ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY OF TOPICAL PREPARATION OF *LANTANA CAMARA* LEAVES

Shylaja.H[1], K.Lakshman*[1], Nabanita kar[1], Vikas maurya[1], G.L. Viswanatha[2]

[1]Department of Pharmacognosy, PES College of Pharmacy, Hanumanthanagar, Bangalore-50
[2]Department of Pharmacology, PES College of Pharmacy, Hanumanthanagar, Bangalore-50

E-mail: drlakshman26@rediffmail.com.

Summary

The present study was undertaken to evaluate the analgesic and anti-inflammatory activity of topical preparation of *Lantana camara* leaves, in literature it is found that leaves of *Lantana camara* having analgesic and anti-inflammatory activity. Due to the toxicity of *Lantana camara* on oral administration, topical formulations containing 1%, 2%, 4%, 6% and 8% of alcoholic extract of *Lantana camara*(AELC) were prepared and screened for analgesic and anti-inflammatory activity. Analgesic activity was evaluated with formalin induced paw licking test in mice using Methyl salicylate as a standard. Anti-inflammatory activity was evaluated with carrageenan induced rat paw edema model in wistar rats using Piroxicam gel as a standard. In preliminary phytochemical investigations the AELC was found to contain alkaloids, carbohydrates, glycosides, saponins, proteins, flavonoids, tannins and phenolic compounds and the pharmacological studies have revealed that preparations containing 4%, 6% and 8% AELC showed significant analgesic and anti-inflammatory activity. In conclusion the formulations containing 4%, 6% and 8% AELC was found to possess good analgesic and anti-inflammatory activity.

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

INTRODUCTION

Inflammation is a chronic disease, which involves (1) increase of vascular permeability resulting in exudation of fluid from blood into the interstitial space (2) infiltration of leucocytes from the blood into the tissues and (3) granuloma formation [1]. Currently drugs like opioid analgesics, corticosteriods, NSAID's and immunosuppressive agents are used to control the symptoms of inflammation and pain [2]. The use of these drugs will produce certain side effects like respiratory depression, sedation, constipation, tolerance, spasm, gastrointestinal disturbances, renal and hepatic damage, bone marrow depression, suppression of response to infection or injury, osteoporosis, development of Cushing's syndrome etc [3].

Natural products in general and medicinal plants in particular are believed to be an important source of new chemical substances with potential therapeutic efficacy. Taking into account that the most important analgesic prototypes (e.g. salicylic acid and morphine) were originally derived from the plant sources, the study of plant species traditionally used as pain killers should still be seen as a fruitful research strategy in the search of new analgesic and anti inflammatory drugs [4].

Lantana camara (verbenaceae) is a native of tropical America, but now naturalized in many parts of India as troublesome prickly weed. It is a large scabling evergreen, strong smelling shrub with stout recurved pricker, leaves opposite, often rugose, scabrid on both sides, flowers small, normally orange but often white to dark red , in heads which are prominently capitate, bracts conspicuous, persistant, fruits fleshy drupe 5mm in diameter, endocarp hard, green when young and blue or black on ripening[5]. 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 [6].

MATERIALS AND METHODS

Drugs and chemicals

All the solvents used for the extraction process are of Laboratory grade. Carbopol 940, Carrageenan (SD Fine chemicals. Mumbai), methyl paraben, propyl paraben (Apex chemicals, Bombay), Cetyl alcohol, NaOH (Lobart Fine chemicals. Surat), Propylene glycol (Otto kemi, Mumbai), Piroxicam gel (Cipla. Ahmedabad).

Plant extraction

The leaves of 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. The % yield of alcoholic extract was found to be 17.1%.

Phytochemical investigation

The alcoholic extract of *Lantana camara* (AELC) was subjected to preliminary qualitative investigations [7].

Formulation of topical preparation

Cream formulations (o/w) were prepared by the following procedure. Carbopol 941 was hydrated in water, using methyl paraben and propylene glycol for 36 h and dispersed with a double bladed mixer (300 rpm) for 30 min., then heated to 60^0C and oily phase containing different amounts of cetyl alcohol, white petrolatum; liquid paraffin oil and Tween 80 were weighed and melted to approximately 60^0C. The aqueous phase was added to the oil phase and mixed with a double bladed mixer (300 rpm) for 30 min. The mixture was neutralized by NaOH to $_P$H 6.2 and then mixed (300 rpm) for 30 min. AELC extract was levigated in propylene glycol and then added to the formulation after neutralizing. Table.No.1 shows the constituents of investigated preparations.

Table 1. Compositions of formulations

Sl.no.	Name of the Ingredient	1% Formulation	2% Formulation	4% Formulation	6% Formulation	8% Formulation
1.	AELC	1gm	2gm	4gm	6gm	8gm
2.	Carbopol-941	500 mg	500 mg	500 mg	500 mg	500mg
3.	Methyl Paraben	200 mg	200 mg	200 mg	200 mg	200 mg
4.	Propyl Paraben	20mg	20mg	20mg	20mg	20mg
5.	Cetyl alcohol	3gm	3gm	3gm	3gm	3gm
6.	White petrolatum	6gm	6gm	6gm	6gm	6gm
7.	Mineral oil	5gm	5gm	5gm	5gm	5gm
8.	Tween 80	1gm	1gm	1gm	1gm	1gm
9.	NaOH ($_P$H 6.2)	1-2ml	1-2ml	1-3ml	1-2 ml	1-3ml
10.	Water	70ml	70ml	65ml	60ml	55ml

Experimental animals

Wistar albino rats of either sex weighing 150–200 gm and albino mice of either sex weighing 20–25 gm were used for anti-inflammatory and analgesic activities respectively. They were housed in standard environmental conditions and fed with standard rodent diet with water ad libitum. All animal procedures were followed in accordance with the approved protocol for use of experimental animals set by the Institutional Animal Ethical Committee. Six groups of six animals were used for each experiment.

Anti-inflammatory activity

Carrageenan-induced rat paw edema

Animals were fasted for 24 h before the experiment with free access to water. Approximately 50 µl of a 1% suspension of carrageenan in saline was prepared 1h before each experiment and was injected into the plantar surface of right hind paw of rat. 0.3g of cream containing 1%, 2%, 4%, 6% and 8% of AELC was applied to the plantar surface of the hind paw by gently rubbing 50 times with the index finger. Rats of the control group received only the cream base. 0.5% Piroxicam gel applied in the same way was used as a standard. Drugs or placebo was applied 1h before the carrageenan injection. Paw volume was measured immediately after carrageenan injection and at 1-, 2-, 3- and 4-h intervals after the administration of the noxious agent by using a plethysmometer (model 7159, Ugo Basile arese, Italy) [8].

Analgesic activity

Formalin induced paw licking test

0.3g of cream containing 1-8% of AELC was applied to the dorsal surface of the left hind paw by gently rubbing 50 times with the index finger. Rats of the control group received only the cream base. Methyl salicylate ointment 30 % applied in the same way was used as standard. Fifteen minutes later, the analgesic activity was determined using formalin test described by Dubuisson and Dennis [9]. Fifty micro liters of 2.5% formalin was injected into the dorsal surface of the left hind paw. The rat was observed for 30 min after the injection of formalin, and the number of lickings during the 30 mins observation period was recorded. The first 10 min post formalin injection is known as the early phase and the period between 15 and 30 min is known as the late phase.

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.

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RESULTS

Phytochemical investigation

It was found that the AELC contains alkaloids, carbohydrates, glycosides, saponins, proteins, flavonoids, tannins and phenolic compounds.

Pharmacological investigations

Analgesic activity

In this test the formulations containing 4%, 6% and 85 of AELC have showed significant inhibition in number of lickings in both early phase (0-10 mins) and late phase (15-30 mins) of the test where as the 1% and 2% formulation of AELC has showed slight inhibition only in the early phase of the test .The results are shown in **Table No.2.**

Table 2. Analgesic effect of the Topical preparation of *Lantana camara* on the Formalin induced paw licking.

Treatment[a]	Total time spent in licking(s)			
	0-10 min	% Inhibition	15-30 min	% Inhibition
Control	159.5±12.01	- - -	342.75±20.1	- - -
Standard	41.75±4.64***	73.82	174.25±18.68***	49.16
AELC-1%	130.0±9.06	18.49	300.25±14.32	12.39
AELC-2%	116.5±5.83**	26.95	279.0±14.29	18.59
AELC-4%	107.25±7.49**	32.75	227.0±4.32***	33.77
AELC-6%	100.5±6.76***	36.99	204.0±8.72***	40.48
AELC-8%	57.5±2.10***	63.94	193.75±19.61***	43.47

[a] 0.3g of preparation were applied to the plantar surface of the right hind paw by gently rubbing 50 times with the index finger.
Values are mean+S.E.M; n=6; ** P< 0.01 and ***p<0.001 as compared to the control

Anti-inflammatory activity

In anti-inflammatory studies all the formulations of AELC has showed significant reduction in paw volume (% Inhibition of paw edema) when compared to the control group receiving only the base cream. The results of anti-inflammatory activity of topical preparation of AELC are given in **Table No. 3**.

Table 3. Anti-inflammatory effect of the Topical preparation of *Lantana camara* on the Carrageenan - induced paw edema.

Treatment[a]	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
Standard	0.225±0.032*** (57.14)	0.2±0.028*** (68.62)	0.1625±0.012*** (77.58)	0.0875±0.024*** (87.27)
AELC-1%	0.375±0.025* (28.57)	0.45±0.02** (29.41)	0.463±0.012*** (36.21)	0.35±0.02*** (49.1)
AELC-2%	0.35±0.02** (33.33)	0.41±0.024*** (35.29)	0.412±0.037*** (43.1)	0.312±0.024*** (54.54)
AELC-4%	0.325±0.032*** (38.09)	0.27±0.032*** (56.86)	0.30±0.02*** (58.52)	0.212±0.012*** (69.09)
AELC-6%	0.31±0.024*** (40.48)	0.22±0.025*** (64.07)	0.237±0.012*** (67.24)	0.162±0.012*** (76.36)
AELC-8%	0.25±0.029*** (52.38)	0.21±0.024*** (66.6)	0.187±0.037*** (74.13)	0.112±0.031*** (83.63)

[a] 0.3g of preparation were applied to the plantar surface of the right hind paw by gently rubbing 50 times with the index finger.

Values are mean±S.E.M; n=6; *p<0.05, ** P< 0.01 and ***p<0.001 as compared to the control.

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 [10]. Centrally acting drugs such as opioids inhibit both phases equally [11] 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 [12]. The effect of topical preparation containing extract on the first and second phase of formalin induced paw licking test suggests that its activity may be because of its central action.

From these results, we can conclude that the topical preparation containing 4%, 6% and 8 % w/w of AELC possesses good analgesic effect; where as the anti-inflammatory studies have revealed that all the formulations (1%, 2%, 4%, 6% and 8%) of AELC possess good anti-inflammatory activity. Preliminary phytochemical investigation of this plant showed the presence of alkaloids, tannins, phenolic compounds, glycosides, phytosterols, flavonoid, carbohydrates and amino acids which might be in part responsible for analgesic and anti-inflammatory effects.

Acknowledgment

The authors are thankful to Prof.Dr.S.Mohan, Principal and management of P.E.S.College of pharmacy for providing all necessary facilities to carry out the research work. Special thanks to Dr.K.P.Sreenath, Reader in Botany, Bangalore University for authentifying the plant material.

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