



EVALUATION OF ANTIANXIETY AND ANTIDEPRESSANT ACTIVITY OF *CESTRUM NOCTURNUM* LEAVES

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Abstract:

The present study was designed to evaluate the antianxiety and antidepressant activity of the ethanolic and aqueous extracts of *Cestrum nocturnum* leaves in rodents. Antianxiety activity was tested by exposing rats to unfamiliar aversion in different methods like elevated plus maze model and actophotometer. The results infer that reduced aversion fear elicits antianxiety activity. The antidepressant activity was tested by using despair swim test and tail suspension test. The results infer that reduced immobility time elicits antidepressant activity. It was concluded that ethanolic and aqueous extracts of *Cestrum nocturnum* leaves having antianxiety and antidepressant activity. Ethanolic extract of *Cestrum nocturnum* leaves showing more significant activity over the aqueous extract.

Keywords: *Cestrum nocturnum*, Antianxiety activity, Antidepressant activity, Elevated plus maze, Actophotometer, Despair swim test.

Introduction:

According to the World Health report [1], approximately 450 million people suffer from mental or behavioral disorders, yet only a small minority of them receives even the most basic treatment. This amounts to 12.3% of the global burden of disease and will rise to 15% by 2020 [2]. In the search for 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 [3]. Anxiety and depression are extremely dramatic and debilitating multifaceted disorders and it is now becoming clear that without knowledge of clinical and biological aspects of anxiety and depression, it is impossible to offer effective treatment strategies for the patients. Over the past decades, there has been intensive study of a variety of neurobiological aspects of depression and anxiety. Currently the most widely prescribed medications for anxiety disorders are benzodiazepines. But the clinical applications of benzodiazepines as antianxiety agents are limited by their unwanted side effects. Therefore the development of new pharmacological agents from plant sources are well justified.

The genus *Cestrum* (Solanaceae) with more than 300 species is globally distributed in tropical and subtropical regions throughout the world including India, southern China, Australia, USA and Bangladesh [4]. Several applications of *Cestrum* have been documented and the toxicity of the species to humans and livestock has been reported [5, 6]. The leaves of *Cestrum* have shown significant analgesic and

bactericidal activity. Also, inhibitory effect on the central nerve system and cardiac arrhythmic effect of *Cestrum* is documented and also used for the treatment of burns and swellings [7]. The oil of these species is known to be mosquito repellent and hence *C. nocturnum* and *C. diurnum* are used to prevent malaria in African Nations [8]. The study with plant extracts of *Cestrum diurnum* showed promising activity to combat against larvae of *Culex quinquefasciatus* (Diptera: Culicidae) and *Anopheles stephensi* [9]. In this study *C. aurantiacum*, *C. nocturnum* and *C. diurnum* were investigated for phytochemicals and determination of their antioxidant and antimicrobial potential.

Materials and Methods:

Plant material

Collection and authentication of plant materials

The leaves of *Cestrum nocturnum* belonging to the family Solanaceae were collected in the month of April 2014 from the local areas of Gondia district, Maharashtra, India. The plant material was identified and authenticated by Department of Botany, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur was preserved Department of Botany, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur, Maharashtra, India.

Processing of sample

Leaves were dried in shade for 25 days and then powdered to get a coarse powder. This powder was stored in an air tight container and used for successive extraction.

Preparation of extracts

Preparation of ethanolic extract of *Cestrum nocturnum* leaves

The leaves of *Cestrum nocturnum* were shade dried and reduced to coarse powder. The standardized coarse powder was evenly packed in the Soxhlet apparatus and subjected to defatting with petroleum ether and ethanol extraction. The extract was filtered and filtrate was concentrated by vacuum distillation. Percentage yield of ethanolic extract was found to be 13.8%.

Preparation of aqueous extract of *Cestrum nocturnum* leaves

The leaves of *Cestrum nocturnum* were powdered and extracted by maceration process by using 300ml of distilled water. In maceration procedure, total amount of 50g of powdered leaves were macerated for 3 days it was occasionally stirred at regular intervals of time. It was filtered and concentrated. Then it was dried by rotary evaporator. The percentage yield of aqueous extract was found to be 11.3%.

Phytochemical analysis

Both the ethanolic and aqueous extracts of *Cestrum nocturnum* were subjected to preliminary phytochemical screening [10].

Experimental animals

Wistar rats (150-200 g) and Swiss albino mice (18-22g) of either sex were procured from Laboratory Animal Centre, Department of Pharmaceutical Sciences, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur and acclimatized at the animal house of Sharad Pawar College of Pharmacy, Wanadongri, Hingna Road, Nagpur. All the animals were maintained under standard conditions, that is room temperature $26 \pm 1^\circ\text{C}$, relative humidity 45 - 55% and 12:12 h light – dark cycle.

Acute toxicity studies

Swiss albino mice of either sex (18-22 g weight) were used for acute oral toxicity study. The study was carried out as per the guidelines set by OECD and animals were observed for mortality and behavioural changes [11].

Ethical approval

The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) of Sharad Pawar College of Pharmacy, Wanadongri, Hingna Road, Nagpur. and all the experiments were conducted according to the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).

Drugs and chemicals

Fluoxetine (Crescent Therapeutics limited, Himachal Pradesh), Diazepam (Ranbaxy Laboratory limited.), Ethanol (Loba Chemicals Mumbai.).

Antianxiety and antidepressant activity

The ethanolic and aqueous extracts of *Cestrum nocturnum* leaves were tested for antianxiety activity using elevated plus maze and actophotometer and antidepressant activity using despair swim test and tail suspension test.

Treatment

Animals were divided into four (I-IV) groups, each group containing six animals

Group I - Control group received distilled water (1ml, p.o).

Group II - Standard group received Diazepam (5mg/kg i.p).

Standard group received Fluoxetine (10mg/kg i.p).

Group III - Test group received ethanolic extract of *Cestrum nocturnum* (500mg/kg p.o).

Group IV - Test group received aqueous extract of *Cestrum nocturnum* (500mg/kg p.o).

ANTI-ANXIETY ACTIVITY

Elevated plus maze (EPM) model

The apparatus comprises of two open arms (35x5cm) and two closed arms (30x5x15cm) that extend from a common central platform (5x5cm). The floor and walls of the closed arms are made of wood and painted black. The entire maze is elevated to a height of 50 cm above the ground level. Rats weighing (150 – 200gms) were housed in a pair of 10 days prior to the test in the apparatus. During this time the rats were handled by the investigator on alternate days to reduce stress. 30 min and 60min after oral administration of the drug treatment, each rat was placed in the center of the maze facing one of the enclosed arms. During five minutes session, number of entries into open arm and time spent in the open arm were noted [12,13]. The procedure was conducted preferably in a sound attenuated environment.

Locomotor activity

The locomotor activity can be easily studied with the help of actophotometer, the rats were grouped and treated with drugs. Each animal was placed individually in actophotometer and the basal activity score of all the animals were recorded for 10 mins after 30 and 60 min of drug treatment [14].

ANTIDEPRESSANT ACTIVITY

Despair Swim Test Apparatus

For the determination of antidepressant activity, forced swim test (FST) protocol was employed. During the test, animals were individually placed in a glass cylinder (20 cm in height, 14 cm in diameter) filled with water up to a height of 10 cm, at $25 \pm 2^\circ\text{C}$. All animals were forced to swim for 5 min and the duration of immobility was observed and measured during the 5 min

interval of the test. Immobility period was regarded as the time spent by the rats to float in water with no struggle and making only those movements necessary to keep its head above the water. In order to check the fitness level of each test animal, a pre-test was carried out 24 h before the FST by subjecting each test animal to a session of 15 min swimming.

Tail suspension test

Tail suspension test was performed based on the method prescribed [15]. The mice were suspended 58cm above the floor by means of an adhesive tape, placed approximately 1cm from the tip of the tail. The total duration of immobility was quantified during a test period of 5min. Mice were considered immobile when they were completely remain motionless.

Results and Discussion:

Acute toxicity

The acute toxicity study revealed the non toxic nature of all the extracts even at a higher dose of 4 g/kg body weight of mice for oral route of administration. For the present study the dose is being selected as 500mg/kg p.o.

Phytochemical analysis

After subjecting to screening, both the ethanolic and aqueous extracts of leaves of *Cestrum nocturnum* revealed the presence of alkaloids, flavonoids, glycosides, tannins and saponins.

Assessment of antianxiety activity

Elevated plus-maze model

In elevated plus-maze test (EPM), the ethanolic and aqueous extracts of *Cestrum nocturnum* leaves at a dose of 500 mg/kg p.o. significantly increased the number of entries and time spent into the open arm. The magnitude of the antianxiety effects 500mg/kg p.o. of ethanolic and aqueous extracts of *Cestrum nocturnum* was comparable to that of diazepam 5 mg/kg i.p. (Figure 1 and 2).

Actophotometer

The average of basal activity scores in the control group after 30 and 60min of administration of ethanolic and aqueous extracts of *Cestrum nocturnum* leaves 500 mg/kg p.o. significantly reduced the locomotor activity. It may be due to the CNS depressant property of the drug. (Table 1)

Assessment of antidepressant activity

Despair swim test apparatus

Table 1: Effect of Ethanolic and Aqueous Extracts of *Cestrum nocturnum* Leaves on Locomotor Activity (Actophotometer) In Rats At Different Time Intervals.

Group	Treatment	Photo cell count		% change in activity	
		30 min	60 min	30 min	60 min
I	Control (Vehicle)	306±5.032	307.1±4.132	NA	NA
II	Diazepam	119±1.581*	84.67±2.789*	61.1	72.45
III	Ethanolic extract	145.7±4.910*	97.33±1.856*	52.4	68.35
IV	Aqueous Extract	167.3±2.404*	118.3±1.453*	45.32	61.53

In despair swim test apparatus, the ethanolic and aqueous extracts of leaves of *Cestrum nocturnum* at a dose of 500 mg/kg p.o. significantly decreased the immobility time. The magnitude of the antidepressant effects of 500 mg/kg p.o. of ethanolic and aqueous extracts of leaves of *Cestrum nocturnum* was comparable to that of fluoxetine 10 mg/kg i.p. (Table 2).

Tail suspension test

In tail suspension test, the ethanolic and aqueous extracts of leaves of *Cestrum nocturnum* at a dose of 500 mg/kg p.o. significantly decreased the immobility time. The magnitude of the antidepressant effects of 500 mg/kg p.o. of ethanolic and aqueous leaves of *Cestrum nocturnum* was comparable to that of fluoxetine 10 mg/kg i.p. (Table 3).

The fear due to height induces anxiety in the animals when placed on the EPM. The ultimate manifestation of anxiety and fear in the animals is exhibited by decrease in the motor activity and preference to remain at safer places. Antianxiety agents are expected to increase the motor activity, which is measured by the time spent by the animal in the open arms. The spontaneous decrease in basal activity score implicates the reduced anxiety recorded using actophotometer. The widespread use of FST is mainly due to its ability to detect a broad spectrum of antidepressant agents. The test is based on the observation that rodents following initial escape-oriented movements develop an immobile posture when placed inside an inescapable cylinder filled with water. The immobility is thought to reflect either a failure of persistence in escape-directed behavior (i.e., despair behavior) or the development of a passive behavior, meaning the loss of the animal's ability to cope with stressful stimuli. Markedly showed a significant decrease in the time spent immobile by rodents. By performing tail suspension test, the reduced immobility time directed the antidepressant effect. The antianxiety and antidepressant effects may be due to the flavonoid [16] content present in both the extracts of *Cestrum nocturnum*. However, further studies are required to identify the phytoconstituents responsible for the observed antianxiety and anti-depressant effect.

NA-Not Applicable, *p<0.001 when compared to control

Table 2: Effect of Ethanolic and Aqueous Extracts of *Cestrum nocturnum* Leaves Despair Swim Test In Rats At Different Time Intervals.

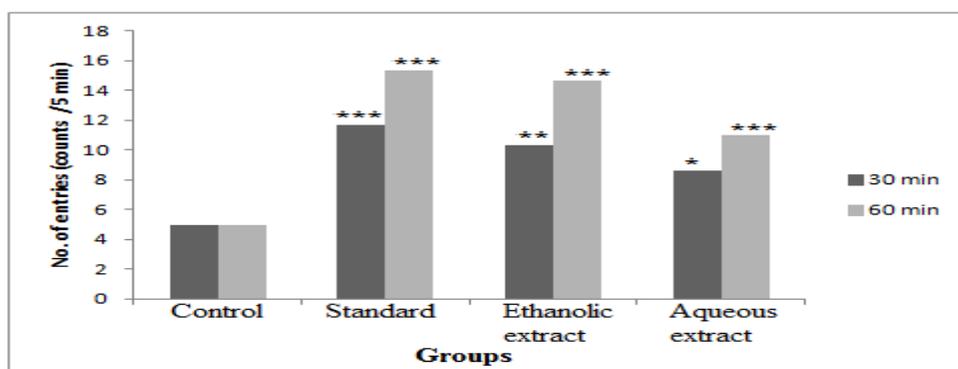
Group	Treatment	Photo cell count (sec/5min)		% change in activity	
		30 min	60 min	30 min	60 min
I	Control (Vehicle)	186.7±4.410	188±4.583	NA	NA
II	Fluoxetine	88.33±1.453*	56.33±1.453*	52.6	70.0
III	Ethanolic extract	98.67±2.028*	68±1.856*	47.15	63.8
IV	Aqueous Extract	111.7±3.338*	83.33±2.404*	55.69	55.69

NA-Not Applicable, *p<0.001 when compared to control

Table 3: Effect of Ethanolic and Aqueous Extracts of *Cestrum nocturnum* Leaves Tail Suspension Test In Rats At Different Time Intervals.

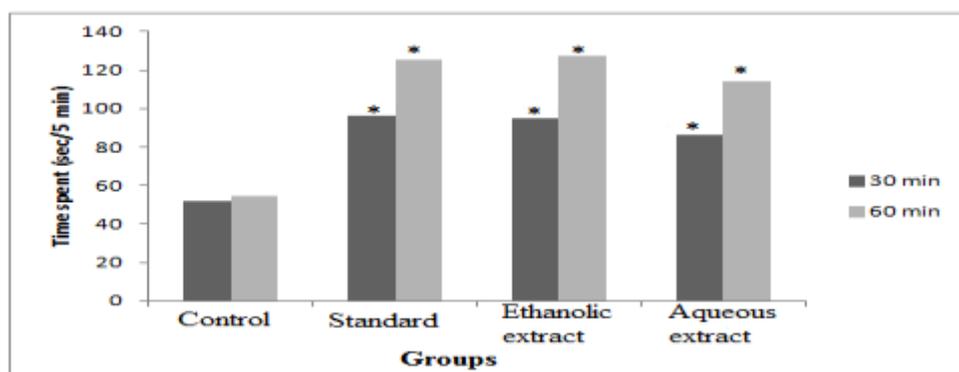
Group	Treatment	Photo cell count (sec/5min)		% change in activity	
		30 min	60 min	30 min	60 min
I	Control (Vehicle)	105.7±3.480	107.3±3.528	NA	NA
II	Fluoxetine	38.3±1.453*	36.6±1.202*	63.73	68.89
III	Ethanolic extract	35.33±1.764*	27.33±2.906*	66.57	74.55
IV	Aqueous Extract	58.67±1.764*	44.33±2.33*	44.50	58.99

NA-Not Applicable, *p<0.001 when compared to control



***p<0.001, **p<0.01 *p<0.1 when compared to Control

Fig.1: Effect of ethanolic and aqueous extracts of *Cestrum nocturnum* leaves on number of entries (open arm) in elevated plus maze.



*p<0.001 when compared to Control

Fig . 2 :Effect of ethanolic and aqueous extracts of *Cestrum nocturnum* leaves on time spent (open arm) in elevated plus maze

Conclusion:

From the results it was concluded that both ethanolic and aqueous extracts of leaves of *Cestrum nocturnum* showed antianxiety and anti-depressant activity. These findings suggest that the ethanolic extract of *Cestrum nocturnum* leaves posses more significant antianxiety and

anti-depressant activity compared to aqueous extract.

Statistical analysis

The results were expressed as mean ± S.E.M. The differences were compared using one way analysis of variance (ANOVA) and subsequently followed by Bonferroni's test.

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