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EVALUATION OF ANXIOLYTIC ACTIVITY OF ETHANOLIC EXTRACT OF *MUEHLENBECKIA PLATYCLADA* IN MICE: A PRELIMINARY EXPERIMENTAL STUDY

Ancy P.*[1], Padmaja V.[2], R. Rajkumar[3], Shiji Kumar P. S.[3] and Nithin Manohar R.[1]

[1]PhD, Research scholar, Meenakshi Academy of Higher Education and Research (Deemed to be University), Chennai, Tamil Nadu.
[2]College of Pharmaceutical Sciences, Medical College, Trivandrum.
[3]Professor, Department of Community Medicine, Meenakshi Medical College, Research Institute, Kancheepuram.
[4]Jamia Salafia Pharmacy College.

***Corresponding Author: Ancy P.**

PhD, Research Scholar, Meenakshi Academy of Higher Education and Research, (Deemed to be University) Chennai, Tamil Nadu.

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ABSTRACT

Objectives: To evaluate the anxiolytic effect of *Muehlenbeckia platyclada* ethanolicextract (MBEE) in mice and to compare the antianxiety activity against standard drug diazepam (2 mg/kg). **Materials and Methods:** After obtaining Institutional Animal Ethics Committee approval, Swiss albino mice (18–25 g) of either sex were randomly divided into five groups of six animals each. The control group, test group, and standard drugs group received saline, MBEE extract (100, and 200 mg/kg), diazepam (3 mg/kg), respectively, by oral feeding. The antianxiety effect was assessed by elevated plus maze (EPM) in mice. **Results:** In EPM, it implied that MBEE at 100 mg/kg and 200 mg/kg significantly ($P < 0.001$) increases the number of entries in open arms compared to control. The time spent in open arms also increased in all the doses of CS extract significantly. **Conclusion:** The current study demonstrates statistically significant dose-dependent antianxiety activity of *Muehlenbeckia platyclada* ethanolic extract (MBEE)

KEYWORDS: Antianxiety effect, *Muehlenbeckia platyclada*, Diazepam, Elevated plus maze, flavonoids.

INTRODUCTION

The primary use of sedative–hypnotic and anxiolytic drugs is to encourage calmness (sedative–hypnotics). All people may be subjected to states of emotional tension and uneasiness. For otherwise healthy individuals, these occasions are usually mild and short term that pharmacological intervention is unnecessary.[1] Anxiety almost invariably accompanies many medical and surgical conditions. When the symptoms become intolerable or interfere with the treatment of the underlying disease, and if counseling is not sufficient, drug treatment can be considered as a means of helping patients cope with their anxiety.[2]

Anxiety that results from fear caused by an acute illness or a stressful event, such as loss of a loved one, is usually self-limiting and can be of relatively short duration. The current options include various kinds of psychotherapy and pharmacotherapy such as benzodiazepines, azapirones, and antidepressants and others.[3]

The recognition of anxiolytic effects of nonbenzodiazepine azapirones agents, which acts as 5-HT1A partial agonists, their therapeutic role in clinical anxiety and mood disorders has further focused attention on the 5-HT1A receptor.[4] 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 for robust anxiolytic compounds that have lesser side effects than benzodiazepines and a more immediate onset of action than currently available 5-HT1A receptor acting drugs.

Nowadays there is revival of interest in the consumption of herbal medicines in the form of standardized extracts, partly due to their multiple side effects, and high cost of patentable chemical drugs.[6,7] The genus Muehlenbeckia is constituted by four species belonging to the family Polygonaceae. Plants of this genus, such as *Muehlenbeckia platyclada* (F. Muell.) Meisn. are found in South America, and have been traditionally used as diuretic, hypotensive, antihemorragic, sedative, antirheumatic, abortive, cicatrizant, antiulcerogenic, antiinflammatory and anthelmintic agents.[8,9] Plant description: Muehlenbeckia or the maidenhair genus is native to the Southern Hemisphere, especially South

America, Papua New Guinea, Australia and New Zealand, and has been introduced both by birds and cultivation to temperate locales north of the equator.[10,11] Some are tiny alpine mat-forming plants whereas others are vigorous vines with masses of dark stems and minimal small bronze-tinged leaves. It is also known by name Homalocladium platycladum (centipede plant, tapeworm plant or ribbon bush). This plant belongs to the knotweed family from New Guinea and the Solomon Islands.[12]

Flavanoids that act on nervous system and regulate various neurotransmitters are reported in the plant. Morin was a novel flavanoid isolated from this plant.[13,14,15]

This experiment was conducted to study the antianxiety (anxiolytic) effect of leaves of the plant CS in mice using elevated plus maze (EPM) test. This is a simple test used to identify the neuroprotective effects[16,17] and anxiety of the given test extracts.

MATERIALS AND METHODS

Collection of plant material

Preparation of ethanolic extract

The plant was collected from Karyavattom campus. The plant was identified and authenticated by a botanist. The plant was shade-dried and coarsely powdered. The dried plant material, 100 g were coarsely powdered and defatted with petroleum ether and extracted with ethanol by continuous hot percolation method.The yield of the extract is 5.9% (w/w). The extract was stored at 4°C and used to treat animals as needed.[18]

Experimental models

Swiss albino mice of either sex weighing approximately 18–25 g (2–2.5-month-old) used for experimental purpose. They were housed in polypropylene cages in the air-conditioned room with the temperature maintained at 25 ± 3°C, and 12 h alternating light and dark cycles. The mice were provided with a nutritionally adequate diet (Hindustan Lever Limited, India) and drinking water *ad libitum* throughout the study. Approval by the Animal Ethics Committee for the experimental procedures obtained.

Acute toxicity study

Acute toxicity was generally carried out for the determination of LD_{50} value in experimental animals. The LD_{50} determination was done in mice by OECD guidelines 423. The aim of performing acute toxicity study is for establishing therapeutic index of a particular drug and to ensure safety *in-vivo*. Acute toxicity test was performed in mice. All animals were fasted overnight before treatment and were given food 1 h after aqueous extract of CS treatment. General behavior was also observed at 1, 8, and 12 h after administration. The number of animals that died after administration was recorded daily for 10 days.[19,20]

Procedure

Elevated plus-maze test

Principle

Elevated plus-maze is the most simple apparatus to study neuroprotective effects[16,17] and anxiolytic responses produced by the test drugs. It is used to test almost all types of anxiolytic agents. Exposure of animals to novel maze alley evokes an approach-avoidance conflict which is stronger in open arm as compared to enclosed arm. Rodents (rats and mice) have an aversion for high and open space and prefer enclosed arm, therefore, spend a greater amount of time in enclosed arm. When animals enter open arm, they freeze, become immobile, defecate and show fear-like movements.[21] Major advantages of this test procedure are: (a) It is simple, fast, and less time consuming, (b) no prior training or noxious stimuli (sound or light) is required, and (c) it is predictable and reliable procedure for studying anxiety response as well as anxiolytic action of drug.[22,23]

Procedure

Animals were weighed, numbered, and divided into five groups, each consisting six mice. One group was used as control (saline), second for standard drug (diazepam) treatment, third, fourth, and fifth group for *Muehlenbeckia platyclada* ethanolic extract treatment (Test - 100, 200 mg/kg). The control group, test group, and standard drugs group received saline, MBEE extract (100, and 200 mg/kg), diazepam (3 mg/kg), respectively, by oral feeding. After one hour animals were placed individually in the center of the maze, head facing toward open arm and stopwatch was started. The following parameters were noted for 5 min. (1) First preference of mouse to open or closed arm. (2) Number of entries in open arm (an arm entry defined as the entry of four paws into the arm). (3) Average time each animal spends in open arm (Average time = total duration in the arm/number of entries) was calculated. Saline and diazepam were injected to the control and standard groups respectively. The extract was injected to the test groups. After 30 min, animals were placed individually in the center of the maze. Finally, we compared the preference of the animals to open or enclosed arm, average time spent in open arm and the number of entries in open arm in each group.[24,25]

Statistical analysis

Data were expressed by mean ± standard error mean. For comparison among the groups, we used analysis of variance with multiple comparisons by *post-hoc* Dunnett *t*-test method. The statistical significance of differences between the control and experimental groups was assessed by Dunnett's two-sided *t*-tests (post-hoc tests). Statistical analysis was done using Statistical Package for the Social Sciences for windows (version 17.0). Statistical significance was considered $P < 0.05$ level.

RESULTS

Table 1: The time spent and number of entries in enclosed and open arms of EPM, (*n*=6)

Treatment	Time spent on (s)		No of entries on	
	Enclosed arm	Open arm	Enclosed arm	Open arm
Control	210.87±2.11	32.76±1.1	5.45±0.23	2.77±0.23
MBEE(100mg/Kg)	152.78±2.63**	64.89±1.5***	6.20±0.30*	3.86±0.17**
MBEE(200mg/kg)	143.36±1.85***	69.72±0.86***	6.52±0.28**	4.50±0.15***
Diazepam (2mg/kg)	133.25±2.2***	79.70±1.1***	7.40±0.31**	6.50±0.13***

Values are expressed as mean ±SEM from 6 rats. Significant at *P<0.05 and **P<0.001, ***P<0.0001. The statistical significance of differences between the control and experimental groups was assessed by Dunnett's two-sided *t*-tests (post-hoc tests). Statistical analysis was done using Statistical Package for the Social Sciences for windows (version 17.0). Statistical significance was considered $P < 0.05$ level.

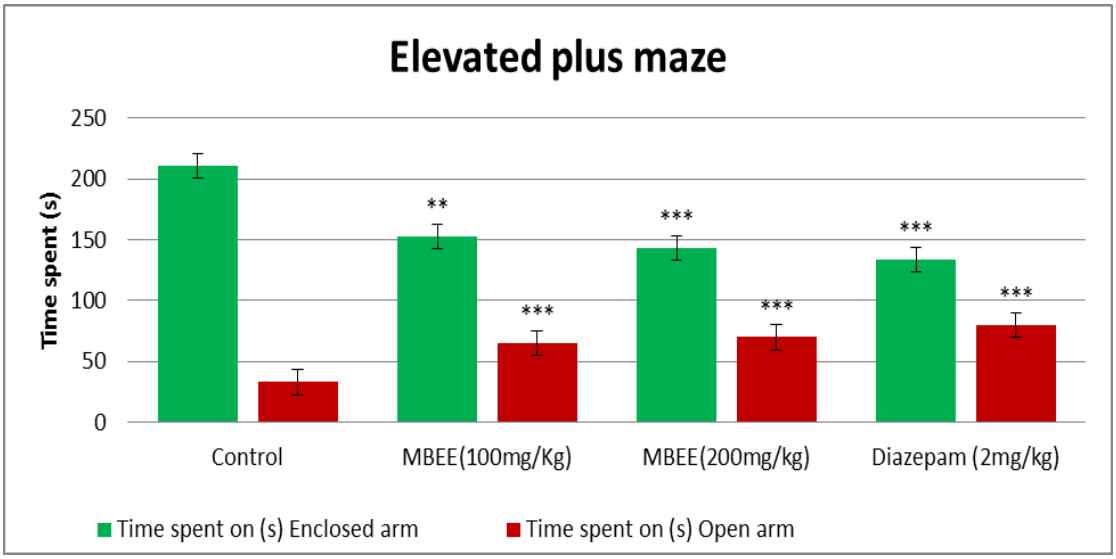

Figure 1: The time spent by mice with *Muehlenbeckia platyclada* on elevated plus maze in closed arm (*n* = 6).

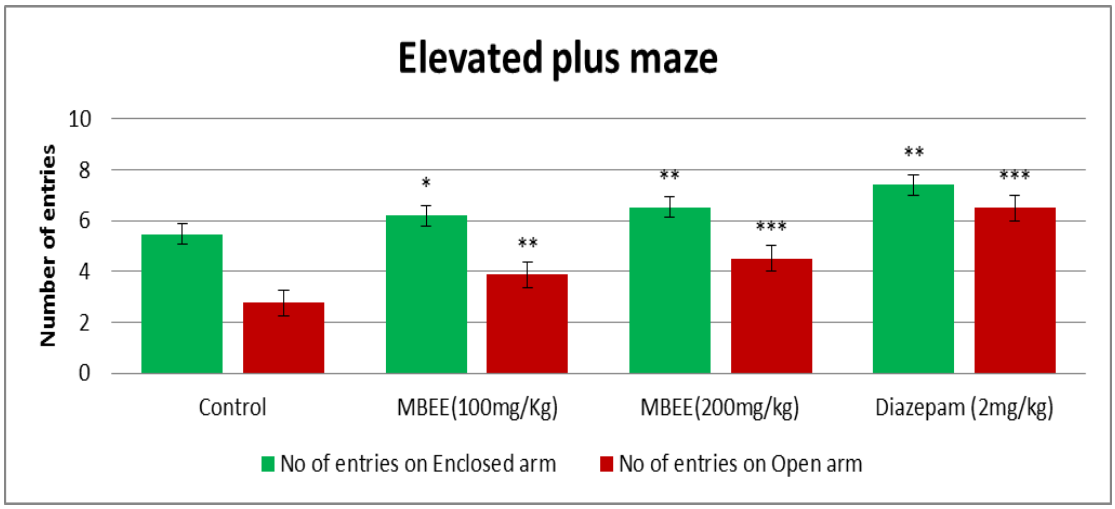

Figure 2: Effect of *Muehlenbeckia platyclada* shows antianxiety effect in total entries.

DISCUSSION

The anxiolytic effect of ethanolic extract of *Muehlenbeckia platyclada* extract (100, 200 mg/kg, o.p) was examined in male albino mice using EPM as an animal model of anxiety. The effects of the extract on spontaneous activity and neuromuscular coordination were assessed using rotarod, apparatus. In the EPM, aqueous extract at 200 mg/kg showed an anxiolytic effect by increasing the time spent on open arms and the percentage of open arm entries, compared to the control group. The extract at 100, and 200 mg/kg significantly reduced spontaneous activity and neuromuscular coordination, compared to the control group. These results suggest that the aqueous extract of CS seed has an anxiolytic effect and may have potential sedative and muscle relaxant effects.[26,27,28,29]

The EPM is currently one of the most widely used models of animal anxiety and has been validated for use with both the sexes of mice. Therefore, this test was chosen to investigate the anxiolytic potential of the aqueous extract of coriander seed. The indices of anxiety in this test are, number of entries in open arm and closed arm. The sensitivity to agents acts via the gamma-aminobutyric acid receptor complex, justifying the use of diazepam as a positive control in this study. Diazepam increased the entries of open arm and the time spent in the open arms confirming its anxiolytic effects.[30,31,32,33]

In our study, *Muehlenbeckia platyclada* ethanolic extract was used in a dose of 50, 100, and 200 mg/kg for identifying antianxiety. In these doses, the extract increases the number of entries in open arm as compared to control in dose-dependent manner effectively and significantly decreased the number of entries in closed arm compared to that of control in a dose-dependent manner. As the dose of increases, the effect also increased, but in all the three doses, there was significant antianxiety effect was seen. Hence, in our study, ethanolic extractof *Muehlenbeckia platyclada* showed significant anxiolytic activity with 100, and 200 mg/kg compared to the control group but has less activity when compared to that of standard drug diazepam.

CONCLUSION

The present study demonstrated that ethanolic extractof *Muehlenbeckia platyclada* possess dose-dependent anxiolytic activity. Further, there is need to isolate, characterize, and screen the active principles from the plant that are responsible for its anxiolytic activity. Furthermore, there is need to find out the exact mechanism by which the plant exerts above effects. Further studies are needed to separate and confirm the active components and its effect on anxiety.

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