Cognitive Enhancing Activity of *Betula platyphylla* Sap in Scopolamine Induced Amnesic Mice

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Abstract – Cognitive enhancing activity of *Betula plantyphylla* sap was determined in scopolamine induced amnesic mice using passive avoidance test. Oral acute administration of the sap effectively reversed memory deficit in a dose dependent manner. Then the sap was standardized on the basis of sugar contents using HPLC combined with refractive index detector. Glucose and fructose were the main sugars present in the sap.
Keywords – *Betula platyphylla* sap, Cognitive-enhancing activity, Passive avoidance test

Introduction

Alzheimer's disease (AD) is a neurodegenerative disease and causes memory loss and dementia, which mostly affects the elderly population (Francis *et al*., 1993). Cognitive impairment in AD is caused mainly by death of cholinergic neurons in basal forebrain (Bartus *et al*., 1982; Collerton, 1986). A deficit of acetylcholine in an AD brain is also well known (Perry *et al*., 1978; Wilcock *et al*., 1982). Impairment of learning and memory can be induced chemically in experimental animals by scopolamine, a cholinergic antagonist known to interfere with acetylcholine transmission in the central nervous system (Misane and Ogren, 2003). The experimental animal model of scopolamine induced amnesia has been extensively used to screen for compounds with potential therapeutic value in dementia (Begar *et al*., 1999; Rubaj *et al*., 2003). Thus, we tried to search for cognitive-enhancing compounds from natural resources by using scopolamine-induced memory impairment in mice.

In the course of screening, the sap of *Betula platyphylla* var. *japonica* (Betulaceae) showed significant cognitive enhancing activity. *B. platyphylla*, birch tree, is widely distributed in Korea, Japan, China, Sahalin and Siberia. In many areas, birch sap has been believed to have health promoting properties, for example, birch sap has been widely used for the treatment of gastroenteric disorders, neuralgia, high blood pressure and pain after childbirth in Korea (Yoon and Jo, 1995). In addition, oral administration of birch sap increased the duration of forced swimming in rats and attenuated stress induced hypertension in rabbits (Drozdova *et al*., 1995). In general, birch sap contains a great deal of water and has been known to be rich in carbohydrates, amino acids, mineral elements and essential oils (Yoon and Jo, 1995). Specially, sap is consisted of monosaccharide such as glucose and fructose. These main monosaccharides in birch sap are absorbed in blood from small intestine and metabolized to generate energy for human system (Terazawa, 1995).

In the present work, the cognitive enhancing activity of the sap was evaluated for the first time using passive avoidance test in scopolamine induced mouse amnesic model. The sap was standardized on the basis of glucose and fructose using HPLC combined with refractive index detector (HPLC-RI).

Materials and methods

Materials and chemicals – Reagents for passive avoidance test, carboxymethylcellulose (CMC) and scopolamine were purchased from Sigma (St. Louis, MO, USA). The sap was provided by SK E&C (Korea). The sap sample was collected in the afforested land of SK E&C, which has more than 450,000 trees of *B. platyphylla* in 167.6 ha of afforestation area in Chungju.

The analytical grade of two monosaccharides, D-glucose and D-fructose, were purchased from Sigma (St.

Louis, MO, USA). HPLC analytical grade solvents and reagents were obtained from BDH chemicals (Poole, UK). Triple deionized water (Millipore, Bedford, MA, USA) was used for all preparations.

Sample preparation – The birch sap was pulverized by evaporation and freeze drying. The pulverized sap powder was suspended in 0.5% CMC to the desired concentration just before use. For HPLC analysis, freeze dried sap was suspended to 80% methanol at the concentration of 0.5 mg/mL. And then suspension was passed gradually to Sep-pak C18. The stock standard solutions of glucose and fructose were prepared in 80% methanol at the concentration of 1.0 mg/mL, respectively. The appropriate amount of each standard solution was mixed and diluted as indicated. All these solutions were filtered through 0.45 μm membrane filter (Millipore, Nylon, 170 mm).

Animals – Male ICR mice (Experimental Animal Breeding Center of Seoul National University, Seoul, Korea), weighing 25 - 30 g, were used for passive avoidance test following a one-week adaptation period (20 to 23 °C; 12 hr light cycle from 9 am to 9 pm; food, Agribrand Purina Korea, and water ad libitum). All experiments were conducted according to the guidelines of the Committee on Care and Use of Laboratory Animals of the Seoul National University.

Passive avoidance test – Training for and testing of passive avoidance performance were carried out in two identical light and dark square boxes (Gemini San Diego Inc., USA) as described in our previous report (Kang et al., 2003). The mice were initially placed in the light chamber and ten seconds later the door between compartments was opened. When mice entered the dark compartment, the door automatically closed and an electrical foot shock (0.1 mA/10 g body weight) for a time period of 2 sec was delivered through the stainless steel rods (one trial training). Ten mice were used per treatment. Mice received 0.5% CMC, test samples (50 and 100 mg/kg, dissolved in 0.5% CMC, p.o.) or donepezil (2 mg/kg, dissolved in 0.5% CMC, p.o.) 120 min before the training trial, respectively. After 90 min, amnesia was induced in mice with scopolamine (1.0 mg/ kg body weight, dissolved in normal saline) given subcutaneously. Twenty-four hr after the training trial, the mice were again placed in the light compartment. The escape latency to enter the dark compartment was measured. If the mice did not enter the dark compartment within 180 sec, the experiment was stopped.

Statistical analysis – All data for passive avoidance tests were analyzed by one-way ANOVA and expressed

as mean ± SEM. The data were considered to be statistically significant if the probability had a value of 0.05 or less.

Chromatographic conditions – Sugar content in the sap was determined by HPLC-RI (Clement et al., 1992). The HPLC equipment consisted of a chromatographic pump, an automated sampler injector, a refraction index detector (Agilent 1100 series, USA). Chromatographic separation was achieved with a column (Super-pak™ 6.5 mm × 300 mm), operating at 70 °C. An isocratic elution using analytical grade water (100%) as a mobile phase was employed to separate glucose and fructose in the sap. The flow rate was 0.4 mL/min and injection volumes were 10 μl.

Results and discussion

To search for cognitive enhancing natural products, passive avoidance test was employed as a screening system in scopolamine induced memory deficit mice model. The step through latency of the scopolamine treated group was significantly shorter than that of the 0.5% CMC treated control group. Donepezil, an acetylcholinesterase inhibitor and the most frequently used among approved AD drugs, was used as a positive control. At a dose of 2 mg/kg body weight, donepezil restored the step through latency by 72.7% compared to the 0.5% CMC treated control group in the passive avoidance test in our system. Freeze dried birch sap powder significantly improved the memory deficit induced by scopolamine in a dose dependent manner at the tested doses, 50 and 100 mg/kg body weight. The potency of the sap at the highest effective dose (100 mg/ kg body weight) seemed to be comparable to that of donepezil recovering the step through latency up to 72.0% of that of control group (Fig. 1).

Natural products should be standardized to acquire consistent biological activity and quality. Active compounds responsible for the biological activity of natural products might be the most reasonable markers for the standardization and quality control. However, in most cases, it is very difficult to identify and analyze the active compounds due to the chemical and biological complexity of natural products. Therefore, major or specific compounds are often used and quantified as markers instead of active compounds. Since the active cognitive enhancing compounds of birch sap have not been determined, we tried to standardize the sap on the basis of some major constituents. Birch sap has been known to be rich in glucose and fructose (Drozdova et al., 1995).
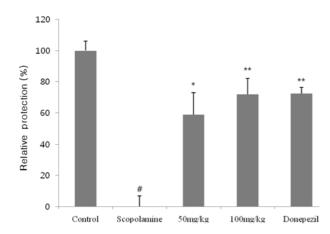
Fig. 1. Effects of oral treatments of birch sap on the scopolamine-induced amnesic mice in the passive avoidance test.

Mice received test sample (p.o.) 120 min before the training trial. After 90 min, amnesia was induced in mice with scopolamine (1 mg/kg body weight, s.c.). Twenty four hours after the training trial, the mice were again placed in the light compartment. The latency time to enter the dark compartment was measured. The values shown are the relative protection (%) ± SEM. Relative protection was calculated as 100 × (escape latency of test sample group - escape latency of scopolamine treated group)/(escape latency of control group - escape latency of scopolamine treated group). Results differ significantly from the value in control group: $P < 0.05^{\#}$. Results differ significantly from the value in scopolamine treated group: $P < 0.05^*$; $P < 0.01^{**}$. The control group was 0.5% CMC and saline treated group (10 mL/kg body weight, p.o.). The donepezil treated group was 2 mg/kg body weight p.o.

Meanwhile, the memory-improving action of glucose has been demonstrated both in human and animal studies (Messier, 2004). The administration of glucose has been shown to improve memory for various learning tasks in rodents. In humans, glucose also increases declarative memory performance in elderly people and in some patients with mild Alzheimer's disease (Micheau *et al.*, 1995).

For this reason, contents of monosaccharides including glucose in the birch sap were analyzed. A variety of chromatographic system including HPLC may be used to separate and analyze the monosaccharides. Generally, the methods for the HPLC analysis of monosaccharides use silica-based column packing materials with RI (Vonach *et al.*, 1997). In agreement with these previous findings, glucose and fructose were predominantly detected and quantified in our experimental condition using HPLC-RI (Fig. 2). The chromatographic conditions were optimized to obtain chromatograms with a good resolution of adjacent peaks. The preferred chromatographic conditions were obtained using Super-pak™ (6.5 mm × 300 mm). An isocratic system with water was employed as a mobile phase. Fig. 2 shows chromatograms of glucose, fructose as standard solution and birch sap sample respectively. The linearity of glucose and fructose was evaluated by seven concentrations of each compound and calculated in the form of y = ax + b, where y and x were the values of
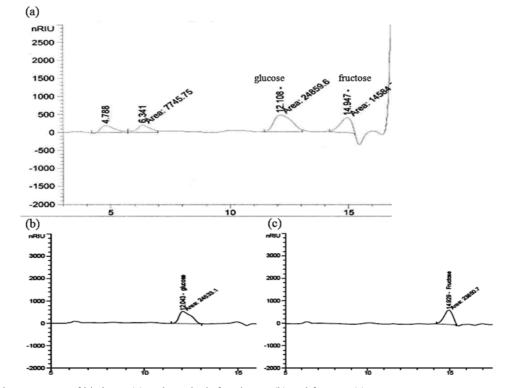

Fig. 2. HPLC chromatogram of birch sap (a) and standards for glucose (b) and fructose (c).

Table 1. Characteristic parameters of calibration curves of sugars in the birch sap

Compound	Linear regression equation[a]	Correlation coefficient (R^2)
Glucose	y = 234.25x + 340.49	0.9999
Fructose	y = 231.25x + 74.548	0.9984

[a]y = peak area, x = concentration (ppm)

Table 2. Sugar contents of the birch sap sample

	Glucose		Fructose	
	Content[a]	RSD[b]	Content[a]	RSD[b]
Birch sap sample	21.4 ± 0.4%	1.84%	13.2 ± 0.6%	4.58%

[a]ppm / ppm × 100
[b]RSD (%) = (SD of amount detected / mean of amount detected) × 100 (n = 5).

peak area and concentration of each compound (x, ppm), respectively. Calibration curves were linear in relatively wide range of concentration with high correlation coefficient values (Table 1). The established method was applied to the determination of the two sugars in the freeze dried birch sap. Five batches of the birch sap sample were used for the determination of two sugars. As shown in Table 2, the contents of the glucose and fructose in freeze dried birch sap were 21.4% and 13.2%, respectively.

Birch sap has been used for the treatment of various diseases in Korean Traditional Medicine. Moreover, various pharmacological effects of the sap have been reported (Drozdova *et al.*, 1995). The present study revealed cognitive enhancing activity of birch sap for the first time. Moreover, the birch sap was standardized on the basis of the contents of two monosaccharides, glucose and fructose using HPLC-RI. These results might provide useful information for the development into utilizing birch sap as a raw material for food, beverage and cosmetics industries.

Acknowledgment

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