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Anti-inflammatory and analgesic activity of *Hypericum empetrifolium* Willd. (Guttiferae)☆

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Abstract

A methanolic extract of *Hypericum empetrifolium* Willd. was evaluated for anti-inflammatory properties in rats (subplantar edema induced by carrageenan) and analgesic effects in mice (hot plate and writhing tests). Our results showed that the methanolic extract exhibits a significant anti-inflammatory activity and analgesic effects only in one of the experimental models (writhing test). Therefore, we may suppose that the methanolic extract of *H. empetrifolium* is active against inflammatory pain. © 2001 Éditions scientifiques et médicales Elsevier SAS

Keywords: *Hypericum empetrifolium* Willd.; Anti-inflammatory; Analgesic effects

1. Introduction

Hypericum is a genus of about 400 species, widespread in warm temperate areas throughout the world and well represented in the Mediterranean area. Some species of genus are used in folk medicine as anthelmintics, diuretics, on wounds, scalds and herpes [1]. *Hypericum perforatum* is a plant well known and successfully used as anti-depressant drug [2].

Hypericum empetrifolium Willd. is a shrub growing in rocky places; it is widespread at low altitudes in the Aegean area, the south part of the Greek mainland and coastal areas of west Turkey. In this paper, we investigate the anti-inflammatory and analgesic effects of *H. empetrifolium* methanolic extract.

2. Experimental

2.1. Preparation of plant extracts

Aerial parts of *H. empetrifolium* were picked during the flowering stage from Parnes mountain, in the Attic region (Greece). Voucher specimens were deposited at the Pharmaco-Biological Department, University of Messina (Italy). The methanolic extract of *H. empetrifolium* aerial parts was used for the biological assays.

50 g of crushed aerial parts of *H. empetrifolium* were macerated with 500 ml of 95% methanol for 4 days. The extract was filtered and the solvent removed in vacuo. The yield was 22.18% (w/w). The methanolic extract was dissolved in propylene glycol before intraperitoneal (i.p.) administration.

2.2. Animals

Male Wistar rats (180–200 g) and Swiss mice (30–35 g) were used in the experiments. The animals were kept under standardized conditions (temperature 22 ± 2°C, humidity 60 ± 4%, natural lighting), fed with a standard diet (S Morini, Mill rat GLP) and water ad libitum.

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The animals were kept fasting 18 h before the experiment, while water was provided ad libitum. In all experiments, the mice were divided into groups of 10 animals each.

2.3. Carrageenan-induced paw edema in rats

Edema was induced in the right hind paw of each rat by subplantar injection of 0.05 ml of a 1% suspension of carrageenan [3]. The animals were divided into three groups. The first group was administered methanolic extract i.p. at a dose of 100 mg/kg, 1 h before carrageenan injection. The second (control group) received only the vehicle. The third group was treated by gavage with indomethacin (Sigma) at a dose of 5 mg/kg.

Paw volume was measured by a water plethysmometer (Basile, 7150), prior to irritant injection and 1, 2 and 3 h after. The percentage of edema inhibition in the treated animals versus control was calculated.

2.4. Hot plate test in mice

The experimental method of Eddy and Leimback was used [4]. The mice were selected randomly for sensitivity. The animals, divided into two groups, received the methanolic extract i.p. (50 and 100 mg/kg).

The reaction time between the moment when the mouse reached the hot plate (50 ± 0.5°C) and that when the animal licked its hind paw, was measured 60 and 180 min after plant extract administration.

2.5. Acetic acid writhing test in mice

The test was carried out by using the technique of Siegmund [5] modified by Koster [6]. Methanolic extract (100 mg/kg) was administered i.p. to mice divided into two groups. One h after treatment the mice were injected i.p. with 0.2 ml of a 3% acid acetic solution (pH 2.87 ± 0.11) to induce the characteristic writhings. The number of writhings occurring between 5 and 15 min after acetic acid injection was recorded.

The response of treated animals was compared with that of the animals receiving vehicle only (control group).

2.6. Statistics

Data were expressed as mean $\pm$ SE of 10 determinations. The results were statistically analyzed by Student's t-test; $P < 0.05$ versus control was taken as significant.

3. Results and discussion

In the experimental model of Winter [3], the methanolic extract administered i.p. (100 mg/kg) showed a significant anti-edemic effect; inhibition was observed from the first hour until the third hour, when the inhibitory effect was greatest (Table 1).

The methanolic extract administered i.p. (50 and 100 mg/kg) did not show any analgesic activity in the hot plate test (Table 2), while in the writhing test, at a dose of 100 mg/kg, it exhibited a significant inhibition of the contractions induced by acetic acid. The methanolic extract produced a significant decrease in the number of writhings in comparison with the controls.

The results demonstrate that the methanolic extract of the aerial parts of *H. empetrifolium* determine significant edema inhibition superior to indomethacin. The greatest effect is at the third hour; therefore, anti-inflammatory activity may be correlated to the inhibition

Table 1
Anti-inflammatory activity: carrageenan-induced paw edema in rat

Treatment	Dose (mg/kg)	% Edema inhibition ($\bar{\chi} \pm$ SE)		
		1 h	2 h	3 h
Indomethacin	5	16.44 ± 0.23	32.89 ± 1.05	$65.05 * \pm 1.01$
MeOH extract	100	$51.52 * \pm 1.23$	$70.90 * \pm 0.23$	$77.40 * \pm 0.44$

* $P < 0.05$.

Table 2
Analgesic effect: hot plate test in mice

Treatment	Dose (mg/kg)	Reaction time ($\bar{\chi} \pm$ SE)			
		Basal	60′	120′	180′
MeOH extract	100	17.41 ± 2.5	11.68 ± 2.0	8.2 ± 1.5	7.7 ± 3.0
MeOH extract	50	16.01 ± 2.0	9.48 ± 1.5	4.1 ± 1.9	4.3 ± 2.0

of the prostaglandin synthesis [7]. Moreover, the methanolic extract shows a significant analgesic activity versus the writhings induced by acetic acid administration, but it does not show any effect against pain induced by thermal stimulus.

Therefore, we may suppose that the methanolic extract of *H. empetrifolium* is active against inflammatory pain. The results indicate an action on endogenous opioid systems, suggesting selective action on κ and δ receptorial subtypes. It was shown [8] that spinal κ and δ receptors do not mediate thermal analgesic stimulus in mice.

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