COMPARATIVE EVALUATION OF ANALGESIC, ANTI-INFLAMMATORY AND ANTIPYRETIC ACTIVITY OF LEAVES, STEM AND ROOTS OF *LANTANA CAMARA*

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Summary

The present study was under taken to compare the analgesic, anti-inflammatory and antipyretic activity of alcoholic extracts of leaves (AELLC), stem (AESLC) and roots (AERLC) of *Lantana camara*. Analgesic activity was evaluated with formalin induced paw licking test in mice, anti-inflammatory activity was evaluated with carrageenan induced rat paw edema model in wistar rats and antipyretic activity was evaluated with yeast induced pyrexia in rats. In preliminary phytochemical investigations the *Lantana camara* was found to contain alkaloids, carbohydrates, glycosides, saponins, proteins, flavonoids, tannins and phenolic compounds and the pharmacological studies have revealed that the leaves of lantana camara possess

Keywords: *Lantana camara;* Anti-inflammatory activity; Analgesic activity; Antipyretic activity.

Introduction

Pain is an unpleasant feeling which may be associated with actual or potential tissue damage and which may have physical and emotional components according to the International Association for the Study of Pain (IASP).The pain can be classified as acute or chronic. Acute pain is defined as short-term but extreme pain that comes on quickly but last only for a brief period of time. Chronic pain defined as pain that persists longer than the normal course of time associated with a particular type of injury.[1]

The major classes of analgesics includes Paracetamol and other NSAIDs, COX2 inhibitors, opiates and morphinomimetics and specific agents like anticonvulsants are used to treat neuropathic pain with modest success and also tricyclic antidepressants.

Inflammation is the first response of the immune system to infection or irritation and may be referred to as the innate cascade. Inflammation is characterized by the following quintent: redness (*rubor*), heat (*color*), swelling (*tumor*), pain (*dolor*) and dysfunction of the organs involved (*functio laesa*). Anti-inflammatory refers to the property of a substance or treatment that reduces inflammation.

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Steroidal anti-inflammatory drugs, many steroids, specifically glucocorticoids, reduce inflammation by binding to corticol receptors. These drugs are often referred to as corticosteroids. Non-steroidal anti-inflammatory drugs (NSAIDs), alleviate pain by counteracting the cyclooxygenase (COX) enzyme.[2]

Fever is considered a temperature above 100.4^0 F (38^0 C). A feverish sensation, however, may occur when the body temperature is above the average normal of 98.6 ^{0} F. (37 ^{0} C). Fever is part of the body's own disease-fighting arsenal. Rising body temperatures apparently are capable of killing off many diseases producing organisms[3]. Antipyretics are drugs that prevent or reduce fever by lowering the body temperature from a raised state. However, they will not affect the normal body temperature if one does not have fever[4].

Since the synthetic drugs produces wide number of side effects and also they are not able to cure the disease completely**.** Hence, the Traditional medical practitioners and Scientists are turning towards medicinal plants for curing these ailments[5].

Lantana camara (verbenaceae) is a native of tropical America, but now naturalized in many parts of India as troublesome prickly weed. This is an erect, branching shrub, 0.5-2m high. Stems are 4-angled, armed with hooked prickles. Leaves opposite, blade ovate, 4-10cm long, with coarse surfaces and toothed margins. Flowers are dense, long stalked, flat-topped, head-like, axillary spike about 2.5cm across. The corolla is sympetalous, with a curved tube and a spreading limb about 8mm wide, yellow, orange, red or pink in color. Fruit is a shiny, dark purple or black, globose drupe, 5-6 mm wide[6]. The plant is vulnerary, diaphoretic, carminative, antispasmodic and tonic. It is useful in tetanus, vitiated condition of vata, malaria, epilepsy and gastropathy. A decoction of fresh root is a good gargle for odontalgia and this is used by the trible peoples for all types of dysentery. Powdered leaves are used for cuts, wounds, ulcers and swellings. An infusion of the leaves is good for bilious fever, vitiated condition of vata and kapha, eczema and eruptions. The fruits are useful in fistula, pustules, tumors and rheumatism[7].

Materials and Methods

Drugs and chemicals

All the solvents used for the extraction process are of Laboratory grade. Paracetamol (Strides Arcolab ltd, Bangalore) and Diclofenac sodium (Micro labs, Bangalore), Carrageenan, Formalin (SD Fine Chem, Mumbai),

Plant extraction

The plant was collected in the month of May – June 2007 and authentified by Dr.K.P.Sreenath, Reader and Taxonomist, Botany Department from Bangalore University. A sample specimen was deposited, bearing voucher number **Coll.LC.no.I.** The shade dried plant material was powdered. The coarse powder was subjected to extraction with alcohol (70%) in soxhlet apparatus.

Experimental animals

Swiss albino mice (18-22g) and Wistar albino rats (150-200 g) of either sex were acclimatized for 7 days under standard husbandry conditions. i.e. room temperature 26 ± 10c. relative humidity 45-55% and light: dark cycle 12:12 h. the experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) of PES College of Pharmacy, Bangalore and conducted according to the guidelines of the Committee for the Purpose of the Control and Supervision on Experiments on Animals (CPCSEA).

Pharmacological Evaluation

Acute toxicity studies

The acute toxicity of AELC was determined in female albino mice (18-22g). After administration with different doses of the extract, the mortality with each dose was noted at 48 hours (acute) and 14 days (chronic). LD50 was calculated as per OECD guidelines 42 [8] using AOT 425 software.

Analgesic activity

A. Formalin induced paw liking test

The formalin test possesses two distinctive phases, which possibly reflecting different types of pain. Diclofenac sodium or vehicle or test drug were given orally to mice. 30 minutes latter 20 µl of 1 % formalin was injected subcutaneously under the dorsal surface of hind paw. Mice were observed in chambers. The number of licks in the injected paw was counted and considered as pain stimuli. The first phase of the nociceptive response normally peaked 5 minute after the formalin injection and the second phase 20-30 minutes after the formalin injections representing neurogenic and inflammatory responses respectively [9].

Anti-inflammatory activity

A. Carragenan induced rat paw edema

The method used was similar to that described by Winter (Winter et al., 1962). Male or female Wistar rats with a body weight between 150-200g are used. The animals are starved overnight. Inject vehicle/test drug /Diclofenac sodium orally. One hour later the rats are challenged by injection of 0.1 ml of 1% carrageenan into the plantar region of the left hind paw. The paw is marked with ink at the level of the lateral malleolus and immersed in mercury up to this mark. The paw volume is measured plethysmographically before carrageenan injection (Initial paw volume) and again at 1, 2, 3, 4 and eventually 24 h after challenge. Compare the mean percent change in paw volume [10].

Antipyretic activity

A. Yeast induced pyrexia

Rats were divided into ten groups of six rats each. The normal body temperature of each rat was measured rectally at predetermined intervals and recorded. Fever was induced as per the method described by Smith and Hambourger (1935) [11]. The rats were acclimatized to remain quiet in a restrained cage. A thermister probe was inserted 3-4cm deep into the rectum and fastened to the tail by adhesive tape. The temperature was measured on a thermometer. After measuring the basal rectal temperature, animals were given a subcutaneous injection of 10ml/Kg BW of 15% (w/v) yeast suspended in saline solution. Rats were then returned to their housing cages. After 19^{th} h of yeast injection, the animals were again restrained in individual cages for the recording of their rectal temperatures as described previously. Nineteen hour after yeast injection the vehicle/ test drug/paracetamol are given orally. Rats were restrained for recording the rectal temperatures at the 19^{th} hour immediately before the administration of vehicle/drug/paracetamol and again at 1 h intervals upto 24 h after yeast injection.

Statistical analysis

Values are expressed as mean ± SEM from 6 animals. Statistical differences in mean were analyzed using one way ANOVA (analysis of variance) followed by Dunnett's test. $p<0.05$ was considered significant.

Results

Plant extraction

The plant parts namely leaves, Stem and roots of *Lantana camara* was subjected to alcoholic extraction process the extractive values are given in the table no.1

Table.no.1. Alcoholic extractive values of Leaves (AELLC) stem (AESLC) and roots (AERLC) OF *Lantana camara.*

Sl.no	Drug	Plant part	Colour of extract	Extractive values
1	***Lantana camara***	**Leaves**	Greenish black and Sticky	17.15%
		Stem	Blackish brown	3.197%
		Roots	Brown	6.23%

Pharmacological Evaluation

Acute toxicity studies

In acute toxicity studies no mortality was observed. Hence the extracts of leaves (AELLC), stem (AESLC) and roots (AERLC) of *Lantana camara* were found to be safe up to 2000 mg/kg.

Analgesic activity

Analgesic activity was studied by using formalin induced paw licking test in mice using diclofenac as a standard (15 mg/kg .p.o.). In this test all the test drugs have shown significant inhibition in number of licking induced by the formalin only in the late phase, where as the AERLC has decreased the number of licking produced by the formalin in early and late phase at lower as well as in the higher dose levels. Tha values are as shown in the table.no.2.

Table.no.2. Effect of *Lantana camara* leaves (AELLC), stem (AESLC) and roots (AERLC) on formalin induced paw licking test in mice

Treatment	Dose (mg/kg)	Number of licking(s)			
		0-10min (early phase)	% Inhibition	15-30min (late phase)	% Inhibition
Control	- - -	89.0±4.18	----------	83.4±3.20	
Diclofenac sodium	15	57.8±2.95**	35.05618	12.2±1.30***	85.3717
AELLC	200	76.8±3.12	13.70787	48.1±2.56**	42.32614
AELLC	400	70.8±3.56	20.44944	42.5±2.31***	49.04077
AESLC	200	78.7±3.56	11.57303	49.7±2.81**	40.40767
AESLC	400	73.42±2.8	17.50562	44±2.14***	47.24221
AERLC	200	69.4±3.57**	22.02247	31.4±2.60***	62.35012
AERLC	400	65.2±3.34**	26.74157	24.2±2.16***	70.98321

Values are Mean ± S.E.M. (n=6); Significance vs. Control group: ***P< 0.001, **P< 0.01 and *P<0.05.

Anti-inflammatory activity

Anti-inflammatory activity was studied by using Carrageenan induced rat paw edema test in rats using diclofenac as a standard (10 mg/kg .p.o.). In this test all the test drugs viz. AELLC , AESLC and AERLC have shown significant inhibition of carrageenan induced rat paw edema at higher dose (400mg/kg.p.o) and also in the lower doses (200 mg/kg .p.o.), but the percentage

inhibition in paw edema showed by the AERLC is more than that of AELLC and AESLC . The values are as shown in the table no.3

Table.no.3. Effect of *Lantana camara* leaves (AELLC), stem (AESLC) and roots (AERLC) on carrageenan induced rat paw oedema in rats

Treatment	Dose (mg/kg)	Paw volume in ml (% Inhibition of Paw Edema)			
		1hr	2hr	3hr	4hr
Control	-----	0.525±0.032	0.6375±0.047	0.725±0.066	0.6875±0.062
Diclofenac sodium	10	0.225±0.032*** (57.14)	0.2±0.028*** (68.62)	0.1625±0.012*** (77.58)	0.0875±0.024*** (87.27)
AELLC	200	0.375±0.025* (28.57)	0.45±0.02** (29.41)	0.463±0.012*** (36.21)	0.35±0.02*** (49.1)
AELLC	400	0.35±0.02** (33.33)	0.41±0.024*** (35.29)	0.412±0.037*** (43.1)	0.312±0.024*** (54.54)
AESLC	200	0.325±0.032*** (38.09)	0.27±0.032*** (56.86)	0.30±0.02*** (58.52)	0.212±0.012*** (69.09)
AESLC	400	0.31±0.024*** (40.48)	0.22±0.025*** (64.07)	0.237±0.012*** (67.24)	0.162±0.012*** (76.36)
AERLC	200	0.25±0.029*** (52.38)	0.21±0.024*** (66.6)	0.187±0.037*** (74.13)	0.112±0.031*** (83.63)
AERLC	400	0.215± 0.06*** (59.04)	0.205±0.024*** (67.77)	0.175 ± 0.012*** (75.86)	0.098 ± 0.024*** (85.74)

Values are Mean ± S.E.M. (n=6); Significance vs. Control group: ***P< 0.001, **P< 0.01 and *P<0.05.

Antipyretic activity

Antipyretic activity was studied by using yeast induced pyrexia in rats using paracetamol as a standard (150 mg/kg .p.o.). In this test AELLC (200 and 400mg/kg), AESLC (200 and 400mg/kg) and AERLC (200 and 400mg/kg) have shown significant reduction in pyrexia induced by yeast where as the antipyretic activity showed by the AERLC is more than that of AELLC and AESLC. The values are as shown in the table no.4

Table. no .4. Effect of *Lantana camara* leaves, stem and roots on yeast induced pyrexia in rats

Groups	Dose (mg/kg)	Rectal temperature (% Reduction in fever)						
		0hr	19hr	20hr	21hr	22hr	23hr	24hr
Control	--------	37.4±0.09	39.2±0.09	39.0±0.1	39.0±0.1	39.1±0.1	39.1±0.1	38.9±0.08
Standard	**150**	37.3±0.04	39.1±0.04	37.8±0.04*** (82.62)	37.8±0.04*** (84.61)	37.6±0.06*** (89.83)	37.4±0.07*** (92.30)	37.45±0.06*** (93.93)
AELLC	**200**	37.4±0.04	39.2±0.08	38.9±0.1 (25.0)	38.9±0.1 (33.3)	38.9±0.1** (50.0)	38.9±0.08** (61.53)	38.925±0.08*** (66.66)
AELLC	**400**	37.4±0.07	39.2±0.09	38.8±0.08 (35.71)	38.7±0.08** (55.55)	38.8±0.1** (57.14)	38.8±0.1*** (68.75)	38.8±0.1*** (75.0)
AESLC	**200**	37.4±0.06	39.1±0.05	38.4±0.03*** (64.0)	38.4±0.02*** (69.23)	38.3±0.02*** (78.57)	38.3±0.02*** (82.14)	38.2±0.02*** (88.23)
AESLC	**400**	37.3±0.08	39.2±0.07	38.2±0.07*** (77.5)	38.1±0.06*** (81.39)	38.1±0.06*** (86.36)	38.0±0.1*** (89.58)	38.1±0.1*** (90.90)
AERLC	**200**	37.3±0.05	39.1±0.04	38.7±0.00 (43.57)	38.5±0.05** (50.0)	38.5±0.05*** (72.72)	38.4±0.02*** (80.0)	38.4±0.02 *** (84.0)
AERLC	**400**	37.2±0.05	39.1±0.06	38.5±0.06** (57.14)	38.5±0.06*** (63.63)	38.4±0.08*** (75.0)	38.4±0.08*** (79.16)	38.3±0.08*** (86.66)

Values are Mean ± S.E.M. (n=6); Significance vs. Control group: ***P< 0.001, **P< 0.01 and *P<0.05.

Discussion

The formalin induced paw licking test is a valid and reliable model for analgesic activity and it is sensitive for various classes of analgesic drugs. Formalin test produces a distinct biphasic response and different analgesics may act differently in the early and late phases of this test. Therefore, the test can be used to clarify the possible mechanism of the antinociceptive effect of a proposed analgesic [12]. Centrally acting drugs such as opioids inhibit both phases equally [13] but peripherally acting drugs such aspirin, indomethacin and dexamethasone only inhibit the late phase. The late phase seems to be an inflammatory response with inflammatory pain that can be inhibited by anti-inflammatory drugs [14]. This study suggested that the AELLC and AESLC are showing analgesic activity only in the late phase this indicates that they are peripherally acting analgesics, where as the AERLC has shown analgesic activity in both early and late phase it indicates that it is having both central and peripheral analgesic action.

In carrageenan induced rat paw edema test is the most commonly used model for screening the anti-inflammatory activity of a drug, in this test all the test drugs namely AELLC, AESLC and AERLC at (200 and 400mg/kg .p.o.) showed significant inhibition in carrageenan induced rat paw edema, where as the AERLC (200 and 400mg/kg) has shown more significant anti-inflammatory activity when compare to AELLC and AERLC (200 and 400mg/kg).

Since antipyretic activity is commonly mentioned as characteristic of drugs or compounds which have an inhibitory effect on prostaglandin-biosynthesis [15], the yeast induced hyperthermia in rat model was therefore employed to investigate the antipyretic activity of leaves of *Lantana camara*. It was found that AELLC, AESLC and AERLC (200 and 400mg/kg) showed a significant decrease in rectal temperature, Among the three extracts the AESLC has showed more significant antipyretic activity then AELLC and AERLC, These results seems to support the view that the plant has influence on prostaglandin biosynthesis because prostaglandin is believed to be a regulator of body temperature [16].

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