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Neuropharmacological screening of *Leucas linifolia* Spreng

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Abstract

Globally, plant research for search of new therapeutic agent, the treatment of neurological disorder has been progressed constantly, indicating the pharmacological effectiveness of different plant species in a variety of animal models. Therefore, the present study was carried out to evaluate the psychological study for methanolic and ethyl acetate extracts of *Leucas linifolia* Spreng whole plant. *Leucas linifolia* Spreng (Lamiaceae) is common herb in India and considered sedative, cynogenetic and stimulant. In the present study, crude methanol (ME) and ethyl acetate (EA) extracts of aerial parts of *Leucas linifolia* have been evaluated for central nervous system (CNS) activities. Significant central and peripheral nociceptive activity ($p < 0.01$) was observed for both extracts. Methanolic and ethyl acetate extracts have also showed significant ($p < 0.01$) decrease in motor activity and fall off time of animals on rotating rod, along with significant ($p < 0.01$) sedative effect by potentiating phenobarbitone-induced sleeping time. In the acute toxicity study, both extracts were found to be safe up to 2500 mg/kg b.w. These results suggested that methanolic and ethyl acetate extracts of *Leucas linifolia* show analgesic, anxiolytic and sedative effects. Further investigations are yet, necessary to explore mechanism(s) of action involved in these pharmacological activities.

Key words: *Leucas linifolia* Spreng, Hot plate test, Writhing test, Locomotor activity, Muscle relaxant activity

Introduction

The Central Nervous System (CNS) comprises of brain and spinal cord, in which the process information mediates with the help of chemical messenger, *viz*., neurotransmitter, neuromodulators, neuroregulators, neuromediators and neurotropic (are the various factors which act *via* precise mechanism to mediate neurotransmission) and neurotransmitter, *viz*., nor adrenaline, adrenaline, dopamine, Gamma Amino Butyric Acid (GABA), glutamate, acetylcholine, 5 hydroxyl tryptamine (5 HT) *etc*. and neuromodulator, *viz*., prostaglandins (PGs), purines and neuropeptides interact with their respective receptor and control the various functions of central nervous functions (Seth, 2005). According to the World Health Organisation report (WHO, 2001), about 450 million people experience from a mental or behavioral disorder, yet only a small minority of them receive even the most basic treatment, so global burden of disease will rise to 15% by 2020 (Ruiz *et al*., 2006). Hence, primitive human was among the first to be discovered the drug, acting in the central nervous system. As drugs acting on CNS produce specific physiological and psychological effects, they are not useful therapeutically, and from the indigenous system of medicine too, many plants have been reported to have activity against CNS disorders and, hence, act as very useful remedies for the alleviation of human distress (Suba *et al*., 2002). So worldwide, plant research for search of new therapeutic product in the treatment of neurological disorder has been progressed constantly, signifying the pharmacological effectiveness of different plant species in a variety of animal models (Ruiz *et al*., 2006). Many standard animal models are there for testing the preliminary CNS related pharmacological activities, which

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afford information about action of constituents present in the plants upon psychomotor performance, motor behaviour and neurotoxicity. The depression activity gives an indication of the excitability of the CNS and this decrease may be related to sedation, resulting from depression of CNS (Franco *et al*., 2005). So *Leucas linifolia* was screened for CNS related activity by various animal models.

Leucas linifolia is a erect, slender and annual herb, 30-60 cm high, found as a weed in field. Phenylethanoid glycosides were isolated from the aerial parts of *Leucas linifolia* and they were found to contain antioxidant activity along with the inhibitory activity against xanthine oxidsase enzyme (Mostafa *et al*., 2007). Methanolic extract of aerial parts of *Leucas linifolia* showed antipyretic activity (Mukherjee *et al*., 2002) and potential reduction in spontaneous activity and cause a significant decrease in exploratory behavioural pattern by the head dip and Y-maze test. The extract shows a remarkable potentiation of pentobarbitone induced sleeping time in mice (Mukherjee *et al*., 2002). Methanolic extract of herb caused a significant reduction of blood glucose levels in streptozotocin induced diabetes and has antitussive activity (Saha *et al*., 2002) as well as wound healing activity (Saha *et al*., 1997).

Materials and Methods

Plant material and preparation of extracts

The whole *Leucas linifolia* Spreng plant was collected in June, 2009 from Ahmednagar District, Maharashtra (India). The plant specimen was authenticated from Botanical Survey of India, Pune (Voucher Specimen No. LRM1). Plant material was dried under shade and coarsely powdered for extraction. The coarsely powdered whole plant (500g) of *Leucas linifolia* was separately subjected to extraction, using ethyl acetate and methanol for 10 days by cold maceration. The methanolic and ethyl acetate extracts were concentrated by rotary vacuum evaporator under reduced pressure and then dried in open air.

Animals

Healthy wistar albino mice of either sex and of approximately the same age, weighing about 30-40 g. were used in this study. All the mice were housed in polypropylene cages, maintained under standard condition (12 h light/12 h dark cycle). All the animals were carefully monitored and maintained in accordance with CPCSEA guidelines on control and supervision of experimental animals. The ethical clearance was obtained from the Institutional Animal Ethics Committee before the experiment.

Analgesic activity

Hot plate test

Central analgesic activity for *Leucas linifolia* whole plant extracts was evaluated, using hot plate method as per described by Woolfe and MacDonald (1944). Albino male mice (30-40 g) were grouped into eight groups of six animals each. Group-I served as control and received only vehicle, Group-II was administered standard drug pentazocine (50 mg/kg, i.p.) and Group-III to Group-VIII were treated with ethyl acetate extract and methanol extract of *Leucas linifolia* whole plant (at concentrations of 150 mg/kg, 300 mg/kg and 450 mg/kg, p.o.), respectively. Mice were placed individually on the hot plate maintained at $55^0C \pm 1^0C$, and latency of nociceptive response such as licking, flicking of a hind limb or jumping was noted. After administration of ethyl acetate extract and methanol extract, the readings were taken at 0, 30, 60, 90, 120, 150 and 180 minutes time interval. The protocol of experiment was terminated by 20 second after their placement on the hot plate to avoid damage to the paws.

Writhing test

Peripheral analgesic activity for *Leucas linifolia* whole plant extracts was evaluated, using acetic acid-induced writhing test (Koster *et al*., 1959). Albino male mice (30-40 g) were grouped into eight groups of six animals each. Group-I serves as control and received a distilled water, Group-II serves as standard and received standard drug paracetamol (50 mg/kg, i.p.), Group-III to Group-VIII animals received methanol and ethyl acetate extracts *Leucas linifolia* whole plant (at concentrations of 150 mg/kg, 300 mg/kg and 450 mg/kg, p.o.), respectively. Mice were placed individually in the glass beakers before intraperitoneal injection of 0.1 ml of 0.6 % solution of acetic acid. Then after administration of acetic acid, the animals were allowed to elapse for 5 minutes. The mice were then observed for the period of 30 minutes and then number of writhes was recorded for each animal.

Locomotor activity

For the assessment of locomotor test, healthy adult albino mice (30-40 g) were firstly divided into eight groups containing six animals each. Group-I serves as control and receives normal saline, Group-II serves as standard and receives diazepam (4 mg/kg i.p.) and Group-III to Group-VIII receives methanol and ethyl acetate extracts of *Leucas linifolia* whole plant (at concentrations of 150 mg/kg, 300mg/kg and 450 mg/kg, p.o., respectively. Thirty minutes later, each animal was individually placed in photoactometer. This test can exhibit a CNS depressant or stimulant activity profile of extract. The animals were allowed to adapt to the new environment for at least 5 minutes and then the locomotor activity was counted. The plant extracts or the standard drug Diazepam 4 mg/kg (i.p.) was administered 30 minutes before the assessment of locomotor activity. Counts were then taken after 30 minutes for 10 minutes (Dewan *et al.*, 2000 and Amos *et al*., 2001).

Motor coordination (Muscle relaxant activity)

Muscle relaxant activity was evaluated for *Leucas linifolia* whole plant extracts. For the assessment of the muscle relaxant activity, albino mice (30-40 g) were divided into eight

groups, containing six animals each. Group-I serves as control and receives normal saline, Group-II serves as standard and receives diazepam (2 mg/kg, i.p.) and Group-III to Group-VIII receives methanol and ethyl acetate extracts of *L. linifolia* whole plant (at concentrations of 150 mg/kg, 300 mg/kg and 450 mg/kg, p.o.), respectively. Rota-rod device was used for the assessment of the experiment. The mice were positioned on a horizontal rotating rod set at a rate of 16 revolutions per minutes. Mice were tested for trials; those who positioned on rota-rod were selected for the experiment. The fall off time from the rotating rod was noted for individual mice. The difference in the fall off time from the rotating rod between the control and the treated mice (with standard as Diazepam, ethyl acetate and methanol extract) was noted at the interval of 30 minutes for 3 hours (Ozturk *et al*., 1996 and Perez *et al*., 1998).

Effect on phenobarbitone sodium sleep

For the assessment of phenobarbitone sodium induced sleeping test, methanolic and ethyl acetate extracts of *Leucas linifolia* were tested on albino mice (30-40 g), and were divided into eight groups, containing six animals each. Group-I received normal saline and serves as control, Group-II received diazepam (1 mg/kg, i. p.) and serves as standard, Group-III to Group-VIII received the methanol and ethyl acetate extracts of *Leucas linifolia* whole plant (at concentrations of 150 mg/kg, 300 mg/kg and 450 mg/kg, p.o.), respectively. Thirty minutes after administration of extracts, phenobarbitone sodium (40 mg/kg b.w.) was administered intraperitoneally to each animal. And all the animals were observed for onset of sleep and duration of sleep, with the condition for sleep being loss of righting reflex (Wambebe *et al.*, 1985 and Rolland *et al*., 1991). The index of hypnotic effect was calculated as interval between loss and recovery of righting reflex (Ramirez *et al*., 1998).

Ethyl acetate and methanol extracts of *Leucas linifolia* were suspended into minimum volume of acacia and then volume is adjusted with water for injection, and administered using a force feeding needle.

Statistical analysis

Statistical analysis was performed using Graph Pad Prism 3. All the results were expressed as Mean ± Standard error of mean ($\overline{x} \pm SE_{\overline{x}}$) and analyzed for ANOVA and post hoc Dunnet's *t*-test (Multiple). Differences between groups were considered significant at $p<0.05$, $p<0.01$ levels.

Results and Discussion

Methanolic and ethyl acetate extracts of *Leucas linifolia* were evaluated for central and peripheral nociceptive activity, locomotor activity, muscle relaxant activity and phenobarbitone induced sleeping time. Methanolic extract showed significant central analgesic activity (Table 1) and peripheral analgesic activity (Table 2) at dose of 300 mg/kg as compare to control ($p<0.01$) and standard ($p<0.01$, $p<0.05$). For peripheral analgesic activity, percentage inhibition at dose 300 mg/kg b.w. was found to be 67.00. As methanolic and ethyl acetate extracts were evaluated for locomotor activity at various doses on photoactometer, both showed stimulating locomotor activity (Table 3). Methanolic extract showed significant ($p <0.01$) reduction in motor activity at doses of 300 mg/kg, when results are compared to control group ($p<0.01$) and standard group ($p<0.01$). Methanolic and ethyl acetate extracts and diazepam also showed significant reduction in fall off time of animals (sec) on rotating rod (Table 4) when results were compared to control group ($p<0.01$) and standard group ($p<0.01$).

For the assessment of phenobarbitone induced sleeping time, methanolic and ethyl acetate extracts were evaluated among that methanolic extract at dose of 300 mg/kg b.w., significantly potentiated phenobarbitone induced sleeping time (Table 5) as compared to control ($p<0.01$) and standard ($p<0.01$).

As remarkable analgesic activity of methanolic extract at dose 200 mg/kg b.w. was seen in Eddy's hot plate method and acetic acid induced writhings. The hot plate test is widely used for assessment of central antinociceptive activities, having tendency to respond to the pain stimuli through neuronal pathways (Chapman *et al.*, 1985 and Morales *et al.*, 2001). In general, acetic acid writhing test is used for evaluation of peripheral antinociceptive activities, as writhing test is useful to differentiate between central and peripheral nociception (Le Bars *et al*., 2001) and acetic acid injection induces peritoneal inflammation, which may triggers a response characterized by writhing (Koster *et al*., 1959). This study demonstrated that acetic acid indirectly induces endogenous release of pain mediators (such as prostaglandins, kinins, histamin, *etc*.) that stimulate the nociceptive neurons, which are responsive to non- steroidal anti-inflammatory drugs and opioids (Derardt *et al*., 1980; Sanchez-Mateo *et al*., 2006 and Sulaiman *et al*., 2008).

The locomotor activity was also assessed for methanolic and ethyl acetate extract by actophotometer but methanolic extract was found to be more potent. As it showed decrease in locomotion and grip strength on rota rod was also decreased (Leewanich *et al*., 1996) which reveals the CNS depressant activity. The CNS depression may be due to the increase in the GABA concentration in brain (Nagarjun *et al*., 2003). The pentobarbitone-induced sleep test, also significantly showed sedative effect for methanolic and ethyl acetate extracts but methanolic extract was found to be potent.

As preliminary phytochemical investigation showed the presence of phenolics, flavonoids, steroids, alkaloids and tannins, which may be responsible for the CNS depressant activity. The above studies indicate that the methanolic extract of *Leucas linifolia* whole plant possesses analgesic, sedative, and anxiolytic activity.

Table 1: Analgesic activity of methanolic and ethyl acetate extracts of *Leucas linifolia* by Eddy's hot plate method

Group	Treatment	Mean reaction time in minutes (Min ± $SE_{\bar{x}}$)					
		0 min	30min	60min	120min	150min	180min
I	Control (Normal saline)	5.34 ± 0.0346	5.53 ± 0.08505	5.48 ± 0.0441	5.58 ± 0.1020	5.79 ± 0.0581	5.8 ± 0.0115
II	Pentazocin (50 mg/kg)	5.2 ± 0.0173	8.15 ± 0.0441	12.55 ± 0.1097	20.31 ± 0.1272	17.53 ± 0.1135	15.31 ± 0.0664
III	ME-150mg/kg	5.36 ± 0.1425	6.69 ± 0.1507*	8.5 ± 0.0960*[a]	11.44 ± 0.0819*[a]	9.44 ± 0.1357*[a]	9.02 ± 0.0152*[a]
IV	ME-300mg/kg	5.84 ± 0.1090	8.48 ± 0.0581*[b]	10.47 ± 0.2194*[a]	13.42 ± 0.1115*[a]	11.48 ± 0.2035*[a]	10.41 ± 0.1322*[a]
V	ME- 450mg/kg	5.75 ± 0.0338	8.91 ± 0.0650*[a]	11.41 ± 0.0938*[a]	14.39 ± 0.1534*[a]	12.48 ± 0.1397*[a]	11.29 ± 0.0896*[a]
VI	EA-150mg/kg	6.16 ± 0.0683	7.42 ± 0.1193*[a]	9.37 ± 0.1017*[a]	11.17 ± 0.1007*[a]	10.58 ± 0.2245*[a]	8.96 ± 0.0873*[a]
VII	EA-300mg/kg	5.34 ± 0.0448	8.81 ± 0.0819*[a]	10.57 ± 0.1017*[a]	13.65 ± 0.1648*[a]	11.61 ± 0.0896*[a]	9.31 ± 0.1041*[a]
VIII	EA-450mg/kg	5.50 ± 0.2118	8.73 ± 0.0990*[a]	12.75 ± 0.0185*[b]	15.56 ± 0.1702*[a]	13.48 ± 0.1419*[a]	11.14 ± 0.1004*[a]

Results are expressed as Min ± $SE_{\bar{x}}$ (n=6). Data processed by one-way-ANOVA followed by Dunnett's test, * $p<0.01$ significant when compared to control group. [a]$p<0.01$, [b]$p<0.05$ significant when compared to standard group.

Table 2: Analgesic activity of methanolic and ethyl acetate extracts of *Leucas linifolia* by acetic acid induced method

Group	Treatment	Number of writhing	Percent inhibition
I	Control (Normal saline)	70.68± 0.2910	-
II	Paracetamol (50 mg/kg)	18.67 ± 0.0606	73.58
III	ME-150mg/kg	31.09 ± 0.1768*[a]	56.01
IV	ME-300mg/kg	23.32 ± 0.0696*[a]	67.00
V	ME- 450mg/kg	20.38 ± 0.2557*[a]	71.16
VI	EA-150mg/kg	35.42 ± 0.1576*[a]	49.8
VII	EA-300mg/kg	24 ± 0.0393*[a]	66.04
VIII	EA-450mg/kg	21.29 ± 0.1405*[a]	69.87

Results are expressed as Min ± $SE_{\bar{x}}$ (n=6). Data processed by one-way-ANOVA followed by Dunnett's test, * $p<0.01$ significant when compared to standard group. [a]$p<0.01$ significant when compared to control group.

Table 3: Effect of methanolic and ethyl acetate extracts of *Leucas linifolia* on locomotor activity

Group	Treatment	Locomotor activity observed for 10 min	
		Before dosing	After dosing
I	Control (Normal saline)	180.68± 0.1097	180.92± 0.1189
II	Diazepam (4 mg/kg)	180.91 ± 0.0819	92.12± 0.0959
III	ME-150mg/kg	180.24± 0.06173	131.13± 0.04667*
IV	ME-300mg/kg	181.41± 0.1322	123.51 ± 0.2227*[a]
V	ME- 450mg/kg	180.45± 0.1054	120.70 ± 0.1433*[a]
VI	EA-150mg/kg	181.08± 0.06119	132.31 ± 0.0642*
VII	EA-300mg/kg	181.17 ± 0.0995	124.52 ± 0.1736*[a]
VIII	EA-450mg/kg	180.55± 0.09838	121.36 ± 0.0318*[a]

Results are expressed as Min ± $SE_{\bar{x}}$ (n=6). Data processed by one-way-ANOVA followed by Dunnett's test, * p<0.01 significant when compared to control group. [a]p <0.01 significant when compared to standard group.

Table 4: Effect of methanolic and ethyl acetate extracts of *Leucas linifolia* on muscle relaxant activity

Group	Treatment	Fall of time in seconds (sec ± $SE_{\bar{x}}$)					
		0 min	30 min	60 min	120 min	150 min	180 min
I	Control (Normal saline)	39.97±	40.38±	40.13±	40.57±	39.82±	40.34±
		0.0448	0.3219	0.0841	0.7850	0.4149	0.0233
II	Diazepam (2 mg/kg)	41.45±	22.06±	18.36±	16.47±	18.19±	23.29±
		0.1106	0.0378	0.0338	0.1020	0.1650	0.0491
III	ME-150mg/kg	40.33±	36.22±	33.14±	29.44±	30.12±	34.29±
		0.0705	0.0811*[a]	0.0480*[a]	0.1365*[a]	0.0202*[a]	0.0536*[a]
IV	ME-300mg/kg	40.53±	35.23±	30.08±	26.17±	27.99±	31.33±
		0.0683	0.0405*[a]	0.0272*[a]	0.0688*[a]	0.0560*[a]	0.0755*[a]
V	ME- 450mg/kg	41.25±	34.18±	29.08±	26.72±	28.44±	30.29±
		0.1734	0.0520*[a]	0.0585*[a]	0.1400*[a]	0.1120*[a]	0.2060*[a]
VI	EA-150mg/kg	40.68±	37.21±	35.27±	31.23±	32.59±	33.04±
		0.1707	0.1162*[a]	0.1719*[a]	0.0800*	0.1186*[a]	0.1186*[a]
VII	EA-300mg/kg	40.32±	35.32±	31.77±	26.94±	29.42±	32.47±
		0.0584	0.1281*[a]	0.0578*[a]	0.0768*[a]	0.0786*[a]	0.0838*[a]
VIII	EA-450mg/kg	40.52±	34.12±	30.20±	26.33±	28.51±	30.22±
		0.1210	0.0272*[a]	0.04667*[a]	0.1069*[a]	0.1141*[a]	0.0441*[a]

Results are expressed as Sec ± $SE_{\bar{x}}$ (n=6). Data processed by one-way-ANOVA followed by Dunnett's test, * p<0.01 significant when compared to standard group. [a]p <0.01 significant when compared to control group.

Table 5: Effect of methanolic and ethyl acetate extracts of *Leucas linifolia* on phenobarbitone induced sleeping time

Group	Treatment	Mean sleeping time in min.
I	Control (Normal saline)	24.39± 0.09018
II	diazepam (1 mg/kg, i. p.)	74.30 ± 0.1186
III	ME-150mg/kg	40.16 ± 0.0393*
IV	ME-300mg/kg	48.32 ± 0.03606*[a]
V	ME- 450mg/kg	50.42 ± 0.1845*[a]
VI	EA-150mg/kg	41.22 ± 0.0928*
VII	EA-300mg/kg	47.36 ± 0.2166*[a]
VIII	EA-450mg/kg	49.38 ± 0.1598*[a]

Results are expressed as Min ± $SE_{\bar{x}}$ (n=6). Data processed by one way ANOVA followed by Dunnett's test, * p<0.01 significant when compared to control group. p <0.01 significant when compared to standard group.

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