THUNBERGIA LAURIFOLIA LINDL. EXTRACT ATTENUATE MOTOR IMPAIRMENT IN ACUTE MPTP MOUSE MODELS OF PARKINSON'S DISEASE

Thanasit Chaiwut[1] and Ranida Quiggins[2] and Chainarong Tocharus[3]

[1]Graduate Student, Department of Anatomy, Faculty of Medicine, Chiang Mai University, thanasitisit@gmail.com

[2]Advisor, Department of Anatomy, Faculty of Medicine, Chiang Mai University,ranida.quiggins@cmu.at.th

[3]Co-advisor.Department of Anatomy, Faculty of Medicine, Chiang Mai University, chainarongt@hotmail.com

---

ABSTRACT

Parkinson's disease (PD) is a neurodegenerative disease that leads to motor impairment. The compound 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), is the specific neurotoxin that affects the dopaminergic neurons in the substantia nigra pars compacta (SNpc), and was used to induce the mice to be models of acute PD. *Thunbergia laurifolia* Lindl. (*TL*) or "Rang chuet" is an herb which is generally used for reducing the toxicity of hazardous chemicals such as pesticides. This study aims to find out whether *TL* extract can improve the motor impairment of the MPTP induced mouse models of PD by using pre *TL* and post *TL* treatments and observing the mouse's latency times on rotating rods. We used an immunofluorescence against tyrosine hydroxylase (TH) antibody to determine the TH immunoreactive (TH-IR) neurons in SNpc. The results showed that latency times of the MPTP + pre *TL* group mouse was not significantly different from that of the control mice. Additionally, the number of TH-IR neurons of the MPTP + pre *TL* group was higher than that of the MPTP + post *TL*, yet was less different ($p < 0.01$) than that of the MPTP + post *TL* ($p < 0.001$) when compared with control. Our results confirmed that the pre-treatment of *TL* extract in the MPTP mouse model of PD reduces motor impairment of the MPTP mouse models of PD. These might shed light on the how to use *TL* leaf extracts to protect the motor impairment of people who have been intoxicated by hazardous chemicals.

Keywords: *Thunbergia laurifolia* Lindl. (*TL*), Parkinson's disease, rotarod test, tyrosine hydroxylase

1. Introduction

Parkinson's disease (PD) is a progressive neurodegenerative in which degeneration of the dopaminergic neurons in the substantia nigra par compacta (SNpc) of the midbrain leads to motor impairment. These motor dysfunctions include rigidity, tremors, bradykinesia, and postural instability. Jackson-Lewis et at, (2012) used 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to induce animals to be a models of PD. Alam G et al. (2017) and Kozina EA et al. (2014) identified a decrease in neurons in SNpc by analyzing immunohistochemistry using tyrosine hydroxylase (TH). The acute MPTP mouse model of PD was induced by intraperitoneal injections (i.p.) of MPTP at 20 mg/kg of body weight (bw), 4 times every 2 hours, as has been done in previous studies (Lee et al.,

2017). The acute model of PD represents the earliest phase of PD which is indicated by having 53% of the TH + cell compared to that of a normal subject (Pain et al., 2013). In addition to MPTP, rotenone, pesticides, and paraquat were also reported to be risk factors of PD by Sherer et al. (2003) and Elbaz et al. (2009), respectively. *Thunbergia laurifolia* Lindl. (*TL*) or "Rang chuet" is a Thai herb which is well known for its detoxification qualities. Usanawarong et al. (2000) indicated that aqueous *TL* leaf extract can reduce toxicity in paraquat intoxicated rats. Tangpongand Satarug (2010) also found that *TL* leaf extract attenuated the neuronal degeneration in the hippocampus and restored memory loss of lead intoxicated rats. There are two treatment strategies which generally can be applied in research of mouse models of PD. Neuroprotective strategies use pre-treatment with protective agents (Lee et al., 2017), while neurorescue or restorative strategies use a post-treatment of therapeutic agents (Zhao et al, 2010). We would like to investigate whether *TL* extract is a protective or therapeutic agent for attenuating the motor dysfunction and neuronal degeneration in the MPTP mouse model of PD.

2. Objectives of the study

This present study aimed at investigating the neuroprotective and neurorescue effects of the TL extracts in MPTP mouse models of PD. Mice were pre-treated or post-treated with TL extracts and determined motor functions and numbers of dopaminergic neurons in SNpc.

3. Materials and methods

Fresh *TL* leaves and its specimens were harvested from an area in Sing Buri Province, Thailand. The scientific name was identified at Ethnobotany and Northern Thai Flora Laboratory, Department of Biology, Faculty of Science Chiang Mai University and then the voucher specimen (QBGNo.104862) was stored at Queen Sirikit Botanic Garden Herbarium (QBG), Ministry of Natural Resources and Environment. The fresh *TL* leaves were thoroughly washed and dried before they were extracted with 100% ethanol. The crude *TL* extract was dried and then diluted with Tween 80 solution before being used as a dilution of 200 mg/kg.bw. A total of 30 male C57BL/6 mice (Nomura Siam International, Thailand), aged 5 weeks weighing between 20-25 g.bw, were used in this study. All mice were caged in controlled conditions having a cycle of 12 hours of darkness and 12 hours of light with temperature set at 25^{o} C. They were allowed free access to food and water. The mice were randomly divided into 5 groups; 6 mice per group. Group 1 was the control group. Group 2 were mice that received Tween 80 as a "vehicle" for one week before receiving intraperitoneal (i.p.) injections of MPTP (Lee et al., 2017). This group was called the "MPTP + pre vehicle group." Group 3 was the mice that were pretreated with *TL* extract injections of 200 mg-/kg.bw/day for 1week before being inducing with MPTP, and was called the "MPTP + pre *TL* group." Group 4 was the mice that received vehicle for 1 week after being inducing with MPTP, and was called the "MPTP + post vehicle group." Group 5 was the mice that were post-treated with *TL* extract for 1 week after being inducing with MPTP. These mice were called the "MPTP + post *TL* group." All mice were tested for their motor movement by

putting on the rotating rod with speed of 4-40 rpm. The latency times of all mice were recorded at day 0 (one day before the pre-treatment), day 7 (one day before the MPTP i.p. injection), day 9 (one day after the MPTP i.p. injection), and day 16 (one week after the MPTP i.p. injection; or one week after treatments). The latency times of pre *TL* and post *TL* treated groups at day 0, 7, 9 and 16 were compared to those of control and pre and post vehicle treated groups. After that, all mice were given a lethal dose of ether and were perfused transcardially with PB solution, followed by a fixative of 4% paraformaldehyde in 0.1 M phosphate buffer (PB), pH 7.4. The brains were then removed from the skulls. All brains were cut into 50 µm thick coronal sections using a vibratome (Leica s 1200). The brain sections were collected from rostral to caudal sections every 5th section in PB. A total of 6 rolls of brain sections were obtained from each brain. One row of each was used for analyzing the number of dopaminergic neurons by immunofluorescence with primary antibodies used against tyrosine hydroxylase (TH) made in mouse (Lot # 106M4874V, Sigma) at a dilution of 1:1500, then reacted with rhodamine anti-mouse made in goat (Catalog No. 31660, Thermo Fisher) at a dilution of 1:100 as the secondary antibody. The TH immunoreactive (IR) cells in the SNpc of all groups were examined under a fluorescent microscope and photos were taken so that the number of TH-IR cells could be counted. All data was presented as mean ± standard deviations (SD). One-way analysis of variance (ANOVA) was completed followed by a post-hoc analysis with a Tukey test for multiple comparisons using an R program. The values of $p < 0.05$ were considered statistically significant.

4. Results

The motor function of the mice was indicated by the latency times on the rotating rod. We found that the latency times of all experimental groups on day 0 and day 7 were not significantly different from that of the control group (Fig. 1). The latency time of all induced groups on day 9 were significantly less than that of the control group ($p < 0.01$). On day 16, the latency time of the MPTP + pre *TL* and the MPTP + post *TL* had increased from those of day 9. There was no significant difference between the latency times of the two groups. However, the latency time of the MPTP + post *TL* groups is still significantly lower than that of the control group ($p < 0.05$). Interestingly, the latency time of the MPTP + pre *TL* group was not significantly different from that of the control.

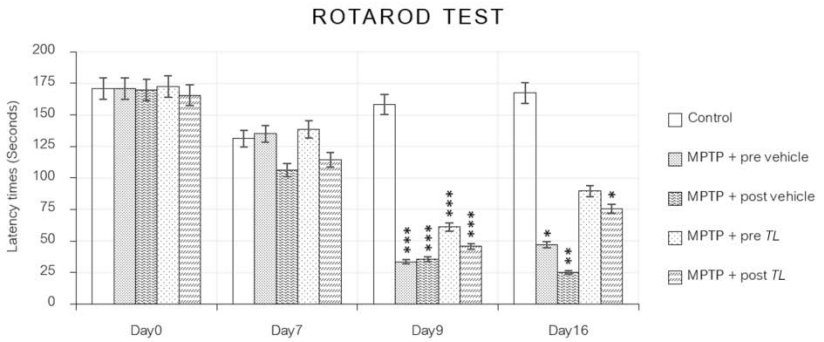

Figure 1. The latency times on the rotating rod of the control group and the four experimental groups. The experimental mice were induced to be acute mouse models of PD with MPTP on day 8. A week before day 8 (day 1-7), two experimental groups of mice were pre treated with vehicle and *TL*, respectively. A week after day 8 (day 9-16), the other two MPTP groups were post treated with vehicle and *TL*, respectively. (Significantly different when compared means to Control (p-value = *0.05, **0.01, ***0.001)

There were the similar outcomes regarding the immunofluorescence TH labeling neurons in the control and the MPTP mouse induced groups. The control group showed the highest density of TH-IR neurons (Fig. 2A) comparing to the rest (Fig. 2B, C, D, and E). The MPTP + pre vehicle, + post vehicle, and + post *TL* groups were found to have significantly lower numbers of TH labeling neurons in SNpc (67±34.4, 58±30.3 and 83±15.6 cells, respectively) than that of the control group, 225±3.6 cells ($p < 0.001$), due to the neurotoxicity of MPTP targeting on the SNpc (Fig. 2B, C, D, and E). However, the number of the TH-IR neurons of the MPTP + pre *TL* group was 106±27.5 cells which were significantly lower than that of the control at $p < 0.01$. Even though the numbers of dopaminergic neurons of the MPTP + pre *TL* group were significantly lower than that of the control, the motor performance of this group was not significantly different compared to those of the MPTP + post *TL* group.

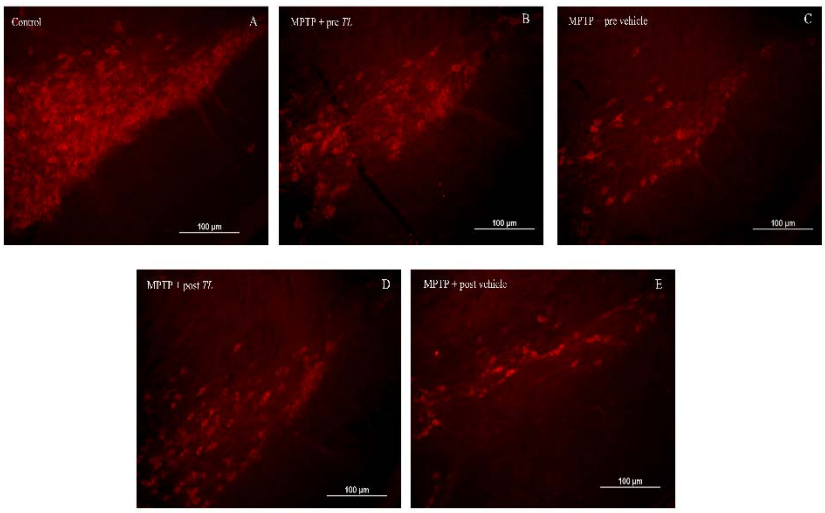

Figure 2. The immunofluorescence against TH antibody demonstrating the TH-IR neurons in SNpc of all groups of mice (A; control group, B; MPTP + pre *TL*, C; MPTP + pre vehicle, D; MPTP + post *TL*, and E; MPTP + post vehicle). The TH-IR, indicated as red color, was labeled as tyrosine hydroxylase in cytoplasm surrounding the nucleus of the dopaminergic neurons.

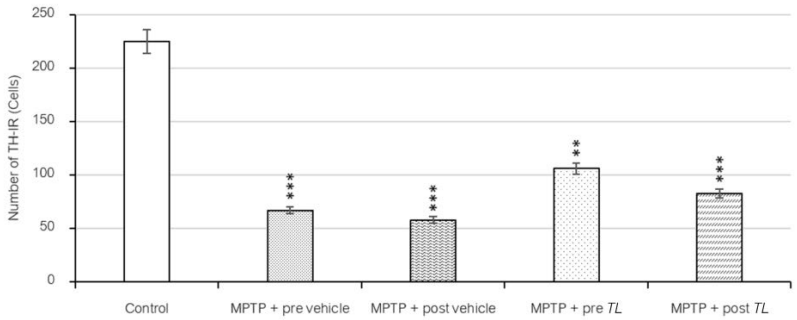

Figure 3. Graph demonstrating the number of TH-IR neurons in control group compared to those of the MPTP induced mice groups. (Significantly different when compared means to Control (*p*-value = *0.05, **0.01, ***0.001)

5. Discussion

This study found that the pre *TL* treatment before inducing with MPTP attenuated the motor dysfunction of the mouse model of PD. Additionally, the neuronal loss in SNpc was comparable to the pre vehicle MPTP group. This study was similar to the study of Tangpong & Satarug (2010) who used the *TL* leaf extract to alleviate the death of the hippocampal neurons of rats poisoned by lead. This study demonstrated that the motor performace of the pre *TL* treatment was better than that of post *TL* treatment of MPTP induced mice models of PD. These findings might be indicate that *TL* extract has neuroprotective effects on MPTP induced mice models of PD. TL extract may

have use in neurorescue or restorative therapies due to its anti-oxidant and anti-inflammation properties (Chan & Lim 2006, Wonkchalee et al., 2012).

6. Conclusion

TL extract treatment before MPTP exposure could more efficiently maintain the loss or reduce the death of dopaminergic neurons in SNpc, which consequently alters the motor impairment of the mouse model of PD than any post *TL* treatment.

Acknowledgements

The authors would like to thank Mrs. Lamaiporn Peeraphapong for her assistance in preparing the fixative solution and perfusion of the animals, and Mr. Thongkhum Taya for his assistance in the fluorescence microscopy. This work was supported by the Faculty of Medicine, Chiang Mai University.

References

Alam, G., Edler, M., Burchfield, S., & Richardson, J. R. (2017). Single low doses of MPTP decrease tyrosine hydroxylase expression in the absence of overt neuron loss. *Neurotoxicology, 60*, 99-106.-doi:10.1016/j.neuro.2017.03.008

Chan, E., & Lim, Y. (2006). Antioxidant activity of Thunbergia laurifolia tea. *Journal of Tropical Forest Science*, 130-136.

Elbaz, A., Clavel, J., Rathouz, P. J., Moisan, F., Galanaud, J. P., Delemotte, B., Tzourio, C. (2009). Professional exposure to pesticides and Parkinson disease. *Ann Neurol, 66*(4), 494-504. doi:10.1002/ana.21717

Jackson-Lewis, V., & Przedborski, S. (2007). Protocol for the MPTP mouse model of Parkinson's disease. *Nat Protoc, 2*(1), 141-151. doi:10.1038/nprot.2006.342

Kozina, E. A., Khakimova, G. R., Khaindrava, V. G., Kucheryanu, V. G., Vorobyeva, N. E., Krasnov, A. N., Ugrumov, M. V. (2014). Tyrosine hydroxylase expression and activity in nigrostriatal dopaminergic neurons of MPTP-treated mice at the presymptomatic and symptomatic stages of parkinsonism. *J Neurol Sci, 340*(1-2), 198-207. doi:10.1016/j.jns.2014.03.028

Lee, Y., Heo, G., Lee, K. M., Kim, A. H., Chung, K. W., Im, E., Lee, J. (2017). Neuroprotective effects of 2, 4-dinitrophenol in an acute model of Parkinson's disease. *Brain research, 1663*, 184-193.

Pain, S., Gochard, A., Bodard, S., Gulhan, Z., Prunier-Aesch, C., & Chalon, S. (2013). Toxicity of MPTP on neurotransmission in three mouse models of Parkinson's disease. *Experimental and toxicologic pathology, 65*(5), 689-694.

Sherer, T. B., Betarbet, R., Testa, C. M., Seo, B. B., Richardson, J. R., Kim, J. H., Greenamyre, J. T. (2003). Mechanism of toxicity in rotenone models of Parkinson's disease. *Journal of Neuroscience, 23*(34), 10756-10764.

Tangpong, J., & Satarug, S. (2010). Alleviation of lead poisoning in the brain with aqueous leaf extract of the Thunbergia laurifolia (Linn.). *Toxicol Lett, 198*(1), 83-88. doi:10.1016/j.toxlet.2010.04.031

Usanawarong, S., Thesiri, T., Mahakunakorn, P., & Parasupattana, S. (2000). Effect of Thunbergia laurifolia Linn. on detoxification of paraquat. *Warasan Wichai Mo-Kho, 5(1),* 11-17.

Wonkchalee, O., Boonmars, T., Aromdee, C., Laummaunwai, P., Khunkitti, W., Vaeteewoottacharn, K., Chamgramol, Y. (2012). Anti-inflammatory, antioxidant and hepatoprotective effects of Thunbergia laurifolia Linn. on experimental opisthorchiasis. *Parasitology research, 111*(1), 353-359.

Zhao, Q., Gao, J., Li, W., & Cai, D. (2010). Neurotrophic and neurorescue effects of Echinacoside in the subacute MPTP mouse model of Parkinson's disease. *Brain Res, 1346*, 224-236. doi:10.1016/j.brainres.2010.05.-018