

Evaluation Of Antiinflammatory And Antinociceptive Properties Of *L.siceraria* Aerial Parts

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ABSTRACT

Purpose: The objective of the present study was to evaluate the anti-inflammatory and antinociceptive activity of the methanol extract of *Lagenaria siceraria* aerial parts (MELS) at the doses of 200 and 400 mg/kg body weight. **Methods:** Hot plate, tail immersion and acetic acid induced writhing response in mice were used to assess antinociceptive activity. Antiinflammatory property was studied using acute inflammatory models (such as, carrageenan, dextran and histamine-induced rat paw oedema) as well as on chronic inflammatory model (cotton pellet granuloma test). **Results:** Potent antinociceptive activity was exerted by the extract. In case of acute inflammatory models, MELS inhibited rat paw oedema in dose dependent manner. In the chronic inflammatory model, daily dosing of 400 mg/kg of the extract significantly suppressed granuloma formation. **Conclusion:** Thus the extract of *L. siceraria* exhibited significant anti-inflammatory and antinociceptive activities.

Keywords: Anti-inflammatory activity, Antinociceptive activity, *L.siceraria*, Cucurbitaceae, carrageenan.

1. INTRODUCTION

Inflammation is a defense mechanism and protective response of vascular tissues to harmful stimuli like allergens, pathogens, irritants, damaged cells [1]. However if it runs unchecked, may lead to onset of diseases such as arthritis, atherosclerosis, Alzheimer's disease, diabetes, cancer and so on [2]. Algesia (pain) is an ill-defined, unpleasant sensation occurs by external or internal noxious stimuli. Pain is a warning signal, excessive pain is unbearable and leads to sinking sensation, sweating, nausea, rise or fall in BP, tachypnoea. Many synthetic drugs are available in market to treat inflammation and pain, leading to side effects [3,4]. As a result search for alternatives is of the utmost importance. The ethnopharmacological uses as well as certain biological activities exhibited by *L. siceraria* indicate it to be a good source of phytomedicine.

Lagenaria siceraria (Mol.) Standley from cucurbitaceae family, popularly known as bottle gourd (English), has wide occurrence throughout India as an edible vegetable. It is a pubescent or trailing herb, with bottle or dumb-bell shaped fruits. Both of its aerial parts and fruits are commonly consumed as vegetable as well as used as traditional medicine in various countries like India, China, European countries, Brazil, Hawaiian island etc [5]. Antihepatotoxic, analgesic and anti-inflammatory, hypolipidemic, antihyperglycemic and antioxidant activities of its fruit extract have been evaluated. *Lagenaria siceraria* fruits are good source of vitamin B complex, ascorbic acid, fibers, proteins, cucurbitacins, saponins, fucosterols and compesterols, polyphenolics, flavones-C-glycoside. Methanol extract of its leaves showed the presence of sterols, polyphenolics, flavonoids, saponin, protein and carbohydrates [6-14]. Traditionally *L. siceraria* has been used in treatment of many types of pain and inflammatory conditions, however no such scientific report is available on its aerial parts. The present study was therefore carried out to investigate the anti-inflammatory and antinociceptive activities of methanol extract of *L.siceraria* aerial parts (MELS).

2. MATERIALS AND METHODS

2.1.Plant material

The aerial parts of *L.Siceraria* were collected in November 2008, from Madanpur, West Bengal, India and identified by the Botanical Survey of India, Howrah, India. A voucher specimen (P/LS/1/08) was retained in our laboratory for further reference.

2.2.Preparation of plant extract

The aerial parts were dried and powdered in a mechanical grinder. The powdered material was extracted by methanol using soxhlet apparatus. This extract was filtered and concentrated in *vacuo* and kept in a vacuum

desiccator for complete removal of solvent. The yield was 18.13% w/w with respect to dried powder. Aqueous suspension of MELS was prepared using 2 % (v/v) Tween-80 and used for the pharmacological studies.

2.3. Animals

Healthy Swiss albino mice (20 ± 2 g) of either sex were taken for acute toxicity study. Healthy Wistar albino male rats ($150 \text{ g} \pm 20$) were used for the antiinflammatory study and healthy Swiss albino male mice (20 ± 2 g) were used for the antinociceptive study. The animals were maintained at standard laboratory conditions and fed with commercial pellet diet (Hindustan Lever, Kolkata, India) and water *ad libitum*. The animals were acclimatized to laboratory condition (temperature $25 \pm 2^\circ\text{C}$) with dark/ light cycle (12 /12 h) for one week prior to the study. Before the experiment, the animals were fasted for 18 h. The experiments were performed following the guidelines of the Institutional Animals Ethical Committee.

2.4. Phytochemical analysis

Preliminary phytochemical screening of the extract was carried out using standard methods [15].

2.5. Acute toxicity study

Healthy Swiss albino mice (20 ± 2 g), starved overnight, were divided into five groups (n=4). Group I-IV animals were orally fed with MELS in increasing dose levels of 0.5, 1.0, 1.5 and 2.0 g/kg b.wt, while group V (untreated) served as control. The animals were observed continuously for first 2 h for any gross change in behavioral, neurological and autonomic profiles or any other symptoms of toxicity and mortality if any, and intermittently for the next 6 h and then again after 24 h, 48 h and 72 h for any lethality or death. One-tenth and one-fifth of the maximum safe dose of the extract tested for acute toxicity, were selected for the experiment [16].

2.6. Antiinflammatory activity

2.6.1. Carrageenan induced rat paw oedema

The rats were randomly divided into four groups (n=6). The rats in different groups were treated orally with MELS (200 and 400 mg/kg b.wt.), the reference drug - indomethacin (10 mg/kg b.wt.), and vehicle (5 ml/kg of 2% v/v Tween -80). The extracts and drugs were administered 30 min prior to subplantar injection of 0.1 ml of 1% w/v freshly prepared suspension of carrageenan in normal saline in the right hind paw of each rat [17]. Using plethysmometer, the paw volume was measured initially (at 0th h) and then at 1, 2, 3 and 4 h after carrageenan injection.

The antiinflammatory activity was calculated by the following equation:

Antiinflammatory activity (% inhibition of rat paw oedema) = $(1-D/C) \times 100$, where, D represents difference in paw volume after extract/drug administration and C represents difference in paw volume in control groups [18].

2.6.2. Dextran induced rat paw oedema

The animals were treated in a manner similar to that of carrageenan induced paw oedema models; dextran (0.1 ml 1% w/v in normal saline) was used instead of carrageenan [17].

2.6.3. Histamine induced rat paw oedema

In this model rat hind paw oedema was induced by subplantar injection of 0.1 ml 1% w/v freshly prepared histamine in normal saline and the paw oedema was measured by the method of Suleyman *et al* [18].

2.6.4. Cotton pellet granuloma

Cotton pellet granuloma was induced according to the method of D'Arcy *et al* [19]. The animals were divided into four groups (n=6). The rats were anesthetized and sterile cotton pellets weighing 10 ± 1 mg were implanted subcutaneously into both sides of the groin region of each rat. Group I served as control and received vehicle (5 ml/kg of 2% v/v Tween -80). The extract, MELS, at the dose of 200 and 400 mg/kg was orally administered to group II and III animals for 7 consecutive days, starting from the day of implantation. Group IV rats received indomethacin (10 mg/kg, p.o.) for the same period. On 8th day, the animals were anesthetized and the pellets together with the granuloma tissues were carefully collected and made free from extraneous tissues. The wet pellets were weighed and then dried in an oven at 60°C until a constant weight was obtained. Increase in the dry weight of the pellets was taken as the measure of the granuloma formation.

2.7. Antinociceptive activity

2.7.1. Hot Plate latent pain response test

The mice were randomly divided into four groups (n=6). Group I served as control and received the vehicle (2% v/v Tween -80), group II and III received MELS at low and high dose (200 and 400 mg/kg, p.o.) respectively, while group IV was treated with standard drug, Pentazocin (5 mg/kg, s.c.), the reference drug. The hot-plate test was carried out according to the method of Eddy and Leimback [20]. Each mouse was placed on a $55^\circ \pm 1^\circ\text{C}$ hot-plate to observe its pain responses (hind-paw-licking or jumping). The latent time was recorded as the analgesic parameter at before (0 min) and 30, 60, 90, 120 and 150 min after administration of test drugs.

Untreated mice with a background latent response time shorter than 5 s or longer than 30 s were excluded from the study.

2.7.2. Tail immersion test

The mice were randomly divided into four groups of six animals in each. Group I served as control and received the vehicle (2% v/v Tween -80), group II and III received MELS at low and high dose (200 and 400 mg/kg, p.o.) respectively, while group IV was treated with standard drug Pentazocin (5 mg/kg, s.c.). The basal reaction time to the heat stimuli was measured by immersing the tip of the tail (1-2cm) in a beaker of warm water maintained at $55^{\circ}\pm 1^{\circ}\text{C}$. The readings were taken after 30 min of administration of test drug [21]. Tail withdrawal was taken as the end point, a cut off point of 15 sec was maintained to prevent the damage to the tail.

2.7.3. Acetic acid induced writhing test

Acetic acid induced writhing test, a chemical visceral pain model, was carried out by the method of Koster *et al* [22]. The mice were randomly divided into four groups (n=6). Group I served as control and received the vehicle (2% v/v Tween -80), group II and III received MELS at low and high dose (200 and 400 mg/kg, p.o.) respectively, while group IV was treated with Indomethacin (10 mg/kg, p.o.). Extracts and the standard drug were administered to the animals 1 h prior to the injection of acetic acid. Intraperitoneal injection of acetic acid (0.7%) at a dose of 0.1 ml/10g of body weight was used to create pain sensation. After acetic acid injection, the mice were placed in a clean box and the number of writhes was recorded for 10 min. Writhing movement was accepted as contraction of the abdominal muscle accompanied by stretching of hind limbs. The analgesic effects were calculated by comparing the number of abdominal writhes of the test group with that of the control group.

2.8. Statistical analysis

The values were expressed as mean \pm S.E.M. The statistical significance was determined by Oneway ANOVA followed by post hoc Dunnett's test. Values of $p < 0.05$ were considered as statistically significant.

3. RESULTS

3.1. Phytochemical analysis

Preliminary phytochemical screening of MELS revealed the presence of polyphenolics, flavonoids, glycosides, triterpenoids, saponin and carbohydrates.

3.2. Acute toxicity study

In acute toxicity study, MELS did not show any toxic effect upto the dose of 2 g/kg b.wt, accordingly 200 and 400 mg/kg b.wt were taken as low and high dose of MELS for the experiment.

3.3. Antiinflammatory activity

Subplantar injection of carrageenan in hind paw induced gradual increase in the paw volume in the control group. Methanol extract of *L.siceraria* at the doses of 200 and 400 mg/kg significantly ($p < 0.05$) inhibited the oedema formation of rat paw after carrageenan challenge (Table.1). The reference drug, indomethacin at a dose of 10 mg/kg was also found to reduce the paw oedema significantly.

The differences in the paw volume after administration of extracts and standard drug indomethacin in dextran inoculated rats were presented in Table.2. At the dose of 200 and 400 mg/kg, MELS exhibited 37.50 and 47.92 % inhibition respectively in dextran induced paw oedema in rats. Significant anti-inflammatory activity produced by the extracts especially at high dose was quite comparable to that of the standard drug.

The antiinflammatory activity of MELS against acute pedal oedema induced by phlogestic agent histamine has been summarized in Table.3. Administration of the extract significantly inhibited the development of paw swelling after histamine injection in a dose dependent manner.

The effects of MELS and indomethacin on the proliferative phase of inflammation are summarized in Table.4. Significant reduction in the weight of cotton pellets i.e., marked reduction in granuloma formation was observed in case of MELS (400 mg/kg) treated rats as compared to that of vehicle treated ones and the effect was equipotent to that of standard drug treated rats. However the degree of reduction was not significant in case of low dose extract treated animals.

3.4. Antinociceptive activity

MELS produced significant analgesic activity at both low and high dose. In case of hot plate latent pain response test, a considerable increase in the reaction time to the heat stimulus was observed with respect to that of control group mice (Table.5). The highest reaction time of MELS (at high dose) was found to be 13.12 ± 0.68 s, however that of the standard drug, pentazocin was recorded as 14.55 ± 0.50 s.

In the tail immersion method, the extract considerably increased the latency of the mice to the heat stimulus. Values were found to be significant and dose dependent. Pre-treatment with MELS showed upto 7.00 ± 0.25 s of latency period while that produced by the standard drug pentazocin was found to be 7.80 ± 0.33 s (Table.6).

In case of the acetic acid induced writhing test, methanol extract of *L.siceraria* at doses of 200 and 400 mg/kg significantly reduced the acetic acid induced abdominal constrictions (Table.7) and the average number of writhes was significantly lowered upto 10.88 (59.25%) by MELS treatment. Analgesic effect of the extract was comparable to that of the standard drug, indomethacin (62.92%).

4. DISCUSSION

The present study explored potent antiinflammatory activity of MELS in both acute inflammatory (carrageenan, dextran, histamine) as well as chronic inflammatory (cotton pellet granuloma) studies in rats.

Carrageenan induced oedema has been commonly used as an experimental animal model for acute inflammation and is believed to be a biphasic event with involvement of different inflammatory mediators [23]. In the first phase (during the first 2 h after carrageenan injection), chemical mediators such as histamine and serotonin play important role, while the second phase (3 – 4 h after carrageenan injection) is sustained by prostaglandins release and mediated by bradykinin, leukotrienes, polymorphonuclear cells and prostaglandins produced by tissue macrophages. Present study revealed that administration of methanol extract of *L. siceraria* inhibited the oedema starting from the first hour and during all phases of inflammation, however, it was found to be more pronounced in later phase. This may be attributed to the inhibition of different aspects and chemical mediators of inflammation by MELS in the system.

Dextran induced oedema is a well-known experimental model in which the oedema is a consequence of liberation of histamine and serotonin from the mast cell [24]. In acute inflammatory model, histamine and serotonin are the important inflammatory mediators and act as vasodilator as well as increase the vascular permeability [3, 25]. Both the extracts were found to effectively suppress the dextran induced rat paw oedema as well as the oedema produced by histamine. The results suggest that the anti-inflammatory activity of the extract is possibly backed by its antihistamine and/or antiserotonin activity.

The cotton-pellet granuloma is widely used to evaluate the transudative and proliferative components of the chronic inflammation. The moist weight of the pellets correlates with transudate, the dry weight of the pellet correlates with the amount of granulomatous tissues [26, 27]. Chronic inflammation occurs by means of the development of proliferate cells.

The methanol extract of *L. siceraria* at high dose showed significant anti-inflammatory activity in cotton pellet induced granuloma, which reflects their efficacy in inhibiting granulocyte infiltration and the increase in the number of fibroblasts and preventing the synthesis of collagen and mucopolysaccharides during granuloma tissue formation and thus found to be effective in chronic inflammatory conditions. However at low dose the extracts could not produce significant effect in the chronic inflammation model.

The extract exerted potent antinociceptive activity in hot plate test and the results were equivalent to that of the standard drug during all the observation times when compared with control values. The hot plate method is considered to be selective for opioid like compounds in several animal species, however other centrally acting drugs including sedatives and muscle relaxants have also shown activity in this test. The results obtained from the present study reveals that the methanol extract of *L. siceraria* aerial parts relieved pain may be through central mechanism.

The extract also had a significant effect on acute pain model, viz. tail immersion test. Centrally acting analgesic drugs elevate pain threshold of animals towards heat and pressure. The effect of the extract on this pain model therefore further potentiates their antinociceptive effect through central mechanism.

Acetic acid induced abdominal constriction method is widely used for the evaluation of peripheral antinociceptive activity [28]. It is very sensitive and able to detect antinociceptive effects of compounds at dose levels that may appear to be inactive in other models, like tail flick test [29]. It has been reported that acetic acid irritates the peritoneal cavity leading to stimulation of local nociceptors located at the surface of the peritoneal cavity. This leads to the release of prostaglandins and other algogens with subsequent stimulation of pain at nerve endings. Local peritoneal receptors are postulated to be partly involved in the abdominal constriction response [30]. Inhibition of acetic acid induced pain by the extract suggests that it probably reduced pain response to acetic acid injection by suppressing the release of local inflammatory mediators like prostaglandin, bradykinin and histamine [31].

5. CONCLUSION

On the basis of above findings it may be concluded that methanol extracts of *L. siceraria* have potent antiinflammatory activity against both acute and chronic phases of inflammation and these may be beneficial in case of cancer, diabetes and rheumatoid arthritis condition by inhibiting the underlying pathological progression of inflammation [32]. Previous investigations showed that MELS possesses significant antioxidant activity [33]. The extract may exert its potent anti-inflammatory activity at least partially through its antioxidant activity. The promising analgesic activity observed in the present study on all the three models indicate that the methanol extracts of *L. siceraria* aerial parts can relieve pain through both central as well as peripheral mechanisms.

However further studies are required to establish the bioactive principle(s) and confirm the mechanism of action for these potent activities of the extract of *L. siceraria* aerial parts.

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Table.1. Effect of methanol extract of aerial parts of *L. siceraria* (MELS) on carrageenan induced rat hind paw oedema

Groups	Mean Paw oedema (% Inhibition)			
	1h	2h	3h	4h
Control (2% Tween 80)	0.58±0.03	0.60±0.05	0.68±0.06	0.70±0.08
MELS (200mg/kg)	0.52±0.06 (10.35)	0.53±0.03 (11.67)	0.49±0.02* (27.94)	0.47±0.06* (32.86)
MELS (400mg/kg)	0.44±0.07* (24.14)	0.46±0.03* (23.33)	0.43±0.09* (36.77)	0.38±0.02* (45.71)
Indomethacin (10 mg/kg)	0.48±0.06* (17.24)	0.43±0.07* (28.33)	0.39±0.05* (42.65)	0.40±0.05* (42.86)

Values are mean ± S.E.M.; n=6; * $p < 0.05$, when compared to control group animals

Table.2. Effect of methanol extract of aerial parts of *L. siceraria* (MELS) on dextran induced rat hind paw oedema

Groups	Mean Paw oedema (% Inhibition)			
	1h	2h	3h	4h
Control (2% Tween 80)	0.48±0.05	0.47±0.05	0.51±0.06	0.48±0.04
MELS (200mg/kg)	0.41±0.07 (14.58)	0.37±0.05 (21.28)	0.32±0.09 (37.26)	0.30±0.01* (37.50)
MELS (400mg/kg)	0.36±0.03* (25.00)	0.33±0.03* (29.79)	0.30±0.05* (41.18)	0.25±0.03* (47.92)
Indomethacin (10 mg/kg)	0.33±0.06* (31.25)	0.27±0.04* (42.55)	0.25±0.08* (50.98)	0.23±0.04* (52.08)

Values are mean ± S.E.M.; n=6; * $p < 0.05$, when compared to control group animals

Table.3. Effect of methanol extract of aerial parts of *L. siceraria* (MELS) on histamine induced rat hind paw oedema

Groups	Mean Paw oedema (% Inhibition)			
	1h	2h	3h	4h
Control (2% Tween 80)	0.54±0.06	0.56±0.05	0.57±0.04	0.58±0.04
MELS (200mg/kg)	0.45±0.05 (16.67)	0.41±0.03* (26.79)	0.40±0.04* (29.83)	0.38±0.05 (34.48)
MELS (400mg/kg)	0.38±0.03* (29.63)	0.33±0.03* (41.07)	0.31±0.05* (45.61)	0.29±0.03* (50.00)
Indomethacin (10 mg/kg)	0.42±0.03* (22.22)	0.37±0.04* (33.93)	0.32±0.05* (43.86)	0.30±0.03* (48.27)

Values are mean ± S.E.M.; n=6; * $p < 0.05$, when compared to control group animals

Table.4. Effect of methanol extract of aerial parts of *L. siceraria* (MELS) on cotton pellet induced granuloma in rats

Groups	Weight (dry) of cotton pellets (mg)	% Inhibition
Control (2% Tween 80)	36.45±2.65	-
MELS (200mg/kg)	33.00±1.56	9.47
MELS (400mg/kg)	21.05±1.33*	42.25
Indomethacin (10 mg/kg)	20.80±1.54*	42.94

Values are mean ± S.E.M.; n=6; * $p < 0.05$, when compared to control group animals.

Table.5. Antinociceptive effect of methanol extract of aerial parts of *L. siceraria* (MELS) on the latency of mice exposed to hot plate

Groups	Latency (s)					
	0 min	30 min	60 min	90 min	120 min	150 min
Control (Tween80)	6.38±0.67	6.44±0.74	6.01±0.35	5.96±0.78	5.52±0.88	6.52±0.58
MELS (200mg/kg)	6.40±0.18	8.70±1.06	11.94±0.70*	10.84±0.98*	9.35±0.58*	8.90±1.25
MELS (400mg/kg)	6.39±0.80	9.50±0.50*	13.12±0.68*	12.98±0.96*	10.88±0.66*	10.00±0.95*
Pentazocin (5 mg/kg)	6.40±0.20	9.95±0.63*	12.98±0.34*	14.55±0.50*	13.04±0.95*	11.90±0.74*

Values are mean ± S.E.M.; n=6; * $p < 0.05$, when compared to control group animals

Table.6. Antinociceptive effect of methanol extract of aerial parts of *L. siceraria* (MELS) on the tail immersion test of mice

Groups	Tail withdrawal time (s)					
	0 min	30 min	60 min	90 min	120 min	150 min
Control (2% Tween 80)	2.90±0.34	2.80±0.60	2.30±0.45	2.50±0.33	2.60±0.80	2.40±0.33
MELS (200mg/kg)	2.33±0.60	4.80±1.30	5.85±0.50*	6.30±0.21*	5.40±1.40	4.77±0.90
MELS (400mg/kg)	2.81±0.33	5.98±0.48*	7.00±0.25*	6.70±0.30*	6.18±0.13*	5.90±0.31*
Pentazocin (5 mg/kg)	2.83±0.40	5.90±0.45*	6.90±0.50*	7.80±0.33*	7.50±0.40*	6.90±0.60*

Values are mean ± S.E.M.; n=6; * $p < 0.05$, when compared to control group animals

Table.7. Antinociceptive effect of methanol extract of aerial parts of *L. siceraria* (MELS) on acetic acid induced writhing in mice

Groups	Number of Writhing	% Inhibition
Control (2% Tween 80)	26.70±1.40	-
MELS (200mg/kg)	19.50±1.00*	26.97
MELS (400mg/kg)	10.88±1.10*	59.25
Indomethacin (10 mg/kg)	9.90±1.50*	62.92

Values are mean ± S.E.M.; n=6; * $p < 0.05$, when compared to control group animals