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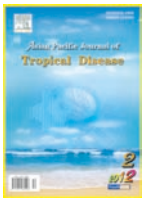

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In vivo anti-nociceptive and anti-inflammatory activities of *Lippia alba*

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ABSTRACT

Objective: To evaluate antinociceptive and anti-inflammatory activities of *Lippia alba* (Mill.) N.E. Brown (Verbenaceae) leaves. **Methods:** Soxhlet extraction method was used to obtain extracts using petroleum ether extracts (PELA); chloroform extracts (CELA); ethanol extracts (EELA) and aqueous extract (AELA). Antinociceptive activity was assessed on rats by tail flick latency using tail immersion method and anti-inflammatory activity was estimated by carrageenan induced paw edema method. PELA, CELA and AELA at a dose of 500 mg /kg.b.wt. and EELA at a dose of 460 mg /kg.b.wt were administered orally. **Result:** Competing to control AELA was found to have a higher range of anti-nociceptive activity and showing maximum (79.66%) response at 60 min, where as CELA and EELA were found to have a maximum range of anti-inflammatory activity and CELA exhibit maximum (19.5%) response at 240 min. **Conclusion:** The results suggest that the extracts of *Lippia alba* possess ant-inociceptive and anti-inflammatory activities, and its help to authenticates the use of the plant in the traditional treatment of ailments associated with pain and inflammation.

1. Introduction

Pain and inflammation are associated with the pathophysiology of various clinical conditions such as arthritis, cancer and vascular diseases. As a result, it has become the focus of global scientific research because of its implication in virtually all human and animal diseases. Inflammatory reactions are not only the response of living tissues to injury and infection, but also are relevant to disease developments, such as asthma, multiple sclerosis, colitis, inflammatory bowel disease and atherosclerosis[1]. Chronic inflammatory diseases remain one of the world's major health problems[2, 3]. It involves a complex array of enzyme activation, mediator release, extravasations of fluid, cell migration, tissue breakdown and repair[4, 5]. The conventional drugs used to ameliorate this phenomenon are either too expensive or toxic and not commonly available to the rural folks that constitute the major population of the world[6, 7]. Drugs from plant sources are being used by about 80% of the world population. Herbal medicines have stood the test of time for their safety, efficacy, acceptability and lesser side effects[8]. Essential oil of *L. alba* have been used for analgesic and antiinflammatory activity[9, 10]. However, the analgesic and anti-inflammatory activity of the leaves of *L. alba* has not been reported. This study therefore seeks to examine antinociceptive effects and anti-inflammatory activity since pain is one of the cardinal signs of inflammation.

Lippia alba (Mill.) N.E. Brown (Verbenaceae), commonly known as Basula (Hindi), a plant of fast growing with a mounding habit and rounded lavender blossoms, Found in central, eastern and southern parts (Western Ghats) of India[11]. Different parts of the plants are traditionally employed as infusion and decoction as a remedy for Gastro intestinal alignments, dysentery, colds, cough and febrifuge as well as sedative and spasmolytic remedies[12]. Previously reported compound was essential oils (geranial, neral, geraniol)[13]. The essential oil had been used for topical anti-inflammatory activity[14]. Present study is undertaken to investigate the antinociceptive and anti-inflammatory potentials of different extracts of *L. alba* scientifically to justify the traditional use.

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2. Materials and method

2.1. Plant material

Fresh leaves of the plant were collected during September, 2007 from the reservoir bank of "Hirakud Dike" situated at Sambalpur, eastern part of India (Orissa), just after the rainy season. The plant was authenticated by Dr. M. S. Mondal, Scientist, Botanical Survey of India Shibpur, Howrah[CNH/I-I/ (193)/2007/Tech.II/288]. The leaves were dried under shade, powdered and stored in a closed air a tight vessels for further use.

2.2. Extraction Procedure

Powdered plant material (600 g) was successively extracted with petroleum ether (PELA) followed by chloroform (CELA), ethanol (EELA) and aquoa (AELA) using soxhlet extraction apparatus by continuous hot percholetion method. Solvents were completely removed after each extraction under reduced pressure and the filtrates were concentrated under reduced pressure using rotary vacuum evaporator and stored in a vacuum desecrator. The yield of the PELA, CELA, EELA and AELA were 2.53%, 4.00%, 9.72% and 10.33% respectively. Phytochemical screenings of the crude extracts were carried out by employing standard procedures[15].

2.3. Animals

Adult male Wistar albino rats weighing 170-200 g were used for the present study. They were housed in a clean polypropylene cage and maintained under standard laboratory conditions (temperature 25℃±2℃ with dark/light cycle 12/12 h). They were fed with standard pellet diet and water ad libitum. The animals were acclimatized to laboratory conditions for one week prior to experiment. All procedures described were reviewed and approved by the University Animal Ethics Committee, Jadavpur University (367001/C/ CPCSEA).

2.4. Acute Toxicity study

The acute oral toxicity of PELA, CELA, EELA and AELA in male Swiss albino mice were studied as per OECD guideline 425[16]

2.5. Anti-nociceptive Activity

2.5.1. Tail Immersion Method

To evaluate the antinociceptive effects of the plant extract animals were divided into six groups (n=6) and marked individually[17]. They were then placed into individual restraining cages leaving the tail hanging out freely. The animals are then allowed to adapt in the cages for 30 minutes before testing. The lower 5 cm portion of tail was immersed in a cup of freshly water of exactly 55℃. Within a few seconds, the animal reacts by withdrawing the tail. The reaction time was recorded in 0.5 seconds by a stop watch. After each determination, the tail was carefully dried. The reaction was observed before oral administration of standard drug (45 mg/kg b.wt.) and extracts[PELA, CELA, EELA (500 mg/kg b.wt.) and AELA (460 mg/kg b.wt.)], which was recorded as time zero readings. After the drug was administered the reaction time was recorded at an interval of 30, 60, 120, 180 minutes. The mean reaction time for each group was found out and compared with the value of the control group.

2.6. Anti-inflammatory Activity

2.6.1. Carrageenan Induced Hind Paw Edema

Anti-inflammatory activity was evaluated using carrageenan induced hind paw edema method. Rats of either sex were divided into six groups (n=6) and numbered individually[18]. The group I served as control and received only vehicle, group II was administered with standard drug Ibuprofen I.P 100 mg/kg.b.w intraperitoneally. The animals of group (III- VI) were treated with PELA, CELA, EELA (500 mg/kg b.wt.) and AELA at a dose of 460 mg/kg.b.w orally. After 30 minutes of above treatment 0.05 ml of 1% w/v Carageenan in normal saline was injected into sub plantar tissue of left hind paw of the animals. The degree of paw edema of the entire group was measured at 0, 30, 60, 120 and 180 minutes by using vernier calipers. The anti inflammatory effect was expressed as percent inhibition of edema. Percentage calculation was done by following equation[19].

C-T/C X 100
Where, C is control,
T is test.

3. Results

Preliminary phytochemical study reveals that the extracts from the tested medicinal plant had a variety of photochemical constituents, namely phytosterol, alkaloids, fixed oils, flavonoids, phenolic compounds, carbohydrates, amino acid and saponins (Table 1). From the LD50 value we have selected the dose for PELA, CELA, EELA was 500 mg/kg b.w. and AELA was found 460 mg/kg b.w. The extracts of the leaves of L.alba when given orally elicited a significant analgesic activity in tail immersion as evidenced by increase in latency time in second (Table 2) as compared with vehicle control. Latency time was noted at 0, 30, 60, 180 and 240 min. after the administration of vehicle, standard and extracts. The result showed in Table 2 indicate that AELA have significant ($P<0.05$) antinociceptive activity as compered with the control. However, maximum effect was found 79.66%, 78.63%, 77.46%, 76.99%, 76.84%, 74.29%, 72.72% and 68.22% at AELA 60 min, AELA 30 min, PELA 120 min, AELA 120 min, PELA 60 min, AELA 180 min, EELA 240 min and PELA 180 min respectively.

The effect of extracts of *L. alba* on carrageenan-induced rat paw edema at a different time intervals was compared to that of control for the evaluation of anti-inflammatory activity assuming percent inhibition of paw edema volume. The experiment featured in Table 3 indicates that CELA and

Table 1.
Result of phytochemical analysis

Extracts	Phytochemicals							
	Phytosterol	Alkaloid	Fixed Oil	Flavonoid	PhenolicCompound	Amino Acid	Carbohydrate	Saponin
PELA	+	−	+	+	−	−	−	−
CELA	+	−	−	+	−	−	−	−
EELA	+	+	−	+	+	−	+	−
AELA	−	+	−	+	−	+	+	+

− = absent; + = present. PELA, CELA, EELA and AELA represent petroleum ether, chloroform, methanol and aqueous extract respectively.

Table 2.
Antinociceptive Activity of Leaves of *Lippia alba* (Mill.) N.E.Brown

Sl. No.	Treatment	Initial Time(s)	Time interval in Sec.				
			30 MIN	60 MIN	120 MIN	180 MIN	240 MIN
1.	Control	1.67 ±0.17	1.70±0.11	1.77±0.81	2.13±0.09	2.14±0.10	2.97±0.05
2.	Diclo. sod.	2.20±0.23	3.23±0.23*	3.37±0.29*	4.18±0.22*	4.07±0.08*	5.43±0.07
3.	PELA	2.10±0.81	2.27±0.42	3.13±0.27*	3.78±0.05*	3.60±0.27*	3.47±0.26
4.	CELA	2.05±0.49	2.18±0.29	2.97±0.52*	3.27±0.43*	3.20±0.46	3.33±0.46
5.	EELA	1.99±0.35	2.40±0.36	2.43±0.52	3.27±0.27*	3.50±0.26*	5.13±0.26*
6.	AELA	2.08±0.67	3.03±1.09*	3.18±1.45*	3.77±1.61*	3.73±1.76*	3.69±2.28

Mean± SEM, "*" indicates $P<0.05$, n=6

Table 3.
Anti-Inflammatory Activity of the Leaves of *Lippia alba* (Mill.) N.E.Brown

Sl. No.	Treatment	Mean Paw Thickness (cm) ± SEM				
		0 MIN	30 MIN	60 MIN	120 MIN	180 MIN
1.	Control	4.70±0.17	5.33±0.21	5.60±0.10	6.00±0.15	6.00 ± 0.12
2.	Ibuprofen	4.33±0s.38**(7.87%)	5.37±0.24**(0.75%)	4.33±0.08(22.68%)	4.20±0.00(30%)	4.03±0.07(32.83%)
3.	PELA	5.47±0.07*(16.38%)	4.93±0.27**(7.5%)	5.80±0.09**(3.71%)	5.57±0.27**(7.17%)	5.20±0.12*(13.33%)
4.	CELA	5.30±0.10(12.78%)	5.27±0.12**(1.13%)	5.30±0.47**(5.36%)	5.07±0.38**(15.5%)	4.83±0.37(19.5%)
5.	EELA	4.73±0.42**(0.63%)	4.90±0.50**(8.07%)	4.67±0.33*(16.61%)	5.20±0.00(13.33%)	5.07±0.30(15.5%)
6.	AELA	5.73±0.18*(21.91%)	5.00±0.32**(6.19%)	4.77±0.34*(14.82%)	5.50±0.35**(8.33%)	5.17±0.41**(13.83%)

Result expressed as Mean paw thickness ± SEM, "*" indicates $P< 0.05$, "**" $P< 0.01$, n=6. Percentage inhibition of oedema is indicated in parenthesis

EELA exhibit significant ($P<0.05$ & $P<0.01$) inhibition of paw volume compared with control. Significant inhibition of paw edema was observed with different doses tested until 180 min. However, maximum inhibition of paw edema was found 19.5% and 16.61% with CELA 180 min and EELA at 120 min reading respectively.

4. Discussion

In the present study, the analgesic and anti-inflammatory activities of different fractions of leaves were investigated applying phytochemical analysis and experimental animal models. This study has shown that the AELA has potent anti-nociceptive effect and thus indicate the presence of analgesic components that might influence the prostaglandin pathways. A significant increase in the reaction time for tail immersion method indicated the analgesic effect by AELA. The phytochemical analysis of leave extract also revealed that it contains flavonoids and flavonoids are well known for their ability to inhibit pain perception[20] by reduced the availability of prostaglandins. Hence, the presence of flavonoids in AELA may also contribute for the analgesic activity. Flavonoids also have anti-inflammatory properties due to their inhibitory effects on enzymes involved in the production of the chemical mediator of inflammation. Flavone, its methoxy derivatives exhibited significant dose-dependent analgesic activity[21]. The carrageenan-induced rat paw edema is a biphasic process. The release of histamine or serotonin occurs in the first phase and the second phase is associated with the production of bradykinin, protease, prostaglandin, and lysosome[22]. Therefore, the inhibition of carrageenan-induced inflammation by the extracts of leave could be due to the inhibition of the enzyme cyclooxygenase and subsequent inhibition of prostaglandin synthesis. The paw edema induced by carrageenan involves several chemical mediators such as histamine, serotonin, bradykinin, and prostaglandins[23]. This effect started from the first hour (60 min.) and was maintained in all the inflammatory phases, suggesting that the main mechanism of action of the tested extracts may involve prostaglandin biosynthesis pathway and may influence other mediators of inflammation. The extract is found to be less active than Ibuprofen even when used in higher doses. As the carrageenan-induced inflammation model is a significant predictive test for anti-inflammatory agents acting by inhibiting the mediators of acute inflammation, these results are an indication that *L. alba* can be an effective for acute inflammatory disorders. The phytochemical analysis of these extract revealed that it contains fixed oils, phytosterol, flavonoid, carbohydrate, alkaloid, phenolic compound, amino acid and saponin. Of

these, flavonoids and saponins are well-known for their ability to inhibit pain perception. Flavonoids also have anti-inflammatory properties due to their inhibitory effects on enzymes involved in the production of the chemical mediator of inflammation[24]. In conclusion, this study has shown that AELA possess significant antinociceptive activity and CELA and EELA possess significant anti-inflammatory effects that may be mediated through inhibition of cell mediators such as bradykinin, and prostaglandins. These studies justify the traditional use of this plant in some painful and inflammatory condition.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Sarker M, Das SC, Saha SK, Mahmud ZA and Bachar SC. Analgesic and Anti-inflammatory Activities of Flower Extracts of Punica granatum Linn. (Punicaceae). *J Appl Pharma Sci.* 2012; **02** (04): 133-136.

[2] Verma N, Amresh G, Sahu PK, Mishra N, Rao CV and Singh AP. Anti-inflammatory and antinociceptive activity of hydroethanolic extract of Woodfordia fruticosa Kurz flowers. *Der Pharmacia Sinica*. 2012; **3**(2):289-294.

[3] Kingsley C, Iwuanyanwu1 P, Eugene N, Onyeike, Ahsana D, *Der Pharmacia Sin.* 2011; **2**(4): 9-16.

[4] Maphosa V, Masika PJ and Moyo B. Investigation of the anti-inflammatory and antinociceptive activities of Elephantorrhiza elephantina (Burch.) Skeels root extract in male rats. *African J Biotech*. 2009; **8** (24): 7068-7072.

[5] Suseem SR and Mary SA. Studies on anti-inflammatory and antipyretic activities of fruiting bodies of pleurotus EOUS in experimental animals. *Pharmacologyonline*. 2011; **1**: 721-727

[6] Kaur S and Jaggi RK. Antinociceptive activity of ahronic administration of different extract of Terminalia bellerica Roxb. and Terminalia chebula Retz. fruits. *Indian J Exp Biol*. 2010; **48**: 925-930

[7] Devraj A and Karpagam. Evaluation of anti-inflammatory avtivity and analgesic effect of Aloe vera leaf extract in rats. *Int Res J Pharma.* 2011; **2**(3): 103-110

[8] Dolai N, Karmakar N, RBS Kumar and Haldar PK. CNS depressant activity of Castanopsis indica leaves. *Orient Pharma Experi Med.* 2012; **12**: 135-140.

[9] Pourmotabbed A, Farshchi A, Ghiasi G, Khatabi PM. Analgesic and Anti-inflammatory Activity of Teucrium chamaedrys leaves aqueous extract in Male Rats. *Iranian J Basic Med Sci*. 2010; **13**: 119-125

[10] Viana GSB, Vale TGD, Rao VSN and Matos FJA. Analgesic and anti-inflammatory effects of two chemotypes of L.alba: a comperative study. *Pharmaceutical Biology*. 1998; **36**(5): 347-351.

[11] Wealth of India: National Institute of Science Communication and Information Resources, CSIR, New Delhi, Vol.4, *First Printed* 1972: 48.

[12] Nanda AK. Text Book of Botany. 14th ed. Kitab Mahal, College Square, Cuttack-753003. 2006-2007:340-242.

[13] Koul O, Walia S and Dhaliwal GS. Essential Oils as Green Pesticides: Potential and Constraints. *Biopestic Int*. 2008; **4**(1): 63-84

[14] Ferreira FS, Brito SV, Saraiva RA, Araruna MKA, Menezes Irwin RA, Costa JGM, et al. Topical anti-inflammatory activity of body fat from the lizard Tupinambis merianae. *J Ethnopharmacol.* 2010; **130**: 514-520

[15] Brain KR, and Turner TD. The Practical Evaluation of Phytopharmaceuticals, Wright scien technica, Bristol; 1975.

[16] Guidelines for the Testing of Chemicals/Section 4: Health Effects Test No. 425: Acute Oral Toxicity: Up-and-Down Procedure. Paris, Organisation for Economic Co-operation and Development Publishing.

[17] Kulkarni SK, Hand Book of Experimental Pharmacology. 2nd ed. New Delhi Vallabh Prakashan. 1993: 49-51.

[18] Panduranga MG, Kumar PTG, Suresh A, Raviashankar HG, Chandrasekhar KB and Lokesh S. Evaluation of ethanolic leaf extract of Dioscorea hispida Dennst. for anti-inflammatory and Analgesic activities. *Int J Pharm & Ind Res*. 2011; **01**(02): 83-87.

[19] Pareek A, Chandurkar N, Saha RN and Payghan R. Evaluation of anti-Inflammatory activity of hydroxychloroquine and simvastatin combination in experimental animals. Res J Pharma, *Biol and Che Sci.*2011; **02**(01): 464-468.

[20] Sannigrahi S, Mazumder UK, Pal DK, Mishra SL and Maity S. Flavonoids of Enhydra fluctuans exhibits analgesic and anti-inflammatory activity in different animal models. *Pak J Pharm Sci.* 2011; **24**(3): 369-375.

[21] Sarker M, Das SC, Saha SK, Mahmud ZA and Bachar SC. Analgesic and anti-inflammatory Activities of Flower extracts of Punica granatum Linn.(Puniaceae). *J Appl Pharma Sci*. 2012; **02** (04): 133-136.

[22] Sahithi B, Rajani GP, Sowjanya K and Deepak Gupta. Antiinflammatory activity of ethanolic and aqueous extracts of Alternanthera sessilis Linn. *Pharmacologyonline*. 2011; **1**: 1039-1043.

[23] George JA and Kabatende J. Antinociceptive and anti-inflammatory activities of leaf methanol extract of Cotyledon orbiculata L. (Crassulaceae). *Adv Pharmacol Sci*. 2012; 862625:6.

[24] Hossain MS, Alam MB, Chowdhury NS, Asadujjaman M, Zahan R and Islam MM, et al. Antioxidant, analgesic and anti-inflammatory activity of the herb Eclipta prostrate. *J Pharmacol Toxicol*. 2011; **6**(5):468-480.