



## Anxiolytic activity of root extracts of *Saussurea lappa* Clark. in mice

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### Abstract

**Objective:** To investigate anxiolytic activity of extracts of roots of *Saussurea lappa* Clark. **Materials and Methods:** Anxiolytic activity in mice was assessed using various parameters like elevated plus maze, open field test and foot shock induced aggression. Diazepam was used as standard anxiolytic agent. **Results:** At 30 mg/kg *Saussurea lappa* petroleum ether extract (SLP) and alcoholic extract (SLA) at 30 and 100 mg/kg showed anxiolytic activity in all the animal model used in this study. Both the extract and diazepam increased the duration of time spend in open arm, decreased time spend in close arm in elevated plus maze, increased the number of squares traveled in open field test and reduced the number of fighting bouts in foot shock induced aggression. **Conclusion:** It is concluded that SLP and SLA possess anxiolytic activity. SLA appears to be active as compared to SLP but less as compared to diazepam.

**Key Words:** *Saussurea lappa*, anxiolytic activity, elevated plus maze, open field test, foot shock.

### 1. Introduction

*Saussurea lappa* Clark of family Compositae is commonly known as kut or kusta. It is a tall robust and perennial plant, which grows on moist slopes of northern Himalayas at a height of 8000-13000 ft. [1] and in Kashmir, Punjab and upper region of Kulu Valley [2]. It has been used in Ayurvedic, Unani and other indigenous system of medicine as an antiasthmatic [3], diuretic, antihelmenthic, in treatment of fever, dyspepsia and leprosy. It is used in 14 out of 30

classical Ayurvedic preparations for treatment of epilepsy.

It is also extensively used in China as a powerful stimulant, carminative and antispasmodic [4]. The phytochemical analysis of roots showed presence of essential oils, alkaloids, tannins, resins, sugars [3], terpenoids [5], costic, palmatic and lanolinic acids, beta setosterol [6] and sesquiterpene lactones [7].

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It is hypothesised that there is a pharmacological link between epilepsy and anxiety. Anxiety and depression appear to be interictal complication in the epileptic population [8]. Clinical as well as experimental data have suggested possible relation between epilepsy and anxiety. On clinical side, several authors have identified anxiogenic syndromes in epileptic patients.

On the experimental side, one of the most common animal models of clinical epilepsy, electrical kindling of the amygdala in the rat, appears to be a good model for human temporal epilepsy and is also known to induce anxiety. Indeed, many molecules such as pentylenetetrazole, picrotoxin or several  $\beta$ -carbolines, that acts on the benzodiazepine site of the GABA<sub>A</sub> receptor and produced convulsions at high doses but became anxiogenic when administered at lower doses [9].

It was therefore thought worthwhile to investigate anxiolytic activity of extracts of roots of *S. lappa*. Although the literature mentioned use of roots for the treatment of epilepsy however, there are no reports about the use of roots in the treatment of anxiety.

## 2. Materials and methods

### 2.1 Preparation of extract

The roots of *S. lappa* were obtained from Regional Research Laboratory, Jammu and authenticated by Agharkar Research Institute, Pune. The powdered roots (200 g) were macerated in 1000 ml of petroleum ether (Merck Ltd, Mumbai) for 48 h. The macerate was then decanted and filtered through cloth and then through Whatman filter paper (No.1) to obtain a clear extract. This process of extraction was repeated again with same volume of petroleum ether.

The macerates were pooled and evaporated in air to give viscous oily yellowish brown liquid

and labeled as SLP. Marc remained after petroleum ether extraction process was dried and extracted with ethyl alcohol (90%) by same procedure to give light brown colour semisolid mass labeled as SLA. The yield of petroleum ether extract and ethanol extract was 2.23% and 5.76% respectively.

### 2.2 Animals

The Institutional Animal Ethical Committee of Poona College of Pharmacy, Pune formed under Committee for Purpose of Control and Supervision of Experimental Animals (CPCSEA) guidelines approved the pharmacological and acute toxicity protocols.

Male albino Swiss mice weighing 22-25 g obtained from Serum Institute of India Ltd, Pune, were housed in group of 6 animals per cage at a temperature of  $24 \pm 1^\circ\text{C}$  and relative humidity of 45-55%. A 12:12 h dark: light cycle was followed during the experiment and the experiment was carried during 10:00 a.m - 4:00 p.m. Animals had free access to food (Standard chaw pellet, Chakan oil mills, Sangli) and water *ad libitum*. Food but not water was withdrawn 3 h before and during the experiment.

### 2.3 Drugs

#### 2.3.1 Dosage form

The dosages were prepared by suspending extracts in Tween 80 (2.5%) and carboxymethyl cellulose (1%). Volume of dosage form administered was 0.05 ml/10g of body weight of animal.

#### 2.3.2 Acute toxicity

Intra-peritoneal acute toxicity study was performed in mice at limit dose of 2000mg/kg as per the OECD Guideline (AOT 425). The extracts (SLP and SLA) were administered at doses of 175, 550, 1000 and 2000 mg/kg.

## 2.4 Assessment of anxiolytic activity

### 2.4.1 Treatment schedule

For elevated plus maze and open field test, 48 mice were used for each experiment by dividing in 8 groups with 6 mice in each group. In foot shock induced aggression 96 mice were divided in 8 groups, consisting 6 pairs of male mice in each group. Group I received vehicle [Tween 80 (2.5%) + Carboxy Methyl Cellulose (CMC) (1%)], Groups II, III and IV received petroleum ether extract (SLP) 30, 100 and 300 mg/kg, i.p respectively. Groups V, VI and VII received ethyl alcohol extract (SLA) 30, 100 and 300 mg/kg, i.p respectively. Group VIII received diazepam (0.5 mg/kg, i.p). All the extracts or vehicle were administered 45 min before the observation.

### 2.5 Elevated Plus Maze (EPM) [10]

EPM apparatus consist of two open arms (25 x 5 cm) crossed with two enclosed arms (25 x 5 x 20 cm). Arms were connected with a central square (open arena) of 5 x 5 cm. The apparatus was elevated to the height of 25 cm. All the observations were carried out in a dimly illuminated room. Mice treated with an extract or vehicle were placed individually at the center of EPM facing an enclosed arm and time spent on open arm and enclosed arm was recorded during 5 min period and number of entries on the arm were noted during this time. An entry was defined as placing all four paws in the arm. EPM was cleaned with hydrogen peroxide after each trial.

### 2.6 Open Field Test [11]

Open-field test apparatus (68 x 68 x 45 cm) was made of plywood. The entire apparatus was painted black except for 6mm thick white line, which divided the floor onto 16 squares. All the observations were carried out in dark room except open field apparatus lighted by a 40 W bulb focused onto the field from a height

of about 100 cm. The entire room except the open field was kept dark during the observations. Each animal was placed at one corner of apparatus and the following behavioral aspects were noted:

1. *Ambulation*: This was measured in terms of the number of squares crossed by the animal.
2. *Activity in the centre*: number of central squares crossed by the animal.

### 2.7 Foot shock induced aggression in mice [12]

A pair of male mice was placed in box with a grid floor consisting of steel rods with the distance of 6 mm. A constant shocker delivered 60 Hz current for 5 sec followed by 5 sec intermission for three minutes. The total number of fights was recorded during 3 min period. The fighting behaviour consisted of vocalization, leaping, running, rearing and facing each other with some attempts to attack by hitting, biting or boxing. Six pairs of mice were used for each treatment.

### 2.8 Statistical Analysis

All observations are presented as Mean  $\pm$  S.E.M. The data was analysed by one way Analysis of Variance (ANOVA) followed by Dunnett's test using GraphPad InStat version 3.05, USA.

## 3. Results

### 3.1 Acute toxicity

Single intra-peritoneal administration of extract SLP and SLA at 175, 550 and 1000 mg/kg dose level did not show any toxic signs during observation period for 24 h in all the mice tested however, 50% mice died at 2000 mg/kg, i.p dose of SLP during short term observation (6 h) and with SLA after 24 h. The LD<sub>50</sub> calculated was 2 g/kg.

### 3.2 Assessment of anxiolytic activity

#### 3.2.1 Elevated Plus Maze

Vehicle treated mice spent  $21.33 \pm 2.30$  sec on open arena and  $278.66 \pm 2.30$  sec in enclosed arm. The number of entries on enclosed arm

was  $4.83 \pm 0.74$ . The results given in Table 1 indicate that, that time spent on open arm and open arena was increased significantly ( $p < 0.01$ ) when compared to vehicle treated group.

Consequently significant decrease in time spent on enclosed arms by SLP (30 mg/kg) and SLA (30 and 100 mg/kg) was observed. The number of entries on enclosed arm was increased significantly ( $p < 0.01$ ) by SLP (30 mg/kg) and SLA (30 and 100 mg/kg). Diazepam showed significant increase in time spent on open arm and numbers of entries on open arm while significant ( $p < 0.01$ ) decrease in time spent on enclosed arm was observed.

### 3.3 Open Field Test

Mice treated with vehicle traveled total  $59 \pm 6.55$  squares. All squares traveled were at wall side only and mice did not enter on squares at center. Compared to vehicle treated group mice treated with SLP (30 mg/kg) and SLA (30 mg/kg) showed significant ( $p < 0.01$ ) increase in number of squares traveled at wall side  $92.50 \pm 5.23$  and  $99.67 \pm 6.75$  respectively, with significant

( $p < 0.01$ ) activity at center by crossing  $13.83 \pm 4.52$  and  $14.67 \pm 2.70$  squares respectively. Diazepam (0.5 mg/kg) showed significant ( $p < 0.01$ ) increase in open field ambulation and activity in the center. (Table 2)

### 3.4 Foot shock induced aggression

Vehicle treated mice fought  $16.33 \pm 1.25$  times during 5 min period of observation. The number of fighting bouts was reduced significantly by SLP (30, 100 and 300 mg/kg) and SLA (100 and 300 mg/kg) when compared to vehicle treated group. Diazepam (0.5 mg/kg) was found to be more potent than extracts. (Table 3)

## 4. Discussion

Anxiety has long been recognized as an intrinsic component of the human condition. The discovery of benzodiazepine in the early 60's and their considerable commercial successes in the treatment of anxiety has feed the development of numerous animal model of anxiety mostly based on pharmacological action of benzodiazepine [13]. Therefore in this study diazepam was used as standard drug.

Table 1.

Effect of extracts of roots of *Saussurea lappa* on behavior of mice in Elevated Plus Maze (EPM).

Treatment (mg/kg)	Time (in Sec) Spent in			No. of Entries	
	Open arm	Open arena	Close arm	Open arm	Open arena
Vehicle	Nil	$21.33 \pm 2.30$	$278.66 \pm 2.30$	Nil	$4.83 \pm 0.74$
SLP (30)	$8.33 \pm 1.05^*$	$37.33 \pm 5.00$	$254.33 \pm 4.91^{**}$	$2.33 \pm 0.71^{**}$	$10.67 \pm 1.60^{**}$
SLP (100)	Nil	$28.83 \pm 3.19$	$271.17 \pm 3.19$	Nil	$7.5 \pm 1.20$
SLP (300)	Nil	$20.5 \pm 2.52$	$279.5 \pm 2.52$	Nil	$7 \pm 1.15$
SLA (30)	$14.67 \pm 4.72^{**}$	$45.67 \pm 4.60^{**}$	$239.67 \pm 5.75^{**}$	$1.67 \pm 0.33^*$	$10.67 \pm 1.31^{**}$
SLA (100)	$24.33 \pm 2.56^{**}$	$57.00 \pm 6.13^{**}$	$218.67 \pm 7.45^{**}$	$1.67 \pm 0.66^*$	$11.17 \pm 1.07^{**}$
SLA (300)	Nil	$17.00 \pm 2.29$	$283.00 \pm 2.29$	Nil	$8.33 \pm 1.31$
Diazepam (0.5)	$24.50 \pm 2.96^*$	$52.50 \pm 6.44^{**}$	$223.00 \pm 7.62^{**}$	$5.17 \pm 0.48^{**}$	$9.67 \pm 0.99^*$

n=6 in each group. Data represented as Mean  $\pm$  S.E.M

Data was analysed by ANOVA followed by Dunnett's test using GraphPad InStat Version 3.05 Software USA.

\* $p < 0.05$ , \*\* $p < 0.01$  as compared to vehicle treated group.



Table 2.

Effect of extracts of roots of *Saussurea lappa* on behaviour of mice in open field test.

Treatment (mg/kg ip.)	Ambulation		
	Wall Side	Central	Total
Vehicle	59.00 ± 6.55	Nil	59.00 ± 6.55
SLP (30)	92.50 ± 5.23**	13.83 ± 4.52**	106.33 ± 7.91**
SLP (100)	43.83 ± 5.35	Nil	43.83 ± 5.35
SLP (300)	21.50 ± 2.54	Nil	21.50 ± 2.54
SLA (30)	99.67 ± 6.75**	14.67 ± 2.70**	114.33 ± 7.61**
SLA (100)	84.33 ± 7.89	8.50 ± 0.92*	92.83 ± 7.75**
SLA (300)	62.67 ± 9.52	6.17 ± 1.22	68.83 ± 8.67
Diazepam (0.5)	105.00 ± 7.33**	10.33 ± 1.33**	115.33 ± 7.35**

n=6 in each group. Data represented as Mean ± S.E.M

Data was analysed by ANOVA followed by Dunnett's test using GraphPad InStat Version 3.05 Software USA. \* $p < 0.05$ , \*\* $p < 0.01$  as compared to vehicle treated group.

Table 3.

Effect of extracts of roots of *Saussurea lappa* on foot shock induced aggression.

Treatment (mg/kg)	No. of fighting behaviour
Vehicle	16.33 ± 1.25
SLP (30)	10.83 ± 0.83**
SLP (100)	11.83 ± 1.47*
SLP (300)	0**
SLA (30)	14.00 ± 1.29
SLA (100)	11.83 ± 1.30*
SLA (300)	8.17 ± 0.46*
Diazepam (0.5)	6.33 ± 0.92**

n=6 in each group. Data represented as Mean ± S.E.M

Data was analysed by ANOVA followed by Dunnett's test using GraphPad InStat Version 3.05 Software USA. \* $p < 0.05$ , \*\* $p < 0.01$  as compared to vehicle treated group.

EPM test is said to be ethologically valid since it utilises fear of balancing on an open and elevated maze arm as a stimulus. This stimulus lead to an approach conflict that is considerably stronger than that evoked by exposure to an enclosed arm of a maze. Thus open/enclosed arm entries provide a measure of fear induced inhibition of exploratory behaviour [14]. These responses are

increased by anxiolytics and reduced by anxiogenic agent [10]. In the present study, significant increase in time spent in open arm, open arena and number of entries and significant decrease in time spent in enclosed arm suggest anxiolytic activity of extracts.

Many authors suggested non-specific nature of anxiety or multiplicity of drug action of anxiolytic agents by different ways of actions in the elevated plus maze with regard to arm entries and time spent on open arm. Diazepam

increases time spent in open arm and entries in open arm. Buspirone a 5-HT<sub>1A</sub> agonist increases time spent in the open arm and entries in open arm but decreased total entries. Various authors reported different effects of the buspirone on the elevated maze test i.e. anxiolytic [15], non-effective [16] and anxiogenic [17].

It is pertinent that anxiety-modulating drug should be carefully assessed on the elevated plus maze test. Therefore in present study elevated plus maze was supplemented by other tests like open field test and foot shock induced aggression.

In open field test, since exposure to a novel environment is associated with emotionality, when animals were taken from their home cages and placed in a novel environment they express their anxiety and fear by decrease in ambulation and exploration. These behavioural changes were attenuated by anxiolytic and augmented by anxiogenic agents [18,19].

In present study significant increase in ambulation and activity in centre indicated the anxiolytic activity of extracts, in elevated plus

maze significant increase in number of entries on both arm suggest increase in ambulation the results obtained in both studies are strengthen the cordence for anxiolytic action.

In foot shock induced aggression test the anxiolytic agent reduce the number of fighting bouts. It is reported that benzodiazepin anxiolytics and buspirone are effective but ondansetron gives negative results [14]. The extracts as well as diazepam reduced the number of fighting bouts supporting the anxiolytic activity. It is concluded that SLP

(30 mg/kg) and SLA (30 and 100 mg/kg) possess anxiolytic activity. SLA appears to be more active as compare to SLP but less active as compared to diazepam.

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