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Study of Analgesic and Anti-inflammatory Activities of *Lagerstroemia lanceolata* wall (seed) extract

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ABSTRACT
Analgesic and anti-inflammatory effects were examined in purified light Petroleum ether (40-60°) extract (LLW Oil) of the *Lagerstroemia Lanceolata* wall seed. The analgesic activity effects of graded doses of extract (in LLW10-200 mg/kg *p.o.*) were evaluated in rat against acetic acid induced writhing (chemically induced pain) and hot-plate method (thermally induced pain). The analgesia produced by extract was compared with the standard analgesics diclofenac sodium (DIS, 5 mg/kg *p.o.*). Acute anti-inflammatory activity of fraction was also evaluated in carrageenan-induced rat paw edema model at the doses 50, 100 and 200 mg/kg *i.p.* and compared with diclofenac sodium (10 mg/kg *i.p.*). In comparison to control group purified Pt. ether extract showed highly significant, dose dependent analgesic activity against thermally as well as chemically induced pain ($p < 0.001$). LLW at the dose of 40 mg/kg has shown highly significant analgesic activity ($p < 0.001$) as compared to diclofenac sodium at the doses employed. In comparison to control, LLW at the employed doses produced marked acute anti-inflammatory activity in rats ($p < 0.001$). The results suggest that the Pt. ether extract (LLW20-200) of seed has active herbal principles which possess significant analgesic and anti-inflammatory potential as reflected by the parameters investigated. Further investigations are, however, necessary to explore mechanism(s) of action involved in these pharmacological activities.

Keywords: *Lagerstroemia Lanceolata, Lythraceae,* Pt. ether extract, analgesic and anti-inflammatory.

INTRODUCTION

The clinically useful drugs against pain and inflammation exhibit many adverse effects; this leads to considerable interest in search of safer drug for these conditions. *Lagerstroemia Lanceolata* wall is a native Indian tree belongs to the family Lythraceae and found from Bombay Southwards to Kerala. [1-2]

The *Lagerstroemia Lanceolata* wall species has been used in the treatment of Asthma, Diabetes Mellitus, Chronic Bronchitis, cold, cough and local application for aphthae of the mouth. [3] Seeds have been documented for its multiple pharmacological activities including narcotic principal. [4] The leaves were also evaluated for potent, anti-inflammatory and antipyretic activities in the rat. [5] Literature revealed that Steroids, Terpenoids,Alkaloids, Antocyanins Ellagic acid and tannins, are the major components in the seeds. [6] The analgesic and anti-inflammatory activity of the oil and its constituents have never been characterized. The present study was planned to explore any possible analgesic and anti-inflammatory potential of the light petroleum ether (40-60°**)** extract obtained from the *Lagerstroemia Lanceolata* seed .

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MATERIALS AND METHODS

Plant materials

The fully mature *Lagerstroemia Lanceolata* wall seeds (Fig. 1) were collected from various parts of Belgaum city in the state Karnataka, India and the seed was identified and authenticated by Dr. Salimath P., Asst. Prof. Dept. Of Botany, R. L. Science College, Belgaum, India. The voucher specimen (KL 469) was deposited in the K. L. E. Society's College herbarium.

Fig. 1: Seeds of *Lagerstroemia Lanceolata* wall

Acute toxicity studies

Acute oral toxicity study [7] was performed as per OECD-423 guidelines (acute toxic class method). Wistar rats (n = 6) of either sex selected by random sampling technique were used for the study. The animals were kept fasting for overnight providing only water, after which the extracts were administered orally at the dose level of 5 mg/kg body weight by intragastric tube and observed for 14 days. If mortality was observed in 2-3 animals, then the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher dose such as 50, 300 and 2000 mg/kg body weight.

Preparation of LLW

Dried coarsely powdered seeds (500 g) were extracted with light Pt. ether (40-60°) using Soxhlet apparatus at 80 ± 2°C for 24 h. The Pt. ether extract were dried over anhydrous sodium sulphate and solvent was removed in vacuum at 40°C to recover the oil by using rotary-evaporator (Rotavapour, Buchii, Switzerland). The Pt ether extract thus separated was preserved and coded as LLW (yield 10 %). The LLW extract was subjected to physicochemical characterization using TLC (thin layer chromatography) on pre-coated silica gel-G plates using petroleum ether: ether: acetic acid (75: 25: 0.1, v/v/v) as mobile phase and iodine as visualizing agent. Phytochemical screening was done for detection of alkaloids, glycosides, saponins and steroids and oil contents were determined for Acid value, Iodine Value, Saponofication value, Hydroxy value , Halphen test and Unsaponifiable matter in LLW. For pharmacological screening LLW oil was emulsified using acacia according to the desired concentration (test dose LLW-10, LLW-20, LLW-40, LLW-50, LLW-100 and LLW-200) just before use.

Analgesic studies

Analgesic activity in mice was assessed in chemically as well as thermally induced pain using acetic acid induced writhing model [8] and hot plate assay [9], respectively.

Acetic acid induced writhing method

Five groups of mice (n = 6) were randomly formed. The groups were treated as control (distilled water, *p. o.*) and standard (DIS 5 mg/kg *p.o.*) while test groups received fraction (LLW) 10, 20 and 40 mg/kg *p.o.* (LLW-10, LLW-20 and LLW-30), respectively. Acetic acid solution 0.6 % v/v (10 mL/ kg) was injected by intraperitoneal route one hour after treatment and number of writhes (i.e. index of pain reaction against chemical stimuli characterized by abdominal muscle contraction together with turning of trunk and extension of hind limbs) was counted over a period of 20 min. Analgesic activity was expressed as percentage of inhibition of writhes with respect to the control group (Table 1).

Hot plate method

Hot plate was maintained at 55 ± 1°C. Albino mice were divided in five groups. The animals were placed on the hot plate and the basal reaction time taken to cause a discomfort (licking of paw or jumping response whichever appeared first) was recorded at 0 min. Cut-off period 15 sec. was established to prevent damage to the paws.

Anti-inflammatory studies

Acute anti-inflammatory activity of LLW at 50,100 and 200 mg/kg was evaluated using carrageenan induced edema in rats described by Winter *et a*l. [10] Five groups of albino rats (n = 6) were randomly distributed in control, standard and test (LLW-50, LLW-100 and LLW-200) groups. The initial paw volumes of each animal were measured by means of a mercury plethysmometer. The standard group was treated with DIS injection (10 mg/kg, *i.p.*) while LLW solution and distilled water (10 mL/kg, *i.p.*) were given to the test and control groups, respectively. Thirty minutes after treatment 0.1 mL of 1 % carrageenan solution was injected in the plantar region of the left hind paw of rats. Paw volumes were again measured 3 h after carrageenan injection. The acute difference in edema volume was calculated in each control, test and standard group and compared with the control group for determination of the percentage of inhibition of the paw edema (Table 3).

$$\text{Percentage inhibition of oedema} = \frac{Vc - Vt}{Vc} \times 100$$

Where, *Vc* is the inflammatory increase in paw volume in control group of animals and *Vt* is the inflammatory decrease inpaw volume in drug-treated animals.

Statistical analysis

Data obtained from pharmacological experiments are expressed as mean ± SEM. Difference between the control and the treatments in these experiments were tested for significance using ANOVA followed by Tukey's comparison test was applied. $P < 0.05$ was considered statistically significant.

RESULTS

Phytochemical screening of the light Pt.ether (40-60°) extract (LLW Oil) obtained from *Lagerstroemia Lanceolata* wall seeds was contains glycoside and steroid principals. Preliminary screening was carried out in mice for four graded doses viz. 5, 10, 20, and 40 mg/kg administered orally as well as intraperi-toneally and indicated no gross behavioral changes, sedation, morbidity and mortality at these doses. Pt.ether (40-60°) extract (LLW Oil)indicated highly significant and dose dependent analgesic activity against both thermally and chemically induced pain. In acetic acid induced writhing method LLW (10, 20, and 40 mg/kg *p.o.*) and standard (DIS 5 mg/kg *p.o.*) treated animals showed significantly reduced number of writhing in 20 min at the rate of 28.82 %, 48.58 %, 75.73 % and 63.77 %, respectively, when compared to that of control group ($p < 0.001$). Analgesia produced by LLW-40 mg/kg was also found to be higher than that of observed for standard diclofenac sodium at the employed doses (Table 1). On hot-plate test, LLW Oil showed significant elevation in pain threshold in comparison to control, as represented in Table 2 and indicated significant analgesic activity ($p < 0.05$) as compared to control at all tested doses.

Acute anti-inflammatory potential of LLW Oil was investigated at its minimal dose producing analgesia (i.e. 50, 100 and 200 mg/ kg, *i.p.*) against carrageenan induced rat paw edema. It was noted that the standard drug diclofenac sodium (DIS, 10 mg/ kg *i.p.*) showed 74.60 % inhibition of edema whereas LLW Oil showed 53.70, 59.70 and 62.60 % inhibition, at dose LLW-50, LLW-100 and LLW-200 respectively with respect to control ($p < 0.001$). In addition, the study also demonstrated lower anti-inflammatory response of LLW at the used doses in comparison to DIS at 10 mg/ kg *i.p.* The results are summarized in Table 3.The extracts were found to significantly inhibit the carrageenan-

Table 1:Analgesic effect of different doses of Pt. ether extract of LLW in acetic acid induced writhings in mice

Treatment	Dose, *p. o.* (mg/kg)	No. of writhes in 20 min The mean ± SE			% Reduction
Control (DW)	10 ml/kg	51.50 ± 0.99			-
DIS	5	18.66 ± 0.88*			63.77
LLW-10	10	36.66 ± 0.95*			28.82
LLW-20	20	26.50 ± 0.84*			48.58
LLW-40	40	12.50 ± 0.76*			75.73
One way ANOVA		F	309.45		
		p	< 0.001		

DIS, diclofenac sodium; DW, distilled water; One way ANOVA followed by multiple Tukeyís comparison test. Values are presented as the mean ± SE (standard error); n = 6 for all groups, df = = 4, 25. * p < 0.05 as compared to control; p < 0.05 as compared to diclofenac group.

Table 2. Analgesic effect of different doses of Pt. ether extract of LLW on hot plate test in mice.

Treatment	Dose, *p.o.* (mg/kg)	Mean reaction time in seconds					
		0 min.		30 min.	60 min.		120 min.
Control (DW)	10mL/kg	3.93 ± 0.04		4.01 ± 0.45	3.96 ± 0.04		3.96 ± 0.04
DIS	5	3.91 ± 0.05		7.46 ± 0.17*	8.06 ± 0.15*		7.61 ± 0.14*
LLW-10	10	3.88 ± 0.05		4.32 ± 0.06	4.91 ± 0.07		4.76 ± 0.11
LLW-20	20	3.91 ± 0.07		4.70 ± 0.09*	5.45 ± 0.08*		5.2 ± 0.06*
LLW-40	40	3.92 ± 0.05		5.32 ± 0.07*	6.12 ± 0.08*		5.75 ± 0.14*
One way ANOVA		F	0.088	178.89	249.7	155.87	
		p	>0.05	<0.01	<0.01	<0.01	

DIS, diclofenac sodium; DW, distilled water;. One way ANOVA followed by multiple Tukey s comparison test. Values are the mean ± SE, n = 6 in each group, df = 4, 25. * p < 0.05 when compared to control group.

Table 3: Anti-inflammatory effect of different doses of Pt. ether extract of LLW on carrageenan induced rat paw edema in rats.

Treatment	Dose, *p.o.* (mg/kg)	Paw oedema volume (ml)			
		60 min	120 min	180 min	240 min
Control	10mL/kg	0.35 ± 0.14	0.49 ± 0.02	0.65 ± 0.01	0.67 ± 0.01
LLW-50	50	0.28 ± 0.16** (20%)	0.39 ± 0.02* (20.4%)	0.39 ± 0.02*** (40 %)	0.31 ± 0.02*** (53.7 %)
LLW-100	100	0.25 ± 0.01** (28%)	0.38 ± 0.02* (22.4%)	0.38 ± 0.02*** 41.5 %)	0.27 ± 0.03*** (59.7 %)
LLW-200	200	0.24 ± 0.01 *** (31.4%)	0.37 ± 0.01** (24.5%)	0.35 ± 0.02*** (46.1 %)	0.25 ± 0.03*** (62.6 %)
DIS	10	0.22 ± 0.01*** (37.1%)	0.36 ± 0.01** (36.1%)	0.27 ± 0.02*** (58.4 %)	0.17 ± 0.10*** (74.6 %)
Difference between the control and the treatments tested for significance using ANOVA					

DIS, diclofenac sodium; Values are mean ± SEM of 6 animals in each group. Comparisons were made between control and the treatments. P- values: *p<0.05, **p<0.01, ***p<0.001. Percentage protection given on Parenthesis.

induced rat paw oedema, a test which has significant predictive value for anti-inflammatory agents acting by inhibiting the mediators of acute inflammation. [11] Carrageenan induced inflammation is useful in detecting orally active anti-inflammatory agents. [12] Oedema formation due to carrageenan in the rat paw is a biphasic event. [13] The initial phase is attributed to the release of histamine and serotonin. [14]

DISCUSSION AND CONCLUSION

In the present investigation, petroleum ether extract of seed of LLW was studied for its analgesic potential in both peripheral (non-narcotic) and central (narcotic) type pain models. Diclofenac sodium (5 mg/kg, *p.o.*) was used as standard drugs for comparing analgesic effects at peripheral and central levels, respectively. LLW pretreatment markedly reduces the painful response produced by acetic acid, manifested as writhing at the employed doses. Pain is a complex process mediated by many physiological mediators e.g. prostaglandins, bradykinins, substance-p etc. In the acetic acid induced writhing model the constrictions induced by acetic acid in mice results from an acute inflammatory reaction with production of PGE2 and PGF2á in the peritoneal fluid. [15-16]

Therefore, it is likely that LLW might suppress the formation of these substances or antagonize their action for exerting analgesic activity. The hot-plate test is commonly used to assess narcotic analgesics or other centrally acting drugs [17] and the present results showed that LLW also significantly elevates the response latency period suggesting centrally mediated analgesic effect. It also showed moderate anti-inflammatory potential at the employed doses. [15] It has been proposed that inflammatory reaction occurs in two phases viz, release of histamine, serotonin and bradykinin in the early or first phase, followed by the release of prostaglandin in the late or second phase. A significant anti-inflammatory activity against carrageenan induced inflammation was also observed in LLW, evaluated by carrageenan induced rat paw edema method, suggesting influence of LLW on release, synthesis or action of the inflammatory mediators. LLW contains Alkaloid, glycoside and steroidal moieties that may be contributing to the observed pharmacological activities. We conclude, the Pt. ether extract of seed of LLW contains active herbal principles and possess potential analgesic and anti-inflammatory activities. Further investigations are required to understand its influence on various pain and inflammatory mediators.

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REFERENCE

1. Said HM. Hambard Pharmacopeia of eastern medicine. Edn 2nd, New Delhi, 1980, 48.
2. Nadkarni KM. Indian material medica. Edn 2, Vol. II, Bombay Popular Prakashan TK., Herbal option, 1997, 723.
3. Graham SA, Weiman RL, Syst. Ecolo J. Biochem.1978; 15(4): 433-9.
4. Goyal MM, Kumar KU, J. Sci. Ind. Res. 1987; 22(1-4):148-51.
5. Barik BR., Kund AB, J. Phytochemistry 1988; 27(11): 3679-80.
6. Chirag MH, Marajkar GH, J. Phytochemistry 1987; 29(7): 2323-4.
7. Ecobichon DJ. The basis of toxicology testing. RC press, New York, 1997, pp. 43-86.
8. Ghosh MN. Fundamentals of Experimental Pharmacology, Ed 2nd, Scientific Book Agency, Calcutta, India 1984, pp. 144.
9. Eddy NB, Leimbach D. J. Pharmacol. Exp. Ther. 1953; 107: 385.
10. Winter CA, Risley EA, Nuss GW. Proc. Soc. Exp. Biol. Med.1962; 111: 544.
11. Mossa JS, Rafatullah S, Galal AM, Al-Yahya MA., Pharmacological studies of *Rhus retinorrhaea*.,Int. J. Pharmacognosy 1995; 166: 96-103.
12. DiRosa M, Giroud JP, Willoughby DA. Studies of the acute inflammatory response induced in rats in different sites by carrageenan and turpentine. J. Pathol. 1971; 104: 15-29.
13. Vinegar R, Schreiber W, Hugo R. Biophasic development of carrageenan oedema on rats. J. Pharmacol. Exp. Ther. 1969; 66: 96-103.
14. Crunkhon P, Meacock SER. Mediators of the inflammation induced in the rat paw by carrageenan., Br. J. Pharmacol. 1971; 42: 393-402.
15. Deraedt R, Jouquey S., Benzoni J. et al.: Arch. Int. Pharmacodyn. Ther. 1976; 224: 30.
16. Deraedt R, Jouquey S, Delevalee F et al.: Eur. J. Pharmacol. 1980; 61:17.
17. Beirith A, Santos ARS, Rodrigues ALS. et al.: Eur. J. Pharmacol. 1998; 345: 233.