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Research Article

EVALUATION OF ANALGESIC ACTIVITY OF *LEPIDAGATHIS CRISTATA* WILLD LEAF EXTRACTS

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ABSTRACT

Aim of the study is to screen the *Lepidagathis cristata* Willd, leaf extracts for analgesic activity, because the plant was screened only for immunosuppressive, antipyretic activities only, now in the present study the analgesic activity of leaf extracts were performed. The ethanolic, ethyl acetate, chloroform extracts were prepared and are used for analgesic activity in two dose level that is 200 and 400 mg/kg body weight per oral in two screening methods, one is Hot Plate (n=5), another is Tail Immersion method (n=5), and the leaf extracts are showed significant analgesic activity. The plant extracts did not exhibit any mortality up to the dose level 4000 mg/kg. The methanol, Chloroform and Ethyl acetate extracts of leaf was evaluated for analgesic activity. . The 400mg/kg dose of leaf chloroform extract has highest activity in both the experimental models with 62.5% protection after 30min and 47.3% after 60 min with the significance of $p< 0.001$ when compared with 0 time interval and after 90 min it was shown 50% of protection and all the extracts has graded dose response.

Keywords: *Lepidigathis Cristata willd*, Analgesic activity, Hot plate, Tail Immersion, Ethanol, Ethyl acetate, Chloroform.

INTRODUCTION

The plant *lepidigathis Cristata* Willd as a common herb in the eastern plains karnataka. The leaves, roots, flowers, seeds and the whole plant of various species of *Lepidigathis cristata* are medicinally useful. The roots of the herb are particularly considered in stomachic and dyspepsia, leaves are used for fever and flower ash is used for burns[1]. In present study *Lepidigathis cristata willd* (Family: Acanthaceae) locally known as nakkapidi, lankapidi by yanadi tribal people at Andhra Pradesh[2]. This yanadi tribal population much widely distributed in Cuddapah, Chittoor, Nellore and Prakasham districs and it is used for burns, wounds, and the tuber ash mixed with coconut oil and used as a lotion. In other parts of India is known as Kollchechutar(Bombay), karappanundu (Malayalam), Bhuyaterada (marathi), and Otdhompo (Santhal). It is a perennial, prostrate, small herb, with woody root stock. Leaves alternate, elliptic – oblong, clothed with sliky pubescence. Flowers blue, solitary or in pairs with long pedicels, axillary. Fruit globose capsule[3,4]. Common weed of open places, roadsides, grasslands and scrub jungles.

MATERIAL AND METHODS

The plant was collected from Sheshachala hills near by Rajiv Gandhi Institute of Medical Sciences Kadapa, Andhra Pradesh India. The plant was identified by Assistant professor Dr.Madhusudana Reddy Department of Botany, Yogivemana University, Kadapa, Andhara Pradesh, india. The collected whole plant was dried at room temperature under shade for fifteen days then the leaves are separated manually. The separated plant material was powdered by using mechanical mixer and sieved(40) material used for extraction and extraction was carried out by using soxhlet of 1000ml capacity round bottom flask. The extractions of leaf part was carried with methanol, ethyl acetate, and chloroform until the solvent becomes colourless in the soxhlet and the extracts were concentrated under reduced pressure. The solvent free substances are mixed with 1%v/v tween 80 and used for the experimentation.

Phytochemical screening

The phytochemical tests are performed to *Lepidagathis cristata* leaf chloroform, ethyl acetate, and methanolic extracts and the results are depicted in the table 1.

Acute toxicity studies

The maximum non lethal dose was found to be 400mg/kg body weight orally. The extracts were showed no mortality. The determination of acute toxicity by adapting fixed dose, the guidelines of CPCSEA and $1/10^{th}$ and $1/20^{th}$ of LD_{50} cute of values of the extracts were taken as a screening doses i.e 200 and 400mg/kg body weight.

Analgesic activity of leaf extracts by eddy's hot plate method

Albino mice weighing between 20 and 25 g were randomly divided into eight groups of 5 rats each and fasted for 12 hours. Animals are individually placed on a hot plate maintained at constant temperature (55^0C) and the reaction time of animals such as paw licking or jumping response is taken as end point. Analgesics increase reaction-time. The cut off time was 10 s. Group I served as control and received normal saline (10 ml/kg b.w), Group II received (12.5 mg/kg standard drug. Group III to Group VIII received orally, the test substances at a dose of 200 and 400 mg/kg b.w) through oral route as mentioned above. The reaction time was recorded for all the mice at 0, 15, 30, 60, and 90.[5,6,7]

Analgesic activity of leaf extracts by tail immersion method

Albino mice were randomly divided into twenty groups of 5 rats each and fasted for 12 hours. About 3-5 cm of the tail of each rat was dipped into a water bath containing warm water maintained at the temperature of 56 ± 2°C and the time taken for a rat to withdraw the tail known as the pain reaction time (PRT) was recorded for all the rats, in 0.01 s units using a stopwatch. The cut off time was 10 s. Group I served as control and received normal saline (10 ml/kg b.w), Group II received (12.5 mg/kg standard drug. Group III to Group VIII received orally, the test substances at a dose of 200 and 400 mg/kg b.w) through oral route. PRT was recorded again as

described earlier for all the rats at 0, 30, 60, 90, and 120. The withdrawal time of untreated animals is between 1 and 5.5 s. the increase in mean reaction withdrawal time was calculated.[6,7]

Table 1 Phytochemical screening

Extracts	Alkaloids	Glycosides	Phenolic compounds	Tannins	Resins	Volatile Oils	Carbohydrate
Leaf Chloroform Extract	+	+	-	-	+	+	-
Leaf Ethyl Acetate Extract	+	+	+	-	-	+	-
Leaf Methanol Extract	+	+	+	+	+	+	+

Table 2: Analgesic activity by eddy' s Hot plate method

Group	Dose (mg/kg)	Reaction time in seconds					
		0 min	15 min	30 min	45 min	60 min	90 min
Control		2.50±0.5	2.75±0.75	3.25±0.25	3.00±0.40	3.00±0.40	2.50±0.28
Diclofenac	12.5	2.75±0.25	6.25±0.25***	6.50±0.5**	4.60±0.40**	4.50±0.47**	4.00±0.91*
LCE	200	2.75±0.47	4.50±0.5	5.50±0.95[c]	5.25±0.62[c]	4.75±0.25[a]	3.50±0.28
LCE	400	2.25±0.62	4.25±0.62[b]	6.00±0.91[c]	5.00±1.15[c]	4.50±0.28[b]	4.50±0.28[b]
LEE	200	2.75±0.47	4.50±0.64[b]	5.00±0.40[c]	4.50±0.28[c]	3.50±0.28	3.75±0.47[a]
LEE	400	2.50±0.50	3.50±0.28	6.25±0.47[c]	4.25±0.25[c]	3.25±0.25	3.50±0.50
LME	200	2.75±0.25	3.00±0.40	5.25±0.62[c]	5.25±0.85[c]	4.25±0.25[b]	4.00±0.70[b]
LME	400	2.25±0.25	3.00±0.57[b]	4.75±0.40[b]	4.25±0.25	4.00±0.47[b]	2.75±0.70[b]

Values are expressed in MEAN±S.E.M. for five animals each group. ANOVA followed by Tukeys multiple comparison test. Values are statically [a]p<0.05, [b]p<0.01, and [c]p<0.001 when compared with 0 min interval

Table: 3 Percentage protection of the *Lepidagathis cristata* leaf extracts by Eddy's Hot plate method

Group	Dose (mg/kg)	% protection				
		15min	30min	45min	60min	90min
Control	-	-	-	-	-	-
Diclofenac	12.5	56	57.6	40.2	38.8	31.25
LCE	200	38.8	50	47.6	42.1	21.4
LCE	400	47	62.5	55	50	50
LEE	200	38.8	45	38.8	21.4	26.6
LEE	400	28.5	60	41.1	23	28.5
LME	200	8	47.6	47.6	35.2	31.2
LME	400	25	52.6	47	43.7	18.1

Table 4: Analgesic activity by tail immersion method

Group	Dose (mg/kg)	Reaction time in seconds				
		0 min	30 min	60 min	90 min	120 min
Control		3.25±0.25	2.25±0.62	3.00±0.40	2.75±0.25	2.75±0.47
Diclofenac	12.5	2.25±0.25	2.50±0.28	3.75±0.75[b]	4.25±0.25[c]	2.75±0.25
LCE	200	3.25±0.25	4.75±0.62[a]	5.25±0.62[a]	5.00±0.25[c]	4.85±0.47[a]
LCE	400	2.5±0.28	3.25±0.25[a]	4.75±0.28[c]	4.50±0.28[c]	3.00±0.40
LEE	200	3.25±0.25	4.50±0.40	5.00±0.40	4.25±0.75[a]	3.75±0.75
LEE	400	3.25±0.47	4.75±0.28	5.50±0.28	5.25±0.25	4.25±0.47
LME	200	3.25±0.28	4.25±0.25	5.0 0±0.40	4.75±0.25	3.50±0.28
LME	400	2.75±0.25	3.75±0.25[c]	4.75±0.47[c]	4.25±0.25	3.25±0.47

Values are expressed in MEAN±S.E.M. for five animals each group. ANOVA followed by Tukeys multiple comparison test. Values are statically [a]p<0.05, [b]p<0.01, and [c]p<0.001 when compared with 0 min interval.

Table 5: Percentage protection of the *Lepidagathis cristata* leaf extracts by tail immersion method

Group	Dose (mg/kg)	% protection			
		30min	60min	90min	120min
Control	-	-	-	-	-
Diclofenac	12.5	10	40	47	18.1
LCE	200	31.5	38	35	32.9
LCE	400	23	47.3	44.4	16.6
LEE	200	27.7	35	23.5	13.3
LEE	400	31.5	40.9	38	23.5
LME	200	23.5	35	31.5	7.1
LME	400	26.6	42.1	35.2	15.3

RESULTS

The phytochemical tests revealed the presence of secondary metabolites the results are presented in the table 1.The analgesic activity of Lepidagathis cristata leaf extracts were performed and results were presented in the tables 2,3,4 and 5.

DISCUSSION

The preliminary phytochemical screening of Chloroform, Ethyl acetate, Methanolic extracts of *Lepidagathis cristata* indicated the presence of alkaloids, glycosides, phenolic compounds, tannins, resins, volatile oils and carbohydrates, thus the activity showed may the alkaloid or glycoside because the maximum activity showed by Chloroform extract(LCE) which contains only alkaloids, glycosides, resins and volatile oils and all the extracts of *Lepidagathis cristata* did not showed any toxicity in mice up to 4000mg/kg body weight hence doses are selected $1/10^{th}$ and $1/20^{th}$ that is 400 and 200 mg/kg body weight p.o. The analgesic activity was performed under thermal models (Eddy's Hot

Plate and Tail immersion method) and all the extracts were found to be significantly effective. The 400mh/kg dose of LCE has highest activity in both the experimental models with 62.5% protection after 30min and 47.3% after 60 min with the significance of $p< 0.001$ when compared with 0 time interval and after 90 min it was shown 50% protection but the standard drug was only 31.25% and with all the extracts there was a graded dose response observed.

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