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Research Article

ACUTE CENTRAL AND PERIPHERAL ANALGESIC ACTIVITY OF ETHANOLIC EXTRACT OF THE LEAVES OF *LEUCAS INDICA* (EELI) IN RODENT MODELS

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ABSTRACT

The aim of the study was to investigate the acute peripheral activity of Ethanolic Extract of the leaves of *Leucas indica* (EELI) by acetic acid induced writhing reflex test in mice and acute central analgesic activity by tail immersion method in rats. Dried powdered leaves of *Leucas indica* were subjected to solvent extraction by using 90 % ethanol. Based on acute oral toxicity study according to Organization for Economic Cooperation and Development (OECD) guidelines No. 423, three doses of the test drug (100, 200 & 400 mg/kg for mice and 75,150 & 300mg/kg for rats) was selected and were subjected to acute analgesic activity. The Ethanolic Extract of the leaves of *Leucas indica* (EELI) showed significant ($p<0.01$) acute peripheral analgesic activity in mice in the dose of 200 mg / kg and moderate analgesic effect ($p<0.05$) in the dose of 400 mg/kg as compared to control but failed to show central analgesic activity by tail immersion method at any of the three doses selected compared to control.

Keyword: Analgesic, Ethanol, *Leucas indica*, Writhing reflex test, Tail immersion test.

INTRODUCTION

Pain, inflammation and fever are very common complications in human beings. Several plants and their products are claimed and proved to possess analgesic and antipyretic property[1]. Pain can be experienced after any tissue injury, but strong stimuli could be also painful even without tissue damage. Pain sensitivity is dynamically changing with the actual physiological status: pain is sometimes absent when tissue damage is obvious and ongoing.[2] On the other hand, inflamed tissues can be more sensitive to painful stimuli (hyperalgesia), moreover even non-noxious stimuli could be "misperceived" as pain (allodynia).[3]

The clinically useful drugs against pain and inflammation exhibit many adverse effects; this leads to considerable interest in search of safer drug for these conditions. [4] The practice of herbal medicine dates back to the very earliest period of known human history. There is evidence of herbs having been used in the treatment of diseases and for revitalizing body system in almost all ancient civilization. Ayurveda, the Science of Life, has provided a rationale basis for treatment of various ailments. Plants are indispensable sources of medicine since time immemorial. Studies on natural product are aimed to determine medicinal values of plants by exploration of existing scientific knowledge, traditional uses and discovery of potential chemotherapeutic agents. Phytochemicals are used as templates for lead optimization programs, which are intended to make safe and effective drugs[5]. The genus *Leucas* comprises of about 80 species [6]. The highest species diversity has been found in East Africa [7]. In India, 43 species are available. Plants of genus *Leucas* (Lamiacae) are widely used in traditional medicine to cure many diseases such as cough, cold, diarrhoea and inflammatory skin disorder. A variety of phytoconstituents has been isolated from the *Leucas* species which include lignans, flavonoids, coumarins, steroids, terpenes, fatty acids and aliphatic long chain compounds. Anti-inflammatory, analgesic, antidiarrhoeal, antimicrobial, antioxidant and insecticidal activities have been reported in the extracts of genus *Leucas***.** Plants of genus *Leucas* are generally shrubs, sub shrubs, annual herbs or perennial herbs with woody root and/or stem-base. Leaves are opposite, entire or with spiky lobes, oval shaped with tapered end, petiolated or sometimes without intervening stalk [8].

MATERIALS AND METHODS

Institutional Animal ethical committee (IAEC) approval was obtained from Yenepoya University before conducting the experiments.

Animals:

Equal number of Swiss albino mice weighing 25-30 g were divided into 5 groups having 10 animals in each group (5 males & 5 females) for acetic acid induced writhing reflex test in mice (Table-1) and equal number of male and female Wistar albino rats weighing 150 - 200 g were taken and divided into 5 groups having 10 animals in each group (5 males & 5 females) for tail immersion test in rats (Table-2). Animals were acclimatized under standard laboratory condition and were kept in 12hr day and night cycle before the start of the experiment for seven days. Animals were handled carefully according to Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines.

Plant Material

The whole plant was collected from rural region of manjanady, in Mangalore region in the month of June – August 2010. It was authenticated by Dr. Krishna Kumar, Chairman, Dept of Applied Botany, Mangalore University, Mangalore. The herbarium of the plant (voucher specimen no YU/LI/2010) has been deposited at Yenepoya University, Mangalore.

Extraction

Leaves of *Leucas indica* were carefully separated, cleaned, shade dried, mechanically grinded and coarsely powdered. The coarse powder was subjected to solvent extraction in Soxhlet extractor using 90 % ethanol. The ethanolic extract was concentrated by vacuum distillation to dryness; the yield obtained was 15.5% w/w with respect to dried leaf. The collected leaf extract was stored in a desiccator. A suspension of the extract prepared in 1% gum acacia was used in experimental studies.

Drugs and chemicals

The drugs and chemicals were Indomethacin, Pentazocin (Yenepoya pharmacy, Mangalore), Gum acacia, Ethanol (Rajesh chemicals, Mumbai)

Acute toxicity studies

As there was no references for the dose of *Leucas indica*, the leaves of EELI was studied for acute oral toxicity study as per revised OECD guidelines. The EELI was devoid of any toxicity in mice when given in doses up to 2000 mg/kg by oral route. Its 1/5th (i.e. 400 mg/kg) and 1/10th (i.e. 200 mg/kg) and 1/20th (i.e. 100 mg/kg) of 2000 mg/ kg were used to test for analgesic activity in mice and 75 mg/kg, 150 mg/kg & 300 mg/kg were used to test for analgesic activity in rats (Dose conversion from mice to rat) [9].

Acetic acid induced writhing reflex of Ethanolic Extract of the leaves of *Leucas indica* (EELI) in mice

In this method, mice in groups of 10 each were treated with vehicle (1% gum acacia, orally) (10ml/kg, orally), EELI (100 mg/kg, 200

mg/kg & 400 mg/kg orally), and Indomethacin (10 mg/kg orally) was used as positive control. Analgesic activity of EELI was assessed by counting the number of writhes induced by 0.6% (10ml / kg Intraperitonially) of acetic acid. Numbers of writhes per animal were counted following 10min. For acute study the animals received the drug 1 hour before conducting the experiment. A writhe was considered when animal showed contraction of abdomen with simultaneous stretching of at least one hind limb. Protection against writhing was taken as an index of analgesia (Table-3). [10]

It is calculated as:

Average number of writhing in control group - Average number of writhing in treated group / Average number of writhing in control group × 100.

Analgesic effects of Ethanolic Extract of leaves of *Leucas indica* (EELI) by tail immersion method in rats

In this method, the rats in each group treated with vehicle (1% gum acacia, orally) 10 ml/kg, EELI (75 mg/kg, 150 mg/kg, & 300 mg/kg orally), and pentazocin (30 mg/kg, intraperitonially) was used as positive control. For acute study the animals received the drug 1 hour before conducting the experiment. They were placed in individual restraining cages leaving the tail hanging out freely.The distal 1-2cm of tail was immersed in water and maintained at 55± 0.5 °C. Time taken by the rat to withdraw the tail from hot water was noted as reaction time. The reaction time noted before the administration of the drug was noted as zero minute. The cut off time to withdraw the tail was kept as 10 seconds. The mean reaction time was recorded for each group and compared between the groups (Table-4).[11]

Statistical Analysis

The observations are mean ± S.E.M. and analyzed by one way ANOVA followed by Dunnett's multiple comparison test. $p < 0.05$ was considered statistically significant.

DISCUSSION

As EELI was devoid of any toxicity in mice when given in doses up to 2000 mg/kg by oral route, its 1/5th (i.e. 400 mg/kg) and 1/10th (i.e. 200 mg/kg) and 1/20th (i.e. 100 mg/kg) of 2000 mg/ kg was used to test for peripheral analgesic activity in mice and 75 mg/kg, 150 mg/kg and 300 mg/kg was used to test for central analgesic activity in rats.

The present study has shown that the Ethanolic Extract of the leaves of *Leucas indica* (EELI) at dose 200mg/kg (Table 3) exhibited significant peripheral analgesic activity compared to control being reported for first time for the species of *Leucas indica*. However it has also shown a moderate peripheral analgesic effect in the dose of 400 mg/kg (Table 4) as compared to control. But, it did not show any significant central analgesic activity by tail immersion method when compared to control and at any of the three doses selected in rats. Preliminary phytochemical screening showed that the genus of *Leucas* possess Phenolic compounds, Steroids, Terpenes, Glycoside, Fatty acids, flavonoids. Flavonoids are known to possess Anti-inflammatory activity by inhibiting the cyclooxygenase responsible for synthesis of inflammatory prostaglandins[8]. As the genus of *Leucas* having flavonoids is known to inhibit cyclooxygenase, this could be the possible explanation for peripheral analgesic activity of Ethanolic Extract of the leaves of *Leucas indica* (EELI). However further studies are needed to evaluate its exact mechanism of action for its peripheral analgesic activity.

Table 1: Shows division of selected animals into 5 groups of 10 mice each (5+5), the dose for each group and the number of days to be housed for acetic acid induced writhing reflex test in mice

Group	Drug / Dose	Animals	Male	Female	No. of days animals were housed
Group I	Control (Gum acasia1%) (3ml/kg)	Swiss albino mice	5	5	10
Group II	Indomethacin (10 mg/kg)	Swiss albino mice	5	5	10
Group III	EELI (100mg/kg)	Swiss albino mice	5	5	10
Group IV	EELI (200mg/kg)	Swiss albino mice	5	5	10
Group V	EELI (400mg/kg)	Swiss albino mice	5	5	10

Table 2: Shows division of selected animals into 5 groups of 10 rats each (5+5), the dose for each group and the number of days to be housed for tail immersion test in rats.

Group	Drug / Dose	Animals	Male	Female	No. of days animals were housed
Group I	Control (Gum acacia 1%) (3ml/kg)	Wistar albino rat	5	5	10
Group II	Pentazocin (30mg/kg)	Wistar albino rat	5	5	10
Group III	EELI (75mg/kg)	Wistar albino rat	5	5	10
Group IV	EELI (150mg/kg)	Wistar albino rat	5	5	10
Group V	EELI (300mg/kg)	Wistar albino rat	5	5	10

Table 3: Showing analgesic effects of Ethanolic Extract of the leaves of *Leucas indica* (EELI) by acetic acid induced writhing reflex in mice (Acute Study)

Treatment	Number of writhing ±SEM	% Inhibition
Control (Gum acacia 1%) (3ml/kg)	29.6±0.81	-
Indomethacin (10 mg/kg)	14.8±0.64***	50
EELI (100 mg/kg)	28.3±0.86*	4.39
EELI (200 mg/kg)	15.2±0.82***	48.64
EELI (400 mg/kg)	24.9±1.90**	15.87

n=10. The observation are mean ± S.E.M. *p> 0.05, **p<0.05, *** p< 0.01 as compared to control (ANOVA followed by Dunnett's multiple comparison test)

EELI- Ethanolic Extract of the leaves of *Leucas indica*

Table 4: Showing analgesic Ethanolic Extract of leaves of *Leucas indica* (EELI) by tail immersion method in rats (Acute Study)

Drugs	Time (0min)	Time (30min)	Time (60min)	Time (120min)	Time (180min)	Time (240min)	Time (360min)
Control (Gum acacia) (3ml/kg)	1.13 ± 0.08	1.12±0.04	1.18±0.09	1.20±0.05	1.30±0.09	1.29±0.09	1.19±0.03
Standard (Pentazocine) (30mg/kg)	1.13±0.02*	5.01±0.11***	5.26±0.15***	5.60±0.39***	5.47±0.15***	5.46±0.21***	4.52±0.11***
EELI 75 mg/kg	1.13±0.03*	1.15±0.04*	1.22±0.09*	1.33±0.07*	1.46±0.06*	1.31±0.08*	1.49±0.08*
EELI 150mg/kg	1.13±0.01*	1.14±0.04*	1.24±0.09*	1.30±0.07*	1.44±0.06*	1.32±0.08*	1.50±0.08*
EELI 300mg/kg	1.13±0.01*	1.32±0.09*	1.36±0.09*	1.47±0.09*	1.30±0.09*	1.32±0.09*	1.48±0.08*

n=10. The observation are mean ± S.E.M. *p> 0.05, *** p< 0.01 as compared to control (ANOVA followed by Dunnett's multiple comparison test)

EELI- Ethanolic Extract of the leaves of *Leucas indica*

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