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Effects of Flos *Sophora japonica* Extract on the Changes of Cerebral Hemodynamics

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ABSTRACT: The purpose of this study was to measure the changes of regional cerebral blood flow(rCBF) and blood pressure(BP) in rats, following the intravenous injection of Flos *Sophora japonica* L.(FSJ) water extract. The measurement was continually monitored by Laser-Doppler Flowmeter and pressure transducer in anaesthetized adult Sprague-Dawley rats for 2 hours to 2 hours and a half through the data acquisition system. FSJ increased the changes of rCBF in rat significantly. The rCBF of FSJ did not change by pretreated propranolol, atropine, L-NNA(N^G-nitro-L-argininine) and indomethacin. But the rCBF of Flos *Sophora japonica* L. was increased by pretreated methylene blue. FSJ decreased the changes of BP, significantly. The BP of FSJ did not change by pretreated propranolol, atropine, L-NNA and indomethacin. But the BP FSJ was decreased by pretreated methylene blue. There results indicated that FSJ can increase the rCBF and decrease the BP, that is related to guanylyl cyclase activity.

Keywords: Flos *Sophora japonica* L., Regional Cerebral Blood Flow, Blood Pressure, Laser-Doppler Flowmetry

INTRODUCTION

The independent contributions of cognitive and genetic risk factors for Alzheimer's disease (AD) have received considerable attention, but less is known about possible intgeractive effects (Christina EW *et al.,* 2012). Cerebral ischemia results in a time-dependent cascade of molecular events including the rapid depletion of intracellular energy stores, anaerobic glycolysis, lactic acidosis, membrane depolarization, glutamate excitotoxicity, intracellular calcium overload, activation of calcium-stimulated enzyme (phospholipases, protein kinases, nitric oxide synthase or NOS, endonucleases), mitochondrial dysfunction, free radical production, activation of the immune system (neutrophils, monocytes/marcrophages), overexpression of genes and neuronal death (Kato H & Kogure K, 1999; Small DL, *et al.*, 1999). In healthy brains, localized increases in neuronal activity are strongly correlated, both spatially and temporally, with localized increases in cerebral blood flow (CBF) and cerebral metabolic consumption of oxygen ($CMRO_2$) (Wesley BB *et al.,* 2012). In transient cerebral ischemia, evidence has accumulated during the past two decades that an overproduction of nitrogen and oxygen free radicals occurs in the early reperfusion and is deleterious by damaging cellular marcromolecules such as lipids, proteins and nucleic acid (Love S, 1999). Thus, in a rat model of focal cerebral ischemia/reperfusion, oxidative injury to DNA was detected as early as 1 min after the onset or reperfusion (Chen J *et al.,* 1997). LDF allows for real time, noninvasive, continuous recordings of local CBF. The LDF method has been widely used to trace hemodynamic changes in the superficial or the deep brain structures in experimental stroke research (Kuroiwa T, 1992). It has also been employed as a useful tool for imaging the instantaneous changes of cortical CBF related to cortical spreading depression (Lauitzen M & Fabricius M, 1995) or tracing stimulation elicited by a local vascular response (Iadecola C & Reis DJ, 1990). Flos *Sophora japonica* L. (Leguminosae), commonly called Scholar Tree, is a traditional Chinese herb used to cool blood, stop bleeding and to treat hemorrhoids with bleeding (Lao CJ *et al.,* 2005). Sophora flowers come from the sophora tree (also known as the Japaness pagoda), a deciduous tree native to east Asia and cultivated in China, Japan and Korea. The flowers of the sophora tree are hermaphrodite and flower in late August and early September. The flowers are usually reaped just before the tree comes into bloom, then fried in the sun for use, either raw or after being parched. According to the principles of traditional Chiness medicine, sophora flowers are bitter and slightly cold, and are affiliated with the liver and large intestine meridians. It is a common component of many Chinese herbal remedies. Its main functions are to remove heat from the blood, stop bleeding, and clear fire from the liver. Among the conditions sophora flower is used to treat are bleeding caused by hemorrhoids, high blood pressure, and headaches. Some practitioners use it to improve eyesight and help resolve the symptoms of conjunctivitis (Chu *et al.,* 2005). The present study thus was carried out to determine the mechanism of action Flos *Sophora japonica* on regional cerebral blood flow, blood pressure by using LDF methods.

MATERIALS AND METHODS

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Sample Preparation

The dried flowers of *S. japonica* (Korean name, Koehwa) were purchased from Geumodang, Jeonju, Jeonbuk, South Korea. *S. japonica* powder was suspended in distilled water (9:1, v/v) and refluxed for 4 h. The extracted solution was filtered, concentrated under reduced pressure (CCA-1100, Eyela, Tokyo, Japan), and finally freeze-dried (PVTFA 10AT, Ilsan, Korea). And the total crude extractive power was 52.3 g. The extract stored in deep freezer when unused, and freshly diluted for experiment.

Animal and Chemicals

Adult male Sprague-Dawley rats at the body weight of 250 g±10 g were obtained commercially (Orient Co., South Korea). All animals were housed under standard conditions of lights and controlled room temperature, and received food and water ad libitum. All chemicals were purchased from Sigma Chemical Corporation (Sigma-Aldrich Korea, South Korea).

Measurement of MABP (Mean Arterial Blood Pressure) and rCBF (Regional Cerebral Brain Flow) in Rats

Rats were anesthetized with urethane (750 mg/kg, i.p.) and placed on a stereotaxic frame. Tracheotomized and entubated, and body temperature was maintained at 37~38℃ with a servo-controlled heating lamp and a rectal thermistor. One femoral artery was used for the monitoring of blood pressure with PE-50 polyethylene catheters and was recorded on an polygraph. The skull was exposed and a hole 5 mm in diameter was frilled in the left side at a site 5 mm lateral and 2 mm posterior to the bregma. A laser-Doppler flowmeter (Transonic Instrument, U.S.A) with a 0.8 mm needle probe was used to determine changes in rCBF. The probe tip was positioned above the surface of the intact dura and fixed to a support attached to the skull.

Statistical Analysis

All experiments were performed in duplicate for three replicates. The data are expressed as the mean±S.E. The differences between groups were analyzed by Student's paired *t*-test. The significance level was set at $p<0.05$.

RESULTS AND DISCUSSION

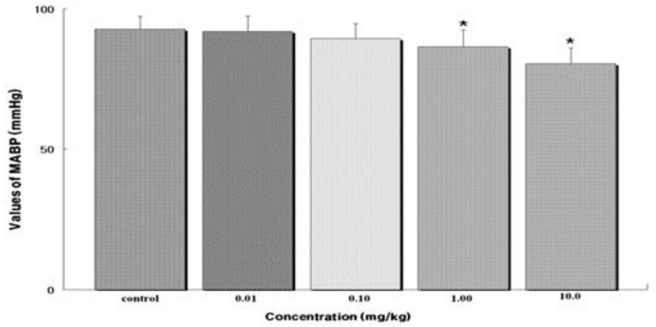

Fig. 1. Effects of *FSJ* extract on the mean arterial blood pressure (MABP) changes in rats. The mean with standard errors were obtained from 10 experiments. If the value of control make 100, the MABP of *FSJ* treated rats can be 99.60, 96.27, 93.27 and 88.74 with the dosage of 0.01, 0.1, 1.0, 10.0; *, statistically significant compared with controlled group.

Blood pressure was recorded on a polygraph with PE-50 polyethylene catheters. MABP showed 92.84±4.2 mmHg vehicle, and decreased to 80.53±5.3 mmHg with the increased of *FSJ* from 0.01 to 10 mg/kg (i.v) Fig. 1.

In brief, *FSJ* administration showed statistical significance on MABP in normal blood pressure rats. The nonselective β-adrenoceptor antagonist propranolol was pretreated and changes of blood pressure were monitored. Propranolol pretreated MABP showed 84.80±3.5 mmHg in the 0.5 mL volume of vehicle, and Flos *Sophora japonica*on from 0.01 to 10 mg/kg (i.v.) treatment showed as Table 1. The parasympatholytic agent (atropine) was pretreated and changes of blood pressure were monitored. Atropine pretreated MABP showed 77.77±2.8 mmHg in the 0.5 mL volume of vehicle, and Flos *Sophora japonica* on from 0.01 to 10 mg/kg (i.v.) treatment showed as Table 1. The nitric oxide synthase inhibitor L-NNA was pretreated and changes of blood pressure were monitored. L-NNA pretreated MABP showed 80.24±3.0 mmHg in the 0.5 mL volume of vehicle, and Flos *Sophora japonica* on from 0.01 to 10 mg/kg (i.v.) treatment showed as Table 1.

The guanylyl cyclase inhibitor methylene blue was pretreated and changes of blood pressure were monitored. Methylene blue pretreated MABP showed 85.06±3.3 mmHg in the 0.5 mL volume of vehicle, and Flos *Sophora japonica* on from 0.01 to 10 mg/kg (i.v.)

Table 1. Effects of pretreatment with propranolol, atrophine, L-NNA, methylene blue and indomethacin on the Flos *Sophora japonica* extract induced in mean arterial blood pressure in rats (%)

Drug mg/kg	Propranolol + Flos *Sophora japonica*	Atropine + Flos *Sophora japonica*	L-NNA + Flos *Sophora japonica*	Methylene blue + Flos *Sophora japonica*	Indomethacin + Flos *Sophora japonica*
Control	100.00±0.05	100.00±0.05	100.00±0.04	100.00±0.04	100.00±0.05
0.01	99.69±0.06	97.80±0.06	98.09±0.04	99.37±0.04	98.00±0.06
0.1	97.08±0.06	96.35±0.06	96.53±0.05	97.28±0.04	96.38±0.07
1.0	95.20±0.06	90.56±0.07	92.41±0.06	94.55±0.04*	90.47±0.07
10.0	91.34±0.07	83.77±0.07	86.43±0.07	91.63±0.05*	83.36±0.07

Values are mean±S.E. (n=10).

: Statistically significance compared with control group ($p<0.05$).

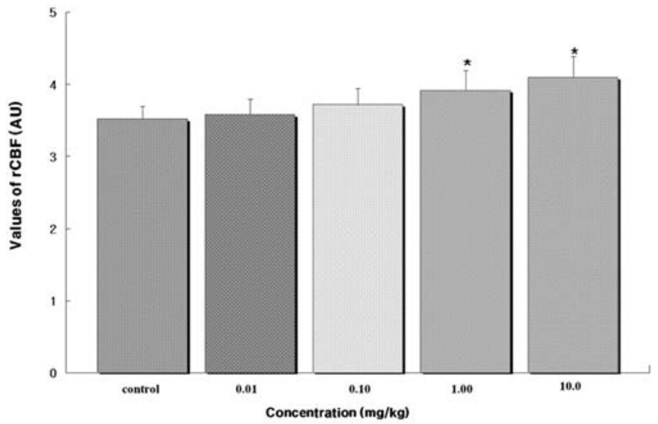

Fig. 2. Effects of *FSJ* extract on regcerebral blood flow (rCBF) in rats. The mean with standard errors were obtained from 10 experiments. If the value of control make 100, the rCBF of *FSJ* treated rats can be 101.52, 105.37, 108.79 and 115.38 with the dosage of 0.01, 0.1, 1.0, 10.0; *, statistically significant compared with controlled group.

treatment showed as Table 1. The cyclooxygenase inhibitor indomethacin was pretreated and changes of blood pressure were monitored. Indomethacin pretreated MABP showed 77.39±4.0 mmHg in the 0.5 mL volume of vehicle, and Flos *Sophora japonica* on from 0.01 to 10 mg/kg (i.v.) treatment showed as Table 1. In brief, Flos *Sophora japonica* on i.v. administration showed no change on MABP in propranolol, atropine, L-NNA and indomtethacin pretreated rats. But Flos *Sophora japonica* on i.v. administration showed statistical significance on MABP in methylene blue pretreated rats. Many trials with interventions using herbal drugs, acupuncture and foods to increase rCBF have been conducted. Samul-tang and Bambusae caulis in liquamen are known prescriptions to increase rCBF by dilating the cerebral artery through cyclooxygenase, a biosynthetic enzyme producing prostaglandin **(**Cho YL & Jeong HW, 2007**)**. rCBF changes were measured with a 0.8 mm needle probe by LDF method. *FSJ* i.v. administration showed significant increase of rCBF as dose dependent manner Fig. 2.

The rCBF value of vehicle treated control group were 3.52±0.05, whereas that of FSJ treated group gradually increase up to 4.10 ±0.20. In brief, *FSJ* i.v. administration showed statistical significance on rCBF in normal rats. Propranolol was pretreated and changes of rCBF were monitored. Propranolol pretreated rCBF showed 4.67 ±0.52 in the 0.5 mL volume of vehicle, and Flos *Sophora japonica* from 0.01 to 10 mg/kg(i.v.) treatment showed as Table 2. Atropine was pretreated and changes of rCBF were monitored. Atropine pretreated rCBF showed 4.66±0.59 in the 0.5 mL volume of vehicle, and Flos *Sophora japonica* from 0.01 to 10 mg/kg(i.v.) treatment showed as Table 2. L-NNA was pretreated and changes of rCBF were monitored. L-NNA pretreated rCBF showed 4.60±0.63 in the 0.5 mL volume of vehicle, and Flos *Sophora japonica* from 0.01 to 10 mg/kg (i.v.) treatment showed as Table 2. Methylene blue was pretreated and changes of rCBF were monitored. Methylene blue pretreated rCBF showed 4.29±0.55 in the 0.5 mL volume of vehicle, and Flos *Sophora japonica* from 0.01 to 10 mg/kg(i.v.) treatment showed as Table 2. Indomethacin was pretreated and changes of rCBF were monitored. Indomethacin pretreated rCBF showed 4.43 ±0.51 in the 0.5 mL volume of vehicle, and Flos *Sophora japonica* from 0.01 to 10 mg/kg (i.v.) treatment showed as Table 2.

In brief, Flos *Sophora japonica* i.v. administration showed no change on rCBF in propranolol, atropine, L-NNA and indomethacin pretreated rats. But Flos *Sophora japonica* i.v. administration showed statistical significance on rCBF in methylene blue pretreated rats. Flos *Sophora japonica* L. decreased the changes of BP, significantly. The BP of Flos *Sophora japonica* L. did not change by pretreated propranolol, atrophine, L-NNA and indomethacin. But the BP of Flos *Sophora japonica* L. was decreased by pretreated methyllene blue. Flos *Sophora japonica* L. increased the changes of rCBF in rats significantsly. The rCBF of Flos *Sophora japonica* L. did not change by pretreated propranolol, atrophine, L-NNA and indomethacin. But the rCBF of Flos *Sophora japonica* L. was increased by pretreated methylene blue. There results indicated that Flos *Sophora japonica* L. can increased the rCBF and decrease the BP, that is related to guanylyl cyclase activity. It is commonly accepted that in a situation of oxidative stress, reactive oxygene molecules (ROS) such as superoxide (O^{2-}, $OOH^{\cdot}$), hydroxyl ($OH^{\cdot}$) and peroxyl ($ROO^{\cdot}$) radicals are generated. The ROS play an important role related to the pathogenesis of various serious diseases, such as neurodegenerative disorders, cancer, cardiovascular disease, atherosclerosis, cataracts, and inflammation (Aruoma OI, 1998). The use of traditional medicine is widespread and plants still present a large source of natural antioxidants that might serve as leads for the development of novel drugs. Several antiinflamamatory, digestive, antinecrotic, neuroprotective, and hepatoprotective drugs

Table 2. Effects of pretreatment with propranolol, atrophine, L-NNA, methylene blue and indomethacin on the Flos *Sophora japonica* extract induced in the regional cerebral blood flow in rats (%)

Drug mg/kg	Propranolol + Flos *Sophora japonica*	Atropine + Flos *Sophora japonica*	L-NNA + Flos *Sophora japonica*	Methylene blue + Flos *Sophora japonica*	Indomethacin + Flos *Sophora japonica*
Control	100.00±0.03	100.00±0.02	100.00±0.02	100.00±0.02	100.00±0.02
0.01	101.81±0.03	103.07±0.03	102.05±0.03	101.55±0.02	102.19±0.02
0.1	105.62±0.05	107.21±0.05	105.85±0.03	104.52±0.03	104.00±0.05
1.0	110.90±0.07	110.41±0.05	109.19±0.06	106.17±0.04*	109.57±0.06
10.0	116.87±0.07	116.70±0.07	115.07±0.05	107.28±0.05*	110.82±0.06

Values are mean±S.E. (n=10).

* : Statistically significance compared with control group at $p<0.05$.

have recently been shown to have an antioxidant and/or antiradical scavenging mechanism as part of their activity (Perry EK *et al.,* 1991; Lin CC & Huang PC, 2002; Repetto MG & Liesuy SF, 2002). In conclusion, the effects of *FSJ* changed of MABP and rCBF was determinate by Laser-Doppler Flowmetry (LDF) *in vivo*. Flos *Sophora japonica* L. decreased the changes of BP, significantly. The BP of Flos *Sophora japonica* L. did not change by pretreated propranolol, atrophine, L-NNA and indomethacin. But the BP of Flos *Sophora japonica* L. was decreased by pretreated methyllene blue. Flos *Sophora japonica* L. increased the changes of rCBF in rats significantsly. The rCBF of Flos *Sophora japonica* L. did not change by pretreated propranolol, atrophine, L-NNA and indomethacin. But the rCBF of Flos *Sophora japonica* L. was increased by pretreated methylene blue. There results indicated that Flos *Sophora japonica* L. can increased the rCBF and decreased the BP, that is related to guanylyl cyclase activity.

REFFERENCES

Aruoma, O. I. (1998). Free radicals, oxidative stress, and antioxidants in human health and disease. *Journal of the American Oil Chemist's Society, 75*, 199-212.

Chen, J., Jin, K., Chen, M., Pei, W., Kawaguchi, K., Greenberg, A., & Simon, R. P. (1997). Early detection of DNA strand breaks in the brain after transient focal ischemia: Implications for the role of DNA damage in apoptosis and neuronal cell death. *Journal of Neurochemistry, 69*, 232-245.

Christina, E. W., Sheena, I. D., David, D. S., Lindsay, R. C., Katherine, J. B., Amy, J. J., Robert, A. R., Thomas, T. L., David, P. S., & Mark, W. B. (2012). Effect of mild cognitive impairment and APOE genotype on resting cerebral blood flow and its association with cognition. *Journal of Cerebral Blood Flow & Metabolism,32*, 1589-1599.

Cho, Y. L., & Jeong, H. W. (2007). Experimental study of Samul-tang extract and *Bambusae caulis* in liquamen on the regional cerebral blood flow and mean arterial blood pressure in normal rats. *Korean Journal of Oriental Physiology & Pathology,21*, 1394-400.

Chu, Q., Fu, L., Wu, T., & Ye, J. (2005). Simultaneous determination of phytoestrogens in different medicinal parts of *Sophora japonica* L. by capillary electrophoresis with electrochemical detection. *Biomedical Chromatography,19*, 149-154.

Iadecola, C., & Reis, D. J. (1990). Continuous monitoring of cerebrocortical blood flow during stimulation of the cerebellar fastigial nucleus: A study by Laser-Doppler flowmetry. *Journal of Cerebral Blood Flow & Metabolism,10*, 608-617.

Kato, H., & Kogure, K. (1999). Biochemical and molecular characteristics of the brain with developing cerebral infraction. *Cell and Molecular Neurobiology,19,* 93-108.

Kuroiwa, T., Vonnekoh, P., & Hossmann, K. A. (1992). Laseer Doppler flowmetry in CA1 sector of hippocampus and cortex after transient forebrain ischemia in gerbils. *Stroke, 23*, 1349-1354.

Lauritzen, M., & Fabricius, M. (1995). Real time Laser-Doppler perfusion imaging of cortical spreading depression in rat neocortex. *Neuro Repor.*, *6*, 1271-1273.

Lao, C. J., Lin, J. G., Kuo, J. S., Chao, P. D., Cheng, C. Y., Tang, N. Y., & Hsieh, C. L. (2005). Microglia, apoptosis and interleukin-1beta expression in the effect of *Sophora japonica* 1. On cerebral infarct induced by ischemia-reperfusion in rats. *The American Journal of Chinese Medicine, 33*, 425-438.

Lin, C. C., & Huang, P. C. (2002). Antioxidant and hepatoprotective effects of *Acathopanax senticosus*. *Phytotherapy Research,14*, 489-494.

Love, S. (1999). Oxidative stress in brain ischemia. *Brain Pathology,9*, 119-131.

Perry, E. K., Pickering, A. T., Wang, W. W., Houghton, P. J., & Perru, N. S. (1991). Medicinal plants and Alzheimer's disease: From ethnobotany to phytotherapy. *Journal of Pharmacy and Pharmacology, 51*, 527-534.

Repetto, M. G., & Liesuy, S. F. (2002). Antioxidant properties of natural compounds used in popular medicine for gastric ulcers. *Brazilian Journal of Medical and Biological Research, 35,* 523-534.

Small, D. L., Morley, P., & Buchan, A. M. (1999). Biology of ischemic cerebral cell death. *Progress in Cardiovascular Diseases,42*, 185-207.

Wesley, B. B., Zhenghui, S., Teruyuki, H., Mary, E. P., Turgut, D., Martin, R., Arjun, G. Y., & Joel, H. G. (2013). Neurovascular coupling varies with level of global cerebral ischemia in a rat model. *Journal of Cerebral blood Flow & Metabolism*, *33*, 97-105.