American-Eurasian Journal of Toxicological Sciences 8 (4): 144-147, 2016
ISSN 2079-2050
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DOI: 10.5829/idosi.aejts.2016.144.147

Evaluation of Antianxiety Activity of *Bauhinia variegata* by Light-Dark Test of Mice

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Abstract: *Bauhinia variegata* has been used traditionally to treat bronchitis, leprosy and tumors. The present study was designed to evaluate the anti-anxiety activity of the leaves and seeds of *Bauhinia variegata* using light-dark test in Swiss albino mice. Albino mice were treated orally with different doses of the extracts (i.e.100, 200 mg/kg) and behavior was observed on the light-dark test apparatus. Buspirone (6mg/kg, p.o) was used as a positive control. Results showed that methanol extract at the dose of 100mg/kg of the methanolic leaf extract of *Bauhinia variegata* and ethanolic seed extract of *B. variegata* seeds (200 mg/kg) markedly increased the time spent in the light area. This effect was comparable to the effect produced by Buspirone. Hence this plant may be used as a potentially useful anti -anxiety agent.

Key words: Anxiety · *Bauhinia variegata* · Buspirone · Light-Dark Test

INTRODUCTION

Now-a-days the changed life style of the people and complexity of daily life in modern society leads to anxiety. In both developed and developing countries, mood and anxiety disorders are associated with chronic pain among medical patients [1, 2].

Benzodiazepines are the most widely used medicines for treating anxiety. But the use of benzodiazepines is linked with the side effects such as psychomotor impairment and dependence liability [3]. This has led to the evaluation of plants which are traditionally used and alternate system of medicine for anxiety disorders and related diseases [4].

Bauhinia variegata (Caesalpiniaceae) also called Mountain Ebony (English), Raktakanchan (Marathi) and Kachnar (Hindi) is a medium-sized, deciduous tree, found throughout India at an altitude of 1800m in Himalayas [5]. *Bauhinia variegata* Linn. is traditionally used in bronchitis, leprosy, tumors and as astringent, tonicand anthelmintic [6, 7]. So, the present study was done to investigate the anti-anxiety activity of different extracts of *Bauhinia variegata* using the Light-dark test apparatus, an exteroceptive behavior animal model.

MATERIALS AND METHODS

Preparation of Extracts: The leaves of *Bauhinia variegata* were washed thoroughly in tap water, shade dried and powdered. This powder was packed into Soxhlet column and extracted with petroleum ether (60°-80°C) for 24 h. The same marc was successively extracted with chloroform (50°-60°C) and later with ethanol (68°-78°C) for 24 h. The extracts were concentrated on water bath (50°C). After concentrated preparation, the dried powder extract was stored at room temperature. The yields of the petroleum, chloroform and methanolic extracts were found to be 0.8, 0.8 and 1.0 % (w/w) respectively. Methanolic extract of *Bauhinia variegata* leaves and seeds were used for the experimental study.

Test Animals: Animals were procured from Central Animal House, MIET, Meerut, India. Animal study was approved by Institutional Animal Ethic Committee (IAEC) of MIET, Meerut. Approval number (711/02/a/CPCSEA) was given for this work. The preferred rodent species included the mice. Swiss Albino strain of young healthy adult of either sex animals in equal numbers per group (n= 6) were taken. At the commencement of the study the weight variations of animals used was kept minimal and

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Table 1: Protocol of the study

Group	Drug	Dose
I	Control (1% gum acacia)	1ml/kg
II	BVMEL	100mg/kg
III	BVMEL	200mg/kg
IV	BVEES	100mg/kg
V	BVEES	200mg/kg
VI	Buspirone i.p.	6mg/kg

Where BVMEL: *Bauhinia variegata* methanolic extract of leaves
BVEES: *Bauhinia variegata* ethanolic extract of seeds

not exceeded ± 20% of the mean weight of each animal. Normal weight of mice was 20-25 gm. The temperature of the experimental animal room was maintained to be 22°C (±3°C). Relative humidity was maintained between 50–60%. Lighting was artificial, the sequence being 12 hours light, 12 hours dark. For feeding, conventional laboratory diets were used with drinking aqueous supplied *ad libitum*. Animals of same group were caged together. Healthy young adult of either sex mice were randomly assigned to the control, standard and treatment groups. The animals were identified uniquely (*i.e.*, via marking at the base of the tail) and acclimatized for not less than 5 days in their cages prior to the start of the study.

Drugs and Chemicals

Drugs: Buspirone was purchased from Sigma Aldrich.

Chemicals: Methanol and ethanol were purchased from Central Drug House Laboratory (CDH).

Analytical reagent grade chemicals were used in the study.

Vehicle: 1% gum acacia.

Study Design: The animals were selected randomly for each experiment and divided into 6 groups of 6 mice each. Drugs (Gum acacia, Buspirone) and BVMEL and BVEES were administered orally (p.o.) for 14 successive days as depicted in (Table 1).

Light-Dark Test: The apparatus consisted of Plexiglas box with two compartments (20cm × 20cm each), one of which was illuminated with a white light while the other remained dark. Each animal was placed at the junction of the light dark, facing the illuminated places, as well as the number of entries in each space, was recorded for 5 minutes. After each test, the box was carefully cleaned up with a wet tissue paper (10% ethanol solution). Single administration (p.o.) of extract and standard was given one hour prior to test [8].

Statistical Analysis: The data were expressed as Mean ± SEM (Standard error of Mean). Statistically difference between the groups were analyzed by using one way analysis of variance (ANOVA) followed by student-'t' test or Dunnett's test. The results were considered statistically significant if $p<0.05$.

RESULTS

Acute Oral Toxicity: *B. variegata*Linn methanolic (Leaf) and ethanolic (Seed) extract, at the doses of 2000 mg/kg (Orally), had no effect on mice behavioral responses and no mortality was observed during 72 hour time period. These results indicate that *B. variegata*Linn methanolic leaf extract and ethanolic seed extracts have no toxicity profile.

Pharmacological Activity

Antianxiety Activity: Light and Dark test.

Table 2: Effect of leaves and seeds extracts of *Bauhinia variegata* Linn in case of light and dark test in rats, after 14 days of treatment

S.No.	Treatment	Dose (mg/kg)	Time spent in light chamber (sec)	Latency
1.	Control	(1% gum acacia 1 ml/kg)	54.833±9.361	4.333± 1.146
2.	BVMEL	100	114 ± 9.101***	9.5 ± 1.348*
3.	BVMEL	200	95.333 ± 9.937*	6.166 ± 1.038
4.	BVEES	100	68.166 ± 5.453	5.333± 1.283
5.	BVEES	200	102.833± 12.113**	7.333 ± 1.045
6.	Buspirone	6 (i.p.)	157 ± 5.126***	112 ± 0.881***

Results are expressed as MEAN±SEM (n= 6), *P<0.05, **P<0.01, ***P<0.001 and ns= not significant; when compared to control group by one way ANOVA followed by Dunnett's test.

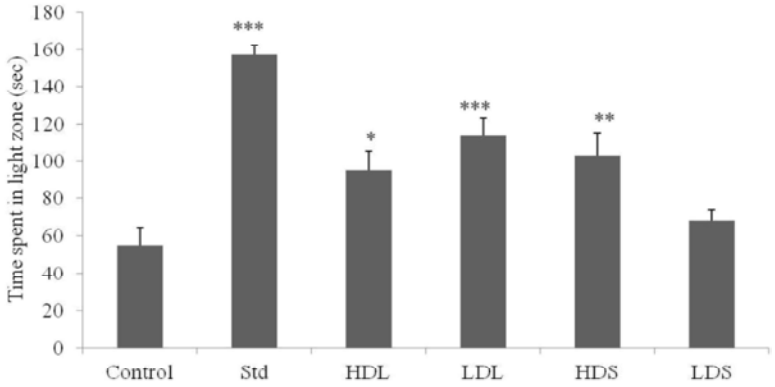

Fig. 1: Effect of *Bauhinia variegata* Linn on time spent in light zone (Sec).

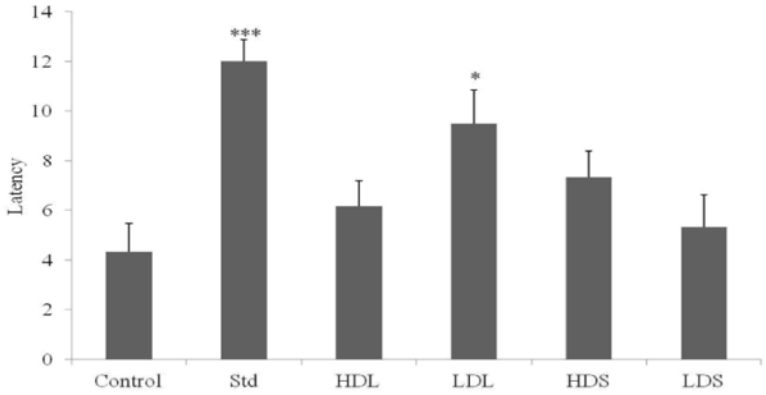

Fig. 2: Effect of *Bauhinia variegata* Linn on Transfer latency.

DISCUSSION

The light dark transition model box is also widely used for rodents as a model for screening anxiolytic or anxiogenic drugs. A good agreement has been observed between relative potency of drugs clinically used in the treatment of anxiety in humans and their ability to facilitate exploratory activity in the light/dark paradigm in mice.

The present data showed that *B. variegata* leaves (100 mg/kg) and *B. variegata* seeds (200 mg/kg) could increase the time spent in the light area, suggesting again these fractions possessed anxiolytic properties. The anxiolytic effects of methanolic extract of *Bauhinia variegata* may be related to their flavonoid content. Flavonoids with anxiolytic activity have been described in many plant species used in folk medicine such as *Passiflora coerulea* [9]. This effect has been attributed to the affinity of flavonoids for the central benzodiazepine receptors [10-12]. However, further studies are required to identify the phytoconstituents responsible for the anxiolytic mechanism.

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