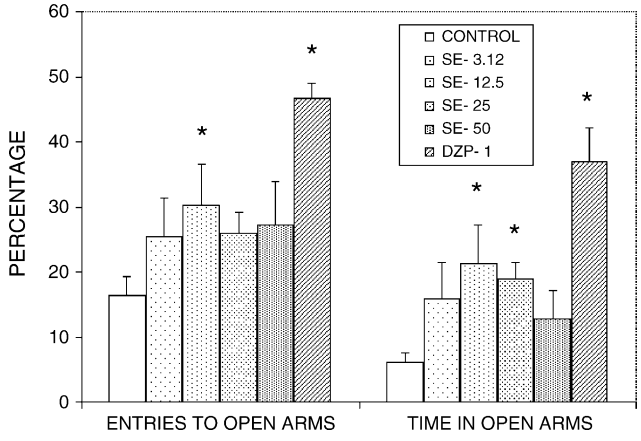

Fig. 1. The effects *of Salvia elegans* hydroalcoholic extract (SE 3.12, 12.5, 25 and 50 mg/kg i.p.) and diazepam (DZP 1 mg/kg i.p.) on the percentage of entries and the time spent in open arms of the elevated plus-maze in rats. Data represent means ± S.E.M. of 10–18 animals during the 5-min test session. Comparisons were made by using a one-way ANOVA followed by post hoc Newman–Keuls's test: * $p < 0.05$ compared with control group.

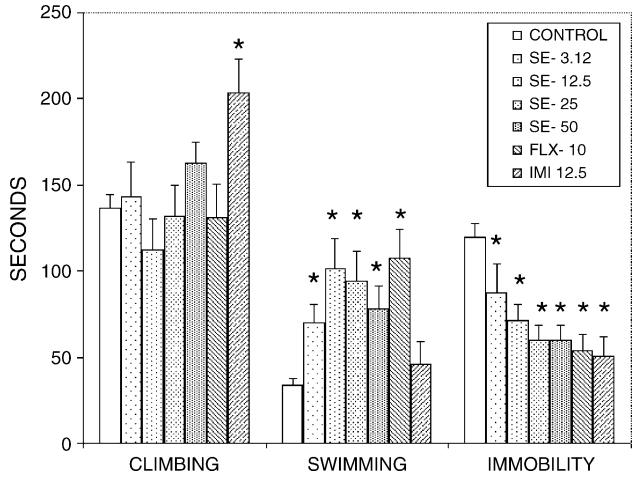

Fig. 2. The effects *of Salvia elegans* hydroalcoholic extract (SE 3.12, 12.5, 25 and 50 mg/kg i.p.), fluoxetine (FLX 10 mg/kg i.p.) and imipramine (IMI 12.5 mg/kg i.p.) on active behaviors in the forced swimming test in rats. Data represent means ± S.E.M. of the duration of climbing, swimming and immobility during the 5-min test session ($n = 10$–18 animals per group). Comparisons were made by using a one-way ANOVA followed by post hoc Newman–Keuls's test: * $p < 0.05$ compared with control group.

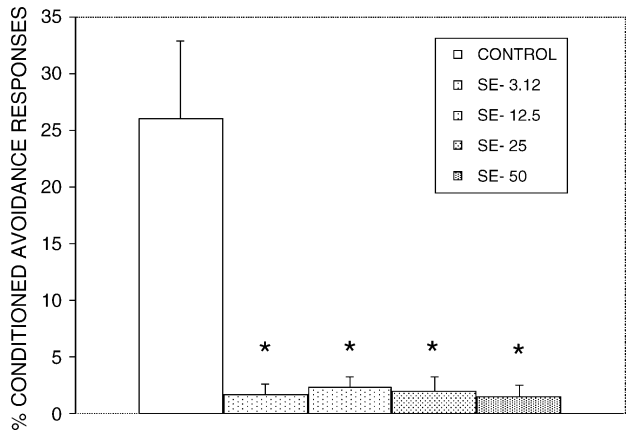

Fig. 3. The effects *of Salvia elegans* hydroalcoholic extract (SE 3.12, 12.5, 25 and 50 mg/kg i.p.) on the acquisition of an active avoidance conditioning task in rats. Data represent means ± S.E.M. of the percent of conditioned responses over a total of 50 trials ($n = 10–18$ animals per group). Comparisons were made by using a one-way ANOVA followed by post hoc Newman–Keuls's test: $^{*}p < 0.05$ compared with control group.

3.5. Passive avoidance conditioning

One-way ANOVA failed to reveal a significant effect of the post-training injection of *Salvia elegans* on the retention of the passive avoidance response, $F(3, 53) = 0, 32$; NS (Data not shown).

4. Conclusions

The present study investigated the putative behavioral effects of the hydroalcoholic extract from the leaves of *Salvia elegans* in rats. The extract was able to induce motor depressant effects after the i.p. injection. Thus, single doses of 12.5, 25 and 50 mg/kg of the extract produced a significant decrease in total motility, whereas all doses decreased locomotor activity, rearing and grooming behavior. These findings indicate a remarkable sedative effect of this plant.

The extract induced a slight anxiolytic-like effect. In fact, only rats treated with *Salvia elegans* 12.5 mg/kg showed a significant increase of both the percentage of entries and the percentage of time spent in the open arms of the elevated plus-maze. On the other hand, we found a more consistent antidepressant-like activity. In the FST all the doses administered were able to reduce immobility time and simultaneously to enhance swimming but not climbing behavior. Reduction of immobility was comparable to that observed after the i.p. administration of the reference antidepressant drugs fluoxetine and imipramine. In agreement with previous reports (Page et al., 1999), the decrease in immobility induced by fluoxetine was accompanied by an increase in swimming, whereas climbing duration was not affected by this drug. Imipramine increased climbing duration without modifying swimming. It has been demonstrated that swimming is sensitive to serotoninergic compounds, such as the selective serotonin reuptake inhibitor fluoxetine, and that climbing is sensitive to tricyclic antidepressants and drugs with selective effects on catecholamine transmission (Detke et al., 1995; Cryan and Lucki, 2000). Then, although other kind of studies are obviously necessary to elucidate the mechanism of action of *Salvia elegans* in the CNS, the pattern of effects observed in the FST may suggest the involvement of serotonergic brain systems on its antidepressant-like effect. It is interesting that other member of the Lamiaceae family, *Salvia divinorum*, used for centuries in healing ceremonies by the Mazatec Indians of Oaxaca, Mexico, has demonstrated beneficial effects in a case of treatment-resistant depression (Hanes, 2001).

Pretraining injection of *Salvia elegans* induced a dramatic disruption on the ability to acquire a conditioned responses. This paradigm is most often used to assess drug effects on learning and memory processes, but in other occasions it have been used to assess aspects that are not directly related to learning and memory per se, such anxiety and learned helplessness (Clinke and Werbrouck, 1993). Changes on avoidance performance induced by *Salvia elegans* could be secondary to cognitive or non-cognitive factors, such as sedation, shock sensitivity and other influences on emotional or motivational aspects (Schultein and Koob, 1993). For many years the ability to inhibit conditioning avoidance responding has been considered as a characteristic effect of almost all drugs which block central dopaminergic (DA) receptors, such as neuroleptics (Janssen et al., 1965).

It has been reported that some extracts of *Salvia* species, such as *Salvia officinalis* and *Salvia elegans*, have CNS cholinergic receptor binding activities that may be relevant to enhance or restore mental functions including memory (Wake et al., 2000). Nevertheless, in our experimental conditions, post-training administration of *Salvia elegans* failed to modify the retention of a passive avoidance response. This procedure is widely used to measure cognitive alterations following drug administration.

One of the main components extracted from the essential oil of *Salvia elegans* is linalool (Makino et al., 1996), a monoterpene compound reported to be a major component of essential oils in various aromatic species. Psychopharmacological in vivo evaluation has shown that linalool possesses dose-dependent marked sedative effects at the CNS including hypnotic, anticonvulsant and hypothermic properties (Jirovetz et al., 1991). This evidence suggests that linalool may take account of at least some of the behavioral effects of *Salvia elegans*.

In conclusion, our results make evident that the hydroalcoholic extract of the leaves from *Salvia elegans* exerts sedative and antidepressant effects in rats. The hypomotility and potentially cognitive impairments could be considered as adverse reactions that must be considered in the future. However, further studies are necessary to confirm and extend these results. Somehow, the findings presented here are relevant because they contribute to validate the traditional medical uses of this plant.

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