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Anti-inflammatory and antinociceptive activities of *Lippia nodiflora* Linn.

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The methanolic extract of leaves of *Lippia nodiflora* Linn. was tested for its anti-inflammatory and antinociceptive activities. The extract showed a significant ($P < 0.001$) anti-inflammatory activity comparable to phenylbutazone against carrageenin-induced paw edema in rats and a significant ($P < 0.001$) antinociceptive activity comparable to diclofenac sodium in acetic acid induced writhing in white albino mice.

Lippia nodiflora Linn. (Verbinaceae) is a creeping perennial herb distributed throughout India, Ceylon, Bangladesh, Baluchistan, Africa and most tropical and subtropical regions. The plant is acrid, cooling, astringent to bowels, stomachic, anthelmintic; useful in diseases of the heart, the blood, the eye; good for ulcers, wounds, burning sensation, asthma (Kirtikar et al. 1987). Raj (1975) reported the anthelmintic action of the alcoholic extracts of *L. nodiflora* against human *Ascaris lumbricoides.* In 1983, Shanmugasundaram et al. reported it as an anti-atherosclerotic Indian drug. The main objective of this study was to evaluate the anti-inflammatory and antinociceptive activities of the methanolic extract of Lippia nodiflora (*L. nodiflora*).

The most widely used primary test for the screening of new anti-inflammatory agents is the carrageenin-induced edema model in the rat hind paw (Winter et al. 1962). The edema formation is a biphasic event. The initial phase, observed during the first hour, is attributed to the release of histamine and serotonin (Vinegar et al. 1969) and the delayed edema is due to the release of bradykinin and prostaglandins (Di Rosa et al. 1971; Flower et al. 1985). It has been reported that the second phase of edema is sensitive to steroidal and non-steroidal anti-inflammatory agents (Di Rosa et al. 1971). The experimental findings from the carrageenin-induced rat paw edema model showed that the methanolic extract of *L. nodiflora* reduced the paw volume significantly ($P < 0.001$) from 1 h to 5 h. The extract showed highest effects at the third hour where the inhibition was about 24% and 42% at the doses of 200 and 400 mg/kg respectively, which were comparable to phenylbutazone, where the inhibition was about 41%. These results tend to suggest that the significant activity of the extracts observed in the first phase of carrageenin induced inflammation may be due to inhibition of early mediators, such as histamine and serotonin and the action on the second phase may be due to the inhibition of bradykinins and prostglandins.

The antinociceptive activity of *L. nodiflora* was tested in the acetic acid-induced writhing model in mice. Acetic acid, which is used to induce writhing causes algesia by liberation of endogenous substances, which then excite the pain nerve endings (Taesotikul et al. 2003). The methanolic extract of *L. nodiflora* produced about 61% and 78% writhing inhibition at the doses of 250 and 500 mg per kg respectively, which was comparable to the standard drug

Table 1: Effect of methanolic extract of *L. nodiflora* on carrageenin induced rat paw edema

Animal group/Treatment	Time after carrageenin injection				
	1 h	2 h	3 h	4 h	5 h
	Edema volume × 1000 ml (Percent inhibition)				
Control 1% Tween-80 10 ml/kg; p.o.	90 ± 0	267.5 ± 1.628	287.5 ± 1.448	225 ± 0.1	183.75 ± 1.85
Positive control Phenylbutazone 100 mg/kg; p.o.	50 ± 1.37* (44.44)	149 ± 2.173* (44.30)	168 ± 2.527* (41.56)	141 ± 2.42* (37.33)	105 ± 1.66* (42.857)
Test group-1 Methanol extract 200 mg/kg; p.o.	75.5 ± 2.306* (19.44)	206 ± 1.996* (22.99)	217 ± 3.39* (24.35)	180 ± 3.299* (20)	165 ± 2.404* (10.20)
Test group-2 Methanol extract 400 mg/kg; p.o.	63 ± 1.581* (30)	168 ± 1.649* (37.19)	165.5 ± 1.48* (42.43)	168 ± 1.648* (25.33)	151.9 ± 1.82* (17.33)

Values are expressed as mean ± S.E.M. *, $P < 0.001$ vs. control, Student's *t*-test. p.o., per oral

Table 2: Effect of methanolic extract of *L. nodiflora* on acetic acid induced writhing in mice

Animal Group/Treatment	Number of writhes (% writhing)	Inhibition (%)
Control 1% Tween-80 solution in water, p.o.	9.4 ± 1.986 (100)	–
Positive control Diclofenac sodium 25 mg/kg, p.o.	2.4 ± 4.169* (25.53)	74.47
Test group-1 Me. extract 250 mg/kg, p.o.	3.7 ± 1.924* (39.36)	60.64
Test group-2 Me. extract 500 mg/kg, p.o.	2.1 ± 1.917* (22.34)	77.66

Values are expressed as mean ± S.E.M. *, $P < 0.001$ vs. control; Me., methanolic. %, percentage. p.o., per oral.

diclofenac sodium where the inhibition was about 74% at the dose of 25 mg/kg. Based on this, it could be concluded that the methanolic extract of *L. nodiflora* possesses an antinociceptive activity and the mode of action might involve a peripheral mechanism.

Experimental

1. Preparation of plant extract

The whole plants were collected in January 2003 from Khulna University Campus and identified by the National Herbarium of Bangladesh (accession no. 29754). One kg of powder of dried aerial parts of the plant was extracted with 90% methanol. The extract was evaporated to dryness and defatted. This extract was used for pharmacological screening.

2. Pharmacology

2.1. Animals

For the anti-inflammatory activity study, Wistar rats of either sex, weighing 180–200 g, purchased from the International Center for Diarrhoeal Disease and Research, Bangladesh (ICDDRB) were used. For the analgesic activity study, young Swiss-albino mice of either sex, weighing 20–25 g, bred in the animal house of the Department of Pharmacy, Jahangirnagar University, were used.

2.2. Anti-inflammatory activity testing

Anti-inflammatory activity of *L. nodiflora* was tested using the carrageenin induced rat paw edema model as described by Winter et al. (1962) and Taesotikul et al. (2003). Rats were randomly divided into four groups, each consisting of six animals. Group I was kept as control and received 1% tween-80 solution in water; group II was kept as 'positive control' and received the standard drug phenylbutazone at a dose of 100 mg per kg of body weight; group III and IV were test groups, treated with extracts at the doses of 200 and 400 mg per kg of body weight, respectively. Control vehicle, standard drug and methanolic extracts were given orally 1 h prior to the injection of 0.1 ml of 1% freshly prepared suspension of carrageenin. The paw volume was measured with a plethysmometer just before and 1, 2, 3, 4, 5 h after the carrageenin injection.

2.3. Antinociceptive activity testing

Antinociceptive activity of the methanolic extract of *L. nodiflora* was tested using the model of acetic acid induced writhing in mice as described by Whittle (1964). Experimental animals were randomly divided into four groups, each consisting of ten animals. Group I was kept as control and received 1% tween-80 solution in water; group II was given the standard drug diclofenac sodium at a dose of 25 mg per kg of body weight; group III and IV were treated with methanolic extracts of *L. nodiflora* at the doses of 250 and 500 mg per kg of body weight, respectively. Control vehicle, standard drug and the methanolic extracts were administered orally, 30 min prior to acetic acid (0.7%) injection. Then after an interval of 15 min, the number of writhes (squirms) was counted for 5 min.

3. Statistical analysis

Student's t-test was used to determine a significant difference between the control group and experimental groups.

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