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Short communications

Acute intake of mulberry leaf aqueous extract affects postprandial glucose response after maltose loading: Randomized double-blind placebo-controlled pilot study

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ABSTRACT

Mulberry leaf extract was investigated as a potent alpha-glucosidase inhibitor, especially for individuals with prediabetes. We evaluated the effect of the ingestion of mulberry leaf aqueous extract (MLAE) on postprandial glucose responses in healthy subjects. We carried out a randomized, double-blind, placebo-controlled trial to determine the intake amount of MLAE (1.25, 2.5, or 5 g) and time necessary to impact postprandial blood glucose levels after 75 g of maltose loading in 50 healthy subjects. Ingestion of MLAE led to a decrease in post-challenge acute glucose levels following intake of 2.5 or 5 g of MLAE at 30 and 60 min ($P = 0.0137$ and 0.0423, respectively). There was no significant difference between pre- and simultaneous administration of MLAE. The ingestion of MLAE resulted in improved postprandial glycemic control in healthy subjects.

1. Introduction

Phytochemical-rich plants play an important role in diet-based therapies for diabetes mellitus. Mulberry contains 1-deoxynojirimycin (DNJ) and some of its derivatives, which are alpha-glucosidase inhibitors (Asai et al., 2011; Asano et al., 2001; Mudra, Ercan-Fang, Zhong, Furne, & Levitt, 2007; Nakamura, Nakamura, & Oku, 2009) that have been used as medicines to treat diabetes mellitus. Alpha-glucosidase enzymes in the intestinal lumen and in the brush border membrane have a main role in carbohydrate digestion of starch and oligosaccharides to monosaccharides before they are absorbed (Standl & Schnell, 2012). Suppressing the activity of such digestive enzymes would delay the degradation of starch and oligosaccharides, which would in turn decrease the absorption of glucose and consequently suppress postprandial blood glucose level elevation (Balfour & McTavish, 1993; Derosa & Maffioli, 2012; Hansawasdi & Kawabata, 2006; Van de Laar, Lucassen, Akkermans, Van de Lisdonk, & De Grauw, 2006; Van de Laar et al., 2005). Recently, several studies in animals and humans have reported that mulberry or sericulture products containing DNJ suppress postprandial increases of glucose (Asano et al., 2001; Kimura et al., 2007; Nakamura et al., 2009).

At present, various mulberry leaf products are available in the market as food supplements; however, these products have different preparations, and there are few assessments of the effects of these products on postprandial glycemic

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control. In our previous studies, we developed a standardized mulberry leaf aqueous extract (MLAE, approximately 3.6 mg DNJ/g MLAE) to investigate the functional food ingredient, and we confirmed that this extract inhibited alpha-glucosidase activity and glucose uptake using *in vitro* and animal models (Kim et al., 2011; Kwon, Chung, Kim, & Kwon, 2011). In addition, we observed that our extract ameliorated insulin sensitivities in high fat or high sucrose diet-induced animal models (Kim et al., 2011).

The present study aimed to determine the intake level of this MLAE product and the time necessary to lower postprandial blood glucose levels by using a maltose tolerance test in healthy subjects.

2. Materials and methods

2.1. Preparation of MLAE

Mulberry leaves (*Morus alba*) were purchased from Hwasun National Agricultural Cooperative Federation (Jeonnam, Korea) and dried. The MLAE was prepared from 1.2 kg of mulberry leaves and 36 L of water at 45 ± 5 °C for 3 h. The extract solution was filtered with a 325 mesh sieve (45 μm). The filtrate was concentrated with an evaporator under thin centrifugal film pilot conditions (189 g) and freeze dried. The dried extract (yield = 13%) was examined for DNJ content (360 mg/100 g) by high performance liquid chromatography method (Kim et al., 2003; Kwon et al., 2011).

2.2. Subjects and study design

This study was carried out to establish the intake amount and time necessary to impact postprandial glucose control with a randomized, double-blind design. Fifty healthy volunteers, aged 20–50 years, were recruited from advertisements on several university websites and internet communities. Subjects with a fasting plasma glucose concentration below 125 mg/dL were included. All subjects were generally healthy as demonstrated by their medical history and laboratory tests. The study protocol was approved by the ethical committees of Ewha Womans University (Institutional Review Board [IRB] No. 2010-3-3, Seoul, Korea). Body weight, blood pressure, and heart rate were measured at every visit (both at the screening and test visit). At the screening visit, blood and urine samples were obtained and analyzed in Seoul Medical Science Institute (SCL, Seoul, Korea). At the test visit, after fasting for 12 h, the subjects were randomly divided into five groups of ten individuals. Among the five groups, the subjects in four groups received a beverage containing 75 g maltose powder (Hayashibara, Okayama, Japan) dissolved in 50 ml water and mixed with 0 (placebo), 1.25, 2.5, or 5 g of MLAE (corresponding to 0, 4.5, 9, and 18 mg of DNJ, respectively). The remaining group took 5 g of MLAE 30 min prior to the intake of the maltose solution. Blood glucose levels were measured from the finger using an Accu-Check (Roche Diagnostics, Manheim, Germany) at 0, 15, 30, 60, 90, and 120 min after maltose administration.

2.3. Statistical analysis

The sample size was designed to detect a difference among groups in postprandial glucose level at 30 min with a 95% confidence interval and 80% power. All results were expressed as the mean ± standard error of the mean (SEM). The Kolmogorov–Smirnov test was used to test for a normal distribution. The postprandial blood glucose levels were analyzed with repeated one-way within-group analysis of variance (ANOVA). ANOVA was used to compare data at individual time points with *post hoc* Duncan's multiple comparison tests. The incremental area under the curve (iAUC) for the glucose response was calculated by the trapezium rule (Wolever, Jenkins, Jenkins, & Josse, 1991). For the comparison of summary measures, such as area under the curve (AUC), peak time, and peak height, one-way ANOVA tests were applied followed by *post hoc* Duncan's multiple comparison tests. When two variables were compared, the Student's *t*-test was applied. Significance was set at $P < 0.05$ for all the statistical analyses and *post hoc* comparisons. All statistical analyses were performed using the Statistical Analysis Systems package version 9.2 (SAS Institute, Cary, NC, USA).

3. Results

Fifty eligible subjects completed this study. The baseline characteristics of the subjects were as follows: mean age 22.7 ± 0.4 years, body mass index (BMI) 21.2 ± 0.4 kg/m^2, fasting blood glucose 90.9 ± 1.1 mg/dL, systolic blood pressure 111.6 ± 1.8 mmHg and diastolic blood pressure 66.1 ± 1.1 mmHg (Table 1).

Table 1 – Clinical characteristics of the study population.

	Control (0 g MLAE)	Low dose (1.25 g MLAE)	Intermediate dose (2.5 g MLAE)	High dose (5 g MLAE)	Pretreatment with high dose (5 g MLAE)
Sex, male:female (%)	40:60	40:60	30:70	30:70	30:70
Age (year)	22.5 ± 0.8	22.5 ± 0.6	22.7 ± 0.8	23.2 ± 0.5	22.5 ± 0.8
Height (cm)	169.6 ± 2.4	169.3 ± 2.3	165.3 ± 2.1	166.9 ± 2.1	169.6 ± 2.4
Weight (kg)	60.5 ± 2.8	60.4 ± 2.9	60.23 ± 2.9	57.6 ± 2.9	60.5 ± 2.8
Body mass index (kg/m^2)	20.9 ± 0.7	20.9 ± 0.7	21.9 ± 0.7	20.5 ± 0.7	20.9 ± 0.7
Fasting blood glucose (mg/dl)	88.7 ± 1.9	92.7 ± 1.8	91.8 ± 1.7	90.3 ± 2.8	88.7 ± 1.9
Systolic blood pressure (mmHg)	122.3 ± 3.4	112.3 ± 2.6	113 ± 3.6	109.7 ± 5.5	112.3 ± 3.4
Diastolic blood pressure (mmHg)	67.3 ± 2.7	66.8 ± 1.5	68.5 ± 2.4	65.3 ± 2.9	67.3 ± 2.7

Values are expressed as mean ± SEM (n = 10 for each group).

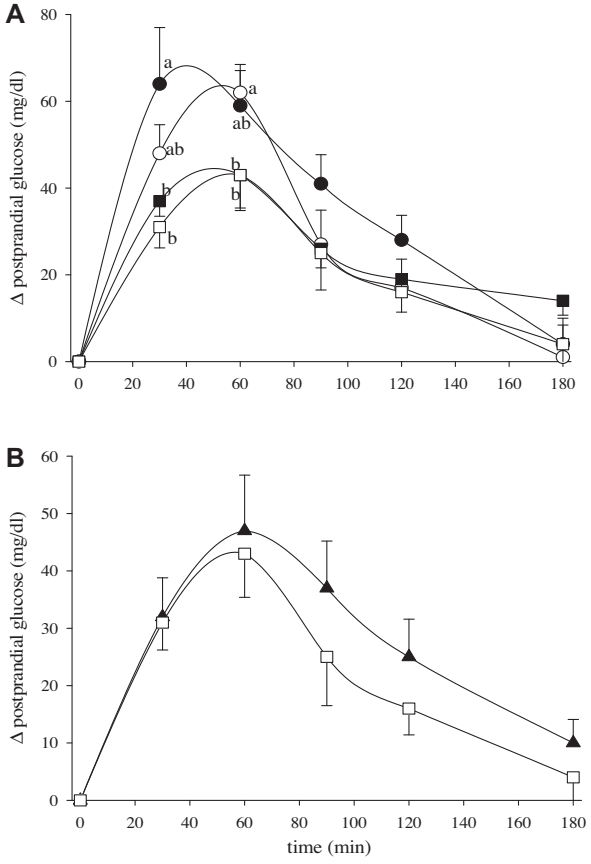

Fig. 1 – Changes in blood glucose concentration from the fasting concentration in healthy subjects after ingestion of 75 g maltose with 1.25 (○), 2.5 (■), 5 g of MLAE (□), or placebo (0 g MLAE) (●) (A). Measurements in the group that ingested 5 g MLAE 30 min prior to maltose loading (▲) are also shown in this figure (B). Values are means with their standard errors. The differences between the MLAE and placebo groups over the time were determined by repeated ANOVA ($P < 0.0001$). Values in each time that do not share a common letter differ significantly in comparisons made by ANOVA followed by Duncan's multiple range test ($P < 0.05$).The postprandial glucose levels were significantly lower in the groups with an intake of 2.5 and 5 g MLAE at 30 and 60 min ($P = 0.0137$ and 0.0423, respectively).

As shown in Fig. 1A, a single intake of MLAE with maltose solution suppressed the elevation in plasma glucose levels in the subjects. There were variations in postprandial blood glucose levels over time ($P < 0.0001$ by repeated ANOVA), and postprandial blood glucose was significantly lower following intake of 2.5 or 5 g MLAE at 30 and 60 min ($P = 0.0137$ and 0.0423, respectively). The iAUC was approximately 0.66-fold following intake of 2.5 or 5 g of MLAE compared to the placebo although no significant differences were observed. In addition, the glucose excursion between 0 and 180 min and C_{max} significantly decreased following intake of 2.5 or 5 g MLAE ($P = 0.007$ and 0.003, respectively, Table 2). Therefore, the effective doses of MLAE were 2.5 and 5 g MLAE (corresponding to 9 and 18 mg of DNJ).

Next, to determine the appropriate intake time, we compared pre- versus simultaneous administration with maltose at the highest dosage (5 g of MLAE). There was no significant difference between pre- and simultaneous administration in the subjects (Fig. 1B). Only the t_{max} was significantly delayed from 51 ± 4.6 to 66 ± 4.0 min by pre-administration of MLAE (Fig. 1B and Table 2, $P = 0.024$ by Student's t-test). Based on this result, we conclude that postprandial glucose uptake was inhibited by the highest MLAE dosage regardless of the intake time.

4. Discussion

We conducted a randomized, placebo-controlled, double-blind pilot study to investigate the effective intake level of MLAE and the time necessary to lower postprandial glucose levels. Ingestion of MLAE containing 0.36% DNJ effectively reduced hyperglycemia after a 75 g maltose challenge in healthy subjects from the initial measurement at 0–180 min. The effective doses were 2.5–5 g of MLAE, which correspond to 9–18 mg of DNJ, respectively.

Several other studies in human subjects have reported the control of postprandial glucose levels by the ingestion of DNJ or mulberry extract containing DNJ. Mudra et al. reported that coingestion of mulberry extract with 75 g sucrose significantly suppressed the increase in blood glucose, although the DNJ content of their extract was not determined accurately (Mudra et al., 2007). Mulberry leaf power containing 1.5% DNJ significantly reduced postprandial glucose levels when sucrose or complex carbohydrates (i.e., boiled white rice) were loaded (Asai et al., 2011; Kimura et al., 2007). A mulberry leaf ethanol extract containing 0.77% DNJ suppressed postprandial blood glucose and insulin levels when ingested with sucrose in the form of confections, such as Mizu-yokan, Daifuku-mochi, and cake (Nakamura et al., 2009).

In this study, the minimum effective dose was 9 mg DNJ, and this result was in agreement with previous results, in which the effective doses were 6–46 mg DNJ (Asai et al., 2011; Kimura et al., 2007; Nakamura et al., 2009). The AUCs of incremental blood glucose in the MLAE administration groups were lower than that of the control group, although the differences were not significant. In addition, the excursions, or the peak-to-through fluctuations in blood glucose, were significantly reduced by administration of 2.5 or 5 g MLAE. These results strongly support a recommended dose in the range of 2.5–5 g MLAE.

The MLAE-induced reduction in blood glucose presumably reflects the ability of mulberry to inhibit intestinal absorption of sucrose or maltose (Oku, Yamada, Nakamura, Sadamori, & Nakamura, 2006). DNJ and its derivatives have been well known their inhibitory activities of sucrose or maltase. The MLAE used in this study was confirmed to have DNJ at 360 mg/100 g and several DNJ derivatives were also identified by Fourier transform ion cyclotron resonance mass spectrometric method. These DNJ derivatives were DNJ monoglycoside, DNJ diglycoside and 2-hydroxymethyl-3,4,5-piperidinetriol (Kim, Chung, Jung, Wee, & Kwon, 2013). In addition, according to our previous study, only mulberry extract, not DNJ alone, showed an inhibitory effect on glucose

Table 2 – Pharmacokinetic parameters of plasma glucose in subjects who consumed different doses of MLAE with 75 g of maltose.

Parameter	iAUC	Excursion	t_{max}	C_{max}
Placebo (0 g MLAE)	4193 ± 594.4	86 ± 11.1[a]	48 ± 4.9	176 ± 11.4[a]
Low dose (1.25 g MLAE)	3268 ± 562.5	78 ± 9.0[a]	57 ± 5.4	164 ± 7.2[ab]
Intermediate dose (2.5 g MLAE)	2959 ± 330.6	49 ± 6.8[b]	51 ± 4.6	141 ± 7.4[bc]
High dose (5 g MLAE)	2773 ± 576.2	53 ± 5.3[b]	51 ± 4.6	129 ± 9.9[c]
Pretreatment with high dose	3502 ± 785.6	50 ± 8.8	66 ± 4.0*	140 ± 8.5

Values are expressed as mean ± SEM ($n = 10$ for each group).
Values within a column that do not share a common superscript letter differ significantly in comparisons made by ANOVA followed by Duncan's multiple range test ($P < 0.05$).
Values of the pretreatment group, which ingested MLAE 0.5 h prior to the intake of the maltose solution, were compared to those of the high dose group using the Student's *t*-test.
* $P < 0.05$.

uptake (Kwon et al., 2011). As mulberry leaf may contain dietary fibers, which are unabsorbable substances, it is reasonable to assume that they may act on the rate of gastric emptying and intestinal absorption (Katsube et al., 2010). Besides DNJ and soluble dietary fiber, flavonoids and related constituents found in mulberry leaf were also described for inhibiting glucose uptake as well as alpha-glucosidase acitivity. These flavonoids were reported chlorogenic acid, rutin and quercetin (Hunyadi, Martins, Hsieh, Seres, & Zupkó, 2012; Thabti, Elfalleh, Hannachi, Ferchichi, & Campos, 2012). Therefore, the lowering of the postprandial glucose response after loading with maltose might be attributed to the additive effect of inhibiting maltase and glucose absorption; however, we did not compare DNJ alone to mulberry extract in this study.

Miyahara, Miyazawa, Satoh, Sakai, and Mizusaki (2004) reported that mulberry leaf ethanol extract suppressed postprandial increases in blood glucose when given 30 min before carbohydrate administration. However, some others researchers have reported inhibitory effects of mulberry leaf extract when it is given simultaneously with sugars (Asai et al., 2011; Kimura et al., 2007; Nakamura et al., 2009). Our previous study also revealed that the pretreatment significantly inhibited glucose absorption compared to the simultaneous-treatment group in experiments in the Caco-2 cell line and in an animal model (Kwon et al., 2011). In contrast, our present results showed that there was no significant difference between pre- and simultaneous administration in humans. The animal and *in vitro* experiments in our previous studies confirmed the capacity for inhibiting glucose absorption only (Kwon et al., 2011), and Miyahara et al. did not compare pretreatment with simultaneous treatment (Miyahara et al., 2004). In this study, we used maltose as a loading sugar instead of glucose and compared the effective treatment time at a high dose of MLAE (5 g) to investigate the mechanism of action of DNJ. Our results demonstrate that postprandial glucose uptake was inhibited by the high dose of MLAE regardless of the intake time.

Some limitations of the present study must be noted. The present study was designed to test the time course and the dosage of MLAE necessary to establish postprandial effects after a single administration, so it is impossible to determine the long-term effects of MLAE. In addition, maltose is not a commonly used sugar. Therefore, larger prospective studies using commonly used sugar with a longer intervention period are needed in the near future. Although this study has a relatively small sample size, the results clearly demonstrate that a single administration of a MLAE may lower postprandial glucose responses. The results of the current investigation provide new insights into the hypoglycemic effect of MLAE in human subjects.

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