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RESEARCH ARTICLE

PHARMACOLOGY

ANTIANXIETY EFFECT OF METHANOLIC EXTRACT OF *Bauhinia racemosa (Lamk) STEM BARK IN MICE*

MRUNAL.S.DAVEY[1]*, CLEMENT ATLEE.W[2], S.R.S ASHOK BHARATHI[1] AND MOHAMED FAROOK[1]

[1]Department of Pharmacology, C.L.Baid Metha College of Pharmacy, Thorraipakam, Chennai, India.
[2]Asst.Professor, Department of Pharmacology, C.L.Baid Metha College of Pharmacy, Thorraipakam, Chennai, India.

MRUNAL.S.DAVEY[1]
Department of Pharmacology, C.L.Baid Metha College of Pharmacy, Thorraipakam, Chennai, India.

ABSTRACT

This study was performed to investigate the anxiolytic-like effects of Methanolic extract of *Bauhinia racemosa* (MEBR) in mice using the elevated plus-maze model (EPM), light dark model, hole board test, foot shock induced freezing behavior. Furthermore, the anxiolytic-like effects of MEBR were compared to a known active anxiolytic drug (Diazepam). The extract administered orally in two different doses of 150mg/kg and 300mg/kg, was able to increase the time spent and the number of arm entries in the open arms of the elevated plus-maze, also increases the time spent by mice in the illuminated side of the light–dark test, showed significant increase in nose poking and decrease locomotion in hole board test, as well as caused significant reduction in freezing time in comparison with control animals. This effect was comparable to that of the benzodiazepine diazepam (2.0mg/kg p.o.). These results indicate that methanolic extract *Bauhinia racemosa* is an effective anxiolytic agent.

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KEYWORDS

Anxiolytic-like effect, *Bauhinia racemosa,* Elevated plus maze, Diazepam.

INTRODUCTION

Anxiety affects one-eighth of the total population world-wide and has become an important area of research interest in psychopharmacology during this decade[1]. Benzodiazepines are the major class of compounds used in anxiety and they have remained the most commonly prescribed treatment for anxiety[2]. 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[3]. The recognition of anxiolytic effects of non-benzodiazepine azapirones agents, which act as $5HT_{1A}$ partial agonists, such as buspirone, gepirone, and ipsapirone and their therapeutic role in clinical anxiety and mood disorders has further focused attention on the $5\text{-}HT_{1A}$ receptor[4]. Although the azapirones interact with other neurotransmitter systems, such as the dopaminergic and noradrenergic, they display nanomolar affinity for $5HT_{1A}$ receptor sites[5]. However, the anxiolytic effects of azapirones follow a time course observed with antidepressants where therapeutic effects are delayed for 3–4 weeks, which is unlike the rapid effects observed with benzodiazepine anxiolytics[5]. Thus, there is a need of robust anxiolytic compounds that have lesser side effects than benzodiazepines and a more immediate onset of action than currently available $5\text{-}HT_{1A}$ receptor acting drugs.

On the basis of these considerations, it was the purpose of this study to characterize the anxiolytic-like activity of a methanolic extract prepared from the stem bark of *Bauhinia racemosa* (MEBR, Caesalpiniaceae). *Bauhinia racemosa* is a small crooked tree with drooping branches, growing up to 3-5m tall, distributed in sub-Himalayan tracts from Ravi eastwards also found in Uttar Pradesh, West Bengal, Central and South India. It has highly astringent, anti-inflammatory (used in glandular inflammation, skin diseases, ulcers), alternative tonic. A previous paper describes its Antimicrobial and antioxidant activity[6]. Furthermore, methanolic extract of *Bauhinia racemosa* stem bark exhibited antitumor effect by modulation lipid peroxidation and augmenting antioxidant defense system in Ehrlich ascites carcinoma (EAC) bearing mice[7]. The alcoholic and aqueous extract of stem bark of the plant *Bauhinia racemosa* showed Antipyretic activity in rats[8]. In addition, it has been shown; the methanolic extract of *Bauhinia racemosa* has a potent hepatoprotective action upon paracetamol and carbon tetrachloride-induced hepatic damage in rats[9]. An experiment was conducted to study the antiulcer effect of dried flower buds of the plant *Bauhinia racemosa* in rats[10].

MATERIALS AND METHODS

Collection of plant material

The stem bark of *Bauhinia racemosa* were collected from the tree present in the place called Tenkasi, Tirunelveli District, Tamilnadu. The plant material was identified and authenticated by Mr. Balakrishnan M. sc., M. Phil (PhD), D.S.M., CAS in Botany, University of Madras, Guindy campus, Chennai.

Extraction and Preliminary Phytochemical Screening

Freshly collected stem barks of *Bauhinia racemosa* were dried in shade and pulverized to get a coarse powder. A weighed quantity of the powder (980gms) was passed through sieve number 40 and subjected to hot solvent extraction in a Soxhlet apparatus using methanol at a temperature range of 60-80^0 C. Before and after every extraction the

powder bed was completely dried and weighed. The filtrate was evaporated to dryness at 40^0C under reduced pressure in a rotary vaccum evaporator. A brownish black waxy residue was obtained. The percentage yield of methanolic extract of bark was 8.9% w/w. The dried extracts were subjected to various chemical tests to detect the presence of different phytoconstituents[11-12] present in them.

Experimental models

Adult male Swiss albino mice (20–30gms) from our own breeding stock were used. They were housed in groups in polypropylene cages (11cm × 17cm × 28cm) with wood shavings as bedding, under controlled conditions of light (12 h light–dark cycle, light on at 8 a.m.) and temperature (22±2^{0}C). The animals had free access to water and food except 1 h before and during the experiments. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) of CPCSEA (Committee for the Purpose of Control and Supervision of Experimental Animals).*IAEC ref. no IAEC/XIII/03/CLBMCP/2009-10 dated 17.12.2009*

Acute toxicity studies[13-14]

Acute toxicity tests were performed in mice. All animals were fasted overnight before treatment and were given food 1 h after MEBR treatment. A single high dose (2000 mg/kg), as recommended by the OECD guidelines, was administered orally to mice (3 males and 3 females, respectively). General behavior was also observed at 1, 3 and 24 h after administration. The number of animals that died after administration was recorded daily for 14days.

Experimental design

The animals were divided into four groups of Swiss albino mice, each comprising six animals. Group I served as a control received 1%w/v CMC orally, Group II mice were administered with standard drug Diazepam (2.5mg/kg body weight administered orally) dissolved in normal saline, Group III animals were administered with (methanolic extract of *Bauhinia racemosa*) MEBR (150mg/kg body weight orally) for 21 days, Group IV animals were administered with (methanolic extract of *Bauhinia racemosa*) MEBR (300mg/kg body weight orally) for 21 days. After 21 days dosing period the animal's anxiety level was observed by screening methods such as elevated plus maze, light dark model, hole board test and foot shock induced aggression.

PHARMACOLOGICAL ASSAY

Elevated plus-maze test[15]***:***

The elevated plus-maze comprised two open (30 cm×5 cm×0.25 cm) and two enclosed (30 cm×5 cm×15 cm) arms that radiated from a central platform (5 cm×5 cm) to form a plus sign. The maze was constructed of black painted wood. A slight raised edge on the open arms (0.25 cm) provided additional grip for the animals. The plus-maze was elevated to a height of 40 cm above floor level by a single central support. The experiment was conducted during the dark phase of the light cycle (9:00–14:00 h). The trial was started by placing an animal on the central platform of the maze facing an open arm. The number of entries into, and the time spent in, each of the two types of arm, were counted during a 5 min test period. The percentage open arm entries and percentage open arm time were used as indices of anxiety. A mouse was considered to have entered an arm when all four paws were on the arm. The apparatus was cleaned thoroughly between trials with damp and dry towels. All behavioral recordings were carried out with the observer unaware of the treatment the mice had received.

Light dark test[16]***:***

The apparatus consisted of two 20 cm×10 cm×14 cm plastic boxes: one was dark and the other was transparent. The mice were allowed to move from one box to the other through an open door between the two boxes. A 100W bulb placed 30 cm above the floor of the transparent box was the only light source in the room. A mouse was put into the light box facing the hole. The transitions between the light and the dark box and time spent in the light box were recorded for 5 min immediately after the mouse stepped into the

dark box. The apparatus was cleaned thoroughly between trials. All behavioral recordings were carried out with the observer unaware of the treatment the mice had received.

The hole-board test[17]*:*

The apparatus was composed of a gray wooden box (50 cm×50 cm× 50 cm) with four equidistant holes 3 cm in diameter in the floor. The centre of each hole was 10 cm from the nearest wall of the box. The floor of the box was positioned 15 cm above the ground and divided into squares of 10 cm×10 cm with a water-resistant marker. An animal was placed in the center of the hole-board and allowed to freely explore the apparatus for 5 min. The total locomotor activity (numbers of squares crossed), and the number and duration of head-dippings were recorded. A head dip was scored if both eyes disappeared into the hole.

Foot shock induced behavior[18]*:*

The animals were placed in a standard conditioning chamber for a 6.5 min session. 2 and 2.5 min after the start of session, a scrambled foot shock (0.5 mA, 0.5 s) is delivered through the grid floor of the chamber. Using an assembly of the push buttons interfaced with an electoral circuit and the time spent or engaged in the freezing (immobility with rigid body posture) behavior was assessed.

STATISTICAL ANALYSIS

Results are expressed as mean ± standard error of the mean (S.E.M.). All data are subjected to analysis of variance (ANOVA) followed by Dunnet's "t" test. P values <0.05(95% confidence limit) was considered statistically significant.

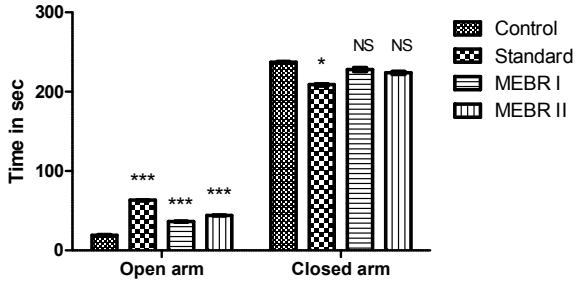
RESULTS

Effect of MEBR on Elevated plus maze

Based on previous studies that were carried out on native plants and in order to determine the effective dose on the EPM, plant doses of 150 and 300 mg/kg were tested. In CMC treated animals the time spent in the open and closed arms, and entries in the open and closed arms were 19.6±1.46 sec, 237.6±5.66 sec, 3.16±0.3 and 15.1±0.92. *Bauhinia racemosa* extract at the dose of 150mg/kg and 300mg/kg showed significant ($p<0.001$) increase in the time spent in the open arms and significant ($p<0.05$) increase in number of entries in open arm (Graph 1 and 2). Diazepam (2.5mg/kg) also increased the time spent and number of entries in open arm ($p<0.001$) as compared to control animals. Furthermore, MEBR 150mg/kg and 300mg/kg had no significant effects on the time spent and number of arm entries in closed arms. However Diazepam showed a significant ($p<0.05$) decrease in time spent and number of entries in closed arm.

Graph 1

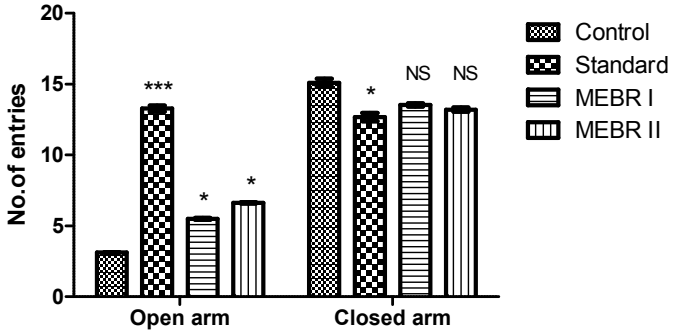
Time spent in Elevated plus maze



The effects of methanolic extract of *Bauhinia racemosa* (150 and 300 mg/kg) and diazepam (2.5 mg/kg) on the time spent in open arms and closed arm of the elevated plus-maze in mice. Data represent means ± S.E.M. of 6 animals during the 5-min test session. Comparisons were made by using a one-way ANOVA followed by Dunnet's "t" test: *p <

0.05; **p<0.001; ***p<0.001; [NS]Non significant compared with control group.

Graph 2
Number of entries in Elevated plus maze



The effects of methanolic extract of *Bauhinia racemosa* (150 and 300 mg/kg) and Diazepam (2.5 mg/kg) on the number of entries in open and closed arm of the elevated plus-maze in mice. Data represent means ± S.E.M of 6 animals during the 5-min test session. Comparisons were made by using a one-way ANOVA followed by Dunnet's "t" test: *p < 0.05; **p<0.001; ***p<0.001; [NS]Non significant compared with control group.

Effect of MEBR on Light dark model

The time spent in light were significantly (p<0.001) increased in animals treated with MEBR 150mg/kg and 300mg/kg when compared to control animals. A significant (p<0.001) increase in time spent in light area was also observed in animals treated with standard (Diazepam 2.5mg/kg) drug. The number of transitions were significantly (p<0.001) increased in animals treated with MEBR 150mg/kg and 300mg/kg and standard drug Diazepam when compared to control animals (Table 1).

Table 1
Effect of MEBR on light dark model

Groups	Time spent in light	No of transitions
Control	6.3±0.3	19.6±0.45
Standard	16.1±0.43***[a]	36.8±0.95***[a]
MEBR I	10.5±0.39***[b]	25.3±0.56***[b]
MEBR II	13.3±0.69***[b]	30.3±0.69***[b]

Values are expressed by mean ± SD of six animals in each group
Comparisons are made between: a- Group II and Group I; b – Group III, IV and Group I
Statistical significance: a, b significant at p<0.001

Effect of MEBR on Hole board test

The number of nose poking was moderately increased in animals treated with MEBR 150mg/kg but statistically non significant and significant (p<0.001) increase in animals treated with MEBR 300mg/kg and standard drug when compared to control. Locomotion of animals treated with MEBR 150mg/kg showed moderate (p<0.05) decrease when compared to control animals. A significant (p<0.001) decrease was observed in animals treated with MEBR 300mg/kg and standard drug treated animals when compared to control (Table 2).

Table 2
Effect of MEBR on Hole Board test

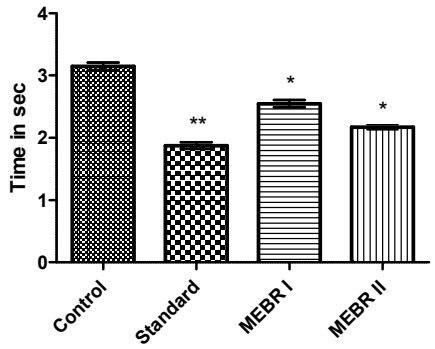
Groups	Nose poking	Line crossing
Control	23.33±1.3	65.1±3.22
Standard	50.1±2.85***[a]	46.5±2.54***[a]
MEBR I	30.6±1.91[NSb]	54.5±1.61*[b]
MEBR II	41.8±2.44***[b]	51.6±2.62***[b]

Values are expressed by mean ± SD of six animals in each group
Comparisons are made between: a- Group II and Group I; b – Group III, IV and Group I
*Statistical significance: [NS] Non significant; * p<0.05; **p<0.01; ***p<0.001*

Effect of MEBR on Foot shock induced freezing behavior

There was a significant reduction in the freezing time (p<0.05) in animals treated with MEBR 150 and 300mg/kg in comparison with control group. The reduction in the freezing time produced by diazepam 2.5mg/kg was significant (p<0.01) in comparison with control group (Graph 3).

Graph 3
Foot shock induced freezing behavior



The effects of methanolic extract of *Bauhinia racemosa* (150 and 300 mg/kg) and Diazepam (2.5 mg/kg) on the freezing time of foot shock induced freezing behavior. Data represent means ± S.E.M of 24 animals during the 6.5min test session. Comparisons were made by using a one-way ANOVA followed by Dunnet's "t" test: *p < 0.05; **p<0.001; ***p<0.001; [NS]Non significant compared with control group.

DISCUSSION

The assessment of anxiety related behavior in animal model is based on the assumption that anxiety in animals is comparable to anxiety in humans. Anxious reaction is an adaptive reaction of an individual when confronted with danger or threat. Behavioral and physiological responses accompanying anxiety prepare an individual to react appropriately to such situation.

One of the most widely used animal models for screening putative anxiolytic is the elevated plus-maze[19]. The EPM is considered to be an etiologically valid animal model of anxiety because it uses natural stimuli, such as a fear of a new, brightly-lit open space and the fear of balancing on a relatively narrow raised platform, moreover it is known that anxiolytic agent increases the frequency of entries and time spent in open arm of the EPM[20]. In agreement with previously published reports, diazepam increased the percentage time spent on open

arms and the number of entries on open arms[21]. Total number of open arm entries and number of closed arm entries are usually employed as measures of general activity. In the present study it is noted that administration of MEBR prolonged the time spent in the open arms and the number of entries into open arms.

The light/dark box is also widely used for rodents as a model for screening anxiolytic or anxiogenic drugs, based on the innate aversion of rodents to brightly illuminated areas and on the spontaneous exploratory behavior of rodents in response to mild stressors, that is, a novel environment and light[22]. It has been reported that simply the measurement of the time spent in the light area, but not the number of transfers, is the most consistent and useful parameter for assessing an anxiolytic action[23]. The present study showed that MEBR (150 and 300 mg/kg) could increase the time in the light area, suggesting again that MEBR possesses anxiolytic properties.

The hole-board test provides a simple method for measuring the response of an animal to an unfamiliar environment and is widely used to assess emotionality, anxiety and/or responses to stress in animals[24]. It has been shown that head-dipping behavior was sensitive to changes in the emotional state of the animal, and suggested that the expression of an anxiolytic state in animals may be reflected by an increase in head-dipping behavior[25]. In the present study MEBR (150 mg/kg) increased head-dip counts and MEBR (300 mg/kg) increased head-dip duration without changing locomotion. These results indicate that MEBR has a significant anxiolytic effect in this paradigm.

Foot shock mediated freezing behavior is a novel model for evaluation of anxiolytics, in which reduction in the freezing time represents a good index of anxiolytic behavior[18]. In the present study MEBR at both the dose level reduced the freezing time indicating its anti-anxiety behavior.

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