

Research Article

Antinociceptive, Anti-inflammatory and Antipyretic Activities of Methanolic Extract of *Lasia spinosa* Leaves

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ABSTRACT

The present study was performed to investigate the scientific basis for the traditional uses of the crude methanolic extract of the leaves of the plant *Lasia spinosa*. The methanolic extract was evaluated for its antinociceptive activity in mice, anti-inflammatory and antipyretic activities in wistar rats. In acetic acid writhing method the crude extract induced significant decrease in the no. of writhes compared to standard diclofenac sodium. In the case of evaluation of anti-inflammatory activity by carrageenan induced rat paw edema, the methanolic extract showed significant inhibition of paw edema as compared to standard (diclofenac) in dose dependent manner. The methanolic extract showed potential lowering of body temperature in yeast induced pyrexia in albino Wistar rats at 200mg/kg and 400mg/kg body weight.

Keywords: *Lasia spinosa*, Antinociceptive, Antiinflammatory, Antipyretic.

INTRODUCTION

Lasia spinosa (Bengali name: Katakachhu Family: Araceae) is a stout intensely prickly marsh plant with creeping rootstock found throughout the country in low-lying shady areas, near water bodies. The plant is recommended for colic, rheumatism and intestinal diseases. Corm is used as a remedy for throat affections. Leaves and corms are given as a cure for piles in Khagrachari¹. The tuber of *Lasia spinosa* (L.) Thwaites are used for treatment of rheumatoid arthritis, constipation, and to purify blood in Rajshahi and Natore district of Bangladesh². *Lasia spinosa* rhizome possessed a wide-ranging antioxidant capacity³, antimicrobial property and cytotoxic activities⁴. The leaves are used as anticestodal agent⁵. Previous investigations on crude root extract demonstrated antinociceptive, anti-inflammatory and antidiarrhoeal activity⁶. Keeping in view of this collection and utilization of different parts of *L. spinosa*, an attempt was made to investigate the pharmacological activities of leaves of *Lasia spinosa* methanolic extract. To the best of the knowledge this is the first report on antinociceptive, anti-inflammatory, and antipyretic properties of

crude methanolic extract of *Lasia spinosa* leaves.

MATERIALS AND METHODS

Collection of plant material and extraction

L. spinosa leaves was collected from Sundarban area, Khulna in May 2011. The plant was identified by an expert taxonomist and a specimen representing this collection has been deposited in the Dhaka University Herbarium, Dhaka (Accession No. DUSH 1940) for further reference. The leaves of the plant were collected in fresh condition. The dried and coarse powder (650g) was extracted with methanol (2.5liters) in an air tight, clean flat-bottomed container for 15 days at room temperature with occasional stirring. The extract was then filtered through a fresh cotton plug followed by a Whatman No. 1 filter paper. The filtrate was concentrated using a rotary evaporator at low temperature (40-45°C) and pressure. The weight of the crude extract was 23gm.

Phytochemical screening

The methanolic extract was subjected to preliminary phytochemical screening for the detection of major chemical groups⁷.

⁸.The results of different chemical tests on the methanolic extract of the leaves of *Lasia spinosa* showed the presence of alkaloids, flavonoids, tannins and glycosides.

Experimental animal

Swiss-albino mice (20-25 g) of either sex, aged 4-5 weeks, and Wistar albino rats of either sex (100-150 g) obtained from the Animal Resource Branch of Jahangirnagar University, Dhaka, Bangladesh were used for the experiment. They were kept in standard environmental condition and fed standard formulated rodent food and water. As these animals are very sensitive to environmental changes, they are kept before the test for at 7 days in the environment where the experiment will take place.

Antinociceptive study

Effect on acetic acid-induced writhing in mice

Analgesic activity was evaluated by the acetic acid induced writhing method in mice⁹. The writhes were induced by intraperitoneal injection of 0.7% acetic acid (v/v) (10 ml kg⁻¹). Two different doses (200 and 400 mg kg⁻¹) of the extract were administered orally to different groups of 5 animals each, 30 min before chemical stimulus. The number of writhes during the following 15 min period was observed after acetic acid injection. Antinociception was expressed as the reduction of the number of abdominal constrictions between control animals (acetic acid treated mice) and mice pretreated with the extract. Diclofenac sodium (50 mg kg⁻¹, i.p.) was used as standard.

Anti-inflammatory study

Carrageenan-induced paw oedema in rats

Inflammation in Wistar rats (150-200 g) was produced according to the method described by Winter¹⁰. An injection (s.c.) was made of 0.1 ml of 1% carrageenan into the right paw of each rat under the sub plantar aponeurosis. The rats were divided into 4 groups (n = 5). The different groups were treated with extract (200, 400 mg kg⁻¹, p.o.), diclofenac (100 mg kg⁻¹, p.o.) and control vehicle per oral. The paw

volume was measured immediately after carrageenan injection and at 1-4 h intervals after the administration of the edematogenic agent using a Plethysmometer (Ugo Basile, Italy) apparatus up to the anatomical hairline on lateral malleolus and compared with the control animals which received only the vehicle. The inhibitory activity was calculated according to the following formula

$$\% \text{ inhibition} = (\text{Vc}-\text{Vt}) / \text{Vc} \times 100$$

Where:

Vc= edema volume of control

Vt= edema volume of test

Antipyretic study

Yeast induced hyperthermia

4 groups of 5 rats each were injected subcutaneously with 10 ml kg⁻¹ body weight of Yeast suspension (15% aqueous suspension) to induce pyrexia after measuring the basal rectal temperature of each animal. About 18h after yeast injection, the rectal temperature was recorded again and animals showing an increase in temperature of at least 0.6°C (or 1°F) were used for further experiment. Then the extract was administered orally at doses of 200 and 400 mg kg⁻¹ body weight to two groups of animals, respectively. About 10% propylene glycol (5 ml kg⁻¹, body weight) was administered orally to the control group of animals. The 4th group of animals received the standard drug paracetamol (100 mg kg⁻¹, body weight.) orally. After the drug was administered, the temperature of all the rats in each group was recorded at 1 hr, 2 hr, and 3hr. The mean temperature was found out for each group and compared with the value of standards drug after yeast injection.

Statistical analysis

All values were expressed as the mean ± standard error of the mean (SEM) and the results were analyzed statistically by one way analysis of variance (ANOVA) followed by Dunnett's test by using SPSS ver.17. P < 0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION

The results from the present study show that the crude extract of *L. spinosa* exhibited activities in various degrees against pain, inflammation and fever. The methanolic crude extract (400 mg/kg b.w.) showed significant antinociceptive activity having 47.65% of inhibition respectively compared to the standard diclofenac (51.67%) (Table 1).

The methanolic extract of *L. spinosa* (4000 mg/kg) reduced the edema induced by carrageenan by 34.43%, 47.78%, 61.92% and 76.53% after 1st, 2nd, 3rd and 4th hr injection of noxious agent carrageenan (Table 2). The rate of anti-inflammatory activity was increased with time and reached the peak level at 4th hour of study which was comparable to standard diclofenac at 100 mg/kg (89.53% inhibition).

The results (Table 3) showed that the leaves extract at doses of 200 mg/kg caused significant lowering of the body temperature at 4 hr. The normal mean temperature 100.26 °F at 0 hr was reduced to 99.04 °F at 4 hr. while maximum lowering of body temperature was noticed at 400 mg/kg of the leave extract, as the mean temperature of 100.46 °F was reduced to 98.7 °F within 3-4hr period in a dose-dependent manner.

In the present study analgesic, anti-inflammatory and antipyretic activities of *L. spinosa* have been established. Acetic acid induced writhing model represents pain sensation by triggering localized inflammatory response. Acetic acid, which is used to induce writhing, causes analgesia by liberation of endogenous substances, which in turn excite the pain nerve endings¹¹. It has been postulated that acetic acid acts indirectly by inducing the release of endogenous mediators, such as PGE2 (prostaglandin E2) and PGE2 α in peritoneal fluids, as well as lipooxygenase products, which stimulate the nociceptive neurons sensitive to NSAIDs^{12,13}. Therefore, the results of the acetic acid-induced writhing strongly suggests that the mechanism of this extract may be linked partly to the inhibition of lipooxygenase and/or cyclooxygenase in the peripheral

tissues, thereby reducing PGE2 synthesis and interfering with the mechanism of transduction in the primary afferent nociceptor.

The development of edema in the paw of the rat after the injection of carrageenan has been described by Vinegar as a biphasic event. The initial phase, observed around 1 hr, is attributed to the release of histamine and serotonin; the second, accelerating, phase of swelling is due to the release of prostaglandin-like substances. It has been reported that the second phase of edema is sensitive to both clinically useful steroidal and non-steroidal anti-inflammatory agents^{14, 15}. The significant activity observed in the suppression of the first and second phases of carrageenan-induced inflammation may due to inhibition of the release of the early mediators such as histamine, serotonin and kinins¹⁶. The action on the second phase may be explained by an inhibition of cyclooxygenase, a prostaglandin derivative¹⁷. Thus, the results obtained for the writhing test using acetic acid are similar to those obtained for the odematogenic test using carrageenan. Therefore, anti-inflammatory substances may also be involved in the peripheral analgesic activity.

Indeed a number of plant extracts modulate enzymes of cyclooxygenase pathway, which inhibit leukotriene and prostaglandins synthesis by inhibiting COX-1 and COX-2 pathways¹⁸. The crude methanolic extracts of *L. spinosa* demonstrated effective antipyretic activity as evident in the inhibition of the temperature elevation in the yeast model. The antipyretic action of the extract may possibly be through inhibition of prostaglandin production, leading to suppression of elevated plasma level especially since the extract had been shown to possess analgesic and anti-inflammatory activities.

CONCLUSION

From these investigations it may be concluded that the crude methanolic extracts of *L. spinosa* showed analgesic, anti-inflammatory and antipyretic effects. It is important to point out that the

phytochemical analysis showed the presence of flavonoids, tannins and glycosides and this might be responsible for observed activities. Further investigations are required in the laboratory to isolate and characterize the specific bioactive components of the plant extract which is responsible for observed pharmacological actions.

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Table 1: Antinociceptive activity of methanolic (crude) extract of *L. spinosa* leave

Group	Number of Writhing (Mean \pm SEM)	% Inhibition of Writhing
Control	29.80 \pm 1.02	-----
Standard	14.4 \pm 0.93*	51.67
Methanol (Crude) extract- (200 mg/kg body weight)	23.2 \pm 1.24*	22.14
Methanol (Crude) extract - (400 mg/kg body weight)	15.6 \pm 1.21*	47.65

Each value represents mean \pm SEM (n= 5). *P < 0.01 compared with control (one way ANOVA followed by Dunnett's test).

Table 2: Effect of oral administration of extracts on carrageenan induced right hind paw oedema in wistar rats

Group	Increase in Paw volume (ml)			
	1 st hr	2 nd hr	3 rd hr	4 th hr
Control	1.12 \pm 0.046	1.162 \pm 0.055	1.192 \pm 0.057	1.234 \pm 0.068
Standard	0.920 \pm 0.034 (34.43)	0.844 \pm 0.057** (60.09)	0.826 \pm 0.030** (66.97)	0.740 \pm 0.035** (89.53)
Crude extract (200 mg/kg)	0.948 \pm 0.01 (16.25)	0.912 \pm 0.017** (33.99)	0.886 \pm 0.013** (44.49)	0.840 \pm 0.020** (64.62)
Crude extract (400 mg/kg)	0.994 \pm 0.026 (34.43)	0.968 \pm 0.062 (47.78)	0.922 \pm 0.080** (61.92)	0.886 \pm 0.059** (76.53)

Percentage inhibitions are indicated in brackets. All values are expressed as mean \pm SEM (n=5). **P < 0.01, * P < 0.05 compared to control.

Table 3: Effect of oral administration of extract on yeast induced pyresis in wistar rats

Group	Treatment	Dose (mg/kg body weight)	Initial Rectal Temp. in °F before Yeast Injection	Rectal Temperature in °F after 18hrs of Yeast Injection				
				0 hour	1 hour	2 hour	3 hour	4 hour
I	Control	----	99.0 \pm 0.20	100.6 \pm 0.30	100.7 \pm 0.277	100.7 \pm 0.187	100.68 \pm 0.191	100.66 \pm 0.194
II	Paracetamol	100	98.56 \pm 0.282	100.56 \pm 0.326	99.82 \pm 0.124*	99.2 \pm 0.192**	98.86 \pm 0.051**	98.32 \pm 0.097**
III	Methanolic extract	200	98.40 \pm 0.173	100.26 \pm 0.275	100.2 \pm 0.259	100.1 \pm 0.19	99.54 \pm 0.121**	99.04 \pm 0.129**
IV	Methanolic extract	400	98.32 \pm 0.227	100.46 \pm 0.216	100.18 \pm 0.229	99.68 \pm 0.198**	98.92 \pm 0.287**	98.7 \pm 0.134**

All values are expressed as mean \pm SEM (n=5). ** P < 0.01, * P < 0.05 compared to control (one way ANOVA followed by Dunnett's test).

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