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Nootropic activity of *n*-butanolic fraction of methanolic extract of leaves of *Ziziphus mauritiana* Lam. in mice

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ABSTRACT

In the present study, we investigated the effects of n-butanolic fraction of methanolic extract of leaves of Ziziphus mauritiana, Lam (BZM 10, 25 and 50 mg/kg) on learning and memory in mice using Elevated Plus Maze, Passive avoidance Paradigm and Object recognition test. Piracetam was used as standard drug. The acute toxicity study shows no mortality up to BZM 2000 mg/kg. Decrease in the transfer latency reported with the treatment of BZM on the elevated plus maze after 24 hrs. and on day-7 and it expressed as inflexion ratio (IR). Significant increase in step down latency was observed on passive avoidance paradigm. The object recognition test report increase in Recognition Index (RI) indicates its nootropic effect. No neurotoxicity observed with all dosage using rota-rod method. BZM 25 mg/kg also antagonises the amnesia produced by scopolamine and hence indicates involvement of central cholinergic mechanism in its effect. Hence, the present study proved nootropic effect of n-butanolic fraction of methanolic extract of leaves of Ziziphus mauritiana.

Key words Nootropic, Learning and memory, *Ziziphus mauritiana*, elevated plus maze.

INTRODUCTION

In recent years, there has been a phenomenal rise in the interest of scientific community to explore the pharmacological actions or to confirm the veracity of claims made about herbs in official book of Ayurveda [1]. Indian systems of medicine emphasize use of herbs, neutraceuticals or life style changes for controlling age related neurodegenerative disorders [2]. Various neurodegenerative disorder including Alzheimer's, Parkinson's and Huntington's diseases are reported to be associated with dementia [3]. Nootropics are agents that enhance the cognitive skills [2]. Indian system of medicine is replete with medicinal plants claimed to promote learning, memory and intelligence (Nootropics). Plants like *Bacopa monniera, Azadirachta indica, Withania somnifera, Hypericum perforatum, Albizzia lebbeck, Vitis vinifera, Panax ginseng* as well as *Ocimum sanctum* [4] have been investigated for their effect on cognitive functions [5]. Saponins from *B. monniera, P. ginseng* and *A. lebbeck* are active principles responsible for enhancing cognitive behavior in experimental animals [1]. Since the leaves of *Ziziphus mauritiana*, Lam (Rhamnaceae) shows a rich presence of saponins, we investigated its nootropic activity [6]. Several existing models for evaluation of learning and memory are based on positive and negative reinforcement behavior. However, recently it have been reported that Elevated Plus Maze (EPM) introduced for measurement of anxiety in rodents, could be used for evaluation of learning and memory in mice, although this method is not based on positive and negative reinforces [7]. Testing animals for avoidance behavior, active and passive, is a classic model based on negative reinforcement for assessment of cognitive performance [3]. The object recognition test is a simple and quick method to test short term memory in rodents [8].

In the present study, we investigated the effects of n-butanolic fraction of Methanolic extract of leaves of *Ziziphus mauritiana*, Lam (Rhamnaceae) on learning and memory in mice using Elevated Plus Maze, Passive avoidance Paradigm and Object recognition test.

MATERIALS AND METHODS

Plant material

The leaves of *Ziziphus mauritiana* were collected from the plants in local area near Aurangabad, Maharashtra, India. Sample was authenticated at the Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad (voucher specimen no. 5640).

Preparation of extract

The leaves were dried under shade and powdered by using grinder mixer. The powdered material was socked in Petroleum ether (60 – 80^{0C}) to remove lipids, filtered it and filtrate was discarded, residue extracted with 95% methanol by soxhlet for 72hr. After extraction the solvent should filter and evaporated in a vacuum, whatever residue may be obtain is dissolved in distilled water and extracted with n-butanol by separating funnel. The filtrate obtained was evaporated to obtain solid dry mass of n-butanolic fraction of methanolic extct of leaves of *Ziziphus mauritiana* (BZM) [9, 10].

Experimental animal

Swiss albino mice of either sex weighing between (20-25 g) were used. They were maintained at temp. of 25 ± 2˚C and relative humidity of 45 to 55% and under standard environmental conditions (12 h. light /12 h. dark cycles). The animals had free access to animal food and water. The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC) of Y.B. Chavan College of Pharmacy Aurangabad (Approval number-CPCSEA/IAEC/P'COL-17/2011-12/41), 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.

Experimental Design

Acute toxicity test

The Acute Toxicity of BZM was performed as per OECD guideline no. 425 for toxicity studies, in the albino mice of either sex (20-25g) maintained under standard dietary conditions. The animals were fasted for 3hr before experiment. Animals were administered with single dose of BZM. Maximum dose of BZM administered was 2000 mg/kg.

Neurotoxicity

In this test, a knurled rod (2.54 cm in diameter) was rotated at a speed of 15 rpm. All animals were trained to remain on the rotating rod for 5 min. A normal mouse could maintain its equilibrium for long periods. In a drug-treated mouse, the neurological deficit was indicated by inability of the mouse to maintain equilibrium for 3 min in each of three trials as described earlier, Dunham and Miya, 1957. BF was administered in doses of 10, 25, or 50 mg/kg, and the animals were tested for neurological deficit [11, 12].

Elevated Plus Maze

The EPM consisting of two open arms (35x6 cm) and two enclosed arms (35x6x15 cm) was elevated to the height of 25 cm. Mice were placed individually at the end of an open arm facing away from the central platform, and the time it took to move from the end to either of the closed arms (transfer latency, TL) was noted [13]. On the first day, mice (n = 5) were allowed to explore the maze for 5 min after the measurement of TL. On the following day, mice received vehicle, piracetam (100 mg/kg ip), scopolamine (0.3mg/kg ip) 30 min before or BZM (10, 25 and 50 mg/kg p.o.) 90 min before the test, and the TL was noted for each animal. The TL was also measured on the seventh day. The TL was expressed as inflexion ratio (IR) using the formula:

$$IR = (L_1 - L_0) / L_0$$

where L_0 = TL after 24 h or on the seventh day and L_1 = initial TL (s) [14, 15].

Passive Avoidance Paradigm

The step-down type of passive avoidance task is based on negative reinforcement. The apparatus consists of transparent acrylic cage (30 x 30 x 40 cm high) with a grid floor, inserted in a semi-soundproof outer box (35 x 35 x 90 cm). The cage was illuminated with a 15W lamp during the experimental period. Electric shock (1Hz, 500 msec, 40V DC) was delivered for 15 sec. Step Down Latency (SDL) was measured. Animals showing SDL of 3-30 sec

during the first training session was preselected for second and retention trials. The second session was carried out 60-90 min after the first. The retention task was carried out 24hr after training. Each mouse was again placed on the platform, and the SDL is recorded with an upper cut-off time of 15min [3, 16].

Object recognition test

A plastic chamber (35cm×35cm×35 cm) was used in low light condition (about 40 lx) during the light phase of the light/dark cycle. The general procedure, as described elsewhere, consisted of three different phases: a habituation phase, an acquisition phase, and a retention phase. On the 1st day (habituation phase), mice were individually subjected to a single familiarization session of 10 min, during which they were introduced in the empty arena, in order to become familiar with the apparatus. On the 2nd day (acquisition phase) animals were subjected to a single 10-min session, during which floor-fixed two objects (A and B) were placed in a symmetric position in the central line of the arena, 10cm from each and 8 cm from the nearest wall (each object occupies approximately 5 cmspace by its size). The two objects, made of the same wooden material with the similar color and smell, were different in shape but identical in size. Mice were allowed to explore the objects in the open field. The exploration time on each object was shown (as seconds) to indicate the exploring activity of mice. On the 3rd day (retention phase), mice were allowed to explore the open field in the presence of two objects: the familiar object A and a novel object C in different shapes but in similar color and size (A and C). A recognition index (for retention session), calculated for each mouse, was expressed as the ratio (TC×100)/(TA + TC), where TA and TC are the time spent during retention phase on object A and object C, respectively. The time spent exploring any object (nose pointing toward the object at a distance≤1 cm, but not mounting on the object or playing with the object) was recorded (using stopwatch) by hand [17].

Statistics

The observations are given as means ± S.E.M. The data was analyzed by one-way ANOVA followed by Dunett's test, $P < .05$ was considered significant.

RESULTS

Acute Toxicity

Animals treated with n-butanol fraction of *Z. mauritiana* (BZM) were free of any toxicity as per acceptable range given by the OECD guidelines no. 425 and no mortality was found up to 2000 mg/kg. Hence three doses 10, 25 and 50 mg/kg were selected for present study.

Neurotoxicity

Mice treated with doses of BF (10, 25 and 50 mg/kg) were able to maintain equilibrium on the rota-rod apparatus for complete duration of 5 min.

Behavioral study

Elevated plus maze test

The transfer latency on the elevated plus maze was expressed as inflexion ratio (IR). Control mice exhibited IR of 1.841±0.657 after 24 hrs and 1.537±0.417 on day-7. Scopolamine (0.3mg/kg) showed significant ($p < 0.05$) decrease in the IR to 0.076±0.030 after 24 hrs and -0.352±0.0564 on the day-7. The IR was significantly ($p < 0.05$) increased by Piracetam (100 mg/kg) to 3.430±0.396 after 24 hrs and ($p < 0.01$) 3.986±0.727 on day-7. It also exhibited significant ($p < 0.05$) antagonism of the effect of Scopolamine after 24 hrs and on the day-7. The treatment with BZM in doses 10, 25 and 50 mg/kg increases IR to 1.313±0.099, 1.899±0.262 ($p < 0.01$) and 1.977±0.4 ($p < 0.01$) after 24 hrs and 2.125±0.238, 2.837±0.524 and 3.768±0.584 ($p < 0.05$) on day-7 respectively. The BZM (25mg/kg) also exhibited significant ($p < 0.05$) antagonism of the amnesic effect of scopolamine (Table 1).

Passive Avoidance Paradigm

The Step Down Latency (SDL) was assessed as inflexion ratio (IR). Piracetam (100mg/kg) showed significant ($p < 0.05$) increase in SDL and IR as compared to control group on after 24 hrs and day-7 and it also exhibited significant ($p < 0.01$) antagonism of the effect of scopolamine (0.3mg/kg). The BZM in all the treatment groups (10, 25 and 50 mg/kg) increased the SDL and IR with 25 and 50mg/kg showing significant ($p < 0.01$) increase and the BZM 25mg/kg had also exhibited significant ($p < 0.01$) antagonism of the effect of Scopolamine (0.3mg/kg) (Figure 1).

Object Recognition Test

The increase in Recognition Index (RI) indicates nootropic effect. The RI of control group was 51.61±1.8 %. Scopolamine (0.3mg/kg) shown significant ($p< 0.05$) decrease in the RI to 30.645 ± 3.2 %. Piracetam (100mg/kg) has significantly ($p<0.01$) increased the RI to 73.913±2.6 % and also exhibited significant ($p < 0.01$) antagonism of the amnesic effect of scopolamine. The BF in the doses 10, 25 and 50 mg/kg increased the RI to 53.73 ± 3.6 %,

69.368 ± 2.9 % ($p < 0.05$) and 70.675 ± 5.6 % ($p < 0.05$) respectively and the mid-dose 25mg/kg has also exhibited significant ($p < 0.01$) antagonism of the effect of Scopolamine with RI of 64.64±4.9% (table 2).

DISCUSSION

Drugs are the potential tools in the study of behavioral and neurobiological basis of learning and memory which may provide critical data for understanding and treating disorders of cognitive dysfunctions [2]. Memory is the ability of an individual to record sensory stimuli, events, information, etc., retain them over short or long periods of time and recall the same at a later date when needed [16]. Cognition, broadly defined, includes perception, learning, memory, and decision making, in other words, all ways in which animals take information about the world through the senses, process, retain, and decide to act on it can be called as cognition [18]. Nootropic drugs belong to the category of psychotropic agents with selective facilitatory effect on intellectual performance, learning and memory. A number of drugs including piracetam have now been introduced in therapy to ameliorate cognitive deficits [1].

In the present study we have investigated the nootrpic activity of n-butanol fraction of *Z. mauritiana* leaves. Animal models have been instrumental in shaping our understanding of the ability of the brain to process information. Simple but explicable models such as the elevated plus maze are available to evaluate learning and memory modulation. The time consumed by the animal to move from the open to the closed arm in EPM is recorded as transfer latency. The cognitive processing of spatial information takes place when the animal navigates the maze at intervals following the first exposure. Re-exposure to the maze would enable the animal to recall places and things reflecting explicit memory [19]. The increase in the inflexion ratio (IR) by BZM has proved that the n-butanolic fraction of *Z. mauritiana* leaves can be regarded as a nootropic agent in the view of its facilitatory effect on the acquired learning and retention [20]. The improvement in IR by BZM on the day-7 indicated its positive effect on long-term memory [15].

The present study demonstrates that in a paradigm of short-term memory, BZM produces improvement in passive avoidance acquisition and memory retrieval [7]. The BZM and piracetam has shown significant increase in step down latency as inflexion ratio and also antagonised effect of scopolamine. This model is predictive of aversion induced motivation [16]. Object recognition test (ORT) was developed for testing non-spatial memory in rats without the need for conventional reinforcers. In the object recognition test animals treated by piracetam and BZM were able differentiate the familiar object (which they explore in trial-1) from the novel object (which was introduced in trial-2) [17]. The experimental paradigm to form the object recognition/location memories is under the condition of relatively low stress or emotional arousal compared with the water maze/radial maze [21]. The present study shows that scopolamine demonstrates memory impairment while, the piracetam and BZM has increased the recognition index significantly. The BZM and piracetam also antagonises the effect of scopolamine. The object recognition learning paradigm allows a rapid evaluation of memory performance in animals [22].

Table 1 Effect of BZM on Elevated Plus Maze

TREATMENT (dose in mg/kg)	TRANSFER LATENCY (Mean ±SEM)			INFLEXION RATIO (Mean ±SEM)	
	DAY-1 (Sec)	DAY-2 (Sec)	DAY-7 (Sec)	DAY-2	DAY-7
Control	56.50±3.58	24.50±4.30	24.66±3.28	1.841±0.65	1.537±0.41
Scopolamine (0.3)	46.66±4.67	43.66±4.73**	73.33±5.85**	0.076±0.03*	-0.35±0.05
Piracetam (100)	77.00±6.52*	17.50±1.08**	16.50±1.52**	3.430±0.39*	3.986±0.72**
BZM (10)	79±3.75*	34.33±1.75*	25.83±1.75**	1.313±0.09	2.125±0.23
BZM (25)	81.83±5.60**	29.00±2.69*	23.50±4.14**	1.899±0.26**	2.837±0.52
BZM (50)	60.66±4.27	21.66±2.17**	13.50±1.58**	1.977±0.40**	3.768±0.58*
Pira. (100) + Scopol. (0.3)	75.33±5.08**	33.00±3.95	26.83±4.37**	1.422±0.273	2.007±0.53*
BZM (25) + Scopol.(0.3)	77.00±6.65**	37.16±4.47	30.50±4.53**	1.318±0.49	1.902±0.60*

Data is presented as mean ±SEM (n=6); one way ANOVA followed by Dunnett's test.
**p < 0.05; **p < 0.01 vs control and scopolamine*

Central cholinergic system plays an important role in learning and memory [23, 24]. Anti-cholinergic drugs like scopolamine impair the learning process and negatively affect the memory performance [21, 22]. Memory impairment in the patients with senile dementia of Alzheimer's type results from a deficiency in cholinergic function in the brain [25]. Present investigation demonstrates the effect of BZM on cholinergic system. The observation shows that BZM has antagonised the amnesic effects of scopolamine, improvement in learning, memory and cognition on the EPM, Passive Avoidance Test and Object Recognition Test. This indicates the action of BZM on cholinergic system, as it has long been known that the stimulation of the cholinergic system improves cognitive processes [26]. But further study to conform its exact mechanism is essential.

Thus, it is concluded that the n-butanolic fraction of methanolic extract of *Z. Mauritiana,Lam* (Rhamnaceae) leaves (BZM), possessed nootropic activity and also indicate the involvement of central cholinergic system in this mechanism.

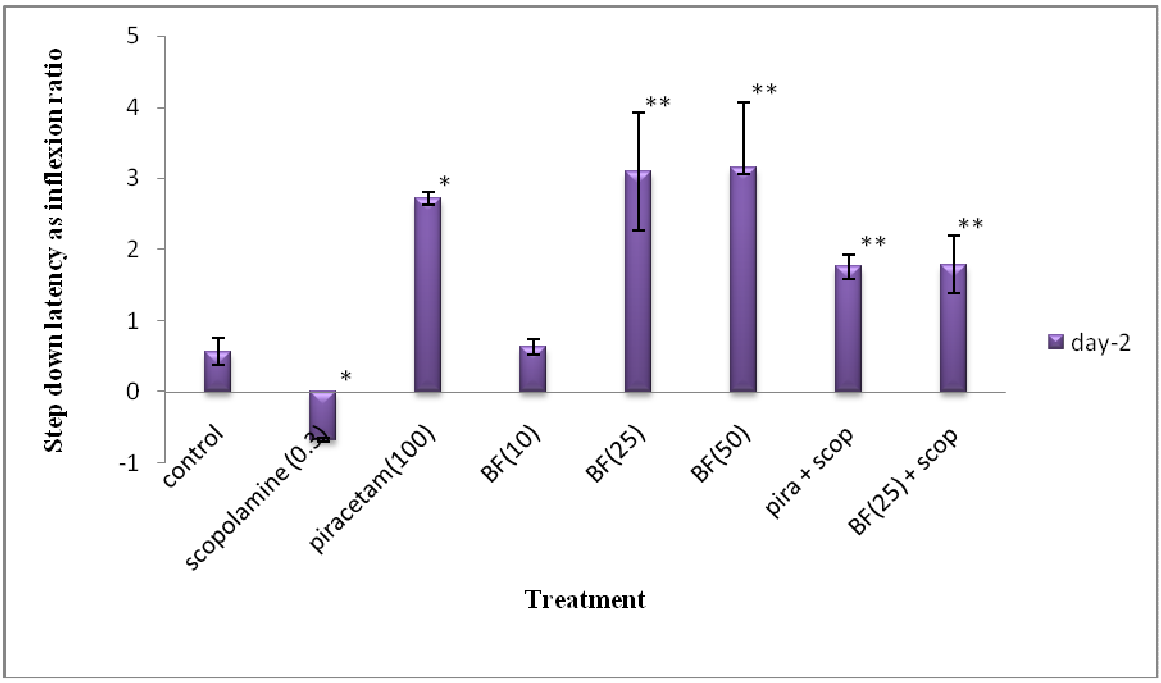

Figure 1 Effect of BZM on step down latency in normal and amnesic mice

Data is presented as mean ±SEM (n=6); one way ANOVA followed by Dunnett's test.
**p < 0.05; **p < 0.01 vs control and scopolamine*

Table 2 Effect of BZM on Object Recognition Test

TREATMENT (Dose in mg/kg)	TRIAL-1 (Mean ± SEM)	TRIAL-2 (Mean ± SEM) Sec.		RECOGNITION INDEX (Mean±SEM) %
	Time spent (Sec)	Time spent Familiar Object	Time spent New Object	
Control	21.00±3.05	10.16±0.47	11.16±1.27	51.61±1.84
Scopolamine(0.3)	31.16±3.84	32±20.98**	14.50±2.48	30.64±3.24*
Piracetam (100)	36.83±4.04	15.83±2.60	42.83±2.27**	73.91±2.65**
BF (10)	22.50±5.09	20.50±2.96	24.33±4.58	53.73±3.69
BF (25)	40.50±4.58	17.50±3.22*	37.66±1.82**	69.36±2.91*
BF (50)	67.16±12.16	23.16±4.15*	58.16±7.98**	70.67±5.63*
Pirace. (100) + Scopol. (0.3)	39.16±5.52	17.00±5.29*	28.83±4.12	64.70±8.28**
BF (25) + Scopol. (0.3)	35.50±7.27	11.00±2.20**	20.00±2.91	64.64±4.98**

Data is presented as mean ±SEM (n=6); one way ANOVA followed by Dunnett's test.
**p < 0.05; **p < 0.01 vs control and scopolamine*

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