

Effect of *Passiflora foetida* on Abnormal Locomotion in MPTP Induced Parkinson-Like Behavior in Mice

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Abstract

Introduction: *Passiflora foetida* L. (passionflower) is a climbing vine that grown in tropical region around the world. In Vietnamese folk medicine, dry leaves are used for sleeping disorder. Other pharmacological activities of this plant have been studied. Relieving abnormal motility in Parkinson-like disease is one of interest. **Methods:** Parkinsonism by 15 mg/kg MPTP (1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine) was performed in C57BL/6 mice. Chronic MPTP injections were administered for 6 weeks. Three main unusual characteristics in these mice were determined using force plate actimeter (FPA). **Results:** Lower number of distance of travelling, higher number of bout of low mobility (BLM), and higher intensity of peaks power spectra at specific frequencies of 9-11 Hz. were found in MPTP mice. 200 mg/kg passion, a granule suspension form of passionflower, significantly reduced the action of MPTP in distance of travelling, bout of low mobility, and power spectra, as well as sinemet did. **Conclusion:** This study suggests that the effect of *P. foetida* on relieving Parkinson's symptoms in mice possibly relate dopaminergic pathway. Therefore, the dopamine levels in the brain of mice treated with *P. foetida* need to be evaluated in the further study.

Keywords: *Passiflora foetida*, Parkinson's disease, MPTP, dopaminergic pathway, force plate actimeter

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1. Introduction

Passiflora foetida Linn. (passionflower; stinking passionflower or Ka-Tok-Rok in Thai) is a climbing vine in a family of Passifloraceae. It origin in the southwestern United States, and has been widely spread to tropical region around the world. The plant tips and young leaves are edible as well as its fruits. In Vietnamese folk medicine, dry leaves are used for helping sleep disorder. The main chemical constituents in the aerial parts of *Passiflora* species are alkaloids, particularly harmine and harmaline (Avula, *et al.*, 2012). b-carbolines, a group of alkaloid includes harmine, harmaline, and tetrahydroharmine, have some properties as monoamine oxidase inhibitors (MAOIs) that inhibit the destruction of dopamine in neuron (Serrano-Duenas, *et al.*, 2001). The study of Splettstoesser *et al.* (2005) showed neuroprotective effect from apoptotic neuronal death of harmaline and harmine.

Parkinson's disease (PD) is a neurodegenerative disorder which affects the ability of movements. The symptoms of PD mostly include bradykinesia, rigidity, tremor, and postural instability (Gelb, *et al.*, 1999). A deficiency of dopamine in the substantia nigra is the characteristic in PD (Moon *et al.*, 2014). To develop Parkinson-like behavior in animal model, toxin-induction is one of common used methods (Terzioglu and Galter, 2008). MPTP (1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine) is a neurotoxin that is discovered to induce several symptoms of PD in animal. After metabolizing into MPP^+ (1-methyl-4-phenylpyridinium ion), it causes PD symptoms by destroying dopaminergic neuron (Moon, *et al.*,

2014). Parkinson-like behaviors in mouse model produced by MPTP are displayed in various abnormal locomotions, such as akinesia, rigidity, body shaking, and other difficulty of movements.

Force plate actimeter (FPA) is used to quantify several type of locomotor activity, for example, startle, ataxia, focused stereotypy, tremor, seizure, and more. It is invented by Dr. Stephen C Fowler from the University of Kansas, USA (Fowler, 2002). FPA comprises of a graphite plate which can detect any forces on the plate, 4 transducers underneath the plate, and one interface. The 4 transducers are used for converting forces to electrical currents, whereas an interface is an instrument for sending those currents to the computer. Then, the currents are analyzed and translated into the numbers and peaks of power spectra FPA analysis program. Three main abnormal motilities induced by MPTP in mice in this study were examined and interpreted as the followings: distance of travelling interpreted as akinesia, bout of low mobility (BLM) interpreted as rigidity, and power spectra interpreted as body shaking or tremor. Therefore, the purpose of this study was to determine the effect of *Passiflora foetida* on Parkinson-like behavior induced by MPTP in mice.

2. Material and Methods

Male C57BL/6 mice were purchased from the National Laboratory Animal Center, Mahidol University, Salaya, Nakhon Pathom. Age of the rats at the beginning of study was 4 months old. They were acclimatized under standard conditions for 1 week with free access of food

and water. All procedures and animal care were approved by Institutional Animal Care and Use Committee of Thailand Institute of Scientific and Technological Research.

The ethanolic extract of aerial parts of passionflower was formulated in granulation form and was then prepared in acacia solution to be suspension form. The dose of granule we used was 1,400 mg/kg (equivalence of 200 mg/kg passionflower extract). This dose was based on the previous study in our laboratory. Sinemet (M&H Manufacturing, Thailand), a standard drug for clinical PD treatment, was also suspended in acacia solution. To prepare acacia solution, 3 g of acacia powder was dissolved in 100 ml of distilled water. It was used as a vehicle. MPTP (Sigma Aldrich, USA.), a neurotoxin, was prepared in 0.9% normal saline solution (NSS). It was used to impair locomotions in mice.

FPA (BASi, USA) were used to monitor locomotor activities in mice. FPA analysis program (BASi, USA) was used for analyzing mice locomotions. Twenty four C57BL/6 mice were used and randomly divided into four groups. Each group of mice was received 15 mg/kg MPTP for 6 injections, except the control group which received 0.9% NSS (normal saline solution). There was a 5-day interval for each injection. Thirty minutes before the 6 injection of MPTP, each group of mice was orally administered as the followings;

- 1) The control group: received nothing
- 2) The MPTP group: 3% w/v of acacia in water (MPTP only)
- 3) The sinemet group: 10 mg/kg sinemet in acacia solution (MPTP + sinemet)

- 4) The passion group: 200 mg/kg passionflower extract in granule form prepared in acacia solution (MPTP + passion)

Immediately after injection, each mouse was place into FPA approximately for 2 hours. Distance of travelling, BLM, and power spectra of each group of mice were recorded and analyzed using FPA analyzing program. Data of distance of travelling and BLM were expressed as mean \pm standard error. Statistical analysis for comparing treatment effects were done by one-way ANOVA. Comparisons among groups were conducted by using the Tukey post-hoc analysis. Statistical significance was defined as $p < 0.05$. The power spectra were showed in peaks of frequency (Hz.), and were compared other treatment groups with the MPTP group.

3. Results and Discussion

Since MPTP destroyed dopaminergic neuron, it produced PD-like symptoms with akinesia and rigidity (Moon, et al., 2014) as shown in Figure 1. Continuous injection of MPTP for 6 times with a 5-day interval produced less movement as showed in significantly lower numbers of distance of travelling in all MPTP-treated groups comparing with the control mice ($p < 0.05$). However, 200 mg/kg passionflower extract could be able to inhibit the action of MPTP. There was a significant increase in the number of distance of travelling ($p < 0.05$) in passion group compared to the MPTP group (Figure 1A). On the other hand, numbers of BLM of all MPTP injected mice were significantly higher than the control ($p < 0.05$). Sinemet, a standard drug, significantly decreased the number of BLM compared to the MPTP only group ($p < 0.05$) as shown in Figure 1B.

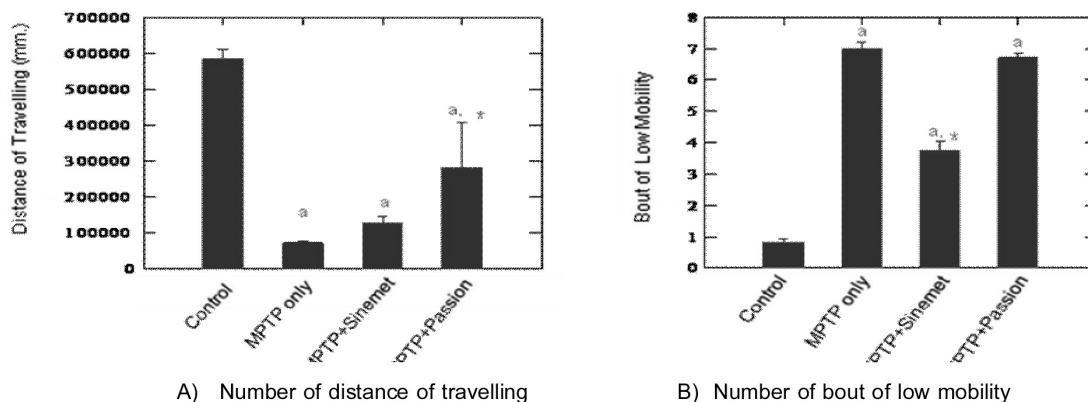


Figure 1: Effect of 10 mg/kg sinemet and 200 mg/kg passionflower (passion) on the number of distance of travelling (A) and number of BLM (B) after 6 injections of MPTP comparing with the control group (^a $p < 0.05$) and the MPTP group (* $p < 0.05$).

The power spectra of the MPTP group were revealed 3-4 main peak forces of body shaking or tremor at a specific range of frequencies at 9-11 Hz. (Figure 2). The power spectra of other groups were analyzed and compared with MPTP

only as shown in Figure 3. Sinemet reduced the intensity of the power spectra at those specific frequencies (Figure 3A) as well as passion did (Figure 3B)

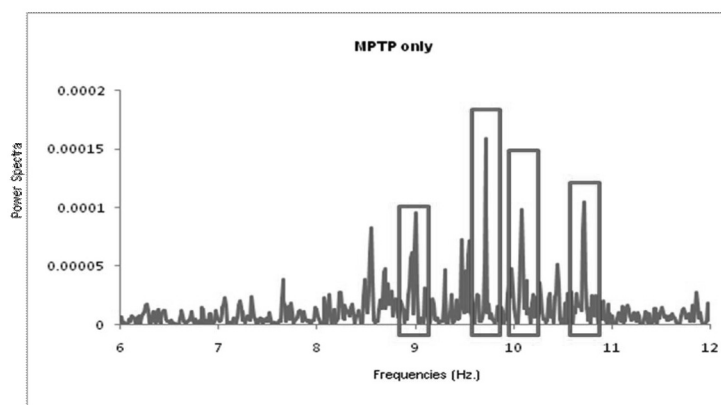
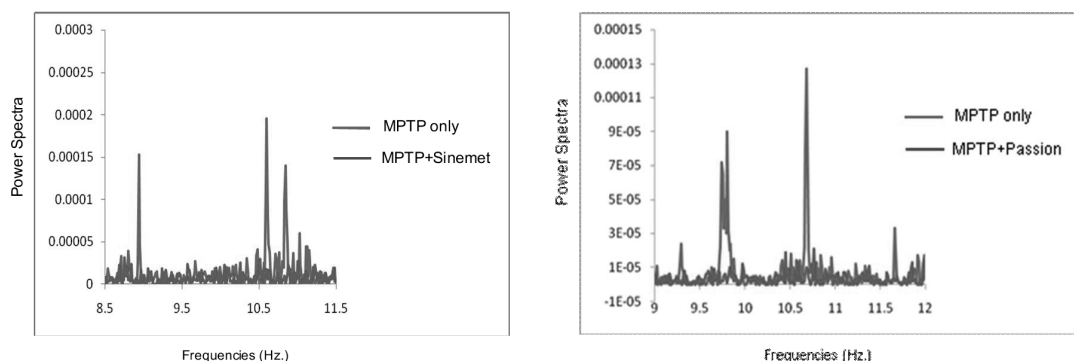


Figure 2: Effect of 15 mg/kg MPTP (MPTP only) on the power spectra. Each box revealed the intensity of peak forces at a specific range of frequencies at 9-11 Hz.



B) The comparison of power spectra between groups of MPTP only and MPTP + sinemet

A) The comparison of power spectra between groups of MPTP only and MPTP + passion

Figure 3: The comparison of power spectra between MPTP only and MPTP + sinemet groups (A) and MPTP only and MPTP + passion groups (B).

4. Conclusion

MPTP produced abnormal motilities in mice including akinesia; displayed a lower number of distance of travelling (Figure 1A), rigidity; displayed a higher number of BLM (Figure 1B), and body shaking or tremor; displayed high intensity of peak power spectra at a specific range of frequencies at 9-11 Hz. (Figure 2). Passionflower as well as sinemet reduced these actions of MPTP by presenting better performance of locomotions. Higher number of distance of travelling was found in passion group and lower number of BLM was found in sinemet group. More importantly, lower intensity of peak power spectra at specific range of frequencies which indicated less power of body shaking were also found in those both groups. These findings imply that the relieving of Parkinson-like behaviors of *Passiflora foetida* L. possibly aid better performance in mice by involving dopaminergic pathway. Therefore, the

dopamine levels in the brain of mice treated with **this plant** need to be evaluated in the further study.

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