Anti-inflammatory Effect of Semecarpus anacardium LINN. Nut Extract in Acute and Chronic Inflammatory Conditions

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The article relates to investigation of the anti-inflammatory effects of Semecarpus anacardium LINN. nut extract (SA), and also an anti-inflammatory drug, indomethacin, on carrageenan-induced paw edema and cotton pellet granuloma tests for their effects on acute and chronic phases of inflammation, respectively. The effect of SA on developing and developed adjuvant arthritis was also evaluated. SA significantly decreased the carrageenan-induced paw edema and cotton pellet granuloma. Indomethacin also decreased the acute and chronic phases of inflammation. SA decreased the adjuvant induced (arthritis) paw edema after the treatment, in both developing and developed adjuvant arthritis. These results indicate that the potent anti-inflammatory effect and therapeutic efficacy of Semecarpus anacardium LINN. nut extract against all phases of inflammation, is comparable to that of indomethacin.

Key words adjuvant arthritis; Semecarpus anacardium; inflammation; carrageenan; granuloma

MATERIALS AND METHODS

Materials Carrageenan and complete Freund’s adjuvant were obtained from Sigma Chemicals, St. Louis, U.S.A. All other chemicals were of analytical grade.

Animals Adult male Wistar rats weighing between 180 and 200 g were maintained in well-ventilated spacious cages. The rats were fed commercial rat feed supplied by Hindustan Lever, Ltd. Mumbai with the name Gold Mohur rat feed. Food and water were given ad libitum.

Formulation of the Drug The drug was formulated as described in the Formulary of Siddha Medicine.11) The drug Serankottai Nei is a siddha preparation prepared by boiling the nuts (200 g) with 500 ml of milk. Decanting the decoction, 500 ml of milk was added to the boiling nuts and again boiled for some time. The decoction was recovered and the process was repeated again with the milk (500 ml). All three portions of the milk nut decoction were mixed with ghee (1.5 kg) and boiled till dehydration, then filtered and stored. (Formulary of Siddha Medicine, 1972). Olive oil was used as a vehicle for the drug.

Investigation of Anti-inflammatory Effects. Carrageenan Induced Paw Edema Anti-inflammatory activity of SA was determined by carrageenan induced paw edema.12) The drug at 150 mg/kg and indomethacin at 10 mg/kg body weight in olive oil were given to rats orally 30 min before carrageenan injection. The same volume of the vehicle was given to control groups. The left rear plantar region of the rats was injected with 0.1 ml of carrageenan (1% in saline). The edema produced was determined by measuring the difference of the paw diameter using an analogic pakimeter (vernier) before carrageenan injection and at 1, 2, 3, 4 h after...
Carrageenan injection.\textsuperscript{13,14}

**Cotton Pellet Granuloma Test** The proliferation phase of inflammation was investigated by the cotton pellet granuloma model.\textsuperscript{15} The drug at 150 mg/kg and indomethacin at 10 mg/kg body weight dissolved in olive oil were given to the rats orally. After 30 min the animals were anesthetised. Sterile cotton pellets of 20 mg each were implanted at an interscapular depth under the skin under sterile conditions. The drug was administered daily for a period of seven days. The rats were sacrificed after a high dose of anaesthesia on the eighth day and the pellets surrounded by granuloma tissues were dissected out, weighed, dried for 24 h at 60 °C temperature and again weighed. The increment between dry and wet pellet weights was taken as a measure of granuloma formation and compared with the control.

**Adjuvant Arthritis in Rats** Two types of investigations were carried out (a) The prophylactic effect was analyzed by dosing immediately before and during the development of arthritis and\textsuperscript{10} (b) the therapeutic effect was analyzed by dosing after the development of arthritis.\textsuperscript{16}

(a) Effect on Developing Adjuvant Arthritis: Three groups of albino rats (150—170 g) in groups of three with six rats each had the arthritic syndrome induced by subcutaneous injection into the plantar surface of the left hind paw by 0.1 ml of Complete Freund’s Adjuvant (10 mg of heat killed mycobacterium tuberculosis per ml of paraffin oil). *Semecarpus anacardium* Linn. nut extract and indomethacin were administered in daily doses of 150 mg/kg body weight in olive oil and 10 mg/kg body weight, respectively, for 28 d from the day of induction. Control animals received the same volume of vehicle over the treatment period. The thickness of the injected foot was measured initially and daily. Changes in thickness over the course were employed as a measure of degree of inflammation.

(b) Effect on Established Adjuvant Arthritis: Adjuvant arthritis was induced as (a) above and the rats were left untreated until the 14th day. From day 14 they were treated daily until treatment was terminated on day 28. Paw thickness was measured daily and the progress of the inflammation was assessed.

**Ulcerogenic Activity** The method was based on that of Cashin et al.\textsuperscript{17} Rats were fasted for 16 h and then *Semecarpus anacardium* extract (150 mg/kg) was administered. Three hours later the animals were sacrificed and the stomachs were removed, cut along the lesser curvature and washed with saline. The ulceration of the gastric mucosa was examined under a microscope, and scored according to the scale. 0=no lesion; 0.5=hyperemia; 1=1 or 2 lesions; 2=severe lesions; 3=very severe lesions; 4=mucosa full of lesions. Indomethacin was used as reference drug. The extent of ulceration of the treated groups was compared with the control groups.

**Statistical Analysis** Results were presented as mean±S.E.M. of six rats. The results were statistically evaluated using Student’s *t*-test and ANOVA.

**RESULTS**

**Carrageenan Induced Paw Edema** Interplantar injection of carrageenan in rats led to a time-dependent increase in paw thickness (Fig. 1); this increase was observed at 1 h and was maximal at 4 h after administration. However, carrageenan-induced paw edema was significantly reduced in all phases of the experiment by treatment with *Semecarpus anacardium* Linn. as well as indomethacin.

**Cotton Pellet Granuloma Test** The drug SA was able to reduce the inflammatory process of granuloma formation in rats after the treatment period in comparison with control rats (Table 1); this was evident from the reduction of both wet and dry weights of the cotton pellets. Treatment with indomethacin also effectively reduced the granuloma. Pus was observed in control groups, but was not found in treated groups.

**Adjuvant Arthritis** Swelling and redness developed over a 24 h period in the foot injected with adjuvant. This inflammatory reaction subsided slightly during the next 8 to 10 d and then increased at that time when disseminated arthritis appeared (Fig. 2). In rats treated from the day of adjuvant injection, the paw swelling was completely suppressed and no secondary increase was seen. The drug treatment begun 14 d after the day of adjuvant injection suppressed the secondary increase in swelling of the injected foot that occurred with the appearance of polyarthritis.

**Ulcerogenic Activity** The ulcerogenic activities of SA and indomethacin are depicted in Fig. 3. No significant ulceration of the gastric mucosa was detected in the animals treated with the extract or in the control animals. However, the gastric mucosa of indomethacin treated animals showed ulceration (ulceration scale: 1.2).
The most widely used primary test for screening of anti-inflammatory agents is carrageenan induced edema in the rat hind paw.\(^4\) The development of edema in the paw of the rat after injection of carrageenan was described by Vinegar et al.\(^{18}\) as a biphasic event. The initial phase observed during the first hour is attributed to the release of histamine and serotonin\(^{19}\); the second phase is due to the release of prostaglandin-like substances.\(^{18}\) Based on this, it could be argued that the suppression of the first phase may be due to inhibition of the release of early mediators, such as histamine and serotonin, and the action in the second phase may be explained by an inhibition of cyclo-oxygenase. The result of the present study indicates that *Semecarpus anacardium* LINN. extract also inhibits monocyte infiltration and fibroblast proliferation. These results indicate the efficacy of *Semecarpus anacardium* LINN. extract which possesses anti-inflammatory activity. In the investigation of adjuvant arthritis in rats, the arthritic rats showed a soft tissue swelling that was noticeable around ankle joints and was believed due to edema of periarticular tissues such as ligaments and joint capsules. The initial reduction of edema and soft tissue thickening at the deposit site is probably due to the effect of the adjuvant, whereas the late occurring disseminated arthritis and flare in the injected foot are presumably immunological events.\(^{27,28}\)

Studies on animal models of inflammation have suggested acute vascular responses: vasodilation and increased vascular permeability resulting from the sequential release of low molecular weight mediators—histamines, serotonin and prostaglandins.\(^{25}\) During these acute vascular changes, polymorphonuclear leukocytes, predominantly neutrophils accumulate slowly in the tissues reaching a significant number after several hours. Prostaglandins greatly potentiate exudates by inducing relaxation of arteriolar smooth muscle cells and increasing the blood supply to the tissue.\(^{29,30}\)

Inhibition of paw edema in adjuvant arthritic rats is a hallmark of anti-inflammatory action. The ability of the drug to reduce edema formation may thus be related to its inhibitory action on prostaglandin synthesis.

Gastric discomfort and ulcers are generally the major side effects related to the currently employed non-steroidal anti-inflammatory agents,\(^7\) which is evident from the data of the present study. But no significant ulceration was found in the animals administered the nut extract.

In conclusion, the results of this study show that *Semecarpus anacardium* LINN. extract has anti-inflammatory activity against early phase (acute paw edema), late phase (cotton pellet granuloma) of inflammation and adjuvant arthritis without any deleterious side effects. The anti-inflammatory activity could be attributed to the presence of the previously reported flavonoids, and other related synergistic components.