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Research Article

CENTRAL NERVOUS SYSTEM ACTIVITY OF DIFFERENT EXTRACTS OF *LEUCAS LONGIFOLIA* BENTH

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ABSTRACT

Leucas longifolia Benth. (Lamiaceae) is commomn plant in India is considered carminative, stimulant and emmenagogue. In the present study, crude petroleum ether (PE), chloroform (CE) and methanol extract (ME) of aerial part of *Leucas longifolia* have been evaluated for analgesic (100, 200 mg/kg, i.p.) and central nervous system (CNS) depressant activity (100, 200 and 400 mg/kg, i.p.).The analgesic activity was assayed in several experimental models: acetic acid induced writhing (chemically induced pain), hot plate, tail flick test (thermally induced pain). The petroleum ether extract and methanolic extract significantly and in dose dependent manner reduce the nociception induced by acetic acid. In hot plate and tail flick test methanolic and petroleum ether extract shows more significant action than chloroform extract. In the study of the CNS-depressant effect, the methanolic extract significantly reduces spontaneous motor activity at higher doses than petroleum ether extract. The fall off time (motor coordination) was also decreased. A potentiation in the pentobarbitone-induced sleep due to the sedative effect of the methanolic extract was observed. The result shows that petroleum ether extract and methanolic extract shows analgesic and CNS-depressant activity may be because of presence of different chemical compounds present in that extracts. Further investigations are, however, necessary to explore mechanism(s) of action involved in these pharmacological activities.

Keywords: *Leucas longifolia,* Analgesic, CNS-depression

INTRODUCTION

Leucas longifolia Benth. (Lamiaceae) is commonly called 'Barumbi or Dudhani' in India. It is perineal herb found in wasted lands and road sides. It is considered as carminative and stimulant [1-2]. In folk remedies its extract used as analgesic and antipyretic. Many of species from Leucas shows antidiabetic, analgesic, antipyretic, anti-inflammatory, antioxidant, CNS depressant and wound healing activity [3-11] .These species shows presence of alkaloids, tannins, saponins, flavonoids, phenolic compounds, glycosides, diterpens [12-16]. According to folk research and as other species from genus Leucas shows analgesic and CNS depressant activities in the present work we evaluate L. longifolia aerial part's petroleum ether, chloroform and methanol extracts for above mentioned activities first time.

MATERIALS AND METHODS

Plant material and preparation of extracts

Leucas longifolia Benth. was collected from hilly region of Ahmednagar district Maharashtra state India in the month of August - September. The plant was identified by Botanical survey of India (BSI), Pune. A voucher specimen **(PRALUL06)** has been deposited for future reference. The aerial part of Leucas longifolia Benth. was dried at room temperature and grounded the powder and passed through 40# sieve. The powder (500 gm) was extracted successively in soxhlet by petroleum ether (40-60^{0}C), chloroform and methanol. The sediments were filtered and filtrate were dried at 40 ^{0} C in an oven to get dried petroleum ether extract (PE), chloroform extract (CE) and methanol extract (ME).

Phytochemical screening of all three extracts were done which sows presence of sterols, fatty acids in PE; sterols, flavonoids in CE; triterpenoids, flavonoids, glycosides, saponins and tannins in ME.

Animals

Male Swiss mice (20–25 g) were obtained from Serum Institute of India, Pune and were maintained at controlled room temperature (21±2 ◦C) on a 12 h light/dark cycle with free access to food and water ad libitum. The experimental protocol was approved by Institutional animal ethical committee (IAEC) and experiments conducted according to CPCSEA.

Drug

A dose of 100 mg/kg; 200 mg/kg and 400 mg/kg of PE, CE and ME were used for activity study. The doses were prepared in 1% aqueous suspension of gum acacia and route of administration was IP.

Acute toxicity

Different doses of PE, CE and ME were administered intraperitonally (i.p.) (10, 100 and 1000 mg/kg) and orally (p.o.) (10, 100 and 1000 mg/kg), The animals were observed during 1 h for signs and symptoms of toxicity and the number of deaths each 24 h during 7 days were recorded.[17]

Activities studied

For the following activities the animals divided into eight groups, each group containing six animals. Group 1 for control, group 2 for standard, group 3 and 4 for PE (100 mg/kg and 200 mg/kg), group 5 and 6 for CE (100 mg/kg and 200 mg/kg), group 7 and 8 for ME (100 mg/kg and 200 mg/kg respectively) In case of CNS depressant activity one more group was added for 400 mg/kg dose of each extract.

Assessment of Analgesic activity

Hot plate method

The parameter evaluated for was the latency time for paw licking and jumping response after exposure on surface of hot plate. The standard used was Pentazocine (10 mg/kg, i.p.) The hot plate temperature was kept at 50^0 ± 1 ^{0}C and the cut off time was 20 sec. [18, 19]

Tail-flick method

The tail flick response of rats was measured by means of tail flick unit. The tail of the rats was placed on a hot wire, and the time taken by the animal to withdraw (flick) its tail from the hot wire was taken as the reaction time. A cutoff time of 20 s was followed to prevent any injury to the tail. The tail flick test was performed after the oral administration of the plant extracts (100 and 200 mg/kg) or the reference drug Pentazocine (10 mg/kg, i.p.) and the mean reaction time was noted. [20, 21]

Acetic-acid induced writhing assay

Analgesic activity of the plant extract was studied by reduction of acetic acid-induced writhing in mice). Thirty minutes after the administration of the plant extracts (100, 200, 400 and 500 mg/kg, orally) or standard diclofenac sodium (10 mg/kg, i.p.), the animals received acetic acid (0.6%, 10 ml/kg i.p.). The number of abdominal contractions (writhing) and stretching with a jerk of the hind limb was counted for 15 min after administering acetic acid, and percent inhibition was calculated as follows

% inhibition = (1 - WT/ WC) × 100

Where WT is the writhings in drug-treated mice and WC is the writhings in control mice. [22, 23]

Assessment of locomotor activity

This test involves placing a number of mice-activity cages, which enables movement of the animal across a light beam to be recorded as a locomotion count. This test can demonstrate a CNS depressant or stimulant activity profile. The animals were allowed to adapt to the new environment for at least 5 min and then the locomotor activity was counted. The plant extract (100,200 and 400 mg/kg, i.p.) or the standard drug

Diazepam 4 mg/kg (i.p.) was administered 30 min before the assessment of locomotor activity. Counts were then taken after 30, 60, 90 and 120 min. [24, 25]

Assessment of skeletal muscle relaxant activity (motor coordination)

Animals remaining on Rota-Rod (16 rpm) 2 min or more in low successive trials were selected for testing; 30 min after the injection of test material or control vehicle the same test was repeated at intervals of 30 min for 3 h. The fall off time from the rotating rod was noted. The difference in the fall off time from the rotating rod between the control and the treated mice (standard-Diazepam/extract) was taken as an index of muscle relaxation. [26, 27]

Assessment of pentobarbital sleeping time

Mice in different groups received 100,200 and 400 mg/kg, i.p. Chlorpromazine (1 mg/kg, i.p.) was used as standard drug. After 30 min mice received an intraperitoneal injection of pentobarbital sodium (40 mg/kg). The time between the loss and recovery of the righting reflex was taken as the sleeping time. [28]

Statistical Analysis

The results were expressed as mean ± S.D. All statistical comparisons were made by Dennett's test after conducting one-way ANOVA.

RESULT

A preliminary acute toxicity study in mice showed that all the three extracts were not toxic (LD50 > 1000mg/kg). However locomotion was reduced in ME treated animals.

Analgesic activity

Hot plate method

The petroleum ether extract and methanol extract (100 and 200 mg/kg, i.p.) shows increase the latency time significantly in dose and time dependent manner to the thermal stimulus. (Table 1)

Tail flick method

In this method also petroleum ether extract and methanol extract shows significant analgesic activity in dose and time dependent manner. Chloroform extract also shows significant action at higher dose. (Table 2)

Acetic-acid induced writhings

In this method the methanolic extract showed significantly reduced number of writhings as compared to control as wll as standard. The percent inhibition was 73.60 ($p < 0.01$) at higher dose (200mg/kg, i.p.). (Table 3)

Locomotor activity

A significant decrease in locomotor activity was observed in case of petroleum ether extract and methanolic extract. A chloroform extract does not shows any decrease in locomotor activity. (Table 4)

Motor co-ordination

The skeletal muscle relaxation activity, rota-roda showed more relaxation in methanolic extract at 400 mg/kg as compared to control. (Table 5)

Phenobarbital sleeping time

Methanolic extract (100, 200 and 400 mg/kg, i.p.) produced a dose dependent potentiation of pentobarbitone sleeping time in mice. (Table 6)

DISCUSSION

In present study three extrats (PE, CE, ME)of shoot part of Leucas longifolia were studied for CNS depressant activity usin several animal models such as analgesic activity by hot plate method, tail flick method (thermal stimui) and acetic acid induced writhing method (chemical stimuli), locomotor activity, muscle relaxant activity and Phenobarbital induced sleeping time.

Pain is centrally modulated via a number of complex processes including opiate, dopaminergic, descending noradrenergic and serotonergic systems [29,30] The hot-plate and tail flick tests are useful in elucidating centrally mediated antinociceptive responses, which focuses mainly on changes above the spinal cord level [31,32]. The hotplate method and tail flick test are considered to be selective to examine compounds acting through opoid receptor; the PE and ME increased mean basal latency which indicates that it may act via centrally mediated analgesic mechanism. Narcotic analgesics inhibit both peripheral and central mechanism of pain, while non steroidal anti-inflammatory drugs inhibit only peripheral pain. [33, 34].

The extracts inhibited both mechanisms of pain, suggesting that the plant extract may act as a narcotic analgesic. It also reported that the inhibition of pain could arise not only from the presence of opioids and/or opiodiomimetics but could also arise from the presence of phenolic constituents [35] and also steroidal constituents [36]. So, it may be due to the similar type of constituents present in the PE and ME of Leucas longofolia which is, exhibited the analgesic activity. The acetic acid induced abdominal constriction method is widely used for the evaluation of peripheral antinociceptive activity [37]. Acetic acid-induced writhing is related to the increase in the peritoneal fluid levels of PGE2 and PGF2a [38] It is therefore possible that PE and ME exerts an analgesic effect probably by inhibiting synthesis or action of prostaglandins. However, the exact mechanism of this action has not been investigated here.

It is generally accepted that the sedative effects of drugs can be evaluated by measurement of pentobarbital sleeping time in laboratory animals [39, 40]. The extracts prolongation of pentobarbital hypnosis is a good index of central nervous system depressant activity [41]. It was found that the i.p. administration of ME (200 and 400 mg/kg) induced sedative effects in mice. However, this test could provide false results, because some substances which interference with the biotransformation of pentobarbital, as Cytochrome P450 complex, could induce apparently the same effects of the depressor CNS drugs [42].

The general depressive activity was confirmed in the spontaneous locomotion test where the ME significantly reduced spontaneous motor activity. Decrease in locomotion reveals depression effect on CNS [43]. The decrease in motor activity gives an indication of the level of excitability of the CNS [44] and this decrease may be related to sedation resulting from depression of CNS [45]. Preliminary phytochemical screening reveals the presence of flavonoids, steroids, tannins and saponins in the plant extract. So, the observed CNS depressant activity may be attributed to these compounds. Further studies are in progress to isolate the active constituents responsible for these activities.

CONCLUSION

Based on the results of the present study, we conclude that the petroleum ether extract and methanol extract possesses strong analgesic and CNS depressant activity in dose dependent manner. However, further studies are necessary to examine underlying mechanisms of analgesic and CNS depressant effects and to isolate the active compound (s) responsible for these pharmacological activities.

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Table 1: Analgesic activity of *Leucas longifolia* by Eddy's hot plate method

Treatment	Dose, i.p.(mg/kg)	Mean reaction time in seconds					
		0 min.	30 min.	60 min.	90 min.	120 min.	180 min.
Control	-	5.13± 0.24	5.4± 0.42	5.44± 0.42	5.6± 0.28	5.68± 0.45	5.53± 0.37
Pentazocine	10	5.60± 0.30	7.07± 0.16*	8.64± 0.87**	10.9± 0.86**	9.87± 0.66**	7.96± 0.01*
PE100	100	5.30± 0.25	5.32± 0.36	7.36± 0.78*	8.33± 0.44*	8.42± 0.51**	6.83± 0.34
PE200	200	4.64± 0.22	6.23± 0.46	7.59± 0.45*	9.08± 0.52**	9.15± 1.12**	6.96± 0.29
CE100	100	4.7± 0.10	4.79± 0.24	4.87± 0.25	6.09± 0.19	6.13± 0.27	5.87± 0.27
CE200	200	4.94± 0.33	4.98± 0.19	5.13± 0.18	6.17± 0.17	6.41± 0.29	6.02± 0.57
ME100	100	5.20± 0.17	6.50± 0.62	6.08 0.39	8.62± 0.98*	7.97± 0.49*	6.35± 0.82
ME200	200	5.28± 0.13	6.64± 0.39	8.10± 0.63*	9.78± 0.58**	10.40± 0.65**	7.38± 1.14

One way ANOVA followed by Dunnet's test. Values are mean ± S.E.M. n = 6 in each group.*P< 0.05 and **P < 0.01 when compared to control.

Table 2: Analgesic activity of *Leucas longifolia* by Tail flick method

Treatment	Dose,i.p. (mg/kg)	Mean reaction time in seconds					
		0 min.	30 min.	60 min.	90 min.	120 min.	180 min.
Control	-	1.46± 0.60	1.64± 0.16	1.40± 0.21	1.49± 0.23	1.53± 0.14	1.58± 0.28
Pentazocine	10	1.66± 0.20	2.28± 0.09	2.90± 0.11**	3.34± 0.17**	3.90± 0.32**	2.31± 0.12
PE100	100	1.30± 0.25	1.95± 0.28	2.30± 0.17*	2.70± 0.33**	2.87± 0.12**	1.98± 0.19
PE200	200	1.64± 0.15	2.21± 0.19	2.68± 0.27**	3.1±0.22**	3.23± 0.41**	2.41±0.19
CE100	100	1.5± 0.55	1.59± 0.20	2.1± 0.28	2.0± 0.19	2.23± 0.18	2.1± 0.64
CE200	200	1.38± 0.09	1.38± 0.27	2.08± 0.25	2.39± 0.56	2.56± 0.33*	2.05± 0.70
ME100	100	1.40± 0.67	2.27± 0.09	2.33± 0.28*	2.57± 0.20*	2.27± 0.16*	2.27± 0.13
ME200	200	1.78± 0.29	2.33± 0.16	2.80± 0.08**	3.17± 0.19**	3.1± 0.01**	2.4± 0.16

Table 3: Analgesic activity of *Leucas longifolia* by acetic acid induced method

Treatment	Dose (mg/kg, i.p.)	Writhings	Inhibition (%)
Control	-	64.1± 1.04	
Diclofenac	10	14.6± 0.67**	77.23
PE100	100	29.2± 0.54*	54.38
PE200	200	20.6± 1.30**	67.82
CE100	100	51.2± 1.22	20.13
CE200	200	40.8± 1.04	36.35
ME100	100	19.4± 0.32**	69.69
ME200	200	16.9± 0.44**	73.60

One way ANOVA followed by Dunnet's test. Values are mean ± S.E.M. n = 6 in each group. *P < 0.05 and **P < 0.01 when compared to control.

Table 4: Effect of different extracts of *Leucas longifolia* on locomotor activity

Group	Dose (mg/kg, i.p.)	Locomotor activity observed for 10 min	
		Before dosing	After30 min of dosing
Control	-	171.23 ± 5.149	173.06 ± 1.052
Diazepam	4	165.75 ± 3.814	91.64 ± 3.129**
PE100	100	176.48 ± 2.659	170.96 ± 5.402
PE200	200	170.33 ± 1.063	159.87 ± 2.714*
PE400	400	168.59 ± 6.328	140.43 ± 4.208**
CE100	100	170.21 ± 8.236	169.52 ± 2.953
CE200	200	177.62 ± 4.950	171.89 ± 8.765
CE400	400	181.54 ± 0.128	178.46 ± 3.588
ME100	100	166.46 ± 5.868	160.34 ± 5.106*
ME200	200	165.41 ± 2.986	115.03 ± 0.685**
ME400	400	163.80 ± 7.655	106.39 ± 5.950**

One way ANOVA followed by Dunnet's test. Values are mean ± S.E.M. n = 6 in each group. *P < 0.05 and **P < 0.01 when compared to control. DW: Distilled water *ad libitum*

Table 5: Effect of different extracts of *Leucas longifolia* on muscle relaxant activity

Group	Dose (mg/kg, i.p.)	Fall of time (sec)						
		0min(before dosing)	30min	60min	90min	120min	150min	180min
Control	-	36 ± 1.568	33 ± 2.651	32 ± 4.582	35±3.256	38± 5.328	37± 5.120	35± 9.045
Diazepam	2	34 ± 6.128	19 ± 2.567*	17 ± 1.025*	15±3.214**	21± 5.487	22 ± 1.571	28 ± 6.948
PE100	100	37 ± 1.025	34 ± 4.325	34 ±8.015	32 ± 2.325	31 ±3.241	33 ± 2.658	35 ± 7.325
PE200	200	35 ± 5.691	32 ± 2.615	30 ± 4.693	29 ± 1.632	31 ± 8.159	34 ± 8.357	33 ± 6.742

PE400	400	37 ± 3.126	35 ± 2.653	32 ± 3.254	30 ± 6.854	32 ± 6.421	29 ± 5.214	34 ± 2.458
CE100	100	38 ± 2.153	37 ± 6.927	35 ± 2.458	38 ± 1.029	36 ± 6.321	33 ± 7.253	37 ± 4.682
CE200	200	40 ± 5.648	35 ± 1.782	38 ± 8.301	32 ± 5.614	33 ± 2.383	35 ± 4.594	37 ± 2.810
CE400	400	39 ± 1.682	38 ± 5.237	33 ± 4.322	30 ± 6.173	31 ± 1.116	35 ± 7.462	37 ± 6.132
ME100	100	36 ± 2.619	33 ± 1.462	31 ± 56.345	28 ± 3.628	29 ± 4.892	31 ± 1.652	35 ± 3.489
ME200	200	37 ± 2.451	30 ± 2.615	25 ± 4.693	23 ± 1.632	26 ± 8.159	32 ± 8.357	30 ± 6.742
ME400	400	34 ± 3.220	21 ± 2.653*	15± 3.254**	18±6.854**	19 ± 6.421*	23 ± 5.214	29 ± 2.458

Table 6: Effect of different extracts of *Leucas longifolia* on pentobarbitone-induced sleeping time in mice

Treatment	Dose (mg/kg, i.p.)	Mean sleeping time ± S.E.M
Control	-	20.3 ± 2.1
Chlorpromazine	1	60.8 ± 3.6**
PE100	100	22.9 ± 5.3
PE200	200	24.4 ± 1.7
PE400	400	29.9 ± 7.4*
CE100	100	21.5 ± 4.9
CE200	200	22.1 ± 2.7
CE400	400	25.6 ± 9.6
ME100	100	28.7 ± 1.9*
ME200	200	40.3 ± 8.2**
ME400	400	56.8 ± 6.3**

One way ANOVA followed by Dunnet's test. Values are mean ± S.E.M; n = 6 in each group. *P<0.05 and **P < 0.01 when compared to control.

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