Rev. Biol. Trop., 47(3): 365-371, 1999
www.ucr.ac.cr. www.ots.ac.cr www.ots.duke.edu

Anti-inflammatory activity of *Urera baccifera* (Urticaceae) in Sprague-Dawley rats

Beatriz Badilla[1], Gerardo Mora[2], Antonio José Lapa[3] and Jose Arthur Silva Emim[3]

1 Instituto de Investigación en Ciencias Farmacéuticas (INIFAR), Facultad de Farmacia.Universidad de Costa Rica. 2060 San José, Costa Rica. Fax (506) 225-35-74. E-mail: bbadilla@cariari.ucr.ac.cr

2 Centro de Investigación en Productos Naturales (CIPRONA) y Facultad de Farmacia Universidad de Costa Rica. E-mail: gamora@cariari.ucr.ac.cr

3 Instituto Nacional de Farmacología (INFAR), Escola Paulista de Medicina, UNIFESP, Sao Paulo, Brasil.

Received 8-VI-1998. Corrected 09-III-1999. Accepted 17-III-1999.

Abstract: On a preliminary test, anti-inflammatory and analgesic dose-related activities on rats were observed for the aqueous fraction of *Urera baccifera*; this extract was bioassay-guided fractionated and the final aqueous fraction was used according the ethnobotanical use. Carrageenan-induced edema (n=6), was used as an assay in the fractionating process. The anti-inflammatory and antinociceptive properties of the final aqueous fraction were studied using *in vivo* models. For the anti-inflammatory activity rat paw edema (n=6), pleurisy induced by carrageenan (n=6) and ear edema induced by topical croton oil (n=6) models were used, and tail-flick test (n=6), abdominal constrictions induced by acetic acid (n=6), and formalin test (n=6), were used for the antinociceptive activity. The tests performed showed an inhibition effect on leukocyte migration, and a reduction on pleural exudate, as well as dose-dependant peripheral analgesic activity, at a range of 25-100 mg/kg i.p. The final aqueous fraction contains most of the anti-inflammatory activity of the plant *U. baccifera*. A possible mechanism of action is discussed and based on the results we conclude that this plant has a potential for both anti-inflammatory and analgesic activity at the clinical level.

Key words: *Urera baccifera*, anti-inflammatory agent, analgesic, antinociceptive, rat paw edema, Urticaceae.

Urera baccifera Gaud (Urticaceae) is known in Costa Rica as "Chichicaste" or "Ortiga brava" and is popularly used for a variety of inflammatory conditions. Information obtained from local communities shows that people use the leaves as an herbal remedy both orally as an infusion and topically applied (J. Poveda 1995 pers. comm.). Costa Rican Amerindians, when crossing high mountains, chastise themselves with the spiny stems to ward off chills; the rubefacient effect is also employed on rheumatic pains (Morton 1981).

The leaves and stems of *Urera baccifera* are covered with stinging hairs, which is why they are commonly named "ortigas" (nettles). Nettles are multipurpose medicinal plants and are often included in naturalistic remedies (Hoffman *et al.* 1992)

In spite of extensive literature search, it was not possible to find any reference concerning the pharmacological actions of this plant. In a preliminary work, an important anti-inflammatory activity was found for the leaves when administered intraperitoneally (i.p.) or subcutaneously (s.c.), but not for the oral administration (p.o.). The present study was, therefore, undertaken to work out the fraction that exerts the anti-inflammatory action and to elucidate its mechanism of action.

MATERIALS AND METHODS

Plant material: *Urera baccifera* was identified at source by Luis Poveda, School of Biology, Universidad Nacional (Heredia, Costa Rica) and a voucher specimen (JVR 6997) was deposited in the Universidad Nacional Herbarium.

The leaves of *U. baccifera* were collected in Guápiles (Limón, Costa Rica) in February 1995. Leaves were chopped and dried for 3 days at about 40 °C. A decoction was prepared in the following proportional manner: 10 g of dried leaves were warmed in 100 ml of distilled water for 30 min at 70 °C. The solution was filtered using a paper filter, rotavapor-concentrated and freeze-dried to obtain the raw aqueous extract (RA) with a yield of 9% on the basis of dry plant material. 14.3 g of RA were dissolved in 100 ml of distilled water and successively extracted with hexane, ethyl acetate, and butanol to yield the hexane fraction (H) (0.34%), the ethyl acetate fraction (EA) (0.42%), and the butanol fraction (B) (4.19%). The residue of this extraction (intermediate aqueous extract, IA) was freeze-dried to a yield of 94.4% on the basis of RA, resuspended on distilled water and filtered through a column of Diaion HP-20 (10 g; 2 cm i.d.) using 50 ml of distilled water and then 50 ml of methanol to give two fractions: a final aqueous fraction (FA) (88.3% of IA) and a methanol fraction (M) (8.8% of IA) .TLC on silica gel (Merck Alufolio) using EtoAc/IPA-/H_2O (6:5:1) or CH_3CN/H_2O (17:3), showed only one spot.

Experimental animals: The animals used were adult male Sprague-Dawley rats (*Rattus norvegicus)* with a corporal weight from 180 g to 220 g, supplied by the Animal Care Unit of the University of Costa Rica, and adult male mice (*Mus musculus*) with corporal weight from 25 g to 35 g supplied by the Pharmacology Department of Escola Paulista de Medicina, Sao Paulo, Brazil. They had free access to food and water and were kept on a 12/12 h light/dark cycle. Before each study, animals were submitted to fasting for at least 12 h.

Drugs and chemicals: Indomethacin, sodium chloride, sulfuric ether, butanol, acetonitrile and sodium bicarbonate, acetic acid (Merck), lambda-carrageenan (Sigma), heparin (Liquemine™ Roche), phentanile (Fentanyl™, Janssen), croton oil (Veado d'Ouro, Sao Paulo , Brazil), dexamethasone (Merck Sharp and Dohme), formalin (Sigma).

Rat paw edema: The anti-edematogenic properties of the fractions were studied using the carrageenan-induced edema model. Groups of six rats were used. Animals were given a saline solution, indomethacin (10 mg/kg intraperitoneally) or a dose of a selected fraction, 1 h before administration of an intradermal injection of carrageenan (0.1 ml of a 1% solution in 0.9% saline solution) into the plantar region of the right hind paw. The contralateral paw was injected with 0.1 ml saline solution. The group treated with the plant extract was injected with 500 mg/kg i.p. or an equivalent dose dissolved in normal saline solution according to the fraction used. The paw volume was measured before the injection and each hour after for a period of 6 h by means of volume displacement methods (Winter *et al.* 1962 ; Di Rosa *et al.* 1971). The difference between the left paw and the right paw volumes indicated the degree of inflammation. The average increase in paw volume of each group was calculated and compared with the control (saline) and the indomethacin groups.

Pleurisy induced by carrageenan: Seven groups of six-animals were used. The rats of the control group were pretreated with the vehicle (0.9% saline solution i.m. or sc.). The experimental groups were pretreated with FA in 0.9% saline solution (25 and 50 mg/kg i.p and 50, 150 and 300 mg/kg s.c.). Thirty min later all animals received an intrapleural injection of 0.25 ml carrageenan (0.2% in saline solution) on the right intrapleural conty. The animals were anaesthetized 3 h later using ether and bled through the portal vein. The pleural exudate was collected and the pleural cavity washed with

1.0 ml saline containing heparin (10 IU/ ml) (Vinegar *et al.* 1973). The number of migrating leukocytes in the exudate was determined with a Newbauer chamber.

Ear edema induced by topical croton oil: Three groups of six mice were used. One group was topically treated 1 h before, on the inner surface of the right ear with 20 ml of FA (100 mg/ml in acetone). Another group of mice was pretreated with 20 µl of dexametasone (4 mg/ml in saline). The third group was pretreated with 20 µl of saline solution. The left ears of all mice were treated with the corresponding solvents (Schiantarelli *et al.* 1982).

Mice were treated then with 20 µl of a fresh solution of croton oil (2.5% v/v in acetone) on the inner surface of their right ear. The left ears were treated with 20 µl of acetone. After 3 h of the administration of croton oil, animals were killed by cervical dislocation and 6 mm circles of the ear tissue were collected and weighed. The results are expressed as the mean weight difference between tissue circles of croton treated ears, and acetone treated ears.

Tail-flick test: The antinociceptive effect was determined in mice using the tail-flick test by caloric focus. The responses were elicited every 30 min the hour before, and 2 h after treatment, with either the fraction under study or the vehicle. Four experimental groups were used. Mice treated with phentanyl (400 µg/kg, s.c.) and saline solution were employed as controls. The experimental groups were treated with FA (25 and 50 mg/kg i.p.) (Jansen *et al.*1963, Riesterer and Jaques 1968, Emim *et al.* 1994).

Abdominal contractions induced by acetic acid: Abdominal muscle contractions were induced by 1% acetic acid solution (0.1 ml /10 g, i.p.) in mice pretreated with saline solution or one of the test samples (Koster *et al.* 1959). The number of abdominal writhings was measured over 30 min at 5 min intervals, and animals treated with indomethacin (10 mg/kg, i.p.) were used as positive controls.

Formalin test: Five groups of six-animals were used. Each group of mice was treated with FA (12.5, 25 or 50 mg/kg i.p. in saline solution), saline solution (0.9% i.p.) or indomethacin (10 mg/kg i.p.). One hour after treatment all animals were injected with 20 µl of a 1% formalin in saline solution into the plantar surface of the left hind paw. Licking time was measured over 30 min divided into two phases. The first phase was from time zero to five min after formalin injection and the second phase was from 20 min to 30 min after formalin injection.

Statistical analysis: The data are expressed as mean ± S.E.M. and the Student's "t" test was used for comparison of the data of the control and standard groups. Probabilities of < 0.05 were considered.

RESULTS

Rat paw edema : The intraplantar injection of the hind paw induced a progressive edema reaching its maximum at 3 h. Animals treated with IA (472.3 mg/kg i.p.) showed edema inhibition in all phases of experimental model and differing from the control group. Animals treated with B (29 mg/kg i.p.) and EA (2.52 mg/kg i.p.) showed edema inhibition only in the early phase; in later phases they were similar to the control group (Fig.1). Subsequently IA was fractionated and animals treated with the fraction M (8.6 mg/kg i.p.), showed no effect. Fraction FA (49.05 mg/kg i.p.) reduced the paw edema in a significantly different manner from the control group (Fig. 2). FA was then used for subsequent tests in order to elucidate its mechanism of action.

Pleurisy induced by carrageenan: The volume of the pleural exudate in subcutaneously vehicle-treated rats was 0.60 ± 0.08 ml and the leukocyte count was (9.60 ± 1.34) x 10^6 /ml cells. The volume of the pleural exudate in intraperitoneal vehicle-treated rats was 0.80 ± 0.07 ml and the leukocyte count was (9.86 ± 0.59) x 10^6 /ml cells. Treatment with subcutaneous FA (50, 150 and 300 mg/kg) did

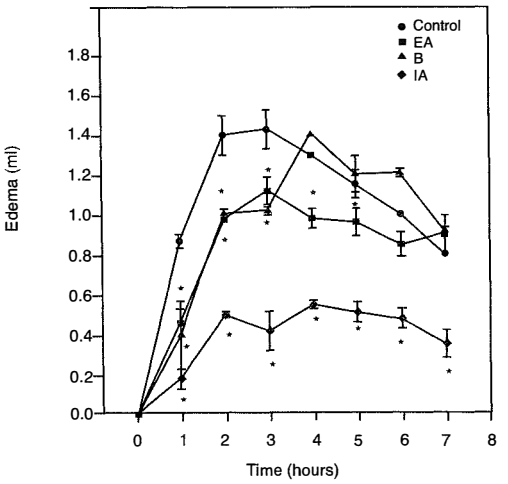

Fig.1. Anti-inflammatory activity. Effect of indomethacine pretreatment (10 mg/kg i.p.) , IA (472.3 mg/kg i.p.), EA (2.52 mg/kg i.p.) and B (29 mg/kg i.p.) compared with the saline control in the rat paw edema induced by carrageenan. Points are means ± S.E.M. (six animals) of the volume difference between the paws injected with phlogistic agent and the contralateral paw injected with normal saline.* p< 0.05

not modify the exudate volume; only the leukocyte count was modified at doses of 150 and 300 mg/kg (6.50 ± 0.60 x 10^6 /ml and 5.46 ± 0.48 x 10^6 /ml respectively) (Fig.3). Treatment with FA intraperitoneally (25 and 50 mg/kg) decreased both the pleural exudate (0.42± 0.07 ml and 0.37 ± 0.07 ml respectively) and leukocyte migration [(5.34 ± 0.91) x 10^6 /ml and (2.50 ± 0.60)x 10^6 /ml respectively] (Fig.4).

Ear edema induced by topical croton oil: The topical treatment with FA (20 µl, 100 mg/ml) did not significantly reduce the auricular edema induced by topical croton oil (70.7 ± 13.4 mg). Dexamethasone (20 µl, 4 mg/ml), (positive control) produced a significant reduction of the edema (30.8 ± 6.4 mg).

Tail flick test: Animals pretreated with fraction FA (25 and 50 mg/kg i.p.) did not show any response different from the control group. In the same conditions as above, phentanyl treated animals showed significantly increased reaction time as compared to the control group.

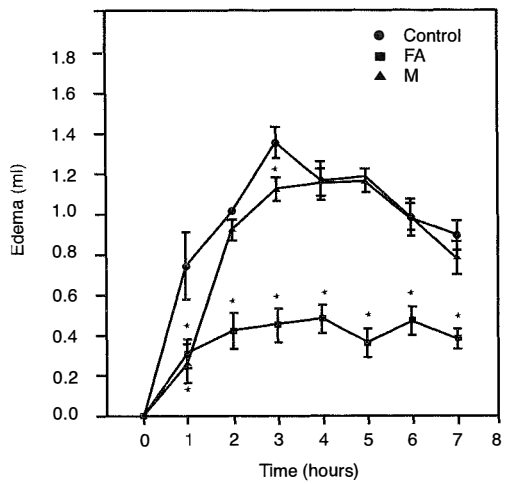

Fig.2. Anti-inflammatory activity Effect of indomethacine pretreatment (10 mg/kg i.p.) , FA (49.05 mg/kg i.p.) and M (8.6 mg/kg i.p.) compared with saline (control), on the rat paw edema induced by carrageenan. Points are means ± S.E.M. (six animals) of the volume difference between the paws injected with phlogistic agent and the contralateral paw injected with normal saline. *p< 0.05

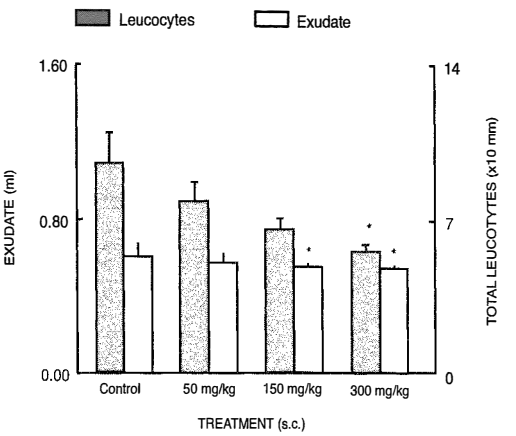

Fig.3. Effects of s.c. FA on pleurisy induced by carrageenan. Volume (ml) of the pleural exudate and leukocyte count in pleurisy induced by carrageenan (0.25 ml, 1% in saline sol. 0.9%) in rats previously treated with normal saline solution 0.9% s.c. (control) and FA (50,150, 300 mg/kg s.c.). The columns represent the mean values ± S.E.M. from six animals. *p< 0.05

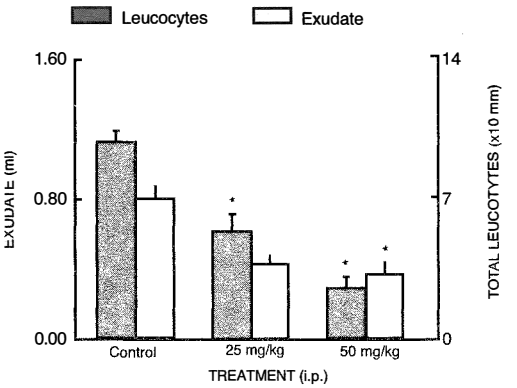

Fig.4. Effects of i.p. FA on pleurisy induced by carrageenan. Volume (ml) of the pleural exudate and leukocyte count in pleurisy induced by carrageenan (0.25 ml, 1% in saline sol. 0.9%) in rats previously treated with saline solution 0.9% s.c. (control) and FA (25 and 50 mg/kg i.p.). The columns represent the mean values ± S.E.M. from six animals per group. *p< 0.05

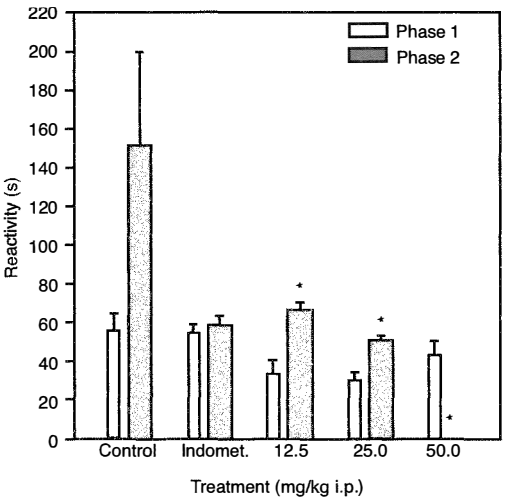

Fig 5. Analgesic activity measured by formalin test. Effects of FA on the first and second phases of mice responses. Mice were treated with saline solution (0.9% i.p.) (control), with indomethacin (indomet) and FA (12.5, 25 and 50 mg/kg i.p.). Licking time is expressed in seconds. The bars represent the mean values ± S.E.M. from six animals per group. µp< 0.05

Formalin test: The response time of control mice in the first and second phases were 55.50 ± 9.07 s and 151.70 ± 48.40 s respectively. FA at low doses (12.5 mg/kg i.p.) inhibited only the second phase (66.60 ± 0.17 s); at medium doses (25 mg/kg i.p) inhibited the first and second phases (26.0 ± 4.80 s and 51.0 ± 2.2 s respectively) and at higher doses (50 mg/kg i.p.) totally inhibited the second phase. Indomethacin (10 mg/kg i.p.), positive control, only reduced the second phase (48.40 ± 5.22 s) (Fig.5).

Abdominal contractions induced by acetic acid: Animals from the control group showed 39.9 ± 4.7 abdominal writhings accumulated at 30 min after acetic acid injection. Animals pretreated with FA (25 and 50 mg/kg i.p.) showed diminished writhings (17.8 ± 3.8 and 9.0 ± 1.4 respectively) in a dose-dependant manner. Animals treated with indomethacin (10 mg/kg i.p.) showed 8.5 ± 0.2 writhings in 30 min (Fig.6).

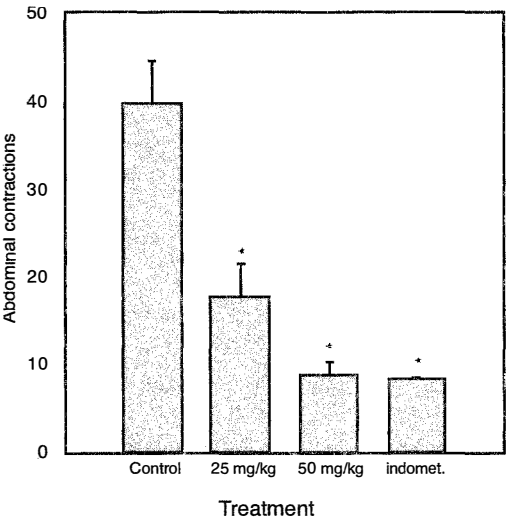

Fig. 6. Abdominal contractions induced by acetic acid. Abdominal contractions induced by i.p. injection of acetic acid (1.2%, 0.1 ml/10 o i.p.) in mice previously treated with saline solution (0.9% i.p.), indomethacin (10 mg/kg i.p.) or FA (25 and 50 mg/kg i.p.). The data are represented as mean abdominal contractions accumulated at 20 min after injection of acetic acid ± S.E.M. from six animals per group. *p< 0.05

DISCUSSION

The most widely used primary test to screen new anti-inflammatory agents measures the ability of a compound to reduce local edema

induced in the rat paw by injection of an irritant agent (Winter *et al.* 1962). This edema depends on the participation of kinins and polymorphonuclear leukocytes with their pro-inflammatory factors including prostaglandins (Damas *et al.*1986). The development of edema in the paw of the rat after the injection of carrageenan has been described by Vinegar *et al.* (1969) as a biphasic event. The initial phase, observed around 1 h, is attributed to the release of histamine and serotonin (Crunkhon and Meacock 1971); the second, accelerating, phase of swelling is due to the release of prostaglandin-like substances (Vinegar *et al.*1969). It has been reported that the second phase of edema is sensitive to both clinically useful steroidal and non-steroidal anti-inflammatory agents (Vinegar *et al.*1969, Di Rosa *et al.*1971)

The biodirected fractionation shows that the final aqueous fraction (FA), in this study and by the methods described, is the main responsible for the anti-inflammatory activity of the plant *Urera baccifera*. The significant activity observed in the suppression of the first and second phases of carrageenan-induced inflammation may due to inhibition of the release of the early mediators such as histamine, serotonin and kinins (Winter *et al.*1962). The action on the second phase may be explained by an inhibition of cyclooxigenase. According to Seibert *et al.* (1994) it could be inferred that FA inhibits cyclooxigenase-2. There are not enough data in this work to make us assume that FA inhibits cyclooxigenase 1 as well.

There are two phases in the formalin test that have different nociceptive mechanisms. It has been suggested that the early phase is due to a direct effect on nociceptors and the prostaglandins do not play an important role during this phase (Hunskaar and Hole 1987). The late phase seems to be an inflammatory response with pain that can be inhibited by anti-inflammatory drugs (Hunskaar and Hole 1987). Despite this, studies have indicated that the endogenous opiod-peptidergic and serotoninergic systems modulate the early and late phases differently (Fasmer *et al.*1985). Central analgesics are known to inhibit the two phases; in contrast, the non-steroidal anti-inflammatory drugs, like indomethacin, inhibit only the late phase (Hunskaar and Hole 1987). The FA fraction is endowed with peripheral analgesic activity which could be related to an inhibition of cicloxygenase.

The tests done with the purified FA fraction showed that it behaves as an inhibitor of leukocyte migration and pleural exudate under both subcutaneous and intraperitoneal administration as occurs with some non-steroidal analgesic anti-inflammatory drugs (Mikami and Miyasaka 1983).

The results also showed that the analgesic and anti-inflammatory activities of the FA fraction are dose-dependant at a range of 25 to 100 mg/kg intraperitoneally, and that it does not exhibit anti-inflammatory activity when topically administered.

Based on the results of this study, we came to the conclusion that the final aqueous fraction (FA) of *Urera baccifera* does have both anti-inflammatory and analgesic activities and have potential pharmaceutical and commercial interest. Studies to define the structure of the active principle are being pursued.

ACKNOWLEDGMENTS

This study was carried out with financial support from Vicerrectoría de Investigación Universidad de Costa Rica N 410-95-561, Consejo Nacional para Investigaciones Científicas y Tecnológicas (CONICIT) FR 198-09 and Programa Iberoamericano de Ciencia y Tecnología para el Desarrollo (CYTED).

REFERENCES

Crunkhon,P. & S.E.R. Meacock. 1971. Mediators of the inflammation induced in the rat paw by carrageenan. Brit. J. Pharmacol. 42:392-402.

Damas,J., G.Remacle-Volon & E. Deflandre.1986. Further studies of the mechanism of counter irritation by turpentine. Arch. Pharmacol.332:196-200.

Di Rosa, M., P.J. Giroud & D.A. Willoughby. 1971. Study of the mediators of acute inflammatory response induced

in rats in different sites by carrageenan and turpine. J. Pathol. 101,15-29.

Emim, J.A.S., R.B. Oliviera & A.J. Lapa. 1994. Pharmacological evaluation of the anti-inflammatory activity of a citrus bioflavonoid, hesperidin, and the isoflavonoids, duartin and claussequinone, in rats and mice. J. Pharm. Pharmacol. 46:118-122.

Fasmer, O.B., O.G. Berge & K.Hole.1985. Changes in nociception after lesions of descending serotoninergic pathways induced with 5,6-dihydroxytryptamine. Different effects in the formalin and tail-flick tests. Neuropharmacology 24:729-734.

Hoffman A., C. Farga, L. Lastra & E. Veghazi. 1992. Plantas medicinales de uso común en Chile. A. Hoffman, Santiago. p 5.

Hunskaar, S. & K. Hole. 1987. The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. Pain 30:103-114.

Jansen P.S.J., C.J.E. Niemegeers & G.H. Dong. 1963.The inhibitory effect of phentanyl and other morphine-like analgesics on the warm water induced tail withdrawal reflex in rats. Arzneim. Frosch. Drug Res. 25: 296-298.

Koster R., M. Anderson & E.J. De Beer. 1959. Acetic acid for analgesic screening Fed. Proc. 18: 412-413.

Mikami T. & K. Miyasaka. 1983. Effects of several anti-inflammatory drugs on the various parameters involved in the inflammatory response in rat carrageenan-induced pleurisy. Europe. J. Pharmacol. 95:1-12.

Morton J. 1981. Atlas of Medicinal Plants of Middle America. Charles Thomas , New York. p 154.

Riesteree L.& R. Jaques. 1968. Anti-inflammatory action of benzylglucofuranoside applied topically. Experimentia 24: 581-582.

Schianterelli P., S. Dacel, D. Acerbi & L. Pavesi. 1982. Anti-inflammatory activity for percutaneous piroxicam Arzneim. Forsch. Drug Res.32: 230-235.

Seibert K., Y. Zhang, K. Leahy, S. Hauser, J. Masferrer, W. Perkins, L. Lee & P. Isakson. 1994. Pharmacological and biochemical demonstration of the role of cyclooxigenase 2 in inflammation and pain. Proc. Natur. Acad. Scie. 91 :12013-12017.

Vinegar R., J F. Truax & J.L. Selph.1973. Some quantitative temporal characteristics of carrageenan-induced pleurisy in the rat. Proc. Soc. Exp. Biol. Med. 143:711-714.

Vinegar, R.,W. Scheirber & R. Hugo. 1969. Biphasic development of carrageenan edema in rats. J. Pharmacol. Exp.Ther.150: 328-334.

Winter, C.A., E.A Risley & G.W. Nuss. 1962. Carrageenan-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. Proc. Soc. Exp. Biol. Med. 111: 544-547.