Sedative and Cardiovascular Effects of *Aloysia citriodora* Palau, on Mice and Rats

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**SUMMARY.** *Aloysia citriodora* Palau, Verbenaceae (“cedrón”) is widely used as infusion or decoction in South America to treat indigestion, tachycardia and anxiety. We previously demonstrated its antispasmodic effect on rat duodenum. Now, its aqueous extract (AEC) from 1 to 10 mg/kg was sedative in mice on the open-field, effect which was potentiated by diazepam and sensitive to flumazenil. In normotensive rats, 1 to 30 mg AEC/kg induced a transitory hypotension, insensitive to atropine and L-NAME. Regarding an effect on α-adrenergic receptors, AEC non-competitively blocked the phenylephrine contraction on vas deferens. In isolated rat hearts, AEC induced negative inotropism, as well as vitexin, the main component. Then, the benzodiazepine-like sedation, negative inotropism and antispasmodic effect preclinically justify its popular use for abdominal cramps and as coadjuvant for anxiety and angor.

**INTRODUCTION**

*Aloysia citriodora* Palau, Verbenaceae (syn. Aloysia triphylla L´Hér.) is a South American plant, popularly known as “cedrón”, “hierba Luisa” and “verbena aromática” around the Río de la Plata, and “cidrão” and “lemon verbena” in Brazil 1. In Argentina it is widely used as an aromatic leaves infusion or as a decoction for abdominal pain, nausea and dizziness 1-4. Its daily consumption as dietary supplement was extended on sale at supermarkets. In Cuba it is used as an expectorant and against insomnia, in Paraguay against tachycardia 5, and also for anxiety and headache 1. We have recently demonstrated the antispasmodic effects of *A. citriodora*, which was mainly attributed to intestinal relaxation by opening of K+ channels 6.

About the composition, verbascoside and other volatile compounds as neral and geranial were identified in the essential oil 5,7,9. A flavonoid, luteolin-7-diglucuronide, it was also identified in the leaves 10,11. As reported in a previous work 6, the HPLC fingerprint of our sample of “cedrón” showed the presence of the flavonoids vitexin and isovitexin, the first of both had antispasmodic properties. Also, it was reported that vitexin has antioxidant effects 12.

On the other side, cardiovascular diseases dependent on stress are very frequent, and need to cronically use medicines and changing of the way of life, especially food. Then, it is important to find phytotherapies which contribute to treat them with less adverse effects than synthetic drugs. In this work, the pharmacological basis for the popular use of “cedrón” for restless and tachycardia was studied from an aqueous extract (AEC) on the spontaneous activity of conscious mice and the blood pressure of normotensive rats. Protocols for the underlying mechanisms were done in isolated preparations, and it was also evaluated whether vitexin was the component responsible for the effects.

*KEY WORDS:* Aloysia citriodora, Cardiac inotropism, Hypotensive, Open-field, Sedative, Vitexin.

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MATERIAL AND METHODS

Plant and extracts

A commercial sample of *Aloysia citriodora* Palau (Verbenaceae) from Paraguay was provided by a local herboristery, and the plant was authenticated by Prof. Dra. Etile Spegazzini, from the Herbarium Museum of Botany and Pharmacognosy, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, Argentina, where the voucher specimen (herbarium number LPE 1039) was kept. An aqueous extract of *A. citriodora* or “cedrón” (AEC) was prepared by boiling 30 g dried leaves in 200 ml distilled water for 20 min, as the ethnomedicinal use. After filtration the decoction was lyophilized, obtaining a 15% w/w yield. The lyophilized extract was diluted in distilled water or Krebs solution, respectively for *in vivo* or *in vitro* tests the day the experiment was carried out. With this procedure, the essential oil is not included in the lyophilized sample.

Animals

Swiss albino mice, weighing 25 to 30 g and Sprague-Dawley rats of 220-260 g weight, kept under controlled conditions (12 h dark-light cycle, 20-25 °C, fed ad-libitum on standard pellets and water) were used. All experiments were conducted in accordance with internationally accepted principles for laboratory animal use and care as was established by US guidelines (NIH publication # 85-23, revised in 1985) 13.

Open-field test in mice

The spontaneous locomotion and exploratory activity of mice was evaluated on the open field, consisted of a 30 x 50 cm white box with walls of 27 cm height divided in 15 squares of 10 cm² by black lines. It was placed in a light and sound-attenuated room. Mice were divided in 7 groups, respectively for the following treatments: saline solution (negative control), 10 mg/kg diazepam (positive control), 0.15, 1 and 10 mg/kg AEC, 1 mg/kg AEC 5 min after receiving 10 mg/kg diazepam (Roche, Argentina), and 1 mg/kg AEC 5 min after receiving 0.5 mg/kg flumazenil (Richmond, Argentina). All drugs were administered by i.p. injections in a volume of 0.1 ml by 10 g of weight. After 40 min, each animal was placed in the same corner of the field, and during 5 min there were counted the number of crossed lines, rearings, grooming and other signs 14. This routine was repeated for every mouse at 80, 120 and 160 min from the administration. The same protocol was done with 4 groups of mice, respectively administered with 33, 100 and 333 mg/kg of vitexin (Extrasynthese, France) or saline solution (negative control), for the times of 30, 60, 90 and 120 min after the administration.

Blood pressure in rats

As in previous works 15, normotensive rats (n = 6) were anesthetized with 1.5 g/kg urethane (Fluka) via i.p. and placed in a supine position with tracheal cannulation. Blood pressure (BP) was directly measured through an heparinized cannula in the internal carotid artery connected to a Bentley 800 pressure transducer. It was continuously recorded on a Beckman polygraph and A/D converted by a National Instruments Ni-DAQ with a PC516 logger to a computer, and expressed in mm Hg. BP pulses were continuously recorded and the heart rate (HR) was calculated. Without treatment, BP remained constant for at least 3 h. Drugs were i.v. infused via the jugular vein in volumes of 0.1 ml separated by not less than 15 min. After 30 min of stabilization, doses of 1, 3, 10 and 30 mg lyophilized/kg were successively administered. After other 30 min stabilization, 1.5 mg/kg atropine (Sigma, USA) was i.v. administered, followed by the same doses series of AEC. Finally, after 30 min, 25 µmol/kg L-NAME (Sigma, USA) was i.v. administered, followed by the same doses series of AEC. A group of 4 rats was similarly treated with saline solution instead of AEC, as a negative control, and other group was treated with 10 µg/kg acetylcholine bromide (Sigma, USA) before and after the infusion of 1.5 mg/kg atropine (positive control).

Rat vas deferens

To evaluate whether the hypotensive effect of AEC was due to an antiadrenergic mechanism, the effect of AEC was evaluated on the dose-response curves (DRC) of phenylephrine (Phe, Sigma, USA) in the rat vas deferens, preparation that has α-1 receptors. Vas deferens was isolated from male rats and submerged in organ-baths containing 20 ml Krebs solution, at 32 °C, preloaded with 0.5 g, and bubbled with 95% O₂-5% CO₂. The Krebs solution composition was (in mM): NaCl 118, NaHCO₃ 25, KH₂PO₄ 1.2, KCl 4.7, CaCl₂ 2.5 y Glu 11.1 (pH 7.4). DRC of Phe was done by cumulatively adding 0.2 ml of the following solutions: 1, 2, 7, 20, 70, 200, 700 y 1000 µg/ml to the chamber bath. Isometric contractions were measured by isometric transducers FORT10 from WPI cou-
pled to a Transbridge TBM 4M preamplifier (WPI, USA) and A/D converted by Eagle software to a computer. AEC was added 10 minutes before the DRC, respectively at 0.20, 0.60 or 2.0 mg lyophilized/ml.

Isolated rat hearts

It was evaluated whether the extract of A. citriodora or vitexin have a direct effect on hearts, and whether the antioxidant properties of the flavonoid protect hearts from the ischemia. Beating hearts were rapidly excised from heparinized (2000 U) rats after anesthesia with pentobarbital sodium overdose, as in a previous work 16. Hearts were perfused by Langendorf method with control Krebs at a constant rate (6 ml/min/g) at 30 °C. The perfusate was a Krebs solution containing (in mM): 1 MgCl2, 120 NaCl, 0.5 NaH2PO4, 6 KCl, 2 CaCl2, 25 NaHCO3, and 6 dextrose, bubbled with 95% O2-5 % CO2 to achieve a pH of 7.3-7.4. Atria were dissected to prevent spontaneous contractions. A latex balloon was placed into the left ventricle and connected to a Bentley 800 pressure transducer for measuring the left ventricular pressure (LVP). Hearts were electrically stimulated with pulses of 5 V-5 ms at 1 Hz. The LVP signals were continuously recorded as described for BP. The maximal pressure developed during a beat (P) was calculated from the recording as a difference between the maximal peak and the basal LVP. Without treatment, P remained constant by 4-5 h. After a 30-min equilibration period (during which optimal diastolic volume was regulated until P reached a steady value), one of the following protocols was done: (a) Perfusion with Krebs and consecutively with 0.2, 0.6, 2, and 6 mg AEC /ml; (b) Perfusion with Krebs and 10 µM vitexin, then exposing hearts to no-flow ischemia (I) during 45 min (by stopping the perfusion), and to reperfusion (R) with Krebs during other 45 min. A negative control group was done with no-pretreatment before the exposition to I-R (non-pretreated hearts).

Statistical analysis

Results were expressed as mean ± S.E.M. Multiple comparisons between treatments which were determined at several times or doses were done by two-way ANOVA tests followed by “a posteriori” all paired Bonferroni tests, considering p < 0.05 for significance. Also, changes in BP were compared against zero by a Student t-paired test. It was used the Prism GraphPad 4.0 program for all tests.

RESULTS

Central effects of A. citriodora and vitexin

The exploratory behaviour and the spontaneous locomotion of mice were measured in the open field. Both properties, respectively measured by the number of rears and crossed lines during 5 min, were dose-dependently decreased by AEC (Fig. 1). There were not significant effects in grooming, which was poor still in control mice (results not shown). Both effects of AEC 1 mg/kg on locomotion and exploration were potentiated by 10 mg/kg diazepam and inhibited by 0.5 mg/kg flumazenil, especially at the start of the test (Fig. 1).

Figure 1. Effects of AEC (aqueous extract of A. citriodora) on spontaneous locomotion (a, number of cross lines during 5 min, two-way ANOVA: by time: F = 10.92, df = 3, p < 0.0001, by treatment: F = 33.83, df = 6, p < 0.0001) and exploratory behavior (b, number of rears in 5 min, two-way ANOVA: by time: F = 5.897, df = 3, p = 0.0008, by treatment: F = 30.65, df = 6, p < 0.0001) of mice vs. time. The negative (saline) and positive (diazepam) controls are shown. Results are expressed as mean ± S.E.M (n = 5 to 7). A posteriori Bonferroni tests: * p < 0.05, a p < 0.01, b p < 0.001 vs. saline solution.
The flavonoid vitexin only increased the spontaneous locomotion at the start of the test and at 100 mg/kg, but not at higher doses or longer periods of testing (Table 1). Moreover, vitexin did not modify the mice exploration (Table 2).

### Table 1. Spontaneous locomotion (number of crossed lines in 5 min) produced by vitexin (Vtx) in mice during the open field test. Two-way ANOVA: by time: F: 32.67, df 3, p < 0.0001; by treatment: F: 8.17, df 3, p < 0.0001; *p < 0.05 vs Saline by Bonferroni test.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (n=8)</td>
<td>122.6 ± 9.4</td>
<td>84.9 ± 11.0</td>
<td>72.4 ± 8.8</td>
<td>52.1 ± 11.8</td>
</tr>
<tr>
<td>Vtx 33 mg/kg (n=8)</td>
<td>148.7 ± 13.4</td>
<td>101.5 ± 14.5</td>
<td>70.0 ± 7.3</td>
<td>73.6 ± 6.7</td>
</tr>
<tr>
<td>Vtx 100 mg/kg (n=10)</td>
<td>177.3 ± 18.5*</td>
<td>123.3 ± 17.4</td>
<td>77.8 ± 12.3</td>
<td>80.2 ± 18.4</td>
</tr>
<tr>
<td>Vtx 333 mg/kg (n=9)</td>
<td>118.5 ± 6.7</td>
<td>85.8 ± 5.6</td>
<td>52.8 ± 10.6</td>
<td>45.0 ± 10.6</td>
</tr>
</tbody>
</table>

### Table 2. Exploratory behaviour (number of rearings in 5 min) produced by vitexin (Vtx) in mice during the open field test. Two-way ANOVA: by time: F: 36.69, df 3, p < 0.0001; by treatment: F: 1.648, df 3, p = 0.1986; NS vs Saline by Bonferroni test.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (n=8)</td>
<td>36.4 ± 3.7</td>
<td>32.2 ± 4.4</td>
<td>19.9 ± 4.5</td>
<td>14.2 ± 4.3</td>
</tr>
<tr>
<td>Vtx 33 mg/kg (n=8)</td>
<td>46.1 ± 4.1</td>
<td>38.0 ± 4.8</td>
<td>25.9 ± 3.3</td>
<td>24.6 ± 4.2</td>
</tr>
<tr>
<td>Vtx 100 mg/kg (n=10)</td>
<td>39.8 ± 3.7</td>
<td>43.4 ± 4.5</td>
<td>31.0 ± 5.2</td>
<td>22.8 ± 5.8</td>
</tr>
<tr>
<td>Vtx 333 mg/kg (n=9)</td>
<td>41.5 ± 4.1</td>
<td>31.7 ± 3.8</td>
<td>22.3 ± 4.6</td>
<td>14.2 ± 3.9</td>
</tr>
</tbody>
</table>

The pre-treatment with 1.5 mg/kg atropine did not significantly change basal BP (ΔBP: 6.9 ± 6.9 mm Hg) nor the effects of AEC, although it reduced the hypotension produced by acetylcholine (Ach) in the positive control group (Fig. 2b). The administration of 25 µmol/kg L-NAME increased basal BP (ΔBP: +33.75 ± 15.3 mm Hg) but did not significantly change the effects of AEC (Fig. 2c). The transitory hypotension of AEC before and after atropine and L-NAME showed a dose-dependence but not differences among the 3 conditions (two-way ANOVA: by doses F = 3.852, df = 3, p = 0.014; by treatments:

**Effects of A. citriodora on the blood pressure**

Basal BP of normotensive rats was 145 ± 27 mm Hg (n = 6). AEC induced a transitory hypotension at 2.1 ± 0.21 s (n = 24), which returned to basal between 60 to 300 s (Fig. 2a).
As a negative control, 4 rats (basal BP: 125.6 ± 25.7 mm Hg) were injected with saline three times separated by 20 min, and the ΔBP was NS from zero (Fig. 2, saline group).

Heart rate (HR) was significantly increased by the pre-treatment with 1.5 mg/kg atropine, but not significantly modified by AEC at any dose, as neither after L-NAME (Table 3).

**DISCUSSION**

In a previous work we demonstrated that the aromatic plant *A. citriodora* Palau ("cedrón")

\[ F = 1.643, \text{df} = 2, \ p = 0.2025. \]  
As a negative control, 4 rats (basal BP: 125.6 ± 25.7 mm Hg) were injected with saline three times separated by 20 min, and the ΔBP was NS from zero (Fig. 2, saline group).

Heart rate (HR) was significantly increased by the pre-treatment with 1.5 mg/kg atropine, but not significantly modified by AEC at any dose, as neither after L-NAME (Table 3).
from Paraguay) has antispasmodic effect on isolated duodenum, which gives support to the use for gastrointestinal disorders. Now, we preclinically evaluated the basis for its use in anxiety and tachycardia. The present results show that AEC reduced the spontaneous locomotion and the exploratory behaviour of mice on the open field, at doses (0.15 to 10 mg lyophilized/kg) equivalents to 1-66 mg leaves/kg, giving support to the soft sedative properties for the “tea of cedrón”. Furthermore, the sedative effect of 1 mg/kg AEC was potentiated by diazepam and was reduced by flumazenil, the antagonist of the benzodiazepine receptor. These results suggest a benzodiazepine-like effect of AEC on the GABA-A receptor. Nevertheless, the flavonoid vitexin previously identified in AEC was not the responsible for such sedative effects, since it did not decrease locomotion. This lack of effect is important when considering that vitexin is a component of sedative and anxiolytic plants from the Passiflora genus, and it is especially the compound used to quantify them. Thus, the reference compound for the qualitative and quantitative analysis is not the sedative active principle. Regarding the sedative component of *A. citriodora*, it still remains undetermined. Some compounds from the “lemon verbena” essential oil were found, such as limonene and citral, which were reported as sedative in the related genus *Lippia*, but it is expected that volatile components may not remain in the AEC after decoction and lyophilization. Luteolin-7-diglucuronide and verbascoside were found in the infusion and leaves of “lemon verbena”. Luteolin-7-diglucuronide has also been isolated from *Lippia alba* and demonstrated to bind the GABA-A receptors at IC50 of 40-100 nM, which could explain the effect that we found with AEC in the open-field. Taking into account the phylogenetic origin of secondary metabolites, it is important to consider that other South American related species (*Aloysia polystachya*) was also sedative in rats and has phenols and monoterpens in its composition. Then, it is possible that a combination of those compounds would be responsible for the important sedative and benzodiazepine-like effect of the aqueous extract of *A. citriodora*.

In normotensive rats, the i.v. administration of AEC produced a transitory hypotension, dose-dependent between 1-30 mg lyophilized/kg. The time-course suggested an effect on muscarinic receptors or on nitric oxide (NO) release. Nevertheless, neither the pretreatment with 1.5 mg/kg atropine or 25 µmol/kg L-NAME (competitive blocker of NO-synthase) changed the hypotensive response to AEC, suggesting that it was not mediated by muscarinic receptors or the NO-synthase. Also, on the isolated vas deferens, a smooth muscle which has α1 receptors, AEC was a non-competitive inhibitor of the phenylephrine dose-response curve, since it decreased the maximal effect. This result sug-
gests that AEC is not a $\alpha_1$-adrenergic antagonist, but it directly affects the smooth muscle. It agrees with the effect of AEC on intestines, where we demonstrated the activation of sarcolemmal K$^+$-channels and guanylate-cyclase, partially due to vitexin. Such a non-competitive blockade of the adrenergic vascular tone could cause at least part of the transitory hypotension seen in normotensive rats. On this connection, the most important component of AEC, vitexin, was reported as hypotensive and bradycardic at doses $>10$ mg/kg i.v., which was not affected by bilateral vagotomy. Another contribution to hypotension could be the negative inotropism of AEC seen on isolated rat hearts, although “in vivo” it did not significantly change the heart rate. If considering that the doses of 1-30 mg/kg assayed on normotensive rats may be equivalent to about 0.067 to 2 mg/ml plasma (since a rat has about 3 ml plasma) and the negative cardiac inotropism was found at the doses of 0.6, 2 and 6 mg/ml, it is expected that AEC could reduce the cardiac demand. Then, both the cardiovascular and sedative effects of AEC give support to the use of the “cedrón tea” as a coadjuvant for the treatment of angor.

By other side, certain flavonoids showed antioxidant activity, potentially giving protection against coronary diseases. When the flavonoid vitexin was evaluated on isolated rat hearts, it did not significantly change the inotropism, which agrees with previous reports. Moreover, in a model of ischemia-reperfusion vitexin slightly prevented hearts from the postischemic diastolic contracture, but not from the decrease in contractility seen in the negative control (regarding non-pretreated reperfused hearts, Fig. 4b). Consequently, vitexin is not the responsible of the negative inotropism of AEC, but neither it is anti-ischemic. Regarding other components, verbascoside was isolated from A. citriodora and demonstrated to be antioxidant. Also, verbascoside induced a positive inotropism in the isolated perfused hearts. Contrarily, our sample of AEC induced a great negative inotropism, suggesting that it would have less verbascoside than other reported specimens. It could be possible that specimens from different regions differ in composition, as it has been described for the essential oil.

CONCLUSIONS

The aqueous extract of A. citriodora produced an important sedative effect on mice, by a benzodiazepine-like mechanism. Also, it induced a transitory hypotension in normotensive rats, which was not due to cholinergic effect, NO-release or $\alpha_1$-adrenergic antagonism. In isolated vas deferens the AEC showed a non-competitive contractile blockade, which agrees with the antispasmodic effect previously seen in intestine. In vivo, AEC did not modify heart rate but in vitro it induced a dose-dependent negative cardiac inotropism, which could contribute to the transitory hypotension, and gives support to the use of A. citriodora (South American “cedrón”) as a coadjuvant for treating angor and anxiety. Nevertheless, the main flavonoid found in this sample and known as hypotensor, vitexin, had not sedative effects or cardiac antiischemic properties.

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REFERENCES