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The antiinflammatory activity of *Icacina trichantha* tuber*

I. U. Asuzu[1], S. Sosa[2] and R. Della Loggia[2]

[1]Department of Veterinary Physiology and Pharmacology, University of Nigeria, Nsukka, Nigeria
[2]DEMREP, University of Trieste, Trieste, Italy

Summary

Five fractions (hexane, chloroform, ethylacetate, methanol and water) of *Icacina trichantha* tuber were obtained by gradient solvent extraction and tested for their ability to inhibit the Croton oil-induced ear edema in mice. The most active fraction was the chloroform one which significantly inhibited ear edema in a dose-dependent manner, showing an ID_{50} (dose giving 50% edema inhibition) of 107 µg/cm^2. The ID_{50} of the reference drug indomethacin was 93 µg/cm^2. The chloroform fraction significantly reduced also the carrageenin-induced paw edema in rats, after oral adiminstration: 50, 100 or 200 mg/kg of the fraction reduced the global edematous response by 15, 20 or 34 %, whereas 10 mg/kg of indomethacin induced 40% inhibition.

Key words: *Icacina trichantha,* tuber extract, inflammation, Croton oil, carrageenin.

Introduction

The ethanol extract of *Icacina trichantha* Pflamzenfam (Icacinaceae) (Dalziel, 1937) has for many years been a major handy household medicine in many homes in Nigeria (Asuzu and Abubakar, 1995a). It is used for treating cases of poisoning, constipation and "yellowness of the eye" which is commonly associated with malaria and hepatitis (Asuzu and Ugwueze, 1990; Asuzu and Abubakar, 1995a). Asuzu and Abubakar (1995a) observed that the crude methanol extract of the tuber produced significant central nervous system-depressant, analgesic and local anaesthetic effects in laboratory animals (Asuzu and Abubakar, 1995a). Further studies showed that the extract significantly protected the liver and the kidneys of carbon tetrachloride-poisoned rats (Asuzu and Abubakar, 1995b). This effect was suspected to be associated with a free radical scavenging activity of the extract, an action possessed by several oriental plant preparations as well as by flavonoids (Robak and Griglewski, 1988; Chen et al., 1990; Saija et al., 1995). Although the chemical composition and the flavonoid presence in *I. trichantha* tuber are not known, scavenging of free radicals, central nervous system depression and analgesia are characteristic effects of many antiinflammatory agents. Therefore, the antiphlogistic properties of *I. trichantha* tuber were investigated. To this aim, the plant material was extracted by solvents of different polarities and the extraction products were evaluated for their topical antiinflammatory activity, measured as inhibition of the Croton oil-induced ear edema in mice (Tubaro et al., 1985). The most active fraction was tested also for its effect on the carrageenin-induced paw edema in rats, after oral administration (Winter et al., 1962). As reference, the non steroidal antiinflammatory drug (NSAID) indomethacin was used.

Materials and Methods

Animals

Male CD-1 Albino Swiss mice (28–32 g) were purchased from Charles River (Milano, Italy). Male Sprague-Dawley rats (110–130 g) were obtained from Harlan-Italy (S. Pietro al Natisone, Italy).

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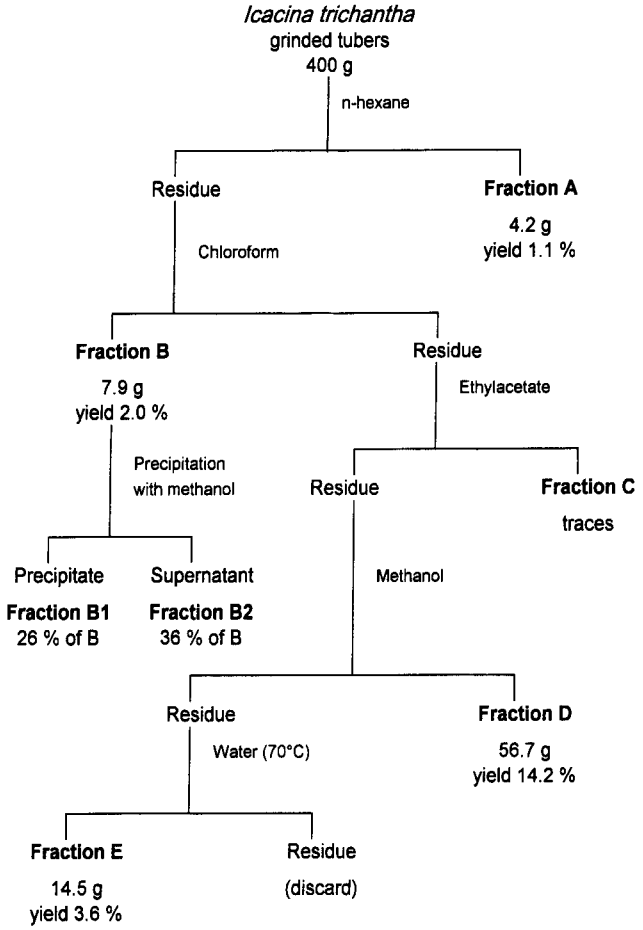

Fig. 1. Extraction procedure of *Icacina trichantha* tuber (Fraction A = hexane fraction; Fraction B = chloroform fraction; Fraction D = methanol fraction; Fraction E = water fraction).

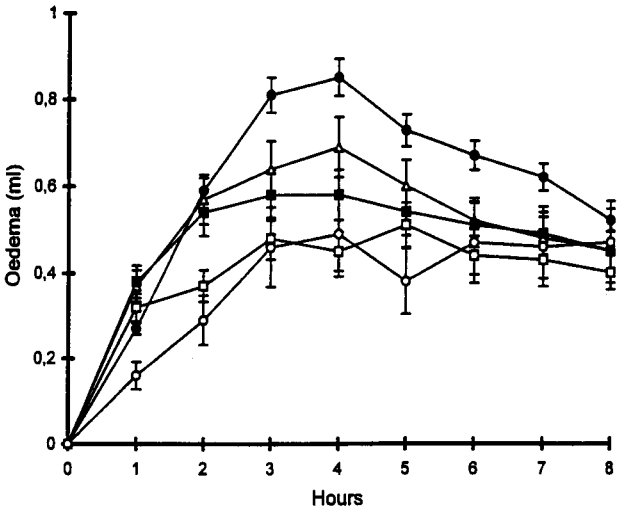

Fig. 2. The effect of fraction B of *Icacina trichantha* tuber on the carrageenin-induced paw edema in rats (● carboxymethylcellulose 10 ml/kg p.o.; Δ Fraction B, 50 mg/kg p.o.; ■ Fraction B, 100 mg/kg p.o.; □ Fraction B, 200 mg/kg p.o.; ○ Indomethacin, 10 mg/kg p.o.).

Chemicals and drugs

The following solvents (Analar grade) were used: *n*-hexane, chloroform, ethylacetate and methanol (Carlo Erba, Milano, Italy). Indomethacin, Croton oil and λ-carrageenin were obtained from Sigma (St. Louis, Missouri, USA).

Plant collection and extraction

Fresh tubers of *Icacina trichantha* were collected from Nsukka in Enugu State (Nigeria) in July, 1995 and identified in the Department of Botany, University of Nigeria, Nsukka by Mr. A. Ozioko. The tubers were washed, sliced into small bits and dried under mild sunshine. The dried tuber slices were reduced to a coarse powder in a mortar.

A preliminary extraction of the dried tuber was performed by stirring 50 g of plant material with 500 ml of aqueous ethanol (70% v/v) over night, at room temperature (22 °C).

A subsequent extraction of *I. trichantha* tuber (400 g) was performed by successive use of solvents of increasing polarity in a soxhlet at 55–70 °C for 3 h. After each extraction step, the solvent was evaporated *in vacuo* by Rotavapor (Büchi, Flawil, Switzerland), obtaining hexane (A), chloroform (B), ethylacetate (C), methanol (D) and water (E) fractions. The fraction B was further separated into fractions B1 and B2 by precipitation of its chloroformic solution with methanol (Fig. 1).

High performance liquid chromatography fingerprint analysis

High performance liquid chromatography (HPLC) analysis was carried out on a Knauer instrument equipped with a DAD Gynkotek UVD S detector and a Vertex Eurospher 100 C-18 column (250 x 4.6 mm; 5 µm). The mobile phase consisted of two solvent systems: A (90% (v/v) 0.45 N phosphoric acid and 10% (v/v) acetonitrile) and B (90% (v/v) acetonitrile and 10% (v/v) 0.45 N phosphoric acid). The gradient elution profile was: 0 min, 0% B; 25 min, 12.5% B; 45 min, 20% B; 55 min, 26% B; 75 min, 60% B; 97 min, 100% B. The column temperature was 22–24 °C with a flow rate of 1.0 ml/min; detection was set at 200, 288 or 340 nm. The injected volume of the sample was 20 µl.

Croton oil-induced ear edema in mice

Cutaneous inflammation was induced on the inner surface of the right ear (surface: about 1 cm^2) of anaesthetized mice (145 mg/kg ketamine hydrochloride, intraperitoneally) by Croton oil application (75 µg) (Tubaro et al., 1985). The substances under testing were applied together with the irritant. Six hours after the induction

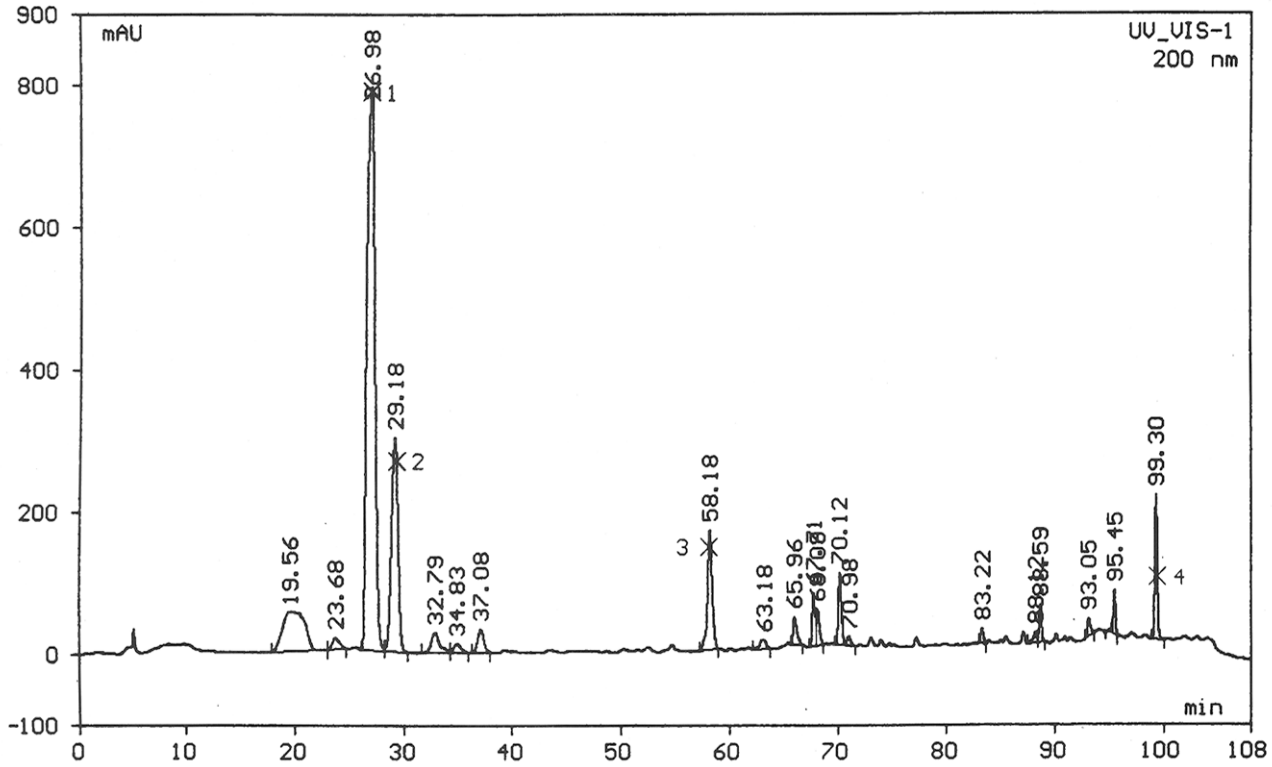

Fig. 3. HPLC profile of the fraction B of *I. trichantha* tuber (detection: 200 nm).

of the inflammation, mice were killed by cervical dislocation and a plug (6 mm ∅) was taken from both the treated and the untreated ears. The edematous response was quantified as weight difference between the two ear plugs. The antiinflammatory activity was evaluated as percent edema reduction in the animals treated with the substances under testing with respect to control animals, treated with the irritant alone (Tubaro et al., 1985).

Carrageenin-induced paw edema in rats

The paw edema was induced in the plantar region of the rat hind paw (Winter et al., 1962). The tested substances, suspended in 1% carboxymethylcellulose, were administered to rats by gastric gavage in a volume of 10 ml/kg body weight. One hour later, the phlogistic agent (100 µl of 0.6% λ-carrageenin in 0.9% sodium chloride) was injected intradermally in the plantar region of the right hind paw.

Edema formation was quantified as foot volume increase and measured by water displacement using a plethysmometer (Ugo Basile, Comerio-Varese, Italy), at 1 hour intervals for 8 hours after carrageenin injection. The antiinflammatory activity was calculated at each time of observation as percent inhibition of edema in the animals treated with the substances under testing in comparison to control animals. The gobal effect on the whole edematous response was quantified as percent reduction of the areas under the edema curves (AUC) till 8 hours.

Statistical analysis

The pharmacological data were analyzed by the Student's *t*-test and significance was assumed at $p < 0.05$.

Results

Extraction of plant material

The preliminary extraction of *I. trichantha* tuber (50 g) with 70% aqueous ethanol yielded 4.1 g of hydroalcoholic extract. A second extraction procedure of the plant material, carried out using different solvents, gave seven fractions which yields are reported in the flow chart (Fig. 1).

Croton oil-induced ear edema in mice

A preliminary evaluation of the topical antiinflammatory activity of *I. trichantha* was performed using the hydroalcoholic extract of the tuber. The extract, applied at a high dose (2000 µg/cm^2), induced a mild but significant edema inhibition (32%) whereas 90 µg/cm^2 of indomethacin provoked 47% reduction of the edematous response (Table 1).

Table 1. Topical antiinflammatory activity of the hydroalcoholic extract.

Substance	Dose ($\mu g/cm^2$)	N°. animals	Edema (mg) mean ± S. E.	% Inhibition
Controls	–	7	7.3 ± 0.4	–
Hydroalcoholic extr.	2000	7	5.0 ± 0.4*	32
Indomethacin	90	7	3.9 ± 0.3*	47

*$p < 0.05$ at the Student's *t*-test.

Table 2. Topical antiinflammatory activity of the main fractions.

Substance	Dose ($\mu g/cm^2$)	N°. animals	Edema (mg) mean ± S.E.	% Inhibition
Controls	–	7	7.2 ± 0.4	–
Fraction A	1000	7	4.6 ± 0.3*	36
Fraction B	1000	7	0.2 ± 0.1*	97
Fraction D	1000	7	4.6 ± 0.6*	36
Fraction E	1000	7	4.4 ± 0.5*	39

*$p < 0.05$ at the Student's *t*-test.

Table 3. Topical antiinflammatory activity of fraction B.

Substance	Dose ($\mu g/cm^2$)	N°. animals	Edema (mg) mean ± S.E.	% Inhibition
Fraction B	0	10	6.9 ± 0.3	–
	25	10	6.3 ± 0.3	9
	50	10	6.0 ± 0.3*	13
	100	10	3.6 ± 0.7*	48
	200	10	1.7 ± 0.4*	75
	400	10	0.4 ± 0.3*	94
	800	10	0.2 ± 0.2*	97
Indomethacin	0	10	6.5 ± 0.2	–
	45	10	4.6 ± 0.4*	29
	90	10	3.5 ± 0.4*	46
	180	10	1.5 ± 0.2*	77

*$p < 0.05$ at the Student's *t*-test.

The subsequent evaluation of the antiinflammatory activity of the plant tuber was carried considering fractions A, B, D and E, applied at the dose of 1000 $\mu g/cm^2$. Fraction C was not tested because of its insufficient amount for the biological assay. All the tested fractions showed significant antiinflammatory activity but, whereas fractions A, D and E reduced the edematous response by about 36–39%, fraction B (chloroform fraction) provoked 97% inhibition, being the most active (Table 2).

Further investigation of the fraction B showed it possesses a dose-dependent antiinflammatory activity: already 50 $\mu g/cm^2$ of the fraction induced 13% edema reduction and more than 90% inhibition was reached at the dose of 400 $\mu g/cm^2$. As reference, indomethacin induced about 29% reduction of the edematous response at the dose of 45 $\mu g/cm^2$ and reached 77% inhibition at 180 $\mu g/cm^2$ (Table 3). The ID_{50} values (dose giving 50% edema inhibition) of fraction B and indomethacin, calculated from their dose-effect relationship, were 107 and 93 $\mu g/cm^2$, respectively. Therefore, fraction B possesses an antiinflammatory potency similar to that of the NSAID.

Fractions B_1 and B_2 were separated from fraction B in a sufficient amount to verify their pharmacological activity after topical application. The obtained results are reported in Table 4. Both the fractions significantly inhibited edema formation, being fraction B_1 the most active. In fact, whereas 200 $\mu g/cm^2$ of fraction B_1 induced 87% edema inhibition, the same dose of fraction B_2 reduced the edematous response only by 39%. Furthermore, the application of a double dose of fraction B_1 almost completely counteracted the edema process. Furthermore, since fraction B_1 represents 26% of fraction

Table 4. Topical antiinflammatory activity of fractions B_1 and B_2.

Substance	Dose ($\mu g/cm^2$)	N°. animals	Edema (mg) mean ± S.E.	% Inhibition
Controls	–	10	7.1 ± 0.4	–
Fraction B_1	200	10	0.9 ± 0.2*	87
	400	10	0.2 ± 0.1*	97
Fraction B_2	200	10	4.3 ± 0.7*	39

*$p < 0.05$ at the Student's *t*-test.

Table 5. Effect of fraction B on the global edematous response induced by carrageenin in the rat paw.

Substance	Dose ($\mu g/cm^2$)	N°. animals	AUC (ml x h) mean ± S.E.	% Inhibition
Controls	–	6	4.81 ± 0.19	–
Fraction B	50	6	4.09 ± 0.25*	15
	100	6	3.83 ± 0.31*	20
	200	6	3.19 ± 0.30*	34
Indomethacin	10	6	2.90 ± 0.29*	40

*$p < 0.05$ at the Student's *t*-test.

B (Fig. 1), it gives the most important contribution to the activity of fraction B. On the contrary, the role of fraction B_2 is of minor relevance, even if it represents 36% of fraction B.

Carrageenin-induced paw edema in rats

Fraction B was obtained in sufficient amounts to evaluate its ability to inhibit the carrageenin-induced paw edema in rats, after oral administration. It provoked a significant dose-dependent inhibition of the edematous response (Fig. 2). At the time of the maximal edematous response (4 h after the induction of inflammation) 23% edema inhibition was observed at the lower administered dose (50 mg/kg), whereas the highest dose (200 mg/kg) reduced the edema formation by 47%. As reference, indomethacin (10 mg/kg) induced 43% inhibition of the edematous response.

In order to evaluate the overall effect of the tested substances on the edema formation, the areas under the edema curves (AUC) reported in Fig. 2 were calculated. Fraction B, at the doses of 50, 100 or 200 mg/kg, induced a global edema reduction of 15, 20 or 34%, whereas indomethacin provoked about 40% inhibition (Table 5).

HPLC fingerprint of fractions B and B_1

Fig. 3 shows the HPLC fingerprint chromatogram of fraction B detected at 200 nm. About twenty peaks with retention times between 19.56 and 99.30 min are evident. The most significant is the peak with a retention time of 26.98 min. This peak is particularly evident also in the chromatogram of fraction B_1, together with other four less pronounced peaks (data not shown). Therefore, this significant peak could correspond to the main component involved in the antiinflammatory activity of fractions B and B_1 as well as of *I. trichantha* tuber.

Discussion

The obtained results indicate that *Icacina trichantha* tuber possess significant antiinflammatory properties both after topical and oral administration. Indeed, the hydroalcoholic extract and the tested fractions inhibited the Croton oil-induced ear edema in mice, after topical application, the chloroform fraction being the most active. The extraction of the antiinflammatory principles by chloroform suggests they should be of lipophilic nature. This characteristic enabled them to cross the skin barrier and to exert their antiphlogistic effect. Moreover, the active components of the chloroform fraction possess a strong antiinflammatory potency since the dose-dependent effect induced by the fraction was comparable to that of the NSAID indomethacin. Therefore, the chloroform fraction of *I. trichantha* tuber could be proposed as a possible antiinflammatory agent for topical use.

The chloroform fraction significantly inhibited also the carrageenin-induced paw edema in rats after oral administration. The effect after systemic rout of administration was dose-dependent and long lasting, suggesting a possible use of the fraction not only in the treatment of skin diseases but also in that of other inflam-

matory-based diseases. The potency exhibited by the fraction after oral administration shows also that the active components are stable and not destroyed during the "first pass effect" in the liver. This explains why the natives who drink alcohol extracts of *I. trichantha* tuber get relief from pain and other symptoms.

In a previous study, an *I. trichantha* extract was observed to exhibit significant analgesic effect (Asuzu and Abubakar, 1995a) implying that it is an antiinflammatory analgesic, like aspirin or indomethacin, which block the cyclooxygenase pathway of arachidonic acid metabolism (Ferreira et al., 1978; Malmberg and Yaksh, 1992). The mechanism by which the extract inhibits inflammation is not yet known, but a free radical scavenging activity could be hypothesized. In fact, previous observations with the *I. trichantha* extract revealed liver protective effects in carbon tetrachloride-poisoned rats that could be due to free radical scavenging effects (Asuzu and Abubakar, 1995b).

In conclusion, the extracts of *Icacina trichantha* tuber exhibited significant antiinflammatory properties after topical or oral administration, confirming the validity of the local use of *I. trichantha* tuber for medicinal purposes. However, further studies have to be done to identify the active component(s) of the plant and to determine their mechanism(s) of action.

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Address

R. Della Loggia, DEMREP, University of Trieste, Via A. Valerio 6, 34100 Trieste, Italy.
Tel.: ++39-040-676 3535; Fax: ++39-040-676 3215;
e-mail: dellalo@univ.trieste.it.