AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Evaluation of Anxiolytic Effect of Chronic Administration of *Mucuna Pruriens* In Wistar Albino Rats

Seema Rai[1], Preethi G Pai*[1], Rajeshwari S[1], Ullal Sheetal D[1], Nishith RS[1], Yogesh Belagali[1]

1.Department of Pharmacology, Kasturba Medical College, Mangalore, Manipal University

ABSTRACT

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 antidepressant activities. Previous studies have reported that *Mucuna pruriens* contains L-DOPA and 5-hydroxy tryptophan (5-HT) as a major constituent with higher concentration in seeds. However, literature search revealed no scientific data on its anxiolytic activity. So the present study was designed to evaluate the anxiolytic activity of *Mucuna pruriens* in a murine model. Wistar albino rats were divided into five groups (n=6). *Mucuna pruriens* administered at doses of 250,500,750mg/kg/day orally, was compared with the standard drug Diazepam (1.0mg/kg/day, oral) fed for 14 days. Three pharmacologically validated models elevated plus maze, bright and dark arena and open field test were used. The data presented was analyzed using one way ANOVA followed by Dunnett's post hoc test. A value of $p<0.05$ was considered as statistically significant. *Mucuna pruriens*at all three doses used significantly reduced the time spent in closed arm, increased the entries into open arm in elevated plus maze ($p < 0.05$). In bright & dark arena model, there was an increase in number of entries, time spent in bright chamber ($p < 0.05$) and in open field test, the time spent and squares crossed in central chambers were increased ($p < 0.05$). To conclude, the present study demonstrates the anxiolytic activity of chronic administration of *Mucuna pruriens* in Wistar Albino rats.

Keywords: Anxiolytic, *Mucuna pruriens*, diazepam, elevated plus maze, bright & dark arena model, open field test.

*Corresponding Author Email: meddocpai2@yahoo.com

Received 30 October 2013, Accepted 04 January 2014

Please cite this article in press as: Pai PG. *et al.*, Evaluation of Anxiolytic Effect of Chronic Administration of *Mucuna Pruriens* In Wistar Albino Rats. American Journal of PharmTech Research 2014.

INTRODUCTION

Anxiety is an unpleasant emotional experience of daily living characterized by a sense of apprehension, uneasiness or impending distress; this feeling is usually associated with changes in the autonomic nervous system and behaviour. It affects one-eighth of the total population worldwide and has become a very important area of research interest in psychopharmacology during this decade[1,2]. Anxiety is a cardinal symptom of many psychiatric disorders and an almost inevitable component of many medical and surgical conditions[3]. It is a normal emotional behaviour; when it is severe and/or chronic and disturbs the day-to-day activities, it becomes pathological and can precipitate or aggravate cardiovascular and psychiatric disorders.Although many drugs are available in modern medicine to treat anxiety disorders, they produce various systemic side effects or exhibit tolerance upon chronic use[4].

*Mucuna pruriens*Linn. (Fabaceae) is commonly known as cowhage plant or kapikacho or kevach in Hindi. It is an annual, climbing shrub grown in tropical regions. In Ayurvedic system of medicine, *Mucuna pruriens* is used in rheumatoid arthritis, diabetes, atherosclerosis, male infertility and nervous disorders[5]. Various activities attributed to this indigenous shrub include anti-parkinsonian[6],anti-tumor[7],neuroprotective[8], antioxidant[9], learning and memory enhancement and anti-diabetic activity[10].*Mucunapruriens* also showed significant antidepressant activity in rodent models[11].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[12]. The recognition of anxiolytic effects of non-benzodiazepine azapirones agents (buspirone, gepirone, and ipsapirone), which act as $5HT_{1A}$ partial agonists and their therapeutic role in clinical anxiety and mood disorders has further focused attention on the $5\text{-}HT_{1A}$ receptor. Although the azapirones interact with other neurotransmitter systems, such as the dopaminergic and noradrenergic, they display nanomolar affinity for $5HT_{1A}$ receptor sites[13].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[14].

With this background we decided to explore the anxiolytic activity of chronic administration of *Mucuna pruriens* in Wistar albino rats, using three pharmacologically validated experimental models namely elevated plus maze, bright and dark arena and the open field test [15]

MATERIALS AND METHOD

Animals

Healthy adult Wistar albino rats of either sex, weighing 150-200 g inbred in our central animal house (KMC, Mangalore)were used for the study. The rodents were housed in clean polypropylene cages, with dust free rice husk as a bedding material; three rats per cage under controlled laboratory conditions (Temperature: 25° ± 2°C, humidity (60% ± 10%) and 12 h light/dark cycle as per CPCSEA guidelines). The experimental animals were fed with standard chow containing fat 4.15%, protein 22.15%, carbohydrates 4% (supplied by Amruth laboratory animal feed manufactured by Pranav Agro industries ltd., Sangli) and water *ad libitum.* The rodents were allowed to acclimatize to these conditions for one week prior to the commencement of the study. Experiments were performed during the light phase of the cycle (10:00-17:00).The study was approved by the institutional animal ethics committee.

Drugs and dosage

Mucuna pruriens Choorna powder was obtained from SDP Remedies & Research Centre, Puttur, D.K. District, Karnataka. Standard anxiolytic drug, Diazepam was obtained from Ranbaxy Laboratories Limited, Gurgaon, Haryana. The doses of each drug were selected on the basis of earlier findings for diazepam[16] and *Mucuna pruriens*[17]. All the drugswere freshly prepared in distilled water andadministered orally in a constant volume of 10 ml/kg. Drugs, dosage and number of animals used per treatment are shown in (Table 1). Drugs/vehicle wereadministered once daily for fourteen days and the last dose was given on the fourteenth day, 60 minutes prior to the experiment.

Table1. Treatment groups

Groups (n=6)	Treatment	Dose
I	Control – Distilled water	10.0 ml/kg
II	Diazepam	1.0 mg/kg
III	*Mucuna pruriens*	250 mg/kg
IV	*Mucuna pruriens*	500 mg/kg
V	*Mucuna pruriens*	750 mg/kg

ANIMAL MODELS FOR TESTING ANXIOLYTIC ACTIVITY:

Elevated plus maze:

The wooden maze consists of two open arms (length 50 cm X breadth 10 cm) and two closed arms of the same size (height 40 cm). The arms of the same type are opposite to each other, with a central square of 10 cm. The maze is elevated to height of 50 cm above the floor.

Bright and dark arena

The apparatus consists of an open top wooden box. Two distinct chambers, a black chamber (20 X30 X 35 cm) painted black and illuminated with dimmed red light and a bright chamber (30 X

30 X 35 cm) painted white and brightly illuminated with a 100 W white light source located 17 cm above the box. The two chambers are connected through a small open doorway (7.5 X 5cm) situated on the floor level at the centre of the partition.

Open field test

The apparatus consists of a large rectangular box (100 X 80 cm) with 60 cm high walls. The floor is made of wire mesh and divided into twenty-five squares (outer 16 and central 9). The box is illuminated with a 100 W bulb placed 60 cm above the centre of the field.

Behavioural assessment

Each animal was tested initially in elevated plus maze and then, in light and dark arena paradigm and then, in open field test in a single setting. On the 14th day of drug or vehicle administration, each animal was placed in the centre square of the plus maze, facing one of the open arms and the observations were made 60 min after administering the last dose. The number of entries into and the time spent in open and closed arms and the number of rears in each arm in a five-minute period was noted.

Following the elevated plus maze test, the animal was placed at the centre of the brightly lit arena in the light and dark box. The number of entries into and the time spent in the bright arena, the number of rears in the bright and dark arenas and the duration of immobility was noted during the 5minutes of observation.

In the open field test, each animal were placed in one of the peripheral corner squares of the box and the number of peripheral and central squares crossed, the time spent in central squares and the number of rears were observed for a five-minute period. Following each trial, the apparatus was cleaned with hydrogen peroxide to mask the odour left by the animal in the previous experiment. Hand operated counters and stop watches were used to score the behaviour of animals.

Statistical analysis:

Statistical analyses were carried out using the software package SPSS (Version 17.0). The data was analysed by one-way ANOVA with drug treatment as the independent factor. Post hoc comparisons were performed by applying Dunnet's multiple comparison test. A value of $P < 0.05$ was considered to be statistically significant

RESULTS AND DISCUSSION:

In the present study, we have studied the effects of chronic administration of *Mucuna pruriens*in three pharmacologically validated experimental models of anxiety –elevated plus maze, bright and dark arena and open field test. All the studied models of anxiety are based on the theory that

unfamiliar, brightly lit and an open environment induces stress which provokes anxiety, thereby inhibiting normal behaviour in rats.

a. Elevated plus maze

In the elevated plus maze, the open arms are more fear provoking, unprotective and anxiogenicthan the closed arms. The animal hence prefers to spend more time and shows normal rearing behaviour in the closed arm. The ratio of entries, time spent and rearing behaviour in open arms to closed arms reflects the safety of closed arms with relative fearfulness of open arms[18]. The reduction in entry, time spent, rearing in open arms, ratio of open arm to total arm entries and increased defecation are the indications of high level of fear or anxiety. Anxiolytic drugs increase the proportion of entries, time spent and rearing in open arms. They also increase the ratio of open arm to total arm entries.

The results as given in table 2 indicate that the diazepam (1.0 mg/kg) treated rats showed a highly significant($p<0.001$) increase in the number of open arm entries, percentage ratio of open arm to total arm entries and time spent in the open arms. They showed a significant ($p<0.05$) reduction in the time spent in the closed arms compared to control group. *Mucuna pruriens* treated rats exhibited a highly significant ($p<0.001$) and significant ($p<0.05$) increase in time spent in the open arms at doses, 500mg/kg, 750 mg/kg and 250mg/kg respectively.The increase in the percentage ratio of open arm to total arm entries was significant ($p<0.05$) at 500mg/kg dose level and highly significant ($p<0.001$)at 750 mg/kg dose used. Significant increase was also noted in the number of open arm entries along with number of rears in open arms at 750mg/kg dose. The time spent in the closed arms was significantly reduced ($p<0.05$).

Table 2: Effect of chronic administration of diazepam and*Mucuna pruriens* on behaviour of rats in elevated plus maze

Groups (n=6)	Number of open arm entries	Percentage of open /total arm entries	Time spent in open arms (seconds)	Time spent in closed arms (seconds)	Number of rears in open arms
Control	4.83±0.30	51.92±3.84	76.33±4.30	222 ± 4.73	7.67±1.02
Diazepam 1.0 mg/kg	8.67±0.55**	76.48±2.42**	211.83±9.00**	88.17 ± 9.0*	11.8±1.30
Mucuna pruriens (250mg/kg)	5.50±0.67	52.38±4.04	109.67±5.27*	273.83±15.1	9.33±1.33
Mucuna pruriens (500mg/kg)	6.33±0.49	64.17±2.03*	156.33±5.78**	290.17±0.6	10.83±1.19
Mucuna pruriens (750mg/kg)	7.00±0.63*	71.19±1.94**	207.33±8.52**	92.67±8.52*	12.33±1.33*

(All values are expressed as mean ± SEM; **P < 0.001 vs. control,*P < 0.05 vs. control)

*Mucuna pruriens*in all the three doses studied, increased the number of entries, time spent and rearing in the open arms compared to control. These behavioural changes were not only comparable to those produced by diazepam but *Mucuna pruriens*at 750 mg/kg showed a significant increase in time spent and rears in open arm than the diazepam treated group. This suggests that *Mucuna pruriens* showed anxiolytic effect in animal models comparable to that of diazepam.

b. Bright and dark arena

In the bright and dark arena, rodents tend to avoid entry into and reduce spontaneous exploratory behaviour in the brightly illuminated area, a natural tendency when a rat is exposed to an unfamiliar environment. Anxiolytics tend to show an increase in the number of entries, time spent and rears in the bright arena.

The results given in table 3 indicate that the diazepam (1.0 mg/kg) treated rats showed a highly significant ($p<0.001$) increase in the number of bright chamber entries, time spent and number of rears in bright arena compared to control group. *Mucuna pruriens* treated rats at all the three doses also significantly increased the time spent in the bright arena (250,500 and 750 mg/kg) compared to control. They also showed significant($p<0.05$) increase in the number of bright chamber entries and rears in bright arena(750mg/kg).Moreover, the diazepam treated rats showed significant increase in the duration of immobility compared tocontrol whereas, the *Mucuna pruriens* treated groups in all the three doses reduced the immobility compared to the diazepam treated groups. Moreover, at the dose of 750mg/kg *Mucuna pruriens* showed a significant increase in the number of entries and time spent in the bright arena compared to diazepam as well.

Table 3: Effect of chronic administration of diazepam and*Mucuna pruriens* on behaviour of rats in Bright and dark arena model:

Groups (n=6)	Number of bright chamber entries	Time spent in bright chamber (seconds)	Number of rears in bright chambers	Number of rears in dark chambers	Immobility
Control	1±0	7.50±1.05	0.33±0.21	5.17±0.47	6.17 ± 3.12
Diazepam 1.0 mg/kg	3.17 ± 0.31**	25.17 ± 2.01**	3.00 ± 0.77*	13.00 ± 1.31**	24.50 ± 2.89**
Mucuna pruriens (250mg/kg)	1.50±0.34	16.67±1.49*	1.33±0.42	6.83±0.47	1.00±0.51
Mucuna pruriens (500mg/kg)	2.00±0.25	21.50±0.76**	1.17±0.40	6.67±0.55	0.67±0.66
Mucuna pruriens (750mg/kg)	2.83±0.30**	25.17±1.13**	1.33±0.49*	12.50±0.99**	0.50±0.34

(All values are expressed as mean ± SEM; **P < 0.001 vs control,*P < 0.05 vs control)

c. Open field test

In open field test, forced confrontational situations, induce anxiety which makes the rodents prefer the periphery, a behaviour called thigmotaxis. An increase in central locomotion or in time spent in the central part of the device without increasing the total locomotion is interpreted as anxiolytic activity.

The results given in table 4 indicate that the diazepam (1.0 mg/kg) treated rats and all the doses of *Mucuna pruriens* (250,500 and 750 mg/kg) showed significant increase in the number of squares crossed, rears and time spent in the central squares. There was also a significant reduction in the number of squares crossed and time spent in the peripheral squares compared to the control group.

Table 4: Effect of chronic administration of diazepam and*Mucuna pruriens* on behaviour of rats in open field test:

Groups (n=6)	Number of Peripheral squaresCrossed	Time spent in Peripheral squares	Number of Central squaresCrossed	Time spent in Central squares	Number of Central squaresRears
Control	56.33±4.78	288.50±3.46	2.17±0.65	11.50±3.46	0.67±0.33
Diazepam 1.0 mg/kg	15.17 ±1.16**	148.67±6.78**	17.00±1.69**	151.33± 6.78**	7.83±0.91**
Mucuna pruriens (250mg/kg)	23.83±3.07*	201.33±9.16	12.00±1.46*	98.67±9.16**	7.00±1.43**
Mucuna pruriens (500mg/kg)	19.83±1.64**	180.83±5.38**	12.33±0.80**	119.17±5.38**	8.50±1.25**
Mucuna pruriens (750mg/kg)	16.33±1.35**	153.00±3.47**	17.00±1.46**	147.00±3.47**	7.00±0.57**

GABAergic and serotoninergic systems are considered among the principal regulatory systems of anxiety. Standard anxiolytics like BZDs act through the GABA A- BZD receptor – Chloride channel complex. Then on-benzodiazepine agents (buspirone) act as $5HT_{1A}$ partial agonists and have therapeutic role in clinical anxiety and mood disorders. Buspirone exhibits properties of both a dopamine agonist and a dopamine antagonist suggesting that dopamine may also be implicated in the etiology and expression of anxiety. Earlier studies have also postulated that dopaminergic agents may also play an important role in the pharmacotherapy of anxiety[13].

Oxidative stress (OS) represents a loss of balance in oxidation-reduction reactions. It is characterized by the reduced ability of the antioxidant defence system to efficiently eliminate the excess of the oxygen-derived species production, eliciting the toxicity of oxygen and its detrimental effects. 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[19]. Hence oxidative metabolism is also regarded as a plausible pathway that can affect the regulation of anxiety.

CONCLUSION:

The results of the present study indicate that *Mucuna pruriens* on chronic administration, showed significant anxiolytic activity in the animal models studied. 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. However, further studies are required to elucidate the targets of action and the exact mechanism of action of *Mucuna pruriens*as an antianxiety drug.

REFERENCES

1. Ban TA, et.al. Therapeutic monograph on anxiolytic-sedative drugs. CMAJ 1981; 124:1439-46.
2. Hwa YC, Jeong HP, Jin TH, Hwan SY, Sukjil S, Bang YH, et.al. Anxiolytic-like effects of Gensinosides on the elevated plus-maze model. Biol. Pharm. Bull. 2005; 28 (9): 1621-25.
3. Baldessarini RS. Drug therapy of depression and anxiety disorders. In: Laurence L Brunton, Lazo JS, Keith L Parker, editors. Goodman & Gilman's The Pharmacological Basis of Therapeutics. New York: McGraw Hill 2006; 452-454.
4. Pari L, Maheshwari JU. Hypoglycaemic effects of *Musa sapientum L* in alloxan induced diabetic rats. J Ethnopharmacol. 1999; 38:1-5.
5. Buckles, D. Velvet bean (*Mucuna pruriens*): A "new" plant with a history. Economic Botany1995: 49(1); 13-25.
6. Katzenschlager, R., Evans A., Manson A. *Mucuna pruriens* in Parkinson's disease: a double blind clinical and pharmacological study. J Neurol Neurosurg Psychiatry 2004;75: 1672-1677.
7. Manyam, B.V., Dhanasekaran M., Hare T.A. Neuroprotective effects of the antiparkinsonian drug *Mucuna pruriens*. Phytother. Res 2004; 18(9): 706-712.
8. Tripathi, Y.B, Upadhyay A.K. Antioxidant property of *Mucuna pruriens* Linn. Current Science 2001; 80(11): 1378.
9. Poornachandra M N, Khanam S, Shivananda BG, Shivananda TN, Dris R.*Mucuna pruriens* (L.) DC – A novel drug for learning and memory retrieval. Journal of Food, Agriculture & Environment; 2005; 3:13 – 15.

10. Bell, EA, Janzen D H. Medical and ecological considerations of L-Dopa and 5-HTP in seeds. Nature 1971; 229:136-137.
11. Pati D, Dilip K P, Mahesh R, Vadiraj K, Hemant RJ. Anti-depressant-like activity of *Mucuna Pruriens*; A traditional Indian herb in todent models of depression Pharmacology online 2010 ;1: 537-551.
12. Daxenbichler, ME, VanEtten CH, Hallinan EA, Earle FR, Barclay AS. Seeds as sources of L-dopa. Journal of Medicinal Chemistry 1971;14: 463-465.
13. Lowry CA, Johnson PL, Hay-Schmidt A, Mikkelsen J, Shekhar A. Modulation of anxiety circuits by serotonergic systems. Stress 2005; 8: 233–246.
14. Taylor DP, Riblet LA, Stanton HC, Eison AS, Eison MS, Temple DL Jr. Dopamine and antianxiety activity. Pharmacol Biochem Behav. 1982;17 (l1): 25-35.
15. Vogel GH, Vogel WH, editors. Drug discovery and evaluation. Pharmacological Essays. New York: Springer (Publishers). 1997; 430-435.
16. H.N.G. Krishna, R.B. Sangha, N. Misra, and M.R.S.M. Pai. Antianxiety activity of NR-ANX-C, a polyherbal preparation in rats. Indian J Pharmacology 2006; 38(5):330-335.
17. Parekar S.S. and Somkuwar A.P. Acute oral toxicity of *Mucuna pruriens* in Swiss Albino mice. Int Res J Pharm 2011; 2(5): 162-63.
18. Pellow G, Chopin P, File SE, Briley M. Validation of open-closed arm entries in elevated plus maze as a measure of anxiety in the rat. J Neurosci method 1985; 14:149-67.
19. Akihiro T, Takeshi S, Futoshi Y, Hideyuki I. Evaluation of antioxidant activity of vanillin by using multiple antioxidant assays. Biochimica et biophysica act.2010;1810:170-7.