ELSEVIER

Journal of Ethnopharmacology 60 (1998) 157–162

Journal of ETHNO-PHARMACOLOGY

Heterotheca inuloides: Anti-inflammatory and analgesic effect

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Received 5 November 1995; received in revised form 10 October 1997; accepted 27 November 1997

Abstract

Heterotheca inuloides Cass. (Asteraceae) is used in the traditional medicine of Mexico. The aqueous extract obtained from the flowers of *H. inuloides* was assessed for anti-inflammatory activity by carrageenan-induced edema test. At 100 mg/kg, i.p, it produced 29% inhibition of inflammation. Ethyl ether (HI-1), butanol (HI-2) and aqueous fraction (HI-3) were obtained from the aqueous extract. The biological assay, by carrageenan-induced edema test, gave the following values (% inhibition): HI-1, 19.9; HI-2, 58.0 and HI-3, 30.0. HI-2 was significantly more effective than HI-1 and HI-3. The dose-effect curve of HI-2 was obtained and the calculated ED_{50} was 29.7 (22.5–39.2) mg/kg. The peritoneal examination after the treatment with HI-2 showed that the anti-inflammatory action of *H. inuloides* was not due to an irritating effect at the injection site. At 50–100 mg/kg, i.p., HI-2 inhibited inflammation induced by dextran (38.9–68.1% inhibition) and arachidonic acid (0–33.9 %). No effect was observed at the same doses for zymosan or C_{16}-paf-induced edema. In addition, HI-2 reduced abdominal constrictions in mice following injection of acetic acid: at 50–100 mg/kg, it gave 73.8–78.2% inhibition. The ulcerogenic assay showed that ulcer indices after HI-2 i.p. treatment were 0.5 ± 0.5 at 50 mg/kg and 1.2 ± 0.4 at 100 mg/kg. The results showed related anti-inflammatory activity and the analgesic effect of HI-2.

Keywords: Heterotheca inuloides; Asteraceae; Anti-inflammatory activity; Analgesic effect

1. Introduction

Heterotheca inuloides Cass. (Asteraceae) grows abundantly in, the cooler temperate regions of Mexico. The flowers of this plant are widely used in traditional medicine of this country. Internally, they are used for the treatment of inflammatory diseases, fever and other disorders; externally, to treat contusions and wounds. Several constituents of *H. inuloides* have been identified, mainly, flavonoids (Willuhn et al., 1983; Jerga et al., 1990a,b), sesquiterpenoids (Bohlmann and Zdero, 1976; Willuhn and Schneider, 1987), triterpenoids and sterols (Willuhn and Schneider, 1987). The

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0378-8741/98/$19.00
PII S0378-8741(97)00155-4

composition of the essential oil has also been described (Willuhn and Rötger, 1980; Willuhn et al., 1985). Recently, four sesquiterpenoids of *H. inuloides* were identified as antimicrobial agents (Kubo et al., 1994). No other pharmacological investigation has been reported.

In this s study, purified fractions obtained from the aqueous extract of *H. inuloides* were tested for anti-inflammatory activity. The active fraction (HI-2) was assessed in various pharmacological models. The anti-inflammatory, analgesic and ulcerogenic effects were evaluated.

2. Material and methods

2.1. Plant material

Dried aerial parts of *H. inuloides* were collected in Cuetzalan (carretera de Zaragoza, Km 1–12), Mexico, in July 1992 and identified by Mrs E. Linares, UNAM, Mexico. A voucher specimen was deposited at the herbarium of BCF (Faculty of Pharmacy, University of Barcelona) under the number 39834.

2.2. Chemicals

Dextran (average molecular weight: 60 000–90 000), zymosan A and C_{16}-paf were obtained from Sigma Chemical (St. Louis, MO). Arachidonic acid (99%) and ι-carrageenan were from Fluka Chemika (Buchs, Switzerland). Indomethacin, dexamethasone, naproxen and methysergide were generous gifts from Uriach S.A., Ciba–Geigy, Syntex Latino and Sandoz (Barcelona, Spain), respectively.

2.3. Animals

Female Wistar rats (130–170 g) and male Swiss CD-1 mice (23–28 g) were purchased from Letica and Panlab (Barcelona, Spain), respectively. Animals were fasted for 16 h, but freely allowed water.

2.4. Edema induced by several agents in rats

Rats were previously hydrated with 30 ml/kg water (p.o.). I.p. administration of HI-2 or reference drug was performed 30 min before subcutaneous injection of the phlogistic agent: ι-carrageenan (1% w/v in normal saline) (Winter et al., 1962), dextran (1% w/v in normal saline) (Merlos et al., 1990), arachidonic acid (0.5% w/v in carbonate buffer 0.2 M, pH 8.5) (DiMartino et al., 1987), zymosan A (1% w/v in normal saline) (Tayrare et al., 1989) or C_{16}-paf (10 μg/ml in 0.25% bovin serum albumin solution in normal saline) (Merlos et al., 1990). Paw volumes were measured using a water plethysmometer (Letica LI-7500) before and at 1, 2, 3, 4 and 5 h after induction of edema. The swelling rates at each time of measurement were calculated in relation to paw volume before injection of phlogogen. From these values, the areas under the curve (AUC) were obtained for each animal by trapezoidal approximation. The global inhibition ratios were obtained from AUC group averages. Naproxen (5 mg/kg), methysergide (1 mg/kg) and dexamethasone (1 mg/kg) were used as reference drugs.

2.5. Acetic acid-induced writhings in mice

The method described by Koster et al. (1957) was used in this experiment. HI-2 (50–100 mg/kg, i.p.) or indomethacin (2.5 mg/kg) was administered intraperitoneally 30 min before acetic acid injection (0.8%, 1 ml/100 g, i.p.). Mice were placed in individual cages. The number of abdominal contractions were observed 10 min after stimulation during a period of 10 min.

2.6. Ulcerogenic and irritant effect in rats

Procedures used here were those of Tsurumi et al. (1986) and Fairlie et al. (1987). A total of 4 h after intraperitoneal injection of HI-2 (50–100 mg/kg, aqueous solution), the peritoneal area was inspected for signs of local irritation, such as, fluid accumulation and membrane redness. Stomachs were removed and examined under dissecting microscope (× 20). The severity of gastric lesion

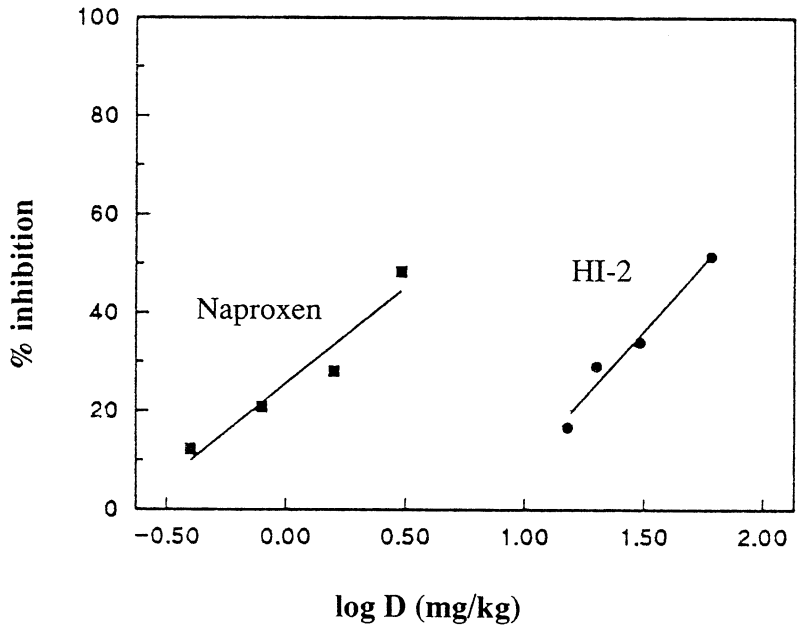

Fig. 1. Dose-effect lines of Naproxen (0.4–3 mg/kg, i.p.) and HI-2 (15–60 mg/kg, i.p.). The data points are means of at least six animals.

was expressed as ulcer index according to the following score: 0 (no lesion), 1 (erythema), 2 (pinpoint ulcers), 3 (small ulcers < 2 mm), 4 (larger ulcers > 2 mm) and 5 (perforation).

2.7. Statistical analysis

Numerical data were expressed as mean $\pm$ standard deviation (S.D.). Analysis of variance (ANOVA) and Sheffé test were used to assess edema-induced assays, whereas Mann–Whitney's non parametrical test was used in the analgesia assay.

2.8. Plant extracts

A total of 400 g dried flowers of *H. inuloides* was divided into four portions of 100 g. Each 100 g of plant material was initially extracted with boiling water (3 l). The extract was concentrated under reduced pressure < 40°C to 1 l. The concentrated aqueous extract was fractionated by successive extraction with ethyl ether (4×600 ml) and butanol (4×600 ml). An ethyl ether (HI-1, 1.2% w/w), a butanol (HI-2, 3.7%) and an aqueous fraction (HI-3, 10.1%) were obtained. Fractions were evaporated under reduced pressure < 40°C, to dryness, then reconstituted as an aqueous suspension and lyophilized. All doses are expressed in terms of fraction weight/body weight.

3. Results and discussions

Pharmacological assays were carried-out to ascertain the potential anti inflammatory activity of *H. inuloides*. The aqueous extract was evaluated using the carrageenan-induced edema test. A single dose of 100 mg/kg, i.p. produced 29.2% inhibition of inflammation ($P < 0.005$).

To purify the active principles, the components of the aqueous extract were separated on the basis of polarity. The biological assay of *H. inuloides* fractions, using carrageenan-induced edema test gave, at 100 mg/kg, i.p., the following values (% inhibition): HI-1, 19.9 (N.S.); HI-2, 58.0 ($P < 0.001$) and HI-3, 30.0 ($P < 0.02$). HI-2 was significantly more effective than HI-1 and HI-3. The

Table 1
Effect of HI-2 and standard drugs (i.p.) on edema induced by several agents

	Treatment	Dose (mg/kg)	Edema (AUC+S.D.)	Inhibition (%)
Carrageenan	HI-2	0	275 ± 35	—
		50	142 ± 30	47.3***
		100	117 ± 42	58.0***
	Naproxen	0	271 ± 36	—
		5	141 ± 36	47.9***
Dextran	HI-2	0	301 ± 42	—
		50	184 ± 36	38.9*
		100	96 ± 29	68.1 ***
	Methysergide	0	249 ± 45	—
		1	108 ± 8	56.7***
Zymosan A	HI-2	0	306 ± 36	—
		50	304 ± 16	—
		100	303 ± 52	—
	Methysergide	0	333 ± 38	—
		1	182 ± 27	45.2***
C_{16}-paf	HI-2	0	157 ± 23	—
		50	126 ± 12	19.4
		100	137 ± 25	12.7
	Dexamethasone	0	171 ± 24	—
		1	125 ± 15	26.7**
Arachidonic acid	HI-2	0	178 ± 23	—
		50	170 ± 18	4.6
		100	119 ± 24	33.4**
	Dexamethasone	0	202 ± 29	—
		1	149 ± 15	26.1*

Data show edema expressed as AUC (mean + S.D.) induced by carrageenan, dextran, zymosan A (l mg/paw), C_{16}-paf (l μg/paw) and arachidonic acid (0.5 mg/kg), and the percent inhibition of inflammation.
The mean values are based on six animals.* $P<0.025$, ** $P<0.005$, *** $P<0.001$ from respective control group (ANOVA and Scheffé test).

dose-effect curve of HI-2 was obtained (Fig. 1) and the ED_{50} calculated value was 29.7 (22.5–39.2) mg/kg.

Peritoneal area was inspected 4 h after administration of 100 mg/kg of HI-2. No animal showed accumulation of exudated liquid or other irritation signs. Thus, the anti-inflammatory action of *H. inuloides* does not seem to be due to an irritating effect at the injection site.

The carrageenan paw edema test produce a non-specific inflammation that results from the sequential action of several mediators. Non-steroidal antiinflammatory drugs (NSAID) show a marked inhibition of carrageenan-induced edema (Burch and DeHaas, 1990).

The anti-inflammatory activity of HI-2 was also evaluated by other phlogistic agent-induced edema tests. At 100 mg/kg, HI-2 markedly inhibited inflammation produced by dextran (68.1% inhibition) and weakly decreased arachidonic acid-induced edema (33.4%). At 50 mg/kg, HI-2 significantly reduced dextran swelling (38.9%). No effect was observed, at the same doses, on zymosan or paf-induced edema (Table 1). It is reported that histamine and 5-OH-tryptamine are the main mediators of dextran-induced inflammation (Nishida and Tomizawa, 1980).

Acetic acid-induced writhings test is a widely used method for evaluation of peripheral anti-nociceptive activity. On this model, HI-2 markedly reduced the number of mouse abdominal contrac-

Table 2
Analgesic effect of HI-2[a] and indomethacin[b] on acetic acid-induced writhing test in mice

Treatment	Dose (mg/kg)	No. of writhings	% Inhibition
HI-2	0	28.0 ± 7.4	—
	50	7.3 ± 5.9	73.8***
	100	6.1 ± 6.2	78.2***
Indomethacin	0	36.5 ± 7.2	—
	2.5	9.9 ± 9.1	72.6***

Data show the number of writhings (mean ± S.D.) produced between 10 and 20 min after the administration of acetic acid (0.8% 1 ml/100 g) and the percent inhibition for each group ($n = 8$).
[a] 50 and 100 mg/kg, i.p.
[b] 2.5 mg/kg, i.p.
*** $P < 0.001$.

tions (% of inhibition): 73.8 at 50 mg/kg and 78.2 at 100 mg/kg (Table 2). The analgesic effect showed by HI-2 at 50 mg/kg is similar to that produced by 2.5 mg/kg of indomethacin.

The gastric mucosa was examined after intraperitoneal administration of HI-2. At active doses, it induced gastric erythema. Table 3 shows that ulcerogenic index after the treatment was 0.5 ± 0.5 at 50 mg/kg and 1.2 ± 0.4 at 100 mg/kg. As HI-2 was intraperitoneally injected, the gastric erythema induced by HI-2 cannot be produced by a direct effect on the mucosa. The ulcerogenic potential is a well-known side effect of NSAID and it is accepted that it is mediated via inhibition of prostaglandin synthesis (Vane and Botting, 1990).

The effect of HI-2 on carrageenan paw edema test and on gastric mucosa suggests that the inhibition of prostaglandin biosynthesis may be involved in the anti-inflammatory activity of *H. inuloides*. Besides, the ability of HI-2 to inhibit dextran-induced swelling indicates that other mechanisms may also take part.

In summary, the results show the marked and dose-related anti-inflammatory activity of fraction HI-2 of *H. inuloides*. Their active principles specifically inhibit certain chemically-induced edemas in rats. Moreover, HI-2 shows analgesic activity on acetic acid-induced writhings test. The chemical study of HI-2 is at present in progress.

Acknowledgements

We thank Mrs E. Linares (UNAM, Mexico) for the botanical identification of *H. inuloides* and Mr R. Rycroft (Linguistic Advice Service of University of Barcelona) for his assistance. This study was supported by the Spanish Comision Interministerial de Ciencia y Tecnologia (CICYT, FAR 90-0501 and project SAF, 1992/0814) and the Programa Iberoamericano de Ciencia y Tecnologia para el Desarrollo (CYTED, project X-1).

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Table 3
Ulcerogenic effect of HI-2[a]

Treatment	Dose (mg/kg)	Ulcer incidence	Ulcer index (mean ± S.D.)
HI-2	0	0/6	0
	50	0/6	0.5 ± 0.5
	100	1/6	1.2 ± 0.4

Results show ulcer incidence and ulcer index (0, no lesion; 1, erythema; 2, pinpoint ulcers; 3, small ulcers <2 mm; 4, larger ulcers >2 mm; and 5, perforation) expressed as mean ± S.D. 4 h after treatment ($n = 6$).
[a] 50 and 100 mg/kg, i.p.

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