ORIGINAL ARTICLE

Anxiolytic activity of *Vitex negundo* Linn. in experimental models of anxiety in mice

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The purpose of this study was to characterize the putative anxiolytic-like activity of an ethanolic extract prepared from the roots of *Vitex negundo* (VN) using the elevated plus maze (EPM) and light–dark exploration test in mice. Male mice were either treated orally with the VN extract or the positive control diazepam, respectively, 1 hour before behavioral evaluation. Oral administration of 100 and 200 mg/kg of VN extract significantly ($P > 0.01$) increased the percentage time spent on and the number of entries into the open arms of the EPM. The effect was comparable to that of the benzodiazepine diazepam (2 mg/kg p.o.). In light–dark exploration test, diazepam-treated rats significantly increased the time spent in light arena and decreased the duration of immobility, while VN treated rats also showed a significant ($P >0.01$) increase in the time spent (100 and 200 mg/kg) in light arena. Diazepam and the VN extracts do not produced any overt motor dysfunction. These results indicate that VN is an effective anxiolytic agent. In conclusion, the action of extract upon the anxiety models tested are in accord with the traditional use of VN L. and could be useful in primary medical care.

Key words: *Vitex negundo*, anxiolytic, elevated plus maze, light–dark exploration, locomotor activity

INTRODUCTION

Anxiety is an exaggerated feeling of apprehension, uncertainty, and fear. It is an unpleasant state of tension with an anticipation of imminent danger.[1] It may be regarded as a particular form of behavioral inhibition that occurs in response to environmental events that are novel. Anxiety affects one-eighth of the total population worldwide and has become a very important area of research interest in psychopharmacology during this decade.[2] There are various ways of explaining the mechanisms of action of anti-anxiety agents because of the involvement of many CNS chemical mediators. The effect of most of the anxiolytic agents is to enhance the response to GABA, by facilitating the opening of GABA-activated chloride channels. GABAA receptors were involved in anxiety and their direct activation would have an anxiolytic effect.[3] Anti-anxiety drugs have also been shown to act on limbic system, hypothalamus, and the brain stem reticular system.[4]

Benzodiazepines are still the most frequently used drugs for the treatment of generalized anxiety disorder despite their undesirable side effects such as muscle relaxation, sedation, physical dependence, memory disturbance, and interaction with other drugs.[5] However, the realization that benzodiazepines present a narrow safety margin between the anxiolytic effect and those causing unwanted side effects has prompted many researchers to evaluate new compounds in the hope that other anxiolytic drugs will have less undesirable effects. [6]

In recent years, the development of new anxiolytics has been an area of interest. It has been established that there are lot of secondary plant 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 such as noradrenaline, serotonin, gamma-aminobutyric acid (GABA), and benzodiazepine (BDZ) neurotransmitters' activities.[7-11] Various types of herbal medicines have been used as anxiolytic agents in different parts of the world.[12] Drugs derived from traditional herbs may have possible therapeutic relevance in the treatment of anxiety.[13] Research has been conducted to investigate natural anxiolytic agents in the search for an alternative, more specific, and perhaps cost-free therapy. Various types of herbal medicines have been used as anxiolytics in different parts of the world. The root of the kava plant from the tropical Pacific region, St. John's wort extract from Europe, and the saponin-containing fraction of the leaves of *A. lebbeck* from India are known to have anxiolytic effects.[14-17]

On the basis of these considerations, it was the purpose of this study to characterize the anxiolytic-like activity of an ethanolic extract prepared from the roots of *Vitex negundo* Linn. (VN). VN (Synonyms-Indian Privet; Nirgundi; Bana), family verbenaceae, is a large aromatic shrub

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with bluish purple flowers widely prevalent in the north-western Himalayan region and has been used for various medicinal purposes in the ayurvedic and unani systems of medicine.[8-20] Almost all the parts are employed, but the leaves and the roots are important as drugs. Analgesic and anti-inflammatory actions of VN seeds[21,22] and fruit[23] have been reviewed thoroughly. Hydroalcoholic extract of VN leaves have been reported to posses analgesic and anti-inflammatory action.[24] Petroleum ether extract of VN leaves has shown significant analgesic activity and the anticonvulsant activity against strychnine and leptazole[25] Dried leaves powder of VN showed anti-arthritic activity in rats.[26] Preliminary evaluation of the ethanolic extract revealed that the extract inhibited passive peritoneal anaphylaxis and mast cell degranulation in rats in a dose-related manner.[27-29] Anti-inflammatory and analgesic properties of mature fresh leaves of VN have also been reported.[30] It has been found to posses anticonvulsant activity particularly against pentylenetetrazol (PTZ)-induced convulsions.[31] VN leaf extract may be used orally as an adjuvant therapy along with standard anti-inflammatory agents.[32]

MATERIALS AND METHODS

Plant Material

The roots of VN were collected from the Sangli region during July 2007 and were authenticated by Dr. A. K. Magdum, Willingdon College, Sangli.

Preparation of Extract

The roots were dried and coarsely powdered. The dried powdered material was then exhaustively extracted with 95% ethanol, concentrated under controlled temperature, and was used for the pharmacological investigation.

Drugs

Diazepam ampoule (10 mg/2 ml; Watson Pharmaceuticals, India) was used as reference drugs. Diazepam was diluted to 1.5 mg/10 ml with distilled water. Two different concentrations (100 and 200 mg/kg) of the VN root extract were prepared by dissolving the extracts in distilled water. All solutions were prepared freshly on test days and administered orally (p.o.) in a volume of 0.1 ml/10 g body weight of mice.

Animals

Swiss albino male mice (22–25 g) were used to study the anxiolytic effect. The animals were housed in groups of six mice per cage and maintained at 24°C ± 1°C, with relative humidity of 45-55% and 12:12 hours dark/light cycle. The experiment was carried out between 10:00 and 17:00 h. The animals had free access to food (Standard chew pellets, Chakan Oil Mills, Sangli) and water ad libitum. Food, not water, was withdrawn 3 hours before and during the experiment. The Institutional Animals Ethics Committee approved all the experimental protocols.

Acute Toxicity Studies

VN ethanolic extract at different doses (50-2000 mg/kg) was administered orally to mice. During the first 4 hours after the drug administration, the animals were observed for gross behavorial changes if any for 7 days. The parameters such as hyperactivity, grooming, convulsions, sedation, hypothermia, and mortality were observed and doses selected were 100 mg/kg and 200 mg/kg, body weight. LD50 dose of VN leaf extract was reported to be 7.58 g/kg, body weight in the previous reported study.[32]

Assessment of Anxiolytic Activity

Treatment schedule

The anxiolytic activity of VN was examined using the elevated plus maze (EPM) and the light and dark model in mice. The animals were divided into four groups, consisting of six mice per group. Group 1 received vehicle (distilled water); Groups 2 and 3 received VN 100 and 200 mg/kg, respectively; Group 4 received diazepam 2 mg/kg.

Elevated plus maze

The plus-maze apparatus, consisting of two open arms (16 × 5 cm) and two closed arms (16 × 5 × 12 cm) having an open roof, with the plus maze elevated (25 cm) from the floor used to observe anxiolytic behavior in mice.[33,34] Each mouse was placed at the center of the elevated plus maze with its head facing the open arm. During the 5-min experiment, the behavior of the mouse was recorded as: (i) preference of the mouse for its first entry into the open or closed arms, (ii) the number of entries into the open or closed arms, and (iii) time spent by the mouse in each of the arms. Ethanolic extracts of VN (100 and 200 mg/kg) were administered orally using a tuberculin syringe fitted with oral canula. During the entire experiment, mice were allowed to socialize. Every precaution was taken to ensure that no external stimuli, other than the height of the plus maze could invoke maze anxiety.

Light and dark exploration test

The apparatus consisted of an open top wooden box. Two distinct chambers, a black chamber (20 × 30 × 35 cm) painted black and illuminated with dimmed red light and a bright chamber (30 × 30 × 35 cm) painted white and brightly illuminated with 100-W white light source, were located 17 cm above the box. The two chambers were connected through a small open doorway (7.5 × 5 cm) situated on the floor level at the centre of the partition.[35] Each mouse was placed individually in the center of the light compartment and observed for the next 5 minutes for the number of crossing between two compartments and time spent in the

light and dark compartments. Diazepam dose of 2 mg/kg, i.p. was used as a reference standard.[36,37]

Measurement of locomotor activity

Since the plus maze experiment was affected by changes in locomotor activity, an additional experiment was carried out with the specific aim of monitoring the activity. Separately from the experiment with the elevated plus-maze, spontaneous locomotor activity was measured using activity cage. Each mouse was placed in the activity cage and after an adaptation period of 10 min, the test compound administration protocol was implemented. Diazepam was administered orally 30 minutes prior to the experiment.

VN extract was administered orally 60 minutes prior to the experiment. Ambulatory activity was measured for 10 minutes after oral administration of the agents. Percentage change in motor activity was measured.[38]

Statistical analysis

All data are presented as mean ± SEM and analyzed by one-way ANOVA, followed by Dunnett's test. The groups treated with extracts were compared with the respective vehicle group. The diazepam-treated group was compared with vehicle. *P* values <0.01 were considered statistically significant.

RESULTS

Locomotor Activity

Locomotor activity was significantly decreased by diazepam (2 mg/kg). Locomotor activity was also decreased in animals pretreated with extract of VN (100 and 200 mg/kg) compared with that in the vehicle group. VN inhibited locomotor activity to a lesser extent than diazepam and thus had a better profile for anxiolytic agents.

Elevated Plus Maze

Oral administration of 100 and 200 mg/kg of VN produced a significant (*P* <0.01, ANOVA followed by Dunnet's test) increase in permanence in the open arms of the maze [Table 1], suggesting an anxiolytic effect of this extract. Animals treated with diazepam (2 mg/kg, i.p.) spent more time in the open arms of the maze.

Light and Dark Box

Diazepam-treated rats significantly (*P* <0.01) increased the time spent in light arena and decreased the duration of immobility. VN-treated rats also showed a significant (*P* <0.01) increase in the time spent (100 and 200 mg/kg) in light arena. The test drug reduced the duration of immobility at the highest dose (200 mg/kg). An increase in the number of entries into light chamber was not significant [Table 2].

DISCUSSION

The elevated plus-maze is a well-established animal model for testing anxiolytic drugs.[32,39] Diazepam, a standard anxiolytic used clinically, is also employed in behavioral pharmacology as a reference compound for inducing anxiolytic-like effects, even when the compound being screened does not act via benzodiazepine receptors.[40]

In the present study, we found that the extract of VN increased the percentage of open arm entries and time spent in open arms and thus showed anxiolytic effects in this model. The anxiolytic effects of drugs such as benzodiazepines are accompanied by decreased locomotor activity and sedation.[41] The extract inhibited locomor activity in this experiment. However, VN inhibited locomotor activity to a lesser extent than diazepam, and thus has a better profile for an anxiolytic agent. There is considerable interest in the development of new anxiolytics

Table 1: Effects of diazepam and *Vitex negundo* root extract on behaviour of mice in elevated plus maze paradigm

Treatment	Dose mg/kg	No. of entries (n)		Time spent in open arm (seconds)	Motor activity
		Open arm	Closed arm		
Control	-	2.8 ± 0.3742	15.2 ± 0.5831	28.6 ± 1.939	126.33 ± 2.06
Vitex negundo	100	7.4 ± 0.6782	11.8 ± 0.5831	37.8 ± 1.356**	114.83 ± 2.774**
extract	200	8.2 ± 0.6633	10.4 ± 0.5099	46.8 ± 1.772**	120.0 ± 2.745**
Diazepam	2	7 ± 0.5477	10.6 ± 0.5099	123.2 ± 1.772**	76.5 ± 3.314**

Data are expressed as mean values ± S.E.M (n = 6); ***P* <0.05 compared with the vehicle-treated control (ANOVA followed by Dunnett's '*t*' test).

Table 2: Effects of diazepam and *Vitex negundo* root extract on behavior of mice in light and dark exploration test

Treatment	Dose mg/kg	No. of entries (n) in the light compartment	Time spent in the light compartment (seconds)	Motor activity
Control	-	8.2 ± 0.8602	23 ± 1.304	126.33 ± 2.06
VN extract	100	12.6 ± 0.5099	49.2 ± 1.744**	114.83 ± 2.774**
	200	15.8 ± 0.8000	52.6 ± 1.749**	120.0 ± 2.745**
Diazepam	2	14.6 ± 0.7486	117.2 ± 1.934**	76.5 ± 3.314**

Data are expressed as mean values ± S.E.M (n = 6); ***P*<0.05 compared with the vehicle-treated control (ANOVA followed by Dunnett's '*t*' test)

that do not induce sedative effects and do not inhibit locomotion.

The effect of most of the anxiolytic agents is to enhance the response to GABA, by facilitating the opening of GABA-activated chloride channels. $GABA_A$ receptors were involved in anxiety and their direct activation would have an anxiolytic effect.[3] It is well documented that pentylenetetrazole-induced convulsions are produced due to diminution of GABA level in brain.[42,43] A recent study showed that VN possesses anticonvulsant activity particularly against pentylenetetrazole-induced convulsion.[31] Therefore, it is likely that VN might possibly be producing anticonvulsant action by increasing the level of GABA, an inhibitory neurotransmitter in the central nervous system. This is in accord with the pharmacological effects of benzodiazepine and highlights the relevance of the putative anxiolytic effect of VN.

In conclusion, the action of extract upon the anxiety models tested are in accord with the traditional use of VN L. and could be useful in primary medical care. In the same way, identification of compound(s) responsible for biological activity could be used as prototype(s) to design new substances with anxiolytic activity. Although further major active components and precise anxiolytic mechanisms need to be identified.

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