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

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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 colletii 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.

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 steril. Chemicals were obtained from the National Laboratory Animal Centre, Mahidol University, Salaya, Nakhon Pathom, Thailand. Rats were housed at a constant 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.

Rothenone-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

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Figure 1 (A) 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.

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<0.001 rotenone+MM extract compared to the activity on day1 of the same animal group.

Results of the 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(8,322) = 16.16,
Effects of L-DOPA compared to day1 (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 day 1 (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 day 1 (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).

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 agregration 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-

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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.

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