Section A-Research paper

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Evaluation of Antidepressant activity of aqueous leaf extract of *Mucuna pruriens*-An Experimental study in wistar albino rats.

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Aim: The aim of this study was to evaluate the antidepressant activity of crude aqueous leaf extract of Mucuna pruriens at doses of 200mg/kg and 400mg/kg using three behavioral parameters: forced swimming test (FST), tail suspension test (TST), and open field test by Photoactometer (OFT/PAM).

Objectives:

- To assess the antidepressant effects of Mucuna pruriens extract at different doses.
- To compare the effects of Mucuna pruriens extract with the standard antidepressant drug fluoxetine.
- To investigate the impact of Mucuna pruriens extract on immobility time in FST, TST, and OFT/PAM.

Results: The results of the study revealed a significant and dose-dependent decline in immobility time in all three behavioral tests (FST, TST, and OFT/PAM) when the animals were treated with the crude aqueous leaf extract of Mucuna pruriens at doses of 200mg/kg and 400mg/kg. This indicates the potential antidepressant activity of Mucuna pruriens extract. Furthermore, the observed effects were comparable to those of the standard antidepressant drug fluoxetine, suggesting that Mucuna pruriens extract may possess similar efficacy in reducing immobility time in these tests.

Keywords: *Antidepressants, monoamine oxidase, forced induced swimming test, tail suspension test, open field test, PAM: Photoactometer.*

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INTRODUCTION:

According to a report from the World Health Organization (WHO), there is a significant burden of depressive and anxiety disorders in lower and middle-income countries, affecting approximately 28.9% and 31% of the population, respectively. Globally, an estimated 129 million people, with a gender distribution of 54.1% female and 45.9% male, are affected by mental or behavioral disorders. However, only a small fraction of these individuals receive adequate care. Depression currently accounts for 15.6% of the global burden of disease, and based on previous WHO reports, it is projected to increase by an additional 15% by 2030. Disturbingly, rates of depression have increased by more than 25% in the first year of the pandemic, surpassing expectations. Suicidal tendencies are frequently observed among patients with depression and have a significant negative impact on cognitive behaviour, as reported by WHO[(1)]. Factors that exacerbate depression include stress, academic pressure, relationship issues, and socioeconomic and family-related problems[(2)]. Deficiencies in neurotransmitters such as glutamate, GABA, norepinephrine, and serotonin have been linked to depression, and current allopathic drugs, such as MAO inhibitors, selective SSRIs, and tricyclic antidepressants, aim to increase these neurotransmitter levels[(3)]. However, the notable drawback of these drugs is the range of side effects they can produce, prompting researchers to explore natural sources for new antidepressants. Several herbs, including Curcuma longa[4] and Echium vulgare[5], have been reported to possess antidepressant properties. Animal models have demonstrated the effectiveness of various plant species, driving the search for novel natural therapeutics for neurological disorders[(6)]. Mucuna pruriens, commonly known as "velvet bean"[(7)] is a perennial plant originating from tropical Asia and cultivated worldwide[(8)], including in various regions of India[(9)]. It features green, ovate leaves, pendulous flowers with five red petals[(10)], and contains various nutritional compounds such as proteins, carbohydrates, lipids, fibers, minerals (K+, Na+, Ca+, Phosphorus, Mg+, Iron, Copper, Zinc), vitamins, and flavonoids[(11)]. Mucuna pruriens has been associated with aphrodisiac, antioxidant, antitumor, antidiabetic, antibacterial, antiprotozoal, anti-snake venom, analgesic, and anti-inflammatory effects[(12)]. However, there is a lack of studies specifically examining the effects of Mucuna pruriens leaf extract on the central nervous system. This knowledge gap inspired the present investigation into the antidepressant activity of Mucuna pruriens leaf extract using established pharmacological models for assessing depression in experimental animals.

Materials and Methods:

In April 2019, fresh leaves of Mucuna pruriens (MP) were harvested from an agricultural field located in Pollachi, Tamil Nadu, India, and subsequently identified as the plant material. The authenticity of the plant was confirmed by Mr. Rambabu V, Head of the Department of Botany at Vikas Degree College (P.G. Course) in Vissannapeta, Krishna District, Andhra Pradesh, India.

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Ethical Approvals:

The research was carried out at the experimental pharmacology laboratory situated within the animal house facility at Karuna Medical College in Palakkad, Kerala, India. Prior to commencing the study, approval was obtained from the Institutional Animal Ethics Committee (IAEC) in accordance with the regulations of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The study was assigned Certificate No. (KMCH/CPCSEA/IAEC/March-2019/1).

Preparation of aqueous extract:

A quantity of 1 kg of Mucuna pruriens (MP) leaves was gathered and subsequently fragmented into small pieces. The leaf fragments were thoroughly cleansed and subjected to a 15-day percolation process in absolute ethanol at room temperature intermittently agitated. The resulting aqueous percolates were filtered using Whitman filter paper and subjected to controlled temperature evaporation (40 °C) under reduced pressure using a rotary evaporator. Once the evaporation process was complete, a brown semisolid residue weighing 1.25 g was obtained.

Phytochemical Analysis of Mucuna pruriens:

1. Carbohydrate Testing:

- Molisch's Test: A few drops of -Naphthol solution in alcohol were added to the 2-3 ml extract, followed by the addition of concentrated H2SO4. The formation of a violet ring indicates the presence of carbohydrates.
- Fehling's Test: Fehling's A and Fehling's B were mixed with the extract and heated in a boiling water bath. The presence of reducing sugars is indicated by the formation of a yellow to brick-red precipitate.
- Benedict's Test: Benedict's reagent was mixed with the extract and heated. The colour change to green, yellow, or red indicates the presence of reducing sugars.
- Barfoed's Test: Barfoed's reagent was mixed with the extract and heated. The presence of monosaccharides is indicated by the observation of a red precipitate.

2. Protein and Amino Acid Testing:

- Biuret Test: The extract was mixed with NaOH and CuSO4. A colour change to violet or pink indicates the presence of proteins.
- Ninhydrin Test: The extract was heated with Ninhydrin solution. The appearance of a purple or blue colour indicates the presence of amino acids.

3. Fats and Fixed Oils Testing:

- The extract was pressed between filter papers, and the presence of oil stains indicates the presence of fixed oils.

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4. Steroid Screening:

- Chloroform, acetic anhydride, and CONH2SO4 were added to the extract. The formation of a purple ring turning green with an acid solution indicates the presence of steroids.

5. Glycoside Testing:

- Keller Kiliani Test: Glacial acetic acid, FeCl3, and conc. H2SO4 were added to the extract. The formation of a reddish-brown layer indicates the presence of cardiac glycosides.
- Legal's Test: Sodium nitroprusside in pyridine and methanolic alkali were added to the extracts. The formation of a pink to red colour indicates the presence of cardiac glycosides.
- Borntrager's Test (Anthraquinone Glycosides): H2SO4 was added to the extract, and after boiling and filtration, benzene or chloroform was added. The formation of a red or pink colour after the addition of ammonia indicates the presence of anthraquinones glycosides.
- Saponin Glycoside Foam Test: Water was vigorously shaken with the extract, and the presence of persistent foam indicates the presence of saponin glycosides.

6. Phenolic Compounds and Tannins Testing:

- FeCl3 was added to the extract, and the formation of a deep blue-black colour indicates the presence of phenolic compounds.

7. Flavonoid Testing:

- Shinoda Test: Magnesium turnings and Con-HCL were added to the extract. The appearance of a pink colour indicates the presence of flavonoids.

8. Alkaloid Testing:

- Dragendroff's Test: The Dragendroff reagent was added to the extract to determine the presence of alkaloids an orange-brown PT was formed. Presence of alkaloids

The analysis of phytochemical constituents in Mucuna pruriens revealed significant levels of saponins and flavonoids, along with a moderate presence of tannins. However, the plant exhibited low levels of phenols and alkaloids.

Pharmacological Analysis of Drugs and Chemicals:

Fluoxetine (15 mg/kg) was utilized as the standard medication and diluted in a 10% solution of dimethyl sulfoxide (DMSO). The volume of each treatment administered ranged from 5 to 10 millilitres per kilogramme.

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Experimental Design for Antidepressant Activity:

The rats were divided into four groups (n = 6) and received drug administration 60 minutes before the study. Group I served as the control, receiving Rat Chew Diet orally. Group II received the standard drug Fluoxetine (15 mg/kg) orally as a positive control. Group III and Group IV were administered Mucuna pruriens orally at doses of 200 mg/kg and 400 mg/kg, respectively.

Acute Toxicity Studies: The acute oral toxicity of a Mucuna pruriens aqueous extract was assessed following revised OECD (2001) guidelines. The animals were observed for behavioral changes for four hours daily over a period of fourteen days. The extract exhibited no toxicity in rats when administered orally at doses up to 5000 mg/kg. Consequently, subsequent studies utilized extract doses ranging from 200 to 400 mg/kg.

Animals and Housing:

Wistar albino rats weighing between 150 and 250 grams were obtained from the animal house facility at Karuna Medical College in Palakkad, Kerala, India. They were fed a standard rat chew diet procured from government veterinary colleges in Mannuthy and Thrissur, Kerala, India. The rats were housed in a well-ventilated room with exhaust and ceiling fans, adhering to a 12-hour light cycle, and maintained at a temperature ranging from 28 to 32 degrees Celsius. The rats were housed in polypropylene cages with a maximum of three rats per cage, segregated by sex. Prior to the study, the rats underwent a one-week acclimation period. The bedding, composed of rice husk, was changed every other day.

Statistical Analysis: The data was subjected to statistical analysis using One-way ANOVA, and multiple comparisons were performed using Dunnett's test. Statistical significance was considered if the P-value was less than 0.05.

Forced Swim Test (FST):

The FST was conducted in two phases. The first phase, known as the "pre-test," was conducted a day before the experiment to select the animals for subsequent research [(13)]. The rats were divided into four groups in the second phase: Control (normal Rat chew diet), standard (Fluoxetine 15 mg/kg), and MP (200 mg/kg and 400 mg/kg) groups. After one hour of medication administration, the rats were subjected to a six-minute swim in a glass tank, and the immobility time was recorded [(14)].

Tail Suspension Test (TST):

Male rats were divided into four groups, and the standard drug (Fluoxetine 15 mg/kg) and MP (200 mg/kg and 400 mg/kg) were orally administered, along with a control group (rat chew diet). The rats were suspended in an inverted position by their tails, and the duration of their immobility was timed [(15)].

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PAM Open Field Test:

Male rats were divided into four groups, and the control group received rat chew diet, while the standard group received Fluoxetine 15 mg/kg. The MP group was administered doses of 200 mg/kg and 400 mg/kg orally. The rats' movements and activity were recorded using PAM sensors, and the duration of mobility was recorded within a five-minute timeframe [(16)]. In this study, three animal models - FST, TST, and PAM - were employed to assess the efficacy of pharmacological antidepressants. Immobility time is a notable behaviour observed in these tests, corresponding to behavioural despondency. The behavioural models used in all of the models' paradigms are well-known tools for assessing the efficacy of pharmacological antidepressants [(17)]. One distinctive behaviour that emerges during these tests is immobility, which corresponds to the behavioural despondency seen in human depression [(17)]. Furthermore, a number of antidepressants are known to reduce the amount of time a rodent spends immobile [(17)]. With a profile resembling that of the conventional antidepressant drug Fluoxetine, *Mucuna pruriens* significantly decreased immobility time in the rat FST, TST, and PAM at doses of 200 and 400 mg/kg. Certain animal models with serotonin reuptake inhibitors are typically reported as active in the TST, it is also believed to be more pharmacologically sensitive than FST [(18)].

RESULTS:

The antidepressant effect of Mucuna pruriens was investigated using two different doses of its aqueous leaf extract (200 and 400 mg/kg) in the Forced Swim Test (FST), and the results are presented in Tables 1 and 2. The control group did not show a significant effect on immobility time and swimming time in the FST compared to the group administered with fluoxetine, which served as a positive control. Therefore, all experimental groups were compared to the control group, and fluoxetine (15 mg/kg) was administered to rats as the positive control. The immobility time of the fluoxetine group (100.0±2.45 sec) exhibited a significant decrease compared to the control group (169.1±7.85 sec), representing a notable 40.86% change in immobility time when compared to the MP extract analyzed with the control group. The MP extracts at doses of 200 and 400 mg/kg also significantly decreased immobility times to 120.9±4.68 sec and 106.4±4.39 sec, respectively. Furthermore, the tail suspension test (TST) was conducted to assess the immobility time, and both the MP extract and standard drug (fluoxetine 15 mg/kg) induced a significant decrease compared to the control group. The immobility times in the control group (167.1±4.55 sec), MP 200 mg/kg group (106.3±2.78 sec), MP 400 mg/kg group (96.38±3.06 sec), and fluoxetine 15 mg/kg group (91.71±4.60 sec) were presented in Tables 3 and 4. The immobility time changes were significant, with fluoxetine showing a 45.12% change, MP 200 showing a 36.56% change, and MP 400 showing a 42.32% change, compared to the control group. In addition, the open field test provided measures of locomotion, exploration, and anxiety. The activity rate was analyzed using PAM, and the results are presented in Tables 5 and 6. The control group showed an activity rate of 64.6±3.17 sec, while the fluoxetine (standard) group exhibited an activity rate of 138.5±3.89 sec, and the MP-200 and MP-400 groups showed activity rates of 125.43±3.59

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sec and 149.52±3.14 sec, respectively. These changes in activity rate were significant ($p<0.0001$) and comparable to those observed in the control group. Please refer to the corresponding tables for detailed data.

FST readings

Table 1: Effects of *Mucuna pruriens* on Duration of Immobility Time (Weekly Difference) in the Forced Swim Test (FST)

Groups treated with	**Concentration**	**Week-1**	**Week-6**	**Week-12**	**Week-18**
Control	q.s	172±17.16	183.5±11.84	162.2±19.54	158.8±15.6
Fluoxetine	15 **(mg/kg)**	103.5±4.32	101±4.18	100.2±6.90	97.83±4.89
Mucuna pruriens	200 **(mg/kg)**	138.5±6.16	127.7±7.63	110±6	107.3±11.81
	400 **(mg/kg)**	122.2±8.02	116.5±7.95	92.5±8.52	94.5±4.31

Table 2: Immobility Time Effects of *Mucuna pruriens* (18 Weeks) in the Forced Swim Test (FST)

Groups treated with	**Concentration**	**Mean and standard deviation of immobility time (sec)**	**Change in %**
Control	q.s	169.1±7.85	0
Fluoxetine	15 (mg/kg)	100.0±2.45	40.86*
Mucuna pruriens	200 (mg/kg)	120.9±4.68	28.50*
	400 (mg/kg)	106.4±4.39	62.92*

TST Average Readings

Table 3: Effects of *Mucuna pruriens* on Duration of Immobility Time in the TST Test (TST)

Treatment Groups	Concentration	Week-1	Week-6	Week-12	Week-18
Control	q.s	166.8±7.53	183.3±9.37	160.5±7.54	157.8±10.05
Fluoxetine	15 (mg/kg)	85.5±15.2	94.8±5.04	87.83±5.82	98.6±8.77
Mucuna pruriens	200 (mg/kg)	106.5±7.76	105.3±2.06	105±6.07	108.5±6.27
	400(mg/kg)	98.8±4.54	101.5±9.33	91.33±3.94	93.83±6.05

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Table 4: Effects of *Mucuna pruriens* on Duration of Immobility Time in the Tail Suspension Test (TST)

Groups treated with	**Concentration**	**Mean and standard deviation of immobility time (sec)**	**Change in %**
Control	q.s	167.1±4.55	0
Fluoxetine	15 **(mg/kg)**	91.71±4.60	45.12*
Mucuna pruriens	200 **(mg/kg)**	106.3±2.78	36.56*
	400 **(mg/kg)**	96.38±3.065	42.32

Locomotor Activity of *Mucuna pruriens*

Table 5: Effects of *Mucuna pruriens* on Duration of Immobility Time in the Photoactometer (PAM) (Locomotor Activity)

Groups treated with	Concentration	Week-1	Week-6	Week-12	Week-18
Control	q.s	62.67±5.54	66.33±8.89	59.67±10.01	67±14.43
Fluoxetine	15(mg/kg)	146±11.01	135.7±10.32	138.8±4.10	133.2±3.87
Mucuna pruriens	200(mg/kg)	139±6.55	125±6.86	137.3±10.85	137.7±9.31
	400(mg/kg)	147.7±4.0	149±3.67	155.5±4.31	145.7±5.05

Table 6: Effects of *Mucuna pruriens* on Duration of Immobility Time in the Photoactometer (PAM)

Groups treated with	**Concentration**	**Immobility time mean and SEM (sec) locomotor activity**
Control	q.s	64.6±3.17
Fluoxetine	15(mg/kg)	138.5±3.89*
Mucuna pruriens	200(mg/kg)	125.4±3.59*
	400(mg/kg)	149.5±2.14*

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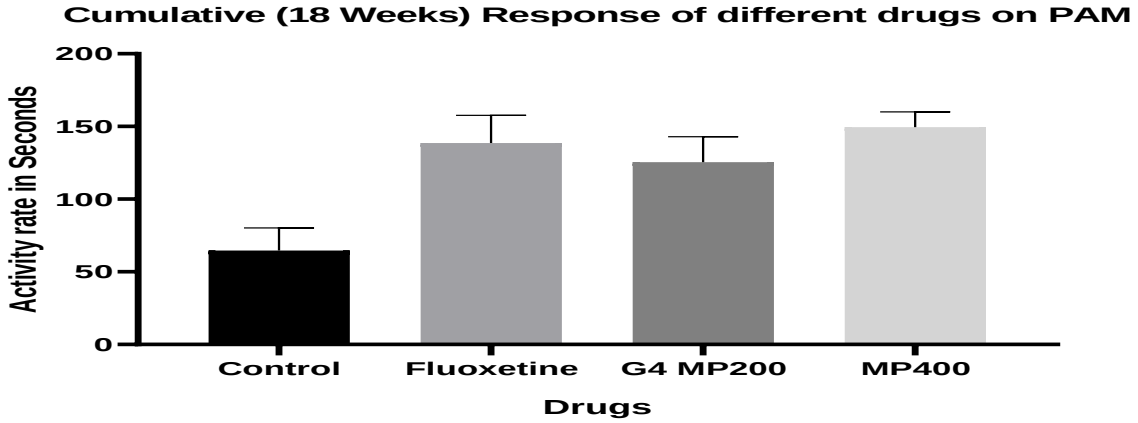

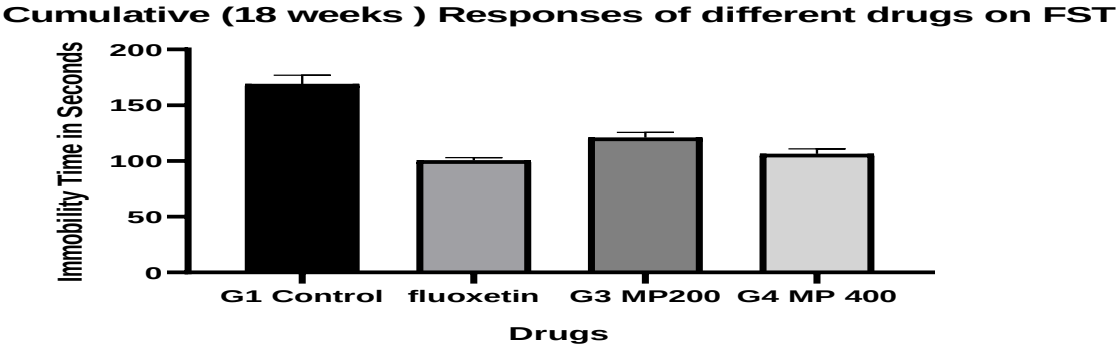

Fig: 1- 18-Weeks Differences in Immobility time on FST, the MP Extract treated group have showed comparable differences with control group, The difference in the immobility time between fluoxetine and *Mucuna pruriens* was not so significant as both are equally producing the same effect.

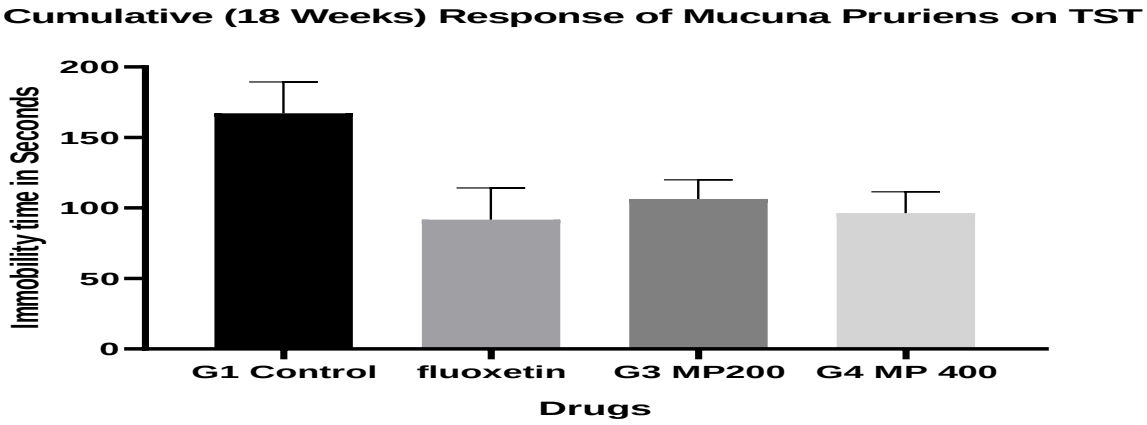

Fig: 1- 18-Weeks Differences in Immobility time on TST, the MP Extract treated group have showed comparable differences with control group, The difference in the immobility time between fluoxetine and *Mucuna pruriens* was not so significant as both are equally producing the same effect.

Fig: 1- 18-Weeks Differences in Immobility time on PAM, the MP Extract treated group have showed comparable differences with control group, The difference in the immobility time between fluoxetine and *Mucuna pruriens* was not so significant as both are equally producing the same effect.

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Discussion:

Depression is a significant mental illness that has detrimental effects on individuals' quality of life and social interactions. It is characterized by biological symptoms such as psychomotor retardation, loss of libido, sleep disturbances, and appetite loss, with major depression occurring when these symptoms are exceptionally severe. Clinical drug therapy for depression involves the use of tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors (SSRIs), selective reversible inhibitors of monoamine oxidase A (RIMAs), and serotonin-norepinephrine reuptake inhibitors (SNRIs) [(19)]. However, these medications come with various side effects, including cardiac toxicity, sexual dysfunction, body weight gain, and sleep disorders [(20)]. Escitalopram, a conventional SSRI, exhibits higher serotonergic activity compared to other SSRIs due to its strong affinity for the presynaptic serotonin transporter (SERT) site. Imipramine, on the other hand, inhibits the reuptake of serotonin and adrenaline, thereby increasing their availability at the synaptic cleft [(21)]. The extract of Mucuna pruriens contains amino acids such as valine, methionine, threonine, cysteine, arginine, histidine, phenylalanine, and tryptophan, all of which have been associated with antioxidant activity [(22)]. Mucuna pruriens is known to contain levodopa as its active constituent and is a source of serotonin, a neurotransmitter responsible for message transmission between nerve cells [(23)]. SSRIs work by blocking the reuptake of serotonin into neurons, increasing its availability at the synaptic cleft and facilitating message transmission between neurons [(24)]. The presence of serotonin in Mucuna pruriens directly enhances serotonin availability and helps alleviate symptoms of depression. The plant is also rich in thiamine, riboflavin, niacin, beta-carotene, and other nutrients. Carotenoids, present in Mucuna pruriens, possess antioxidant properties and act as precursors to vitamin A [(25)].

Phytochemical analysis has revealed that Mucuna pruriens contains flavonoids and phenolic compounds, which have been associated with various biological effects, including the treatment of central nervous system disorders [(26)], anti-inflammatory and analgesic activities [(27)], and antiulcerogenic properties [(28)]. There is a reciprocal association between ulcers and depression, as both conditions have shown a bidirectional relationship.

Conclusion:

In conclusion, this study provides initial evidence that the aqueous leaf extract of Mucuna pruriens significantly reduces depressive symptoms in the Forced Swim Test (FST), Tail Suspension Test (TST), and PAM depression models. Further investigations are necessary to elucidate the mechanism of action of Mucuna pruriens in depression.

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