Chronic Effect of *Thunbergia laurifolia* Extract and Cocaine in Rats Using Behavior Model of Addiction

Watchareewan Thongsaard PhD*,
Ratirat Sangpayap BSc*, Charles Marsden PhD**

** Department of Physiology, Faculty of Medicine, Srinakharinwirot University, Bangkok, Thailand*
*** School of Biomedical Sciences, Faculty of Medicine and Health Sciences, University of Nottingham, Nottingham, UK*

Background: *Thunbergia laurifolia Linn. (TL) is a herbal medicine which has been used as a component of a mixture of crude extracts to treat drug addicted patients. TL extract was reported to increase dopamine levels significantly in the rat brain both in vitro and in vivo studies similar to that seen with cocaine and TL stimulated in the same brain area that amphetamine stimulated.*
Objective: *The study aims to investigate whether long term treatment with TL can cause addiction or not by comparing with known addicted drug, cocaine, using the conditioned place preference test.*
Material and Method: *Chronic oral administration (30 days) of crude water extract of TL (1 and 10 g/kg, orally) and cocaine (1 mg/kg, orally) were performed to determine the alteration of addiction behavior using a conditioned place preference (CPP) test.*
Results: *Rats received chronic treatment of cocaine became addicted. While, both doses of TL (1 and 10 g/kg, orally) did not cause significantly in the time spent in both compartment between pre- and post-drug treatment.*
Conclusion: *When treated long-term and tested with CPP test of addiction, rats received chronic treatment of cocaine became addicted. On the other hand, both doses of crude water extract of TL (1 and 10 g/kg, orally) did not cause significant changed in the time spent in both compartment between pre- and post- drug treatment indicating that TL did not cause addiction.*

Keywords: *Addiction, Cocaine, Conditioned place preference (CPP) test, Thunbergia laurifolia*

J Med Assoc Thai 2015; 98 (Suppl. 9): S48-S52
Full text. e-Journal: *http://www.jmatonline.com*

Thunbergia laurifolia Linn. (TL), named Rang Jued and babbler's bill in Thai and English, respectively, is a herbal medicine used for several purposes in Thai traditional medicine. TL plant phytochemical profile composes of phenolic content, gallic acid equivalent (GAE) for water, ethanol and acetone extract, respectively. Caffeic acid and apigenin such as delphinidin 3:5-di-O-β-d-glucopyranoside, apigenin-7-O-β-d-glucopyranoside and chlorogenic acid are primary constituents of water extracts which identified by HPLC analysis[1]. TL extract used as an antidote for several poisonous agents including insecticide, ethyl alcohol, arsenic and strychnine poisoning[2,3]. Anti-inflammatory, antipyretic, antioxidant and anti-hemolytic effects have also been reported[4-7]. TL leaf extract (0.1 mg/ml) prevents renal toxicity induced by cadmium in rats[8]. Catalytic activity of caspase-3 enzyme in the rat brain induced with lead has been reduced[9]. Herbal medicine drugs for addicted patients have a component of a mixture of TL crude extracts[10]. Moreover, TL and amphetamine were reported to significantly increase potassium-stimulated dopamine release from rat striatal slices in vitro[11] and increase extracellular dopamine levels in the rat nucleus accumbens in vivo using microdialysis similar to that seen with cocaine[12]. TL has been shown to increase cerebral activity in the anesthetized rat brain detected by functional magnetic resonance imaging (fMRI) on similar area of rat brain with amphetamine[13], indicating that TL exerts similar effects on dopaminergic function to cocaine and amphetamine in the mesolimbic dopaminergic system responsible for the reward mechanisms associated with addiction[12,13]. The study was performed to investigate the possible addictive effect of chronic TL treatment compared to the known addicted drug, cocaine, using the CPP test.

Correspondence to:
Thongsaard W, Department of Physiology, Faculty of Medicine, Srinakharinwirot University, Sukhumvit 23, Wattana, Bangkok 10110, Thailand.
Phone: +66-2-6495921, Fax: +66-2-2601533
E-mail: watchare@swu.ac.th

Material and Method

Animals

Male Wistar rats, weighing 220-250 g, were obtained from the National Laboratory Animal Center, Mahidol University, Thailand. They were housed in groups of 4-6 in a room with a 12 hours light: 12 hours dark cycle and allowed free access to laboratory pellets (National Laboratory Animal Center, Thailand) and water. All animals were cared for in accordance with the International Guiding Principles for Biomedical Research Involving Animals provided by the National Research Council of Thailand (Animal license No. 18/2557).

Crude extraction of TL

Thunbergia laurifolia (TL) was obtained from Wutythamawech botanical garden and the identification confirmed by comparison with the herbarium specimens in the Botany Section, Technical Division, Department of Agriculture, Ministry of Agriculture and Co-operative, Thailand. The crude water extract of TL was prepared freshly in the morning and was administered orally to the rat at a volume of 2 ml/kg. TL at the concentration of 1 g/kg was prepared as follows. Dried TL leaves were chopped and boiled in distilled water at a volume of 5 g/100 ml water. The mixture was boiled at low heat until the volume was slightly over 10 ml. The extracted were then filtered using thin cloth and filter paper No. 1 (Whatman, UK) and boiled until the final volume was 10 ml leaving the final concentration of 5 g/10 ml or 1 g/2 ml. The extract was left to cool down to room temperature before administered to the animals. The concentration of 10 g/kg TL was prepared in the similar manner as above but the TL 50 g was boiled with 400 ml water until the final volume was 10 ml leaving the final concentration of 50 g/10 ml or 10 g/2 ml.

Drugs

Cocaine hydrochloride (Macfarlan Smith Limited, UK) was used to represent a drug of addiction acting on dopaminergic neurons. The authors hold the official license for handling and experimenting on drugs of addiction under the Faculty of Medicine, Srinakharinwirot University, Bangkok, Thailand issued by Office of the Narcotics Control Board, Thailand.

Conditioned place preference (CPP) test

The animals were explored in the conditioned place preference (CPP) apparatus for 15 minute in the first day to evaluate the preferred and non-preferred side of each rat. The rats were divided into 4 groups. The control group received water (2 ml/kg, orally), TL groups received water extract of TL (1 and 10 g/kg, orally) and cocaine group received cocaine (1 mg/kg, orally). The behavioral study using CPP apparatus consisted of two black Perspex compartments (30x30x40 cm each) separated by a guillotine door. Each compartment had different visual cues in the form of thick (2.5 cm) or thin (1.0 cm) horizontal black and white lines. During pre-test phase, animals were placed in the middle of the apparatus without the guillotine door and allowed to explore it for 15 minute. The amount of time spent in each compartment was recorded. The rats were treated with crude water extract of TL (1 g/kg and 10 g/kg, orally) or cocaine (1 mg/kg, orally) for 30 days. During conditioned phase, rats were placed in their less preferred compartments with guillotine door in place for 10 minute, 30 minute after administration of water, TL (1 g/kg and 10 g/kg, orally) or cocaine (1 mg/kg, orally). During the testing phase, the guillotine door was removed and the animals were placed at the intersection of the two compartments of the apparatus and left for 15 minute. The time spent in each compartment during pre-test and test sessions were analyzed. The diagram illustrating the protocol of the CPP test was shown in Fig. 1.

Statistics

The data were presented as mean $\pm$ SEM and analyzed using ANOVA and a post-hoc (Newman-Keuls multiple comparison) test with $p<0.05$ considered significant.

Results

The effect of oral administration of *Thunbergia laurifolia* Linn. (TL), 1 and 10 g/kg and cocaine (1 mg/kg) on time spent in non-preferred compartment before and after drug training compared with control was shown in Fig. 2. The rats given cocaine 1 mg/kg, orally for 30 consecutive days significantly ($p<0.05$) spent more time in the non-preferred compartment compared to the time spent before drug treatment. The results demonstrated that rats received chronic treatment of cocaine become addicted and preferred to stay in the least preferred compartment even though the cocaine was not administered on the test date. On the other hand, both doses of TL (1 and 10 g/kg) oral administration for 30 consecutive days did not cause significant changes in the time spent in both compartment between pre- and post- drug treatment indicating that TL did not cause addiction.

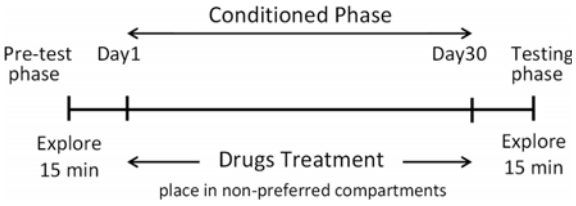

Fig. 1 Diagram illustrating the protocol of the conditioned place preference test. The drug was administered during the conditioned phase and withdrawn on the test phase.

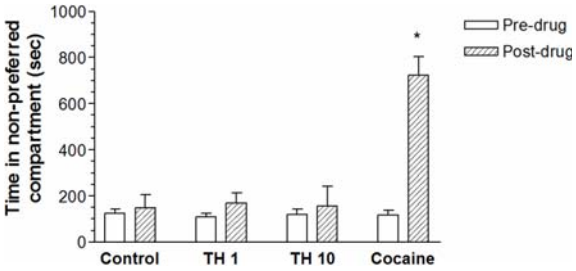

Fig. 2 Effect of *Thunbergia laurifolia* (TL) 1 g/kg (n = 9) and 10 g/kg (n = 9) and cocaine (1 mg/kg, n = 9) on time spent in non-preferred compartment before and after drug training compare with control (n = 16) using the conditioned place preference test. Data are presented as mean ± SEM. * $p<0.05$ using ANOVA and post-hoc (Newman-Keuls multiple comparison) test.

Discussion

The CPP procedure is a useful model of craving in the absence of the drug, based on the approach to conditioned stimuli associated with the drug and has been an interesting tool in studying the neuroanatomy and neurochemistry of drug rewards[14]. Drug-inducing CPP is based on the principle that when a primary reinforcer is paired with contextual stimuli, the contextual stimuli can acquire secondary reinforcing properties[15]. In general the methodology of CPP is established by paring some distinct set of contextual stimuli (visual, tactile, olfactory) with a drug. The drug is typically administered prior to placing the animal into context.

The nucleus accumbens has been suggested to play a key role in brain reward processes[16-19]. There is considerable evidence in the literature to support such a role in drug reinforcement mechanisms[20-22]. Intravenous self-administration studies utilizing 6-hydroxydopamine lesioning and microinjection techniques suggest that the nucleus accumbens is a site initiating the rewarding effects of the psycho-stimulants[23-25].

The results from the first two experiments revealed that TL exhibits similar effects as some addictive drugs such as amphetamine and cocaine on the dopaminergic system by increasing dopamine release from the brain responsible for the reward mechanism[11,13]. However, TL itself did not show significant effect in addiction using behavioral studies.

Conclusion

The present study demonstrates the non-addictive effect of chronic treatment of TL using the behavioral model of addiction, CPP. On the contrary, TL exerts similar effect with cocaine on dopaminergic levels in the rat nucleus accumbens both in vitro and in vivo. TL increases cerebral activity in the anesthetized rat brain detected by functional magnetic resonance imaging (fMRI) on similar area of rat brain with amphetamine. Further study to evaluate the effect of TL on the functional changes in the dopaminergic neuron in brain area responsible for the reward mechanisms associated with addiction is required.

What is already known on this topic ?

TL activates similar brain area as amphetamine and exhibits similar effects as some addictive drugs do.

What this study adds ?

Chronic treatment of TL exhibits the non-addictive effect in rats using the behavioral model of addiction.

Potential conflicts of interest

None.

References

1. Oonsivilai R, Cheng C, Bomser J, Ferruzzi MG, Ningsanond S. Phytochemical profiling and phase II enzyme-inducing properties of *Thunbergia laurifolia* Linn. (RC) extracts. J Ethnopharmacol 2007; 114: 300-6.
2. Ussanawarong S, Thesiri T. Effect of *Thunbergia laurifolia* Linn. on detoxication of parathion in rat. KKU Res J 2001; 6: 3-13.
3. Ussanawarong S, Thesiri T, Mahakunakorn T, Parasupattana S. Effect of *Thunbergia laurifolia* Linn. on detoxication of paraquat. KKU Res J 2000; 5: 11-7.
4. Khobjai W, Jaihan U, Watcharasamphankul W, Somsak V. Protective effect of *Thunbergia laurifolia* extract on hemolysis during Plasmodium

berghei infection. Parasitol Res 2014; 113: 1843-6.
5. Palipoch S, Punsawad C, Suwannalert P. *Thunbergia laurifolia*, a new choice of natural antioxidant to prevent oxidative stress-related pathology: a review. J Med Plant Res 2013; 7: 698-701.
6. Chan EWC, Eng SY, Tan YP, Wong ZC, Lye PY, Tan LN. Antioxidant and sensory properties of thai herbal teas with emphasis on *Thunbergia laurifolia* Linn. Chiang Mai J Sci 2012; 39: 599-609.
7. Tejasen P, Thongthapp C. The study of the insecticide antitoxicity of *Thunbergia laurifolia* Linn. Chiang Mai Med Bull 1980; 19: 105-14.
8. Chattaviriya P, Morkmek N, Lertprasertsuke N, Ruangyuttikarn W. Drinking *Thunbergia laurifolia* Linn. Leaf extract helps prevent renal toxicity induced by cadmium in rats. Thai J Toxicology 2010; 25: 124-32.
9. Tangpong J, Satarug S. Alleviation of lead poisoning in the brain with aqueous leaf extract of the *Thunbergia laurifolia* (Linn.). Toxicol Lett 2010; 198: 83-8.
10. Wutythamawech W. Encyclopedia of Thai herbs. Bangkok: Odient Store Printing House; 1998.
11. Thongsaard W, Marsden CA. A herbal medicine used in the treatment of addiction mimics the action of amphetamine on in vitro rat striatal dopamine release. Neurosci Lett 2002; 329: 129-32.
12. Thongsaard W, Marsden C. Effect of *Thunbergia laurifolia* extract on extracellular dopamine level in rat nucleus accumbens. J Med Assoc Thai 2013; 96 (Suppl 1): S85-9.
13. Thongsaard W, Marsden CA, Morris P, Prior M, Shah YB. Effect of *Thunbergia laurifolia*, a Thai natural product used to treat drug addiction, on cerebral activity detected by functional magnetic resonance imaging in the rat. Psychopharmacology (Berl) 2005; 180: 752-60.
14. Tzschentke TM. Measuring reward with the conditioned place preference paradigm: a comprehensive review of drug effects, recent progress and new issues. Prog Neurobiol 1998; 56: 613-72.
15. Bardo MT, Rowlett JK, Harris MJ. Conditioned place preference using opiate and stimulant drugs: a meta-analysis. Neurosci Biobehav Rev 1995; 19: 39-51.
16. Wise RA, Rompre PP. Brain dopamine and reward. Annu Rev Psychol 1989; 40: 191-225.
17. McBride WJ, Murphy JM, Ikemoto S. Localization of brain reinforcement mechanisms: intracranial self-administration and intracranial place-conditioning studies. Behav Brain Res 1999; 101: 129-52.
18. Carelli RM. The nucleus accumbens and reward: neurophysiological investigations in behaving animals. Behav Cogn Neurosci Rev 2002; 1: 281-96.
19. Ikemoto S. Dopamine reward circuitry: two projection systems from the ventral midbrain to the nucleus accumbens-olfactory tubercle complex. Brain Res Rev 2007; 56: 27-78.
20. Koob GF, Nestler EJ. The neurobiology of drug addiction. J Neuropsychiatry Clin Neurosci 1997; 9: 482-97.
21. Wise RA. The role of reward pathways in the development of drug dependence. Pharmacol Ther 1987; 35: 227-63.
22. Wise RA. Neurobiology of addiction. Curr Opin Neurobiol 1996; 6: 243-51.
23. Caine SB, Koob GF. Effects of mesolimbic dopamine depletion on responding maintained by cocaine and food. J Exp Anal Behav 1994; 61: 213-21.
24. Pettit HO, Ettenberg A, Bloom FE, Koob GF. Destruction of dopamine in the nucleus accumbens selectively attenuates cocaine but not heroin self-administration in rats. Psychopharmacology (Berl) 1984; 84: 167-73.
25. Roberts DC, Koob GF, Klonoff P, Fibiger HC. Extinction and recovery of cocaine self-administration following 6-hydroxydopamine lesions of the nucleus accumbens. Pharmacol Biochem Behav 1980; 12: 781-7.

ผลของการให้สารสกัดรางจืดและโซเซนอย่างต่อเนื่องในหนูขาวโดยการทดสอบด้วยโมเดลการวัดพฤติกรรมการติดสารเสพติด

วัชรีวรรณ ทองสะอาด, รติรัตน์ แสงพยัพ, Charles Marsden

ภูมิหลัง: *รางจืดเป็นพืชสมุนไพรไทยที่ใช้เป็นส่วนประกอบของยาที่ใช้รักษาผู้ติดยาเสพติด มีรายงานจากการทดลองทั้งแบบ in vitro และ in vivo พบว่าสารสกัดรางจืดมีผลเพิ่มระดับสารสื่อประสาทโดปามีนในสมองของหนูขาว คล้ายฤทธิ์ของโคเคนและกระตุ้นสมองบริเวณเดียวกันกับแอมเฟตามีน*

วัตถุประสงค์: *เพื่อทดสอบผลของการให้สารสกัดสมุนไพรรางจืดอย่างต่อเนื่องต่อภาวะการติดสารเสพติด เปรียบเทียบกับโคเคนโดยใช้โมเดลการทดสอบพฤติกรรมการติดสารเสพติด*

วัสดุและวิธีการ: *ป้อนสารสกัดรางจืดด้วยน้ำ (1 และ 10 g/kg, orally) หรือโคเคน (1 mg/kg, orally) เป็นเวลา 30 วันต่อเนื่องกัน และนำมาทดสอบพฤติกรรมการติดสารเสพติดโดยใช้โมเดล Conditioned place preference*

ผลการศึกษา: *หนูกลุ่มที่ได้รับการป้อนโคเคนเป็นเวลา 30 วันแสดงพฤติกรรมการติดสารเสพติดอย่างชัดเจน ในขณะที่หนูกลุ่มที่ในสารสกัดรางจืดทั้ง 2 ขนาดไม่แสดงพฤติกรรมการติดสารเสพติด*

สรุป: *ผลการทดลองสรุปได้ว่าการให้สารสกัดรางจืดอย่างต่อเนื่องเป็นเวลานานไม่มีผลทำให้เกิดการติดสารเสพติด เมื่อเปรียบเทียบกับการให้โคเคน แม้ว่ารางจืดจะมีรายงานว่าออกฤทธิ์ในการเพิ่มการหลั่งสารสื่อประสาทโดปามีนเช่นเดียวกับสารเสพติดโคเคน และออกฤทธิ์กระตุ้นสมองบริเวณเดียวกับแอมเฟตามีนก็ตาม ผลของรางจืดต่อเซลล์สมองบริเวณที่เกี่ยวข้องกับการควบคุมพฤติกรรมความพึงพอใจจะต้องมีการศึกษาต่อไป*