

Determination of Total Chiro-inositol Content in Selected Natural Materials and Evaluation of the Antihyperglycemic Effect of Pinitol Isolated from Soybean and Carob

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Abstract Pinitol and chiro-inositol exert insulin-like effect by mediating post-receptor signaling pathway. Total chiro-inositol concentrations, including pinitol, chiro-inositol, and their derivatives, were determined in 115 natural and food materials to identify economical sources for mass production of pinitol. Carob pod, *Bougainvillea*, soy whey, and soybean oligosaccharides were rich sources of chiro-inositol. Pinitol was isolated from soy whey and carob pod, considered as economically viable sources, by chromatographic separation using activated carbon. Soy and carob pinitols had same chemical structure as that of reference pinitol based on HPLC and NMR results. Oral administration of soy pinitol and carob pinitol (10 mg/kg) significantly decreased blood glucose at 2–6 hr in streptozotocin-induced diabetic rats. These results suggest pinitol isolated from soy whey and carob pod could be beneficial in controlling blood glucose in animal model of diabetes mellitus.

Keywords: pinitol, chiro-inositol, isolation, soy, carob

Introduction

Pinitol (D-3-O-methyl-chiro-inositol) exerts an insulin-like effect due to its involvement downstream of the cellular signal transduction mediated by the insulin receptor (1). Binding of insulin to the insulin receptor on a cell membrane of a target tissue activates phospholipases C and D, which hydrolyze glycosylphosphatidylinositol into inositolphosphoglycan - an insulin mediator-and diacylglyceride on the cell membrane (2). There are two types of inositolphosphoglycan insulin mediators: one being a mediator consisting of myo-inositol and glucosamine, and the other consisting of chiro-inositol and galactosamine (3, 4), among which the chiro-inositol mediator promotes glucose transport and activates enzymes that catalyze both the oxidative and nonoxidative metabolisms of glucose (5). Pak et al. (6) demonstrated the *in vivo* conversion of the myo-inositol mediator into the chiro-inositol mediator. The importance of the chiro-inositol insulin mediator in the insulin signal pathway has been demonstrated in many studies (7-10). In type 2 diabetes mellitus, a defect in the synthesis of the chiro-inositol insulin mediator is one possible mechanism leading to the failure in blood glucose control (11). In addition, chiro-inositol deficiency could result in insulin resistance (12-14). Pinitol, a natural component of foods and botanical materials, is converted into D-chiro-inositol *in vivo* (15). Pinitol extracted from *Bougainvillea spectabilis* reduced the blood glucose concentration in alloxan-induced diabetic rats (16) and streptozotocin (STZ)-induced diabetic mice (1). Soybean-

derived pinitol treatment resulted in an improved glucose tolerance in diabetic patients (15). Furthermore, administration of pinitol or chiro-inositol could help normalize the synthesis of the chiro-inositol mediator, which results in the control of blood glucose (7).

Bougainvillea (11), soybean (17, 18), carob pod (19), and pine wood (20) have been reported to contain relatively high concentrations of pinitol. Although pinitol is a natural hypoglycemic agent, wide application of pinitol is limited due to its relatively high price. Therefore, it is important to find economical sources of pinitol for mass production. However, to date, no systematic analyses of the pinitol content of natural products have yet been reported in the literature. The objectives of the present study were thus to determine the total chiro-inositol content, including pinitol, chiro-inositol, and their derivatives, in 115 natural and food materials, identify good sources for mass production of pinitol, and isolate pinitol from economically potential viable sources. Soy whey pinitol and carob pod pinitol were isolated, their structures were identified, and their antihyperglycemic effects were verified in animal model of diabetes mellitus.

Materials and Methods

Materials Standard pinitol and chiro-inositol, STZ, hydrochloric acid, and sodium hydroxide were purchased from Sigma Co (St. Louis, MO, USA).

Pretreatment of botanical materials and determination of total chiro-inositol content The sample was thermally hydrolyzed in 6 N HCl solution at 100°C for 48 hr. Under this condition, all derivatives of chiro-inositol, including pinitol and glycosylated pinitol, are converted into free

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chiro-inositol. The resulting total chiro-inositol content of the sample was measured using a high-performance liquid chromatograph (Dionex Co., Sunnyvale, CA, USA) equipped with an electrochemical detector and a Dionex Carbopak MA-1 column. The mobile phase was 60 mmol NaOH solution/L at a flow rate of 0.4 mL/min. Total chiro-inositol contents were expressed as means of four replicates.

Isolation and identification of pinitol from soy whey and carob pod Pinitol was isolated from soy whey by chromatographic separation using activated carbon, crystallization, and drying (21). Pinitol in carob pod was extracted with water, and the same isolation process as described above was applied to yield pure pinitol. Pinitol isolated from the samples was identified by HPLC and NMR. The NMR spectra were obtained on a Bruker Avance 400 instrument (9.4 T; Karlsruhe, Germany) in D₂O. For the ¹H-NMR experiment, 32 transients were acquired with a 1-s relaxation delay using 32 K data points, and 90° pulse was 9.8 μs with a spectral width of 4000 Hz.

Animal experiment Male Sprague-Dawley rats weighing between 230 and 260 g were purchased from Bio Genomics, Inc. (Seoul, Korea). All rats were fed a pelletized commercial chow diet *ad libitum* for 7 days after arrival. The animals were rendered diabetic by intraperitoneal injection of STZ (60 mg/kg) in citrate buffer, pH 4.5. Blood samples were taken from the tail tip after 7 days, and the blood glucose concentration was measured with a glucometer (Glucotrend; Roche Diagnostics, Indianapolis, IN, USA). Animals were considered diabetic if their fasting blood glucose concentration was higher than 200 mg/dL. All animals were continuously fed a commercial chow diet. The rats ($n = 27$) were randomly

divided into three groups. After being starved overnight, the animals were administered carob pinitol or soy pinitol (10 mg/kg) dissolved in physiological saline by gastric intubation; the controls received vehicle only (3 mL/kg). Blood samples were collected from the tail tip, and glucose was measured at 1-hr intervals up to 6 hr. Feed was withheld during the test.

Statistical analyses The blood glucose data, expressed as the percentage change from time zero, were reported as means ± SDs. One-factor analysis of variance and Tukey's test of multiple comparisons were used to determine statistical differences between the groups at each time point. Individual time points were compared with time zero by Student's paired *t* test. All statistical analyses were performed with the use of SAS (version 8.02; SAS Institute, Cary, NC, USA). The limit of significance was set at $P < 0.05$.

Results and Discussion

The content of total chiro-inositol content in various natural sources Of the 115 natural sources and food materials tested, only four—carob pod, *Bougainvillea*, soy whey, and soybean oligosaccharides—were found to be rich sources of chiro-inositol, containing more than 10 g/kg (Table 1). Fifteen sources tested had chiro-inositol contents of 1–10 g/kg. Silkworm, which is consumed in some parts of Southeast Asia and Korea as an antidiabetic product, was the only animal source that contained more than 1 g/kg of chiro-inositol. Among the remaining 96 sources containing less than 1 g/kg of chiro-inositol, no chiro-inositol was detected in 84 sources.

Diabetes is among the five leading causes of death in most developed countries (22), and, by the year 2010,

Table 1. Total chiro-inositol content of various natural materials*

	(g/kg)		(g/kg)
Carob pod	40.0	Buckwheat	1.55
Bougainvillea	20.0	Rice	0.50
Soybean (USA)	6.75	Wheat	0.17
Soybean (China)	4.82	Arrowroot	2.32
Soybean (Korea)	4.36	Pistachio	0.11
Soybean germ	7.05	Burdock	0.34
Soybean leaf	8.25	Thistle	0.85
Soybean pod	2.59	Blackberry	0.17
Soy whey (dried)	20.0	Grape	0.51
Soybean oligosaccharides	17.4	Lemon	0.61
Defatted soybean	6.45	Lime	0.19
Pine nut	1.74	Orange	1.57
Pine needle	6.96	Korean raisin	4.04
<i>Rhynchosia molubilis</i>	2.02	(<i>Hovenia dulcis</i> Thunb)	
Ginseng	1.05	Honey	0.18
Siberian ginseng	4.80	Silkworm	1.02
(<i>Eleutherococcus senticosus</i>)		Carob	0.45
		Yeast	0.12

Not detected

Sunflower seed, barley, kidney bean, mung bean, adzuki bean, pea, peanut, walnut, foxtail millet, oats, wheat bran, rice bran, corn, corn stump, almond, Brazil nut, cashew, coconut, hazelnut, pecan, sesame, garlic, ginger, lotus root, yam, beet, potato, sweet potato, sugar beet, carrot, onion, tomato, beet leaf, alfalfa, avocado, asparagus, broccoli, cabbage, celery, cucumber, egg, apple, lettuce, kale, mushroom, parsley, pepper, spinach, pumpkin, apricot, plum, pear, apple, banana, melon, cherry, fig, kiwi, mango, jujube, watermelon, peach, pineapple, strawberry, tangleweed, laver, brown seaweed, green laver, chicken, pork, beef, oyster, shrimp, sardine, tuna, milk, yogurt, cheese, coffee, cocoa, gelatin, green tea, maple syrup, molasses, yeast

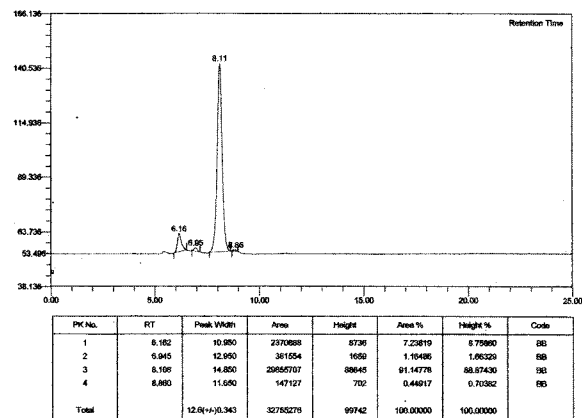
*Values are means of 4 replicates.

complications from diabetes are estimated to surpass both heart disease and cancer as the leading cause of death. Evidence from prospective randomized clinical trials suggests that achieving near-normal glycemic control in patients with diabetes mellitus is associated with sustained decreased rates of diabetic complications (23, 24). Although there have been enormous improvements in anti-diabetic medications, many diabetic patients still have difficulty achieving glycemic control. Therefore, numerous studies have been carried out to evaluate natural products, including plant materials, as alternative treatments for diabetes to be used in addition to the conventional treatments (25). Pinitol, an insulin sensitizer, could be one such alternative hypoglycemic agent.

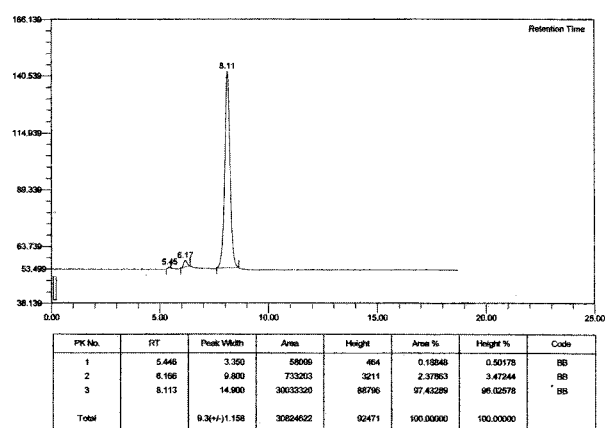
The relatively high price of pinitol could limit its wide application. Thus, we determined the total chiro-inositol concentration of a variety of natural materials in an effort to identify economically viable sources. Considering their annual production worldwide and price, soy whey and carob pod are the two sources that are likely to be most competitive as raw materials for the mass production of pinitol. Carob has been used mainly as a raw material for locust bean gum production. Carob bean consists of seed, which accounts for only 10% of the total weight of the carob bean, and carob pod, which accounts for 90% of the total weight of the bean. Despite its large production volume, the carob pod-40% of which is composed of sugars-has had only limited application in uses such as animal feed. Only recently has carob pod been used as a chocolate substitute or a source of dietary fiber. Approximately 99% of total chiro-inositol in carob pod exists as pinitol. As the various beneficial effects of pinitol become recognized by the public, the economic value of carob pod will further increase, because it is the richest natural source of pinitol. Soy whey is the wastewater produced in large volumes during the production of tofu. Approximately 80% of total chiro-inositol in soy whey exists as pinitol. Because of the high solubility of pinitol in water, almost all of the pinitol present in soybean is recovered in the soy whey. The main advantage of soy whey as a raw material for pinitol production is that it is available almost free of charge.

Confirmation of the chemical structures of soy pinitol and carob pinitol Chromatograms from the high-performance liquid chromatographic analysis (Fig. 1) and spectra from $^1\text{H-NMR}$ (Fig. 2) and $^{13}\text{C-NMR}$ (Fig. 3) showed that the pinitol samples isolated from soy whey and carob pod were identical to the pure reference D-pinitol. The $^1\text{H-NMR}$ spectrum showed seven peaks at 3.48, 3.90, 3.23, 3.90, 3.67, 3.67, and 3.56 ppm, which were assigned as methoxy group, C-2, C-4, C-6, C-3, C-5, and C-1, respectively. The $^{13}\text{C-NMR}$ spectrum showed seven peaks at 60.7, 70.7, 71.5, 72.4, 72.6, 73.0, and 83.7 ppm, which were assigned as methoxy group, C-2, C-4, C-6, C-3, C-5, and C-1, respectively. These results indicate that both soy pinitol and carob pinitol possess hypoglycemic effect. Purities of pinitol isolated from soy whey and carob pod were over 98.0%.

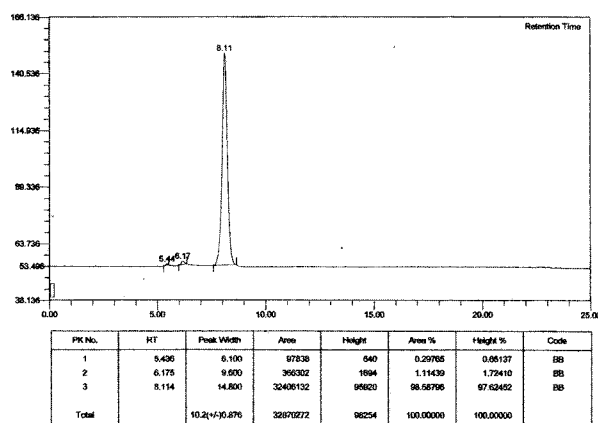
The antihyperglycemic effect of soy pinitol and carob pinitol The hypoglycemic effects of soy pinitol and



A



B



C

Fig. 1. High-performance liquid chromatogram of pinitol from different sources. A: Pinitol isolated from soy whey; **B:** pinitol isolated from carob pod; **C:** reference pinitol

carob pinitol were determined *in vivo* to assess possible therapeutic use. Changes in blood glucose concentration after an oral dose of pinitol are shown in Fig. 4. Fasting blood glucose concentrations at time zero in the control, soy pinitol, and carob pinitol groups were 368.3 ± 30.4 , 374.2 ± 38.6 , and 371.6 ± 42.2 mg/dL, respectively. In the control group, the relative blood glucose concentration at 5

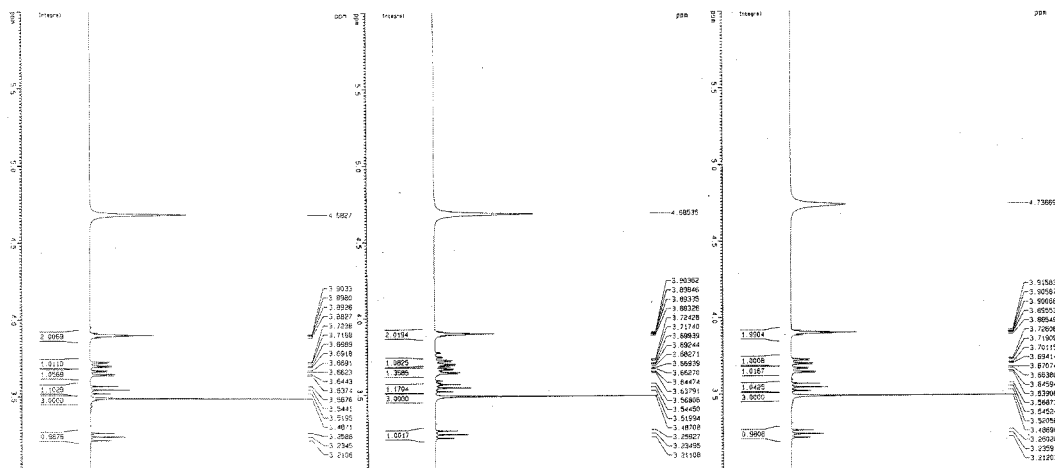


Fig. 2. ¹H-Nuclear magnetic resonance data for pinitol from different sources. A: Pinitol isolated from soy whey; B: pinitol isolated from carob pod; C: reference pinitol.

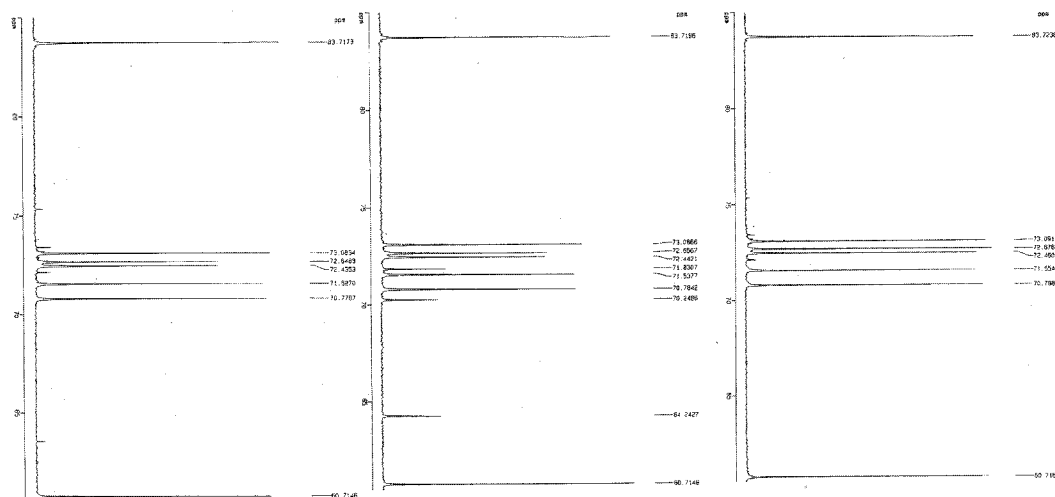


Fig. 3. ¹³C-NMR data for pinitol from different sources. A: Pinitol isolated from soy whey; B: pinitol isolated from carob pod; C: reference pinitol.

hr decreased significantly from that at time zero by 96.9±3.3% ($p < 0.05$); at 6 hr the percentage change from time zero was 93.7±2.5% ($p < 0.001$). In both soy pinitol and carob pinitol groups, blood glucose concentrations decreased significantly from time zero, starting at 2 hr ($p < 0.01$ at 2 hr, $p < 0.001$ at 3-6 hr). The percent changes in relative blood glucose concentrations in the soy pinitol and the carob pinitol groups at 6 hr were 79.6±4.8 and 80.5±4.3 %, respectively; however, no significant difference was observed between both groups at any time point.

The acute oral administration of either soy pinitol or carob pinitol significantly decreased blood glucose in STZ-injected rats within 2 hr after administration. In the body, pinitol from either source could be converted into D-chiro-inositol, which is a component of an inositol phosphoglycan-one of the mediators involved in the actions of insulin (15). D-chiro-inositol-containing phosphoglycan has been shown to stimulate both glycogen synthase and pyruvate dehydrogenase phosphatase (3, 26). Thus, pinitol administered in this study could mimic the action of insulin, stimulating the uptake of glucose into insulin-sensitive tissues, such as muscle and fat, and inhibiting the release of glucose from the liver. Bates et al. (1) reported

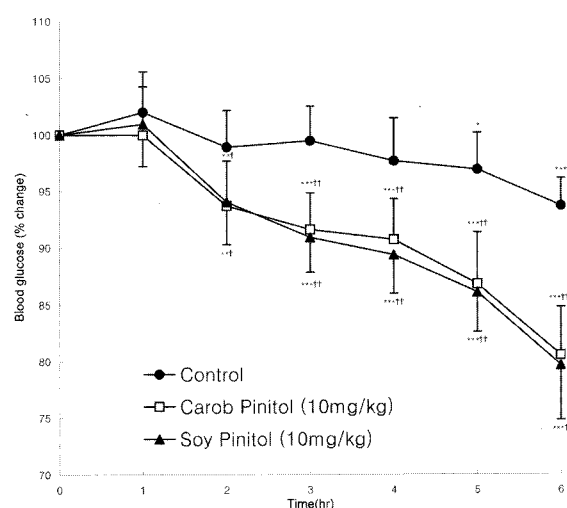


Fig. 4. Acute effects of carob pinitol and soy pinitol administration on blood glucose in streptozotocin-induced diabetic rats. Data are expressed as the mean (± SD) percentage change from time zero. $n = 9$. Significantly different from time zero: $p < 0.05$, $**p < 0.01$, $***p < 0.001$. Significantly different from the control group at the same time: $+p < 0.01$, $++p < 0.001$.

the acute feeding of commercial pinitol lowered blood glucose in STZ-diabetic mice. In the present study the acute feeding of soy pinitol and carob pinitol resulted in an antihyperglycemic effect in STZ-diabetic rat.

In conclusion, our results clearly show that pinitol extracted from soybean and carob has the same chemical structure as that of the reference pinitol. Both soy pinitol and carob pinitol showed an antihyperglycemic effect in STZ-induced diabetic rats. Chronic feeding of soy pinitol improves glycemic control and insulin sensitivity in patients with type 2 diabetes mellitus (27). Thus, soy pinitol could be beneficial in controlling hyperglycemia in both patients with types 1 and 2 diabetes. However, the clinical importance of pinitol as a hypoglycemic agent for patients with type 1 diabetes requires further study.

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