Antinociceptive activity of *Hypericum caprifoliatum* and *Hypericum polyanthemum* (Guttiferae)

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Abstract

The aim of the present study was to assess the analgesic activity of the aerial parts of two *Hypericum* species native to Southern Brazil, *H. caprifoliatum* and *H. polyanthemum*. The antinociceptive effect of the *H. polyanthemum* cyclohexane extract (POL; 180 mg/kg) and of the *H. caprifoliatum* methanol (MET) and cyclohexane (CH) extracts (90 mg/kg) was evaluated in the hot-plate (*ip* and *po*) and writhing (*po*) tests using male Swiss CF1 mice weighing 22-27 g (N = 10 per group). All extracts displayed antinociceptive effects in the hot-plate test (MET *ip* = 48%, MET *po* = 39%, CH *ip* = 27%, CH *po* = 50%, POL *ip* = 74%, and POL *po* = 49% compared to control). Pretreatment with naloxone (2.5 mg/kg, *sc*) abolished the effects of CH and POL, and partially prevented the analgesia induced by MET administered by the *ip* (but not by the *po*) route. POL and CH (*po*) significantly reduced the number of writhes induced by acetic acid, while MET was ineffective in this regard. We conclude that the antinociceptive effects of the *H. caprifoliatum* (CH) and *H. polyanthemum* (POL) hexane extracts seem to be mediated by the opioid system. Moreover, the antinociceptive activity of the *H. caprifoliatum* MET extract seems to depend on at least two chemical substances (or groups of substances) with distinct pharmacokinetic profiles and mechanisms of action. Only the naloxone-insensitive component of MET activity showed good bioavailability following oral administration.

The chemical investigation of the genus *Hypericum* (Guttiferae), which comprises approximately 400 species (1), has led to the isolation of more than 100 compounds from about 20 species with various different biological activities, especially antiviral, antimicrobial and antidepressant properties. *H. perforatum* extracts are widely used in Europe, in the United States, and also in Brazil, for the treatment of mild to moderate depression (2).

The Southern Brazilian *Hypericum* species *H. brasiliensis* and *H. connatum* are popularly used for relief of disorders such as angina, cramps and oral and pharyngeal inflammations, which suggests an analgesic property for this genus (3).

Previous reports published by our group
have shown interesting biological activities for the *Hypericum* species native to the State of Rio Grande do Sul, Brazil. A crude lipo-
philic extract of *H. caprifolium* induces an anti-immobility effect in the forced swim-
mig test (4), which is considered to indicate an antidepressant action (5), as well as an
antinociceptive effect in the hot-plate test

(6). *H. caprifolium, H. pirtai* and *H. polo-
anthemum* showed *in vitro* monoamine ox-
dase A-inhibitory activity (7). The aim of
the present study was to investigate further the
antinociceptive effects of *H. caprifolium*
and to start the characterization of the anti-
nociceptive properties of *H. polyanthemum*.

Air-dried and powdered aerial parts of *H.
caprifolium* and *H. polyanthemum* were
extracted with cyclohexane using an ultra-
turax apparatus (3 x 5 min; plant/solvent
ratio 1:10, w/v), yielding extracts termed CH
and POL, respectively. In order to obtain an
extract rich in polar substances, *H. capri-
folium* was also extracted consecutively in a
Soxhlet apparatus with petroleum ether, chlo-
roform and methanol (MET) and only the
MET extract was used. All solvents were
evaporated to dryness under reduced pres-
sure. The extract was dissolved in saline
solution containing 2.5% (w/v) polysorbate
80. The pH of the final solutions was 6.5 to
7.0. The volume administered was 1 ml/100

g body weight for the analgesic tests.

Male Swiss CF1 mice (22-27 g) from the
breeding colony of Fundação Estadual de
Pesquisa e Ensino em Saúde (FEPPS, RS,
Brazil) were used. The animals were housed
in plastic cages, 5 to a cage, under a 12-h
light/dark cycle (lights on at 7:00 h) at con-
stant temperature (23 ± 1°C), with free ac-
cess to standard certified rodent diet and tap
water. The experiments were performed ac-

     *P<0.005 compared to the latency of the same mouse before treatment (paired Student *t*-test).

<table>
<thead>
<tr>
<th>Treatment (administration)</th>
<th>Latency (s)</th>
<th>% Analgesia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before treatment</td>
<td>After treatment</td>
<td></td>
</tr>
<tr>
<td>Control (saline + 2.5% polysorbate 80)</td>
<td>12.1 ± 0.8</td>
<td>12.0 ± 1.4</td>
</tr>
<tr>
<td>MOR (6 mg/kg, <em>sc</em>)</td>
<td>10.2 ± 1.2</td>
<td>27.9 ± 1.9*</td>
</tr>
<tr>
<td>MET (90 mg/kg, <em>ip</em>)</td>
<td>12.0 ± 1.8</td>
<td>20.5 ± 3.7*</td>
</tr>
<tr>
<td>CH (90 mg/kg, <em>ip</em>)</td>
<td>9.4 ± 1.5</td>
<td>14.2 ± 1.8*</td>
</tr>
<tr>
<td>POL (180 mg/kg, <em>ip</em>)</td>
<td>10.2 ± 1.8</td>
<td>23.1 ± 3.9*</td>
</tr>
<tr>
<td>NAL (2.5 mg/kg, <em>sc</em>) + MOR (6 mg/kg, <em>sc</em>)</td>
<td>10.3 ± 1.3</td>
<td>10.8 ± 1.5</td>
</tr>
<tr>
<td>NAL (2.5 mg/kg, <em>sc</em>) + CH (90 mg/kg, <em>ip</em>)</td>
<td>11.4 ± 1.3</td>
<td>11.2 ± 2.0</td>
</tr>
<tr>
<td>NAL (2.5 mg/kg, <em>sc</em>) + MET (90 mg/kg, <em>ip</em>)</td>
<td>9.7 ± 1.4</td>
<td>15.8 ± 2.4*</td>
</tr>
<tr>
<td>NAL (2.5 mg/kg, <em>sc</em>) + POL (180 mg/kg, <em>ip</em>)</td>
<td>12.37 ± 1.3</td>
<td>12.6 ± 3.1</td>
</tr>
<tr>
<td>CH (90 mg/kg, <em>po</em>)</td>
<td>9.2 ± 0.9</td>
<td>17.8 ± 1.7*</td>
</tr>
<tr>
<td>MET (90 mg/kg, <em>po</em>)</td>
<td>10.5 ± 0.8</td>
<td>17.4 ± 2.1*</td>
</tr>
<tr>
<td>POL (180 mg/kg, <em>po</em>)</td>
<td>12.26 ± 1.8</td>
<td>20.7 ± 2.5*</td>
</tr>
<tr>
<td>NAL (2.5 mg/kg, <em>sc</em>) + CH (90 mg/kg, <em>po</em>)</td>
<td>12.8 ± 1.5</td>
<td>11.1 ± 0.6</td>
</tr>
<tr>
<td>NAL (2.5 mg/kg, <em>sc</em>) + MET (90 mg/kg, <em>po</em>)</td>
<td>10.2 ± 1.0</td>
<td>16.6 ± 2.7*</td>
</tr>
</tbody>
</table>

Data are reported as mean ± SEM. MOR: morphine; NAL: naloxone.

mg/kg POL (ip and po). Treatment-induced changes in responsiveness to the hot plate were determined 30 and 45 min after ip and po administration, respectively. The negative control group received an equal volume of vehicle (saline + 2.5% (w/v) polysorbate 80). Morphine (6 mg/kg, sc) was administered to the positive control group. To determine the possible involvement of opioid-mediated mechanisms, some groups of animals were pretreated with naloxone (2.5 mg/kg, sc), a nonspecific opioid receptor antagonist, immediately after evaluating baseline responsiveness, 10 min before extract administration.

For the hot-plate test, mice were placed on a metal surface kept at 53 ± 1°C. The time elapsed until the animal licked one of its hind paws or jumped was recorded (latency time, in s) and considered to be the reaction time in both exposures. Mice that presented baseline reaction times of more than 15 s in the first session were not used. In the second session, a maximum latency time of 30 s was imposed in order to avoid tissue damage.

The data were analyzed by the paired Student t-test, considering the animal as its own control (second measure vs first measure). The results obtained in the hot-plate test are reported as the mean ± SEM absolute latency time or as the percent of antinociceptive effect relative to morphine (6 mg/kg, sc) according to the following formula: % analgesia = (test_after - test_before)/(morphine_after - morphine_before) x 100.

The animals were treated with CH, MET (90 mg/kg, po) or POL (180 mg/kg, po) for the writhing test 45 min before receiving an ip injection of 0.8% acetic acid. Mice were then placed individually in glass observation chambers and the number of abdominal writhes was counted over a period of 15 min. The control group received an equal volume of vehicle (saline + 2.5% (w/v) polysorbate 80, po). Dipyone (150 mg/kg, po) was the positive control treatment. Previous experiments carried out in our laboratory have revealed that none of the extracts caused any signs of pain or writhes per se, in mice, when injected ip (8). The results obtained in the writhing test are reported as median values and their respective interquartile intervals, and were analyzed by the Kruskal-Wallis test.

All extracts displayed antinociceptive effects in the hot-plate test (Table 1). Pretreatment with naloxone abolished the effects of CH and POL, indicating that these effects are produced by opioid-mediated mechanisms. Conversely, antinociception produced by MET administered ip was only partially prevented by naloxone, whereas the po antinociceptive activity was not modified, indicating that opioid-like substances present in this extract were not absorbed by the gastrointestinal tract or suffered single-pass inactivation by the liver. In addition, the percent of analgesia was higher when MET was administered by the ip route compared to the po route.

Administration of CH and POL significantly reduced the number of abdominal writhes induced by acetic acid, whereas MET did not have a significant effect (Table 2). Interestingly, the magnitude of the antinociceptive effect of CH in the writhing test (oral route) was similar to that observed in the

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of writhes*</th>
<th>% Reduction of abdominal writhing compared to control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>58 (45-65)</td>
<td>-</td>
</tr>
<tr>
<td>DIP</td>
<td>0 (2-16)*</td>
<td>100</td>
</tr>
<tr>
<td>MOR</td>
<td>0 (0-3)*</td>
<td>100</td>
</tr>
<tr>
<td>CH</td>
<td>24 (0-34.5)*</td>
<td>58.6</td>
</tr>
<tr>
<td>MET</td>
<td>40.5 (16-59)</td>
<td>30.2</td>
</tr>
<tr>
<td>POL</td>
<td>0 (0-10)*</td>
<td>100</td>
</tr>
</tbody>
</table>

Control (saline + 2.5% polysorbate 80); DIP (dipyrone, 150 mg/kg, po); MOR (morphine, 10 mg/kg, po).

*Values are reported as medians (interquartile intervals).

*P<0.001 compared to control (Kruskal-Wallis, H = 30.235).
References