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Evaluation of anxiolytic activity of ethanolic extract of *Mucuna pruriens* (L.) DC. seeds in experimental animals

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ABSTRACT

Mucuna pruriens (L.) is a popular Indian medicinal plant, which has been used in ayurvedic system for neurodegenerative diseases including Parkinsonism. Traditionally, *Mucuna pruriens* is commonly used as histaminic, carminative, hypertensive and hypoglycemic agent. The present work is to evaluate the anxiolytic activity of an ethanolic extract of *Mucuna pruriens* (EEMP) in mice using the elevated plus-maze (EPM) and Y-maze models. The efficacy of the extract (200 and 400 mg/kg p.o.) was compared with the standard anxiolytic drug Diazepam (2 mg/kg). EEMP was significantly ($p<0.05$) able to increase the time spent and the number of entries in the open arms of the elevated plus-maze model and decrease the number of visits in the Y-maze by the experimental animals. These results indicate that EEMP may be possessing anxiolytic activity.

Keywords: *Mucuna pruriens*, Anxiolytic, Elevated plus-maze, Y-maze.

INTRODUCTION

Anxiety is an emotional state commonly caused by the perception of real or perceived danger that threatens the security of an individual. According to WHO approximately 450 million people suffer from mental or behavioral disorders. Anxiety disorders, as a group, are the most commonly occurring psychiatric disorders. Hence, became a very important area of research interest in psychopharmacology [1]. Currently, the most widely prescribed medications for anxiety disorders are the benzodiazepines. 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 hence interest in alternative medicine and plant-derived medications that affect the 'mind' is growing. Various types of herbal medicines have been used as anxiolytic drugs in different parts of the world and researchers are exploring the traditional remedies to find a suitable cure for 'mind affecting diseases'. Many researchers have been evaluating new compounds from herbs which are possessing anxiolytic activity such as *Bacopa monnieri* Linn, Ginseng, *Ginkgo biloba* Linn, *Piper methysticum* forst, and *Salvia officinalis* Linn. Etc.

Mucuna pruriens (L.) DC. Belongs to family Fabaceae. Popularly known as "Konch" in Hindi, "Velvet bean" in English and "Khaajkuiri" in Marathi. *Mucuna pruriens* (L.) has been used for centuries in the Indian traditional medicine [2]. The main constituents found in *Mucuna pruriens* include alkaloids, dopamine, fatty acids, flavones, gallic acid, glutamic acid, glutathione, glycine, histidine, hydroxygenistein, 5-hydroxytryptamine, mucunadine, mucunain, mucunine, trypsin, tryptamine, histamine and it possesses various pharmacological activities like histaminic activity, estrogenic activity [3], Antitoxin activity against Cobra and krait venom [4, 5], Anti-inflammatory [6], Diuretic [6], Antibacterial activity [6], Hypoglycemic and Hypolipidemic activity, Anticholesterol activity [7] and antioxidant activity [8].

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A literature survey on *Mucuna pruriens* (L.) has revealed only a few pharmacological reports and no major investigation reports were found for its CNS activity. Therefore the aim of the present study was to investigate the anxiolytic effects of the EEMP on the elevated plus-maze (EPM) and on the Y-maze model.

MATERIALS AND METHODS

Chemicals

Diazepam used as standard was procured from Ranbaxy Laboratories Ltd., India (Calmpose). Ethanol was purchased locally and was of analytical grade. Distilled water was used as vehicle.

Plant material

The seeds of *Mucuna pruriens* L. (Family: Fabaceae) were obtained commercially from M/s. Gopal Govind Lokhande (Dealers in Ayurvedic and Unani medicines), Pune, Maharashtra, India. The specimen was authenticated by A.S. Upadhye, scientist, Plant drug authentication service, botany group, Agharkar research institute, Pune, Maharashtra, India and a voucher specimen (MCP/IA/Plants/2011/99) was deposited.

Preparation of the EEMP

Freshly collected seeds of *Mucuna pruriens* were dried in shade and pulverized to get a coarse powder. A weighed quantity of powder (1000 g) was passed through sieve no.40 and subjected to hot solvent extraction in soxhlet apparatus using ethanol, at temperature range of 40-80° C. Before and after every extraction the marc was completely dried and weighed. The filtrate was evaporated to dryness at 40°C under reduced pressure in rotary vacuum evaporator. A brownish black waxy residue was obtained. The percentage yield of EEMP was found to be 19.2%w/w.

Preparation of Test Doses

The EEMP were suspended in the vehicle in concentrations 200 and 400 mg/kg p.o.

Animals

Animals were purchased from National Institute of Biosciences, Pune,

Maharashtra, India. Healthy swiss albino mice weighing about 18–22 g were used for the present study. The animals were housed in well ventilated cages, maintained under standard conditions of light, temperature and humidity (12 h light and 12 h dark cycle; 25 ± 3 ^{0}C; 35–60% humidity) and were fed with standard diet and water ad libitum. The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC) and all the permitted species of animals involved in the research were handled and experimented in strict accordance with guidelines and procedures for animal experimentation as prescribed by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India. (IAEC Reference number: MCP/IAEC/27/2011 dated 22-10-2011).

Acute Toxicity Study

The procedure was followed as per OECD 423 guidelines. The extract was administered orally at a dose 2000 mg/kg body weight to different groups of mice and observed for signs of behavioral, Neurological toxicity and mortality in 14 days [9].

Elevated Plus Maze Model

The plus-maze apparatus, consisting of two open arms (16 x 5 cm) and two closed arms (16 x 5 x 12 cm) having an open roof. The EEMP (200 and 400 mg/kg p.o.) and vehicle were administered for 7 days once daily and the last dose was given on the 7th day, 60 min prior to experiment. The standard drug was given (2 mg/kg i.p.) 60 min before starting the experiment. After proper treatment each mouse was placed at the center of the maze with its head facing the open arm. During the 5 min experiment, the behavior of the mouse was recorded as the number of entries into the open or closed arms and time spent by the mouse in each of the arms. An arm entry was defined as the entry of all four paws into the arm [10, 11].

Y – Maze Model

Y- Maze is made of black painted wood or grey plastic. Mice were treated with the EEMP (200 and 400 mg / kg p.o.) or vehicle for 7 days once daily and the last dose was given on the 7th day, 60 min prior to experiment and kept individually in one arm of the apparatus. The standard drug was given (2 mg/kg i.p.) 60 min before starting the experiment. For a period of 10 min. the total numbers of visits to different arm were measured [12].

Statistical Analysis

The data were expressed as mean ± standard error mean (SEM). The significance of differences among the groups was assessed using one way analysis of variance (ANOVA). The test was followed by Dunnett's test, $p< 0.05$ were considered as significance.

RESULTS

Acute toxicity study

As no mortality, no adverse changes in behavior of animals as well as no abnormalities were detected at necropsy in experimental mice at the dose rate of 2000 mg/kg body weight, the EEMP plant was assigned to class 5 (LD_{50} > 2000 mg/kg), which were recommended by OECD.

Elevated Plus Maze Model

The results showed that the number of open arm entries and time spent in the open arms were increased and number of closed arm entries and time spent in the closed arms were decreased significantly in the EEMP treated groups which was comparable with the standard Diazepam (Table 1 figure 1a-b).

Table 1: Effect of EEMP on Mice in EPM Model

Groups	Treatment (mg/kg)	Time spent in the open arm (S)	Time spent In the enclosed arm (S)	No. of entries in open arm Mean±SEM	No. of entries in enclosed arm Mean±SEM
I	Control	14.58 ±1.28	248.00±4.30	4.83±1.01	14.66±0.71
II	Diazepam (2)	91.17±2.23	143.23±7.72	12.5±1.72	05.16±0.87
III	EEMP (200)	28.76±2.09**	211.67±3.42**	10.33±1.45*	9.83±1.30**
IV	EEMP (400)	52.84±4.17**	202.05±8.44**	12.16±1.57**	10.83±1.07*

*Values expressed as mean ± SEM., n=6, One way ANOVA followed by Dunnett's test. * p< 0.05, ** p< 0.01 considered significant when Compared to control.*

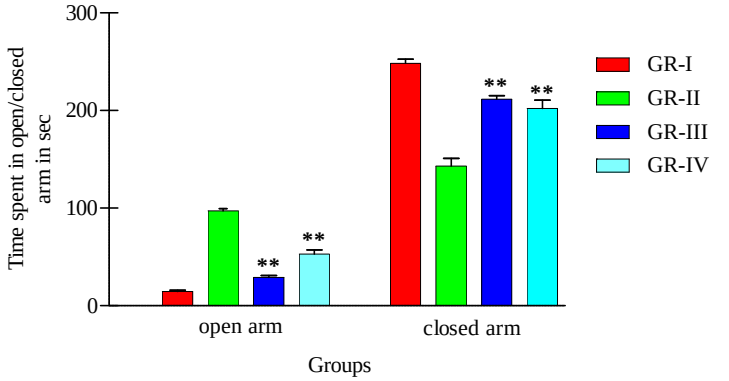

Fig. 1a Effect of EEMP in time spent in open/closed arm in EPM model.

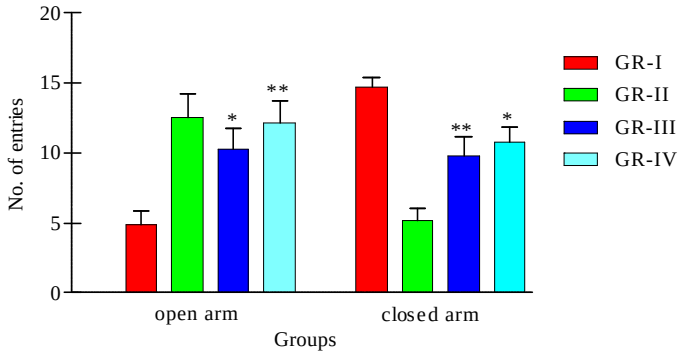

Fig.1 b Effect of EEMP in no. of entries in open/closed arm in EPM model.

Y-Maze Model

A significant decrease in the number of visits in the three arms of the Y-maze was observed in the Diazepam treated animals as compared to the control animals. Both the doses of EEMP showed a significant decrease in the number of visits in the three arms of the Y-maze which was comparable with the standard Diazepam (Table 2, figure 2).

Table 2: Effect of EEMP on Mice in Y-maze Model

Groups	Treatment (mg/kg)	No. of visits
I	Control	51.33±5.63
II	Diazepam (2)	12.33±6.69
III	EEMP (200)	24.50±6.50**
IV	EEMP (400)	19.16±1.51**

*Values expressed as mean ± SEM., n=6, One way ANOVA followed by Dunnett's test. ** p< 0.01 considered significant when Compared to control.*

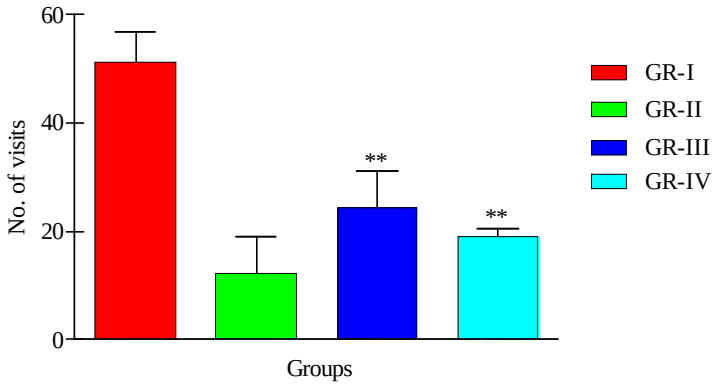

Fig.2 Effect of EEMP on mice in Y-maze model

DISCUSSION

The etiology of most anxiety disorders are not fully understood, but various studies has shown the involvement of GABA, serotonergic neurotransmission in etiology and treatment of anxiety [13]. The adrenergic and dopaminergic systems have also been shown to play a role in anxiety [14]. Major drug classes for the treatment of anxiety disorders are Benzodiazepines (BZDs), Selective serotonin-reuptake inhibitors (SSRIs), Tricyclic antidepressant, ß-blockers, and Azapirones. All this drug classes currently used are associated with side effects and severity. Like BZDs produce undesirable effects such as drowsiness, ataxia, and sedation, muscle relaxation, insomnia and hepatotoxicity.

The results of the present study also demonstrate that EEMP has an anxiolytic like effect in the elevated plus-maze and Y-maze model. In this study, we observed that EEMP (200 and 400mg//kg p.o.) induced significant increase in the both number of entries and time spent in the open arms, compared to the closed arm. In the Y-maze model showed that the number of visits in the three arms decreased significantly by all groups when compared to the control animals. Many researchers have been evaluating herbs that possess anxiolytic activity due to presence of flavonoid [14, 15]. *Bacopa monnieri* L., *Ginseng*, *Ginkgo biloba* Linn, *Piper methysticum forst*, and *Salvia officinalis* L. having the anxiolytic effect. It has been found that flavones bind with high affinity BZD site of the $GABA_A$ receptor. [16, 17]. The well known GABA is an inhibitory neurotransmitter which plays an important role in regulatory and inhibitory effect on the serotonin, nor epinephrine and dopamine. $GABA_A$ receptors are ligand-gated ion channels. When GABA binds to the $GABA_A$ receptor, neuronal excitability is reduced, leading to reduction of anxiety and causing sedation. Phytochemical tests of EEMP revealed the presence of flavonoids. The anxiolytic effects of EEMP may be related to their flavonoid content. It may possible that the mechanism of anxiolytic action of EEMP could be due to the binding of phytochemical to the $GABA_A$-BZD receptor.

CONCLUSION

From the above observations we can conclude that EEMP possesses anxiolytic activity at both the doses which is comparable with the standards. Further study is to find out the actual mechanism of action of anxiolytic with isolation of active constituents.

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