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Anxiolytic property of *Lactuca sativa*, effect on anxiety behaviour induced by novel food and height

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ABSTRACT

Objective: To study anxiolytic property of hydro alchohol extract and to estimate polyphenols present in the extract by HPLC. **Methods:** To evaluate anxiolytic property two animal models were used viz. Elevated T maze and hyponeophagia. 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 locomotor and anxiolytic activities were performed. Polyphenols was estimated using HPLC. **Results:** The HPLC analysis of the polyphenols revealed the presence chlorogenic acid, vanillin, epicatechin, caffeic acid, rutin hydrate, sinapic acid, quercetin-3-rhamnoside, p-coumeric acid and quercitin. Time spent and number of entries into the open arm was improved in 30 days treated animals than that of 15 days treated groups, 200 and 400 mg/kg body weight treated group showed significant results when comparing with the control group. **Conclusions:** The hydro alcohol extract rich in Polyphenols and other secondary metabolites is a potent anxiolytic agent.

1. Introduction

Although anxiety is an important protective mechanism, it can become maladaptive and disruptive[1-3]. Pathological anxiety, as manifested in anxiety disorders, is an anxious response that occurs out of proportion to the threat, becomes disruptive to daily life and causes suffering. Anxiety has been implicated in a number of psychiatric disorders, such as generalized anxiety disorder, depression, panic-attacks, phobias, obsessive-compulsive disorders and posttraumatic stress disorders. Anxiety alleviates the levels of intracellular reactive oxygen species[4]. Anxiety induces stress and activates the sympathetic nervous system, stimulating the release of catecholamines from the adrenal medulla as a response to stressors fight or flight[5,6]. Benzodiazepine, the most commonly prescribed treatment for anxiety disorders, has side effects such as sedation, myorelaxation, ataxia, amnesia and pharmacological dependence[7]. Hence various plants are used in complementary and alternative medicines for management of anxiety[8].

Lactuca sativa (*L. 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, neurosis, dry coughs, rheumatic pain, etc.[9]. The whole plant has also been used for the treatment of stomach problems, to stimulate digestion and to enhance appetite and relieve inflammation[10]. The latex from *L. sativa* contains 15 oxalyl and 8 sulfate conjugates of the guaianolide sesquiterpene lactones, lactucin, deoxylactucin and lactucopicrin[11,12]. Lettucenin-A is highly antimicrobial[13]. Antioxidant activity of lettuce has been reported to prevent chronic diseases related to oxidative stress such as cancer[14]. *L. sativa* gives protection against D-galactose induced oxidative stress and reduces accumulation of lipofuscin granules[15].

However, the leaves of *L. sativa* has not been thoroughly studied with respect to its anxiolytic properties. Anxiety may be regarded as a particular form of behavioural

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inhibition that occurs in response to environmental events that are novel. It has been established that there are lot of plant secondary metabolites like polyphenols and flavonoids 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[16,17]. Considering the varied important activities reported in traditional system of medicine about *L. sativa*, it was planned to study the effects of the extract of L. sativa leaves on anxiolytic properties in mice.

2. Materials and methods

Extraction- 250.0 g of crushed *L. 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.

2.1. 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 ℃) and was maintained in 12 h light/ dark cycle with free access to food and drinking water *ad libitum*.

2.2. Experimental design

The extracts of the leaves of *L. 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 (1 mg/kg body weight). The doses of extracts were calculated to administer 0.25 mL of the suspension of extracts to the mice.

2.3. HPLC analysis of the polyphenols

Phenolic compounds were identified using a Diode Array Detector (JASCO Pu-1580 HPLC system) on reverse phase C_{18} column (150 mm × 4.5 mm). The mobile phase used consisted of two solvents 0.1% formic acid (A) and 100% methanol (B). The total run time was 60 mins. The eluting compounds were detected by monitoring at 270 nm. The phenolic compounds were identified by comparing the retention time of the unknown with the standards. The lyophilized plant extract was redissolved in 0.1% formic acid for HPLC analysis. 20 μL of the sample and the standards were injected into the column.

2.4. Elevated T-maze test

The test procedure and scoring methodology for the elevated T-maze test have been described by Viana *et al*[18] and Graeff *et al*[19]. In brief, the apparatus composed of two open (30 cm × 5 cm × 0.25 cm) and one enclosed (30 cm × 5 cm × 15 cm) arms that radiated from a central platform (5 cm × 5 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 T-maze was elevated to a height of 40 cm above floor level by a single central support. The mice were injected 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 arm, were counted during a 5 min test period. The open-arm entries and open-arm time were used as indices of anxiety.

2.5. Hyponeophagia

A modified procedure based on Deacon[20] was employed to carry out hyponeophagia test. The open field apparatus is made up of black plexi glass. Remove all food from cage the evening before testing, novel food like paneer (cheese) was used to induce hyponeophagia. 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. Measure the latency to eat and the behavioral aspects were noted in the next 5 min. The apparatus was cleaned thoroughly between trials with damp and dry towels. All behavioral recordings were carried out using ANY MAZE software.

2.6. 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.

3. Results

The HPLC analysis of the polyphenols revealed the presence of chlorogenic acid, vanillin, epicatechin, caffeic acid, rutin hydrate, sinapic acid, quercetin-3-rhamnoside, p-coumeric acid and quercitin (Figure 1). The retention time of the phenolic compounds identified was chlorogenic acid-21.6, vanillin-26.6, epicatechin-23.8, caffeic acid-24.7, rutin hydrate-36.96, sinapic acid-32.86, quercetin-3-rhamnoside-39.9, p-coumeric acid-30.1 and quercitin-45.7. However, a few of the polyphenols could not be identified due to lack of standards.

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 (Table 1). 70% ethanol extracts of *L. sativa* showed increase in time spent in open arm and in the number of entrances into the open arms compared to untreated group. The dose at 400 mg/kg body wt. was comparable to that of diazepam drug adminisistered group. Time spent and number of entries into the open arm was improved in 30 days treated animals than that of 15 days treated groups, 200 and 400 mg/kg body weight treated group showed significant results when comparing with the control group (Table 2).

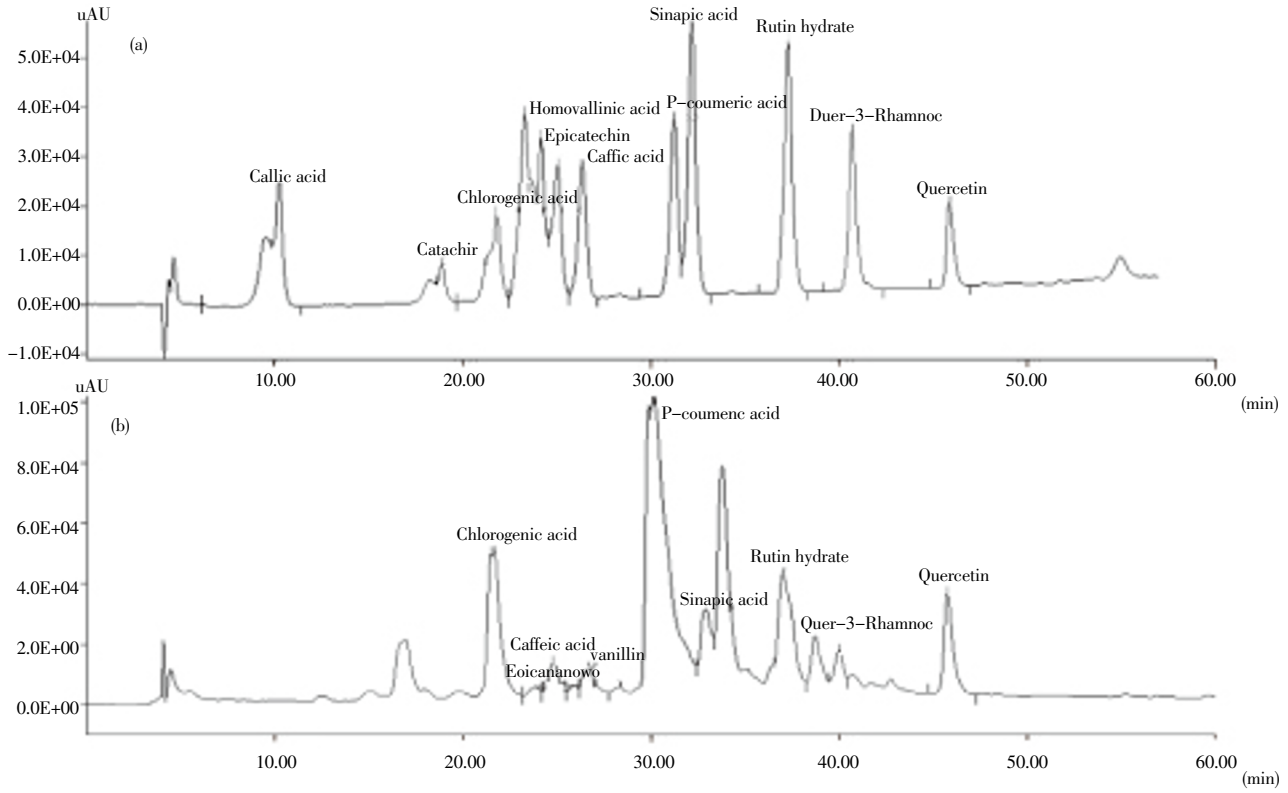

Figure 1. The HPLC analysis of the polyphenols. (a) indicate standard polyphenols and (b) indicate polyphenols of hydro alcohol extract of *L. sativa*.

Table 1

Effect of ethanol extract *L. sativa* extracts on the elevated T maze test for 15 and 30 days duration courses. The plant extracts, diazepam or control, were injected 60 min prior to test.

Groups	Dosage	15 days treatment			30 days treatment		
		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	vehicle	51.68±9.60	41.67±11.80	8.75±5.10	54.33±10.90	44.72±15.30	12.50±5.10
Extract	100mg/kg bwt.	86.32±10.30	45.25±9.70	19.50±4.60	97.97±15.30*	53.02±4.20	20.50±7.40
Extract	200mg/kg bwt.	97.80±10.07*	70.72±13.00*	24.00±6.30*	116.15±7.90*	79.60±14.60*	25.50±5.50*
Extract	400mg/kg bwt.	169.88±12.90**	97.82±15.00*	30.50±8.50*	173.93±12.60**	94.40±21.40*	31.25±8.50*
Diazepam	1mg/kg bwt.	186.97±9.70**	121.45±10.00**	38.50±7.50**	197.02±10.30**	113.82±8.20**	38.50±7.50**

Data are presented as mean values (±SD.) from group of six mice. *$P < 0.05$ and **$P < 0.001$indicates significant difference from control.

Table 2
Effect of *L. sativa* extracts on the hyponeophagia for 15 and 30 days duration courses.

Groups	Dosage	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	vehicle	7.50±3.80	52.13±13.20	23.00±5.20	7.50±3.80	62.75±9.90	27.70±7.20
Extract	100 mg/kg bwt.	11.75±3.50	45.35±14.80	39.40±8.20	14.00±2.30	45.70±17.70	40.90±6.80
Extract	200mg/kg bwt.	14.00±3.50*	19.80±5.10*	53.10±19.02*	14.25±3.10*	25.43±5.60**	58.00±21.70*
Extract	400mg/kg bwt.	17.25±4.50*	18.40±3.00**	54.80±10.02*	20.75±4.03*	19.56±1.30**	62.50±14.60*
Diazepam	1 mg/kg bwt.	20.75±3.30*	12.86±1.60**	62.70±14.60*	25.00±4.20**	15.65±2.30**	72.30±7.80**

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

4. Discussion

Elevated T maze pharmacological studies provided evidence that the open-arm avoidance and escape behaviours are expressions of emotional states akin to the generalized anxiety and panic disorder, respectively[21-25]. Rodents encountering a desirable food in a novel environment will consume very limited quantities after considerable investigation. Mice tend to avoid exploration of novel open environments, yet are motivated to approach and consume palatable food. This inhibition of feeding behavior 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[26]. Number of entries, time spent in rat zone and latency to first entry to food zone was measured. Here the experiments was conducted by using Paneer (cheese), not only this result in the feeding latency being 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. There was a significant improvement in the activity in 30 days treated animals than that of 15 days treated groups. 200 and 400mg/kg bwt treated groups showed significant result when compared to control.

In conclusion the anxiolytic properties of extracts of *L. sativa*, locomotor activity and exploratory behaviour of mice were studied using elevated T maze and hyponeophagia models. The anxiolytic property was higher in the group of mice fed with 400 mg/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-a-vis the drug, diazepam. The Polyphenols present in the extract may possess anxiolytic activity. In view of these results, it is suggested that hydro-alcohol extract of *L. sativa* rich in polyphenols possess potent anxiolytic property.

Conflict of interest statement

We declare that we have no conflict of interest.

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