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Antiinflammatory Evaluation of *Leucas lavandulaefolia* Rees. Extract

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Abstract – The antiinflammatory activity of the methanol extract of *Leucas lavandulaefolia* Rees was evaluated on different experimental models of inflammation in rats. The extract has been found to possess significant, inhibitory activity against carrageenin, histamine, serotonin, and dextran induced hind paw oedema in rats. The effect produced by extract was comparable to that of phenylbutazone and a prototype, nonsteroidal antiinflammatory agent.

Key words – *Leucas lavandulaefolia*; antiinflammatory; activity; phenylbutazone.

Introduction

Leucas lavandulaefolia Rees. Syn. *Leucas linifolia* Spreng. (Family-Labiatae) is a herbaceous annual weed found in pastures and waste lands throughout India. Plants are 30-60 cm long; leaves are opposite, linear-lanceolate, entire or sparingly serrate (Anonymous, 1962). All parts of this plant are medicinally important in traditional system of medicine in India and have been extensively used by rural people of Mithila region (Bihar, India) in human and cattle ailments, such as cough, cold, fever, inflammation, skin diseases, headache etc. (Kirtikar and Basu, 1975; Nadkarni, 1992; Chopra *et al.*, 1958; Kamat *et al.*, 1994). The antibacterial efficacy of this plant extract have been reported (Saha *et al.*, 1995). Presently it has come to our notice that tribal people of Tripura, India use the juice of this plant against inflammation and get cure of it.

In the light of above information the present study was undertaken to evaluate the antiinflammatory activity of the methanol extract of this plant and is being reported in the present communication.

Materials and Methods

Plant material – *Leucas lavandulaefolia* herbs were collected from Khatra, Bankura District of West Bengal, India and was identified by Botanical Survey of India, Shibpur, Howrah. Plants were dried under shed, pulverized by a mechanical grinder and passed through 40 mesh sieve.

Preparation of extract – The powdered herbs (500 g) were extracted with methanol in a soxhlet extractor. The extract was then distilled under reduced pressure and a brownish green colored semisolid mass was

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obtained (yield 16.3% w/w with respect to dry powdered material). It was passed through a silica gel (60-120 mesh) column using chloroform: methanol (1:1) as eluting solvent. A yellowish brown coloured band seperated out. This fraction was concentrated under reduced pressure which showed the presence of steroidal and alkaloidal substances.

The concentrated solid mass (yield 6.5% w/w) was stored in a desiccator and subjected to further experiments. The extract was administered at a dose of 200 and 400 mg/kg (i.p.) by dissolving the extract in normal saline.

Animals used – Male Wistar albino rats with body weight of 180-200 g were purchased from M/S B.N. Ghosh & Company, Calcutta. They were housed in an animal room at an ambient temperature of 25±1°C under normal lighting conditions. The animals were used after an acclimatization period of at least 7 days to the laboratory environment. During this period the animals were fed on standard food and water *ad libitum*.

Carrageenin induced rat paw oedema – 1% solution/suspension of carrageenin was prepared. 0.1 ml of this solution was injected into the right hind paw of the rats (Winter *et al.*, 1962). The extract (200 mg and 400 mg/kg), phenylbutazone (100 mg/kg) and control vehicle were injected intraperitoneally (i.p) 30 min. prior to the injection of carrageenin. The paw volume was measured just before and 3 h after administration of carrageenin by the volume displacement method (Bhatt *et al.*, 1977).

For differentiating "counterirritant" activity from "true" antiinflammatory activity, the extract was mixed with carrageenin (Mixture I contained 50 mg of extract and 0.1 ml of 1% carrageenin; Mixture II contained 40 mg of extract and 0.1 ml of 1% carrageenin) and was injected into the right hind paw of rats (shanahan, 1968) and the paw volume was measured as before (Bhatt *et al.*, 1977).

Mediator induced inflammation – The antiinflammatory activity of the extract was measured with some phlogestic agents acted as mediators of the inflammation to study the selectivity of the leaf extract. 0.1 ml solution of histamine base (10^{-3} g/ml), serotonin (10^{-3} g/ml) and Dextran were injected into the right hind paw and the oedema volume was determined. The extract at dose of 400 mg/kg was injected along with the mediators which served as drug treated group and the others injected only with the mediators served as control group (Parmer and Ghosh, 1978). The paw volume was measured 30 min. after injection of the phlogestic agents.

In all the above experimental models the oedema rate and inhibition rate was calculated as follows (Lin *et al.*, 1995)

$$\text{Oedema rate (E)\%} = \frac{V_r}{V_l} \times 100$$

$$\text{Inhibition rate (I)\%} = \frac{E_c - E_t}{E_c} \times 100$$

Where V_l is the contralateral paw volume of rat (left hind paw, without carrageenin at t hour); V_r is the right hind paw volume of rats (with carrageenin) at t hour; E_c is the oedema rate of control group and E_t the same of treated group.

Chronic test – The rats were aneasthetised and 10 mg of sterile cotton pellets were inserted one in each axilla of rats. Extract (200 and 400 mg/kg), phenylbutazone (100 mg/kg), and control vehicle were administered (i.p.) for 7 consecutive days starting from the day of cotton pellet implantation. The animals were aneasthetised again on the 8th day and cotton pellets were removed surgically, freed from extraneous tissue, incubated at 37°C for 24 hrs, and dried at 60 °C to constant weight. Increment in the dry weight of the pellets was taken as a measure for granuloma formation (Winter and Porter, 1957).

Results and Discussion

The antiinflammatory activity of *L. lavand-*

ulaefolia against carrageenin induced acute pedal oedema has been shown in Table 1. The extract showed significant antiinflammatory activity which was comparable to that of phenylbutazone, prototype of nonsteroidal antiinflammatory agent. Carrageenin induced oedema is commonly used as an experimental animal model of acute inflammation and is believed to be biphasic. The first phase is due to release of histamine and serotonin the second phase is caused by the release of bradykinin, protease, prostaglandin and lysosome (Castro *et al.*, 1968). It has been reported that the second phase of oedema is sensitive to most clinically effective antiinflammatory agents (Smucker *et al.*, 1967). Carrageenin rat paw oedema is a suitable test for evaluating antiinflammatory drugs which has been frequently used to assess the anti-oedematous effect of natural products (Della Loggia *et al.*, 1986; Alcaraz *et al.*, 1988). Simultaneous subplantar administration of a mixture of the extract with carrageenin showed no counterirritant activity and showed clear-cut local antiinflammatory activity which has been explained in Table 2.

Depending on the above concept the effect of the extract against inflammations produced by different individual mediators were studied. The extract effectively suppressed the inflammation produced by histamine, serotonin (5HT). So, it may be suggested that it's anti 5HT activity possibly is responsible for it's antiinflammatory activity. The extract also reduced the oedema produced by dextran which is known to be mediated both by histamine and serotonin (Ghosh *et al.*, 1963) (Table 3) though the results obtained with dextran was statistically insignificant.

The effect of the extract on granuloma pouch in rats has been shown in Table 4. It was observed that the LLF extract significantly inhibited granuloma formation in rats. Multiplation of small blood vessels as well as proliferation of fibroblasts are the characteristics features at the repair phase of inflammation. Such proliferating cells penetrate the exudate, producing a highly vas-

Table 1. Effect of methanolic extract of *L. lavandulaefolia* on carrageenin induced pedal oedema in rats (Values are Mean±SE from six animals in each group)

Treatment	Dose (mg/kg)	Paw oedema volume in units	Percentage of inhibition
Control	-	48.5±3.2	-
Methanolic extract	200	31.3±2.8[a]	35.5
Methanolic extract	400	25.1±2.5[b]	48.2
Phenylbutazone	100	23.5±2.6[b]	51.5

(P-value was calculated by comparing with control by Student's t-test; [a]p<0.01; [b]p<0.001).

Table 2. Effect of *L. lavandulaefolia* extract mixed with carrageenin (values are Mean±SE from six animal in each group)

Drug	Dose (mg/kg)	Paw volumes in units	Inhibition percentage
Control	-	46.8±3.5	-
Extract	40	29.3±3.7[a]	37.39
Extract	50	27.4±3.8[a]	41.45

P-value was calculated by comparing with control by student's t-test; [a]p<0.01.

Table 3. Effects of *L. lavandulaefolia* extract on mediator induced pedal oedema in rats (N= 10; Mean±SE)

Treatment	Paw volume in units	% inhibition
Histamine (control)	45.3±2.6	-
Histamine with extract (400 mg/kg)	33.5±2.3[a]	26.0
Serotonin (control)	43.8±3.1	-
Serotonin with extract (400 mg/kg)	32.8±2.7[b]	25.0
Dextran (control)	39.2±2.2	-
Dextran with extract 400 mg/kg)	35.3±2.4[c]	9.9

P-Value was calculated by comparing with control by student's t-test; [a]p<0.01; [b]p<0.05; c not significant.

Table 4. Effect of *L. lavandulaefolia* extract on granuloma pouch in rats (N=10, Mean±SE)

Treatment	Dose (mg/kg)	Weight (mg)	Inhibition (%)
Control	-	46.8±2.1	-
Extract	200	38.2±1.9[a]	18.37
Extract	400	24.3±1.5[b]	48.1
Phenylbutazone	100	19.3±1.2[b]	58.7

P-value was calcullated by comparing with control by Student's t test; [a]$p<0.05$; [b]$p<0.001$.

cularized reddened mass known as granulation tissue (Swingle, 1974). The LLF extract effectively and significantly reduced cotton pellet granuloma suggesting its activity in the proliferative phase of the inflammation process.

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