Anti-Depressant-Like Activity of Mucuna Pruriens; A Traditional Indian Herb in Rodent Models of Depression

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Summary

The anti-depressant-like effects of *Mucuna pruriens* (MP) were studied in validated models of depression. Psycho-pharmacological investigations involved acute and chronic treatment (14 days) of mucuna in forced swim test (FST), tail suspension test (TST) in mice and olfactory bulbectomy in rats, respectively. Dose response study in mice FST and TST revealed the initial anti-depressant-like effect of Mucuna (10-20 mg/kg i.p.). Interaction studies revealed that, mucuna (10 mg/kg) significantly enhanced the anti-depressant action of fluoxetine and bupropion in FST and TST respectively. Potentiation of 5-Hydroxytryptophan induced head twitches response (in mice) and reversal of reserpine induced hypothermia (rats) were observed at same dose level. Further, the behaviour anomalies exhibited by olfactory bulbectomised rats (OBX) were attenuated by chronic mucuna treatment as observed in open field. In conclusion, this behavioural study depicts the anti-depressant-like effect of mucuna in acute and chronic model of depression.

Keywords: *Mucuna Pruriens*; Reserpine; Forced swim test; Olfactory bulbectomy, Anti-depressant

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Introduction

Depression is a chronic mental disorder clinically characterized by a pervasive low mood, loss of interest or pleasure in daily activities, low self-esteem, and a high suicidal tendency [1 – 4]. The disease, which is common incidence worldwide, affects the quality of life of many people, and has become a major cause of suicidal death [5]. Despite the advent of new molecule in the pharmacotherapy of depression, it is unfortunate that this disorder goes undiagnosed and untreated [6-9]. Antidepressant drugs are widely available in the pharmaceutical market. However, because multiple pathogenic factors are involved in depression, many synthetic antidepressant drugs show low response rates and even produce adverse side-effects such as cardiotoxicity, hypertensive crisis, sexual dysfunction and sleep disorder in depressed patients [10- 12]. On the other hand, drugs obtained from natural sources are perceived to have the least low risk and low side effect profiles, while having the ability to cure psychiatric disorder in much the same way as their synthetic counterparts. One such plant, which claims various medicinal properties, is Mucuna Pruriens Linn., a popular and important medicinal plant extensively used in Ayurveda, which is a holistic alternative science traditionally practiced in India [13]. All parts of this annual legume possess valuable medicinal properties and there is a heavy demand of MP in Indian and International drug markets. Previous studies reported that, MP has anti-parkinsonism activity [14-16], anti-tumor activity [17], neuroprotective activity [18], antioxidant activity [19], learning and memory enhancement and anti-diabetic activity [20]. Previous studies reported that, MP contains L-DOPA and 5-hydroxy tryptophan (5-HTP) as a major constituent with higher concentration in seeds [21,22]. Some of the other reported constituents of MP include: 1-methyl-3-carboxy-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinolone, 5-hydroxy tryptamine (serotonin), 5-methoxy-n,n-dimethyltryptamine-n-oxide, 5-oxyindole-3-alkylamine, 6-methoxyharmane, β-carboline and nicotine [23] confirmed through HPLC , paper chromatography, LC-ESI/MS and potentiometric methods [24,25]. Captivating the recent knowledge of MP constituents, the current study was undertaken (i) to investigate the effects of MP in validated animal models of depression (ii) to explore the possible underlying mechanism of anti-depressant like activity of MP.

Material and Methods

Animals

Experiments on animals were approved by the Institutional Animal Ethics Committee of Birla Institute of Technology & Science, Pilani, India (Protocol No. IAEC/RES/4/1). Male Swiss Albino mice (18–25g) and Male Wistar rats (200–250 g) were obtained from Hissar Agricultural University, Haryana, India and maintained in standard laboratory conditions with food (standard pellet chow feed) and filtered water *ad libitum*. The animals were used only once for each experiment.

Drugs

Mucuna Pruriens in the form of seed powder was procured as a gift sample from Vedic Life Sciences (Mumbai, India). Escitalopram (ESC), Fluoxetine (FLX) and Bupropion (BUP) were procured from Glenmark Pharmaceuticals and Ranbaxy Research

Laboratories (India) respectively, as generous gift sample. Reserpine was purchased from Sisco Research Laboratories Pvt. Limited (India). Pargyline and 5-hydroxy tryptophan (5-HTP) were purchased from Sigma chemical (USA). Parthenolide (PTN) was purchased from Tokris (U.K). The drugs for anesthesia namely, ketamine and xylazine were purchased from Reidel Neon Labs, Indian Immunologicals (Mumbai, India). The drugs were freshly prepared in distilled water and administered per oral (p.o.) or intraperitoneally (i.p.) (as specified) in a constant volume of 10 ml/kg. For interaction studies, the antidepressants/ligands and parthenolide were administered i.p., 45 and 30 min respectively before testing in forced swim or tail suspension tests as per the protocol adopted earlier in our laboratory [26]. In the chronic treatment schedule, the drugs were administered p.o. once a day for 21 days. All the drug administrations were carried out between 10:00 and 15:00 h.

Interaction studies

The interaction study with marketed antidepressants was carried out in mice using the FST/TST. Mice were treated individually with a single dose (p.o.) of vehicle, fluoxetine (20 mg kg-1) and bupropion (20 mg kg-1) post 15 min of MP administration. The doses of standard antidepressants were obtained from pilot studies or from previous studies carried out in our laboratory (Mahesh et al 2007; Ramamoorthy et al 2008). Thirty minutes after the antidepressant injection, mice were subjected to FST/TST. All the equipments used to assess the rodent's behaviour were sprayed with alcohol and wiped thoroughly between trials to eliminate the residual odour.

BEHAVIOURAL ASSAYS

Spontaneous locomotor activity

The spontaneous locomotor activity was assessed using an actophotometer [27] . The animals were individually placed in a square arena (30 cm × 30cm), with walls painted black and after an initial 2 min familiarization period, the digital locomotor scores were recorded for the next 10 min in a dimly lit room. The arena was cleaned with dilute alcohol and dried between trails.

Forced swim test

The forced swim test was carried out as described in Porsolt etal. [28] with slight modifications. Mice were dropped individually into a plexi-glass cylinder (height: 30 cm, diameter: 22.5 cm) filled with water to a depth of 15 cm and maintained at 23–25 °C. In this test, after an initial vigorous activity (2 min), the mice acquire an immobile posture which was characterized by motionless floating in the water, making only those movements necessary to keep the head above the water. The duration of immobility which reflects the state of depression was recorded during the last 4 min of the 6 min test. The swimming episodes were recorded as number of quadrants (demarcated at the base of the cylinder) crossed. The mice were subjected to 15 minute training session under similar conditions, 24 h before the test.

Tail suspension test

The mice were individually suspended by the tail to a horizontal bar (distance from floor was 50 cm) using scotch tape (distance from tip of tail was approximately 1 cm). Typically, mice exhibited several escape-oriented behaviour interspersed with temporary increasing bouts of immobility [29]. The duration of immobility (in seconds) during the 6-min test session was recorded.

5-Hydroxytryptophan-induced head twitch response

The method mentioned elsewhere **Martin et al**. [30] was adopted with slight modifications. Fifteen minutes after 5-HTP (5 mg kg-1) administration, the number of head twitches exhibited by the mice (vehicle or drug treated) during the next 15 min was recorded as head twitch scores. The head twitch response was characterized by abrupt lateral movements, which may be accompanied by body twitches and hind limb retraction.

Reserpine induced hypothermia test

The rats were gently hand-restrained, and the lubricated digital thermometer probe was inserted into the rectum. The rectal temperature of the rats treated with reserpine (1 mg/kg, i.p)/ MP (10-20 mg/kg, i.p.)/ ESC (10 mg/kg, i.p) was recorded at 30, 60, 90 and 120 min after the drug administration. Reserpine was injected 15 min post drug administration. The difference in the rectal temperature between the baseline and 120th min values were tabulated. On the day preceding the experiments, the rectal temperature of the rats were assessed in a similar manner in order to habituate the animals to the experimental procedures [31].

Rat olfactory bulbectomy

Bilateral olfactory bulbectomy was performed as described earlier [32, 26] with slight modification as mentioned below. Briefly, the rats were anaesthetized with xylazine (5 mg kg-1) and ketamine (75 mg kg-1, i.p.). The head of the rat was fixed in a stereotaxic frame and the skull was exposed by a midline sagittal incision. Burr holes (2 mm in diameter) were drilled 8 mm anterior to bregma and 2 mm on either side of the midline at a point corresponding to the posterior margin of the orbit of the eye. The olfactory bulbs were removed by suction, the holes were then filled with haemostatic sponge to control excessive bleeding and the scalp was sutured. To prevent post-surgical infection, the rats were given Sulprim injection (each mL containing 200 mg of sulfadiazine and 40 mg of trimethoprim) intramuscularly (0.2 mL/300 g) once a day for 3 days, post-surgery. Sham-operated rats were treated in the same way, including piercing of the dura mater, but their bulbs were left intact. Post 28 days of olfactory bulbectomy open field test was done (as given below). Treatment and bahavioural test was done as shown in Table.1.

Open field exploration

The OBX and sham rats were subjected to an open field test on the 29th day post surgery and the 15th day of chronic drug/vehicle administration. The open field exploration was conducted as described by Kelly et al. [33] with slight modifications. The apparatus consisted of a circular (90 cm diameter) arena with 75-cm-high aluminium walls and a floor equally divided into 10-cm squares. A 60 W light bulb was positioned 90 cm above the base of the arena, which was the only source of illumination in the testing room. Each rat was individually placed in the centre of the open field apparatus and the ambulation scores (number of squares crossed) was noted for 5 min.

Table 1: Surgery and treatment schedule to assess the effect of mucuna on olfactory bulbectomised rats

Day 0	0th -1st day	1st -14th day	15th - 28th day	29th day Behavioural Assessments
Surgery	Recovery from surgery (continuous care)	Rehabilitation period (Daily handling and observation)	Drug/vehicle treatment (Once a day p.o. administration for 14 days)	Modified open field exploration

Statistical Analyses

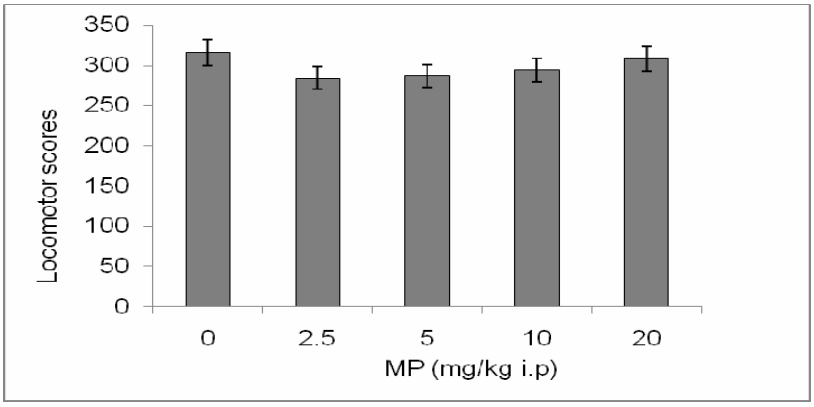
All the data were expressed as mean ± S.E.M and analysed using one-way analysis of variance (ANOVA) followed by post hoc Dunnett's test. The level of statistical significance was fixed at $P<0.05$.

Results

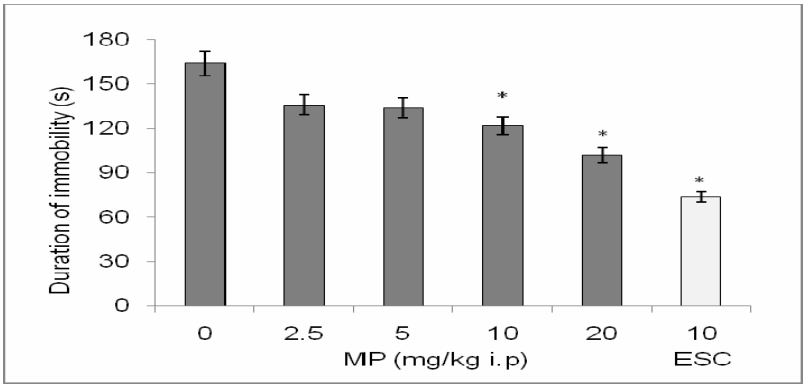
Locomotor scores, forced swim and tail suspension tests

Fig.1A displays the effects of MP on locomotor activity in mice. MP (2.5-20 mg/kg i.p) treatment had no influence on the mice locomotor activity when compared to control group.

FST and TST for depressive-like behaviour quantitates the duration of immobility which reflect the behavioural despair. *Post-hoc* analysis revealed that, MP (10-20 mg/kg) induced a significant ($p<0.05$) reduction of immobility time as compared to control group in mice FST (Fig. 1B). The positive control ESC (10 mg/kg), also induced a significant change in immobility. In TST, mucuna and BUP treatment significantly ($p <0.05$) decreased the duration of immobility as compared to control group (Fig.1C).



1.A



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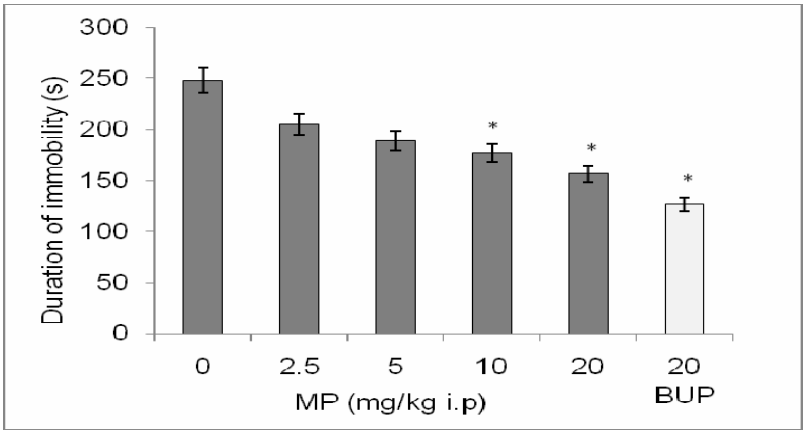
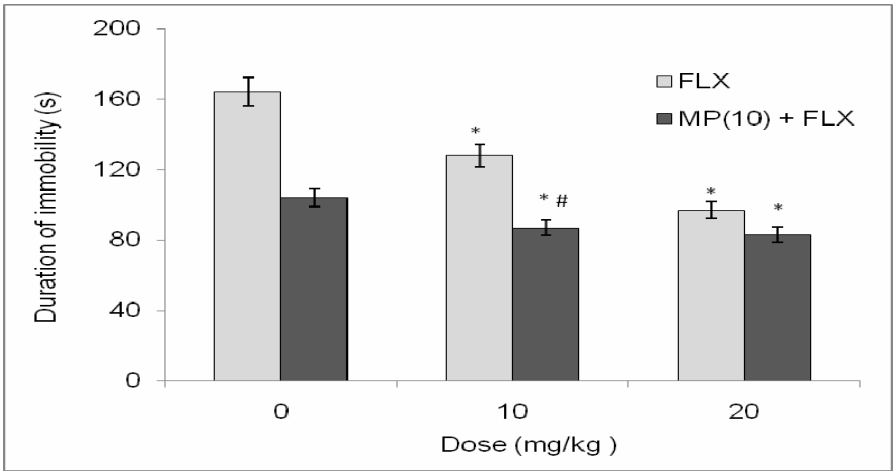


1.C

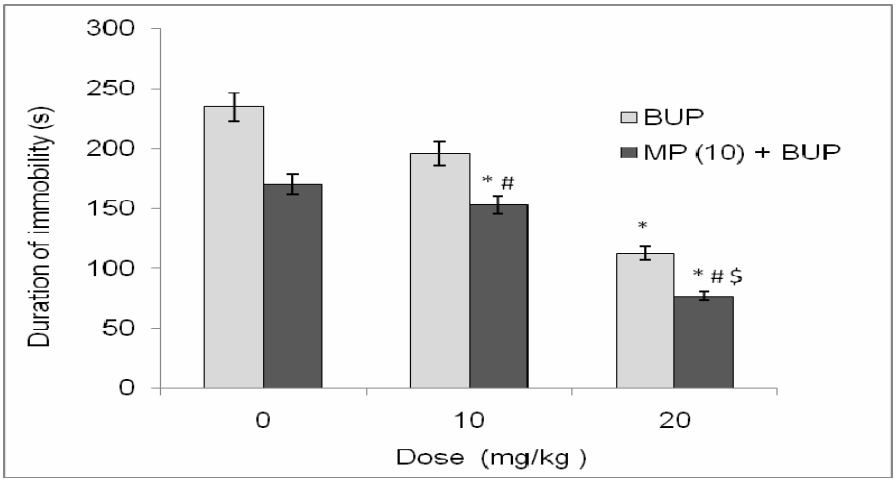
Fig.1. A. Effect of MP on spontaneous locomotor activity of mice. The columns represent mean locomotor scores recorded in a 10-min observation period. The error bars indicate s.e.m., n = 6 per group. B. Effect of MP on duration of immobility of mice in forced swim test. The columns represent mean duration of immobility in seconds (s) and error bars indicate s.e.m., n = 6 per group. *P < 0.05 compared with vehicle treated group. C. Effect of MP on duration of immobility of mice in tail suspension test. The columns represent mean duration of immobility (s) and error bars indicate s.e.m., n = 6 per group. *P < 0.05 compared with vehicle-treated group

Interaction studies

The peak dose of MP (10 mg/kg) showed significant ($p < 0.05$) decrease in the duration of immobility was selected for the interaction studies. The anti-depressant-like effects of MP were weaker than that of fluoxetine (FLX 10 mg/kg i.p). Pre-treatment with MP significantly ($p < 0.05$) enhanced the anti-depressant action of FLX i.e. decreased duration of immobility ($p < 0.05$) in mice FST (Fig. 2A). Co-administration of MP (10 mg/kg) and BUP significantly ($p <0.05$) augmented the anti-depressant activity of BUP in mice TST, when compared to BUP alone (Fig.2B). Parthenolide significantly induced the depressant-like effect characterized by increased duration of immobility ($p<0.05$) in mice FST. Mucuna significantly reversed the depressant-like effect of parthenolide (fig.3)



2.A



2.B

Figure.2. Effect of MP (10mg/kg) pre-treatment on anti-depressant effects of FLX (10 and 20mg/kg) and BUP (10 and 20 mg/kg) in mice FST and TST respectively. Columns represent mean of duration of immobility in mice FST (A) and TST (B). Error bars represent mean ± S.E.M. *p< 0.05 vs control group, #p< 0.05 vs FLX and BUP treated group and $ p<0.05 vs MP treated group. n= 6 per group

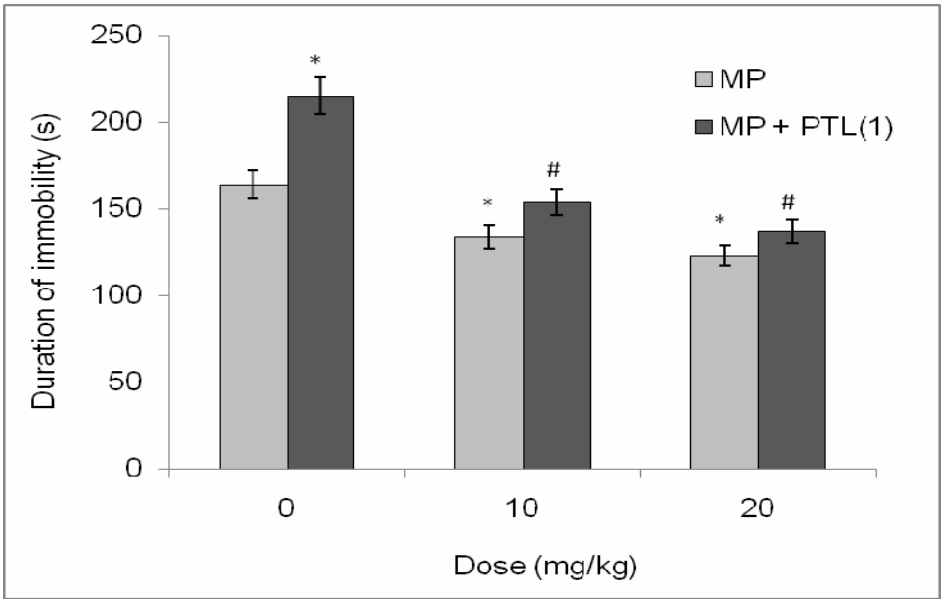


Fig.3 Effect of MP (10-20 mg/kg i.p) pre-treatment on depressant effect of PTN (1 mg/kg i.p) in mice FST. Columns represent mean of duration of immobility. Error bars represent mean ± S.E.M. *P< 0.05 vs control group, #p< 0.05 vs PTN treated group. n= 6 per group

5- HTP induced head twitches response

The co-administration of pargyline and 5-HTP (75 + 5 mg/kg) induced the characteristic head twitch response. Pre-treatment with MP (10-20 mg/kg) and FLX significantly ($p < 0.05$) potentiated the head twitch response as compared to combination of pargyline and 5-HTP (Fig. 4).

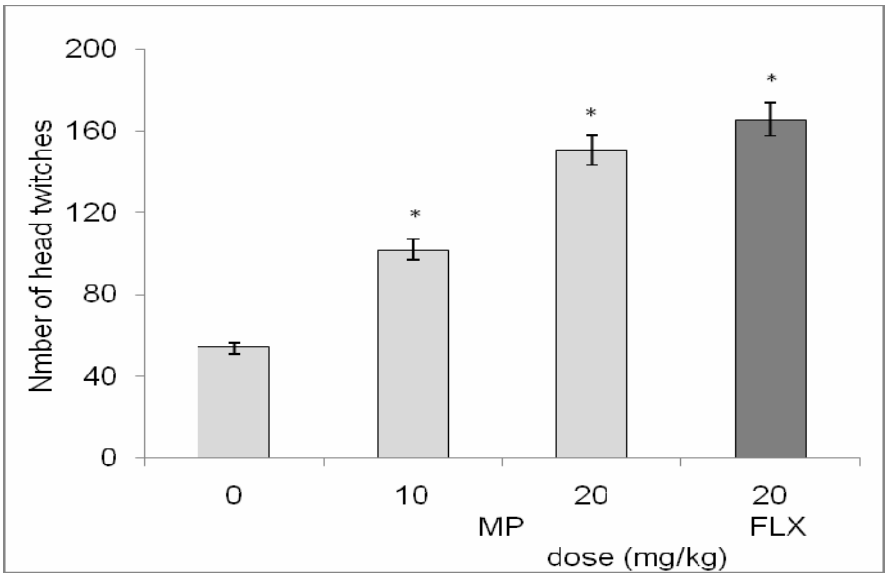


Fig.4. Effects of MP treatments on 5-HTP/PRG induced head twitches in mice. The columns represent the mean number of head twitches during a 15 min period. All the animals were administered with 5-HTP (5 mg/kg i.p) + PRG (100 mg/kg i.p) Error bars represent S.E.M. *p< 0.05 versus control group, n= 6 per group.

Reserpine induced hypothermia

Administration of reserpine (1m/kg i.p) elicited a pronounced decrease ($p < 0.05$) in core body temperature of rats. This effect was significantly ($p < 0.05$) attenuated by MP. Similarly, FLX reversed the hypothermic effect of reserpine [Fig. 5].

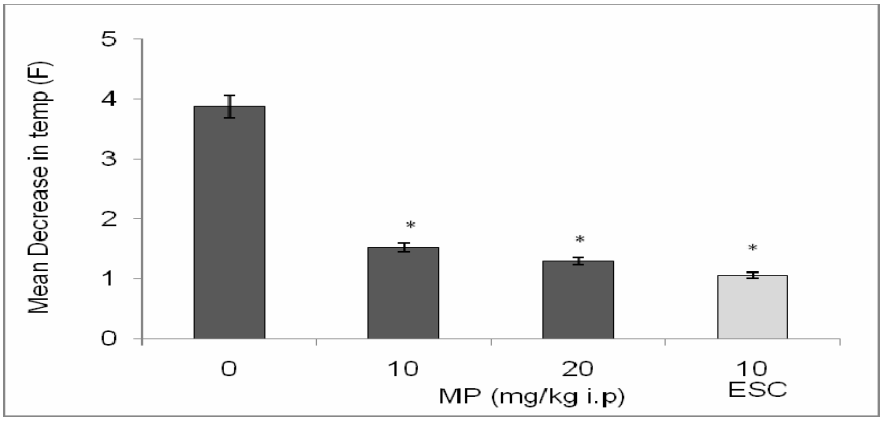


Fig.5. Effects of ESC (10mg/kg p.o) and MP (10-20 mg/kg p.o) treatment on reserpine induced hypothermia in rats. The columns represent the mean decrease in rectal temperature (F). Error bars represent mean ± S.E.M. *p< 0.05 versus 2nd hour value of vehicle treatment. n=6 per group.

Open field test

The effects of MP on the behaviour of OBX/sham rats were analyzed in different circumstances as shown in Table-2. Removal of the olfactory bulbs produced a characteristic hyperactivity in the OBX rats when compared to sham rats in the open field test. Chronic (21 days) treatment with MP significantly (p< 0.05) reduced the ambulation, rearing and fecal pellets (p <0.05) in OBX rats as compared to the vehicle treated OBX rats. MP (10 mg/kg) exhibited anti-depressant-like effects and escitalopram was the most effective among all treatments.

Table 2: Effect of MP and escitalopram on the behaviour of OBX rats in modified open field

Groups	Dose (mg/kg)	Crossing	Rearing	Fecal Pellets
Sham Control	0	115.33±7.75	12.5±1.34	3±0.45
Sham + Mucuna Pruriens	10	118±6.54	10±1.37	2.33±0.42
Sham + Escitalopram	10	92.16±6.05	8.5±0.56	1.83±0.48
OBX Control	0	212.16±8.58 *	24.5±1.71*	6.17±0.60*
OBX + Mucuna Pruriens	10	166.33±15.51 #	10.83±1.08#	4.33±0.92#
OBX + Escitalopram	10	138.33±5.66 #	10±0.86#	2.17±0.60#

Values represent mean±S.E.M. *n* = 6 in each group. Drugs were administered (p.o.) once day for 21 days. **P < 0.05* compared with vehicle treated sham rats, *#P < 0.05* compared with vehicle treated OBX rats.

Discussion

Anti-depressant effect of herbs has been paid more and more attention gradually because of increasing incidence of depression and predominance of traditional herbs in therapy. Previous research reports indicate that herbs including St. John's wort, morinda root, curcuma, and the Chinese traditional herbal decoctions including Banxia *Houpu-Tang* [34], *Chaihu-Shugan-San* [35], *Chaihu jia Longgu muli-tang* [36], *Xiaoyao-san, Sini-san, Baihe dihuang-tang*, etc. exert anti-depressant-like effect in animal models [36,37,38].

In the present study, the anti-depressant like effects of MP were evaluated in animal models of depression. The practice of using whole animal assay is considered to be a rapid method for the identification of neuro-psychopharmacological effect of novel compounds. This investigation encompassed acute (forced swim and tail suspension tests) and mechanistic (reserpine induced hypothermia in rats) and 5-HTP induced head twitches in mice and chronic animal model of depression, Olfactory bulbectomy were employed to examine the effects of mucuna. The predictive validity of the aforementioned anti-depressant assays is already reported to be adequate.

While interpreting the anti-depressant-like effect of any test substance based on swimming and exploratory behaviour of rodents, the influence of the test substance in baseline locomotion in animal is of prime concern [27]. In the present study MP (10- 20 mg/kg i.p) significantly decreased the duration of immobility in mice FST and TST. Reduction in duration of immobility reflects the anti-depressant properties of drugs. The anti-depressant-like effect of mucuna seems not be associated with any motor effects, since it did not show significant change in locomotion of mice. Interactions with SSRIs (Selective serotonin reuptake inhibitors) are necessary for conclusive assessment of anti-depressants (ADs) potential [39]. MP (10 mg/kg) significantly enhanced the antidepressant action of fluoxetine. Due to species dependent variation, BUP failed to exhibit anti-depressant effects in FST with *swiss* Albino mice [40]. Hence, interaction study in TST was expected to throw some light on the influence on dopaminergic system. In the present study, MP pre-treatment was found to augment the anti-depressant effects of bupropion (10 and 20mg/kg) in TST indicating the influence on dopaminergic system. Depressant-like effect induced by parthenolide was considered as a model to identify anti-depressants acting through serotonergic mechanism [41]. MP significantly reversed the depressant-like effect of parthenolide, possibly through serotonin release.

One of the pharmacological mechanisms of anti-depressants is the enhancement of synaptic concentrations of monoamines, particularly serotonin. 5-HTP being the immediate precursor of 5-HT, its administration was reported to increase the serotonergic transmission inducing a characteristic head twitch response in mice [42, 41, 43]. In the present study, the PRG and 5-HTP induced head twitch responses were significantly potentiated by FLX and MP pre-treatment. In this regard, the anti-depressant-like effect of mucuna appears to be modulated by an increase in serotonin concentrations in the synapse [41].

Depletion of biogenic amines (NE, 5-HT, DA) in the brain has been observed to induce catalepsy, ptosis and the most recorded parameter, hypothermia. The decrease of body temperature induced by reserpine is proved to be antagonized by anti-depressants that act by increasing the amount of biogenic amines at the synaptic cleft [31]. In the current study, MP (10-20 mg/kg body weight) and ESC (10mg/kg body weight) prevented the decrease in rectal temperature at 120th minute after reserpine challenge indicating anti-depressant effect in this sensitive model.

Olfactory bulbectomy was proposed as an agitated hypo-serotonergic model of depression [44] and used to explore the novel agents for their anti-depressant potential [45]. OBX rats exhibited a specific abnormal behavioural pattern in the open field test [46] characterized by increased ambulation, rearing and fecal pellets [47] and this abnormal behaviour was reversed by anti-depressants [48]. In the open field test, MP (10-20 mg/kg p.o.) and escitalopram significantly reversed the hyperactivity exhibited by OBX rats.

The present neuro-behavioural studies showed anti-depressant- like effects of mucuna pruriens, a traditional herb, in animal models of depression. The precise mechanism by which MP produced anti-depressant-like effect is not understood. However, but the presence of l-DOPA [21,22] a precursor of dopamine and 5-HTP, a precursor of serotonin in brain and the results obtained in FST ,TST, potentiation of head twitch responses and reversal of reserpine induced hypothermia suggests that, mucuna produced anti-depressant-like effect by increasing the concentration of neurotramitter [49]. Reversal of parthenolide induced depression-like behaviour indicates the involvement of serotonin in anti-depressant like action of MP.

In conclusion, MP exerts anti-depressant-like effect in all the animal models studied here, and these effects may be mediated. by the central monoaminergic neurotransmitter systems. The convergence of these findings suggest that mucuna pruriens may be useful as a powerful, natural anti-depressant agent.

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