

# Hypoglycemic and antioxidant effects of Daraesoon (*Actinidia arguta* shoot) in animal models of diabetes mellitus

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**BACKGROUND/OBJECTIVES:** The primary objective of the treatment of diabetes mellitus is the attainment of glycemic control. Hyperglycemia increases oxidative stress which contributes to the progression of diabetic complications. Thus, the purpose of this study was to investigate the hypoglycemic and antioxidant effects of Daraesoon (*Actinidia arguta* shoot) in animal models of diabetes mellitus.

**MATERIALS/METHODS:** Rats with streptozotocin-induced diabetes received an oral administration of a starch solution (1 g/kg) either with or without a 70% ethanol extract of Daraesoon (400 mg/kg) or acarbose (40 mg/kg) after an overnight fast and their postprandial blood glucose levels were measured. Five-week-old C57BL/6J mice were fed either a basal or high-fat/high-sucrose (HFHS) diet with or without Daraesoon extract (0.4%) or acarbose (0.04%) for 12 weeks after 1 week of adaptation to determine the effects of the chronic consumption of Daraesoon on fasting hyperglycemia and antioxidant status.

**RESULTS:** Compared to the control group, rats that received Daraesoon extract (400 mg/kg) or acarbose (40 mg/kg) exhibited a significant reduction in the area under the postprandial glucose response curve after the oral ingestion of starch. Additionally, the long-term consumption of Daraesoon extract or acarbose significantly decreased serum glucose and insulin levels as well as small intestinal maltase activity in HFHS-fed mice. Furthermore, the consumption of Daraesoon extract significantly reduced thiobarbituric acid reactive substances and increased glutathione levels in the livers of HFHS-fed mice compared to HFHS-fed mice that did not ingest Daraesoon.

**CONCLUSIONS:** Daraesoon effectively suppressed postprandial hyperglycemia via the inhibition of  $\alpha$ -glucosidase in STZ-induced diabetic rats. Chronic consumption of Daraesoon alleviated fasting hyperglycemia and oxidative stress in mice fed a HFHS diet.

Nutrition Research and Practice 2015;9(3):262-267; doi:10.4162/nrp.2015.9.3.262; pISSN 1976-1457 eISSN 2005-6168

**Keywords:** *Actinidia arguta* shoot,  $\alpha$ -glucosidase, glucose, antioxidant, diabetes

## INTRODUCTION

Diabetes mellitus is a metabolic disease that manifests due to insulin insufficiency and/or insulin resistance and has become a serious health problem worldwide. In 2010, approximately 285 million adults between 20 and 79 years of age in the world were affected by diabetes and it is expected that 439 million adults will have diabetes by 2030 [1]. The primary goals for the management of diabetes include the tight regulation of glucose levels in the blood and the prevention of diabetic complications [2]. Hyperglycemic control is crucial for the prevention and delay of the progression of diabetic complications [3].

One approach used to control hyperglycemia in patients with diabetes is the use of agents such as acarbose, which inhibits  $\alpha$ -glucosidase activity and subsequently impairs the digestion of carbohydrates and delays glucose absorption after a meal [4].  $\alpha$ -Glucosidase inhibitors such as acarbose are prescribed as

an oral hypoglycemic agent for patients with type 2 diabetes [5].  $\alpha$ -Glucosidase inhibitors are also used to control hyperglycemia in conjunction with insulin therapy in type 1 diabetic patients [6]. However, acarbose can induce undesirable side effects such as flatulence, vomiting, and diarrhea [5]. Therefore, studies have focused on the identification of plants that may contain substances that inhibit  $\alpha$ -glucosidase activity to develop possible hypoglycemic agents and establish functional foods for patients with diabetes [7]. Among them, guava leaf showed  $\alpha$ -glucosidase inhibitory activity *in vitro* [8,9]. Consumption of guava leaf extract at 250 mg/kg effectively flattened postprandial glucose response in normal mice, demonstrating its inhibitory effect against  $\alpha$ -glucosidase *in vivo* [10]. Chronic consumption of guava leaf extract at 250 mg/kg alleviated fasting hyperglycemia in db/db mice, an animal model of type 2 diabetes [10]. Guava leaf polyphenol, polymerized polyphenols composed of ellagic acid, cyanidin, and other low-molecular-weight polyphenols,

This work was supported by the 2013 Inje University research grant.

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Received: January 19, 2015, Revised: February 11, 2015, Accepted: March 11, 2015

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has been identified as the active component responsible for  $\alpha$ -glucosidase inhibition [11]. Guava leaf extract has been accepted as health functional food in Korea [12].

In diabetic patients, hyperglycemia induces oxidative stress, which, in turn, plays a pathological role in the development and progression of diabetic complications, including macro- and microvascular symptoms [13]. Thus, the identification of natural sources of agents, such as edible plants, that can inhibit  $\alpha$ -glucosidase activity and act as antioxidants will aid in the development of beneficial dietary guidelines for diabetic patients as well as potential anti-diabetic agents with fewer side effects.

*Actinidia arguta* is a perennial vine that belongs to the family Actinidiaceae and bears grape-sized edible fruits (hardy kiwi-fruits) [14,15]. The shoot of *A. arguta* (Daraesoon) is a traditional Korean food; raw Daraesoon is collected only in the spring but it is difficult to store for a long period of time due to its high moisture content. Therefore, the majority of raw Daraesoon is blanched and then air-dried to make mucknamul, which can be stored for extended periods and is commercially available year round [16]. Mucknamul is soaked in water, boiled, and then re-soaked in cold water before being prepared as namul, a cooked Korean vegetable dish with seasonings.

The polyphenol fraction of *A. arguta* inhibits the activity of  $\alpha$ -glucosidase both *in vitro* and *in vivo* through the action of isoquercitrin, one of its active components [17]. Chronic consumption of isoquercitrin at 0.15% of the diet improved fasting hyperglycemia via  $\alpha$ -glucosidase inhibition in KK-A<sup>y</sup> mice, an animal model of type 2 diabetes [17]. The stem of *A. arguta*, which is not used as a food ingredient, inhibits  $\alpha$ -glucosidase and exerts antioxidant effect *in vitro* [18]. Other study has found that Daraesoon inhibits  $\alpha$ -glucosidase activity *in vitro* [19]. In addition, Daraesoon acts as a 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenger *in vitro* and is a good source of phenolic compounds [20]. Thus, Daraesoon has the potential to benefit patients with diabetes via its inhibition of  $\alpha$ -glucosidase and antioxidant effects, but these capabilities have yet to be fully characterized using an *in vivo* model. Therefore, the purpose of the present study was to investigate the hypoglycemic and antioxidant effects of Daraesoon in rodent models of diabetes mellitus. The effect of Daraesoon on postprandial hyperglycemia was determined in rats with streptozotocin (STZ)-induced diabetes. Previously, the extracts of guava leaf [9] and *Saururus chinensis* Baill with  $\alpha$ -glucosidase inhibitory activity *in vitro* administered at a dose of 500 mg/kg improved postprandial hyperglycemia in STZ-induced diabetic rats [21]. Oral administration of the extract of seed of *Holarrhena antidysenterica* (400 mg/kg) was reported to suppress the blood glucose response after carbohydrate loading in normal rats [22]. We examined the acute effect of Daraesoon extract (400 mg/kg) on blood glucose response after carbohydrate loading in STZ-induced diabetic rats and compared its effect with that of acarbose (40 mg/kg) to investigate the inhibitory effect of Daraesoon on  $\alpha$ -glucosidase *in vivo*. The effect of chronic consumption of Daraesoon on fasting hyperglycemia and oxidative status was determined in C57BL/6J mice fed the high-fat/high-sucrose (HFHS) diet, an animal model of type 2 diabetes that shows insulin resistance and hyperglycemia [23,24].

## MATERIALS AND METHODS

### *Preparation of the Daraesoon extract*

Daraesoon in the form of mucknamul was purchased from National Forestry Cooperative Federation in Yeosu, Korea in 2013. Daraesoon was prepared using a standard procedure prior to cooking, as described previously by Ahn *et al.* [16]. Briefly, the Daraesoon was soaked in cold water for 16 h, boiled in water for 30 min, re-soaked in cold water for 1 h, and then the excess water was removed by squeezing. The pretreated Daraesoon was freeze-dried, powdered, and then twice extracted using 20 volumes of 70% ethanol at room temperature over a 24-h period. The solution was filtered (Whatman No. 1 paper; GE Healthcare, UK) and the filtrate was removed with a rotary evaporator (Eyela; Tokyo, Japan).

### *Determination of total polyphenol content*

The total polyphenol content of the Daraesoon extract was measured by the method developed by Folin and Denis [25] using tannic acid as standard. Briefly, 1 mg of the extract was dissolved in 1 mL of methanol, and centrifuged at 1,500 g for 10 min. The supernatant (400  $\mu$ L) was mixed with 50% Folin-Ciocalteu's phenol reagent (200  $\mu$ L), and then incubated for 3 min. After the solution was mixed with 2% Na<sub>2</sub>CO<sub>3</sub> (400  $\mu$ L) and allowed to incubate for 60 min, the absorbance at 760 nm was measured.

### *Oral carbohydrate loading in diabetic rats*

Male Sprague-Dawley rats weighing 220-250 g (Bio Genomics, Inc.; Seoul, Korea) were fed with commercial chow (Samyang Co.; Korea) during an initial 2-week adaptation period. The animals were individually housed in a room maintained at 21  $\pm$  2°C and 55  $\pm$  5% relative humidity under a 12 : 12 h light : dark cycle. To induce diabetes, the rats were intraperitoneally injected with STZ (65 mg/kg) in a citrate buffer (pH 4.5). One week later, fasting blood samples were obtained from the tip of the tail and glucose levels were measured using a glucometer (Glucotrend, Roche Diagnostics; Lewes, UK). Rats with blood glucose levels between 200 and 400 mg/dL (n = 21) were included in the present study.

For the carbohydrate-loading test, the rats were administered soluble starch (1 g/kg body weight) with or without the extract of Daraesoon (400 mg/kg) or acarbose (40 mg/kg; Glucobay; Bayer Korea Ltd.; Seoul, Korea) by gastric intubation after an overnight fast. Blood samples were collected from the tip of the tail at 0, 30, 60, 120, 180, and 240 min after the administration of the starch. Glucose levels in the blood were measured using a glucometer (Glucotrend) and the incremental blood glucose level at each time point and area under the response curve (AUC) were calculated.

### *Assessments of fasting blood glucose levels and antioxidant status in mice with insulin resistance*

Five-week-old male C57BL/6J mice (n = 28) were obtained (Bio Genomics, Inc.; Seoul, Korea) and allowed a 1-week adaptation period. The mice were then randomly divided into four groups: the control group was fed a basal diet, the high-fat/high-sucrose (HFHS) group was fed a HFHS diet, the Daraesoon group was

fed a HFHS diet containing 0.4% Daraesoon extract, and the acarbose group was fed a HFHS diet containing 0.04% acarbose *ad libitum* for 12 weeks (Table 1). The basal diet contained 5.0% corn oil and 65.0% cornstarch. The HFHS diet was formulated based on previous studies showing that the long-term consumption of this diet resulted in the development of type 2 diabetes in C57BL/6J mice [23,24]. Consumption of HFHS diet containing 3.0% corn oil, 33.0% lard, 10.1% cornstarch, 27.0% sucrose, 5.0% AIN-76 mineral mixture, and 1.4% AIN-76 vitamin mixture for 12 weeks induced insulin resistance and hyperglycemia in these animals [24]. Following the 12th week from the initiation of their respective diets, the mice were sacrificed by a heart puncture procedure after an overnight fast and blood, small intestine, and liver samples were collected.

The blood was centrifuged at 1,500 g for 15 min and serum glucose levels were measured by enzymatic methods [26] using an assay kit (Yeongdong Co.; Seoul, Korea) while insulin levels were measured using an enzyme-linked immunosorbent assay (ELISA) kit (Mercodia; Uppsala, Sweden). The homeostasis model assessment for insulin resistance (HOMA-IR) index, a surrogate indicator of insulin resistance, was calculated as follows: fasting glucose (mmol/L) × fasting insulin (μU/mL)/22.5 [27].

After the duodenum was removed, the proximal one-third of the jejunum was cut longitudinally and the lumen was flushed out with ice-cold saline. The mucosa was scraped off with a glass slide, homogenized in four volumes of cold saline, and then centrifuged at 12,000 g for 30 min. The maltase activity of the supernatant was determined according to the method described by Dahlqvist [28] and the protein content was measured according to the method described by Lowry *et al.* [29]. One unit of maltase activity was defined as μmoles of malate substrate hydrolyzed per minute.

Lipid peroxidation in the liver was determined by measuring the production of thiobarbituric acid reactive substance (TBARS)

according to the method described by Ohkawa *et al.* [30]. Briefly, a portion of the liver tissue was homogenized in five volumes of 10 mM sodium phosphate buffer (pH 7.4), and the homogenate was mixed with a solution of 15% trichloroacetic acid (TCA), 0.4% thiobarbituric acid (TBA), and 2.5% HCl. After being heated at 100°C for 45 min, the reaction mixture was cooled on ice and centrifuged at 1,500 g for 15 min. The absorbance of the supernatant was measured at 532 nm and the results were expressed as nmol of malondialdehyde (MDA)/mg protein. Glutathione (GSH) levels in the liver were measured according to the method described by Ellman [31] and expressed as nmol/mg protein. Briefly, the liver homogenates were prepared with nine volumes of 0.1 mM phosphate buffer (pH 7.4) and centrifuged at 10,000 g for 30 min at 4°C. The supernatant was incubated with 10 mM 5,5-dithiobis-2-nitrobenzoic acid (DTNB) in 0.1 M phosphate buffer (pH 8.0) for 15 min and the absorbance was measured at 534 nm. The protein content of the liver was measured according to the method described by Lowry *et al.* [29]. All assays were performed in triplicate. All animal experimental procedures were carried out in accordance with the guidelines of animal experimentation as approved by the Animal Resource Center at Inje University, Korea (Approval no. 2013-43 and 2014-18).

#### Statistical analysis

All results were expressed as the mean ± standard error of the mean (SEM). Differences among the groups were assessed by a one-way analysis of variance (ANOVA) followed by a post hoc Tukey test ( $P < 0.05$ ) using the SAS software (ver. 9.2, SAS Institute Inc.; Cary, NC, USA).

## RESULTS

#### Total polyphenol content of the Daraesoon extract

The extraction yield of the Daraesoon extract was 8.1%. The total polyphenol content of the Daraesoon extract was  $99.7 \pm 1.0$  mg tannic acid equivalent/g extract.

#### Effects of Daraesoon on postprandial hyperglycemia in carbohydrate-loaded diabetic rats

The changes in blood glucose levels at each timepoint after a single oral administration of only starch (control group), starch with Daraesoon extract (400 mg/kg), or starch with acarbose (40 mg/kg) in STZ-induced diabetic rats are provided in Fig. 1. The consumption of Daraesoon resulted in a significant reduction of blood glucose levels at 30, 60, and 120 min ( $P < 0.05$ ) compared to the control group. The blood glucose levels of the acarbose group exhibited significant decreases at 30, 60, 120, and 180 min ( $P < 0.01$ ) compared to the control group, but these values did not differ significantly from those of the Daraesoon group. Additionally, the AUCs for the glucose responses were significantly suppressed in the Daraesoon and acarbose groups compared to the control group ( $P < 0.01$ ; Fig. 1).

#### Effects of Daraesoon on fasting hyperglycemia and oxidative stress in diet-induced diabetic mice

The C57BL/6J mice that were fed a HFHS diet for 12 weeks exhibited significantly increased final body weight, enhanced

**Table 1.** Composition of the experimental diets

Ingredient	Group			
	Control	HFHS <sup>1)</sup>	Daraesoon <sup>2)</sup>	Acarbose <sup>3)</sup>
	(%)			
Casein	20.0	20.0	20.0	20.0
Corn starch	65.0	10.1	9.7	10.06
Sucrose	-	27.0	27.0	27.0
Cellulose	5.0	-	-	-
Corn oil	5.0	3.0	3.0	3.0
Lard	-	33.0	33.0	33.0
Vitamin mixture <sup>4)</sup>	1.0	1.4	1.4	1.4
Mineral mixture <sup>5)</sup>	3.5	5.0	5.0	5.0
D,L-Methionine	0.3	0.3	0.3	0.3
Choline bitartrate	0.2	0.2	0.2	0.2
<i>Tert</i> -butyl hydroquinone <sup>6)</sup>	0.001	0.007	0.007	0.007
Daraesoon extract			0.4	-
Acarbose	-	-	-	0.04

<sup>1)</sup> Mice fed a high-fat/high-sucrose diet

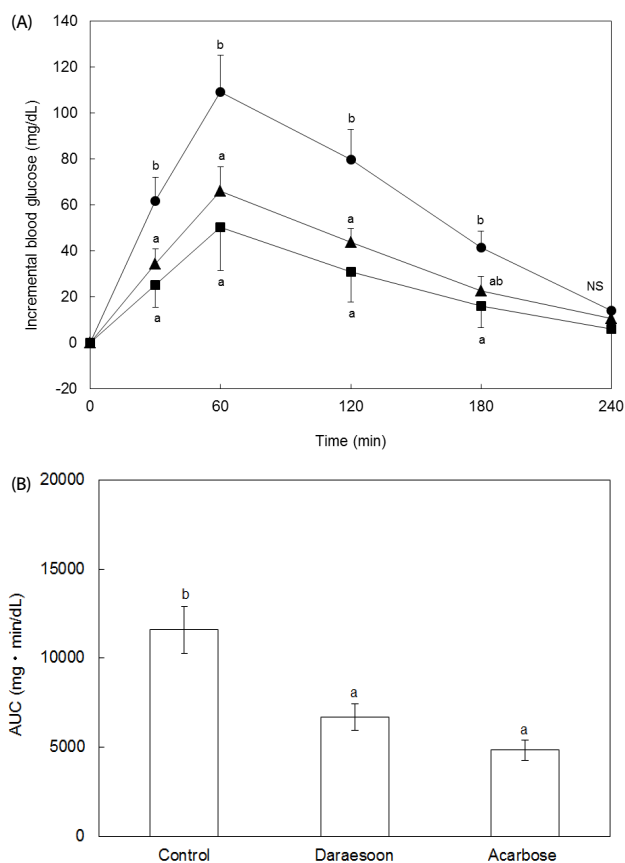
<sup>2)</sup> Mice fed the HFHS diet containing 0.4% Daraesoon extract

<sup>3)</sup> Mice fed the HFHS diet containing 0.04% acarbose

<sup>4)</sup> AIN-76 vitamin mixture

<sup>5)</sup> AIN-76 mineral mixture

<sup>6)</sup> Antioxidative agent, 0.01 g/50 g lipids



**Fig. 1.** Incremental blood glucose levels (A) and area under the glucose response curve (B) of STZ-induced diabetic rats. Control group (●): Starch (1 g/kg) was administered orally to STZ-induced diabetic rats after an overnight fast, Daraesoon group (▲): Starch (1 g/kg) with 70% ethanol extract of Daraesoon (400 mg/kg) was administered orally to rats after an overnight fast, Acarbose group (■): Starch (1 g/kg) plus acarbose (40 mg/kg) was administered orally to rats after an overnight fast. Values represent means ± SEM (n = 7). Means that do not share a common letter are significantly different at \*  $P < 0,05$  or \*\*  $P < 0,01$ .

**Table 2.** Body weight, food intake, and feed efficiency ratio in mice fed the experimental diets

Group <sup>1)</sup>	Control	HFHS	Daraesoon	Acarbose
Initial body weight (g)	19.5 ± 0.6 <sup>NS3/4)</sup>	19.3 ± 0.6	19.4 ± 0.7	19.5 ± 0.7
Final body weight (g)	26.8 ± 0.8 <sup>a</sup>	40.2 ± 1.1 <sup>b</sup>	39.7 ± 1.3 <sup>b</sup>	39.0 ± 1.5 <sup>b</sup>
Weight gain (mg/day)	87.4 ± 4.3 <sup>a</sup>	249.3 ± 9.7 <sup>b</sup>	241.3 ± 9.3 <sup>b</sup>	232.7 ± 11.5 <sup>b</sup>
Food intake (g/day)	3.38 ± 0.14 <sup>NS</sup>	3.09 ± 0.10	2.97 ± 0.10	3.02 ± 0.12
FER <sup>2)</sup>	2.63 ± 0.20 <sup>a</sup>	8.11 ± 0.32 <sup>b</sup>	8.17 ± 0.36 <sup>b</sup>	7.79 ± 0.48 <sup>b</sup>

<sup>1)</sup> Control, mice fed the basal diet; HFHS, mice fed a high-fat/high-sucrose (HFHS) diet; Daraesoon, mice fed the HFHS diet containing 0,4% Daraesoon extract; Acarbose, mice fed the HFHS diet containing 0,04% acarbose

<sup>2)</sup> Feed efficiency ratio (FER, %) = (Body weight gain (g/day)/food intake (g/day)) × 100

<sup>3)</sup> Values are means ± SEM (n = 7). Means in the same row not sharing a common letter are significantly different at  $P < 0,01$ .

<sup>4)</sup> NS, not significant

weight gain, and heightened feed efficiency ratios (FERs) compared to the control group ( $P < 0,01$ ; Table 2). However, there were no significant differences in final body weight, weight gain, food intakes, or the FERs among the HFHS, Daraesoon, and acarbose groups.

**Table 3.** Serum glucose, insulin, and HOMA-IR values in mice fed the experimental diets

Group <sup>1)</sup>	Control	HFHS	Daraesoon	Acarbose
Glucose (mg/dL)	122.3 ± 6.0 <sup>a3)</sup>	162.1 ± 7.3 <sup>b</sup>	136.0 ± 5.3 <sup>a</sup>	128.1 ± 6.2 <sup>a</sup>
Insulin (μU/mL)	17.5 ± 0.9 <sup>a</sup>	36.9 ± 2.3 <sup>c</sup>	27.4 ± 2.6 <sup>b</sup>	26.1 ± 2.0 <sup>b</sup>
HOMA-IR <sup>2)</sup>	5.2 ± 0.2 <sup>a</sup>	14.7 ± 1.1 <sup>c</sup>	9.2 ± 0.8 <sup>b</sup>	8.2 ± 0.7 <sup>b</sup>

<sup>1)</sup> Control, mice fed the basal diet; HFHS, mice fed a high-fat/high-sucrose (HFHS) diet; Daraesoon, mice fed the HFHS diet containing 0,4% Daraesoon extract; Acarbose, mice fed the HFHS diet containing 0,04% acarbose

<sup>2)</sup> Homeostasis model assessment for insulin resistance (HOMA-IR) = Fasting glucose (mmol/L) × fasting insulin (μU/mL)/22,5

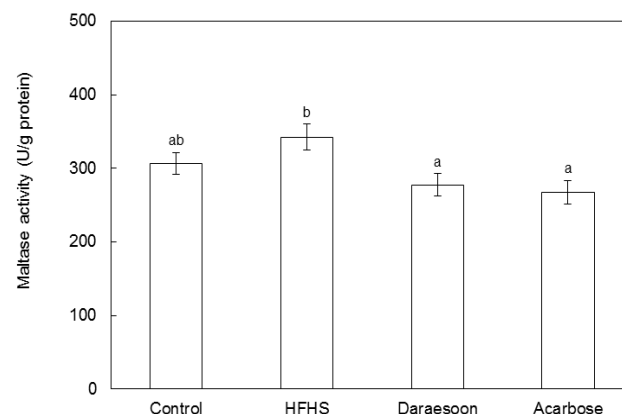
<sup>3)</sup> Values are means ± SEM (n = 7). Means in the same row not sharing a common letter are significantly different at  $P < 0,05$ .

**Table 4.** Lipid peroxide and glutathione levels of the liver in mice fed the experimental diets

Group <sup>1)</sup>	Control	HFHS	Daraesoon	Acarbose
TBARS (nmol MDA/mg protein)	0.635 ± 0.039 <sup>a2)</sup>	0.849 ± 0.052 <sup>b</sup>	0.649 ± 0.042 <sup>a</sup>	0.832 ± 0.048 <sup>b</sup>
GSH (nmol/mg protein)	25.9 ± 1.3 <sup>b</sup>	19.5 ± 1.2 <sup>a</sup>	24.4 ± 1.0 <sup>b</sup>	19.8 ± 0.8 <sup>a</sup>

<sup>1)</sup> Control, mice fed the basal diet; HFHS, mice fed a high-fat/high-sucrose (HFHS) diet; Daraesoon, mice fed the HFHS diet containing 0,4% Daraesoon extract; Acarbose, mice fed the HFHS diet containing 0,04% acarbose

<sup>2)</sup> Values are means ± SEM (n = 7). Means in the same row not sharing a common letter are significantly different at  $P < 0,05$ .



**Fig. 2.** Maltase activity in the small intestine in mice fed the experimental diets. Control, mice fed the basal diet; HFHS, mice fed a high-fat/high-sucrose (HFHS) diet; Daraesoon, mice fed the HFHS diet containing 0,4% Daraesoon extract; Acarbose, mice fed the HFHS diet containing 0,04% acarbose. Values represent means ± SEM (n = 7). Bars that do not share a common letter are significantly different at  $P < 0,05$ .

The serum glucose and insulin levels and HOMA-IR values were significantly elevated in the HFHS group compared with the control group ( $P < 0,05$ ; Table 3). However, the mice that consumed either Daraesoon or acarbose along with the HFHS diet showed significant reductions in insulin levels and HOMA-IR values compared with the mice that were fed a HFHS diet ( $P < 0,05$ ). Additionally, compared to the HFHS group, there were significant decreases in the serum glucose levels of the Daraesoon and acarbose groups to levels that approached those of the control group. The maltase activities of the Daraesoon and acarbose groups exhibited significant reductions compared to the HFHS group ( $P < 0,05$ ) but these values did not differ significantly from those of the control group (Fig. 2).

The mice that were fed a HFHS diet exhibited significantly

increased hepatic TBARS levels and decreased GSH concentrations compared with the mice that were fed a basal diet ( $P < 0.05$ ; Table 4). The Daraesoon group had significantly lower TBARS levels and significantly higher GSH levels than the HFHS and acarbose groups ( $P < 0.05$ ), but these levels were not significantly different from the control group.

## DISCUSSION

The ingestion of a 70% ethanol extract of Daraesoon (400 mg/kg) effectively flattened the increase in postprandial blood glucose after starch-loading and reduced the AUC in STZ-induced diabetic rats; the hypoglycemic effects of Daraesoon were as effective as those of acarbose (40 mg/kg). Because  $\alpha$ -glucosidase in the small intestinal brush-border catalyzes the final step of digestion of carbohydrates,  $\alpha$ -glucosidase inhibitors can attenuate postprandial hyperglycemia [4]. Control of both fasting and postprandial hyperglycemia is crucial in achieving overall glycemic control [32]. Daraesoon strongly inhibits  $\alpha$ -glucosidase activity *in vitro* via its active compound, pinorensin diglucoside [19]. Thus, Daraesoon may be beneficial in management of diabetes by exerting  $\alpha$ -glucosidase inhibitory activity.

The chronic consumption of a HFHS diet induces obesity and causes the downregulation of insulin signaling which results in insulin resistance and type 2 diabetes in C57BL/6J mice [33]. Mice that were fed a HFHS diet in the present study exhibited obesity, hyperinsulinemia, and hyperglycemia, which agree with the findings of previous studies [23,24,33]. However, the consumption of Daraesoon extract (0.4%) or acarbose (0.04%) reduced the serum insulin levels and HOMA-IR values of mice fed a HFHS diet; this is indicative of improved insulin resistance. In the present study, the average daily intakes of Daraesoon extract and acarbose in the Daraesoon and acarbose groups were 404.3 and 41.8 mg/kg, respectively. The Daraesoon extract also reduced fasting serum glucose levels in mice with HFHS diet-induced diabetes to levels similar to those of the control group. Importantly, the ameliorating effect of Daraesoon (0.4%) on fasting hyperglycemia was as effective as those of acarbose (0.04%). The consumption of Daraesoon or acarbose also decreased maltase activity in the small intestine. Liu *et al.* reported that STZ-induced diabetic rats exhibited reductions in small intestinal  $\alpha$ -glucosidase activity following the chronic consumption of acarbose [34]. They suggested decreased activities of disaccharidases could contribute to a decrease of fasting blood glucose in these animals.

Acarbose decreases the demand for insulin by delaying the rise in blood glucose levels after a meal and the attenuated postprandial glucose and insulin responses are thought to improve insulin sensitivity [35]. The chronic consumption of an  $\alpha$ -glucosidase inhibitor improves insulin sensitivity in an animal model of diabetes [36] and patients with diabetes [37]. Therefore, in the present study, Daraesoon could have improved *in vivo* insulin resistance and fasting hyperglycemia via the inhibition of  $\alpha$ -glucosidase activity.

The mice that were fed a HFHS diet exhibited elevations of lipid peroxide levels in the liver compared with the control group but Daraesoon reduced hepatic TBARS to levels comparable with those of the control group. Daraesoon has

been shown to effectively scavenge DPPH free radicals *in vitro* [20] and the Daraesoon extract exerted antioxidant effects in the present *in vivo* investigation. Additionally, although there was a reduction in hepatic GSH levels due to the ingestion of a HFHS diet, they were restored to values comparable to non-diabetic mice by the consumption of Daraesoon extract. GSH is thiol-containing tripeptide that can protect the body against reactive oxygen species (ROS) [38]. Daraesoon extract contained total polyphenol at 99.7 mg tannic acid equivalent/g. Polyphenols are strong antioxidant agents which can directly scavenge free radicals and induce antioxidant enzyme systems [39]. Antioxidant effect of Daraesoon could have been mediated by polyphenols in this study. Moskaug *et al.* reported that dietary plant polyphenols, particularly flavonoids, can induce the expression of  $\gamma$ -glutamylcysteine synthetase ( $\gamma$ GCSH), which is the rate-limiting enzyme in the synthesis of GSH [40]. Daraesoon is a good source of total polyphenols and flavonoids [20] and, thus, Daraesoon may increase GSH levels via the induction of  $\gamma$ GCSH. Future studies investigating the effects of Daraesoon on the gene expression of  $\gamma$ GCSH could be necessary.

In the present study, Daraesoon improved postprandial hyperglycemia via the inhibition of  $\alpha$ -glucosidase in STZ-induced diabetic rats. In addition, chronic consumption of Daraesoon alleviated fasting hyperglycemia and oxidative stress in mice fed a HFHS diet. Thus, Daraesoon could be considered a potential anti-diabetic agent.

## REFERENCES

1. Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Res Clin Pract* 2010;87:4-14.
2. Rahati S, Shahraki M, Arjomand G, Shahraki T. Food pattern, lifestyle and diabetes mellitus. *Int J High Risk Behav Addict* 2014;3:e8725.
3. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. The Diabetes Control and Complications Trial Research Group. *N Engl J Med* 1993;329:977-86.
4. Standl E, Baumgartl HJ, Fuchtenbusch M, Stempler J. Effect of acarbose on additional insulin therapy in type 2 diabetic patients with late failure of sulphonylurea therapy. *Diabetes Obes Metab* 1999;1:215-20.
5. Hanefeld M. The role of acarbose in the treatment of non-insulin-dependent diabetes mellitus. *J Diabetes Complications* 1998;12:228-37.
6. Raptis SA, Dimitriadis GD. Oral hypoglycemic agents: insulin secretagogues, alpha-glucosidase inhibitors and insulin sensitizers. *Exp Clin Endocrinol Diabetes* 2001;109 Suppl 2:S265-87.
7. Kumar S, Narwal S, Kumar V, Prakash O.  $\alpha$ -Glucosidase inhibitors from plants: a natural approach to treat diabetes. *Pharmacogn Rev* 2011;5:19-29.
8. Wang H, Du YJ, Song HC.  $\alpha$ -Glucosidase and  $\alpha$ -amylase inhibitory activities of guava leaves. *Food Chem* 2010;123:6-13.
9. Mai TT, Chuyen NV. Anti-hyperglycemic activity of an aqueous extract from flower buds of *Cleistocalyx operculatus* (Roxb.) Merr and Perry. *Biosci Biotechnol Biochem* 2007;71:69-76.
10. Deguchi Y, Osada K, Uchida K, Kimura H, Yoshikawa M, Kudo T, Yasui H, Watanuki M. Effects of extract of guava leaves on the development of diabetes in the db/db mouse and on the postprandial

- randial blood glucose of human subjects. *Nippon Nogeikagaku Kaishi* 1998;72:923-31.
11. Deguchi Y. Effect of guava tea on postprandial blood glucose and diabetes. *Assoc J Jpn Soc Med Use Funct Foods* 2006;3:439-45.
  12. Ministry of Food and Drug Safety (KR). Health functional food [Internet]. Cheongju: Ministry of Food and Drug Safety; 2014 [cited 2014 November 7]. Available from: <http://www.foodnara.go.kr/hfoodi/>.
  13. Kaneto H, Nakatani Y, Kawamori D, Miyatsuka T, Matsuoka TA, Matsuhisa M, Yamasaki Y. Role of oxidative stress, endoplasmic reticulum stress, and c-Jun N-terminal kinase in pancreatic  $\beta$ -cell dysfunction and insulin resistance. *Int J Biochem Cell Biol* 2005;37: 1595-608.
  14. Nishiyama I. Fruits of the actinidia genus. *Adv Food Nutr Res* 2007;52:293-324.
  15. Williams MH, Boyd LM, McNeillage MA, MacRae EA, Ferguson AR, Beatson RA, Martin PJ. Development and commercialization of 'Baby Kiwi' (*Actinidia arguta* planch.). *Acta Hort* 2003;610:81-6.
  16. Ahn H, Kim J, Kim J, Auh J, Choe E. In vitro  $\alpha$ -glucosidase and pancreatic lipase inhibitory activities and antioxidants of Samnamul (*Aruncus dioicus*) during rehydration and cooking. *Food Sci Biotechnol* 2014;23:1287-93.
  17. Kurakane S, Yamada N, Sato H, Igarashi K. Anti-diabetic effects of *Actinidia arguta* polyphenols on rats and KK-Ay mice. *Food Sci Technol Res* 2011;17:93-102.
  18. Lee J, Sowndhararajan K, Kim M, Kim J, Kim D, Kim S, Kim GY, Kim S, Jhoo JW. Antioxidant, inhibition of  $\alpha$ -glucosidase and suppression of nitric oxide production in LPS-induced murine macrophages by different fractions of *Actinidia arguta* stem. *Saudi J Biol Sci* 2014;21:532-8.
  19. Kwon D, Kim GD, Kang W, Park JE, Kim SH, Choe E, Kim Ji, Auh JH. Pinoreosinol diglucoside is screened as a putative  $\alpha$ -glucosidase inhibiting compound in *Actinidia arguta* leaves. *J Korean Soc Appl Biol Chem* 2014;57:473-9.
  20. Kwak CS, Lee JH. In vitro antioxidant and anti-inflammatory effects of ethanol extracts from sprout of evening primrose (*Oenothera lacinata*) and gooseberry (*Actinidia arguta*). *J Korean Soc Food Sci Nutr* 2014;43:207-15.
  21. Joo HJ, Kang MJ, Seo TJ, Kim HA, Yoo SJ, Lee SK, Lim HJ, Byun BH, Kim Ji. The hypoglycemic effect of *Saururus chinensis* Baill in animal models of diabetes mellitus. *Food Sci Biotechnol* 2006;15: 413-7.
  22. Ali KM, Chatterjee K, De D, Jana K, Bera TK, Ghosh D. Inhibitory effect of hydro-methanolic extract of seed of *Holarrhena antidysenterica* on  $\alpha$ -glucosidase activity and postprandial blood glucose level in normoglycemic rat. *J Ethnopharmacol* 2011;135:194-6.
  23. Surwit RS, Kuhn CM, Cochrane C, McCubbin JA, Feinglos MN. Diet-induced type II diabetes in C57BL/6J mice. *Diabetes* 1988;37: 1163-7.
  24. Choi HN, Kang MJ, Lee SJ, Kim Ji. Ameliorative effect of myricetin on insulin resistance in mice fed a high-fat, high-sucrose diet. *Nutr Res Pract* 2014;8:544-9.
  25. Folin O, Denis W. On phosphotungstic-phosphomolybdc compounds as color reagents. *J Biol Chem* 1912;12:239-43.
  26. Raabo E, Terkildsen TC. On the enzymatic determination of blood glucose. *Scand J Clin Lab Invest* 1960;12:402-7.
  27. Haffner SM, Miettinen H, Stern MP. The homeostasis model in the San Antonio Heart Study. *Diabetes Care* 1997;20:1087-92.
  28. Dahlqvist A. Assay of intestinal disaccharidases. *Scand J Clin Lab Invest* 1984;44:169-72.
  29. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951;193:265-75.
  30. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 1979;95:351-8.
  31. Ellman GL. Tissue sulfhydryl groups. *Arch Biochem Biophys* 1959;82: 70-7.
  32. Woerle HJ, Neumann C, Zschau S, Tenner S, Irsigler A, Schirra J, Gerich JE, Göke B. Impact of fasting and postprandial glycemia on overall glycemic control in type 2 diabetes: Importance of postprandial glycemia to achieve target HbA1c levels. *Diabetes Res Clin Pract* 2007;77:280-5.
  33. Yang ZH, Miyahara H, Takeo J, Katayama M. Diet high in fat and sucrose induces rapid onset of obesity-related metabolic syndrome partly through rapid response of genes involved in lipogenesis, insulin signalling and inflammation in mice. *Diabetol Metab Syndr* 2012;4:32.
  34. Liu L, Yu YL, Yang JS, Li Y, Liu YW, Liang Y, Liu XD, Xie L, Wang GJ. Berberine suppresses intestinal disaccharidases with beneficial metabolic effects in diabetic states, evidences from in vivo and in vitro study. *Naunyn Schmiedebergs Arch Pharmacol* 2010;381:371-81.
  35. Carrascosa JM, Molero JC, Fermín Y, Martínez C, Andrés A, Satrustegui J. Effects of chronic treatment with acarbose on glucose and lipid metabolism in obese diabetic Wistar rats. *Diabetes Obes Metab* 2001;3:240-8.
  36. Fujita H, Yamagami T. Fermented soybean-derived Touchi-extract with anti-diabetic effect via  $\alpha$ -glucosidase inhibitory action in a long-term administration study with KKAy mice. *Life Sci* 2001; 70:219-27.
  37. Meneilly GS, Ryan EA, Radziuk J, Lau DC, Yale JF, Morais J, Chiasson JL, Rabasa-Lhoret R, Maheux P, Tessier D, Wolever T, Josse RG, Elahi D. Effect of acarbose on insulin sensitivity in elderly patients with diabetes. *Diabetes Care* 2000;23:1162-7.
  38. Townsend DM, Tew KD, Tapiero H. The importance of glutathione in human disease. *Biomed Pharmacother* 2003;57:145-55.
  39. Stevenson DE, Hurst RD. Polyphenolic phytochemicals-just antioxidants or much more? *Cell Mol Life Sci* 2007;64:2900-16.
  40. Moskaug JØ, Carlsen H, Myhrstad MC, Blomhoff R. Polyphenols and glutathione synthesis regulation. *Am J Clin Nutr* 2005;81:2775-83S.