Anxiolytic activity of *Ziziphus mauritiana* Lam. leaves

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ABSTRACT

The present study was designed to evaluate the anxiolytic effects of ethanolic extract of leaves of Ziziphus mauritiana Lam (ZME). It contains flavonoids, saponins, alkaloids and tannins as major chemical constituents. The experimental model used was elevated plus maze and light and dark box paradigm. The effect of extract on neurotoxicity was studied using rota-rod apparatus. The result of the study showed that treatment with diazepam and ZME significantly (p < 0.05) increased the time spent and the number of entries in open arms and decreased the time spent in closed arms compare to normal control in elevated plus maze. On the light and dark box paradigm the treatments with diazepam and ZME significantly (p < 0.05) increased the time spent in bright arena, number of entries in bright arena and decreased the time spent in the dark arena. The extract shows absence of neurotoxicity on rota-rod. It is concluded that the ethanolic extract of Ziziphus mauritiana Lam. leaves possesses anxiolytic effects.

Key words: Anxiety, elevated plus maze, light and dark box paradigm, *Ziziphus mauritiana*.

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INTRODUCTION

The complexity of daily life in modern society frequently leads to varying degree of anxiety [1]. Human anxiety is a feeling of apprehension, uncertainty or tension stemming from the anticipation of imaginary or unreal threat. Anxiety is generally caused by a number of reasons important one is chronic medical conditions and stress in the daily life. Anxiety affects one-eighth population worldwide and has become an important research area in the field of psychopharmacology [2].

Currently, the most widely prescribed medications for anxiety disorders are the benzodiazepines (BZD). However, the clinical uses of benzodiazepines are limited by their side effects such as psychomotor impairment, potentiating of other central depressant drugs and dependence liability [1].

Ziziphus mauritiana Lam. plant is widely used in folk medicine. The various parts of *Z. mauritiana* Lam. like leaves, fruits and seeds also used in folk medicine for various purposes. The *Z. mauritiana* Lam. leaves contains flavonoids, saponins, alkaloids and tannins as major chemical constituents [3, 10]. Hence the present study was designed to evaluate the anxiolytic activity of *Ziziphus mauritiana* Lam. leaves.

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MATERIALS AND METHODS

Plant material
The fresh leaves *of Zizyphus mauritiana* were collected from Aurangabad, Maharashtra, India and air-dried in shade at room temperature. The plant was identified and authenticated at the Herbarium of Botany Department Dr. Babasaheb Ambedkar Marathwada University, Aurangabad. The dried leaves were powdered mechanically and kept separately in airtight container till the time of use.

Preparation of extract
The powdered leaves were defatted with petroleum ether (60-80^{0}C) in soxhlet's extractor. The mark was dried and extracted with ethanol. The ethanolic extract was evaporated to dryness in vacuum. The mass was stored in a refrigerator and considered as the extract (ZME).

Experimental animals
Albino rats (Wistar), weighing between 180-230 g. of either sex were used in this study. The animals were allowed to acclimatize to laboratory condition for 10 days after their arrival. The animals were housed into group of six under standard housing conditions. The animals were fed with standard animal feed and allowed water *ad libitum*.

All the procedures were performed in accordance with the Institutional Animal Ethics Committee (IAEC) constituted as per the direction of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), under Ministry of Animal Welfare Division, Government of India, New Delhi, India. (CPCSEA/IAEC/P'col/19/43)

Experimental Design
Acute toxicity
Acute toxicity study was carried out as per the Organization for Economic Co-operation and Development (OECD) guideline 423. ZME were administered orally in doses of 300, 1000 and 2000 mg kg $^{-1}$body weight to groups of female rats (n=3). Animal were observed individually after dosing at least once during the first 30 min, periodically during the first 24 h, with special attention given during the first 4h, and daily thereafter, for total of 14 days. Percentage mortality and toxic reactions were noted [6].

Elevated plus maze
The wooden maze consisting of two open arms (length 50 cm X breadth 10 cm) and closed arms of the same size (Height 40 cm). The arms of the same type were opposite to each other, with a central square of 10 cm. The maze was elevated to a height 50 cm above the floor. Rats were placed individually in the center of the plus maze facing an enclosed arm. The time spent by rat during the next 5 min. on the open arm and closed arms was recorded along with the number of entries into the open and closed arms [5, 7].

Light and dark box
The apparatus consist of an open top wooden box. Two distinct chambers, a black chamber (20 X 30 X 35 cm) painted black and illuminated with dimmed red light and a bright chamber (30 X 30 X 35 cm) painted white and brightly illuminated with 100 W white light sources, were located 17 cm above the box. The two chambers were connected through a small open doorway (7.5 X 5 cm) situated on the floor level at the center of the partition [7-8].

Following the elevated plus maze test, the animal was placed at the center of the brightly lit arena in the light and dark box. The number of entries into and the time spent in the bright and dark arena were noted.

Neurotoxicity
Neurotoxicity was studied using Rota-rod test. A knurled rod was rotated at a speed of 15rpm. All the rats used were trained to remain on the rotating rod for 5 min. After the drug treatment the rats again placed on rotating rod. The neurological deficit was indicated by inability of the rat to maintain equilibrium for 3 min in each of three trials as described earlier, Dunham and Miya, 1957 [4]. ZME was administered in doses of 50, 100 or 200 mg/kg.

Statistics
The observations are given as means ± S.E.M. The data was analyzed by student's *t* test, and compared with standard treatment group. P < 0.05 was considered significant.

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RESULTS

Acute toxicity study

The rats treated with ZME 300, 1000 and 2000 mg kg $^{-1}$ orally exhibited normal behavior, i.e. they were alert, with normal grooming, touch response and pain response. There was no sign of passivity and secretary signs were normal. No mortality was reported for any dose.

Elevated plus maze:

Time spent by normal control rats in open arms was 33.50 ± 4.88 sec.; and in close arms was 253.33 ± 5.98 sec. Number of entries in open and close arms was 3.33 ± 0.61 and 4.17 ± 0.30 respectively. Treatment with diazepam and ZME significantly ($p < 0.05$) increased the time spent in open arms and decreased the time spent in closed arms compare to normal control. Significant increase in the number of entries in open arms was also reported.

Table 1: Effect of Extracts on behavior of rats in Elevated plus maze

Treatment	Time spent (s)		Number of entries	
	Open arm	Close arm	Open arm	Close arm
Normal Control	33.50 ± 4.88	253.33 ± 5.98	3.33 ± 0.61	4.17 ± 0.30
Diazepam (1mg kg $^{-1}$)	$58.17 \pm 5.61^*$	$223.00 \pm 6.07^*$	$6.00 \pm 0.52^*$	$3.17 \pm 0.60^*$
ZME (50 mg kg $^{-1}$)	$45.33 \pm 6.78^*$	$224.50 \pm 8.30^*$	3.17 ± 0.48	4.17 ± 0.87
ZME (100 mg kg $^{-1}$)	$52.67 \pm 9.32^*$	$209.00 \pm 8.30^*$	5.67 ± 0.80	4.33 ± 0.76
ZME (200 mg kg $^{-1}$)	$62.50 \pm 9.96^*$	$208.83 \pm 11.79^*$	$5.17 \pm 1.08^*$	3.67 ± 0.56

n=6, $^*p< 0.05$vs. Normal control (student's t test)

Light and dark box:

Normal control rats spent 18.76 ± 3.98 sec. in bright arena and 278.17 ± 4.17 sec. in dark arena. Number of entries noted in the bright arena was 3.00 ± 0.52. Diazepam significantly ($p < 0.05$) increased the time spent in bright arena i.e. 86.67 ± 7.52 sec and 6.00 ± 0.73 number of entries. There was significant ($p < 0.05$) decrease in the time spent in dark arena by the diazepam treatment. All the treatments with ZME also significantly ($p < 0.05$) increase the time spent in bright arena, number of entries in bright arena and decreased the time spent into the dark arena.

Table 2: Effect of Extracts on behavior of rats in bright and dark arena paradigm

Treatment Dark	Time spent in chamber (s)	No. of entries in	
		Bright	bright chamber
Normal Control	278.17 ± 4.17	18.67 ± 3.98	3.00 ± 0.52
Diazepam (0.5 mg kg $^{-1}$)	$209.17 \pm 7.59^*$	$86.67 \pm 7.52^*$	$6.00 \pm 0.73^*$
ZME (50 mg kg $^{-1}$)	263.83 ± 5.64	$30.67 \pm 4.02^*$	3.17 ± 0.48
ZME (100 mg kg $^{-1}$)	$220.17 \pm 6.05^*$	$77.17 \pm 4.41^*$	$6.17 \pm 0.90^*$
ZME (200 mg kg $^{-1}$)	$214.33 \pm 4.65^*$	$82.00 \pm 4.65^*$	$10.83 \pm 0.75^*$

n=6, $^*p<0.05$vs. respective control (student's t test)

Neurotoxicity

The rats treated with doses of ZME (50, 100 and 200 mg/kg) were able to maintain equilibrium on the rota-rod apparatus for complete duration of 5 min.

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DISCUSSION

The present study was designed to evaluate anxiolytic effects of *Ziziphus mauritiana* Lam. leaves extracts in rats.

The two experimental model of anxiety, elevated plus maze and bright and dark arena, are based on the assumption that unfamiliar, non-protective and brightly lit environmental stress provokes inhibition of normal behavior. This normal behavioral inhibition is further augmented in the presence of fear of anxiety like state.

The elevated plus-maze (EPM) is considered as one of the valid ethological animal models of anxiety since it employs natural stimuli (fear of a novel, brightly lit open space and fear of balance of a relatively narrow and raised platform) capable of inducing anxiety in humans. This test has been described as bi-directionally sensitive to both anxiolytic drugs, particularly benzodiazepines and anxiogenic agents used in humans [3]. The number of entries in open and close arm reflects the safety of closed arms with relative fearfulness of open arms. The reduction in entry, time spent and increased defection is indications of high level of fear or anxiety. Anxiolytic drugs increases the number of entries and time spent in open arms [7, 9].

The treatment with diazepam and ZME significantly increased the time spent and number of entries into open arms and reduced the time spent in enclosed arms.

 In the light and dark box paradigm, the brightly lit environment is a noxious environment stressor that inhibits the exploratory behavior of rodents. Reduction in number of entries, time spent and rearing behavior in the light chamber is regarded as markers of anxiety [7, 9]. The result shows that treatment with diazepam and ZME significantly increased the time spent in bright arena and reduced the time spent in dark arena. The treatment groups also showed increase in the number of entries in the bright arena.

The result of both the experimental models proved the anxiolytic effects of ethanolic extract of *Ziziphus mauritiana* Lam. leaves.

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