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Inhibitory Effects of *Viburnum dilatatum* Thunb. (Gamazumi) on Oxidation and Hyperglycemia in Rats with Streptozotocin-Induced Diabetes

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The fruit of *Viburnum dilatatum* Thunb. (gamazumi) was found in a previous study to have strong radical scavenging activity. The present study investigated the antioxidative functions of gamazumi crude extract (GCE) in rats having diabetes induced by the administration of streptozotocin. In rats given water (H_2O group), plasma levels of glucose, total cholesterol, and lipid peroxide (TBARS) and erythrocyte levels of TBARS increased with time over the experimental period of 10 weeks. These increases were inhibited in rats given GCE (GCE group). After 10 weeks, hepatic, renal, and pancreatic TBARS in the GCE group were significantly lower than those in the H_2O group. GCE contains a high concentration of polyphenols, and it is expected that they are the active components. These results demonstrate that GCE has an inhibitory effect on the oxidative stress induced by diabetes and suggest that GCE may be useful for the prevention of diabetic complications. Furthermore, as the increase of plasma glucose and total cholesterol was inhibited in the GCE group, GCE may also have anti-hyperglycemic activity in diabetes.

KEYWORDS: ***Viburnum dilatatum*** **(gamazumi); diabetes; lipid peroxidation; antioxidation; hyperglycemia; streptozotocin**

INTRODUCTION

Viburnum dilatatum Thunb. (gamazumi), a wild deciduous low tree, is found widely in the hills of northern Japan. Its small crimson fruit ripens in autumn (*1*). Until recently, this fruit had not been marketed and used as a food material. However, cultivation of *V. dilatatum* is increasing, and the harvested fruit is used for squeezed juice.

We found that a squeezed extract of *V. dilatatum* fruit had strong antioxidant and radical scavenging activities as measured by the XYZ-dish method (*2*) and electron spin resonance (*3*). The physiological actions of *V. dilatatum* fruit were studied in rats, which were given gamazumi crude extract (GCE) and subjected to water immersion restraint stress. In the study, GCE significantly depressed gastric ulcer formation, increase of tissue lipid peroxidation, and organ functional damage in rats subjected to stress (*3*). The effect of GCE was similar to or more pronounced than that of (−)-epigallocatechin gallate, which is the main antioxidative component in teas. Furthermore, although hepatic antioxidant efficiency increased in normal rats given GCE, induction of antioxidative enzymes was not observed. These observations suggested that antioxidant components in GCE were absorbed into the body and prevented oxidation induced by stress (*4*).

The present study was performed to investigate the antioxidative effect of GCE on oxidative damages in rats with experimental diabetes induced by the administration of streptozotocin (STZ). Generation of reactive oxygen and free radicals is accelerated in diabetes (*5*), which increases oxidative stress and decreases the activities of the radical scavenging system (*6*, *7*). Furthermore, it is well-known that specific complications, such as disorders of microcirculation and cellular function, arise in diabetes (*8*, *9*). In this study, STZ-induced diabetic rats (*10*) were administered GCE, water, or aminoguanidine (AMGN), which has been reported to depress the oxidation induced by STZ (*11*, *12*). The time-coursed levels of plasma glucose and lipid peroxidation in blood were investigated in these diabetic rats. Moreover, concentrations of lipid peroxidation in tissues were also determined at the endopoint of this experiment.

MATERIALS AND METHODS

Reagents and Preparation of GCE. STZ and AMGN were purchased as biochemical grade from Sigma Chemical Co. (St. Louis, MO). The highest available grade of all other reagents was used.

GCE was prepared by condensing and freeze-drying an extract of *V. dilatatum* fruit obtained by squeezing the fruit with an extractor

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followed by filtration through filter cloths without addition of any solvent (*3*). The yield of extract solution from 1 kg of fruit was 600 mL, and 50.4 g of GCE was obtained from the solution. GCE contained 53.0 mg/g of gallic acid equivalent polyphenols by the Folin−Denis method described by Gao et al. (*13*). GCE solution was prepared at 16.8 mg/mL in distilled water, and 1 mg/mL AMGN solution was prepared with distilled water.

Animals. Male Sprague−Dawley rats were purchased from Clea Japan Inc. (Tokyo, Japan). This study was carried out under the control of the guidelines for animal experiments of Notification 6 of the Cabinet Office of the Japanese government. Animals were divided into normal, H_2O, AMGN, and GCE groups of five rats each. All rats, except for the normal group, were administered 50 mg/kg of STZ intraperitoneally (*11*). STZ solution of 50 mg/mL was prepared by dissolution in 50 mM citrate buffer (pH 4.5). The normal group was injected intraperitoneally with 2 mL/kg of 50 mM citrate buffer (pH 4.5).

Urine was collected from 24 to 48 h after administration of STZ, and diabetic status was identified by measurement of urinary glucose level by pretest 10 (Wako Pure Chemical Industries Ltd., Osaka, Japan). Then, all rats were given free access to a commercial diet (type MF; Oriental Yeast Co. Ltd., Tokyo, Japan) and the experimental solutions as drinking water. The normal and H_2O groups were given tap water, the AMGN group was given 1 mg/mL AMGN solution, and the GCE group received 16.8 mg/mL GCE solution.

Blood was collected with heparinization from the tail artery before after weekly after the administration of STZ. Plasma and red blood cells were immediately separated from blood by centrifugation at 15000 rpm for 1 min.

After the experimental period of 10 weeks, all rats were fasted and received tap water for 20 h. Then, blood was collected from the abdominal artery under ether anesthesia. The heparinized blood was immediately separated into plasma and red blood cells by centrifugation at 3500 rpm for 10 min. After blood collection, 0.15 M NaCl solution containing 0.16 mg/mL of heparin was perfused, and then the liver, kidney, and pancreas were removed and weighed. Plasma and these tissues were stored at −70 °C until assay.

Biochemical Determination. The concentrations of glucose, total cholesterol, and triglyceride in rat plasma were measured by the Fuji Dri-Chem 3000V system (Fuji Medical System Co., Tokyo, Japan) after suitable dilution of plasma with 0.15 M NaCl solution.

The concentration of glycated hemoglobin A_1 in the blood of fasted rats after experimental feeding for 10 weeks was measured with the glycated hemoglobin kit (Sigma Chemical Co.).

Insulin concentrations in nonfasted rat plasma obtained before the administration of STZ and at 3, 6, and 9 weeks after the administration of STZ were measured with the rat−insulin ELISA kit (Mercodia AB, Uppsala, Sweden).

Protein concentrations in plasma, red blood cells, and tissues were measured by the BCA protein assay kit (Pierce Chemical Co., Rockford, IL) with bovine serum albumin as a standard (*14*). Samples were suitably diluted with 0.1 N NaOH solution containing 0.19 M disodium carbonate.

Measurement of Lipid Peroxidation. The level of lipid peroxidation in plasma, which was collected weekly, was measured as thiobarbituric acid reactive substance (TBARS) using the LPO Test Wako (Wako Pure Chemical Industries Co.) (*15*).

TBARS in erythrocytes was measured by the following method described by Ihm et al. (*11*). Red blood cells were suspended in 1.0 mL of 10 mM phosphate buffered saline (pH 7.4), and 25 μL of 8.8 mg/mL butylated hydroxytoluene was added. Then, 0.5 mL of 1.84 M trichloroacetic acid solution was added and mixed, and samples were stored on ice for 2 h. After centrifugation of the mixture at 12000 rpm for 10 min, 75 μL of 0.1 M ethylenediaminetetraacetic acid solution and 0.4 mL of 69.4 mM TBA solution were added to 1.0 mL of the supernatant and mixed. The sealed solution was boiled at 95 °C for 15 min, and then the dual absorbance of the cooled solution was measured at 532 and 600 nm with a spectrophotometer.

Liver, kidney, and pancreas were homogenized in a 1.5-fold volume of cold 0.15 M potassium chloride solution. TBARS in these homogenates was measured by the fluorescence method (*16*) with 1,1,3,3-tetraethoxypropane as a standard.

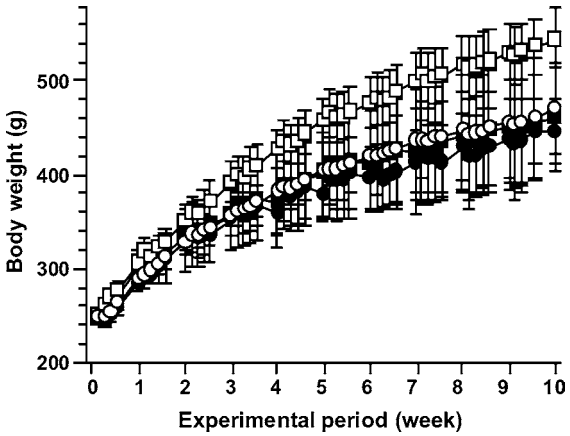

Figure 1. Changes of body weight of normal and diabetic rats during experimental feeding for 10 weeks: normal (□); H_2O (●); AMGN (■); GCE (○). Data represent the mean ± SD of five rats.

Statistical Analysis. Data were obtained as the mean and standard deviation (SD) and analyzed by the Scheffe test after one-way analysis of variance (ANOVA) using the Stat View System (SAS Institute Inc., Cary, NC). Significant differences in the mean values were tested at $P < 0.05$.

RESULTS

Effect on Growth of Rats. Figure 1 shows changes in the body weight of rats for 10 weeks after the administration of STZ. Although all groups showed increased body weight during feeding, the body weights of the three groups that were injected with STZ were lower than that of the normal group, which was not injected with STZ. The difference of body weight between the normal group and diabetic groups increased with the experimental period. There was no significant difference in body weight among the three diabetic groups, but the GCE group tended to have a higher body weight than the H_2O and AMGN groups. Moreover, decreased body weight gain was found in all groups with aging.

Table 1 shows the body weight, tissue weight, and the tissue-to-body weight ratio of liver, kidney, and pancreas in rats fasted for 20 h after the experimental period of 10 weeks. There was no significant difference in fasted body weight among the four groups, but the diabetic groups tended to have a lower weight than the normal group. There was no difference in liver and kidney weights among the groups. The pancreas weight of the H_2O and AMGN groups was decreased significantly compared with the normal group. The GCE group also showed a lower pancreas weight, but it was not significantly different from that of the normal group. The weight ratios of liver and kidney to body weight were increased by administration of STZ. All ratios in the H_2O group and the ratio of liver in the AMGN group were significantly higher than those in the normal group. There was no difference in the ratios among the three diabetic groups. Differences in the weight ratio of the pancreas were not found among the four groups.

Total body weight gain, food and drink intakes, and food efficiency are shown in **Table 2**. Body weight gain was decreased by administration of STZ, and that of the H_2O and AMGN groups was significantly lower than that of the normal group. On the other hand, the food and drink intakes of the H_2O group increased significantly from those of the normal group. Although the drink intake of the GCE group increased, the food intake of the GCE group was not significantly different from that of the normal group. The food efficiency of the diabetic groups was significantly lower than that of the normal

Table 1. Fasted Body Weight, Tissue Weight, and Ratio of Tissue-to-Body Weight after Experimental Feeding for 10 Weeks[a]

	normal	H_2O	AMGN	GCE
final body wt (g)	516.0 ± 29.9	410.7 ± 53.0	429.2 ± 77.5	445.8 ± 79.0
liver wt (g)	15.90 ± 1.07	16.83 ± 1.24	17.06 ± 2.80	16.62 ± 2.31
kidney wt (g)	3.513 ± 0.328	3.982 ± 0.512	3.545 ± 0.219	3.420 ± 0.240
pancreas wt (g)	1.082 ± 0.096	0.840 ± 0.046[c]	0.853 ± 0.137[c]	0.895 ± 0.106
tissue-to-body wt ratio[b]				
liver (g/100 g)	3.082 ± 0.114	4.138 ± 0.490[c]	3.996 ± 0.384[c]	3.733 ± 0.297
kidney (g/100 g)	0.681 ± 0.058	0.988 ± 0.219[c]	0.847 ± 0.157	0.776 ± 0.110
pancreas (g/100 g)	0.210 ± 0.015	0.213 ± 0.033	0.201 ± 0.034	0.201 ± 0.015

[a] Rats were fasted for 20 h after the experimental period of 10 weeks. Data represent the mean ± SD of five rats. [b] Tissue weight per 100 g of body weight. [c] Significant difference from the normal group at $P < 0.05$.

Table 2. Total Body Weight Gain, Food and Drink Intakes, and Food Efficiency of Rats during Experimental Feeding for 10 Weeks[a]

	normal	H_2O	AMGN	GCE
body wt gain (g)	294.6 ± 26.9	200.1 ± 37.6[c]	211.4 ± 53.8[c]	221.9 ± 39.9
food intake (g)	1385 ± 71	1941 ± 327[c]	1689 ± 275	1500 ± 154
drink intake (mL)	2259 ± 283	7154 ± 2665[c]	5824 ± 2377	4144 ± 970
food efficiency[b] (%)	21.24 ± 1.20	10.82 ± 3.88[c]	13.16 ± 5.02[c]	15.06 ± 3.67

[a] Rats were fasted for 20 h after the experimental period of 10 weeks. Data represent the mean ± SD of five rats. [b] Food efficiency was calculated from the following formula: efficiency (%) = body wt gain/food intake × 100. [c] Significant difference from the normal group at $P < 0.05$.

group. However, the GCE group showed the highest efficiency among the three diabetic groups injected with STZ.

Concentration of Glucose, Total Cholesterol, and Triglyceride in Plasma. Figure 2 shows the concentrations of glucose, total cholesterol, and triglyceride in plasma, which was obtained from the tail artery of nonfasted rats during experimental feeding for 10 weeks.

Glucose concentrations were the same in all groups before the administration of STZ, and the normal group showed the same and constant level during the experiment. On the other hand, the three diabetic groups showed a higher concentration of glucose than the normal group at 1 week after the administration of STZ. The level in the H_2O group continued to increase gradually and was significantly higher than that in the normal group after the fourth week. The AMGN group showed an increase of plasma glucose level until 2 weeks, and after that showed a lower level than the H_2O group, although the glucose level in the AMGN group was not significantly different from that in either the normal or H_2O group. Although the glucose level of the GCE group increased gradually, it was lower than that of the AMGN group. Following the seventh week, the GCE group showed a significantly lower glucose level than the H_2O group (**Figure 2A**).

Plasma total cholesterol concentrations of the H_2O and AMGN groups increased gradually and were significantly higher than that of the normal group at the endpoint of the experiment. However, the levels of the normal and GCE groups remained constant during the experiment. The GCE group showed the same or lower concentration of total cholesterol than the normal group and showed a significantly lower level than the H_2O group after the fourth week (**Figure 2B**).

Although there was no significant difference in plasma triglyceride concentration among all groups, the H_2O group tended to show a high triglyceride level. The other three groups showed similar levels (**Figure 2C**).

The concentrations of glucose, total cholesterol, and triglyceride in plasma and the hemoglobin A_1 level of fasted rats after feeding for 10 weeks are shown in **Table 3**. The glucose

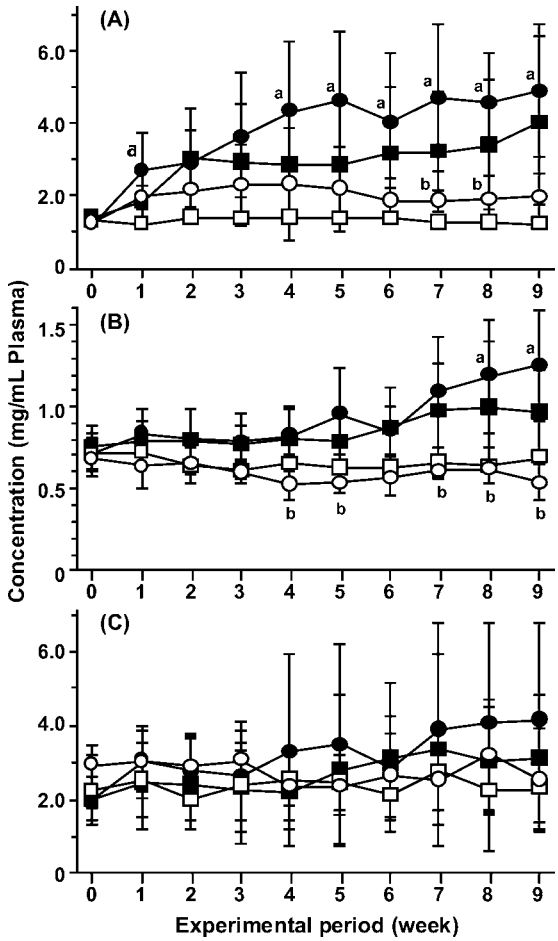

Figure 2. Changes of plasma glucose (**A**), total cholesterol (**B**), and triglyceride (**C**) in normal and diabetic rats during experimental feeding for 10 weeks: normal (□); H_2O (●); AMGN (■); GCE (○). Data represent the mean ± SD of five rats. Significant difference is indicated from the normal (a) and H_2O (b) groups at $P < 0.05$.

concentration of the H_2O group was the highest among the groups, but not significantly so, and that of the AMGN and GCE groups was similar to that of the normal group.

The plasma total cholesterol concentration of the H_2O group was significantly higher than that of the normal group. The AMGN group showed a lower concentration than the H_2O group but tended to show a higher level than the normal group. On the other hand, the total cholesterol concentration of the GCE group was similar to that of the normal group and significantly lower than that of the H_2O group.

There was no significant difference in plasma triglyceride concentration among the groups, but the H_2O group showed a

Table 3. Plasma Concentrations of Glucose, Total Cholesterol, and Triglyceride and Hemoglobin A_1 of Fasted Rats after Experimental Feeding for 10 Weeks[a]

	normal	H_2O	AMGN	GCE
glucose (mg/mL)	1.31 ± 0.26	2.09 ± 0.46	1.51 ± 0.46	1.51 ± 0.46
total cholesterol (mg/mL)	0.64 ± 0.11	1.12 ± 0.35[b]	0.90 ± 0.27	0.58 ± 0.06[c]
triglyceride (mg/mL)	1.30 ± 0.34	1.60 ± 0.22	1.45 ± 0.68	1.29 ± 0.63
hemoglobin A_1 (%)	2.08 ± 0.10	3.31 ± 0.81[b]	2.82 ± 0.75	2.49 ± 0.39

[a] Rats were fasted for 20 h after the experimental period of 10 weeks. Data represent the mean ± SD of five rats. [b,c] Significant difference from the normal (*b*) and H_2O (*c*) groups at $P < 0.05$.

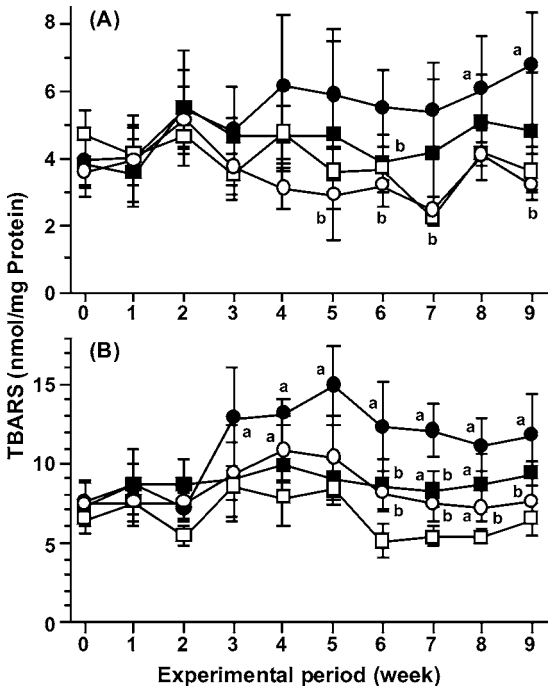

Figure 3. Changes of lipid peroxidation (TBARS) in plasma (**A**) and red blood cells (**B**) of diabetic rats during experimental feeding for 10 weeks: normal (□); H_2O (●); AMGN (■); GCE (○). Data represent the mean ± SD of five rats. Significant difference is indicated from the normal (a) and H_2O (b) groups at $P < 0.05$.

tendency to a high level, and the GCE group showed a level similar to that of the normal group.

The hemoglobin A_1 level increased in the diabetic rats, and the H_2O group showed a significantly higher level than the normal group, which was not injected with STZ. However, the GCE group showed the lowest level among the three diabetic groups.

Changes of Lipid Peroxidation Levels in Plasma and Red Blood Cells during Feeding. Figure 3 shows the changes of TBARS in plasma and red blood cells during feeding for 10 weeks.

Before the administration of STZ, plasma TBARS was almost the same in all of the groups. Although there was some variability, plasma TBARS of the normal group remained almost constant during the experimental period. TBARS of the H_2O group increased gradually until the fourth week, at which time it was the highest of all groups. After that, it remained at the same level, and it was significantly higher than the TBARS of the normal group at the sixth and seventh weeks. The AMGN group showed a lower TBARS than the H_2O group after the fourth week, but the difference was not significant. On the other hand, the TBARS of the GCE group remained at a level similar to that of the normal group and was significantly lower than that of the H_2O group after the fourth week (**Figure 3A**).

Table 4. TBARS in Plasma, Red Blood Cells, Liver, Kidney, and Pancreas of Fasted Rats after Experimental Feeding for 10 Weeks[a]

	TBARS (nmol/mg of protein)			
	normal	H_2O	AMGN	GCE
plasma	0.051 ± 0.013	0.075 ± 0.013[b]	0.056 ± 0.008	0.047 ± 0.008[c]
red blood cells	0.055 ± 0.011	0.095 ± 0.013[b]	0.061 ± 0.013[c]	0.055 ± 0.012[c]
liver	0.331 ± 0.110	0.364 ± 0.077	0.327 ± 0.021	0.252 ± 0.120
kidney	0.378 ± 0.101	0.957 ± 0.475[b]	0.605 ± 0.216	0.451 ± 0.093
pancreas	0.039 ± 0.016	0.062 ± 0.016	0.042 ± 0.017	0.040 ± 0.021

[a] Rats were fasted for 20 h after the experimental period of 10 weeks. Data represent the mean ± SD of five rats. [b,c] Significant difference from the normal (*b*) and H_2O (*c*) groups at $P < 0.05$.

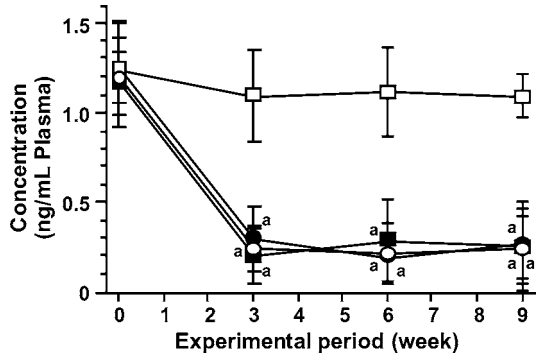

Figure 4. Changes of plasma insulin concentration in diabetic rats during experimental feeding for 10 weeks: normal (□); H_2O (●); AMGN (■); GCE (○). Data represent the mean ± SD of five rats. Significant difference is indicated from the normal (a) and H_2O (b) groups at $P < 0.05$.

Erythrocyte TBARS levels of all groups were similar before the administration of STZ. The TBARS level of the normal group remained almost constant, with some variability. The three diabetic groups showed a gradual increase of TBARS in red blood cells, which reached a significantly higher level than in the normal group at the fourth week. Subsequently, the TBARS of the H_2O group remained at the same level, higher than that of the normal group. On the other hand, the AMGN and GCE groups showed a trend to decrease TBARS in red blood cells. After the sixth week, their TBARS levels were higher than that of the normal group but significantly lower than that of the H_2O group.

Lipid Peroxidation in Plasma, Red Blood Cells, and Tissues after Experimental Feeding. TBARS in plasma, red blood cells, liver, kidney, and pancreas of rats after experimental feeding for 10 weeks are shown in **Table 4**. The H_2O group showed significantly higher TBARS in plasma, red blood cells, and kidney than the normal group and showed a tendency to higher pancreatic TBARS. On the other hand, although renal TBARS of the AMGN group was higher than that of the normal group, other tissue TBARS levels of the AMGN group were similar to those of the normal group. Erythrocyte TBARS of the AMGN group was significantly lower than that of the H_2O group. TBARS levels in all tissues of the GCE group were similar to those of the normal group. Notably, the GCE group showed a tendency to a lower hepatic TBARS than the normal group. Plasma and erythrocyte TBARS of the GCE group were significantly lower than those of the H_2O group.

Insulin Concentration in Plasma. Figure 4 shows the insulin concentration in nonfasted plasma before and at 3, 6, and 9 weeks after the administration of STZ. Although all groups

showed a similar insulin level before the administration of STZ, the three diabetic groups showed significantly lower levels than the normal group at 3 weeks after the administration of STZ. The plasma insulin level in the diabetic rats was less than half that in the normal group and continued at this level to the end of the experiment.

DISCUSSION

Administration of STZ to rats depressed growth and increased intakes of food and drink. It is generally found in diabetic animal models after the administration of STZ. In the diabetic rats, increased volumes of and glucose in urine were also observed, and the weight ratios of liver and kidney to body weight increased. The decrease of pancreatic weight suggests that the β cell in the pancreas is affected by STZ (*10*). These observations confirm that administration of STZ caused diabetes in our experimental animals. Ingestion of GCE did not show improvement of the depressed growth in the diabetic rats. This is similar to probucol (*17*) and taurine (*18*), which caused improvement of lipid metabolism but had no hypoglycemic action. Although the intakes of food and drink decreased by ingestion of GCE, this phenomenon has also been observed in normal rats given GCE, and rats given GCE showed growth similar to that of normal rats given water (*3*, *4*). STZ depressed increase of body weight by a mechanism not involving reduced intake of food and drink in rats given GCE. Although as a result the food efficiency of GCE-treated rats was higher than that of diabetic rats given water, it appears that ingestion of GCE would not improve the growth in diabetic rats. Moreover, although hypertrophy of the liver and kidney and atrophy of the pancreas were found in the diabetic rats, these effects were comparatively less in the GCE group than in the H_2O group. Therefore, it appears that GCE has no accelerating effect on the suppression of growth in diabetic rats.

Experimental hyperglycemia is induced by the administration of STZ, which impairs the action of pancreatic β cells and produces an insulin-deficient state (*10*). In the H_2O group, the gradual elevation of plasma glucose level was found, but it was clearly attenuated in the GCE group. In this experiment, supplementation with GCE was started after the identification of the diabetic status in rats by determination of the urinary glucose level at 48 h after the administration of STZ. Moreover, a decreased plasma insulin concentration was found in all diabetic rats at 3 weeks after the administration of STZ. Therefore, it is unlikely that the inhibition of the elevated plasma glucose level in the GCE group is a result of the promotion of the insulin secretion by GCE.

Tea catechin has been reported to have inhibitory actions on sugar-degrading enzymes (*19*), and there are studies that anthocyanins and flavonoids inhibit α-glucosidase and sugar absorption (*20*, *21*). Most of these polyphenols are known to have antioxidant actions. Because GCE contains high concentrations of polyphenols, 53 mg/g (2.67 mg/g of fruits) as gallic acid equivalent, the possibility that the activity of GCE results from an inhibitory effect of polyphenols on sugar metabolic enzymes was expected. Moreover, we identified anthocyanins as antioxidative components of GCE (*22*), and they might also participate in the inhibition of the increase in plasma glucose level. Although identification of other phenolic compounds in GCE is progressing, the sum of these compounds is 44% of polyphenols in GCE. These observations suggest that GCE may be useful for control of blood glucose level in diabetes.

It is known that total cholesterol and triglyceride are increased in diabetes as the result of secondary metabolic disease and that the administration of insulin lowers these lipid levels (*23*). Furthermore, the administration of antioxidative agents has also been reported to improve dyslipidemia (*24*); similar hypolipidemic activity has been found in vitamin E (*25*), curcumin (*26*), and kaikasaponin (*27*). In agreement with these reports, the concentrations of total cholesterol and triglyceride in the plasma of diabetic rats increased gradually in this experiment. Furthermore, in the diabetic rats given GCE, elevation of the plasma total cholesterol level was not found and triglyceride also showed a tendency to be lower than that of the H_2O group. Because diet affects plasma lipid level, these results under nonfasted conditions might have been caused by decreased dietary intake and inhibition of lipid absorption related to GCE ingestion. However, the GCE group also showed lower total cholesterol and triglyceride levels than the H_2O group under fasted conditions (**Table 3**). This result suggests that some components of GCE also exert effects on lipid metabolism.

Diabetes increases oxidative stress (*6*, *7*, *28*), and increased glycosylated hemoglobin, which is caused by glycation of proteins as a result of hyperglycemia, is a feature of diabetes (*29*). In our study, increased hemoglobin A_1 was found in diabetic rats, but the ingestion of GCE suppressed the hemoglobin A_1 level (**Table 3**). Because the administration of vitamin E also decreases the glycated hemoglobin level in diabetes (*30*), this result suggests that GCE can depress glycosylation of hemoglobin in diabetic rats via its antioxidative actions on oxidative stress.

The inhibitory effect of GCE on oxidation was investigated in STZ-induced diabetic rats given GCE for 10 weeks. Reactive oxygen species not only contribute to the symptoms of diabetes but also induce some of the complications of diabetes by promoting oxidative injury (*5*−*9*). In the diabetic rats, TBARS increased gradually in plasma and red blood cells over the experimental period, in parallel to the impaired growth and increased plasma glucose level.

It has been reported that AMGN has an inhibitory effect on the progression of oxidative status induced by diabetes, even though AMGN has no anti-hyperglycemic activity (*11*, *12*); our observations agree with these reports. Moreover, ingestion of GCE showed an effect similar to that of AMGN on the oxidative damage. The inhibitory effect of GCE on oxidation was found not only for the increase of plasma and erythrocyte TBARS during the experimental period but also for the increase of tissue TBARS, especially in kidney and pancreas. In all tissues, TBARS levels in diabetic rats given GCE were similar to those of normal rats, and GCE was more effective than AMGN in this respect (*11*). Although the doses of GCE and AMGN were different, the respective polyphenol content in GCE solution was 0.9 mg/mL, and half of the content was antioxidative phenolic compounds (*22*). Therefore, it is probable that the active component in GCE is more potent than AMGN. GCE has previously been shown to possess strong antioxidant activity, in particular an inhibitory effect on the oxidative injury induced by stress (*3*, *4*). In this study, we found that GCE ameliorates oxidative damage in STZ-diabetic rats, an effect that may be useful for the prevention of diabetic complications. Furthermore, we showed that GCE tended to inhibit the elevation of plasma glucose level, an effect not shown by AMGN.

There are few studies on the structural analysis of chemical composition in the fruits of *V. dilatatum*, although the existence of the polyphenolic compounds quercetin and kaempferol has been reported (*31*). We have so far identified chlorogenic acid (unpublished data), and the presence of anthocyanins (*22*) and flavonoids is also likely. Antioxidant activity and inhibitory

actions on glucosidases have been reported for anthocyanins and flavonoids (*20*, *21*), and these compounds are expected to participate in the color of fruits. The identification of other phenolic compounds is currently under way, and future studies will investigate the effects of these compounds on oral glucose tolerance and inhibition of the sugar metabolic enzymes. The antioxidant and anti-hyperglycemic effects of *V. dilatatum* fruits may be useful for the prevention of diabetes and its complications.

ABBREVIATIONS USED

GCE, gamazumi crude extract; STZ, streptozotocin; AMGN, aminoguanidine; TBARS, thiobarbituric acid reactive substance.

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