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

Studies have been continuing on anti-inflammatory drugs to treat inflammatory diseases in various countries. In spite of the discovery of several newer agents, the search for better anti-inflammatory drugs continues because they have many known side effects and none of them is suitable for prolonged use. The side effects of the anti-inflammatory drugs are one of the major problems in developing medicine today.[1] Therefore, development of new and more powerful drugs with fewer side effects is needed. Large numbers of herbal drugs are in use for the treatment of arthritis by Ayurvedic and Siddha practitioners.

This article reports the anti-inflammatory effect of *Semecarpus anacardium* (SA) Linn. nut extract. *Semecarpus anacardium* Linn. (Family: Anacardiaceae) also called the "marking nut" has found many applications in Indian medicine in the treatment of gout, rheumatic pain and cancer.[2] Phytochemical studies of the milk extract have shown flavonoids, phenols and carbohydrates among its contents and the drug was found to be effective against adjuvant arthritis at the dose level of 150 mg/kg body weight.[3] Pillai[4] reported the presence of phenolic compounds like semicarpol and bhilawanol in the nuts, and the milk extract of the nut was found to inhibit acute tuberculin reaction in sensitized rats and also the primary phase of adjuvant arthritis.[5] Previous studies with this drug have been shown to be effective against breast cancer,[6] and rheumatoid arthritis (RA).[3] Its pharmacological activities have been attributed to some flavonoids present in the drug, particularly those related to their anti-inflammatory properties.[7]

Acute and chronic inflammations are complex processes that can be induced by a variety of means. Anti-inflammatory agents exert their effect through a spectrum of different modes of action.[8] In the screening of the new anti-inflammatory compounds, carrageenan-induced edema in the hind paw (acute inflammation) and cotton pellet granuloma (chronic inflammation) is widely employed.[9] Adjuvant induced arthritis is also an often used model of inflammation.[10]

This study investigated the anti-inflammatory effects of *Semecarpus anacardium* Linn. extract on acute and chronic phases of inflammation using carrageenan induced paw edema and the cotton pellet granuloma test, respectively, and also investigated the anti-inflammatory effect of the drug against adjuvant induced arthritis. Further, the ulcerogenic index was evaluated during administration of the drug SA, which is the effective dosage level for the treatment of RA[3] and compared to that of indomethacin.

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 decotion 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

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carrageenan injection.[13,14]

Cotton Pellet Granuloma Test The proliferation phase of inflammation was investigated by the cotton pellet granuloma model.[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 anesthesia 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[10] (b) the therapeutic effect was analyzed by dosing after the development of arthritis.[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.*[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.

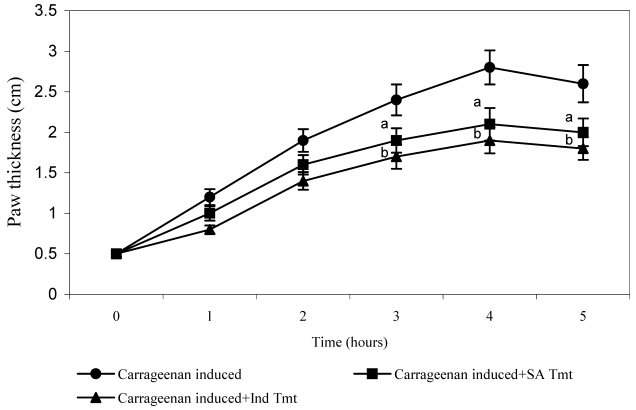

Fig. 1. Anti-inflammatory Effect of SA and Indomethacin on Carrageenan Induced Paw Edema in Animals

Values are expressed as mean±S.D. of six animals. a, b; denote significance at $p<0.05$ when compared with carrageenan induced group, respectively (ANOVA).

Table 1. Anti-inflammatory Effect of SA and Indomethacin on Cotton Pellet Granuloma in Control and Experimental Animals

Weight of cotton pellets (mg)	Control	SA Tmt	Indomethacin Tmt
Wet wt.	271.4±7.15	147.25±8.06[a)]	138.74±7.86[b)]
Dry wt.	73.28±3.47	46.18±2.29[a)]	43.37±2.08[b)]

Values represented as mean±S.D. of six animals. *a*) denote significance at the level of $p<0.05$. Control *vs.* SA Tmt. *b*) denotes significance at the level of $p<0.05$. Control *vs.* Ind Tmt.

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).

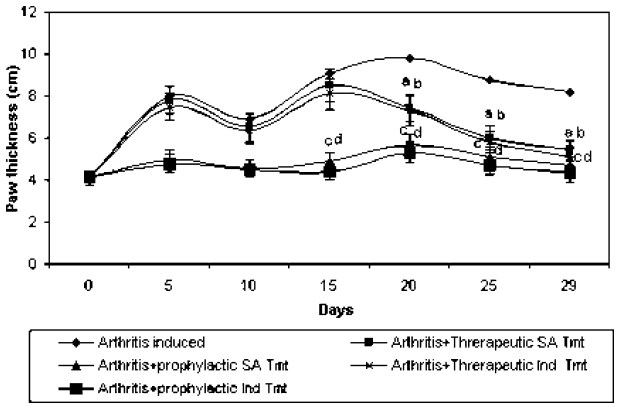

Fig. 2. Anti-arthritic Effect of SA and Indomethacin on the Changes in Paw Edema of Control and Experimental Animals

Values represented as mean±S.D. of six animals. a, c; denote significance at $p<0.05$ when SA treated groups are compared with arthritic group. b, d; denote significance at $p<0.05$ when Ind treated groups are compared with arthritic group (ANOVA).

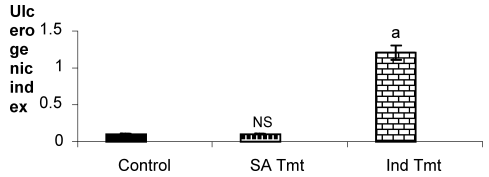

Fig. 3. Ulcerogenic Activity of SA and Indomethacin in Control and Experimental Animals

Values represented as mean±S.D. of six animals. a; denote significance at the level of $p<0.05$. Control *vs.* Ind Tmt. NS; denotes non significance at the level of $p<0.05$. Control *vs.* SA Tmt.

DISCUSSION

The most widely used primary test for screening of anti-inflammatory agents is carrageenan induced edema in the rat hind paw.[12] 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 and indomethacin play a crucial role as protective factors against the carrageenan-induced acute inflammation. The significant suppressive activity of the drug (SA) in both phases shows its potent anti-inflammatory effect which can be attributed to the presence of flavonoids, a component of the preparation.[20]

The cotton pellet granuloma method has been widely employed to assess the transudative, exudative and proliferative components of chronic inflammation and is a typical feature of established chronic inflammatory reaction. The fluid absorbed by the pellet greatly influences the wet weight of the granuloma and the dry weight correlates well with the amount of granulomatous tissue formed.[21,22]

Semecarpus anacardium Linn. extract decreased both wet and dry weights of the cotton pellets when compared to control groups (Table 1). Monocyte infiltration and fibroblast proliferation rather than neutrophil infiltration and exudation take place in chronic inflammation.[23,24] This proliferation becomes widespread by proliferation of small vessels or granuloma.[23] Nonsteroidal anti-inflammatory drugs (NSAIDS) decrease the size of granuloma which results from cellular reaction by inhibiting granulocyte infiltration/inflammation, preventing generation of collagen fibers and suppressing mucopolysaccharides.[25,26] It is possible 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.[12] 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.

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