ANTI-INFLAMMATORY ACTIVITY OF *SEMECARPUS ANACARDIUM* LINN. A PRELIMINARY STUDY*

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Semecarpus anacardium Linn. (N.O.—Anacardiaceae) is a deciduous tree growing on the sub-Himalayan and tropical parts of India. It is commonly known by the name of '*marking nut*' and in the vernacular as *Bhallataka*. The fruits (seeds) and oil have been claimed to be highly efficacious in the treatment of neuritis, arthritis, leprosy, helminthic infection and venereal disorders by the protagonists of Ayurvedic system of medicine (3, 4, 5, 7, 14, 15).

Pillay and Siddiqui (11) reported the presence of phenolic compounds like *Semicarpol* and *Bhilwanol* in the nut. Sharma and Chaturvedi (14) have found the oil to be effective in hookworm infection. Bose *et al.* (2) have reported an action similar to known histamine liberators. No scientific work seems to have been done to evaluate the anti-inflammatory activity of the plant which presumably forms the main basis of its principal uses in arthritis and neuritis. The present work has been carried out to screen it for anti-inflammatory and anti-arthritic activity in experimental animals. Since a milk extract of the nut (*Bhallatak ksheerapak*) is a favourite preparation indicated by the Ayurvedic physicians in these conditions (7), and the same has also been reported to be clinically effective in cases of sciatica (10, 12), the drug has been used in the same form in the present studies.

MATERIALS AND METHODS

The milk extract of the drug was prepared by boiling a known weight of the crushed nut of *S. anacardium* in milk diluted with equal quantity of water. After boiling, the volume of the milk extract was adjusted to 2.5 *mg.* nut/*ml.* This milk extract, when chemically analysed, was found to contain traces of phenolic compounds.

Albino rats of either sex weighing approximately 100 to 150 *gm.* were used for the experiment. Five or six animals were used in each group. The milk extract was administered orally in the dose of 2 *ml.*/100 *gm* body weight. The control animals received milk in a dose of 2 *ml.*/100*gm* body weight. Betamethasone was used (50 *mcg.*/100*gm.*) orally as a known anti-inflammatory agent for comparison.

For inducing inflammation, both non-immunological and immunologcial methods were emplo ed.

A. *Non-immunological* :—

1. Acute inflammation was evoked by the following phlogistic agents injected intradermally into the plantar surface of both hind paws :—

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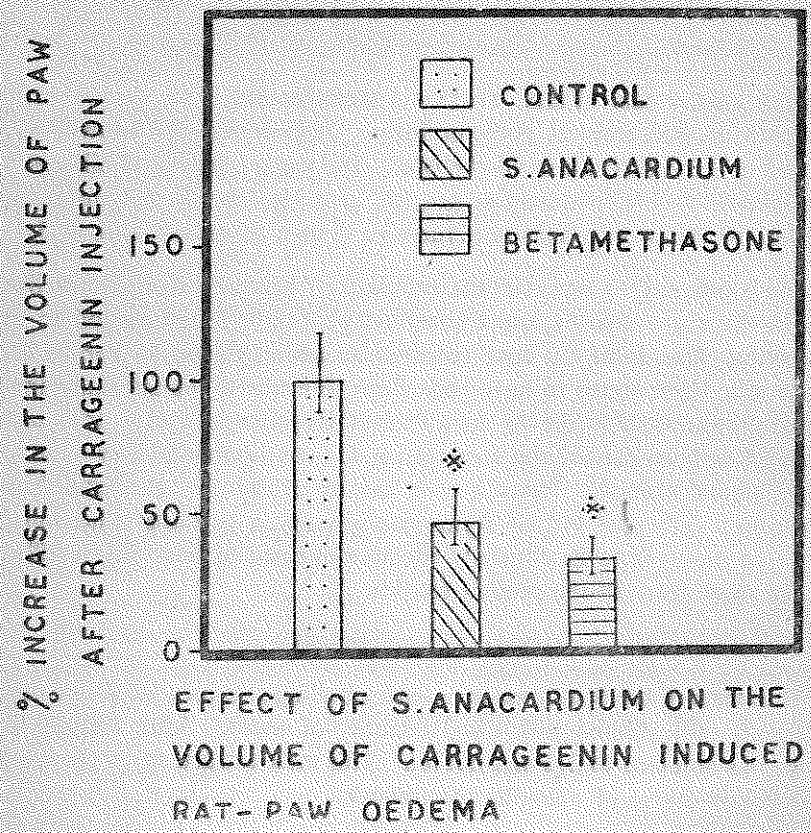
(*i*) Carrageenin 1% (16)

(*ii*) 5-Hydroxytryptamine 0.1% (6).

(*iii*) Formaldehyde 3% (6).



GRAPH 1
(*denotes statistically significant difference)

The drug was administered orally one hour before the injection of the phlogistic agent. The volume of the hind paws was measured by causing displacement of mercury. For this, a 10 *ml.* syringe containing mercury and mounted on an adjustable stand was connected to a transparent polythylene tubing fixed on a scale and mounted on another adjustable stand. The ankle joint of the rats was marked with a skin pencil before dipping the paw into the syringe. The paw was dipped up to the mark on the ankle joint which was made to coincide with a prefixed line kept constant on the syringe. The level of the mercury was everytime brought to the level of this line by adjusting the height of the scale and the reading was taken. The difference in the readings before and after injection of the phlogistic agents gave the difference in the paw-volume.

In the case of carrageenin-induced oedema, readings were taken after 3 hours while in the case of the other two phlogistic agents, measurements were taken 1½ hours, 4 hours, and

24 hours after the injection. The drugs administered orally were diluted to a fixed volume of 5 *ml.* with distilled water to ensure uniform hydration of all the rats thus minimizing the variability of the oedematous response.

2. Subacute Inflammation

(*i*) *Granuloma pouch technique*(13)—2% croton oil in arachis oil was used. The drugs were administered orally for 6 days. The rats were anaesthetized with ether on the 6th day and the volume of the exudate in the pouch was measured and examined macroscopically.

(*ii*) *Implantation of Carrageenin pellets in adrenalectomized rats* (1) —Albino rats were adrenalectomized and on the same day, carrageenin pellet of known weight (40 *mg.*) was implanted by a separate incision subcutaneously in the dorsum just 2 *cms.* above the tail in each rat. Each pellet consisted of carrageenin 19.8 *mg*, magnesium stearate 0.4 *mg.*, and sucrose 19.8 *mg.* compressed into a pellet (1), the dry weight of each pellet being 40 *mg.* The drugs were administered orally for 5 days. On the 5th day, the rats were anaesthetized with ether and the granulation tissue around the pellet was dissected out and the wet weight of each pellet with granulation tissue was recorded.

B. *Immunological*

1. *Adjuvant arthritis* (8, 9) : The arthritic syndrome was induced by an intradermal injection of 0.05 *ml.* of a fine suspension of dead tubercle bacilli in liquid paraffin (concentration 5 *mg./ml*) into the plantar surface of the left hind paw. The tubercle bacilli were derived from the human strain DT which was grown for 8 weeks, killed by steam and dried in a vacuum oven. The drugs were administered orally for 14 days starting one day prior to the injection of the "adjuvant" into the footpad. The volume of the paws was measured daily. On the 13th day after the injection of the adjuvant, the weight of each rat was recorded, the volume of both the paws measured and the severity of the secondary lesions assessed as nil, mild, moderate or severe, depending on the lesions found in the uninjected hind foot, the forepaws, and nodule formation in the ears and the tail.

2. *Tuberculin sensitivity test* : This was performed on the fourteenth day after injecting mycobacterial adjuvant. Purified tuberculin (P.P.D.) was injected intradermally (0.01 *ml.* of 1:10 dilution) into the flanks of the rats which were previously depilated. The diameter of the tuberculin reaction was measured 24 hr. and 48 hr. after injection. The drug was administered 3 hr. before injecting P.P.D.

Toxicity Studies : Since the drug was used in the form of milk extract, detailed toxicity studies were not conducted. However, all the albino rats which were administered the drug in the adjuvant arthritis group were placed under observation for 14 days and their food intake, changes in body-weight and toxic manifestations, if any, were recorded daily. After the fourteenth day, animals from each group were sacrificed and sections were taken from liver, spleen and kidney and examined histologically.

RESULTS

Carrageenin-induced oedema :—The effect of the drug *S. anacardium* on carrageenin-induced oedema of rat's paw in comparison to betamethasone has been summarized in graph I. The dose of the drug was decided after conducting a few pilot studies which showed that a dose less than 2 *ml.*/100 *mg* body weight was not effective whereas higher doses were found to be toxic. As such, a dose of 2 *ml.*/100 *mg.* body weight was employed in all subsequent experiments. With this dose, *S. anacardium* was found to suppress the oedema induced by carrageenin. This inhibition was highly significant statistically ($p < 0.005$). In case of betamethasone also the inhibition was highly significant ($p < 0.001$).

Inflammation induced by 5-HT and formaldehyde : The effect of the drug on the inflammation induced by the injection of 5-HT and formaldehyde after 1½ hours, 4 hours and 24 hours interval has been summarized in Table I. In the case of 5-HT induced oedema, the drug was found to inhibit the inflammation 1½ hours, as well as 4 hours after the injection of 5-HT. In formaldehyde-induced inflammation, *S. anacardium* produced statistically significant inhibition only after 4 hours interval.

TABLE I

Effect of S. anacardium on 5-HT and formaldehyde induced inflammation

Group	*Volume of the Rat's paw (mm)**		
Control	1½ hr.	4 hr.	24 hr.
5-HT	4.6±1.2	3.6±0.42	0.9±0.3
Treated 5-HT	2.0±1.2	1.9±0.6	0.8±.02
	P<0.05	P<0.05	P>0.05
Control Formaldehyde	3.5±1.2	5.8±1.1	7.6±1.2
Treated Formaldehyde	2.5±1.9	3.0±0.8	6.5±1.3
	P>0.05	P<0.05	P>0.05

*Mean ± standard error of the mean

Granuloma Pouch : Table II summarizes the effect of *S. anacardium* and betamethasone on the inflammatory exudate of granuloma pouch in albino rats. While rats treated with betamethasone showed marked reduction in the exudate (to 15%), *S. anacardium* was not found to reduce the volume of the exudate to any significant degree ($p > 0.05$).

TABLE II

Effect of S. anacardium on the inflammatory exudate in rats

(Granuloma-pouch)

Drugs	*Dose*	*No. of animals*	*Exudate per rat (in mls)*	*t.*	*p.*
Control	Milk (2*ml*/100*gm*)	5	0.61±0.18*		
Betamethasone	50 *mcg.*/100*gm*	5	0.09±0.034	3.5	<0.001
S. anacardium	Milk extract 2 *ml.*/100*gm.*	5	0.32±0.25	0.9	>0.05

*Mean ± Standard Error of the mean.

Carrageenin-pellet implantation in adrenalectomized rats : Table III summarizes the effect of *S. anacardium* and betamethasone on the wet weight of the granulation tissue produced by implantation of carrageenin-pellets in adrenalectomized rats. Betamethasone was

TABLE III

Weight of carrageenin granulomata in adrenalectomized rats on the 4th day

	No. of Animals	*Mean wet weight (gms)* ± *S.E.*	*t.*	*p.*
Control	3	1.16+0.47		
S. anacardium	3	1.06+0.37	1.69	>0.05
Betamethasone	3	0.12+0.1	2.4	<0.05

Fig. 1 : *Adjuvant arthritis* (*primary phase*) : 3*rd* day; *Untreated arthritic control rat* : Note the *marked oedema in the* left hind paw.

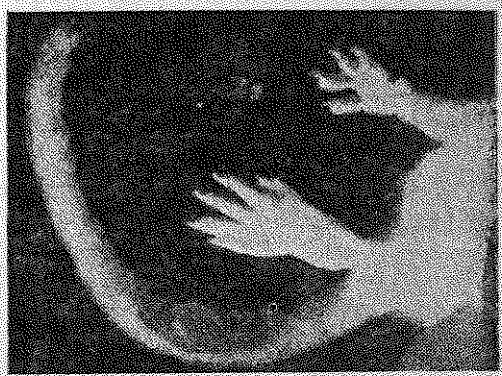


Fig. 2. : *Adjuvant arthritis* (*Secondary lesions*) : 13th day; *Untreated arthritic control rat* : Note the *arthritic changes* in both the hind paws and the *ulcerating nodules* on the tail.

found to reduce the wet weight of the granulation tissue to a highly significant degree whereas *S. anacardium* was not found to suppress the formation of granulation tissue in adrenalectomized rats.

Adjuvant Arthritis

The effect of *S. anacardium* and betamethasone on the arthritic syndrome induced by the mycobacterial adjuvant in albino rats is summarized in Table IV. In the control animals, acute inflammation of the injected paw occurred during the first three days, reaching maximum on the 3rd day (Fig. 1). Thereafter, the swelling gradually subsided. On or after the 10th day again inflamed lesions (known as secondary lesions) could be detected on the uninjected (right) hind paw which increased in volume, and in the fore-paws, ears and tail (Fig. 2). In the ear, lesions were first seen as small patches of dilated capillaries mostly on the 10th day which turned into reddish nodules by the 13th day, thereafter subsiding by the 20th day. A number of nodules were noticed on the tail also by about the 10th day, which later ulcerated. The tail, by about the 20th day, had become noticeably thicker. All these features were comparable with those described by Newbould (8, 9).

TABLE IV

Effect of S. anacardium on adjuvant arthritis in rats

Drug	*%Change in Foot Volume*				*Change in weight***	*Secondaries*
Control (Milk 2*ml*/100*gm*)	1st day 100	3rd day 116.02±1.34*	4th day 112.6±2.1	13th day 125±2.2	−18±2.0	Severe
Betamethasone (50*mcg.*/100*gm*)	100	104.1±1.07 t=6.21 p<0.001	100.3±1.9 t=4.3 p<0.005	106.8±2.8 t=5.2 p<0.001	−16±1.8	Mild
S. anacardium (5 *mg*/100*gm*)	100	103.7±1.8 t=5.48 p<0.001	102.2±2.1 5t=3.60 p<0.005	119.1±2.6 t=1.88 p<0.1	+15±2.2	Severe

*Mean±Standard Error
**Grams per rat (Mean±Standard Error)

During the course of the development of the arthritic syndrome the control rats invariably lost weight.

In contrast to the control group, betamethasone treated group was found to suppress not only the primary inflammation but also to modify the secondary lesions in the tail, ear and the paws after the 10th day. Only 20% of the animals treated with betamethasone developed nodules in the tail or sewelling of the fore paw. No ear lesion was detectable in any of the animals. In case of the animals treated with *S. anacardium*, however, while the primary inflammation in the injected foot was effectively suppressed (Fig. 3), the drug did not show any inhibitory effect on the delayed secondary lesions occurring in the remote parts of the body.

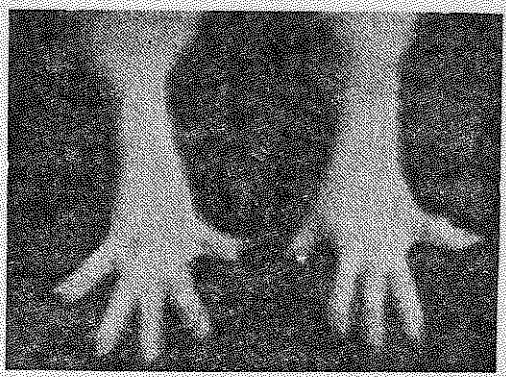


Fig. 3. : *Adjuvant arhritis* (primary phase) : 3rd day; *S. anacardium-treated rat* : Note the *absence of edema* in the left hind paw

Tuberculin-sesitivity test

The result of the tuberculin-sensitivity test performed on the 14th day after injecting the adjuvant is summarized in Table V. A marked tuberculin reaction developed 24 hours after injecting 0.1 *ml.* of 1:10 dilution of P.P.D. in the control group. *S. anacardium* showed marked activity in suppressing the tuberculin reaction after 24 hours and 48 hours interval.

TOXICITY STUDIES

Loss of weight in the animals during development of arthritic syndrome was almost similar in control and betamethasone treated rats. However, in case of *S. anacardium* treated group, there was an average gain of weight (15*g*/rat) on the 13th day of the experiment (Table IV).

TABLE V

Action of S. anacardium on Tuberculin-reactin Diameter of Tuberculin reaction (Mean±S.E.)

Group	24 hr. (mm)	48 hr. (mm)
S. anacardium	6.5±2.1	3.7±1.2
Control	12.6±1.9	11.6±3.6
t	2.26	2.24
p	<0.05	<0.05

It was noted that 20% of the animals treated with *S. anacardium* developed alopecia and gangrene of the limbs as well as the tail and ears. Histological section showed cloudy swelling of the kidneys with total occlusion of the Bowman's capsule in the treated group. The spleen showed congestion in the same group while the liver was normal.

DISCUSSION

Preliminary screening of *S. anacardium* for anti-inflammatory activity revealed significant suppressing action of the drug on acute inflammation induced by carrageenin, 5-HT, and formaldehyde in rat's hind paw. The drug was further found to inhibit acute tuberculin reaction in sensitized rats as also the primary phase of adjuvant arthritis without having any significant effect on the development of the secondary lesions induced by the adjuvant. *S. anacardium* was ineffective in inhibiting granuloma formation induced either by croton oil or by carrageenin pellet implantation in adrenalectomized rats. Betamethasone, on the other hand, was found to be more effective in suppressing acute, subacute and chronic phases of both immunologically and non-immunologically induced inflammations. Nevertheless, its was found that betamethasone had marked catabolic action since all the animals treated with it invariably lost weight, whereas rats treated with *S. anacardium* showed an average gain in weight. *S. anacardium* has been reported to be effective clinically in the treatment of sciatica (10, 12) as well as in acute cases of rheumatoid arthiritis (12). The present experimental study supports, to some extent, the efficacy of the drug in acute inflammatory disorders. The mechanism of action of *S. anacardium*, however, must await further detailed chemical and pharmacological studies.

SUMMARY

Semecarpus anacardium Linn. was screened for its anti-inflammatory activity by various methods in albino rats :

1. The drug was found to effectively suppress acute inflammation induced by carrageenin, 5-HT and formaldehyde.
2. It was found to have no inhibitory effect on granuloma formation induced by granuloma pouch technique as well as by carrageenin pellet implantation in adrenal ectomized rats.
3. *S. anacardium* was found to suppress the acute primary inflammation of adjuvant arthritis in albino rats but had no effect on the delayed secondary lesions which are presumably due to a generalized immunological response. However, it effectively suppressed the tuberculin reaction in sensitized rats.
4. While most of the animals treated with *S. anacardium* showed an average gain in weight, 20% of the animals were found to develop alopecia as well as gangrene of the limbs, tail and the ears.
5. Further work is in progress with different chemical fractions of the drug in order to isolate the active principle and also to work out the mechanism of anti-inflammatory activity.

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