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Analgesic and Anti-Inflammatory activities of *Lagenaria siceraria* Stand. fruit juice extract in rats and mice

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ABSTRACT- Present work was undergone to investigate analgesic and anti-inflammatory effects of *Lagenaria siceraria* (Molina) Stand. fruit juice extract (LSFJE) in rats and mice. LSFJE was studied for its analgesic effect on acetic acid-induced writhing and formalin pain tests in mice. The anti-inflammatory effects were investigated employing the acute inflammatory models, i.e. ethyl phenylpropionate-induced ear edema, carrageenin- and arachidonic acid-induced hind paw edema, and also the albumin-induced paw edema in rats. LSFJE was also studied for its preliminary phytochemical screening and acute toxicity studies. LSFJE (150-300 mg/kg, p.o.) exhibited a dose-dependent inhibition of writhing and also showed a significant ($P<0.001$) inhibition of both phases of the formalin pain test, but with a less intense effect on the first than on the second phase. The effects of the extract were significantly ($P<0.01$) lower than those produced by morphine (10 mg/kg) and aspirin (300 mg/kg) in the same tests. LSFJE elicited significant ($P<0.05$) inhibitory effect on the ear edema formation at 30 min, 1 h and 2h after EPP-injection. In other acute inflammatory models, the extract significantly inhibited carrageenin- and arachidonic acid-induced hind paw edema. LSFJE also caused inhibition of albumin-induced paw edema over a period of 90 min. The extract did not produce mortality in dose up to 4.2 g/kg, p.o. and 0.29 g/kg, i.p. Preliminary phytochemical screening revealed the presence of flavonoids, cucurbitacin, saponins, proteins, and carbohydrates. The results obtained suggest marked analgesic and anti-inflammatory activity of the LSFJE. The results support the traditional use of this plant in some painful and inflammatory conditions.

KEYWORDS- *Lagenaria siceraria*, Cucurbitaceae, Analgesic, Ant-inflammatory, Acute and chronic models

INTRODUCTION

Lagenaria siceraria (Molina) Standley syn. *L. leucantha* Rusby; *L. Vulgaris* Ser. (Family: Cucurbitaceae) is commonly known as Bottle gourd, an excellent fruit in the nature having composition of all the essential constituents that are required for normal and good health of humans (1). *L. siceraria* fruits are traditionally used for its cardioprotective, cardiotonic, general tonic, aphrodisiac and acts as alternate purgative, diuretic (2, 3). It also cures pain, ulcers, fever, and used for pectoral cough, asthma and other bronchial disorders (2). The fruits are edible and considered as good source of vitamin C, β-carotene, vitamin B-complex, pectin and also contain highest choline level- a lipotropic factor (1, 4, 5). Modern phytochemical screening methods showed the presence of triterpenoid cucurbitacins B, D, G, H (4, 6, 7) fucosterol, campesterol (8) and flavone C-glycosides (9). *L. siceraria* seeds are used in migraine type headache and pain and reported to contain saponins, essential fixed oils, vitamins (1, 5). Lagenin- a novel ribosome inactivating protein has been isolated from the lyophilized water extract of seeds which is known to possess immunosuppressive, antitumour, antiviral, antiproliferative and anti-HIV activities (10).

The purpose of the present study was, therefore, to evaluate the analgesic and anti-inflammatory effects of the *L. siceraria* fruit juice extract (LSFJE) using different acute and chronic models of pain and inflammation in mice and rats. The extract was also studied for its acute toxicity effects and preliminary phytochemical screening.

MATERIALS AND METHODS

Plant material

The fresh fruits of *Lagenaria siceraria* Stand. (Mol.) were collected from Wardha district of Maharashtra state, India. Plant was authenticated by the authority of Botany Department, Nagpur University, Nagpur, India where a voucher specimen is deposited for future reference. The fresh, rind fruit (500g) of *L. siceraria* was squeezed to obtain juice extract. The extract was evaporated to dryness in *vacuo* (40°C). The yield of LSFJE was obtained 22.5 % w/w.

Preliminary phytochemical screening

LSFJE was studied for its preliminary phytochemical screening (6) for the detection of various plant constituents.

Preparation of test drugs

All test drugs were suspended in 10% v/v propylene glycol except in the ethyl phenyl propionate-induced ear edema model, where test drugs were dissolved in acetone.

Experimental Animals

Swiss albino mice (18–20 g) and Wistar rats (100-150 g) of either sex kept at the Laboratory Animal Center of the Institute of Pharmaceutical Education and Research, Wardha, India were used. The experimental protocol was initially approved from the Institutes animal ethics committee and then experimental studies were undergone according to their rules and regulations. The animals were housed under standard environmental conditions and had free access to standard pellet diet (Goldmohar brand, Lipton India Ltd.) and water *ad libitum*.

Acute toxicity studies

The 50% lethal dose of the LSFJE was estimated by the up- and -down method in mice (11). Doses were adjusted by a constant multiplicative factor viz. 4, for this experiment. The dose for each successive animal was adjusted up and down depending on the previous outcome.

Mouse writhing assay

The method of Koster et al. (12) was used. The LSFJE (150-300 mg/kg, p.o.) or acetylsalicylic acid (300 mg/kg, s.c.) was administered to mice 60 and 30 min, respectively, before intraperitoneal injection of acetic acid (0.6%, v/v in normal saline, 10 ml/kg). 10% v/v propylene glycol was used as the control. The number of writhes was counted for 15 min.

Formalin test

The method used was similar to that described previously by Shibata et al. (13). Twenty microlitre of 1% v/v formalin was injected subcutaneously into the right hind paw of mice. The time (in seconds) spent in licking and biting responses of the injected paw was taken as an indicator of pain response. Responses were measured for 5 min after formalin injection (first phase) and 15–30 min after formalin injection (second phase). The LSFJE (150-300 mg/kg, p.o.) and acetylsalicylic acid (300 mg/kg, s.c.) were administered 60 and 30 min, respectively, before formalin injection. Control animals received 10% v/v propylene glycol (10 ml/kg).

Ethyl phenylpropionate (EPP)-induced ear edema in rats

Male rats weighing 100-150 g were used. Ear edema was induced by topical application of EPP at a dose of 1 mg/20 µl per ear to the inner and outer surfaces of both ears by means of an automatic micriliter pipette (14). Test drugs were applied topically in volumes of 20 µl just before the irritant. The control group received vehicle only. Before and at 30min, 1h and 2h after edema induction, the thickness of each ear was measured by vernier calipers. The percent inhibition of the edema formation of test substances was calculated.

Carrageenin- and arachidonic acid-induced paw edema in rats

Male rats weighing between 100-150 g were used. Paw edema was induced by an intradermal injection of carrageenin (1% in normal saline solution) (15) or arachidonic acid (0.5% in 0.2 M carbonate buffer, pH 8.4) into the plantar surface of the right hind paw of the rats, at a volume of 0.05 or 0.1 ml, respectively. The edema volume was determined using a plethysmometer (16) prior to and 1, 3 and 5 h after carrageenin injection, or 1 h after arachidonic acid injection. Test drugs were given 1 h prior to carrageenin or 2 h prior to arachidonic acid injection. The control group received vehicle only.

Egg albumin-induced paw edema in rats

This test was performed by a modification of Winter et al. (17) as described by Akah and Nwambie (18). Four groups of male and female wistar rats were pre-treated as follows, group 1, 10% propylene glycol; group 2 and 3, 150 and 300 mg/kg, p.o. of LSFJE; and group 4 with Diclofenac sodium (100 mg/kg, p.o.). After 30 min, each group was injected with 0.5 ml raw egg albumin sub-plantar to the left hind-paw. A digital plethysmometer was used to measure volume of paw oedema for a period 120 min, with readings taken at 30 min intervals, i.e. at 30, 60 and 90 min after albumin administration.

Statistical analysis

All data were expressed as mean±SEM and analyzed statistically by using Paired *t*-test and Dunnett's *t*-test. A difference was considered significant at P value less than 0.05.

RESULTS

Acute toxicity studies

In the acute toxicity test, signs of toxicity included lethargy, jerk, convulsions and death. The LD_{50} value of orally and intraperitoneally administered LSFJE in mice

was estimated to be 4.2 g/kg and 0.29 g/kg, respectively.

Preliminary phytochemical screening

Preliminary phytochemical screening of the LSFJE showed the presence of flavonoid glycosides, cucurbitacin saponins, proteins and carbohydrates.

Analgesic studies

The effect of LSFJE on acetic acid induced writhing is demonstrated in Table 1. The LSFJE (150 and 300 mg/kg, p.o.) showed the significant ($P<0.01$) reduction in the number of writhes induced by acetic acid in a dose-dependent manner. Aspirin (300 mg/kg, p.o.) exhibited inhibitory effect on writhing response. There was a significant, dose-dependent inhibition of both phases of the formalin induced pain response in mice, with a more potent effect on the second than the first phase (Table 2).

Anti-inflammatory studies

The activity of LSFJE on EPP-induced ear edema in rat is shown in Table 3. The LSFJE dose-dependently elicited significant ($P<0.05$) inhibitory effect on the edema formation at 30 min, 1 h, 2 h after EPP injection. Phenylbutazone, a COX –inhibitor, at a dose of 1 mg/kg also showed significant reduction of ear edema.

The result of LSFJE against carrageenin-induced paw edema is shown in Table 4. The result shows that the LSFJE (150-300 mg/kg, p.o.) gave the significant ($P<0.01$) reduction of rat paw edema at all assessment times. Aspirin, a COX-inhibitor at the dose of 300 mg/kg, p.o. significantly reduced the paw edema.

The activity of the LSFJE on arachidonic acid-induced hind paw edema in rats is shown in Table 5. LSFJE (150 and 300 mg/kg, p.o.) and also phenidone (60 mg/kg), a dual blocker of cyclooxygenase and lipooxygenase, exerted significant reduction of the paw edema whereas aspirin (300 mg/kg) did not show any effect.

The effect of LSFJE on egg albumin-induced hind paw edema in rats is shown in Table 6. The result showed that the LSFJE caused dose-dependent inhibition of albumin-induced edema over a period of 90 min.

DISCUSSION

In this work, we have demonstrated the effect of LSFJE (150-300 mg/kg; p.o.) on acetic acid-induced writhing and formalin pain tests in mice. The methods for investigating analgesic effects of LSFJE were selected such that both centrally and peripherally mediated effects were investigated. The acetic acid-induced writhing response method elucidated peripheral activity, while the formalin test investigated both peripheral and central activity. Four different animal models (i.e. ethyl phenylpropionate-induced ear edema, carrageenin- and arachidonic acid-induced hind paw edema, and also the albumin-induced paw edema in rats) were employed to investigate the potential anti-inflammatory activity of the LSFJE in this study.

The mouse writhing assay is a useful test to evaluate mild analgesic non-steroidal anti-inflammatory agents. Acetic acid causes algesia by liberating endogenous substances including serotonin, histamine, PGs, bradykinin and substance P which stimulate pain nerve endings (19). Local peritoneal receptors are postulated to be partly involved in the abdominal constriction (writhing) response (20). The method has been associated with prostanoids in general, i.e. increased levels of PGE_2 and $PGF_{2\alpha}$ in peritoneal fluids (21) as well as lipoxygenase products by some researchers (22). Therefore, the LSFJE might inhibit the synthesis and/or release of these endogenous substances (Table 1).

The formalin pain test is very useful for evaluating the mechanism of pain and analgesia. The formalin injection into rat paw produces localized inflammation and pain. The effect is biphasic in nature: an early neurogenic component followed by a late tissue-mediated response (23).Drugs which act mainly centrally, such as narcotic analgesics, inhibits both phases of pain in this model while peripherally acting drugs, such as aspirin or indomethacin, only inhibit the late phase (24). The formalin pain test assesses the way an animal responds to moderate, continuous pain generated by injured tissue (25). The LSFJE inhibited both phases of the formalin-induced pain with more potent effects on the second than the first phase (Table 2).

EPP-induced ear edema test provides a skin inflammation model suitable for evaluation of topical and systemic anti-inflammatory agents. The majority of its activities appear to involve or depend on arachidonic acid release and metabolism and interaction with protein kinase C (26). It has a good predictive value to screen anti-inflammatory agents. Inflammatory mediators such as kinin, serotonin, and PGs are released by EPP (14). It was found that the LSFJE elicited significantly inhibitory effect on the edema formation at all assessment times. Phenylbutazone, a COX-inhibitor, showed a marked reduction of the ear edema (Table 3).

Table 1: Effect of LSFJE on Acetic Acid induced writhing

Group(s)	Dose (mg/kg)	No. of writhes (per 15 min)	Inhibition (%)
Control	---	37.0±0.70	---
LSFJE	150	31.6±0.48[a]	14.50
	300	29.8±0.56[a]	19.50
Aspirin	300	11.8±0.83[a]	68.10

Values are expressed as mean±SD. [a] P<0.01; significantly different from control; Dunnett's t-test (n=6).

Table 2: Effect of LSFJE on Formalin Induced Pain

Group(s)	Dose (mg/kg)	0-5 min	Inhibition (%)	15-30 min	Inhibition (%)
Control	---	96.60±0.57	---	91.16±0.70	---
LSFJE	150	89.33±0.55[b]	7.52	85.33±0.40[a]	6.39
	300	84.66±0.61[b]	12.70	70.50±1.28[b]	22.66
Morphine	10	62.33±0.71[b]	33.47	49.16±0.87[b]	46.07

Values are expressed as mean±SEM. [a]P<0.0006, [b]P<0.0001; Significantly different from control; Paired t-test (n=6).

Table 3: Effect of LSFJE and Phenylbutazone on EPP-Induced Ear Edema in Rats

Group(s)	Dose (mg/kg)	Time after topical application of EPP					
		30 min		1h		2h	
		ED (um)	EDI (%)	ED (um)	EDI (%)	ED (um)	EDI(%)
Control	---	290±12.0	---	288±12.6	---	224±10.4	---
LSFJE	0.5	261±10.02	10.0	257±8.6[b]	10.76	164±9.9[a]	26.78
	1.0	14±6.3[a]	26.20	205±6.3[a]	28.81	153±6.7[a]	31.69
PBZ	1.0	197±5.6[a]	32.06	168±4.6[a]	41.66	127±5.8[a]	43.30

Values are mean±SEM. [a]P<0.01, [b]P<0.05; Significantly different from control; Dunnett's t-test (n=6); PBZ: Phenylbutazone; ED: edema thickness; EDI: edema inhibition.

Table 4: Effect of LSFJE and Aspirin on Carrageenin-Induced Hind Paw Edema in Rats

Group(s)	Dose (mg/kg)	Time after Carrageenin injection					
		1h		3h		5h	
		EV (ml)	EI (%)	EV (ml)	EI (%)	EV (ml)	EI (%)
Control	---	0.48±0.06	--	0.72±0.08	---	0.76±0.03	---
Aspirin	200	0.20±0.04[a]	58.33	0.28±0.03[a]	61.11	0.31±0.01[a]	59.21
LSFJE	150	0.39±0.03	18.75	0.52±0.02	27.77	0.55±0.03[a]	27.63
	300	0.28±0.02[a]	41.66	0.38±0.08[a]	47.22	0.41±0.04[a]	46.05

Values are expressed as mean±SEM. [a]P<0.01; Significantly different from control, Dunnett's t-test (n=6); EV: edema volume; EI: edema inhibition.

Table 5: Effect of LSFJE, Phenidone and Aspirin on Arachidonic Acid Induced Hind Paw Edema in Rats

Group(s)	Dose (mg/kg)	Time (1h) after arachidonic acid injection	
		EV (ml)	EI (%)
Control	---	0.72±0.03	---
PhenidoneA	60	0.49±0.02[b]	31.94
spirin	200	0.56±0.01	05.55
LSFJE	150	0.64±0.04[a]	15.27
	300	0.61±0.03[b]	16.66

Values are expressed as mean±SEM. [a]P<0.01, [b]P<0.05, Significantly different from control; Dunnett's t-test (n=6); EV: edema volume; EI: edema inhibition.

Table 6: Effects of LSFJE and Diclofenac Sodium on Egg Albumin-Induced Hind Paw Edema in Rats

Group(s)	Dose (mg/kg)	Time after albumin injection					
		30 min		60 min		90 min	
		EV (ml)	EI (%)	EV (ml)	EI (%)	EV (ml)	EI(%)
Control	---	0.39±0.04	---	0.48±0.02	---	0.54±0.03	---
LSFJE							
	150	0.31±0.04	20.51	0.37±0.02[b]	22.91	0.39±0.02[a]	27.77
	300	0.29±0.03	25.64	0.32±0.01[a]	33.33	0.35±0.04[a]	35.18
Diclofenac	200	0.22±0.01[a]	43.58	0.28±0.01[a]	41.66	0.29±0.01[a]	46.29

Values are expressed as mean±SEM (n=6); [a]P<0.01, [b]P<0.05; Significantly different from control; Dunnett's t-test; EV: edema volume; EI: edema inhibition

The paw edema induced by the subplantar injection of carrageenin in rats is biphasic, the first phase (1 h) involves the release of serotonin and histamine while the second phase (over 1 h) is mediated by prostaglandins, the cyclooxygenase products and the continuity between two phases is provided by kinins (27). The LSFJE might have inhibitory effects on the release and/or synthesis of inflammatory mediators especially the cyclooxygenase products (Table 4).

Metabolism of arachidonic acid mediated through the lipooxygenase pathway is necessary for the edema formation (28). The LSFJE (150-300 mg/kg) as well as phenidone (60 mg/kg) a dual blocker of cyclooxygenase and lipooxygenase, and aspirin (200 mg/kg) exerted significant reduction of the paw edema. This suggests that the activity of LSFJE might be mediated via the cyclooxygenase and/ or lipooxygenase pathway (Table 5). The egg albumin-induced hind paw edema method is useful to detect activity in acute inflammation. The LSFJE also caused marked inhibition of egg albumin-induced hid-paw edema in rats (Table 6).

The co-existence of analgesic and anti-inflammatory effects seen with this extract is well-defined for various non-steroidal anti-inflammatory drugs (NSAIDs) particularly salicylates and their derivatives. It's therefore interesting that the extract behaved like NSAIDs in this study which correlates well with the traditional application of the plant.

The LSFJE does possess significant analgesic and anti-inflammatory effects in laboratory animals at the doses investigated. The results support the traditional use of this plant in some painful and inflammatory conditions and also suggest the presence of biologically active principals i.e. flavonoid glycosides, cucurbitacin saponins, proteins and carbohydrates. Several flavonoids and saponin glycosides isolated from medicinal plants have been discovered to possess significant analgesic and/ or anti-inflammatory effects (29). It is, therefore, possible that both the analgesic and anti-inflammatory effects observed with LSFJE may be attributed to its flavonoids and saponin glycoside components, shown to be present during phytochemical screening. Further studies are in progress to isolate and characterize the active principles of the extract.

The oral and intraperitoneal LD_{50} obtained with this plant extract also suggest that it may have a

reasonable safety margin with regards to acute toxicity further justifying its wide application in various communities and lack of any reported side effects with the traditional use of this plant.

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