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Research Article

Evaluation of Antianxiety Effect of *Mucuna Pruriens* (L) –An Experimental Study in *Wistar Albino Rats.*

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Abstract: *Mucuna Pruriens* (MP) is a plant legume growing well in tropical countries like Africa and Asia. Various parts of this plant are used in traditional medicine to treat diseases like Parkinsonism and male infertility and as anabolic agents. These effects are attributed to the biologically active components with antioxidant properties. Many studies are highlighting the antiparkinsonian effect of MP. Twenty-four Wistar albino rats were used in this study to investigate the antianxiety activity. The animals were separated into four groups of six (Control, Diazepam-5mg/kg, MP-200mg/kg, and MP-400/kg). All animals were tested for 18 weeks, with a six-week interval beginning with week one. This study aims to observe the effect of MP on anxiety on the Elevated Plus Maze (EPM). Effects of test drug and control were noted by observing the time spent in the Open & Closed arm of EPM and the number of entries into the open & closed arm and compared among the four groups. Observations were analyzed using One-way ANOVA & Dennett's multiple comparisons tests. Animals treated with Mucuna Pruriens (200, 400mg/kg) produced a significantly increased number of entries and time spent in the open arm in the EPM ($p<0.0001$) compared with the control group. *Mucuna Pruriens*, used in this study at doses of 200mg/kg & 400mg/kg, shows an antianxiety effect comparable to that of the standard reference drug diazepam. Diazepam appeared to have a soothing effect, and no sedation was observed with MP powder based on the above results in this study.

Keywords: *Mucuna Pruriens*, Anti-Anxiety effect, Elevated Plus Maze, Traditional Medicine, Plant Extract, Anti-anxiety drugs.

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1. INTRODUCTION

Anxiety is a common everyday experience and is usually associated with stress. However, some people may develop high anxiety as a personality trait, causing physical and emotional symptoms such as excessive fear, restlessness, and insomnia [1, 2]. Physical symptoms may present as sweating, palpitations, or gastrointestinal disorders. Panic disorder, phobic states, and generalized anxiety disorder are serious conditions. Early stages of anxiety can be treated successfully by non-pharmacological methods, but significant disorders influencing lifestyle and work may need medical treatment [3]. The current therapeutic agents include benzodiazepines, Azapirone, beta-blockers, SSRIs, and SNRIs. Most of these drugs reduce some anxiety symptoms but may produce adverse effects with regular use. Benzodiazepines have sedation; frequent use leads to dependence and memory problems [4]. Cognitive impairment is a problem in the elderly. Azapirone does not cause dependence but can lead to interactions with other drugs [5]. Beta-blockers reduce the physical symptoms of adrenergic overactivity, but psychological symptoms remain present[6]. So searching for suitable agents to treat anxiety is required. This study is undertaken to observe the effects of a herbal plant extract, MP leaf extract, and its use in controlling anxiety. The current study on selected herbal preparations was conducted on laboratory animals using a well-accepted method to observe behavioral effects. *Mucuna Pruriens* (MP) is widely cultivated in Asian countries and Africa. It is commonly known as velvet bean, cowage, and monkey tamarind. MP belongs to the plant kingdom of Trichophytes, the family Fabaceae, the genus Mucuna, and the species Pruriens [7]. As an herbal product, MP is used in Ayurvedic medicine and has many active ingredients like flavonoids, alkaloids, amines, polyphenols, and fatty acids [8]. It is a rich source of dopamine and is used as a prodrug in treating PD [9]. Some publications mention the use of this substance in male infertility. According to current research, there is no conclusive evidence of *Mucuna Pruriens* leaf extract's anti-anxiety activity. Several research studies have demonstrated the anti-anxiety activity of Mucuna pruriens seed extract [10], which inspires me to conduct a study on the leaf extract because this plant is highly safe for long-term use and is widely used in the Indian subcontinent. It helps the body fight against physical, chemical, or biological stress because stress raises the chance of anxiety. Because of those mentioned above, it was determined to do the current experiment to evaluate whether *Mucuna Pruriens* had an antianxiety effect.

2. MATERIALS & METHODS

2.1. Ethical Approvals

The study was conducted in the experimental pharmacology laboratory attached to the animal house facility of Karuna Medical College in Palakkad, Kerala, India. IAEC approval was obtained from CPCSEA before this study, Certificate No. (KMCH/CPCSEA/IHEC/March-2019/1).

2.2. Animals used and Housing

The animals were fed the standard rat chew diet, procured from Govt Veterinary College, Mannuthy, Thrissur, Kerala. The animals were kept in a room with adequate ventilation, exhaust, and ceiling fans and subjected to a 12-hour light cycle. The temperature was between 28 and 32 degrees Celsius. The food was delivered in pellet form. Drugs were administered in the drinking water at a certain time during the day, between 10 a.m. and 4 p.m., as intended in the study. However, the animals were deprived of water for two hours before administering the medication. Animals were housed in polypropylene cages, with just three rats per cage. Rats of both sexes were kept in separate cages; they were acclimatized for one week in advance to use them for the study. The bedding material was rice husk, which was replaced every other day.

2.3. Plant collection and authentication

Leaves of *Mucuna Pruriens* were collected from an agricultural field in April 2019 in Pollachi, Tamil Nadu, India. Mr. Rambabu V, Head of the Department of Botany, Vikas Degree College (P.G. Course), Vissannapeta, Krishna District, Andhra Pradesh, India, authenticated the plant.

2.4. Extraction and phytochemical evaluation

After being finely pulverized and stored in an airtight container, the leaves of MP were shade-dried for ten days at room temperature. Approximately 500 g of finely powdered dried leaf powder was used. The powder was continuously heated and percolated through various solvents with varying degrees of polarity, including pet ether, chloroform, acetone, ethanol, and water. After drying the extracts in the rotary evaporator, the percentage yield of the extracts was determined. Then, several phytochemical components, including alkaloids, flavonoids, glycosides, phenols, saponins, sterols, tannins, proteins, and carbohydrates, were examined in the extract to determine their presence. First, the primary phytoconstituents of the plant extracts were analyzed using conventional qualitative techniques. Next, the plant extracts were examined for several chemicals, including glycosides, alkaloids, flavonoids, phenolic compounds, saponin, steroids, quinine, and tannin [11].

2.5. Phytochemical Analysis

1. **Test for alkaloids: (**Dragendroff's reagents): Solution A: 20 ml of water and 0.6 g of bismuth sulfate; Solution B: 50 mL of water was used to dissolve 6 g of potassium iodide. After being combined, solutions A and B were left to strand. Then, the supernatant was decanted from potassium iodide to make up to 100 mL.
2. **Test for flavonoids**: Add a few drops of neutral $FeCl_3$ to 1 ml of stock alcohol solution and 5 ml of an extract to 1 ml of alcohol before doing the ferric chloride test.
3. **Test for Phenols:** Combine 1 mL of an extract with 5 mL of alcohol and a few drops of neutral FeCl3 to check for phenolic chemicals.
4. **Test for tannins** using a minimum of 1 ml of extract and H20. Filter, then add a few drops of $FeCl_3$ solution to the filter.
5. **To check for saponins:** Stir 1 mL of extract into 20 mL of distilled water for 15 minutes.
6. **To test for steroids**, 1 ml of the extract was mixed with 1 ml of the drug's methanolic extract, 1 ml of chloroform, 2–3 ml of acetic anhydride, and 1–2 drops of concentrated H2SO4.
7. **To test for quinine**, 1 ml of the extract was mixed with a few drops of alcoholic KOH.
8. **To test for glycosides**, mix 1 g of powder with 2–3 ml of distilled water and add 2–3 drops of a 1% alcohol–naphthol solution to one side of the test tube.

Table 1: Results of Phytochemical analysis of Mucuna Pruriens with different solvents

Phytochemical analysis of Mucuna Pruriens									
S.No	Solvents	Phytochemicals							
		F	St	A	S	PC	Q	G	T
1	Ethyl Acetate	✓	×	✓	×	✓	×	×	✓
2	Ethanol	✓	×	✓	×	✓	×	✓	✓
3	Acetone	✓	×	✓	×	✓	×	×	✓
4	Hexane	✓	×	✓	×	✓	×	×	✓
5	Petroleum ether	✓	×	✓	×	✓	×	✓	✓
6	Chloroform	✓	×	✓	×	✓	×	✓	✓
7	n-butanol	✓	×	✓	×	✓	×	✓	✓
8	Methanol	✓	×	✓	×	✓	✓	✓	✓
9	Water	✓	×	✓	×	✓	×	✓	✓
10	n-propyl alcohol	✓	×	✓	×	✓	×	✓	✓
11	Diethyl ether	✓	×	✓	×	✓	✓	✓	✓

F-Flavonoids, St-Steroids, A-Alkaloids, PC-Phenolic Compounds, Q-Quinine, G-Glycoside and T-Tannins

2.6. Drugs and Dosage

Diazepam (5 mg/kg) was given orally mixed in drinking water; Powder leaf extract of *Mucuna Pruriens* obtained from Himalaya Natural Products (Himalaya, Greenland Trading Company, Delhi, (Ayush License Number: 1.3319E+13) and authenticated) was given orally mixed with rat chow diet with the supervision of a veterinarian to ensure proper administration of doses of *Mucuna Pruriens*. The control group received a rat chow diet. The test group included two doses (200 mg/kg and 400 mg/kg) of MP; diazepam was given at 5 mg/kg as the reference standard for testing the antianxiety effect.

Group-1: Control Rat Chow diet (q.s)
Group-2: Diazepam 5mg/kg (Reference Standard)
Group-3: *Mucuna Pruriens* 200mg/kg (Test drug[1])
Group-4): *Mucuna Pruriens* 400mg/kg (Test drug[2])

2.7. Study design for anti-anxiety activity

The animals used in this study were healthy adult Wistar albino rats. Four groups of six rats each were used for this study. It includes one control group and one group for a testing reference standard (diazepam). Two groups of six animals each were used to study the effect of the test drug (*Mucuna Pruriens* powdered leaf extract 200 & 400 mg/kg). These drugs were given daily for 18 weeks, and the animals were tested to observe the effects every six weeks up to the 18th week (1st, 6th, 12th, and 18th). Anti-anxiety effects of the test drug were observed using the Elevated Plus Maze (EPM) [12]. This apparatus contains four arms: two open and two closed. The behavioral model is based on the fact that animals prefer to remain in a closed or open arm. Animals naturally remain in closed arms and avoid reaching the open arm due to fear and anxiety of falling. Therefore, the proportion of time spent in the open arm and the total entries into both the closed and open arms are used to calculate the antianxiety effect.

2.8. Statistical analysis

Results of the data were analyzed statistically by one-way ANOVA, multiple comparisons made by Dennett's test, and the comparison between the control group and the standard (Diazepam 5 mg/kg) group, MP-200, and MP-400. In addition, the difference between the control group and the standard and MP-treated groups was analyzed. Finally, the results observed between these groups were compared. The p-value of (< 0.05) was considered significant.

3. RESULTS

Observations on time spent in the closed and open arms of EPM are presented in (Table 1 and Figure 1). The mean values of six rats in each group are calculated each week and presented for comparison. The control and test groups were observed regularly throughout the study (1 to 18 weeks). All groups were on a similar diet and moved around freely.

3.1. a. The minimum and maximum time spent in the open arm of EPM during 6–18 weeks, in seconds: (Table:1)

- Control group: (91.5 to 102.83)
- Diazepam group: (105.66 to 133.66)
- MP-200 group: (122.16 to 134.33)
- MP-400 group: (126.50 to 141.83)

3.1. b. The minimum and maximum time spent in the closed arm of EPM during 6–18 weeks, in seconds: (Table:1)

- Control group: (154 to 174)
- Diazepam group: (94.66 to 120.66)
- MP-200 group: (92.66 to 109.16)
- MP-400 group: (94.16 to 107.83)

Rats given diazepam and the test powder of MP showed a decrease in time spent in the closed arm from week one onward.

Table 2: Mean values of the time spent in open & closed arms with different drugs from week 1 to week-18 (SE ±9.4 open & ± 9.6 closed) 95% CI

Time spent in Open & Closed Arms						
		week 1	Week 6	week 12	week 18	Average
Control	Open	99.33	102.83**	91.66	91.5*	**96.33**
	Closed	174**	154*	162	166	**164.00**
Diazepam	Open	105.16*	129.16	133.66**	130.83	**124.70**
	Closed	94.66*	96.16	101.16	120.66**	**103.16**
MP-200	Open	122.16*	133.83	134.33**	130.83	**130.29**
	Closed	92.66*	107.33	109.16**	107.33	**104.12**
MP-400	Open	132	138.66	141.83**	126.5*	**134.75**
	Closed	107.83*	98.33	97	94.16*	**99.33**

Range of timings during the weeks of the study indicated with: Minimum*, Maximum** timings in seconds.

When the Overall time spent in the open and closed arms of animals was observed, the control group preferred to spend (96.33 Sec) in the open arm and (164Sec) in the closed arm, while the Diazepam group preferred to spend (124.70 Sec) in the open arm and (103.16 Sec) in the closed arm. When the results were carried over to the MP-200 and 400 groups, the MP-200 group preferred to spend (130.29 Sec) in the open arm and (104.12 Sec), the MP-400 Group preferred to spend (134.75 Sec) in the open arm and (99.33 Sec) in the closed arm. Even though there was very little difference in the time spent in open-arm readings of MP extract compared to diazepam, as far as the control group is concerned, the difference in the time spent with the control group is very high. (Table 2 & Figure 3). The time spent in the closed arm was reduced, and the time spent in the open arm was increased after diazepam and the test powder. These changes were observed from week one and continued to follow the same pattern during the ensuing period of study. The averages for each week were calculated and compared. Statistical analysis showed the difference to be significant (p 0.001) between the control and drug-treated groups. (Figure 1).

3.2. *Observations on the number of entries in the closed and open arms of EPM*

3.2. a. *The minimum and maximum Number of entries in the open arm of EPM during 6–18 weeks, in seconds:*

- Control group:(3.5 to 5.3)
- Diazepam group:(4.33 to 6.33)
- MP-200 group:(5 to 6.33)
- MP-400 group:(4 to 6.5)

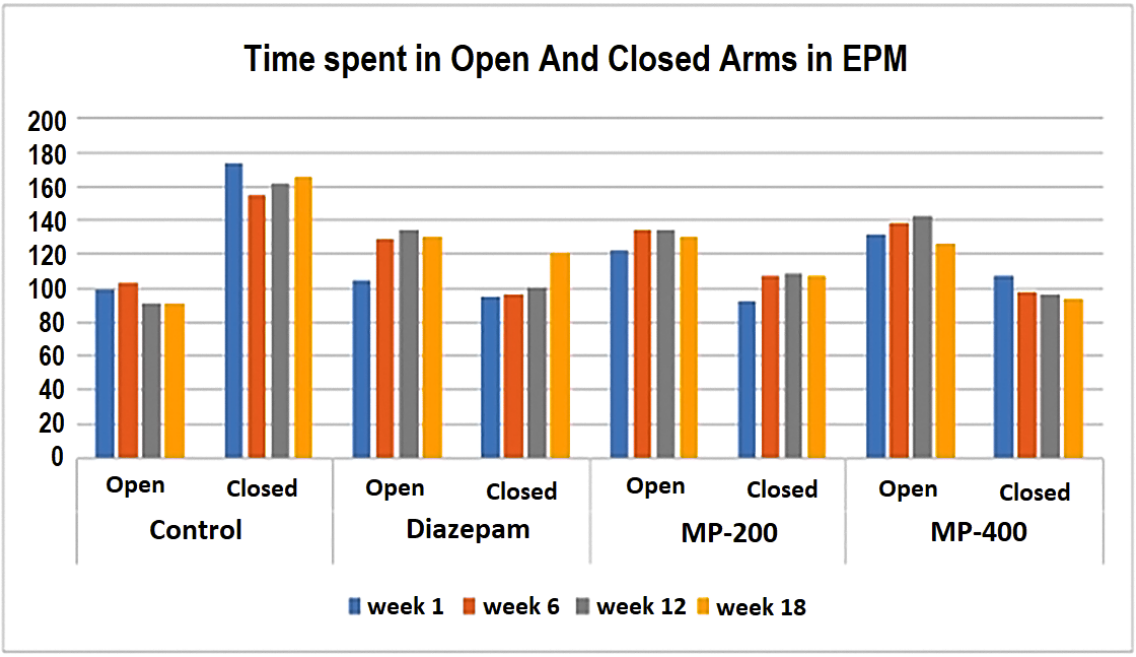

Fig 1: Mean differences in time spent in open and closed arms with different drugs and the duration in different weeks.

X-axis: Open & Closed Arms with different drugs
Y- axis: The time spent in seconds

Table 3: Mean values of the number of entries in open & closed arms with different drugs from week 1 to 18 (SE ±0.6 open & ± 0.2 closed) 95% CI

No of entries in Open Arm & Closed Arm on EPM						
		week 1	Week 6	week 12	week 18	**Average**
Control	Open	4.6	4.3	5.3**	3.5*	**4.425**
	Closed	4.5*	6.83	5.83	8**	**6.29**
Diazepam	Open	4.33*	6.16	6.33**	4.83	**5.4125**
	Closed	3.33**	3.16	3*	3.66	**3.2875**
MP-200	Open	6.33**	5.16	5*	5.5	**5.4975**
	Closed	3	3.16**	2.83	2.5*	**2.8725**
MP-400	Open	6.5**	5.6	6.33	4*	**5.6075**
	Closed	2.33	2.16*	3.66**	2.33	**2.62**

Range of timings during the weeks of the study indicated with: Minimum*, Maximum** timings in seconds.

3.2. b. *The minimum and maximum Number of entries in the closed arm of EPM during 6–18 weeks, in seconds:*

- Control group:(4.5 to 8)
- Diazepam group:(3 to 3.66)
- MP-200 group:(2.5 to 3.16)
- MP-400 group:(2.16 to 3.66)

When the Overall number of entries in the open and closed arms of animals was observed, the control group preferred to enter (4.42 times) in the open arm and (6.29 times) in the closed arm. In contrast, the Diazepam group preferred to enter (5.41) in the open arm and (3.28) in the closed arm. When the results were carried over to the MP-200 and 400 groups, the MP-200 group preferred to enter (5.49 times) in the open arm and (2.87 times), the MP-400 Group preferred to enter (5.60 times) in the open arm and (2.62) in the closed arm. Even though there was very little difference in the number of entries in open-arm readings of MP extract compared to diazepam, as far as the control group is concerned, the difference in the number of entries with the control group is very high. Considering the above results, the MP leaf extract produced anti-anxiety activity comparatively potent as diazepam. (Table 3 & Figure 4). The changes in the number of entries were noted after one week and continued in a similar pattern for the following weeks. The diazepam-treated rats were less active than the control rats, but the test drug did not cause any decrease in activity compared with the reference standard diazepam. The less active rats administered diazepam looked tired and sedated, while the MP powder group didn't produce tiredness or a soothing effect. The timings were significantly different between the diazepam and the control. Two doses of MP-200 and 400 also showed a significant increase in time spent with open arms compared to the control. The effect of two doses of the herbal powder was similar to that of diazepam. These results indicate that the powdered leaf extract of *Mucuna Pruriens* is equally effective as the reference standard with the added advantage of non-sedative action. These results show that diazepam and the test powder showed an increase in the number of entries into the open arm and a decrease in entries into the closed arm. The maximum increase in the open arm was observed with MP 400 mg/kg, and the maximum decrease in the number of entries into the closed arm was also observed with MP 400 mg/kg. (Figure 2).

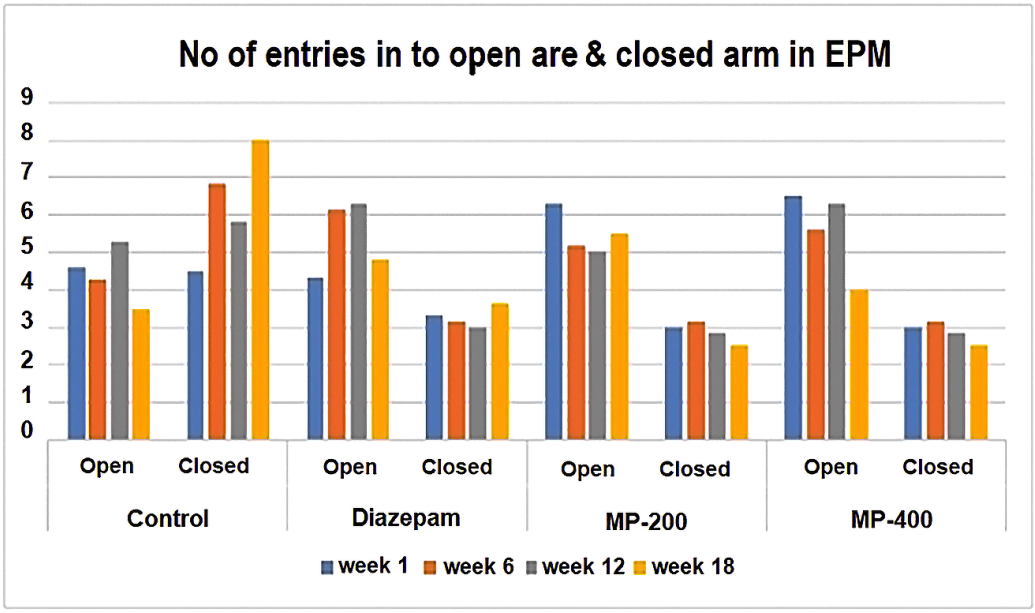

Fig 2: Mean differences in the number of entries into the open and closed arms with different drugs and the duration in different weeks.

X-axis: Open & Closed Arms with different drugs
Y- axis: The number of entries into the open and closed arms

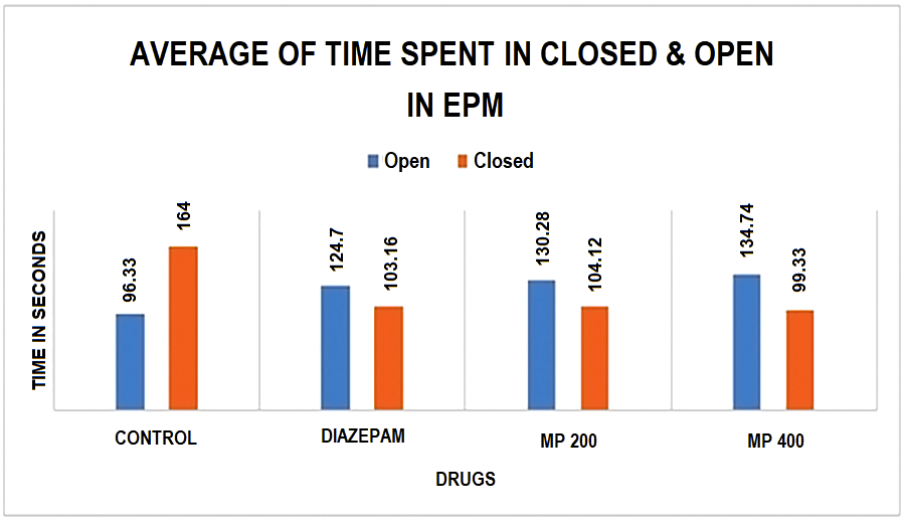

Fig 3: Graph indicates 18 weeks' average of time spent in both open as well as closed arms with different drugs; the animals treated with MP-200 & 400 have increased the duration of time spent in the open arm compared with the closed arm, all the readings of the drugs (O&C) were compared with the control group.

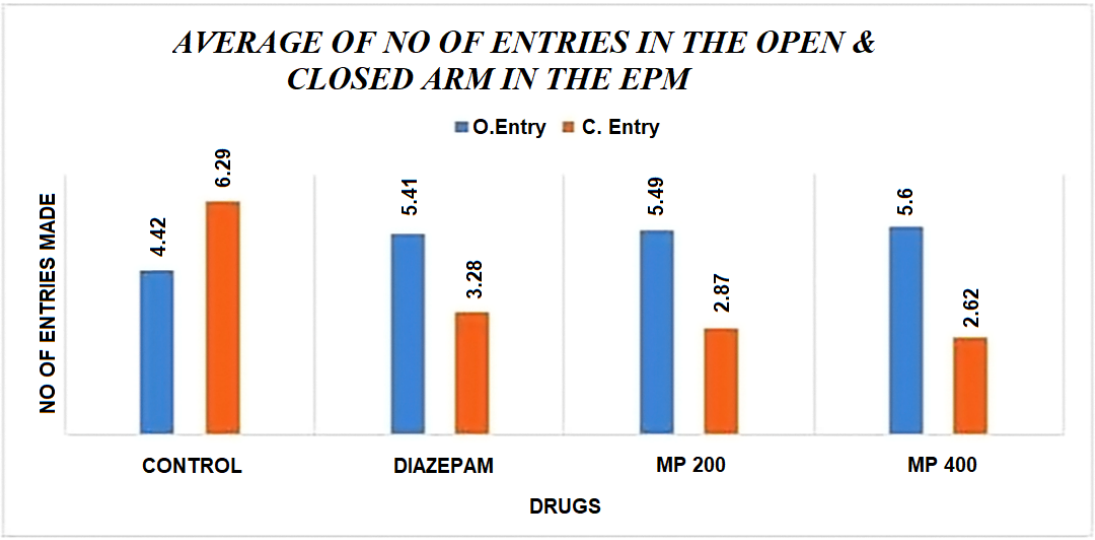

Fig 4: Graph indicates 18 weeks' average of the number of entries in both open as well as closed arms with different drugs; the animals treated with MP-200 & 400 have increased the Number of entries in the open arm compared with the closed arm.

4. DISCUSSION

The present study was aimed at evaluating the anti-anxiety effect of MP in comparison with the control and standard groups in Wistar albino rats by using the elevated plus maze method. Generally, rodents are anxious to enter the open arm; they spend more time in dark and closed environments [12]. The animals treated with anti-anxiety drugs tend to spend time with open arms[13], as they are not anxious due to the influence of the drugs. Based on the same phenomenon, we tested the anxiolytic activity of MP with graded doses of 200 mg/kg and 400 mg/kg, and the results were compared with the control group and the standard drug. In the EPM model, the MP group at doses of 200 and 400 mg/kg showed a significant (p 0.0001) increase in time spent in the open arm and a gradual decrease in time spent in the closed arm. The anxiolytic effect of MP was as effective as the standard antianxiety drug diazepam at 5 mg/kg. Previous research has discovered that *Mucuna Pruriens* contains a high concentration of L-DOPA [14, 15] and 5-hydroxytryptophan (5-HTP) [16], commonly associated with controlling anxiety. Previous research has suggested that dopamine is key in treating anxiety [14, 15]. The presence of L-DOPA and 5-hydroxytryptophan (5-HTP) as key constituents in *Mucuna Pruriens* may imply that dopaminergic and serotonergic pathways play a role in its anxiolytic properties [14-16]. Anxiety disorders may also be caused by free radical damage to the GABAergic and serotoninergic systems [17]. Recent research has found that patients with anxiety disorders had increased levels of enzyme activity, such as superoxide dismutase and glutathione peroxidase, as well as higher lipid peroxidation activity [17, 18]. As a result, oxidative metabolism is viewed as a possible mechanism that can influence anxiety control [18]. The phytochemical screening of *Mucuna Pruriens* indicates the presence of flavonoids. Flavonoids are well known for their anti-anxiety properties. Therefore, activating benzodiazepine receptors by flavonoids has been hypothesized as a mechanism for the antianxiety reactions elicited by certain flavonoids [19]. As a result, we hypothesized that the flavonoids included in *Mucuna Pruriens* extracts acted via benzodiazepine and GABA chloride channel receptors. However, more research is required to determine the mechanism responsible for anti-anxiety action. The toxicity studies have indicated that oral doses up to 2000 mg/kg of MP showed no significant toxicity. The acute toxicity studies per the OECD guidelines

2010 have already reported the safety of using this herbal product [20].

5. CONCLUSION

Anxiety is a major factor in developing a behavioral illness, which is the leading cause of many other diseases. It might affect the thinking behavior of patients suffering from these types of behavioral disorders. Diseases can be prevented or at least have their effects reduced with early identification and treatment. Anxiety is linked to increased mortality and morbidity due to changes in behavioral aspects. *Mucuna Pruriens* demonstrated an antianxiety effect comparable to the standard reference drug diazepam at doses of 200 mg/kg and 400 mg/kg. Diazepam appeared to have a soothing effect, but no sedation was observed with MP powder. The study's objective has thus been met based on the above results. The leaf extract of *Mucuna Pruriens* produces its antianxiety activity by different mechanisms, including its ability to lower sedation; this is a very good contributing factor and beneficial to mankind. Sedation greatly impacts daily life, as most anti-anxiety drugs lower anxiety by causing sedation as an adverse event. To understand the clear-cut mechanisms, there is a need for additional studies on its action on the changes happening in the brain tissues and the chemical changes happening in the mentioned region.

6. ABBREVIATIONS

MP: *Mucuna Pruriens*
EPM: Elevated Plus Maze
ANOVA: Analysis of Variance
SSRI: Selective Serotonin Reuptake Inhibitor
SNRI: Selective Norepinephrine Reuptake Inhibitor
PD: Parkinson's Disease
CPCSEA**:** Committee for Control and Supervision of Experiments on Animals
IAEC: Institutional Ethics Committee
qs: "Quantity Sufficient."
p.o.: per oral
OECD: Organization for Economic Co-operation and Development
GABA is gamma-aminobutyric acid.

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8. AUTHORS CONTRIBUTION STATEMENT

The authors confirm their contributions to the paper as follows: Study conception and design: Mr. Indla Ravi, Dr. Manivannan E, Dr. Kothai Ramalingam, and Dr. Regina Roy; data collection: Mr. Indla Ravi; analysis and interpretation of results: Dr. E Manivannan, Dr. V. Sivasankari, Dr. Kothai Ramalingam, draught manuscript preparation: Mr. Indla Ravi and Dr. Regina Roy, Mr. Arbind Kumar Choudary, and all the other authors reviewed the results and approved the final version of the manuscript.

9. DECLARATION OF COMPETING INTEREST

The authors declare that they do not have any known competing financial interests or personal relationships that could have influenced the work reported in this paper.

10. CONFLICT OF INTEREST

Conflict of interest declared none.

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