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Research Article

Potential Anti-Anxiety Effect of *Mucuna pruriens* in Experimental Model of Swiss Albino Mice

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ABSTRACT

The usage of benzodiazepines, the major class of anxiolytic drugs is invariably accompanied by many side-effects like sedation and muscle relaxation leading to incoordination of movements. Search for novel anxiolytic agents have identified flavonoids as potential compounds devoid of these adverse effects. Mucuna pruriens commonly known as cowhage plant has been claimed to possess various beneficial effects like anti-parkinsonian, anti-tumor, neuroprotective, antioxidant, anti-diabetic and antimicrobial activities. Previous studies have reported that Mucuna pruriens contains LDOPA and 5-hydroxy tryptophan (5-HT) as a major constituent with higher concentration in seeds. Present study was designed to evaluate the anxiolytic activity of Mucuna pruriens extract in Swiss albino mice. The study was conducted on 30 male Swiss albino mice. Three doses of Mucuna pruriens (100, 200, 400 mg/kg, p.o.) and standard dose of diazepam (2 mg/kg, i.p.) were used for treatment. The pharmacologically validated model elevated plus maze (EPM) was used to take as a measure of antianxiety effect. Mucuna pruriens at the doses of 200 mg/kg and 400 mg/kg significantly reduced the time spent and no. of entries in closed arm, increased the time spent and entries into open arm in elevated plus maze ($p<0.05$) as compared to control group. Present study demonstrates the anxiolytic activity of Mucuna pruriens in Swiss albino mice.

Key words: Anxiolytic, Diazepam, Elevated plus maze, *Mucuna pruriens*, Side effects, Swiss Albino Mice.

INTRODUCTION

Anxiety usually refers to the experience of fear, apprehensiveness, nervousness, panic, restlessness, tension, and agitation. Manifest symptoms include trembling, fainting, headaches, and sweating, possibly elevated blood pressure, and changes in other psychophysiological indices such as heart rate, muscle tone, and skin conductance.[1] Anxiety affects most of the population nearly one-eighth of the total population world-wide.[2] Benzodiazepines are the major class of compounds commonly prescribed for treating anxiety. However, their use is associated with side effects like psychomotor impairment, potentiating of other central depressant drugs and addiction liability.[3] A number of plants are being investigated in complementary alternative medicines for anxiety.[4,5] Research has been conducted in the search of an alternate, more specific and cost-effective therapy.[6]

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Neurotransmitters involved in anxiety generation include serotonin, dopamine, noradrenaline, GABA, Corticotropin releasing factor (CRF), Melanocyte stimulating hormone (MSH), neuropeptides and neurosteroids.[7] The recognition of anxiolytic effects of non-benzodiazepine azapirones agents, which act as 5HT1A partial agonists and their therapeutic role in clinical anxiety and mood disorders has further focused attention on the 5-HT1A receptor.[8] Previous studies have reported that Mucuna pruriens contains L-DOPA and 5-hydroxy tryptophan (5-HTP) as a major constituent with higher concentration in seeds.[9] The recognition of anxiolytic effects of non-benzodiazepine azapirones agents (buspirone, gepirone, and ipsapirone), which act as 5HT1A partial agonists and their therapeutic role in clinical anxiety and mood disorders has further focused attention on the 5-HT1A receptor. Although the azapirones interact with other neurotransmitter systems, such as the dopaminergic and noradrenergic, they display nanomolar affinity for 5HT1A receptor sites.[10] Buspirone appears to only interact with the dopaminergic system with reasonable potency and exhibits properties of both a dopamine agonist and a dopamine antagonist. This suggests that dopamine is implicated in the etiology and expression of anxiety. Based on earlier studies it has been postulated that dopaminergic agents may play an important role in the pharmacotherapy of anxiety.[11]

Mucuna pruriens is a popular medicinal plant of India, which has long been used in Ayurvedic system of medicine. Numbers of studies have

shown beneficial effects of Mucuna pruriens as aphrodiastic, anti-parkinsonism, hypoglycemic, antioxidant, antibacterial, antifungal, and anticancer agents.[12-18] Phytochemical evaluation on the seeds revealed the presence of 5-indolic compounds, especially tryptamine and 5-hydroxytryptamine, alkaloids like flavonoids, mucunine, mucunadine, prurine and prurienine.[19,20] Flavonoids are an important group of polyphenolic compounds derived from nature. These compounds have been shown to possess many useful biological activities like antioxidant, anti-inflammatory, cytotoxic[21] and anti-nociceptive properties.[22] Some of the naturally occurring flavonoids and their synthetic derivatives have been reported to selectively bind to the central benzodiazepine receptors and to exert anxiolytic and other benzodiazepine like effects in animals.[23] Therefore present study was planned with the objectives to evaluate anti-anxietyeffect of Mucuna pruriensusing elevated plus maze in mice.

MATERIAL AND METHOD

Selection of Animals

The study was conducted in the Department of Pharmacology, King George's Medical University (KGMU), Lucknow. Prior permission was sought from the Institutional Animal Ethics Committee (IAEC) for conducting the study [Project no. 47/IAEC/2013]. Adult healthy male Swiss Albino mice, of similar physical constitution (in terms of age, body weight), weighing 20-30 g had been use dinstudy. Animals had been obtained from animal house of Indian Institute of Toxicology Research, Lucknow, which is certified by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) for breeding and housing of animals. The animals were housed in our Institutional Animal facility under temperature, humidity and light and dark cycle-controlled environment [25 ± 2°C, 70%, 12 hrs. cycle) and were given standard pellet diet and water ad libitum. The maintenance of the animals and the experimental procedures were in accordance with the 'Guide for the Care and Use of Laboratory Animals' (Latestrevisionin 2011) and the guiding principles of IAEC which strictly adhered to the guidelines of CPCSEA.[24]

Drugs, Dosage and Treatment Groups

Mucuna pruriens (MP) seed extract was procured from Himalaya drug company, Bangalore, India. The standard drug Diazepam (NEON Laboratories) was purchased from government authorized medical store. It was administered in a dose of 2 mg/kg, i.p.[25] MP dissolved in distilled water and administered per-orally (p.o.). Total 30 mice were divided randomly into control and experimental groups (n=6). Group 1 received the Distilled water and served as the control group, group 2 received the standard drug diazepam (2 mg/kg) i.p., groups 3, 4 and 5 received the test drug (MP) in doses of 100, 200 and 400 mg/kg, per-orally.

ELEVATED PLUS MAZE

The elevated plus maze model is well established animal model for testing anxiolytic drugs.[26] The ratio of enteries, time spent and rearing behaviour in open arms to close arms reflects the safety of close arms with relative fearfulness of open Arms. Exploring behaviour in the open arm is anatural tendency when a mouse is exposed to a new environment. The plus maze apparatus constructed of wood consisting of two open arms (16x5 cm) and two closed arms (16 x 5 x 12 cm) and a central platform (5×5 cm), arranged in such a way that the two arms of each type were opposite to each other to give the apparatus a plus sign appearance. The entire maze was elevated to a height of 25 cm above the floor.[27] Animals were brought to the testing room 1 hr. prior to testing. Each mouse was placed at the centre of the elevated plus maze with its head facing the open arms. The dose administration schedule was so adjusted that each mice was having its turn on the elevated plus maze apparatus 30 min after Diazepam (2 mg/Kg i.p.) and 60 min after the oral administration of the extracts doses and vehicle. Each mouse was observed for a total of 5 minutes at approximately 2 m distance from the apparatus. Entry into an arm was defined as the point when the animal places all four paws into the arm.

STASTISTICAL ANALYSIS

All result are expressed as mean ± S.D. Statistical analyses were carried out using the software package SPSS (Version 17.0). Data were analyzed using one way analysis of variance (ANOVA), to assess the comparability of the groups assigned to the treatment groups followed by Tukey's multiple comaparison tests. P values <0.05 were considered significant.

RESULT

In EPM distilled water treated animals the time spent in the open and closed arms, and entries in the open and closed arms were compared with Mucuna pruriens extract at the dose of 100 mg/kg, 200 mg/kg and 400 mg/kg & also Diazepam (2 mg/kg). MPE at the dose of 200 mg/kg and 400 mg/kg showed significant ($p<0.05$) increase in the time spent in the open arms and at the dose of 400 mg/kg it showed significant ($p<0.05$) increase in number of entries in open arm (Graph 2). Furthermore, MPE 200 and 400 mg/kg had decrease in time spent and number of entries in closed arm (Table 1) as compared to control group showed a significant ($p<0.05$) in elevated plus-maze.

MPE has shown dose dependent increase in time spent and no. of entries in open arm. Among the groups which received different doses of MPE, the maximum time spent in open arm is seen at dose of 400 mg/kg (Table 1 and Figure 1&2), followed by dose of 200 mg/kg and then at 100 mg/kg. As compared to control group, it is significant at the dose 200 mg/kg and 400 mg/kg of MPE and with diazepam.

Table 1: Effect of MPE on EPM in mice

GROUP	Time spent in seconds (Mean±SD)		No. of entries (Mean±SD)	
	Open arm	Close arm	Open arm	Close arm
Distilled water	17.67±1.51	200.83±13.58	3.50±1.05	14.67±2.38
Diazepam (2 mg/kg)	91.33±8.76**	135.67±6.10**	14.00±1.37**	7.67±1.63**
***Mucuna pruriens* (100 mg/kg)**	25.00±4.37	186.60±9.50	4.00±.90	12.50±1.87
***Mucuna pruriens* (200 mg/kg)**	30.08±4.26**	182.15±7.79*	5.17±1.47	11.33±1.51*
***Mucuna pruriens* (400 mg/kg)**	78.83±8.79**	137.50±5.96**	10.50±1.38**	8.33±0.82**
p-value	< .01##	< .01##	< .01##	< .01##

(p< .05) ## (p< .01) (ANOVA), * (p< .05), ** (p< .01), (Tukey's multiple comparison test).

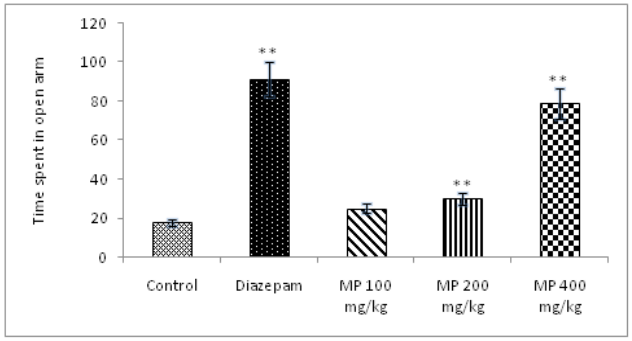

Figure 1: Effect of *Mucuna pruriens* (MP) and Diazepam (2 mg/kg) in EPM.

The column represents the mean of the time spent in open arm recorded in a 5 min observation period.

*(p< .05), **(p< .01), (Tukey's multiple comparison test), compared with control group.

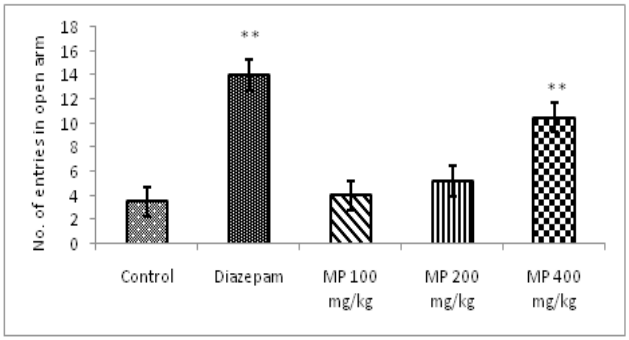

Figure 2: Effect of *Mucuna pruriens* (MP) and Diazepam (2 mg/kg) in EPM.

The column represents the mean of no. of entries in open arm recorded in a 5 min observation period.

*(p< .05), **(p< .01), (Tukey's multiple comparison test), compared with control group.

DISCUSSION

Traditional Indian Medicines are widely beneficial to mankind. Numbers of studies have shown beneficial effects of Mucuna pruriens. However, studies pertaining to antianxiety action of Mucuna pruriens are lacking so far. Toxicity study to determine the safe oral dose of Mucuna pruriens was not done in our study because acute toxicity tests, as per OECD 423 guidelines (2010) have already been conducted in many previous studies, reported that extract of Mucuna pruriens did not produce any toxicity, or significant behavioural change, or mortality upto an oral dose of 2000 mg/kg in albino mice.[28]

In the present study we have evaluated the anti-anxiety effect was assessed by using elevated plus maze. In the elevated plus maze, rodents tend to avoid entry into open arm. The animal hence prefers to spend more time and shows normal rearing behaviour in the closed arm. Anxiolytics tend to show an increase in the number of entries, time spent and rears in the open arms. They also increase the ratio of open arm to total arm entries. Hence, there is dose dependent increase in effect of drugs. Other workers have also reported similar results regarding same drug but at different doses.

MPE at the dose of 200 mg/kg and 400 mg/kg showed significant ($p<0.05$) increase in the time spent in the open arms and at the dose of 400 mg/kg it showed significant ($p<0.05$) increase in number of entries in open arm (Figure 2). Furthermore, MPE 200 and 400 mg/kg had decrease in time spent and number of entries in closed arm (Table 1) as compared to control group showed a significant ($p<0.05$) in elevated plus-maze. Antianxiety effect of MPE at the dose of 400 mg/kg is comparable to the antianxiety effect of diazepam.

Previous studies have reported that Mucuna pruriens contains L-DOPA and 5-hydroxy tryptophan (5-HTP) as a major constituent with higher concentration.[29] GAB Aergic and serotoninergic systems are most frequently associated with anxiety. Earlier studies have postulated that dopamine also plays an important role in the pharmacotherapy of anxiety.[30] The presence of L-DOPA and 5-hydroxy tryptophan (5-HTP) as a major constituent in Mucuna pruriens may suggest the role of dopaminergic and/or serotonergic pathways in its anxiolytic activities. Anxiety disorders can also be due to free radical induced damage to GABA ergic and serotoninergic system. Recent studies have shown that patients with anxiety disorders have higher activity levels of the enzymes like superoxide dismutase and glutathione peroxidase as well as higher lipid peroxidation activity.[31] Hence oxidative metabolism is also regarded as a plausible pathway that can affect the regulation of anxiety.

Phytochemical screening of Mucuna pruriens confirms the presence of flavonoids.[32,33] Flavonoids are known for their antianxiety effects. Benzodiazepine receptors activation by flavonoids has been proposed for the antianxiety responses produced by different flavonoids.[34] Therefore we hypothesize that flavonoids present in the extracts of Mucuna pruriensmay have acted through benzodiazepine GABA chloride channel receptors. However further studies are needed to know the exact mechanism responsible for antianxiety activity.

CONCLUSION

Due to wide benefits of the traditional Indian medicine to the mankind, many studies have shown the importance of the Mucuna pruriens. The Phytochemical screening of Mucuna pruriens confirms the presence of flavonoids and their antianxiety effects. Here we hypothesize that flavonoids present in the extracts of Mucuna pruriens may have acted through benzodiazepine GABA chloride channel receptors. However further studies are needed to know the exact mechanism responsible for antianxiety activity.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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