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Research Article

EVALUATION OF ANTIPARKINSONIAN ACTIVITY OF *MUCUNA PRURIENS* AND *AEGLE MARMELOS*: AN ANIMAL MODEL BASED STUDY

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Abstract: **Introduction:** Parkinson's disease is a progressive, neurodegenerative disorder that affects movement, muscle control, and balance as well as numerous other functions. Mucuna Pruriens (Fabaceae), commonly known as cow hage plant or kapikacho or kevach in Hindi, contains high concentrations of levodopa, a direct precursor of the neurotransmitter dopamine. *Aegle marmelose* has antioxidant property. Both are used in traditional Ayurvedic Indian medicine for diseases including Parkinson's disease. In this study the potential neuroprotective effects by *Mucuna Pruriens* extract alone, *Aegle Marmelose* alone and in combination were investigated. **Materials and methods:** It was a six months experimental based study done at Pinnacle Biomedical Research Institute, Bhopal & NIMS Medical College, Jaipur to evaluate the antiparkinsonian activity of *Mucuna pruriens* and *Aegle Marmelos* on various behavioural activities of male albino wistar rats. **Results:** There were significant differences in the behaviours of line crossing between the animals of the *Mucuna Pruriens* treated and Combination treated groups. Pretreatment with *Mucuna Pruriens* extract or combination of *Mucuna Pruriens* and *Aegle Marmelos* significantly reduced tremulous jaw movements and the number of bursts induced by Tacrine; suppressed the haloperidol induced vacuous chewing movements and tongue protrusions and significantly reduce the oxidative stress. **Conclusion:** The inhibition of haloperidol induced catalepsy, tacrine induced vacuous chewing movements and haloperidol induced dyskinesia by *Mucuna pruriens* and *Aegle marmalose* strongly indicate that both the plants have potential as Anti-parkinsonism agents. Further studies are necessary to establish the safety of the combination of both these herbs.

Key words: Parkinson's disease, *Mucuna pruriens*, *Aegle Marmelos*.

INTRODUCTION

Parkinson's disease is a progressive, neurodegenerative disorder that affects movement, muscle control, and balance as well as numerous other functions. The drugs used in treatment of Parkinson's disease are levodopa, carbidopa, bromocriptine, pergolide, amantadine, benztropine, trihexyphenidyl, selegiline. *Mucuna Pruriens* (Fabaceae), commonly known as cowhage plant or kapikacho or kevach in Hindi, contain high concentrations of levodopa, a direct precursor of the neurotransmitter dopamine. It has long been used in traditional Ayurvedic Indian medicine for diseases including Parkinson's disease. Aegle Marmelos is a slow-growing, medium sized tree, up to 12-15 m tall with short trunk, thick, soft, flaking bark, and spreading, sometimes spiny branches, the lower ones drooping. It has also been used in treatment of parkinson's disease. Since very few studies are available on effect of *Mucuna pruriens* and *Aegle Marmelos* on parkinson's disease, the present study was undertaken to evaluate the antiparkinsonian activity of *Mucuna Pruriens* and *Aegle Marmelos.*

Material and methods:

It was a six months experimental based study done at Pinnacle Biomedical Research Institute, Bhopal & NIMS Medical College, Jaipur, to evaluate the antiparkinsonian activity of *Mucuna pruriens* and *Aegle Marmelos* on various behavioral activities of male albino wistar rats.

Collection of Plant material: The seeds of *Mucuna pruriens* and fruits of *Aegle marmelos* were procured locally from Bhopal. The materials were authenticated, and the voucher specimens (Herbarium Voucher No. V.S.N./2010/137,136) were deposited in the Department of Botany (Sarojini Naidu Govt. Girls P.G. autonomous College Shivaji Nagar Bhopal).

Preparation of extract: Seeds were washed twice using tap water and then washed again in distilled water to remove the dust. The seeds of *Mucuna pruriens* (MP) and fruits of *Aegle marmelose* (AM) were dried in the shade, and then crushed into coarse powder. Later, it was transferred for maceration with acetone and methanol (1:1) for 10

days, and then filtered with muslin cloth. The filtrate was then filtered by Whatman filter paper. The filtrate was kept on water bath for concentration at 40^0C.

Phytochemical screening of acetone and methanolic extract of *Mucuna Pruriens* and *Aegle Marmelos* by standard methods.[1]

Animals for experiment:

Swiss albino rats were obtained from animal house of Pinnacle Biomedical Research Institute. The experiment was conducted as per the permission of Institutional Animal Ethical Committee (IAEC) of PBRI (Reg No. 1283/c/09/CPCSEA). All conditions were maintained according to CPCSEA norms. The 4-months old animals of either sex were selected randomly of uniform weight 120±20 gm from animal house. The room temperature was maintained 22±2°C with food (Lipton India Ltd. pellets) and water ad libitum. The animals were transferred to the laboratory at least 1h before the start of the experiment. The experiments were performed during day (08:00-16:00 h).

Evaluation of Antiparkinsonian activity

The rats were divided randomly into four groups, each having six rats each. The drug was administered intraperitoneally (ip) to the rats 30 minutes before the experiment.

Group A – The vehicle was given ip to rats 30 minute before the experiment and all the behaviour parameter were observed. The vehicle used in the group was Dimethyl Sulfoxide (DMSO).

Group B- Mucuna pruriens (100mg/kg ip) was given to the rats 30 minute before the experiment and all the behavioural parameters were observed.

Group C- *Aegle Marmelos* (100mg/kg ip) was given to the rats 30 minute before the experiment and all the behaviour parameters were observed.

Group D- Combination of Mucuna pruriens and *Aegle marmelos* 1:1, 100 mg/kg ip was given to the rats 30 minute before the practical and all the behaviour parameters were observed.

Effect on gross behaviour using Irwin schedule[2]

The behaviour activity viewed under this method were rearing, grooming, searching, exploratory behaviour activity, calm, sleep, teeth chattering, head twitching, face washing, licking, paw licking, and writhing.

Open field locomotor activity[3, 4]

The open field apparatus was constructed with plywood and measured 72 x 72cm with 36cm walls [(93)]. Then the rats were allowed to explore in the apparatus, the number of line crossing done by the rat was noted as locomotors activity.

Haloperidol induced catalepsy[5, 6]

After 30 min. the rats in each group were administered haloperidol (1mg/kg ip) and the forepaw of rat were placed on wooden bar elevated 6 cm above the ground. The duration for which the rat retained the forepaw on the elevated bar was noted at 0, 30, 60, 90, and 120 min. the cut off time was recorded.

Tacrine induced jaw movement[7, 8]

After 20 min the rats in each group was administered tacrine (2.5 mg/kg, i.p.) and the number of tremulous jaw movements and bursts were measured for 60 min.

Haloperidol induced tardive diskynesia[9]

Haloperidol (1 mg/kg i.p.) was given chronically to rats for a period of 21 days to induce oral dyskinesia (Pattipati S. Naidu, et al 2003). All the behaviour assessments were carried out 24 hr after the last dose of haloperidol.

Action against oxidative stress in brain

Dissection and homogenization

On the 22nd day of haloperidol treatment, the animals were sacrificed by decapitation immediately after behavioural assessment. The brains were removed, forebrain was dissected out and rinsed with isotonic saline and weighed. A 10% (w/v) tissue homogenate was prepared in 0.1 M phosphate buffer (pH 7.4). The post nuclear fraction for catalase assay was obtained by centrifugation of the homogenate at 1000×g for 20 min, at 4 ^{0}C and for other enzyme assays centrifuged at 12,000×g for 60 min. at 4 ^{0}C.

Lipid peroxidation assay

The quantitative measurement of lipid peroxidation in forebrain was performed according to the method of Wills (1966).

Estimation of reduced glutathione

Reduced glutathione in the forebrain tissue was estimated according to the method of Ellman (1959).

Enzyme assays

Superoxide dismutase activity

Superoxide dismutase activity was assayed according to the method of Kono (1978).

Catalase activity

Catalase activity was assayed by the method of Luck (1971).

Results:

There were significant ($P < 0.05$) differences in the behaviours of line crossing, between the animals of the Mucuna Pruriens treated and Combination treated groups. There were no significant changes observed in the behaviours of line crossing between the vehicle treated and *Aegle marmelos* groups (Table 1).

Table 1: Effect of Mucuna Pruriens, *Aegle Marmelos* and Combination on locomotor activity.

Group	Number of line crossing MEAN±SEM
Vehicle	55.5±8.563
Mucuna pruriens	27.5±2.742[a]
Aegle marmelose	57.33±4.387[b]
Combination	30.5±4.209[a,b]

No. of Animals = 24 (each groups contains 6 animal)

a- Significant variation as compare to vehicle treated group P<0.05

b- Significant variation as compare to Mucuna Pruriens P<0.05

Following the administration of Vehicle, at the 0 minute, and after 30, 60, 90, and 120 minute, Mean (in sec) and SEM were observed to be 5.33 ± 0.42, 177.5 ± 16.53, 152.3 ± 12.64, 155.7 ± 14.79, 154.5 ± 13.11 respectively. Following the administration of Mucuna Pruriens at the 0 minute and after 30, 60, 90, and 120 minute Mean (in sec) and SEM were observed 3.33±0.42, 119.0 ± 21.48, 87.17 ± 4.19, 88.17 ± 3.6, 86.0 ± 5.65 respectively. Following the administration of Aegle Marmelos, at the 0 minute, and after 30, 60, 90, and 120 minute Mean (in sec) and SEM were observed 3.83 ± 0.30, 119.3 ± 7.0, 124.0 ± 8.8, 129.5 ± 8.3, and 109.7 ± 8.717 respectively (Table 2).

Table 2: Effect of Mucuna Pruriens, Aegle Marmelos and Combination on duration of catalepsy.

Group	Duration of catalepsy in sec (Mean ± SEM)				
	0 min	**30 min**	**60 min**	**90 min.**	**120 min**
Vehicle	5.33 ± 0.42	177.5 ± 16.53	152. ± 12.64	155.7 ± 14.79	154.5± 13.11
Mucuna Pruriens	3.33±0.4216[a]	119 .0 ± 21.4[ef]	87.17±4.19[g]	88.17 ± 3.6[jl]	86.0 ± 5.6m
Aegle Marmelos	3.83±0.30	119.3±7.0[f]	124.0±8.81[i]	129.5 ± 8.3	109.7±8.71[m]
Combination	1.5 ± 0.61[acb]	57.33 ± 8.25[fe]	51.83±4.9[gih]	51.83 ± 5.9[jlk]	48.0 ± 8.4[mon]

At zero minute.

a- Significant variation as compare to vehicle treated group P<0.05

b- Significant variation as compare to Mucuna PruriensP<0.05

c- Significant variance as compare to Aegle Marmelose P<0.05

At 30 minute.

d- Significant variation as compare to vehicles treated group P<0.05

e- Significant variation as compare to Mucuna PruriensP<0.05

f- Significant variance as compare to Aegle Marmelos P<0.05

At 60 minute.

g- Significant variation as compare to vehicles treated group P<0.05

h- Significant variation as compare to Mucuna PruriensP<0.05

i- Significant variance as compare to Aegle Marmelos P<0.05

At 90 minute.

j- Significant variation as compare to vehicles treated group P<0.05

k- Significant variation as compare to Mucuna PruriensP<0.05

l- Significant variance as compare to Aegle Marmelos P<0.05

At 120 minute.

m- Significant variation as compare to vehicles treated group P<0.05

n- Significant variation as compare to Mucuna PruriensP<0.05

o-Significant variance as compare to Aegle Marmelos P<0.05

Pretreatment with Mucuna Pruriens extract (100 mg/kg) or combination of *Mucuna Pruriens* and *Aegle Marmelos* (1:1, 100 mg/kg) significantly reduced tremulous jaw movements induced by acute administration of Tacrine (2.5 mg/kg) (P<0.002 vs. vehicle). Moreover, *Mucuna Pruriens* extract, *Aegle Marmelos* (100 mg/kg), and combination of Mucuna Pruriens and Aegle Marmelos pretreatment (1:1, 100 mg/kg) significantly reduced the number of bursts induced by Tacrine (P<0.001 vs. vehicle).

Table 3: Effect of *Mucuna Pruriens, Aegle Marmelos* and Combination on jaw movement and burst.

Treatment group	Effect on Jaw movement and Burst	
	Jaw movement (Mean ± SEM)	Burst (Mean ± SEM)
Vehicle	178.2±19.23	48.33±4.835
Mucuna pruriens	119.8±14.07a	5.285±5.285c
Aegle marmelose	67.83±10.36a	4.897±4.897c
Combination	63.83±8.581ab	1.874±1.874cde

For jaw movement
a- P<0.05 significant variation as compare to vehicle
b- P<0.05 significant variation as compare to Mucuna pruriens
For burst
c- P<0.05 significant variation as compare to vehicle
d- P<0.05 significant variation as compare to Mucuna Pruriens
e- P<0.05 significant variation as compare to Aegle Marmelose.

Effect of Mucuna Pruriensextract (100 mg/kg), Aegle Marmelos (100 mg/kg), and combination of both on haloperidol induced vacuous chewing movements and tongue protrusions are shown in Table 4. Chronic haloperidol (1 mg/ kg) treatment significantly increased the vacuous chewing movements (VCMs) and tongue protrusions frequency in rats as compared to vehicle treated controls. Chronic co-administration of Mucuna Pruriens and combination along with haloperidol suppressed the haloperidol induced vacuous chewing movements and tongue protrusions.

Table 4: Effect of Mucuna Pruriens, Aegle Marmelos and Combination on haloperidol induced jaw movement and tongue protrusion.

Treatment group	Haloperidol induced	
	Jaw movement Mean±SEM	Tongue protrusion Mean±SEM
Vehicle	140.5 ± 22.02	6.66 ± 0.61
Mucuna pruriens	78.17 ± 9.12a	4.0 ± 0.85d
Aegle Marmelos	96.83 ± 11.06	4.5 ± 0.76
Combination	25.17 ± 3.38acb	1.0 ± 0.25dfe

For jaw movement
a- P<0.05 significant variation as compare to vehicle treated group
b- P<0.05 significant variation as compare to Mucuna Prurienstreated group.
c- P<0.05 significant variation as compare to Aegle Marmelos treated group.
For tongue protrusion
d- P<0.05 significant variation as compare to vehicle treated group
e- P<0.05 significant variation as compare to Mucuna Prurienstreated group.
f- P<0.05 significant variation as compare to Aegle Marmelos treated group.

Table 5 shows the effect of Mucuna Pruriens extract (100 mg/kg), Aegle Marmelos (100 mg/kg), and combination of both on oxidative stress. Mucuna Pruriens extract (100 mg/kg), Aegle Marmelos (100 mg/kg), and combination of both showed significant variation in concentration of LPO, GSH, and SOD.

Table 5: Effect of Mucuna Pruriens, Aegle Marmelos and Combination on Oxidative stress.

Group	LPO(nmol MDA/mg protein) Mean±SD	GSH (nmol/mg protein) Mean±SD	SOD(Unit/mg protein) Mean±SD
Vehicle	1.25±0.138	20.08 ±0.608	8.2866 ±0.6451
Vehicle+haloperidol	3.655 ±0.098[a]	9.05 ±0.216[b]	4.8666 ±0.73393[c]
M.P. +haloperidol	2.8383 ±0.1225[a]	16.166 ±0.56[b]	6.655 ±0.3731[c]
A.M.+haloperidol	2.453 ±0.1256[a]	14.9 ±0.9757[b]	5.8933 ±0.4588[c]
Combination+haloperidol	2.42 ±0.24[a]	14.9333± 0.7763[b]	5.475± 0.4213[c]

LPO- Lipid peroxidation assay
MDA- Malondialdehyde
SOD- Superoxide dismutase

a- Significant variations as compare to vehicle treated group P< 0.05
b- Significant variations as compare to vehicle treated group P< 0.05
c- Significant variations as compare to vehicle treated group P< 0.05

DISCUSSION

Despite the demonstrated efficacy of treatment with levodopa, some physicians are cautious when prescribing the drug because of its association with the emergence of motor complications[10]. The first randomized clinical trial of conventional levodopa (ELLDOPA), which was carried out relatively recently, showed that high doses of levodopa/DDCI are a factor in the development of motor complications. Complications can emerge as early as 5 to 6 months after treatment initiation with doses ≥600 mg/day[11]. Wearing-off and dyskinesia associated with long-term conventional levodopa therapy can result in disability and have a significant impact on a patient's quality of life[12]. In a limited number of cases, usually young, severely-affected patients, motor complications can outweigh the functional benefits provided by treatment. Consequently, initiation of levodopa may be postponed in an attempt to delay the onset of these complications. There is some evidence to suggest that high doses of antioxidants may reduce the oxidative stress caused by dopachrome and other toxic indoles that can be produced by the metabolism of L-DOPA[13]. Mucuna pruriens seeds are currently used in Indian ayurvedic medicine in the treatment of PD[14] and Aegle marmelose used as antioxidant[15]. MP seed extract is known to contain, among other components, the dopamine precursor L-DOPA, which is thought to underlie the anti-PD effects of the substance[16]. In this study, the efficacy of selected doses of MP extracts with Aegle marmelose in parkinsonian motor deficits was evaluated. The potential neuroprotective effects by MP extract alone and in combination with Aegle marmelose were assessed and rewarding effects of this combination were investigated.

After the intraperitoneal administration of vehicle (DMSO), MP (100mg/kg), AM (100mg/kg) and Combination of both (1:1, 100mg/kg), behavioral signs were observed. Grooming, writhing, teeth chattering, calm and sleep were reduced, but face washing, raring, licking, paw licking, head twitch, and searching were increased as compared to vehicle treated group. All the parameters indicated that MP, AM, Combination of both has the CNS activity that may be excitatory or inhibitory.

The open field experiments have indicated that vehicle and AM did not caused any deterioration of motor performance in rats, while MP and combination decreased locomotion, stereotypic events, and coordination. The behavioral effects were closely linked to the degree of dopamine dysfunction. Typical neuroleptic agents such as chlorpromazine, haloperidol and reserpine induce a cataleptic state in rodents which is widely used as a model to test the extrapyramidal side effects of antipsychotic agents. Neuroleptic-induced catalepsy has been linked to a blockade of postsynaptic striatal dopamine D1 and D2 receptors[17]. Despite of this evidence, several other neurotransmitters such as acetylcholine, serotonin, angiotensin, adenosine or opioids have also been implicated in the catalepsy induced by neuroleptic agents[18]. In addition to various neurotransmitters, many preclinical and clinical studies have also proposed role of reactive oxygen species as causes of haloperidol-induced toxicity[19]. Evidence indicates that drugs which potentiate or attenuate neuroleptic catalepsy in rodents might also aggravate or reduce the extrapyramidal signs respectively in human being[20]. In the present study, combination of MP and AM protected rats from catalepsy induced by haloperidol as effectively as the standard drugs (MP). The protective effect of combination against haloperidol induced catalepsy is consistent with activation of dopaminergic neurons[21]. Thus, the anticataleptic effect of combination might be due to both its dopamine agonist and antioxidant properties.

Mucuna pruriens extract (100 mg/kg), and combination of *Mucuna pruriens* and *Aegle marmelos* (1:1, 100mg/kg) also reduced tacrine-induced tremulous jaw movements, a correlate of parkinsonian tremor, suggesting that *Mucuna pruriens* extract (100 mg/kg), and combination of Mucuna pruriens and Aegle Marmelos (1:1, 100mg/kg) may also be effective on this symptom of PD. The rat model of tacrine-induced jaw movements has been widely used as model of PD tremor due to the good correlation between inhibition of jaw movements and potency of anti-PD drugs showed that L-DOPA produced significant inhibition of tacrine-induced jaw movements at a dose of at least 50 mg/kg i.p., whereas in our study Mucuna pruriens extract (100 mg/kg) and combination of Mucuna pruriens and Aegle Marmelos (1:1, 100mg/kg) significantly inhibited Tacrine induced jaw movements. Mucuna Pruriens and Aegle Marmelos have the potent antioxidant activity. Mucuna pruriens extract (100 mg/kg) and combination of Mucuna pruriens and Aegle Marmelos (1:1, 100mg/kg) significantly reduced the LPO which has measured by the MDA

level. Mucuna pruriens extract (100 mg/kg) and combination of Mucuna pruriens and Aegle Marmelos (1:1, 100mg/kg) significantly increased the GSH level that showed it has antioxidant activity. Mucuna pruriens extract (100 mg/kg) and combination of Mucuna pruriens and Aegle Marmelos (1:1, 100mg/kg) significantly increased the SOD level.

In the present study chronic haloperidol treated animals showed increased frequencies of vacuous chewing movements and tongue protrusions as compared to vehicle treated control animals. Treatment with Mucuna pruriens extract (100 mg/kg) and combination of Mucuna pruriens and Aegle Marmelos (1:1, 100mg/kg) significantly attenuated the induction of haloperidol- induced vacuous chewing movements and tongue protrusion. Thus in the present study it is convincingly established that both *Mucuna pruriens* and *Aegle marmalose* has anti-parkinsonism activity in various animal models of PD.

Since *Mucuna pruriens* has a well established place in phytotherapy of PD, Mucuna pruriens served as its own control and synthetic levodopa was not used as a reference standard. Previous studies have shown that Mucuna pruriens has better activity in both animals and Humans. However, safety of *Mucuna pruriens* and *Aegle marmalose* in long term use should be established. Molecular basis of their activity needs to be explored.

Conclusion:

The inhibition of haloperidol induced catalepsy, tacrine induced vacuous chewing movements and haloperidol induced dyskinesia by *Mucuna pruriens* and *Aegle marmelos* strongly indicate that both the plants have potential as Anti-parkinsonism agents. Further studies are necessary to establish the safety of the combination of both these herbs in long term use.

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