Antipyretic, Hypothermic and Antiinflammatory Activities and Metabolites from Solanum ligustrinum Lodd.

C. Delporte, N. Backhouse, R. Negrete, P. Salinas, P. Rivas, B. K. Cassels and A. San Feliciano

1Laboratorio de Farmacognosia, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Casilla 233, 1-Santiago, Chile.
2Facultad de Ciencias, Universidad de Chile. Casilla 653, Santiago, Chile.
3Facultad de Farmacia, Universidad de Salamanca, Avda. Campo Churro s/n, 37007, Salamanca, Spain

The acute toxicities of the global methanol extract of Solanum ligustrinum (Solanaceae) and the crude steroidal glycoalkaloids mixture were determined. The antipyretic, hypothermic and antiinflammatory activities of aqueous, global methanol, petroleum ether, dichloromethane and methanol extracts and crude steroidal glycoalkaloids mixture of the aerial parts were evaluated.

All the extracts and the crude steroidal glycoalkaloids mixture were inactive in the hypothermic activity assay, nevertheless, they showed antipyretic and antiinflammatory activities. Scopoletin and β-sitosterol 3-O-β-D-glucoside were isolated and identified from the dichloromethane extract. The impure coumarin fraction showed antipyretic and antiinflammatory activities and β-sitosterol glucoside exhibited antiinflammatory activity. In the light of the results of several NOE experiments, the H-5 and H-8 signals in the 1H-NMR spectra of scopoletin were reassigned.

The methanol global extract yielded a crude steroidal glycoalkaloids mixture with antipyretic and antiinflammatory effects. © 1998 John Wiley & Sons, Ltd.

In Chile herbal remedies are used frequently to treat a large variety of ailments and symptoms, such as fever and inflammatory conditions, but little is known about the efficacy and innocuousness of these plants. Solanum ligustrinum is a native species which grows in central Chile and is used in folklore medicine for the treatment of fever as an infusion, decoction and macerate of the aerial part, without scientific data to support this use and its lack of toxicity (Montes and Wilkomirsky, 1987; Muñoz et al., 1981). Steroidal glycoalkaloids such as solamnine and the genins solasodine, tomatillidine and dihydrotomatillidine were isolated in earlier studies from this species (Bianchi et al., 1960, 1961; 1965).

The literature describes antifungal properties and a certain degree of toxicity for steroidal glycoalkaloids found in species of Solanum (Keeler et al., 1990; Roddick and Drysdale, 1984). It was demonstrated that aqueous macerated samples of Solanum ligustrinum inhibited the growth of Candida albicans, Aspergillus niger and Achromomium falciforme (Lazo, 1987; Giron et al., 1988; Kusano et al., 1987).

MATERIALS AND METHODS

General experimental procedures. Silica gel 60 was used for TLC and flash column chromatography (CC). TLC spots were detected under UV (254 and 365 nm), spraying with Dragendorff reagent and heating the plates to 110°C after spraying with Liebermann–Burchard (LB) reagent, AlCl₃ and NH₃. 1R spectra (KBr disc) were recorded using a Perkin-Elmer 1310 infrared spectrometer. The NMR spectra were run in CDCl₃ or C₅D₅N solutions with TMS as an internal standard and recorded at 500 MHz (1H) and 300/75 MHz (1H/13C) in a Bruker spectrometer. The high resolution mass spectrum was recorded using a Kratos MS-50 spectrometer. The melting point was determined on a Kofler hot stage.

Plant material. Solanum ligustrinum Lodd. Solanaceae was collected in the upper Maipo valley (Cajón del Maipo), SE of Santiago, latitude 34°S Chile, in early summer (December), and identified by Professor Carla Delporte. Voucher specimens are kept in the herbarium of the Escuela de Química y Farmacia, University of Chile (SQF: 17045).

Extraction and isolation. The dried and powdered material (1 kg) was extracted with methanol at room temperature. This global methanol extract (GME) (140 g)
was used for the acute toxicity studies and for the antinflammatory and antipyretic assays, after removing the solvent under vacuum in a rotary evaporator. An aqueous extract or infusion (INFU), was prepared from dried and ground material, adding boiling water to a weighed amount, to obtain 20% and 40% (w/v) extracts for the antinflammatory and antipyretic assays respectively. A decoction (DECO) was prepared by heating a weighed amount of plant material with water for 5 min, to obtain 20% and 40% (w/v) extracts. Macerates (MACR) were obtained at 20% and 40% w/v for the antinflammatory and antipyretic assays respectively and were prepared with a weighed amount of the plant material with water and allowed to stand for the next 24 h.

As the GME showed antipyretic and antinflammatory activities, and in order to continue with the chemical study, a new amount of *S. lugens* (5 kg) was subjected to successive extractions with petroleum ether, dichloromethane and methanol, to afford the extracts PEE (110 g), DM (140 g) and ME (1200 g), respectively. All were subjected to antinflammatory, hypothermic and antipyretic assays at doses of 600 mg/kg to monitor potential activities. The ME and DM extracts were submitted to CC eluting with mixtures of increasing polarity, and testing the fractions for bioactivity. Fractionation of ME (100 g) eluting with methanol-CH$_2$Cl$_2$ (50:50), afforded an alkaloid concentrate M-1 (30.5 g) containing an orange odour with Dragendorff reagent. Eluting with 100% methanol, a concentrate M-2 (8.5 g) rich in flavonoids was obtained, giving a positive reaction with NH$_3$ and AlCl$_3$.

The M-1 and M-2 fractions were submitted to successive fractionation in CC, yielding a crude steroid glycoalkaloids mixture: CGA (20 g) when eluting with methanol: CH$_2$Cl$_2$ (40:60) and 50:50), and a flavonoid mixture: FLAV (8.5 g) when eluting with methanol: CH$_2$Cl$_2$ (90:10), respectively.

Fractionation of DM (70 g) eluting with hexane-CH$_2$Cl$_2$ (80:20), yielded a fraction (FSI) (5 g), rich in a compound S-1 accompanied by chlorophyll, showing an intense light blue fluorescence at 366 nm, and dark blue at 254 nm. Fractions eluted with hexane-CH$_2$Cl$_2$ (50:50) gave the concentrate named ImpS-2 containing a major compound S-2 giving a purple colour with the LB reagent, which was submitted to successive fractionation in CC yielding S-2, purified by successive crystallization and identified for a complete spectral study.

**Animals.** Pirbright guinea-pigs (220-300 g) of both sexes were used for the antiinflammatory study and for antipyretic and hypothermic studies adult female New Zealand rabbits (2-3 kg) were used. The animals were kept under standard housing conditions at the Animal Maintenance Unit (UMA) of the Chilean Public Health Institute (ISP), and fasted overnight before the day of the experiments.

**Acute toxicity.** Groups of 10 CF-1 mice of either sex (20-25 g), were kept under laboratory conditions and allowed free access to water. GME and CGA, suspended in propylene glycol, were administered orally, via gastric catheter. Animals were observed and weighed daily for a week. The lethal dose 50 (LD$_{50}$) was calculated with the Morgan scoring method (1992).

**Antipyretic activities.** Antipyretic activity was determined using three animals for each dose (modified from USP XXII, 1990) and repeating each experiment three times (nine results were obtained for each dose). Pyrexia was induced by i.v. injection of *Escherichia coli* endotoxin (prepared in sterile saline) at a dose of 13 ng/kg. Rectal temperatures were recorded continuously for 180 min, with an Ellab Pyrogentester (model Z12DP) immediately after pyrogen injection.

The mean areas under curves temperature vs time obtained for each pyrogen-treated animal, with and without previous oral administration of the samples, were compared and the antipyretic effect (E) was calculated according to the following equation:

\[
%E = \left(1 - \frac{\text{area}_{\text{pyr+sample}}}{\text{area}_{\text{pyr}}}\right) \times 100
\]

where area$_{\text{pyr+sample}}$ represents the mean area under the curve obtained after plotting temperature vs time in minutes for sample-tested rabbits, and area$_{\text{pyr}}$ is the corresponding mean area for animals treated only with pyrogen. These mean areas were calculated for the two time intervals: from 0-90 and 90-180 min. The experiment was carried out in this way, since active principles with fast absorption and elimination could be present in the sample being studied, showing an effect only in the first 90 min of the assay. However, the active principles could have a slow absorption and the effect would only be seen after a 90-180 min interval (Delporte et al., 1996). The mean areas were calculated using a computer program developed in our laboratory for this purpose.

**Antinflammatory activity.** The antinflammatory activity was evaluated in groups of 10-15 animals for each dose, using the carrageenan-induced paw oedema described by Winter *et al.* (1963). Paw volume was measured with an Ugo Basile plethysmometer (model 7150) immediately, and 3 h after injecting 0.1 mL of sterile saline $\lambda$-carrageenan (1%). Antinflammatory activity (% A) was evaluated as:

\[
%A = \left(\frac{I_d - I_s}{I_d}\right) \times 100
\]

where $I_d$ is the mean inflammation reached in control guinea-pigs which received only the vehicle (37.7% ± 1.3% for 96 animals), and $I_s$ is the average inflammation in drug-treated animals, expressed as:

\[
%I = \left(\frac{V_t - V_i}{V_i}\right) \times 100
\]

where $V_t$ and $V_i$ are final and initial paw volumes, respectively, averaging %I over all the animals used in each test (Backhouse *et al.*, 1994).

In both assays, the samples were administered orally 1 h before the carrageenan or the endotoxin injection, by means of an intragastric catheter, suspended in propylene glycol. Sodium naproxen (SN) (from Laboratorios Saval, Chile) was used as a positive control and was suspended in the same vehicle. $\lambda$-carrageenan was purchased from Sigma. *E. coli* endotoxin was obtained from Chilean Health Institute (Backhouse *et al.*, 1994).

The significance of the drug-induced changes was estimated using the Wilcoxon test for independent data for the antinflammatory assays and Wilcoxon tests for dependent data for antipyretic and hypothermic assays (Hollander and Wolfe, 1973). The Sm (SD/√n) values were calculated for mean % $I_d$ and % $I_s$ values and for the mean areas under the curve for treated and untreated animals in each assay.
Hypothermic activity. The hypothermic activity was evaluated in groups of three animals for each dose and each experiment repeated three times (nine results were obtained for each dose). Rectal temperatures were recorded continuously for 180 min, with an Ellab Pyrogentester (model Z12DP) immediately after the oral administration of the extracts studied. The mean areas under curve temperature vs time obtained for each with and without oral administration of the extracts were compared and the hypothermic effect (H) was calculated according to the following equation:

\[ \%H = \left[ 1 - \frac{\text{area}_{\text{sample}}}{\text{area}_{\text{normal}}} \right] \times 100. \]

RESULTS AND DISCUSSION

Acute toxicity

GME and CGA showed no toxic effect at a dose of 2.4 and 8 g/kg in mice. Common side effects including mild diarrhoea, loss of weight and depression were not recorded. The LD₅₀ for each sample was not determined as no deaths were observed, and due to the consistency of the extracts, higher doses could not be administered. It is important to carry out toxicological studies in other animal species in order to demonstrate its innocuousness.

DM showed antipyretic and antiinflammatory activities and was selected for further study. Its fractionation led to the isolation of two pharmacologically active compounds S-1 (30 mg) and S-2 (100 mg). The first compound was identified as scopoletin by spectral comparison. It crystallized as needles from methanol, MP = 204°C, and presented a coumarin characteristic light blue intense fluorescence at UV 366 nm. The mass spectrum showed a M⁺ at 192.4, which could correspond to both scopoletin (7-hydroxy-6-methoxy coumarin) and isoscopoletin (6-hydroxy-7-methoxy coumarin) according to bibliographic data (Aplin and Page, 1967).

The ¹H-NMR spectrum also showed similarity with those of scopoletin and isoscopoletin (Aplin and Page, 1967). The ¹H chemical shifts values (δ) did not allow us to differentiate both isomers. Signals for H-3 (6.30 ppm, doublet, alpha to the CO group), H-4 (7.68 ppm, doublet, beta to the CO group), OCH₃ (3.95 ppm) and OH (6.15 ppm) were easily assigned by comparison with bibliographic data. This was not the case for the signal appearing at 6.90 and 6.85 ppm, which may correspond to H-5 or H-8 indistinctly. It was necessary to carry out some NOE difference experiments to ascertain their assignment. On irradiation of the OCH₃ signal (3.95 ppm) a NOE was observed for the signal at 6.85 ppm. The same signal was enhanced by NOE on irradiating the signal at 7.68 ppm, which unequivocally corresponds to H-4. These results confirm both the location of the methoxy group at C-6 and the correct assignment of H-5 (6.85 ppm) and H-6 (6.90 ppm) (Table 1 and Fig. 1), which differ from those performed previously (Aplin and Page, 1967).

S-2 was identified as β-sitosterol 3-O-β-D-glucoside by comparison with an authentic sample by IR and ¹H-NMR and ¹³C-NMR. The ¹H and ¹³C signals of the NMR spectra were assigned by comparison with the bibliographic data (Villar and Payá, 1981).

The antipyretic activity of sodium naproxen is dose-related and in our experiments the maximum effect at both time intervals (0-90 min: 51% and 90-180 min: 81.1%) was reached at a dose of 25 mg/kg (Delporte et al., 1996). The antiinflammatory response to sodium naproxen is also a dose-related effect, with a maximum

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dose (mg/kg)</th>
<th>0-90 min</th>
<th>90-180 min</th>
<th>% Antipyretic effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>INFU</td>
<td>800</td>
<td>28.7 ± 3.5/26.5 ± 3.1</td>
<td>86 ± 9.8/93.3 ± 7.9</td>
<td>0/0</td>
</tr>
<tr>
<td>DECO</td>
<td>800</td>
<td>39.9 ± 3.4/63.1 ± 3.8</td>
<td>19.0 ± 1.7/21.7 ± 1.7</td>
<td>25.0*</td>
</tr>
<tr>
<td>MACE</td>
<td>800</td>
<td>38.8 ± 4.5/43.5 ± 4.9</td>
<td>116.5 ± 12.8/117.5 ± 12.5</td>
<td>13.0</td>
</tr>
<tr>
<td>GME</td>
<td>600</td>
<td>15.3 ± 1.1/21.0 ± 1.0</td>
<td>6.4 ± 1.9/65.8 ± 1.1</td>
<td>27.1*</td>
</tr>
<tr>
<td>PEE</td>
<td>600</td>
<td>21.6 ± 4.7/47.7 ± 4.0</td>
<td>21.6 ± 2.9/14.3 ± 1.8</td>
<td>54.7*</td>
</tr>
<tr>
<td>DM</td>
<td>600</td>
<td>13.8 ± 2.9/31.4 ± 3.9</td>
<td>28.9 ± 4.7/18.1 ± 2.3</td>
<td>56.1*</td>
</tr>
<tr>
<td>ME</td>
<td>600</td>
<td>16.0 ± 3.0/17.0 ± 4.1</td>
<td>13.3 ± 2.5/27.7 ± 3.0</td>
<td>90.6*</td>
</tr>
<tr>
<td>CGA</td>
<td>50</td>
<td>8.9 ± 0.7/37.3 ± 3.8</td>
<td>30.4 ± 4.9/39.2 ± 3.2</td>
<td>76.4*</td>
</tr>
<tr>
<td>FLAV</td>
<td>50</td>
<td>58.6 ± 4.1/59.3 ± 1.7</td>
<td>63.5 ± 3.1/64.2 ± 4.0</td>
<td>0/0</td>
</tr>
<tr>
<td>FS1</td>
<td>50</td>
<td>23.4 ± 2.1/26.9 ± 3.7</td>
<td>64.8 ± 2.6/63.4 ± 3.4</td>
<td>80.5*</td>
</tr>
<tr>
<td>SN</td>
<td>25</td>
<td>15.6 ± 2.9/31.8 ± 3.7</td>
<td>9.5 ± 1.2/50.5 ± 8.0</td>
<td>51.0*</td>
</tr>
</tbody>
</table>

*p ≤ 0.05

INFU: inducement; DECO: decoction; MACE: maceration; GME: global methanol extract. PEE, DM, ME: petroleum ether, dichloromethane and methanol extracts; CGA: crude steroidal glycoalkaloids mixture; FLA: crude flavonoids mixture; FS1: fraction rich in scopoletin; SN: sodium naproxen; Sm = SD/√n; n number of animals in each assay.
Table 3. Antiinflammatory effect (% A) of different extracts and metabolites from Solanum ligustrinum and sodium naproxen

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dose (mg/kg)</th>
<th>% ± Sm</th>
<th>% Antiinflammatory effect</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>800</td>
<td>37.7 ± 1.3</td>
<td>0</td>
<td>96</td>
</tr>
<tr>
<td>INFU</td>
<td>800</td>
<td>27.1 ± 2.8</td>
<td>28.1*</td>
<td>38</td>
</tr>
<tr>
<td>DECO</td>
<td>800</td>
<td>25.8 ± 0.02</td>
<td>31.6*</td>
<td>17</td>
</tr>
<tr>
<td>MACE</td>
<td>800</td>
<td>28.5 ± 1.9</td>
<td>24.2*</td>
<td>22</td>
</tr>
<tr>
<td>PEE</td>
<td>600</td>
<td>19.8 ± 2.0</td>
<td>47.5*</td>
<td>24</td>
</tr>
<tr>
<td>DM</td>
<td>600</td>
<td>31.7 ± 3.2</td>
<td>16.0*</td>
<td>23</td>
</tr>
<tr>
<td>ME</td>
<td>600</td>
<td>19.9 ± 2.4</td>
<td>48.5*</td>
<td>18</td>
</tr>
<tr>
<td>CGA</td>
<td>30</td>
<td>28.3 ± 2.0</td>
<td>24.8*</td>
<td>16</td>
</tr>
<tr>
<td>FS1</td>
<td>50</td>
<td>13.3 ± 1.4</td>
<td>65.0*</td>
<td>12</td>
</tr>
<tr>
<td>S-2</td>
<td>30</td>
<td>19.1 ± 5.7</td>
<td>49.3*</td>
<td>7</td>
</tr>
<tr>
<td>SN</td>
<td>4</td>
<td>17.1 ± 0.8</td>
<td>54.6*</td>
<td>15</td>
</tr>
</tbody>
</table>

*p < 0.05

%l, mean inflammation in sample-treated animals. INFU, infusion; MACE, macerate; PEE, DM, ME, petroleum ether, dichloromethane and methanol extracts; CGA, crude steroidal glycoalkaloids mixture; FS1, fraction rich in scopoletin; S-2, 3β-sitosterol-3-O-D-glucoside; SN, sodium naproxen; Sm = SD/√n. n, number of animals in each assay.

Table 4. Antiinflammatory effects (% A) of GME from Solanum ligustrinum at different dose and sodium naproxen

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>% ± Sm</th>
<th>% A</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>37.7 ± 1.3</td>
<td>0</td>
<td>96</td>
</tr>
<tr>
<td>400</td>
<td>24.0 ± 2.3</td>
<td>36.3*</td>
<td>30</td>
</tr>
<tr>
<td>600</td>
<td>18.3 ± 1.5</td>
<td>51.3*</td>
<td>32</td>
</tr>
<tr>
<td>800</td>
<td>10.2 ± 1.3</td>
<td>73.0*</td>
<td>15</td>
</tr>
<tr>
<td>1000</td>
<td>25.1 ± 2.1</td>
<td>33.4*</td>
<td>18</td>
</tr>
<tr>
<td>SN 4</td>
<td>17.1 ± 0.8</td>
<td>54.6*</td>
<td>15</td>
</tr>
</tbody>
</table>

*p < 0.05

%l, mean inflammation in sample-treated animals. GME, global methanol extract; SN, sodium naproxen; Sm = SD/√n. n, number of animals in each assay.

effect at 4.0 mg/kg of 54.6% (Backhouse et al., 1994) (Tables 2 and 3). INFU showed no antipyretic effect, but exhibited an antiinflammatory effect (28.1%) at a dose of 800 mg/kg in the first interval. DECO gave a 25% antipyretic and 24.2% antiinflammatory effect respectively at a dose of 800 mg/kg. MACE at a dose of 800 mg/kg showed no antipyretic effect, but exhibited a 24.2% antiinflammatory effect (Tables 2 and 3). Only DECO, of the aqueous extracts, presented an antipyretic effect and the highest antiinflammatory effect, leading us to propose that the active principles would have been extracted in a major quantity when the plant material was heated with boiling water. Assays performed on GME showed a dose-related antiinflammatory effect with a maximum of 73% at 800 mg/kg and an antipyretic effect of 27.1% at 600 mg/kg (Tables 2 and 4). PEE exhibited 54.7% antipyretic and 47.5% antiinflammatory effects. DM gave 56.1% antipyretic and 16% antiinflammatory effects. ME showed 90.6% antipyretic and 19.9% antiinflammatory effects (Tables 2 and 3). As can be observed, all successive extracts showed both activities, possibly due to the inhibition of the release of prostaglandins by the active principles of these extracts, that could explain both effects observed (Kluger, 1991; Weissmann, 1987).

The CGA proved to have marked antipyretic (76.4%) and slight antiinflammatory (24.8%) activities at doses of 50 and 30 mg/kg respectively (Tables 2 and 3). These crude steroidal glycoalkaloids mixture would be responsible in part for the effects shown by GME and ME, which gave an antiinflammatory and a stronger antipyretic effect for the latter. One of the components of this mixture is solanine, a glycoside of solasodine (Bianchi et al., 1961). Solasodine exhibited antiinflammatory effects in arthritis caused by kaolin, similar to that of cortisone at the same doses (0.25 mg/kg) (Müller-Dietz, 1972). For this reason, solanine could be one of the active principles responsible for the pharmacological effects found.

The fraction rich in scopoletin showed both activities at doses of 50 mg/kg: 60% antipyretic and 60% antiinflammatory effects (Tables 2 and 3). Scopoletin must be one of the active principles of this species, since this simple coumarin inhibited prostaglandin synthetase, and its use as an antiinflammatory topical application has been reported (Hussein and Samuelsson, 1992). Besides, unsubstituted coumarin is antipyretic (Ritschel et al., 1984). The steroid β-sitosterol 3-O-β-D-glucoside gave an antiinflammatory effect (49.3%) at 20 mg/kg (Table 3). This is another of the active principles isolated from DM. According to the literature, the simple genin, β-sitosterol itself, exhibited antiinflammatory and antipyretic activities at doses of 155.6 and 160 mg/kg respectively (Gupta et al., 1980).

It is important to note that in the assays of the antipyretic activity, the effects were seen only at the first time interval for all the samples in study, differing from the positive control that presented an effect at both time intervals. This may reflect substantial pharmacokinetic differences between sodium naproxen and the active principles of S. ligustrinum. All the extracts studied showed no hypothermic effects, they were especially effective in lowering an elevated temperature but did not affect appreciably a normal temperature. In view of the results, the present study demonstrates that S. ligustrinum exerts significant antipyretic and antiinflammatory in vivo activities.

Acknowledgements

We wish to extend our gratitude to I.F.S. (Grant F/1494-1), and especially to Dr Julio Maddonado of the Chilean Public Health Institute for the experimental animals and facilities. We also acknowledge to Dr Luis Rodriguez, for the collaboration in the treatment of the statistical data.
REFERENCES


