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Comparative analgesic activity of leaves, stems and roots of *Leucas linifolia* spreng. (Lamiaceae)

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ABSTRACT

All the extracts from the leaves, stems and roots of *Leucas linifolia* (Lamiaceae) were investigated for their analgesic. The effect of pet ether, ethyl acetate and methanol extracts of various parts of *Leucas linifolia* on nociceptive response using hot plate method and writhing test in mice was examined. All the extracts of *Leucas linifolia* showed significant central and peripheral analgesic activity in hot plate method and acetic acid-induced writhing test, respectively, at the dose of 50mg/kg intraperitoneally. Pet ether extract of stems of *Leucas linifolia* showed highest increase in reaction time in hot plate method while methanol extract of stems of *Leucas linifolia* showed more inhibitory effect on writhing induced by acetic acid as compare to extracts of leaves and root.

KEYWORDS

Leucas linifolia;
Lamiaceae;
Hot plate method;
Acetic acid induced writhing test.

INTRODUCTION

Leucas linifolia is erect, slender and annual herb 30-60cm high found as a weed in field. The leaves are linear-lanceolate, entire serrate and flowers are white with oblong and pale brown nutlets[1,2]. Acacetin and chrysoeriol are isolated from aerial parts. A new flavonoid compound linifolioside was isolated and characterized asisopimara -8, 15-diene -7-keto-3-o-α-L-rhamnopyranosyl (1→2)-β-D-glucopyranoside[3]. The aromatic plant used as flavouring leaves eaten as a potent herb, decoction of leaves used as a sedative, stomachic and vermifuge[4]. Poultice of fresh leaves applied to old sores and dermatitis. It is also used as stimulant and diaphoretic, used in rheumatism and snake bite. The root, stem and leaves are cynogenetic[5]. The *Leucas linifolia* is also shows hypoglycemic activity[6].

MATERIAL AND METHOD

Plant material

The leaves, stems and roots of *Leucas linifolia* were collected from Ahmednagar district, Maharashtra (India) in August 2007. The plant specimen was authenticated from Botanical Survey of India, Pune (Voucher specimen no. - LRM1).

Preparation of extracts

Dried and coarsely powdered stem, leaf and root parts (500g, each) of *Leucas linifolia* were separately subjected to successive extraction using petroleum ether, chloroform, ethyl acetate and methanol in Soxhlet extractor. The extracts of various parts were concentrated by vacuum distillation and then dried in open air.

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TABLE 1: Effect of various extracts of leaves, stems and roots of *Leucas linifolia* L. on thermic stimulus-induced pain in mice (Hot plate test)

Treatment	Latency to lick the paw (Sec)±SEM						
	Predrug reaction time	30 min	60 min	90 min	120min	150min	180min
Vehicle	12.45±0.64	13.8±0.79	14.0±1.23	13.8±0.79	12.8±0.69	11.57±0.59	11.78±0.60
Pentazocine	14.71±1.1	12.30±0.66#	13.58±0.74*	20.01±2.93#	16.30±1.79	8.19±0.21	11.79±0.61*
PEL	13.5±0.71#	18.30±2.51*	20.00±2.92	19.52±0.72#	17.38±2.3*	10.71±0.41	8.57±0.29
EAL	10.96±0.53	16.50±1.82	19.92±2.56#	14.18±3.49	20.00±2.95	9.74±0.31#	13.67±0.78*
MEL	13.62±0.76*	15.03±1.4#	10.49±0.36#	12.76±0.68	19.81±2.53*	12.55±0.67	14.84±1.24
PES	11.5±0.71#	16.30±2.51*	19.10±2.92	16.92±0.72#	15.33±2.3*	9.71±0.41	7.97±0.29
EAS	10.96±0.53	17.05±1.82	19.92±2.56#	15.10±3.49	12.00±2.95	10.02±0.31#	14.97±0.78*
MES	12.12±0.76*	16.03±1.4#	11.04±0.36#	13.46±0.68	18.98±2.53*	12.32±0.67	15.88±1.24
PER	13.9±0.71#	19.98±2.51*	21.89±2.92	20.52±0.72#	19.38±2.3*	12.07±0.41	9.57±0.29
EAR	11.09±0.53	17.05±1.82	20.02±2.56#	14.98±3.49	19.98±2.95	10.24±0.31#	14.23±0.78*
MER	11.62±0.76*	14.93±1.4#	11.14±0.36#	14.66±0.68	20.81±2.53*	11.55±0.67	14.14±1.24

All the values are expressed as mean±SEM; n=6, #P<0.05, *P<0.0001 significant compared to control. All the extracts and pentazocine were given intraperitoneally at 50 mg/kg dose. PEL, EAL, MEL, PES, EAS, MES, PER, EAR, MER are petroleum ether, chloroform, ethyl acetate extract of leaves, stems and roots of *L.linilifolia* respectively

Animals

Healthy wistar albino mice of either sex and of approximately the same age, weighing about 20-25gm were used for study of analgesic activity. They were housed in polypropylene cages maintained under standard condition (12hour light/12 hour dark cycle; 30+4°C, 36-60 humidity).The experimental protocol was subjected to the scrutiny of the Institutional Animal Ethical Committee and was cleared by the same before starting.

Chemicals

Drugs: Pentazocin (Ranbaxy, Ahmedabad), Paracetamol (Heilenlab, Goa), Chemicals: Pet ether (PCL, Pune), Ethyl acetate (PCL, Pune), Methanol A.R (PCL, Pune), DMF (PCL, Pune), Saline water (Nurilife, Ahmedabad).

Analgesic activity

1. Hot plate method

Central analgesic activity of petroleum ether, ethyl acetate, and methanol extract of leaves, stem and roots were evaluated using hot plate method[7]. The mice of either sex were divided into thirty three groups of six animals each. The first group served as control and received only vehicle (2% DMF), second group was administered standard drug pentazocine lactate (50mg/kg, i.p.) dissolved in 2% DMF in water for injection. The animals of third to eleven groups were treated with petroleum ether, ethyl acetate, and methanol extracts (40mg/kg, 50mg/kg, 60mg/kg, i.p.) suspended in 2% DMF in saline water. Mice were placed individually on the hot plate maintained at 55±1^{0}C and the latency to lick paws was noted. The basal reaction time was noted before and 30, 60, 90, 120, 150, 180min after the administration of each extract at 40mg/kg, 50mg/kg, 60 mg/kg,

The experiment was terminated 20sec after their placement on the hot plate to avoid damage to the paws. 0 min readings are the predrug reaction time.

2. Acetic acid-induced writhing test

Peripheral analgesic activity was evaluated using acetic acid-induced writhing test[8,9]. Mice of either sex were prescreened 48 hrs before the actual experiment and those sensitive to acetic acid-induced writhing were divided into eleven groups, of six animals each. The animals received petroleum ether or ethyl acetate or methanol of *Leucas linifolia* of stem, leaves and roots (50mg/kg, i.p.) in 2% DMF or standard drug paracetamol (50mg/kg, i.p.) or vehicle as 2% DMF, 30min before intraperitoneal injection of 0.1ml of 0.6% solution of acetic acid. Mice were placed individually into glass beakers after administration of acetic acid and five minutes were allowed to elapse. The mice were then observed for the period of 30 minutes and then number of writhes recorded for each animal.

Statistical significance

The results were analyzed for statistical significance

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TABLE 2: Effect of various extracts of leaves, stems and roots of *Leucas linifoila* L. on acetic acid-induced writhing in Mice

Treatment (50mg/kg)	Number of writhing
Vehical	63.66±0.729*
Paracetamol	42.06±0.726
PEL	32.06±0.621*
EAL	39.7±0.722*
MEL	19.3±0.711*
PES	28.06±0.621*
EAS	38.00±0.722*
MES	17.3±0.711*
PER	30.06±0.621*
EAR	40.00±0.722*
MER	18.7±0.711*

All values are expressed as mean±SEM (n=6), *P<0.05 significant as compared to control. PEL, EAL, MEL, PES, EAS, MES, PER, EAR, MER are petroleum ether, ethyl acetate and methanol extract of leaves, stems and roots of *L. linifolia* respectively

using student 't' test. P<0.05, *P<0.0001 was considered significant.

RESULT AND DISCUSSION

The pet ether extract stems of *Leucas linifolia* showed significant analgesic activity at 50mg/kg, i.p. dose (TABLE 1) as compare to leaves and roots. Analgesic activity was comparable with standard drug pentazocine. Among all the extracts, petroleum ether of *Leucas linifolia* of all the parts showed highest increase in reaction time. In case of analgesia, prostaglandins and bradykinins were suggested to play an important role in the pain process[10]. Some sterols and triterpenes are responsible for analgesic activity[11]. As phytochemical tests showed presence of these constituents in petroleum ether extracts, they may be responsible for the activity.

All the extracts of *Leucas linifolia* stems at dose of 50mg/kg, i.p., significantly attenuated the number of writhing and stretching induced by intraperitoneal 0.1ml 0.6% acetic acid (TABLE 2) as compare to leaves and roots. Methanol extract of stems of *Leucas linifolia* (50mg/kg,) showed more inhibitory effect on writhing induced by acetic acid as compared to other extracts as well as standard drug paracetamol. Result shows that peripheral analgesic activity is in descending order like methanol, ethyl acetate and pet ether for all the parts of *Leucas linifolia*. Tannins, flavonoids and sterols were detected in above extract respectively[12]. These compounds are having good analgesic activity by inhibiting Prostaglandin synthesis[13]. Thus this supports peripheral analgesic activity of above extracts and the activity may be because of PG synthesis inhibition.

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