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Effects of Silkworm Extract on Disaccharidase Activities of Small Intestine and Blood Glucose-Lowering in C57BL/6J Mice

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Abstract

This study examined the anti-diabetic effect of a silkworm extract in C57BL/6J mice, an ob/db model, fed a high fat diet for 8 weeks. The body weight was not significantly different with the silkworm-extract supplement, nor did food intake and body weight gains also did not differ significantly among the high-fat diet groups. However, the water intake by the silkworm-extract supplemented groups increased significantly compared with that by the distilled-water supplement group, nonetheless, the FER did not differ significantly. For all groups, the blood glucose increased the most after 30 minutes and yet returned to a fasting level within 90 minutes. The fasting time and resulting glucose tolerance for the silkworm-extract supplemented groups were significantly decreased compared to that for the high fat diet with distilled water supplement group, while the level of blood glucose in silkworm-extract supplemented groups was significantly decreased compared with than in the diabetic control group. The HbA1c and insulin levels were no different among the groups. The sucrase and lactase activities in the proximal small intestine were significantly decreased in the silkworm-extract supplement groups compared to that in the diabetic control group. There was no significant difference in the glycogen contents in the liver and muscle among the groups. In conclusion, it was found that the silkworm-extract supplement repressed the disaccharidase activity in the small intestines mucosa of the C57BL/6J mice.

Key words: C57BL/6J mice, silkworm, blood glucose, disaccharidase activities

INTRODUCTION

Due to recent dietary changes involving a higher fat intake, there has been a rapid increase in the occurrence of many chronic diseases, such as obesity, hyperlipidemia, atherosclerosis, diabetes, and hypertension. (1,2) Among these diseases, diabetes mellitus is ranked 4th as a cause of death, and more than 90% of diabetic patients have type-2 diabetes. The cause of type-2 diabetes, which usually occurs in developed countries, is long-term obesity and is characterized as hyperinsulinemia in relation to insulin resistence. (3-5) If hyperglycemia is not controlled, several complications can arise, including neuropathy, hypertension, and atherosclerosis, due to non-enzymatic glucose combined with cellular protein. (6) Therefore, the level of blood glucose must be controlled to prevent hypertension and diabetic complications. (7) Recently, various studies have focused on the development of anti-diabetic functional foods utilizing natural phytotherapeutic agents from mulberry leaves (8, 9), mulberry fruit (10), green tea catechin (11,12), *Alisma canaliculatum* (13), *Polygonatum odoranum* (14), sea-tangle (15), and *Platycodon grandiflorum* (16). In addition, silkworms have also received some attention.

The silkworm is the larva of the silkworm moth, as registered in Shin-nong medical herbs phytology, but recent studies on silkworms have discovered the presence of deoxynojirimycin (DNJ) (17-19), which is known to be a blood glucose-lowering substance and powerful competitive-glucosidase inhibitor (18). Clinical studies have reported that silkworms have a hypoglycemic effect in patients with hepatitis and hardened livers, lowering blood glucose by 29% and 62%, respectively (19). However, despite previous studies related to the hypoglycemic effect of silkworms on type-1 diabetes, there has been no study on the anti-diabetic effect in an obesity/diabetic type-2 model. Therefore, the current study investigated the effects of a silkworm extract on the disaccharidase activities in the small intestine and blood glucose level in C57BL/6J mice high-fat diet induced diabetes.

MATERIALS AND METHODS

Preparation of plant materials

The silkworms (bombyx mori) used in this experiment were grown in the fields of the Youngcheon

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Silkworm Culture Agricultural Co-operative Association, Youngcheon, Korea. Silkworms which were aged five years were freeze-dried for three days, pulverized, measured according to concentrations, brought to a 1 L volume in water and steeped at 80°C for 1 hour. The resulting extracts were filtered, concentrated under a vacuum at 60°C and stored at 4°C until used. They were divided into difference quantity and mixed to be offered each groups *ad libitum*.

Experimental animals and diets

Four-week old male C57BL/6J mice were purchased from Bio Genomics (Seoul, Korea). The animals were maintained on a chow diet (Jeil-jedang, Suwon, Korea) for 1 week (17.2 ± 0.4 g) and then randomly divided into one diabetic control group and three high fat diet groups given silkworm extract in their drinking water (n=20). The four groups were fed experimental diets for 8 weeks and the diabetic groups were classified to a high fat diet with distilled water supplement group (D-C group), high fat diet with 0.125% silkworm extract supplement group (D-SL group), high fat diet with 0.25% silkworm extract supplement group (D-SM group), high fat diet with 0.5% silkworm extract supplement group (D-SH group), according to the level of silkworm extract supplemented (Table 1). Research Diets (New Brunswick, NJ) manufactured the diets (60% fat kcal) were used. The animals were housed in a temperature-controlled environment with a 12 hour light/dark cycle.

Table 1. Classification of experimental diet (g/kg diet)

Ingredient	High fat diet[1]			
	D-C[2]	D-SL[3]	D-SM[4]	D-SH[5]
Casein	20.00	20.00	20.00	20.00
L-Cystine	0.30	0.30	0.30	0.30
Corn starch	-	-	-	-
Maltodextrin	12.5	12.5	12.5	12.5
Sucrose	6.88	6.88	6.88	6.88
Cellulose	5.00	5.00	5.00	5.00
Soybean oil	2.50	2.50	2.50	2.50
Lard	24.50	24.50	24.50	24.50
Mineral mix	1.00	1.00	1.00	1.00
Dicalcium phosphate	1.30	1.30	1.30	1.30
Calcium carbonate	0.55	0.55	0.55	0.55
Porassium citrate	1.65	1.65	1.65	1.65
Vitamin mixture	1.00	1.00	1.00	1.00
Choline bitartrate	0.20	0.20	0.20	0.20
Total (%)	100	100	100	100
Administration of silkworm extracts		0.125%	0.250%	0.500%

[1]AIN-93 diet (60% fat kcal).
[2]D-C: mice were fed with high-fat diet and distill water.
[3]D-SL: mice were fed with high-fat diet and 0.125% silkworm extract water.
[4]D-SM: mice were fed with high-fat diet and 0.25% silkworm extract water.
[5]D-SH: mice were fed with high-fat diet and 0.5% silkworm extract water.

Sample collection and preparation

Food was removed 12 hours before sacrificing. After blood was drawn from the orbital vein and allowed to coagulate for 30 minutes at room temperature, serum was obtained by centrifuging at 1,500 × g for 15 minutes to use measurement of blood glucose and insulin level. The serum was stored at 80°C after being fast frozen. The livers, small intestine and muscle were excised, and washed in 9 g/L of NaCl. The liver and muscle were frozen rapidly in lipid nitrogen and stored at -80°C until used.

Oral glucose tolerance test

After 8 weeks the mice were fasted for 3 hours and initial blood glucose assayed in blood drawn from the tail veins. A 50% glucose solution (0.1 g glucose/100 g b.w.) was orally administered to mice using a metal gavage tube. Blood glucose levels which extracted from blood on vein of tail were determined on 30, 60, 90 and 120 minutes using a blood glucose test meter (Acctrend GC, Boehringer Mannheim, Germany).

Measurement of blood glucose

Blood glucose concentrations in the mouse were measured after a 12-hr fast using the enzymatic kit AM 201K (Asan Co., Korea) and absorbance was read at 500 nm.

Measurement of HbA1c

HbA1c concentrations in mice were measured after a 12-hr fast using the Helena laboratories kit by micro-column chromatography.

Measurement of insulin

Insulin levels were measured after a 12-hr fast using the ^{125}I-labeled insulin RIA (Mediagnost, USA) and a Gamma Scinitillation Counter.

Measurement of maltase, sucrase and lactase activities in intestinal mucosa

The activities of maltase, sucrase and lactase were measured according to Dahlqvist method (22). The small intestine was excised and washed in 9 g/L of NaCl on ice in order to remove the duodenum. After being washed clearly with 9 g/L of NaCl, the organs were kept in cold storage after being cut open. The duodenums were divided into proximal, middle and distal section as three equal parts, and then water was removed by a cheese cloth. The mucosa was scraped off with a glass microscopic slide and weighed. The mucosa was then homogenized with four volumes of cold distilled water. The 0.1 mL of diluted enzymes was mixed with 0.056 M a disaccharide solution/0.1 M sodium malate buffer

(pH 6.0) 0.1 mL, and incubated at 37°C for 60 min. The solutions were then cooled in tap water after being added to 0.8 mL of distilled water and incubated in boiling water for 2 min. A 0.5 mL aliquot of the test sample was then added to 3 mL of glucose oxidase. It was vortexed and incubated at 37°C for 60 min and absorbance was read at 420 nm. The protein contents of the intestinal mucosa were measured with the standard substance of bovine serum albumin according to Lowry et al. method (23).

Measurement of glycogen content in the liver and muscle

The glycogen contents of liver and muscle were determined according to the method of Lo et al. (24). About 70~80 mg of the muscle or 10~20 mg of the liver abrasion filled with liquid nitrogen in poison cup and added to the 1 mL of KOH (30%). After mixing it was incubated at 100°C for 30 min. It was then left for more than 12 hours at 0~5°C after mixed with 1.5 mL ethanol (95%). After it was centrifuged at 3,000 rpm for 25~30 min, 350 μL of the resultant was put into the mixture of distilled water, 0.5 mL of 5% phenol, and 2.5 mL of Na_2SO_4 after being mixed with 3 mL of distilled water. The absorbance was measured at 490~492 nm.

Statistical analysis

All data were assessed by analysis of variance (ANOVA). If significance was found by ANOVA, comparisons among group means were made by Tukey's Honestly Significant Difference test (25).

RESULTS

Food and water intake, body gains and food efficiency ratio

Food and water intakes and FER are shown in Table 2 and body weight gains in Fig. 1. The body weight gains during 8 weeks for induced ob/db C57BL/6J mice which fed a high fat diet (60% fat kcal) were not significant difference among the experimental groups (Fig. 1). There was also no significant difference in the food intakes between the groups. However, the water intake of the silkworm-extract supplemented groups was significantly higher than that in the distilled-water supplement group. The FER were not significantly different.

Oral glucose tolerance test (OGTT)

The fasting time and resulting glucose tolerance (Fig. 2) in the silkworm-extract supplemented groups were significantly decreased compared with those in the D-C group. For all groups, the glucose level was highest after

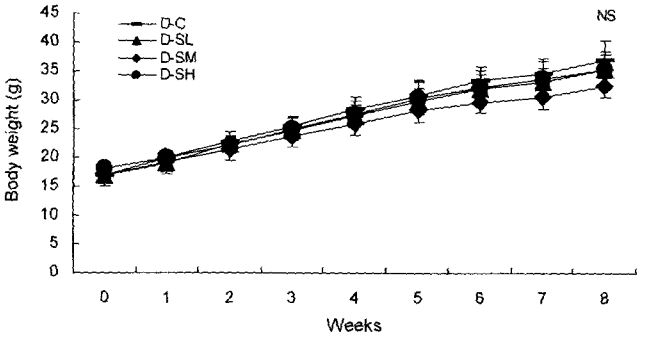

Fig. 1. Effects of silkworm extracts on the body weight (g) in C57BL/6J mice. Not significantly different by Tukey's test. Abbreviations are the same as Table 1.

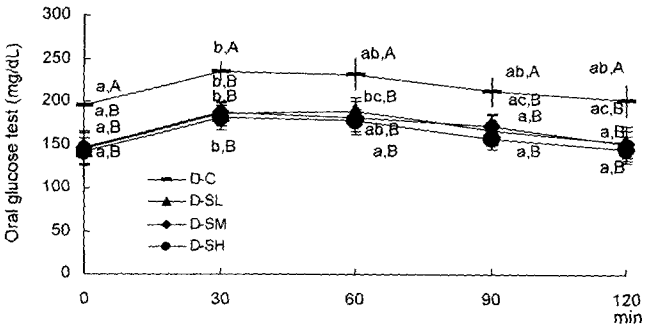

Fig. 2. Effects of silkworm extracts on oral glucose tolerence test in C57BL/6J mice. Different small superscripts in the same column indicate significantly different at $p<0.05$ by Tukey's test. Different capital superscripts in the same row indicate significantly different at $p<0.05$ by Tukey's test. Abbreviations are the same as Table 1.

Table 2. Food intake, drinking water intake, body weight gain and food efficiency ratio (FER) in C57BL/6J mice during 8 weeks

Groups[1)]	Food intake (g/day)	Drinking water intake (g/day)	Body weight gain (g/mice)	FER (%)
D-C	5.37±0.33[2)NS3)]	5.58±0.40[b4)]	20.10±3.31[NS]	3.62±0.56[NS]
D-SL	5.34±0.22	6.69±0.31[a]	18.10±2.61	2.71±0.17
D-SM	5.21±0.33	7.17±0.37[a]	15.45±3.52	2.66±0.32
D-SH	5.43±0.10	6.42±0.23[a]	17.80±2.91	2.78±0.17

[1)]Groups are the same as Table 1.
[2)]All values are mean±SE (n=20).
[3)]Not significant.
[4)]Values within a column with different superscripts are significantly different at $p<0.05$ by Tukey's test.

30 minutes, yet all groups returned to a fasting level within 90 minutes.

Blood glucose, HbAlc and insilin levels

The level of blood glucose in silkworm-extract supplement groups was significantly decreased compared with that in the diabetic control group. But there were no significant differences among the silkworm supplemented groups (Fig. 3). Level of HbA1c and insulin were no significantly different among groups (Table 3).

Intestinal mucosa maltase, sucrase and lactase activities

The results of intestinal mucosa disaccharidase activities were shown in Fig. 4. The maltase activity was not significant difference in each groups by part (Fig. 4). The sucrase activities in the proximal and middle part of the intestine in the silkworm supplement groups were significantly lower than that of the D-C group. However, there was no significant difference in the distal part (Fig. 4). The lactase activities as α-glucosidase of the proximal part in the silkworm supplemented groups were significantly lower than that of the D-C group. Especially in the D-SM group, it was significantly decreased compared to the D-C group. The middle part in the silkworm supplement groups was significantly lower than that of the D-C group. There were no significant differences between the experimental groups in lactase activity of the distal (Fig. 4).

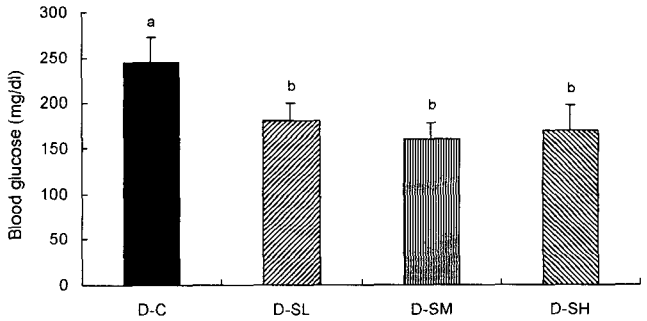

Fig. 3. Effects of silkworm extracts on blood glucose in C57BL/6J mice. Bars within different letters are significantly different at $p<0.05$ by Tukey's test. Abbreviations are the same as Table 1.

Table 3. Effects of silkworm extracts on HbA1c and plasma insulin in C57BL/6J mice

Groups[1)]	HbA1c (%)	Plasma insulin (μU/mL)
D-C	5.70±0.45[2)NS3)]	42.8±2.4[NS]
D-SL	5.55±0.07[4)]	39.4±2.3
D-SM	5.30±0.30	39.1±1.1
D-SH	5.30±0.37	39.1±2.1

[1)]Groups are the same as Table 1.
[2)]All values are mean±SE (n=20).
[3)]Not significant.
[4)]Not significantly different by Tukey's test.

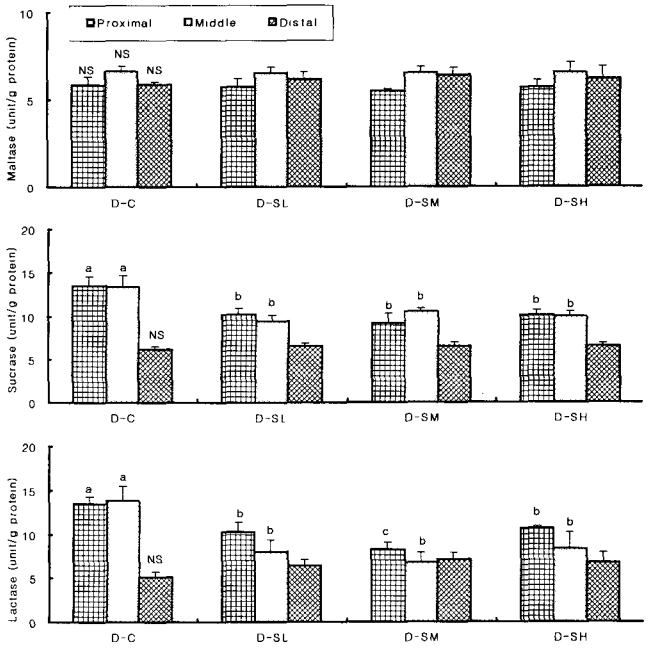

Fig. 4. Effects of silkworm extracts on disaccharidase activity in C57BL/6J mice. Bars within different letters are significantly different at $p<0.05$ by Tukey's test. Abbreviations are the same as Table 1. NS: not significant.

Contents of glycogen in liver and muscle

The contents of glycogen (Table 4) in the liver and muscle were not significantly different between any groups.

DISCUSSION

The purpose of this study was to investigate the glucose-lowering effects of silkworm-extract in type-2 obesity/diabetes-induced experimental mice.

During the 8-week study period, the body weights of the silkworm-extract supplemented groups were decreased compared with those of the diabetic control group, although the differences were not significant. There was also no significant difference in the food

Table 4. Effects of silkworm extracts on glycogen contents of C57BL/6J mice

Groups[1)]	Liver glycogen (mg/g)	Muscle glycogen (mg/g)
D-C	33.12±2.38[2)NS3)]	5.96±0.89[NS]
D-SL	33.94±2.56[4)]	6.03±0.92
D-SM	35.94±2.56	6.47±0.74
D-SH	34.70±1.67	6.62±0.43

[1)]Gruops are the same as Table 1.
[2)]All values are mean±SE (n=20).
[3)]Not significant.
[4)]Not significantly different by Tukey's test.

intakes between the groups. However, the water intake by the silkworm-extract supplemented groups was significantly higher than that by the distilled-water groups. Meanwhile, the body weight gains decreased in the silkworm-extract supplement groups, yet the differences were not significant. The fasting time and resulting glucose tolerance in the silkworm-extract supplement groups were significantly decreased compared with those in the D-C group. For all groups, the glucose level was highest after 30 minutes, yet all groups returned to a fasting level within 90 minutes. Also the blood glucose levels in the silkworm-extract supplemented groups were significantly lower compared to that in the diabetic control group. However, there was no significant difference among the silkworm-extract supplement groups.

According to a recent study, the blood glucose-lowering effect of mulberry leaves and silk worms is due to an inhibition of the disaccharidase activity in the small intestinal mucosa (26). In the present study, there was significant correlation between the blood glucose and HbA1c levels, as the HbA1c reflects the changes in the blood glucose during a hemoglobin half-life (27,28). The level of HbA1c in the silkworm-extract supplement groups decreased compared with that in the diabetic control group, although there was no significant difference.

In an insulin-resistant state, the β-cells of the pancreas secrete more insulin to maintain a normal metabolism. However, when an insufficient amount of insulin is secreted, this induces a saccharo-metabolism or non insulin-dependent diabetes mellitus (NIDDM). As such, NIDDM is a characteristic of insulin-resistance and hyperergasia in the β-cell of the pancreas (29). In the present study, the blood-insulin levels were not significantly different. This result was also reported by Ryu et al. (30), where silkworms were found to lower the blood glucose and improve the saccharo-metabolism without stimulating the secretion of insulin by the β-cells in the pancreas.

The lowering of blood glucose and the secretion of insulin are both considered to delay the digestion and absorption of glucose in the intestine by competitive α-glucosidase activities, where the polysaccharide is dissolved into monosaccharides in the small intestine (31, 32). The sucrase and lactase activities of the proximal segments in the silkworm-extract supplement groups were significantly decreased compared to that in the diabetic control group.

The change in the enzyme activity in the small intestine controlled the blood glucose level, which rapidly increased after a meal. Therefore, the α-glucosidase inhibitor protected against an increase in blood insulin levels.

The glycogen contents in the liver and muscle were not significantly different among the groups, which was similar to the results previously reported by Kim et al. (26), where a silkworm-extract was found to inhibit the disacharidase in the small intestine, yet had no influence on the glycogen content in the liver and muscle. In that study, it was considered that the silkworm extract had a tissue-specific character as regards the enzymes in the small intestine.

In conclusion, the present study found that the silkworm-extract decreased the disaccharidase activity in the intestine proximal segments of C57BL/6J mice, which is an obesity/diabetic type-2 model, thereby demonstrating that the extract may be effective in protecting against changes in the saccharo-metabolism, such as a rapid increase in blood glucose and insulin due to diabetes.

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