Effect of *Thunbergia laurifolia* Extract on Cerebral Blood Flow in Rats

Watchareewan Thongsaard PhD*, Amporn Jariyapongskul PhD*, Chonticha Areebambud MSc**

** Department of Physiology, Faculty of Medicine, Srinakharinwirot University, Bangkok, Thailand*
*** Biomedical Science Program, Faculty of Medicine, Srinakharinwirot University, Bangkok, Thailand*

Background: *Thunbergia laurifolia Linn. (TL) is a herbal medicine which has been used as an antidote for several poisonous agents including insecticides and as a component of a mixture of crude extracts to treat drug addicted patients. The previous study using Functional Magnetic Resonance Imaging (fMRI) technique found that oxyhemoglobin: deoxyhemoglobin increased in amygdala, caudate and nucleus accumbens by TL. These areas represent reward and addiction behavior.*
Objective: *The present study aim to investigate the direct effect of TL (100, 200 and 300 mg/kg) on cerebral blood flow, mean arterial blood pressure, partial pressures of oxygen and carbondioxide in normal rats.*
Material and Method: *Methanol extract of TL (100, 200 and 300 mg/kg, n = 12 in all groups) was administered intraperitoneally to rats. Cerebral blood flow, mean arterial pressure, pH, PO_2 and PCO_2 were measured at 5 min before, 5, 10, 20 and 30 min after injection.*
Results: *The results showed a significant increase in cerebral blood flow and mean arterial blood pressure in a dose-related manner, but partial pressures of oxygen and carbondioxide were not affected.*
Conclusion: *The finding explains that TL cause the increase in oxyhemiglobin: deoxyhemoglobin by increasing cerebral blood flow and mean arterial blood pressure.*

Keywords: *Cerebral blood flow, Mean arterial blood pressure, Thunbergia laurifolia*

J Med Assoc Thai 2017; 100 (Suppl. 8): S13-S18
Full text. e-Journal: *http://www.jmatonline.com*

Thunbergia laurifolia Linn. (TL) or Rang Jued, in Thai, is a herbal medicine used as an antidote for several poisonous agents in Thai traditional medicine. Different parts of the plant are used for various medicinal purposes including an antidote for insecticide, ethyl alcohol, arsenic and strychnine poisoning, an anti-inflammatory agent and antipyretic[1-3]. There are no published clinical trials on the use of TL in the treatment of drug addiction, but this plant has been used extensively as a component of a mixture of crude extracts to treat drug addicted patients[4].

The scientific report demonstrated that TL potentiated the effect of amphetamine on potassium-stimulated dopamine release from rat striatal slices[5]. The results indicated that TL may stimulate dopamine release in a similar manner to amphetamine. There is extensive evidence linking changes in dopamine function to the pharmacology of addictive drugs and the development of drug dependence[6]. Mapping of functional MRI (fMRI) signals in response to saline or TL (200 mg/kg, ip) indicating changes in signal intensity in amygdala nucleus, caudate nucleus and nucleus accumbens[7]. The fMRI utilizing the innate paramagnetic difference between oxyhemoglobin and deoxyhemoglobin called blood oxygen level-dependent (BOLD) contrast as a proposed functional correlate of neural activity[8-10]. Because oxyhemoglobin and deoxyhemoglobin have different magnetic properties, changes in metabolic activity that alter the ratio of oxygenated to deoxygenated hemoglobin are revealed by changes in magnetic resonance signal intensity[9]. Therefore, the present study was performed to investigate the cause of changes in hemodynamic parameters in response to TL in order to explain the effect of TL on neuronal activity.

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

Material and Method

Animals

Male Wistar rats, weighing 220 to 250 g, were obtained from the National Laboratory Animal Center, Mahidol University, Thailand. They were housed in

groups of 4 to 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. SWU-MED 15/2557).

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 method of extraction was the same as in previous reports[11,12]. TL at the concentration of 100, 200 and 300 mg/kg was prepared freshly in the morning and was administered intraperitoneally to the rat at a volume of 2 ml/kg.

Animal preparation

The animals were divided into groups of 12 and the preparation was performed according to the previous study[13]. On the day of experiment, the rat was anesthetized with pentobarbital sodium (60 mg/kg bw, ip). After a tracheotomy was performed, the rat was ventilated mechanically with room air supplemented by oxygen. A femoral artery was cannulated for the measurement of blood pressure and analysis of blood gas. The blood pressure was monitored throughout the experiment using a pressure transducer (PowerLab System, AD instruments). The blood gas and pH levels were maintained within the physiological limit, where PCO_2 = 35 to 45 mmHg, PO_2 = 100 to 120 mmHg, and pH = 7.35 to 7.45 by using a blood gas analyzer (Model 278, CIBA CORNING, England).

Cerebral blood flow (CBF)

A craniotomy was prepared to expose the anterior cerebral cortex and dura was opened. A stainless metal frame with a circular window was fixed to the cranial bone. An artificial cerebrospinal fluid (ACSF; compositions: NaCl 118.0, KCl 4.0, $MgSO_4$ 1.2, $CaCl_2$ 1.5, NaH_2PO_4 1.2, $NaHCO_3$ 25.0, glucose 5.0 in mM) was superfused. The CBF was monitored in the intracranial space by laser Doppler flowmetry (DRT4, Moor instruments). The fiber optic needle probe of laser Doppler flowmetry was mounted on a micromanipulator and connected to a laser Doppler flow perfusion monitor (Moor software). The CBF data were collected from 2 locations of cranial space and recorded 3 times in each location. The CBF values were the average values of the mean of each location. The CBF was presented in perfusion unit (PU).

Rats were divided into 4 groups, control (Sterile normal saline), 100, 200, and 300 mg/kg, ip of TL, n = 12 in all groups. CBF was measured 5 minutes before injection of vehicle control or TL and 5, 10, 20, and 30 minutes after injection.

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 TL (100, 200 and 300 mg/kg, n = 12) on cerebral blood flow compared with control was shown in Fig. 1 and Table 1. TL 100, 200 and 300 mg/kg significantly ($p<0.001$) increased CBF from 5 minutes after injection onward when compared with control. Systolic and diastolic blood pressure were increased by TL resulted in a significant ($p<0.001$) increased of mean arterial pressure with time in every doses of TL when compared with control. The results were shown in Fig. 2 and Table 2. TL in all concentration used have no significant effect on pH, PaO_2 and $PaCO_2$ (Table 3).

Discussion

The previous finding using fMRI demonstrate a reduction in the concentration of deoxyhaemoglobin, leading to an increase in the BOLD signal seen in activation[7]. Overall, the increases in BOLD following TL administration indicate that the plant extract produced enhanced neuronal activity in specific brain regions *i.e.*, amygdala, caudate and nucleus accumbens similar to the action of cocaine[14]. The results of this study demonstrate the increase in hemodynamic parameters by TL in these areas. There is report that dopamine induced dissociation of BOLD[15] which explained the involvement of TL on dopamine release[11] and the increase in the BOLD signal and the increase in blood flow leads to an overshoot of blood flow in comparison to cerebral blood volume, oxidative metabolism and a fall in the oxygen extraction fraction[16]. The hemodynamic parameters changes such as increased cerebral blood flow, cerebral blood volume[17] and increased oxygen levels[18-19] represent the increase in brain activities. The blood gas and pH

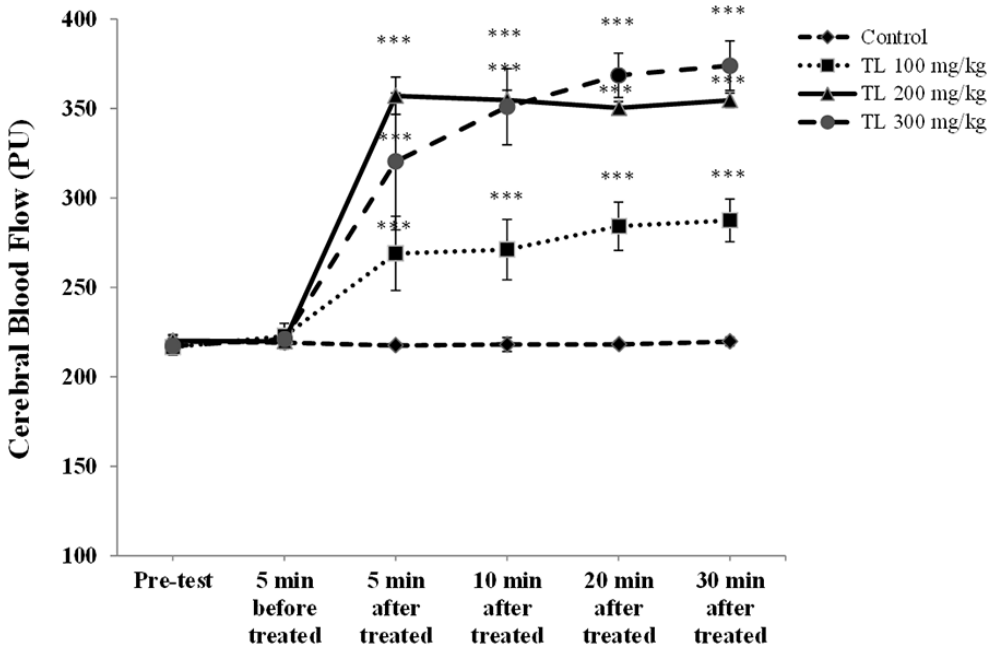

Fig. 1 Effect of TL (100, 200 and 300 mg/kg, n = 12 in all groups) on cerebral blood flow (PU) at various times. Data are presented as mean ± SEM. ***p<0.001 using ANOVA and post-hoc (Newman-Keuls multiple comparison) test when compared with control.

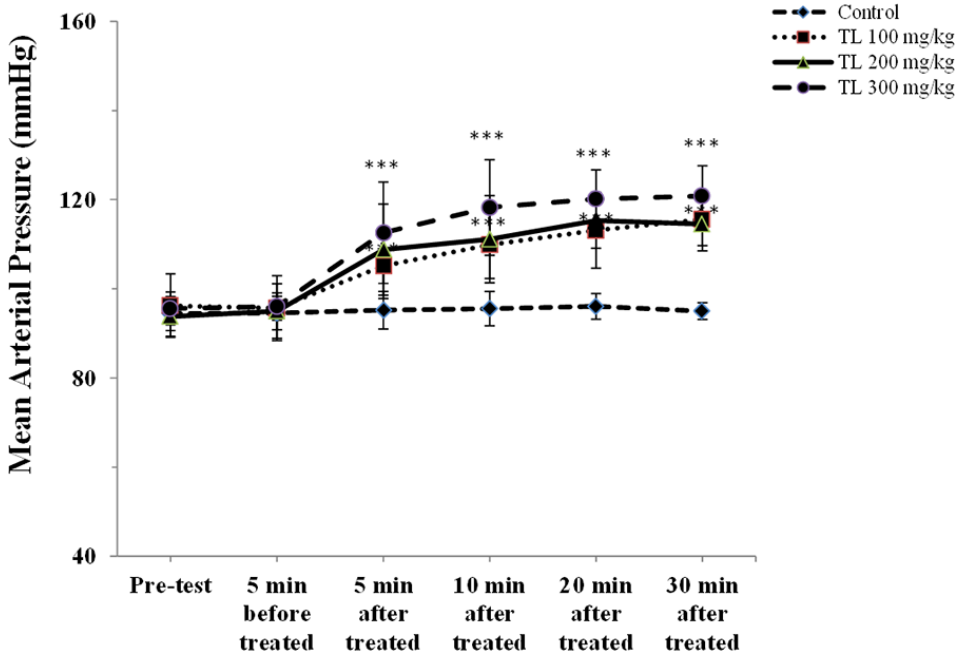

Fig. 2 Effect of TL (100, 200 and 300 mg/kg, n = 12 in all groups) on mean arterial pressure (mmHg) at various times. Data are presented as mean ± SEM. ***p<0.001 using ANOVA and post-hoc (Newman-Keuls multiple comparison) test when compared with control.

Table 1. Effect of TL (100, 200 and 300 mg/kg, n = 12 in all groups) on cerebral blood flow (PU) at various times

	Cerebral blood flow (PU)			
	Control	TL 100 mg/kg	TL 200 mg/kg	TL 300 mg/kg
Pre-test	219.81±3.23	216.63±4.46	220.18±3.40	217.28±1.84
5 min before experiment	219.11±2.75	222.78±7.08	219.83±2.94	221.10±1.47
5 min after experiment	217.54±1.67	268.96±20.78***	357.10±10.41***	320.41±38.21***
10 min after experiment	218.07±3.92	271.15±16.91***	354.78±5.41***	350.96±21.27***
20 min after experiment	218.11±1.75	284.15±13.50***	350.29±3.56***	368.58±12.34***
30 min after experiment	219.76±2.43	287.48±11.94***	354.62±3.99***	373.92±13.83***

Data are presented as mean ± SEM
*** $p<0.001$ using ANOVA and post-hoc (Newman-Keuls multiple comparison) test when compared with control

Table 2. Effect of TL (100, 200 and 300 mg/kg, n = 12 in all groups) on mean arterial pressure (mmHg) at various times

	Mean arterial pressure (mmHg)			
	Control	TL 100 mg/kg	TL 200 mg/kg	TL 300 mg/kg
Pre-test	94.44±3.79	96.22±7.10	93.78±4.42	95.58±3.67
5 min before experiment	94.56±3.76	95.67±7.26	95.00±6.15	96.04±3.04
5 min after experiment	95.22±4.20	105.17±7.36***	108.78±10.22***	112.58±11.37***
10 min after experiment	95.56±3.85	109.89±7.56***	111.11±9.76***	118.25±10.75***
20 min after experiment	96.06±2.86	113.11±8.53***	115.28±6.19***	120.17±6.52***
30 min after experiment	95.00±1.87	115.56±5.87***	114.50±5.95***	120.83±6.76***

Data are presented as mean ± SEM
*** $p<0.001$ using ANOVA and post-hoc (Newman-Keuls multiple comparison) test when compared with control

levels were maintained within the physiological limit throughout the experiment.

Conclusion

The present study demonstrates that TL significantly enhanced CBF and mean arterial pressure in rats. From the previous finding, TL significantly increase activity in specific brain areas i.e. amygdala nucleus, caudate nucleus and nucleus accumbens which involved in drug addiction by increasing various hemodynamic parameters. Importantly, in view of the anecdotal reports that TL extracts can be used to treat both drug toxicity and addiction, the increases in activity are particularly found in brain areas richly innervated by dopamine, which is associated with both drug reward and addiction[20,21]. There is increasing interest in the development of drug treatments for addiction, and the present study suggests that a possible action of such drugs is through the increase in hemodynamic parameters in the same brain region i.e., same neurotransmitter system as that involved in addiction.

Acknowledgements

This research was performed under the support from the research grant from Faculty of Medicine, Srinakharinwirot University (No. 204/2554).

What is already known on this topic?

TL activates certain brain areas involved in drug addiction as indicated by fMRI which represent the difference between oxyhemoglobin and deoxyhemoglobin.

What this study adds?

TL increases various hemodynamic parameters such as cerebral blood flow and mean arterial blood pressure.

Potential conflicts of interest

None.

Table 3. Effect of TL (100, 200 and 300 mg/kg, n = 12 in all groups) on pH, PaO_2 and $PaCO_2$ at various times

	pH			
	Control	TL 100 mg/kg	TL 200 mg/kg	TL 300 mg/kg
Pre-test	7.33±0.04	7.32±0.05	7.33±0.06	7.24±0.03
10 min after experiment	7.36±0.03	7.37±0.03	7.30±0.02	7.24±0.10
30 min after experiment	7.39±0.05	7.32±0.03	7.32±0.01	7.29±0.06
	pO2 (mmHg)			
	Control	TL 100 mg/kg	TL 200 mg/kg	TL 300 mg/kg
Pre-test	107.75±4.84	111.78±4.24	120.04±4.65	114.66±3.06
10 min after experiment	110.30±4.27	111.8±8.92	116.92±5.92	121.26±11.49
30 min after experiment	110.02±6.04	112.5±4.55	117.48±4.27	116.36±3.80
	pCO2 (mmHg)			
	Control	TL 100 mg/kg	TL 200 mg/kg	TL 300 mg/kg
Pre-test	38.05±3.03	37.56±1.12	38.10±2.59	36.73±2.73
10 min after experiment	38.47±2.77	38.78±2.18	38.68±2.55	36.76±3.15
30min after experiment	37.67±4.45	38.94±1.07	38.20±2.55	35.76±2.05

Data are presented as mean ± SEM using ANOVA and post-hoc (Newman-Keuls multiple comparison) test

References

1. Tejasen P, Thongthapp C. The study of the insecticide antitoxicity of *Thunbergia laurifolia* Linn. Chiang Mai Med Bull 1980; 19: 105-14.
2. Ussanawarong S, Thesiri T, Mahakunakorn T, Parasupattana S. Effect of *Thunbergia laurifolia* Linn. ondetoxication of paraquat. KKU Res J 2000; 5: 11-7.
3. Ussanawarong S, Thesiri T. Effect of *Thunbergia laurifolia* Linn. ondetoxication of parathion in rat. KKU Res J 2001; 6: 3-13.
4. Wutythamawech W. Encyclopedia of Thai herbs. Bangkok: Odient Store Printing House; 1998.
5. 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.
6. Koob GF, Nestler EJ. The neurobiology of drug addiction. J Neuropsychiatry Clin Neurosci 1997; 9: 482-97.
7. 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.
8. Ogawa S, Lee TM, Nayak AS, Glynn P. Oxygenation-sensitive contrast in magnetic resonance image of rodent brain at high magnetic fields. Magn Reson Med 1990; 14: 68-78.
9. Ogawa S, Lee TM, Barrere B. The sensitivity of magnetic resonance image signals of a rat brain to changes in the cerebral venous blood oxygenation. Magn Reson Med 1993; 29: 205-10.
10. Bandettini PA, Wong EC, Hinks RS, Tikofsky RS, Hyde JS. Time course EPI of human brain function during task activation. Magn Reson Med 1992; 25: 390-7.
11. 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.
12. Thongsaard W, Sangpayap R, Marsden C. Chronic effect of *Thunbergia laurifolia* extract and cocaine in rats using behavior model of addiction. J Med Assoc Thai 2015; 98 (Suppl 9): S48-52.
13. Jariyapongskul A, Pathumraj S, Niimi H. Effects of Yahom on the regional cerebral blood flow in

rat using fluorescence videomicroscopy. Clin Hemorheol Microcirc 2006; 34: 139-44.
14. Luo F, Wu G, Li Z, Li SJ. Characterization of effects of mean arterial blood pressure induced by cocaine and cocaine methiodide on BOLD signals in rat brain. Magn Reson Med 2003; 49: 264-70.
15. Zaldivar D, Rauch A, Whittingstall K, Logothetis NK, Goense J. Dopamine-induced dissociation of BOLD and neural activity in macaque visual cortex. Curr Biol 2014; 24: 2805-11.
16. Ericksson L, Roland P, Hahlbom M, Blomqist G, Widen L. Coupling between regional cerebral oxidative metabolism and regional cerebral blood flow during physiological stimulation. J Cereb Blood Flow Metab 1989; 9 (Suppl 1): S578.
17. Fox PT, Raichle ME. Focal physiological uncoupling of cerebral blood flow and oxidative metabolism during somatosensory stimulation in human subjects. Proc Natl Acad Sci USA 1986; 83: 1140-4.
18. Fox PT, Mintun MA, Raichle ME, Miezin FM, Allman JM, Van Essen DC. Mapping human visual cortex with positron emission tomography. Nature 1986; 323: 806-9.
19. Fox PT, Raichle ME, Mintun MA, Dence C. Nonoxidative glucose consumption during focal physiologic neural activity. Science 1988; 241: 462-4.
20. Koob GF. Neurobiology of drug addiction. In: Kandel D, editor. Stages and pathways of drug involvement: Examining the gateway hypothesis. New York: Cambridge University Press; 2002: 337-61.
21. Koob GF. Neuroadaptive mechanisms of addiction: studies on the extended amygdala. Eur Neuro psychopharmacol 2003; 13: 442-52.

ผลของสารสกัดรางจืดต่อการไหลเวียนเลือดในสมองหนูขาว

วัชรีวรรณ ทองสะอาด, อัมพร จาริยพงศ์สกุล, ชลธิชา อารีบำบัด

ภูมิหลัง: *รางจืดเป็นพืชสมุนไพรที่ใช้อย่างแพร่หลาย มีสรรพคุณเป็นยาถอนพิษ แก้พิษสุรา ยาฆ่าแมลง อีกทั้งเป็นส่วนผสมสำคัญในสมุนไพรที่ใช้ถอนพิษยาเสพติด มีรายงานการศึกษาด้วยเทคนิค Functional Magnetic Resonance Imaging (fMRI) พบว่าสารสกัดรางจืดมีผลทำให้ปริมาณ oxyhemoglobin: deoxyhemoglobin เพิ่มขึ้นใน amygdalla, caudate และ nucleus accumbens ซึ่งเป็นสมองส่วนที่ตอบสนองต่อความพึงพอใจ (reward) และพฤติกรรมการติดสารเสพติด*

วัตถุประสงค์: *งานวิจัยนี้จึงมุ่งศึกษาผลทางตรงของสารสกัดรางจืดความเข้มข้น 100, 200 และ 300 mg/kg ต่อ cerebral blood flow, mean arterial blood pressure, partial pressure ของออกซิเจนและคาร์บอนไดออกไซด์ในหนูปกติ*

วัสดุและวิธีการ: *ฉีดสารสกัดรางจืดด้วยเมธานอล (100, 200 และ 300 mg/kg จำนวน 12 ตัวในทุกกลุ่ม) เข้าทางช่องท้องของหนูขาว และวัด cerebral blood flow, mean arterial blood pressure, partial pressure ของออกซิเจนและคาร์บอนไดออกไซด์ที่ 5 นาทีก่อนฉีดและ 5, 10, 20 และ 30 นาทีหลังฉีด*

ผลการศึกษา: *ผลการศึกษาพบว่าสารสกัดรางจืดเพิ่ม cerebral blood flow และ mean arterial blood pressure อย่างมีนัยสำคัญทางสถิติตามความเข้มข้นที่มากขึ้นเมื่อเทียบกับกลุ่มควบคุมแต่ค่า partial pressure ของออกซิเจนและคาร์บอนไดออกไซด์ไม่มีการเปลี่ยนแปลง*

สรุป: *ผลการทดลองสามารถอธิบายได้ว่าสาเหตุที่สารสกัดรางจืดเพิ่ม oxyhemoglobin: deoxyhemoglobin นั้นเนื่องมาจากการเพิ่มการไหลเวียนเลือดและความดันเลือดในสมอง*
