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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.

Key words
- *Hypericum caprifoliatum*
- *Hypericum polyanthemum*
- Analgesia
- Antinociception
- Hot-plate test
- Writhing test

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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 lipophilic extract of *H. caprifoliatum* induces an anti-immobility effect in the forced swimming test (4), which is considered to indicate an antidepressant action (5), as well as an antinociceptive effect in the hot-plate test (6). *H. caprifoliatum*, *H. piriai* and *H. polyanthemum* showed *in vitro* monoamine oxidase A-inhibitory activity (7). The aim of the present study was to investigate further the antinociceptive effects of *H. caprifoliatum* and to start the characterization of the antinociceptive properties of *H. polyanthemum*.

Air-dried and powdered aerial parts of *H. caprifoliatum* and *H. polyanthemum* were extracted with cyclohexane using an ultra-turrax 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. caprifoliatum* was also extracted consecutively in a Soxhlet apparatus with petroleum ether, chloroform and methanol (MET) and only the MET extract was used. All solvents were evaporated to dryness under reduced pressure. 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 constant temperature (23 ± 1ºC), with free access to standard certified rodent diet and tap water. The experiments were performed according to the guidelines of the National Ethics Committee on Research, Brazilian National Health Council. Ten mice per group were used for all experiments.

Before actual testing on the hot plate, the mice were habituated to the nonfunctioning apparatus for 1 min. Thirty minutes later, the animals were placed on the functioning hot plate (Ugo Basile, Comerino, Italy) to determine baseline responsiveness 10 min before treatment with 90 mg/kg CH or MET or 180

Table 1. Antincociceptive effect of cyclohexane (CH) and methanol (MET) extracts of the aerial parts of *Hypericum caprifoliatum* and of the cyclohexane extract of aerial parts of *H. polyanthemum* (POL) in mice submitted to the hot-plate test.

Treatment (administration)	Latency (s)		% Analgesia
	Before treatment	After treatment	
Control (saline + 2.5% polysorbate 80)	12.1 ± 0.8	12.0 ± 1.4	0
MOR (6 mg/kg, *sc*)	10.2 ± 1.2	27.9 ± 1.9*	100
MET (90 mg/kg, *ip*)	12.0 ± 1.8	20.5 ± 3.7*	48.4
CH (90 mg/kg, *ip*)	9.4 ± 1.5	14.2 ± 1.8*	27.3
POL (180 mg/kg, *ip*)	10.2 ± 1.8	23.1 ± 3.9*	73.8
NAL (2.5 mg/kg, *sc*) + MOR (6 mg/kg, *sc*)	10.3 ± 1.3	10.8 ± 1.5	3.1
NAL (2.5 mg/kg, *sc*) + CH (90 mg/kg, *ip*)	11.4 ± 1.3	11.2 ± 2.0	0
NAL (2.5 mg/kg, *sc*) + MET (90 mg/kg, *ip*)	9.7 ± 1.4	15.6 ± 2.4*	30.8
NAL (2.5 mg/kg, *sc*) + POL (180 mg/kg, *ip*)	12.37 ± 1.3	12.6 ± 3.1	1.5
CH (90 mg/kg, *po*)	9.2 ± 0.9	17.8 ± 1.7*	50
MET (90 mg/kg, *po*)	10.5 ± 0.8	17.4 ± 2.1*	39.2
POL (180 mg/kg, *po*)	12.26 ± 1.8	20.7 ± 2.5*	48.6
NAL (2.5 mg/kg, *sc*) + CH (90 mg/kg, *po*)	12.8 ± 1.5	11.1 ± 0.6	0
NAL (2.5 mg/kg, *sc*) + MET (90 mg/kg, *po*)	10.2 ± 1.0	16.6 ± 2.7*	36.5

Data are reported as mean ± SEM. MOR: morphine; NAL: naloxone.
*P<0.005 compared to the latency of the same mouse before treatment (paired Student *t*-test).

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 ± 1ºC. 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 = $(test_{after} - test_{before})/(morphine_{after} - morphine_{before}) \times 100$.

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*). Dipyrone (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 2. Analgesic effects of methanol (MET) and cyclohexane (CH) extracts of aerial parts of *Hypericum caprifoliatum* (90 mg/kg, *po*) and of the cyclohexane extract of aerial parts of *H. polyanthemum* (POL, 180 mg/kg, *po*) on writhing induced by 0.8% acetic acid (*ip*) in mice.

Treatment	Number of writhes#	% Reduction of abdominal writhing compared to control
Control	58 (55-65)	–
DIP	0 (2-16)*	100
MOR	0 (0-3)*	100
CH	24 (0-34.5)*	58.6
MET	40.5 (16-59)	30.2
POL	0 (0-10)*	100

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).

hot-plate test, while the effect of POL was more pronounced in the writhing test. Thus, the antinociceptive properties of POL might be due to actions on both central and peripheral pain systems. Apparently, the antinociceptive activity is not correlated with the antidepressant activity previously reported. Although lipophilic extracts of *H. caprifoliatum* were active in the Porsolt test (1,6), the same was not true for MET or POL (4,7).

The CH extract is rich in phloroglucinol (7) and the POL extract contains benzopyrans as its main constituent (9). Phloroglucinol derivatives may be responsible for the opioid-like effect since Simmen et al. (10) have reported that hyperforin - a phloroglucinol isolated from *H. perforatum* - inhibited binding to opioid receptors. With respect to the benzopyrans as well as the flavonoid derivatives, which are present in MET (Dall'Agnol R, Ferraz A, Schapoval ES and von Poser G, unpublished data), we are unaware of any previous reports on their influence/action on opioid systems. None of the extracts (CH, POL or MET) contains hypericin (11).

In conclusion, extracts obtained from both species, *H. caprifoliatum* and *H. polyanthemum*, contain compounds with substantial antinociceptive properties related, at least in part, to activation of opioid-mediated mechanisms. Further studies are in progress in order to elucidate the mechanisms underlying the antinociceptive effects of these species.

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