EFFECTS OF *MUCUNA MACROCARPA* SEED EXTRACT ON ROTENONE-INDUCED PARKINSONISM RATS

Puttichai Siriwatananukul[1], Ratchanee Rodsiri[1, *], Nijsiri Ruangrungsi[2, 3]

[1]Department of Pharmacology and Physiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand, [2]College of Public Health Sciences, Chulalongkorn University, Bangkok 10330, Thailand, [3]Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand

ABSTRACT: *Mucuna macrocarpa* (MM) seed extract contains high content of L-DOPA, an intermediate precursor of dopamine synthesis. Therefore an anti-parkinsonian effect of MM water extract was evaluated in this study. Rats were induced the motor symptoms of parkinsonism using chronic rotenone treatment (3 mg/kg s.c. 14 days). Then either L-DOPA (6 mg/kg i.p.) or MM extract (equivalent to L-DOPA 6 mg/kg i.p.) was given to rotenone-induced parkinsonism rats for 10 days. Three behavioral tasks were used to examine motor symptoms including the open field study, stepping test and postural instability test. Chronic rotenone exposure impaired motor performance in all behavioral tasks while L-DOPA significantly improved motor symptoms in rotenone-induced parkinsonism rats seen in all behavioral tasks. In contrast, parkinsonism rats received MM extract showed the improvement of locomotor activity in the open field and the performance in postural instability test but not in stepping test indicating lower efficacy of MM extract compared to L-DOPA. Other chemical constituents of the water extract of MM seed and the mechanism underlying the anti-parkinsonian effect should be further investigated.

Keywords: *Mucuna macrocarpa*, parkinsonism, rotenone

INTRODUCTION

Parkinson's disease (PD) is a chronic, progressive neurodegenerative disorder caused by loss of dopaminergic neurons in the nigrostriatal dopaminergic pathway. The main motor symptoms of PD include resting tremor, rigidity, bradykinesia and postural disturbance. It is estimated that PD affected approximately 700,000 people in South-East Asia [1]. L-DOPA, an intermediate precursor of dopamine, is the current standard medication for PD which aimed to increase dopamine synthesis. Dopamine receptor agonists and monoamine oxidase-B inhibitors are used as an adjunctive therapy.

Mucuna macrocarpa Wallich, Leguminosae (synonyms *Mucuna collettii* Lace) has been recorded in Thai traditional medicine and currently used to treat male sexual dysfunction [2]. L-DOPA is normally found in Mucuna seeds (*Mucuna pruriens*, *Mucuna macrocarpa* and *Mucuna interupta*). *Mucuna pruriens* seed was used to treat the symptoms of tremor and akinesia as described in an Indian ayurvedic texts [3]. Recently a study by Kasture et al. [4] showed that *Mucuna pruriens* seed extract can improve motor symptoms in rodent models of Parkinson's disease. In Thailand, it was found that *Mucuna macrocarpa* seed extract contains the highest levels of L-DOPA compared to other Mucuna seeds. Therefore the present study aimed to evaluate an antiparkinsonian effect of *Mucuna macrocarpa* seed extract using rotenone-induced parkinsonism rat model.

[*] *Correspondence to: Ratchanee Rodsiri*
E-mail: Ratchanee.R@chula.ac.th

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats weighing 200-250 g were obtained from the National Laboratory Animal Centre, Mahidol University, Salaya, Nakhon Pathom, Thailand. Rats were housed at a constant ambient temperature (25±2°C) and humidity (50-60%) on a 12/12 h light/dark cycle with free access to food and water. All experiment procedure was approved by the Institutional Animal Care and Use Committee, Faculty of Pharmaceutical Sciences, Chulalongkorn University.

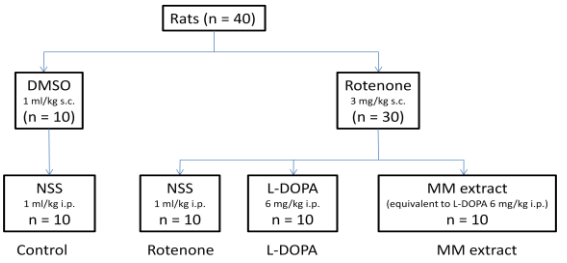
Chemicals

Rotenone (Sigma; 3 mg/ml) was dissolved in 5% DMSO (Sigma) and injected subcutaneously to induce Parkinsonism in rats. Crude water extract of *Mucuna macrocapa* (MM) was prepared from MM seeds. MM seeds were cut into small pieces then heated with 70°C sterile water for 40 min. The supernatant was lyophilized to dry powder. L-DOPA content in the MM extract was quantified using capillary electrophoresis. L-DOPA (Sigma) was used as a positive control. L-DOPA standard (6 mg/ml) and MM extract (equivalent to L-DOPA 6 mg/ml) were dissolved in normal saline solution (NSS).

Rotenone-induced parkinsonism

Chronic rotenone administration was used to induce parkinsonism in rats (modified from Cannon et al. [5]). Rats were firstly divided into 2 groups

(A)



(B)

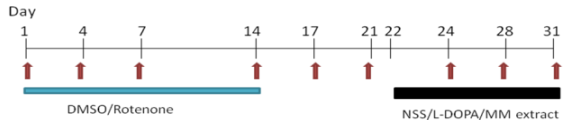
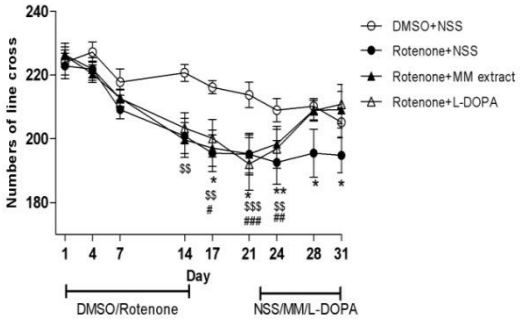


Figure 1 The picture shows four treatment groups of rats including control, rotenone-induced parkinsonism, L-DOPA-treated (positive control) and MM extract-treated groups (A) and experimental timeline (B). The arrows in figure 1B indicated behavioral testing days.

(A)



(B)

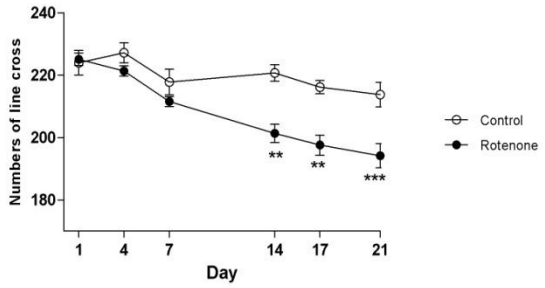


Figure 2 (A) The open field study showed effects of chronic rotenone administration (3 mg/kg s.c. 14 days) on numbers of line cross in the open field study. **p<0.01, ***p<0.001 compared to control at the same time point. (B) Effects of MM extract and L-DOPA on numbers of line cross in rotenone-induced parkinsonism rats. *p<0.05, **p<0.01 rotenone+NSS compared to the activity on day1 of the same animal group, $^{\$\$}$p<0.01, $^{\$\$\$}$p<0.001 rotenone+MM extract compared to the activity on day1 of the same animal group, $^{\#}$p<0.05, $^{\#\#}$p<0.01, $^{\#\#\#}$p<0.001 rotenone+L-DOPA compared to the activity on day1 of the same animal group.

receiving either 5% DMSO (1 ml/kg s.c.; n=10) as a control group or rotenone (3 mg/kg s.c.; n=30) to induce parkinsonism for 14 days and allowed 7 days free-drug period. On day 22, rats in the control group (n=10) were given NSS (1 ml/kg i.p.) for 10 days while rotenone-treated rats received either NSS (1 ml/kg i.p.; n=10), L-DOPA (6 mg/kg i.p.; n=10) or MM extract (equivalent to levodopa 6 mg/kg i.p.; n=10) (Figure 1A). Rats were performed three behavioral tasks including the open field study, stepping test and postural instability test on day 1, 4, 7, 10, 14, 17, 21, 24, 28 and 31 (Figure 1B).

Open field study

Change in locomotor activity was observed in an open field arena immediately after each injection on the behavioral testing day. The arena comprised of 1-m diameter round smooth surface area surrounded by dark cylindrical wall (70-cm high). The inner circle line (80-cm diameter) was drawn on the arena surface and 12 radii were made from the inner circle line to the wall. Rats were freely acclimatized in the open field for 5 min and numbers of line cross were recorded.

Stepping test

Bradykinesia symptom in parkinsonism rats was assessed using stepping test modified from Olsson et al. [6]. A smooth surface open track with 20-cm width and 70-cm length was used. After set the rat at one edge of the track, the experimenter held the rat hindlimbs and slightly rising from the ground and left the forelimbs touching track's surface. The investigator let the rat moving forward and recorded distance from the starting point within 5 seconds.

Postural instability test (PIT)

Postural instability test was used to assess the impairment of an ability to make motor adjustment in response to involuntary changes in center of gravity [7]. The experimenter gently lifted the rat hindlimbs and one forelimb off the surface and pushed the rat slowly forward along the track (70-cm). Rat will make the "catch-up step" using the remaining forelimb to maintain center of gravity and numbers of "catch-up step" were counted.

Statistical analysis

Data were expressed as mean + SEM and analyzed using two-way ANOVA with treatment and time as main factors, followed by Bonferroni post-hoc test. For all measurements, significant difference was considered as p<0.05.

RESULTS

Open field study

Rats were allowed to move freely in an open field arena immediately after treatment. Locomotor activity was recorded as numbers of line cross within 5 min. Two-way ANOVA revealed an overall main effect of time ($F_{(8,323)}$ = 16.16,

$p<0.0001$) and treatment ($F_{(3,323)} = 11.40$, $p<0.0001$) in numbers of line cross. Chronic rotenone treatment (3 mg/kg s.c. 14 days; n=30) significantly decreased rats locomotor activity on day 14, 17 ($p<0.01$) and 21 ($p<0.001$) compared to control (DMSO 1 ml/kg s.c.; n=10) (Figure 2A). After chronic rotenone treatment, rats that received NSS showed continuously decreased locomotor activity on day 24 ($p<0.01$), 28 ($p<0.05$) and 31 ($p<0.05$) compared to day1 (figure 2B). In contrast, rats that received either MM extract or L-DOPA showed no significant different in numbers of line cross on day 28 and 31 compared to day1 (Figure 2B) indicating that MM extract and L-DOPA treatment can improve locomotor activity in parkinsonian rats.

Stepping test

Rats were performed in the stepping test 15 min after treatment followed postural instability test. Stepping distance within 5 seconds was recorded. There were effects of time ($F_{(8,323)} = 61.95$, $p<0.0001$), treatment ($F_{(3,323)} = 112.1$, $p<0.0001$) and time x treatment interaction ($F_{(24,323)} = 8.79$, $p<0.0001$) in stepping distance. Chronic rotenone treatment plus NSS significantly decreased stepping distance from day 14 to 31 compared to control ($p<0.001$) (Figure 3). L-DOPA significantly increased stepping distance in parkinsonism rats compared to rotenone+NSS group on day 24 ($p<0.01$), 28 ($p<0.001$) and 31 ($p<0.001$) while MM extract failed to improve motor symptom in parkinsonism rats as there was no significant different in stepping distance between rotenone+MM extract and rotenone+NSS groups on day 24, 28 and 31 (Figure 3).

Postural instability test (PIT)

There were effects of time ($F_{(8,323)} = 76.45$, $p<0.0001$), treatment ($F_{(3,323)} = 100.9$, $p<0.0001$) and time x treatment interaction ($F_{(24,323)} = 9.49$, $p<0.0001$) in numbers of cat-up step. Chronic rotenone treatment plus NSS significantly decreased numbers of catch-up step from day 14 to 31 compared to control ($p<0.001$) (Figure 4). After chronic rotenone treatment, administration of either L-DOPA or MM extract significantly increased numbers of catch-up step in parkinsonism rats compared to rotenone+NSS group on day 28 ($p<0.05$ rotenone + L-DOPA; $p<0.001$ rotenone + MM extract) and 31 ($p<0.001$) (Figure 4).

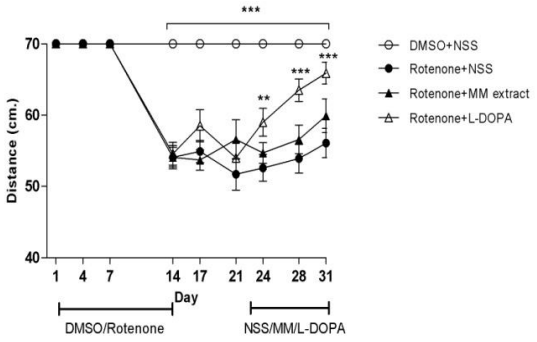


Figure 3 The stepping test showed effects of L-DOPA but not MM extract on stepping distance in rotenone-induced parkinsonism rats. ***p<0.001 compared to rotenone+NSS at the same time point.

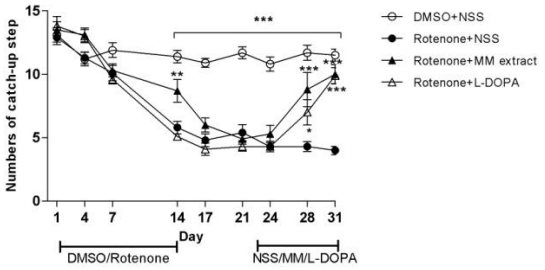


Figure 4 The postural instability test showed effects of MM extract and L-DOPA on numbers of cat-up step in rotenone-induced parkinsonism rats. *p<0.05, ***p<0.001 compared to rotenone+NSS at the same time point.

DISCUSSION

Mucuna macrocarpa seed extract is known to contain L-DOPA, a precursor of dopamine synthesis therefore its anti-parkinsonian effect was investigated preclinically in the present study using rotenone-induced parkinsonism rat model.

Rotenone is a potent inhibitor of mitochondrial complex I and previously shown a selective damage to dopaminergic neurons [7]. Chronic rotenone exposure caused parkinsonian symptoms, degeneration of dopaminergic neuron in the substantia nigra, decreased dopamine levels in the striatum and α-synuclein aggregration in rats [5, 7] which imitated the pathology of PD in human. In the present study, chronic administration of rotenone permanently reduced rat motor performance in the open field study, stepping test and postural instability test indicating a successful modeling of PD motor symptoms in rats.

L-DOPA, a standard medication for the treatment of PD, was used in this study as a positive control. It was shown that motor deficits in chronic rotenone-treated rats were improved by L-DOPA treatment seen in all behavioral tasks. The effect of L-DOPA on motor symptoms in parkinsonism rat is probably due to an increase of dopamine synthesis as L-DOPA is an intermediate precursor of dopamine.

Mucuna macrocarpa seed extract revealed a significant increase in locomotion and performance in the postural instability test but not in the stepping test indicating the lower efficacy of MM extract compared to standard L-DOPA treatment at the same dose. In contrast to other studies using *Mucuna pruriens* (MP), it was found that *Mucuna pruriens* seed produced higher anti-parkinsonian efficacy compared to L-DOPA in 6-

hydroxydopamine-induced parkinsonism rats [4, 8]. Previous studies suggested that MP seed extract exhibited the neuroprotective effect via an anti-oxidative activity [9] and the increase of mitochondrial complex I activity [10]. An antioxidant compound, genistein, was also found in the hexane, ethyl acetate and methanolic extracts from tubers of *Mucuna macrocarpa* [11]. However there was no study in the anti-oxidative effect of the water extract from *Mucuna macrocarpa* seeds.

CONCLUSION

Mucuna macrocarpa seed extract alleviated the motor symptoms in rotenone - induced parkinsonism rats. Other chemical constituents of the water extract of MM seed and the mechanism underlying the anti-parkinsonian effect should be further investigated.

ACKNOWLEDGEMENTS

The authors express their gratitude to the Graduate School, Faculty of Pharmaceutical Sciences, Chulalongkorn University and Ratchadaphiseksomphot Endowment Fund of the Research Affairs of Chulalongkorn University for financially support. Thanks are also due to Herbal Remedies and Alternative Medicine Task Force of STAR: (Special Task Force for Activating Research) under 100 years Chulalongkorn University for partially supported.

REFERENCES

1. World Health Organization. Neurological disorders: Public health challenges. Geneva: WHO; 2006.
2. Cherdshewasart W, Sutjit W, Pulcharoen K, Panriansaen R, Chulasiri M. Antimutagenic potential of the Thai herb, Mucuna collettii Lace. J Ethnopharmacol. 2008; 115: 96–103.
3. Manyam BV. Paralysis agitans and levodopa in ‘‘Ayurveda’’: ancient Indian medical treatise. Mov Disord. 1990; 5:47–8.
4. Kasture S, Pontis S, Pinna A, Schintu N, Spina L, Longoni R, et al. Assessment of Symptomatic and Neuroprotective Efficacy of Mucuna Pruriens Seed Extract in Rodent Model of Parkinson’s Disease. Neurotox Res. 2009; 15:111–22.
5. Cannon JR, Tapias V, Na HM, Honick AS, Drolet RE, Greenamyre JT. A highly reproducible rotenone model of Parkinson's disease. Neurobiol Dis. 2009; 34(2): 279-90.
6. Olsson M, Nikkhah G, Bentlage C, Bjarklund A. Forelimb akinesia in the rat Parkinson model: differential effects of dopamine agonists and nigral transplants as assessed by a new stepping test. J Neurosci. 1995; 15: 3863-75.
7. Betarbet R, Sherer TB, MacKenzie G, Garcia-Osuna M, Panov AV, Greenamyre JT. Chronic systemic pesticide exposure reproduces features of Parkinson’s disease. Nature Neuroscience. 2000; 3(12): 1301-6.
8. Hussain G, Manyam BV. Mucuna pruriens proves more effective than L-DOPA in Parkinson’s disease animal model. Phytother Res. 1997; 11: 419–23.
9. Dhanasekaran M, Tharakan B, Manyam BV. Antiparkinson drug – Mucuna pruriens shows antioxidant and metal chelating activity. Phytother Res. 2008; 22: 6–11.
10. Manyam BV, Dhanasekaran M, Hare TA. Neuroprotective effects of the antiparkinson drug Mucuna pruriens. Phytother Res. 2004; 18: 706–12.
11. Chukeatirote E, Saisavoey T. Antimicrobial property and antioxidant composition of crude extracts of Pueraria mirifica, Butea superba and Mucuna macrocarpa. Maejo Int J Sci Technol. 2009; 3(01): 212-22.