Effects of *Lactuca sativa* extract on exploratory behavior pattern, locomotor activity and anxiety in mice

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**Article info**

**Abstract**

**Objective:** To evaluate antianxiety property of *Lactuca sativa*, an important and commonly used leafy vegetable known for its medicinal properties belongs to Asteraceae family. **Methods:** Elevated plus maze (EPM), open field test (OFT), rat exposure test, hyponeophagia and marble burying test were performed in mice models to assess the exploratory behaviour and to assess anxiolytic property of hydro–alcohol extract of *Lactuca sativa*. Diazepam (1 mg/kg body wt.) served as the standard anxiolytic agent for all the tests. The dried extract of the plant leaf in doses of 100, 200 and 400 mg/kg body weight was administered orally to mice for duration of 15 or 30 days and evaluated exploratory behaviour, locomotor and anxiolytic activities. **Results:** Time spent and number of entries into the open arm was measured in EPM followed by total locomotor activity in OFT and latency to enter the food zone in hyponeophagia. **Conclusions:** The study suggested that hydro–alcohol extract of *Lactuca sativa* leaves possess potent anxiolytic property.

**1. Introduction**

*Lactuca sativa* belonging to Asteraceae family is an important leafy vegetable known for its medicinal properties. As per traditional knowledge, it is used in the treatment of insomnia, anxiety, neuritis, dry coughs, rheumatic pain, etc. [1]. The whole plant has also been used for the treatment of stomach problems, to stimulate digestion and to enhance appetite and relieve inflammation [2]. The latex from *Lactuca sativa* contains 15 oxalyl and 8 sulfate conjugates of the guaianolide sesquiterpenolactones, lactucin, deoxylactucin and lactucopicrin [3]. Lettucenin–A is highly antimicrobial [4]. Antioxidant activity of lettuce has been reported to prevent chronic diseases related to oxidative stress such as cancer [5]. Analgesic potency of *Lactuca sativa* opium extracts has been reported [6]. *Lactuca sativa* gives protection against D–galactose induced oxidative stress and reduces accumulation of lipofuscin granules [7].

However, the leaves of *Lactuca sativa* has not been thoroughly studied with respect to its anxiolytic properties. Anxiety may be regarded as a particular form of behavioural inhibition that occurs in response to environmental events that are novel. It has been established that there are lot of plant secondary metabolites being employed in the treatment of psychotic disorders especially for anxiety in traditional medicine practice, most of which directly or indirectly affect the central nervous system [8, 9]. Considering the varied important activities reported in traditional system of medicine about *Lactuca sativa*, it was planned to study the effects of the extract of *Lactuca sativa* L. leaves on exploratory behaviour pattern, locomotor activity and anxiolytic properties in mice.

**2. Materials and methods**

**2.1. Extraction**

250.0 g of crushed *Lactuca sativa* was used for extraction. This sample was soaked overnight in 70% ethyl alcohol and filtered using Whatman No.1 paper. Process was repeated twice by adding fresh solvent every time. The pooled extract
was subjected to flash evaporation followed by lyophilization. The lyophilized sample was further analyzed for its anxiolytic property.

2.2. Animal experiment

Animal studies were conducted according to the institute animal ethical committee regulations approved by the committee for the purpose of the control and supervision of experiments on animals. Male mice weighing 25–30 g were selected from the stock colony, Defence Food Research Laboratory, Mysore, India, housed in an acryl fibre cage in a temperature controlled room (temperature 25±2°C) and was maintained in 12 h light/dark cycle with free access to food and drinking water ad libitum.

2.3. Experimental design

The extracts of the leaves of *Lactuca sativa* were separately suspended in a vehicle comprising 1% (w/v) Tween 20 in distilled water. The grouping of mice was done 6 animals/group and the extracts were administered for 15 days and 30 days treatment courses. Group 1 received vehicle which served as Control. Groups 2, 3 and 4 received hydro–alcohol extract at the doses of 100, 200, 400 mg/kg body wt. respectively and Group 5 received diazepam (1mg/kg body wt.). The doses of extracts were calculated to administer 0.25 ml of the suspension of extracts to the mice.

2.4. Behavioural study

2.4.1. Elevated plus–maze test

The test procedure and scoring methodology for the elevated plus–maze test have been described by Kulkarni [10]. In brief, the apparatus composed of two open (30x5x0.25 cm) and two enclosed (30x5x15 cm) arms that radiated from a central platform (5x5 cm) to form a plus sign. A slightly raised edge on the open arms (0.25 cm) provided an additional grip for the animals. The maze floor and the closed arms were covered with black adhesive tape. The plus–maze was elevated to a height of 40 cm above floor level by a single central support. The mice were administered with drugs or vehicle and sixty minutes later, the trial was started by placing the animal on the central platform of the maze facing an open arm. The number of entries into, and the time spent, in each of the two types of arms were counted during a 5 min test period. The open–arm entries and open–arm time were used as indices of anxiety.

2.4.2. Open Field test

Spontaneous motor activity was evaluated in open field test as per the method of Bhattacharya & Satyan [11]. The open field apparatus is made up of black plexi glass and consisted of a square 56 cm x 56 cm. The entire apparatus was divided the floor into 16 square of identical dimension. The entire room, except the open field was kept dark during the experiment. One hour after the treatment of vehicle/standard/extract each animal was placed at one corner of the apparatus and the behavioural aspects were noted in the next 5min [12].

2.4.3. Novel object test

The open field test apparatus was used for this experiment. Novel objects e.g., mega colour blocks structure was used to induce anxiety. The entire room, except the open field was kept dark during the experiment. One hour after the treatment of vehicle/standard/extract each animal was placed at one corner of the apparatus and the behavioural aspects were noted in the next 5min [12].

2.4.4. Marble Burying Test

A modified procedure based on Yamada et al. [13] was employed. Mice were placed individually in plastic cages with the designated bedding material for 30 min (habituation period) and then placed into waiting cages. Twelve glass marbles were then evenly spaced 3 cm apart on a 4–cm layer of bedding material in the habituation cages. Mice were then reintroduced into the same cage in which they had been habituated. After 30 min, the marble burying period was terminated by removing the mice, and the number of marbles that were more than two-thirds covered with bedding material was counted.

2.4.5. Rat exposure test

The open field apparatus was used for this experiment, 20cm x 10cm cylinder made of wire was kept in one corner; rat was placed in that cylinder. The entire room, except the open field was kept dark during the experiment. One hour after vehicle/standard/extract treatment each animal was placed at one corner of the apparatus and the behavioural aspects were noted in the next 5min [14].

2.4.6. Hyponeophagia test

A modified procedure based on Deacon [15] was employed to carry out hyponeophagia test. The open field apparatus was used for this experiment. The animals were fasted on the previous night. Novel food like sweet corn was used to induce hyponeophagia. The entire room, except the open field was kept dark during the experiment. One hour after the treatment of vehicle/standard/extract each animal was placed at one corner of the apparatus. The latency to eat and the behavioural aspects were measured in the next 5min.

2.5. Statistical analysis

All data are presented as mean±SD and was analysed by one–way ANOVA. The groups treated with extracts were compared with the respective vehicle (control) group. The diazepam treated group was compared with control and P values <0.05 and <0.001 were considered statistically significant. All behavioural recordings were carried out using ANY MAZE software from Columbus Instruments, Ohio, USA.

3. Results

Hydro–alcohol extract of *Lactuca sativa* showed increase in time spent in open arm and in the number of entrances into the open arms compared to untreated group. Though the lower doses did not affect the number of entries and the time spent in open arms, the 200 and 400mg/kg body weight treated group
showed significant increase which is comparable to diazepam drug administered group of mice (Table 1).

The values of the group treated with L. sativa at 400 mg/kg body weight were higher than that of the treatment with lower doses. However, the standard drug diazepam showed higher locomotor activity comparable to that of 400 mg/kg body weight in open field test (Table 2).

Number of entries, time spent in object zone and latency to first entry to object zone was measured. 200 and 400 mg/kg body weight treated groups exhibit a significant result when compared to the control (Table 3).

Feeding at the dosage levels of 200 and 400 mg/kg body weight resulted in decrease in number of marbles buried as compared to the control (Table 4).

### Table 1
Effect of hydro-alcohol extract *Lactuca sativa* extracts on the elevated plus maze test for 15 and 30 days treatment.

| Groups              | 15 days | 30 days |
|---------------------|---------|---------|
|                     | Time in open arm (s/5min) | Time mobile in open arm (s/5min) | No of entries to open arm | Time in open arm (s/5min) | Time mobile in open arm (s/5min) | No of entries to open arm |
| Control             | 53.00±11.2 | 33.12±10.9 | 15.75±5.3 | 66.95±10.1 | 35.00±15.08 | 16.00±6.4 |
| 100mg/kg bwt. (LS)  | 87.72±12.2 | 49.15±10.7 | 22.25±5.4 | 86.90±10.8 | 47.05±13.06 | 27.75±4.1 |
| 200mg/kg bwt. (LS)  | 113.32±13.3 | 76.20±12.7 | 28.25±3.3 | 114.57±11.9 | 78.42±12.1 | 31.50±4.3 |
| 400mg/kg bwt. (LS)  | 145.32±11.4 | 82.27±12.8 | 33.75±4.4 | 155.27±13.9 | 102.32±5.6 | 40.50±7.1 |
| 1mg/kg bwt. (D)     | 192.90±12.2 | 116.25±13.04 | 37.25±7.6 | 180.72±14.6 | 117.75±14 | 46.00±11.5 |

The plant extracts, diazepam or control, were injected 60 min prior to test. Data are presented as mean values (±SD.) from group of six mice. *P < 0.05 and **P < 0.001 indicates significant difference from control. LS represent *Lactuca sativa* and D represents diazepam.

### Table 2
Effect of hydro-alcohol extract *Lactuca sativa* extracts on the open field test for 15 and 30 days treatment.

| Groups              | 15 days | 30 days |
|---------------------|---------|---------|
|                     | Time mobile in open arm (s/5min) | Line crossing | Time mobile in open arm (s/5min) | Line crossing |
| Control             | 80.93±13.5 | 72.75±12.2 | 75.08±10.3 | 68.82±12.8 |
| 100mg/kg bwt. (LS)  | 118.50±9.3 | 132.75±15.3 | 121.58±6.4 | 110.25±10.9 |
| 200mg/kg bwt. (LS)  | 142.15±22.0 | 171.00±15.8 | 134.00±12.6 | 171.00±15.8 |
| 400mg/kg bwt. (LS)  | 184.58±11.5 | 206.50±15.5 | 172.55±15.5 | 210.25±11.1 |
| 1mg/kg bwt. (D)     | 190.95±14.7 | 244.65±12.3 | 205.45±12.2 | 251.65±18.5 |

The plant extracts, diazepam or control, were injected 60 min prior to test. Data are presented as mean values (±SD.) from group of six mice. *P < 0.05 and **P < 0.001 indicates significant difference from control. LS represent *Lactuca sativa* and D represents diazepam.

### Table 3
Effect of hydro-alcohol extract *Lactuca sativa* extracts on the novel object test for 15 and 30 days treatment.

| Groups              | 15 days | 30 days |
|---------------------|---------|---------|
|                     | Time spent in object zone (s/5min) | Time mobile in object zone (s/5min) | Latency for 1 entry to object zone (s) | Time spent in object zone (s/5min) | Time mobile in object zone (s/5min) | Latency for 1 entry to object zone (s) |
| Control             | 18.63±8.3 | 16.58±6.7 | 54.05±14.6 | 20.83±7.4 | 16.58±6.7 | 55.10±9.2 |
| 100mg/kg bwt. (LS)  | 25.60±9.2 | 18.20±5.1 | 37.12±12.6 | 31.30±7.3 | 19.20±4.5 | 41.67±15.4 |
| 200mg/kg bwt. (LS)  | 54.85±15.7 | 20.00±9.05 | 72.02±13.6 | 60.70±11.6 | 27.33±8.5 | 31.47±10.6 |
| 400mg/kg bwt. (LS)  | 87.73±11.9 | 32.83±6.5 | 21.27±4.2 | 94.35±12.9 | 46.88±10.1 | 27.20±5.7 |
| 1mg/kg bwt. (D)     | 109.40±14.2 | 34.40±4.3 | 16.52±7.7 | 106.00±11.8 | 58.48±11.3 | 17.77±5.8 |

The plant extracts, diazepam or control, were injected 60 min prior to test. Data are presented as mean values (±SD.) from group of six mice. *P < 0.05 and **P < 0.001 indicates significant difference from control. LS represent *Lactuca sativa* and D represents diazepam.

### Table 4
Effect of hydro-alcohol extract *Lactuca sativa* extracts on the marble burying test for 15 and 30 days treatment.

| Groups              | 15 days | 30 days |
|---------------------|---------|---------|
|                     | No of marbles buried | No of marbles buried |
| Control             | 4.25±0.9 | 5.00±0.8 |
| 100mg/kg bwt. (LS)  | 3.50±1.2 | 3.25±1.7 |
| 200mg/kg bwt. (LS)  | 2.50±0.5 | 2.50±1.2 |
| 400mg/kg bwt. (LS)  | 2.25±0.9 | 1.75±0.9 |
| 1mg/kg bwt. (D)     | 1.75±0.9 | 1.25±0.5 |

The plant extracts, diazepam or control, were injected 60 min prior to test. Data are presented as mean values (±SD.) from group of six mice. *P < 0.05 and **P < 0.001 indicates significant difference from control. LS represent *Lactuca sativa* and D represents diazepam.
Rat exposure test has proven useful to determine strain difference in defensive behaviors and relative dosage levels of anxiety in the response to predators. Number of entries, time spent in rat zone and latency to first entry to rat zone was measured. In this test the feeding of L. sativa at the dosage levels of 200 and 400mg/kg body weight showed significant increase in the time spent in rat zone when compared to the control (Table 5) demonstrating anxiolytic effect.

Number of entries, time spent in food zone and latency to first entry to food zone constitutes the behavioral pattern. We have conducted the experiment using sweet corn which resulted in the feeding latency, confounded by exploratory time. It also introduces an additional facet of anxiety, i.e. open field central field aversion, a classic measure of open field mediated anxiety. At the dosage levels of 200 and 400mg/kg body weight, mice showed significant increase in the time spent in food zone when compared to the control (Table 6).

### Table 5
Effect of hydro–alcohol extract Lactuca sativa extracts on the rat exposure test for 15 and 30 days treatment.

| Groups       | 15 days treatment | 30 days treatment |
|--------------|-------------------|-------------------|
|              | No of entries to rat zone | Latency for 1 entry to rat zone (s/5min) | Time in rat zone (s/5min) | No of entries to rat zone | Latency for 1 entry to rat zone (s/5min) | Time in rat zone (s/5min) |
| Control      | 8.75±5.9          | 32.05±6.8         | 14.80±9.7       | 11.50±4.6          | 42.90±11.9       | 18.57±5.7 |
| 100mg/kg bwt. (LS) | 12.00±5.0         | 22.97±7.2         | 19.87±8.1       | 12.00±2           | 32.57±9.4       | 26.17±10.2 |
| 200mg/kg bwt. (LS) | 13.50±5.7         | 20.07±5.9         | 20.95±5.1       | 16.25±3.9         | 24.25±2.4       | 38.52±10.5 |
| 400mg/kg bwt. (LS) | 17.50±2.6         | 13.77±6.6         | 38.30±12.1      | 18.75±3.5         | 15.72±4.1       | 55.52±8.4   |

The plant extracts, diazepam or control, were injected 60 min prior to test. Data are presented as mean values (±SD.) from group of six mice. *P < 0.05 and **P < 0.001 indicates significant difference from control. LS represent Lactuca sativa and D represents diazepam.

### Table 6
Effect of hydro–alcohol extract Lactuca sativa extracts on the Hyponeophagia (sweet corn) for 15 and 30 days treatment.

| Groups       | 15 days treatment | 30 days treatment |
|--------------|-------------------|-------------------|
|              | No of entries to food zone | Latency for 1 entry to food zone (s/5min) | Time in food zone (s/5min) | No of entries to food zone | Latency for 1 entry to food zone (s/5min) | Time in food zone (s/5min) |
| Control      | 1.25±0.5          | 112.67±7.7        | 8.63±3.4        | 2.00±0.8          | 143.5±10.3       | 8.63±3.4 |
| 100mg/kg bwt. (LS) | 3.00±0.8          | 65.80±11.7        | 12.40±5.1       | 3.50±1.3          | 92.10±12.9       | 12.40±5.1 |
| 200mg/kg bwt. (LS) | 8.00±4.1          | 58.72±12.2        | 29.18±11.5      | 8.75±3.5          | 59.12±10.2       | 29.18±11.5 |
| 400mg/kg bwt. (LS) | 10.00±3.7         | 24.72±5.2         | 33.40±6         | 11.75±3.8         | 26.20±5.9        | 31.98±6.6   |

The plant extracts, diazepam or control, were injected 60 min prior to test. Data are presented as mean values (±SD.) from group of six mice. *P < 0.05 and **P < 0.001 indicates significant difference from control. LS represent Lactuca sativa and D represents diazepam.

when compared to the control (Table 6).

### Discussion

EPM is considered one of the most widely validated tests for assaying new benzodiazepine–like anxiolytic agents [11]. It is well known that the anxiolytic agents increase the motor activity which is measured by time spent by the animal in the open arms [16, 17]. Animals treated with diazepam showed a significant increase in the time spent in the open arms and decreased time spent in closed arms, as well as an increase in the number of entries in the open arms. Open–field test shows the total distance travelled in the whole of the open–field arena by the mice. Though this parameter does not reflect changes in emotional behavior, it is important for evaluating the total locomotor activity of the animals during the 5–minute trial. The two major behavioral variables evaluated in the open field test were the time spent in the zone of the field and the number of line crossings across the entire zone. Novel object test model investigates the approach avoidance behaviors of mice in response to novel stimuli, a useful method for measuring anxiety [12]. The marble burying test is a useful model of neophobia [18] and obsessive–compulsive behaviour [19]. It has also been proposed that the test may have predictive validity for the screening of novel antidepressants [20] and anxiolytic agents [21]. This behaviour is probably a type of defensive burying typical of rodents [22]. Rat exposure test utilizes the natural defensive ‘avoidance’ behavioral response of mice to signs of potential danger, such as natural predators (e.g. rats). The defensive behaviors measured by this test are sensitive to anxiolytics, making this paradigm useful in pharmacological screening [14]. The inhibition of feeding behaviour has been termed hyponeophagia and is robust in both rats and mice. Treatment with a variety of drugs used to manage anxiety in humans reliably reverses this decrement in feeding, reducing the latency to the first taste and increasing the total amount of food consumed [23, 24].

Reports state that, the ethanol extract of Lactuca sativa is rich in sesquiterpene lactones [25], carotenoids [26].

In this work, the anxiolytic properties of extracts of Lactuca sativa, locomotor activity and exploratory behaviour of mice were studied. The anxiolytic property was higher in the group of mice fed with 400mg/kg body weight than the other doses.
Generally there was a significant increase in the locomotor activity by feeding the extract for a period of 30 days than 15 days. This dose demonstrated a near to equivalent anxiolytic behaviour vis-à-vis the drug, diazepam. In view of these results, it is suggested that hydro-alcohol extract of *Lactuca sativa* possess potent anxiolytic property.

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