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Neuro Pharmacological Effect of Cyclohexane 1, 2, 3, 4, 5, 6-Hexol and (2Z, 6E) 3, 7, 11-Trimethyldodeca-2, 6, 10-Trien-1-Ol , Isolated from *Launaea Pinnatifida* Cass in Mice

Naglapur S[1], Kamble AK[1], Khan LA[2] and Swamy PJ[1]*

[1]Department of Biochemistry, Gulbarga University, India
[2]Luqman college of pharmacy, Kotnoor (D) Ring Road, India

***Corresponding author:** Paramjyothi Swamy, Department of Biochemistry, Gulbarga university, Gulbarga, 585106, Karnataka, India, Email: paramjyothiswamy@gmail.com

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Abstract

Cyclohexane 1,2,3,4,5 6-hexol and (2Z, 6e) 3,7, 11-trimethyldodeca-2, 6 10-trien-1-ol, isolated from *Launaea Pinnatifida* Cass were evaluated for their neuro pharmacological properties in mice in terms of analgesic and sedative effects. The compounds exhibited potent analgesic effect at the dose of 10 mg/kg in Eddy's hot plate test similar to pentazocin, the standard drug. Significant sedative effect was exhibited by the test samples in mice, subjected to both the loco motor activity and marble burying activity compared with the standard drugs chlorpromazine and diazepam respectively at the dose of 10mg/kg. This study confirms at least partly the ancient use of *Launaea Pinnatifida* Cass as a medicine that cures neuro pharmacological disorders.

Keywords: *Launaea Pinnatifida* Cass; Cyclohexane 1, 2,3,4,5, 6-Hexol 2Z, 6E00 3, 7, 11-Trimmethylodeca-2 6; 10-Trien-1-Ol; Analgesic; Sedative

Abbreviations: SEM: Standard Error of Mean; LPEE: *Launaea Pinnatifida* Cass Ethanolic Extract.

Introduction

Pain, stress and depression are interlinked primordial concept of neuro pharmacology. Analgesics relieve pain as a symptom, without affecting its cause [1]. Currently available analgesic drugs such as opiates and NSAIDs are not useful in all cases due to their adverse effects. Stress plays the main role in pathogenesis of mental disorders [2]. Anxiety and depression are extremely common, dramatic and debilitating multifaceted disorders and it is now becoming clear that without knowledge of both clinical and biological aspects of anxiety and depression, it is impossible to offer effective treatment to the patients [3]. Since mice and humans share more than 90% of their genes; all animal models seem to be a useful tool in biomedical sciences, as evidenced by a notable increase in the number of active laboratories working in the field of neurobiology [4]. However, owing to the unfavorable risks produced by classical analgesics, anxiolytics or anti anxiolytic drugs, the development of new effective but less potent to induce adverse reactions is necessary. Thus, considerable attention has been given to the plant-derived therapeutics by the scientific community and the pharmaceutical industry [5].

Over the past decades, there has been intensive study of a variety of neurobiological disorders like pain, depression and anxiety [6,7]. In the search of new therapeutic products for the treatment of neurological disorders, medicinal plant research, worldwide, has progressed constantly,

demonstrating the pharmacological effectiveness of different plant species in a variety of animal models [8]. Concurrently, phyto chemicals identified from traditional medicinal plants are presenting an exciting opportunity for the development of new types of therapeutics. This has accelerated the global effort to harness and harvest those medicinal plants that bear substantial amount of potential phyto chemicals showing multiple beneficial effects in combating diseases. For various reasons in recent years, the popularity of complementary medicine is increased. Dietary measures and traditional plant therapies as prescribed by indigenous systems of medicine are used commonly in India [9].

Launaea Pinnatifida Cass is found along the coastal regions from Bengal to Ceylon and Chennai to Malbar where it serves as sand binders with other plants. It is an edible plant, commonly called as paathri, kneekhowa, almirao, etc. It is reported to possess tonic, soporific, diuretic and used as substitute for taraxacum. Leaves are eaten during famine and herbs are fed to buffaloes as a galactagogue. It is extremely applied in rheumatic affections combined with the oil of *Pongamia galbara* [10]. The *Launaea Pinnatifida* Cass leaves are commonly used for relieving pain and anti-anxiety purpose by tribal practitioners without any proper scientific investigations. Therefore, the primary objective of this study was to assess the neuro pharmacological properties of *Launaea Pinnatifida* Cass.

Materials and Methods

Instruments, Drugs and Chemicals

Instruments like Actophotometer (INCO, Ambala, India) and Eddy's hot plate analgesiometer (Incomedicraft, S.No: A-10-042) were used in studies. Diazepam, Chlorpromazine and Pentazocin (Ranbaxy Laboratories Ltd. Mumbai, India) and all other chemicals used were of the analytical grade.

Plant Material

The fresh leaves of *Launea pinnatifida* Cass (Asteracease) were collected from the farmland of Saradagi village, 24 km south of Gulbarga district, Karnataka (India). The plant was identified and authenticated by Prof. Y.N. Seetaram, Department of Botany, Gulbarga University, Gulbarga. A voucher specimen HGUG/SN-76 is deposited in this department.

Experimental Animals

Male albino mice (25-30 g) procured from Mahaveer Enterprises, Hyderabad (India) were used for the studies. All the experiments were conducted according to the protocols approved by the Institutional Animal Ethics Committee (IAEC Reg.No:346/CPCSEA). The rats were housed in polypropylene cages lined with husk, renewed every 24 h under 12/12 h light/dark cycles at 22±20C and at 45%-55% relative humidity. The animals were fed with a standard pellet diet supplied by Lipton India Ltd. and allowed to free access of water ad libitum. After randomization into various groups, the animals were acclimatized for a period of 7 days. Animals described as fasting had been deprived of food for at least 16 hr but had been allowed free access to drinking water before the experiment was carried out.

Preparation of Extracts and Isolation of Compounds

The shade dried leaves of *Launaea Pinnatifida* Cass were powdered to 22 mesh size and subjected to successive soxhlet extraction using non-polar to polar solvent system, with increasing polarity (petroleum ether, chloroform, ethanol and distilled water). Based upon the preliminary phyto chemical screening, the *Launaea Pinnatifida* Cass Ethanolic Extract (LPEE) was subjected to column chromatography to isolate the various fractions. The elution was carried out into the column containing silica gel-C (60-120 mesh), using various percentages of polar and non-polar solvents. Compounds 1 and 2 (C1 and C2) obtained from chloroform: methanol fraction (60:40and10:90 respectively) were again purified by eluting through the freshly prepared column using the respective solvent ratios. The isolated compounds were subjected for recording IR spectra (Shimadzu IR-450) with KBr pellets (cm-1). The 1H NMR spectra (Bruker DRX-500) was carried out in 300 MHz CdCl3 using TMS as reference.

Classification of Animals in Groups

Male albino mice (20-25 g) were divided into five groups (n=6). Group I: Normal control (Tween-80, 0.1 ml/20 g), Group II: Standard, Group III: LPEE (250 mg/kg), Group IV: C1 (10mg/kg) and Group V: C2 (10 mg/kg).

Drugs

Launaea Pinnatifida Ethanolic Extract (LPEE), C1 (Cyclohexane 1, 2, 3, 4, 5, 6-hexol), C2 (2Z, 6E) 3, 7, 11-trimethyldodeca-2, 6, 10-trien-1-ol) and standard drugs were suspended in 1% Tween- 80 immediately before their oral administration.

Acute Toxicity Studies

Healthy adult male albino rats were subjected for oral acute toxicity studies. The animals fasted overnight were divided into several groups (n=6) and were orally fed with LPEE, C1 and C2 respectively [11]. The animals were

observed continuously for 2 h for behavioral, neurological and autonomic profiles and after 24 and 72 hrs for any lethality [12].

Antinociceptive Activity using Eddy's Hot Plate Method

The animals were individually placed on the Eddy's hot plate maintained at constant temperature of 55 ±0.50 C, the cut off time was selected as 30 sec. The parameter evaluated was latency time of licking of legs and jumping response after exposure to the hot plate surface. The reaction times were determined at 0 hr, 1 hr, 2 hr, 4 hr and 8 hr [13,14]. Mice were administered with pentazocin (10 mg/kg), i.p as a standard drug.

Locomotor Activity using Actophotometer

Male albino mice (20-25 g) were subjected for locomotor activity to be evaluated by digital Actophotometer. The animals were placed individually in Actophotometer immediately after administration of test samples and ambulation was recorded at 0 hr, 01 hr, 02 hr, 04 hr, and 08 hr. Mice were administered with chlorpromazine (10 mg/kg) i.e. as a standard drug. The locomotor activity was expressed in terms of total photo beam count/5 min/animal [15].

Marble-Burying Activity

Twenty-five clear glass marbles (20 mm diameter) were used for each individual test. Opaque cages (30 X 36 X 13 cm) of smooth, opaque plastic with a vinyl ceiling containing air holes, and a 5 cm layer of sawdust were constructed. Mice were placed individually in these cages for 15 min (habituation trial) and then returned to their home cage.25 marbles were evenly spaced 5 cm apart on a 5 cm layer of sawdust in the habitation cages. Mice were then reintroduced (each test mouse was returned to the same cage in which they had been habituated). After 15 min, the test was terminated by removing the mice and counting the number of marbles that were more than two-thirds covered with sawdust. After each trial, the sawdust was replaced, and the test apparatus and glass marbles were washed with water and cleaned with 70% alcohol [16,17]. Male albino mice were administered with diazepam (10 mg/kg) i.p. as a standard drug.

Statistical Analysis

All the results were expressed as Mean ± Standard Error of Mean (SEM) with one-way ANOVA instant graph pad (U.S.A), followed by Tukey's Kramer's multiple comparison tests. The test was considered to be significant at $P<0.05$.

Results and Discussion

LPEE resulted into successful isolation of various compounds of which the two compounds viz. cyclohexane 1, 2, 3, 4, 5, 6-hexol (C1) and (2Z, 6E) 3, 7, 11-trimethyldodeca-2, 6, 10-trien-1-ol (C2) depicted in Fig 1 and 2 were used for studies. The test samples LPEE, C1 and C2 obtained were subjected to pharmacological screening. Based upon the continuous monitoring and observations for 72 hours, oral acute toxicity studies revealed the non-toxic nature of the LPEE at 250 mg/kg and of C1 and C2 at 10 mg/kg body weight.

Table 1 shows the antinociceptive activity of *Launaea Pinnatifida* Cass leaves using Eddy's hot plate in mice. Administration of the test samples significantly increased the latency in mice in licking of legs and jumping on the hot plate from 1st hr up to 2nd hr ($p<0.01$). However, they almost retained normalcy at 8th hr with immediate response to the heat stimuli. C2 was found to be more effective compared to other test samples with significant result till 2nd hr.

Group	Treatment	Zero hr	30 min	1st hr	2nd hr	4th hr	8th hr
I	Tween-80	3.000 ± 0.036	3.766 ± 0.212	2.883 ± 0.212	2.500 ± 0.210	1.866 ± 0.210	2.200 ± 0.208
II	Pentazocin	2.616 ± 0.153	4.066 ± 0.714	5.283 ± 0.552**	4.866 ± 0.280**	2.400 ± 0.143 ns	3.050 ± 0.080 ns
III	LPEE	3.283 ± 0.116	3.950 ± 0.204	4.950 ± 0.477**	5.333 ± 0.525**	3.033 ± 0.384 *	2.566 ± 0.304 ns
IV	C-1	2.700 ± 0.212	3.600 ± 0.219	4.400 ± 0.139*	4.616 ± 0.183**	3.816 ± 0.216 *	2.900 ± 0.253 ns
V	C-2	2.483 ± 0.218	3.830 ± 0.574*	4.900 ± 0.398**	5.466 ± 0.482**	2.933 ± 0.405*	3.500 ± 0.305**

Values are expressed as Mean ± SEM. ***p <0.001, **p < 0.01; *p<0.05 ns –Non significant, when compared to group-II.
Vehicle control = Tween- 80
LPEE = *Launaea Pinnatifida* Cass ethanolic extract
C-1 = Cyclo-1, 2, 3, 4, 5, 6-hexol.
C-2 = (2Z, 6E) -3, 7, 11-trimethyldodeca-2, 6, 10-1-ol.

Table 2 represents the loco motor activity of *Launaea Pinnatifida* Cass leaves in mice using Actophotometer. This test was performed to check the potentiality of the test compound exhibiting its effect as CNS stimulant or CNS depressant. Significant decrease in the locomotor activity was found in all test samples for first two hrs. However, increase in motor activity was retained in the mice at 4th hr by the test samples.

Group	Treatment	zero	01hr	02hr	04hr	08hr
I	Tween-80	290.66 ± 2.20	260.83± 1.45	256.83± 3.03	227.5± 2.74 ns	288.3± 2.43
II	Chlorpromazine	310.11± 2.18	181.5± 5.73**	101.33 ± 3.91**	88.167± 16.042 **	180.56± 2.74ns
III	LPEE	372.66± 3.64	242.17± 3.97**	184.83± 1.58	221.17± 21.489**	260.86± 1.39 ns
IV	C-1	292.500 ± 6.48	172.666 ± 4.52*	161.166 ± 1.45**	195.000 ± 4.65*	303.000 ± 2.46 ns
V	C-2	340.666 ± 4.22	198.833 ± 3.97*	191.500 ± 3.40*	230.17 ± 2.57 **	275.330 ± 4.19 **

Values are expressed as Mean ± SEM. ***p <0.001, **p < 0.01; *p<0.05 ns –Non significant, when compared to group-II.
Vehicle control = Tween- 80.
LPEE = *Launaea Pinnatifida* Cass Ethanolic Extract.
C-1= Cyclo-1, 2, 3, 4, 5, 6-hexol.
C-2= (2Z, 6E) -3, 7, 11-trimethyldodeca-2, 6, 10-1-ol.

Table 3 shows the effect of *Launaea Pinnatifida* Cass leaves in mice on marble burying activity. The crude LPEE exhibited uniform and slow burying activity in mice from 1st hr up to 4th hr. However, normalcy in burying was witnessed in mice administered with C1 and C2 when compared to diazepam. Variation in marble burying at specific time interval could be due to potentiality and effectiveness of the test sample.

Group	Treatment	Zero hr	01hr	02hr	04hr	08hr
I	Tween-80	18.166 ± 0.909	18.000 ± 0.577	16.166 ± 0.945	12.666 ± 0.614	8.666 ± 1.764
II	Diazepam	13.166 ± 2.286	1.666 ± 0.421***	0.000 ± 0.000***	0.000 ± 0.000***	0.000 ± 0.000***
III	LPEE	16.333 ± 0.667	8.666 ± 1.256***	7.333 ± 1.116***	6.500 ± 0.763***	13.666 ± 0.8433*
IV	C-1	13.000 ± 0.632	3.833 ± 1.223 *	1.833 ± 0.7491ns	0.500 ± 0.341***	11.666 ± 0.988 ns
V	C-2	10.000 ± 0.516	3.166 ± 0.401*	0.500 ± 0.341***	0.000 ± 0.000***	10.833 ± 0.654 ns

Values are expressed as Mean ± SEM. ***p <0.001, **p < 0.01; *p<0.05 ns –Non significant, when compared to group-II.
Vehicle control = Tween 80.
LPEE = *Launaea Pinnatifida* Cass Ethanolic Extract.
C-1= Cyclo-1, 2, 3, 4, 5, 6-hexol.
C-2= (2Z, 6E) -3, 7, 11-trimethyldodeca-2, 6, 10-1-ol.

The animal behavior in response to stimulus depends upon the functioning of the central and peripheral nervous system. Due to the exposure of various stress conditions an aberrant behavior by individual in response to the environmental situations was exhibited. Hence the neuro pharmacological property of *Launaea Pinnatifida* Cass by three different activities explicitly exhibited analgesic and sedative effects. Each parameter carried out in the study works on their specified principles.

The hot plate method test is considered to be selective to examine the compounds acting through opoid receptors; with an increase in the mean basal latency indicating that they may act via centrally mediated analgesic mechanisms. Narcotic analgesics inhibit both peripheral and central mechanisms of pain, while non-steroidal anti-inflammatory drugs inhibit only peripheral pain [18].

In case of the spontaneous motor activity in actophotometer, the animal which exhibited the locomotion depends upon the measurement of the level of excitability of the CNS [19]. The reduction in spontaneous activity may be closely related to sedation resulting from the depression of the central nervous system [20]. Thus the reduction in the spontaneous motor activity could be due to the inhibitory effects of the treatment samples.

The marble burying activity holds the principle of defensive burying, a behavior that can be elicited in rodents in response to aversive stimuli and inhibited by diazepam

or chlordiazepoxide. Glass marbles provide an effective unconditional stimulus which provokes burying and this model has been used for screening of anxiolytic drugs with the diminution of the burying activity indicating an anxiolytic-like effect [21]. The sedative effect of the test samples inhibited the effective unconditional stimulus which reduced the burying of glass marbles by exhibiting the anxiolytic effect.

The neuropharmacological results obtained during the study suggest that animals fed with the test samples of *Launaea Pinnatifida* Cass possess the adaptogenitcity. The significant results exhibited by crude LPEE for its analgesic and sedative property becomes quite difficult to specify the specific bioactive compound and mechanism of action involved in it due to the presence of various phytoconstituents present in it. Nevertheless, it may be attributed to the presence of C1 and C2. Compound C1 cyclohexane 1, 2,3,4,5, 6-hexol(inositol) has been successfully used in the clinical trials to control many psychiatric disorders without deleterious effects to kidney, liver or heart functioning. These disorders include depression, panic and obsessive-compulsive disorder that suggest the therapeutic benefits for the spectrum of illness responsive to the serotonin selective reuptake inhibitors [22]. Epi- inositol has been successfully used in mice to treat anxiety and is effective at reversing lithium effects [23] D-chirol inositol is effective in preventing the folate resistant mouse neural tube defects (Cogram.2002) and scyllo- inositol for treating and preventing AD-like symptoms in TgCRND8 MICE [24].

Compound C2 i.e. (2Z,6E) 3,7, 11-trimethyldodeca-2, 6, 10-1-ol has also contributed equally rather better by possessing significant neuro pharmacological activity suggesting that it is better analgesic and sedative compound when compared to other test samples. It has been reported earlier that 24-hydroxytormetric acid, a triterpenoid isolated from the bark of *Ocotea suaveolens* was used in relieving the pain thereby exhibiting the antinoceptive properties without side effects [25] Eulosmanolide santonin, a phyto constituent commonly found in Asteraceae exhibited analgesic effect in hot plate test in mice [26]. Recently it has been demonstrated that the sesquiterpene lactones exert anti-inflammatory response by inhibiting the central transcription factor and nuclear factor-k B(NF-kB) which are the major factors responsible for expressing multiple inflammatory genes [18]. Thus, we propose that the compounds C1 and C2 might have mimicked the similar mode of action as other polyphenols or terpene molecules.

Conclusion

On the basis of these investigations on *Launaea Pinnatifida* Cass, it may be concluded that cyclohexane 1, 2,3,4,5, 6-hexol (C1) and (2z, 6E) 3, 7, 11-trimethyldodeca-2, 6, 10-trien-I-ol(C2) possesses analgesic and sedative proprieties, which are quite efficient than the standard drugs. The experimental observations confirm at least partly the ancient use of *Launaea Pinnatifida* Cass as a medicine that relives pain in injury and reduces the anxiety and depression which serves as an herbal remedy for neuro pharmacological disorders. The mechanisms involved in and their action are not completely understood and further studies seem to be carried out necessarily.

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