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ARTICLE

Protective and Anticataract Effects of the Aqueous Extract of *Cleistocalyx operculatus* Flower Buds on β-Cells of Streptozotocin-Diabetic Rats

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The aim of the present study was to examine whether an aqueous extract of *Cleistocalyx operculatus* flower buds (COB) had protective and anticataract effects on β-cells in experimental streptozotocin (STZ)-induced diabetes in rats. After 9 weeks of COB supplementation (500 mg/kg bw), the COB group had a significantly more stable insulin level as compared with the control diabetic group. Increased staining of insulin and preservation of islet cells were apparent in the COB-treated diabetic rats, whereas islet cell degeneration and weak insulin immunohistochemical staining were observed in the control diabetic rats. In addition, COB significantly delayed diabetic cataract formation and caused significant reductions in the glucose, sorbitol, and fructose levels in diabetic rat lenses. Furthermore, as compared to the control diabetic group, the COB group also showed antihyperglycemic effects (reductions in plasma glucose and HbA1c levels).

KEYWORDS: β-Cell damage; cataract; *Cleistocalyx operculatus*; diabetes

INTRODUCTION

Diabetes mellitus is a pathological condition that results in severe metabolic imbalances and nonphysiological changes in many tissues, and oxidative stress plays an important role in its etiology. Diabetic patients and experimental animal models exhibit high oxidative stress due to persistent and chronic hyperglycemia, which exhausts the antioxidative defense system and thus promotes free radical generation (*1*). Streptozotocin (STZ), an antibiotic produced by *Streptomyces achromogenes*, is a drug that is commonly used to induce experimental diabetes (*2*). In STZ-induced type 1 diabetes, destruction of the pancreatic β-cell causes an acute insulin shortage. The hyperglycemia and increased oxidative stress in STZ-induced diabetic rats have been implicated in the etiology and pathology of disease complications (*1*).

Chronic elevation of blood glucose in diabetes represents a serious risk factor for the development of cataracts, which is one of the earliest secondary complications of diabetes mellitus. Free radical production is increased in the diabetic lens, and natural antioxidant defenses are compromised, which results in increased oxidative stress (*3*, *4*). Human studies, as well as in vitro and animal experiments, have strongly suggested that there is an association between increased oxidative stress and the development of cataracts (*5*).

Polyphenols, especially flavonoids, are a group of naturally occurring compounds that are ubiquitous secondary metabolites in the plant kingdom. They have been shown to possess interesting clinical properties, such as anti-inflammatory, antiallergic, antiviral, antibacterial, and antitumoral activities. Welihinda et al. (*6*) and Broadhurst et al. (*7*) have reported that some tropical plants possess an insulin-releasing activity. Anderson and Polansky (*8*) and Wu et al. (*9*) have shown that polyphenols in green tea enhanced insulin activity in vitro and in vivo. Flavonoids with antioxidant and free radical scavenging activities were shown to protect pancreatic islets against the cytotoxic effects of STZ or alloxan, another agent that induces experimental diabetes (*10*). Antioxidant polyphenols were found to inhibit the development of cataracts in rats with experimentally induced diabetes (*11*, *12*). Furthermore, flavonoids from herb plants reduced the accumulation of sorbitol in STZ-induced diabetic rat lenses (*13*). Poly(ADP-ribose) synthetase inhibitors protected cells from the alloxan- and STZ-induced decreases in islet NAD levels and the inhibition of islet functions (*14*).

Cleistocalyx operculatus flower buds (COBs) (which belong to the Myrtaceae family) are used as a phytotherapic in folk medicine to treat gastrointestinal and respiratory disturbances and as an anti-inflammatory medicine in Vietnam. COBs have been reported to possess beneficial antitumor effects (*15*), antioxidant activity in vitro (*16*, *17*), and cardiotonic effects (*18*). The high polyphenol and flavonoid contents of COBs that inhibit the activities of carbohydrate-hydrolyzing enzymes and have antihyperglycemic effects were shown by Mai and Chuyen (*19*), and the antioxidant and antihyperlipidemic effects of the COBs on STZ-induced diabetic rats were also shown in our previous report (*20*).

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The present study was undertaken to determine the ability of COB administration to protect pancreatic cells against STZ damage in rats by immunohistochemical staining and to examine the accompanying changes in insulin release status. Moreover, we also determined whether COB has an anticataract effect in STZ-diabetic rats, and the effects on blood glucose, the serum lipid profile, and oxidative stress were observed.

MATERIALS AND METHODS

Plant Materials and Aqueous Extraction of COB. COBs were purchased from local markets in the Red River Delta area in Vietnam in the summer of 2005. One hundred grams of ground dried flower buds was extracted by boiling for 30 min in 2 L of distilled water. After centrifugation, the extracted solution was lyophilized, and a yellow-brown powder was obtained and stored at −80 °C until use. The yield of the aqueous extract (w/w from the dried material) was 19.6%.

Animal Experiments. Twenty-one male Wistar rats (7 weeks old, 180−200 g) were purchased from Nippon Clea Co. The animals were fed a standard diet (CE-2, Nippon Clea Co.) and tap water ad libitum. All rats were fed commercial CE-2 (CLEA Japan, Tokyo, Japan) with free access to water for 1 week to adapt to the new environment. The control diet, CE-2, contained (per 100 g) 8.9 g of moisture, 25.4 g of protein, 4.4 g of fat, 4.1 g of fiber, 6.9 g of ash, 50.3 g of carbohydrate, and sufficient vitamins and minerals to maintain the health of the rats. [The diet contained (per 100 g diet) 1517 IU of vitamin A, 250 IU of cholecalciferol, 7.0 mg of vitamin E, 1.7 mg of thiamine, 1.3 mg of riboflavin, 1.2 mg of pyridoxine, 3.4 μg of vitamin B-12, 19 mg of vitamin C, 3.7 mg of pantothenate, 16.7 mg of niacin, 0.2 mg of folic acid, 195 mg of choline, 48.4 μg of biotin, 549 mg of inositol, 1.18 g of Ca, 1.03 g of P, 0.29 g of Mg, 1.06 g of K, 0.26 g of Na, 10.57 mg of Mn, 26.0 mg of Fe, 1.25 mg of Cu, 6.38 mg of Zn, 0.13 mg of Co, 45.5 μg of I, 1.15 Ca/P, 4.07 Ca/Mg, and 4.08 K/Na.] The animals were maintained at 25 ± 2 °C and a relative humidity of 50 ± 15% with a 12 h light−dark cycle. After 2 weeks of feeding, 14 rats, which now weighed 300−310 g, were injected with a single dose of STZ (50 mg/kg bw) dissolved in saline. Seven days after the STZ injection, rats with serum glucose levels higher than 180 mg/dL were considered to be diabetic and were used for the subsequent experiment. Normal rats and the diabetic rats were each randomly assigned to three groups, and each group consisted of seven animals: normal, control, and COB. This experiment was conducted in accordance with the Guidelines for Animal Experimentation No. 6, established by the Prime Minister's Office of Japan in 1980, and the regulation on animal experimentation at Japan Women's University.

Monitoring during Animal Feeding. The normal rats and control diabetic rats were each given 1.0 mL of saline, whereas the COB-treated normal rats and COB-treated diabetic rats received 1.0 mL of the COB extract (500 mg/kg bw in saline) once a day for 9 weeks via oral gavage. Blood was periodically taken from all animals that had been fasted for 14 h (given tap water ad libitum only), before the treatment and 3, 6, and 9 weeks after the treatment to assess the changes in the levels of blood glucose, insulin, and HbA1c.

Cataracts were graded once a week until dissection. Eyes were inspected with a hand-held light focused close to the rat's eyes, using the modified Sippel rating (*21*): Sippel stage 0 is a normal lens; in stage 1, one-half of a thin band of vacuoles appeared in the periphery; in stage 2, one vacuole occupied up to one-third of the lens; in stage 3, one and a half vacuoles occupied two-thirds of the lens; and in stage 4, two vacuoles reached the center of the lens, and the vacuoles began to liquefy. The results of each analysis were averaged to obtain the cataract rating.

After 9 weeks of treatment, all rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (Abbott Laboratories, IL) (50 mg/kg body weight). Blood was obtained from the aorta ventralis in tubes containing heparin and then centrifuged to obtain the plasma, which was then stored at −80 °C until analysis. Harvested pancreatic organs were fixed in 10% neutral formalin solution.

Blood Biochemical Analysis. The blood glucose level was measured with a glucose test kit (Wako Pure Chemical Industries, Osaka, Japan). Plasma insulin levels were measured with the Rat Insulin ELISA kit (AKRIN-010, Shibayagi, Gunma, Japan).

Hemoglobin A1c, total cholesterol, triglyceride, total fatty acid, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein cholesterol, total protein, creatinine, blood urea nitrogen (BUN), glutamic oxaloacetic transaminase (GOT), and glutamic-pyruvic transaminase (GPT) were measured enzymatically using an Olympus AU5200 automatic analyzer (Olympus, Tokyo, Japan) at the clinical laboratory of Mitsubishi Kagaku BCL Co., Ltd. (Tokyo, Japan).

The levels of vitamin A and vitamin E in the serum were determined with an high-performance liquid chromatography (HPLC) method. After collection, blood samples were allowed to stand for 20 min before centrifugation at 700*g* for 30 min. The serum obtained was frozen until it was analyzed for α-tocopherol and retinol. Before analysis, the frozen serum samples were thawed at room temperature while being protected from light (20 min). The serum was deproteinized using ethanol, water, and extracted by *n*-hexane two times. The supernatant obtained was dried by nitrogen gas and then dissolved in *n*-hexane. The HPLC was performed by Shimadzu SCL-10A, with a Senshu Pak NH_2-1251-N column (4.6 mm × 250 mm); the mobile phase was *n*-hexane:2-propanol (97:3), and fluorescence was detected with an excitation at 297 nm and emission at 327 nm, with retinol and α-tocopherol as standards, respectively. The level of vitamin C in the serum was determined with an HPLC method [Shimadzu SCL-10A, Senshu Pak column (4.6 mm × 250 mm)]; the mobile phase was 0.05 M phosphate buffer at pH 2.5 containing 0.05 M *meta*-phosphoric acid, and L-ascorbic acid was used as a standard.

Lipid Peroxide in Rat Serum and Rat Lenses. Lipid peroxidation (LPO) in the rat serum was estimated using the thiobarbituric acid reactive substances (TBARS) measurement method of Buege and Aust (*22*). LPO in the rat lenses was determined by the method of Ohkawa et al. (*23*). A malondialdehyde (MDA) standard was prepared from 10 nmol of 1,1,3,3-tetraethoxypropane (Sigma Chemicals Co., St. Louis, MO). TBARS values in the rat serum were expressed in nmol MDA/L and in the lens were expressed in nmol MDA/g tissue weight.

Glucose, Fructose, and Sorbitol Concentrations in Lenses. Glucose, fructose, and sorbitol concentrations in the lenses were measured according to the procedures described by Haraguchi et al. (*13*).

Urinary 8-Hydroxy-2′-deoxyguanosine (8-OHdG) Measurement. At the end of the animal experiment, urine from normal rats and diabetic rats was obtained. The urinary 8-OHdG was measured using the New 8-OHdG Check ELISA kit (Japan Institute For the Control of Aging, Shizuoka, Japan).

Histopathological Procedures. Pancreatic tissues were harvested from the sacrificed animals, and pieces of the tissues were fixed in a 10% neutral formalin solution, embedded in paraffin, and then stained with hematoxylin and eosin at the Sapporo General Pathology Laboratory Co., Ltd. (Sapporo, Japan).

Immunohistochemical Procedures. The immunocytochemical reactions described below were performed at the Sapporo General Pathology Laboratory Co., Ltd. The system was composed of a PC, hardware and software (PCD and WORDROOF) for image acquisition and analysis, a Spot Insight QE (OLYMPUS A x 80T) camera, and optical microscope. Preparations were evaluated with a bright field microscope and photographed. All Langerhans islets from each rat were observed and counted. The intensity of staining with anti-insulin antibodies was semiquantitatively scored as weak, moderate, and strong. The following markers were used in the experiments: guinea pig antiswine insulin IgG primary antibody (Dako, Carpinteria, CA) and biotin-labeled goat anti-guinea pig IgG secondary antibody diluted at 1:150 (Vector Laboratories, CA).

The method requires the preliminary software procedures of spatial calibration (micrometer scale) and color segmentation setup for quantitative color analysis. Fifty islets of Langerhans from each group were chosen randomly. The areas of the insulin immunoreactive cells in the islets of Langerhans were measured.

The percentage of the insulin immunoreactive cells was calculated according to these results. The investigator who performed these measurements was unaware of the experiment.

Statistical Analysis. The data were expressed as the means ± standard deviations (SDs). Differences among groups at various times of the experiment were subjected to a one-way analysis of variance followed by Duncan's multiple range *t* test. Statistical comparisons between the two groups were analyzed using the independent sample *t* test. Cataract ratings were compared using a χ-squared test and analyzed using repeated measures of variance. For the analysis of the immunohistochemical, GPT,

and GOT data, a nonparametric test (Kruskal−Wallis) was used. Differences were considered statistically significant if $p < 0.05$.

RESULTS

Protective Effect of COB against Insulin Deficiency. The body weight, urine volume, and dietary intake were different in the experimental diabetic rats (**Figure 1**). The body weight of the COB-treated diabetic rats tended to remain stable, whereas that of the control group decreased. The rats treated with the COB extract produced a slight weight gain, but the increase was not statistically significant. Moreover, we found that the volume of urine from the diabetic rats treated with COB was significantly lower after 9 weeks than in the control diabetic rats. Similarly, a marked increase in food intake was observed in the control diabetic group (55.5 g/rat/24 h), while the COB-treated group maintained the same dietary intake for 9 weeks (47.3 g/rat/24 h). **Table 1** shows the effect of the COB extract on the insulin secretion in the STZ rats during the 9 weeks of treatment. Initially, the insulin level did not differ significantly between the diabetic rats with and without the COB treatment (0.76 and 0.80 ng/mL, respectively). After COB treatment, the insulin values in the treated diabetic rats were stable in weeks 3, 6, and 9 of the treatment (0.89, 0.94, and 1.01 ng/mL, respectively), whereas those of control diabetic rats decreased (0.63, 0.48, and 0.51 ng/mL, respectively). **Table 2** shows the effect of the COB extract on the blood glucose levels in the STZ rats during the 9 weeks of treatment. Initially, the blood glucose levels did not differ significantly between the diabetic rats with and without the COB treatment. However, the blood glucose values in the treated diabetic rats were significantly lower in weeks 3, 6, and 9 of treatment ($p < 0.01$, $p < 0.01$, and $p < 0.05$, respectively). This observation agreed with the results shown in our previous report (*19*). **Table 3** shows the effects of the COB on levels of blood glucose, plasma insulin, and HbA1c of different experimental groups. After 9 weeks, the diabetic control rats showed significant increases in the levels of blood glucose and hemoglobin glycosylation with a marked decrease in the level of plasma insulin. By contrast, oral administration of COB to diabetic rats significantly reversed these biochemical changes.

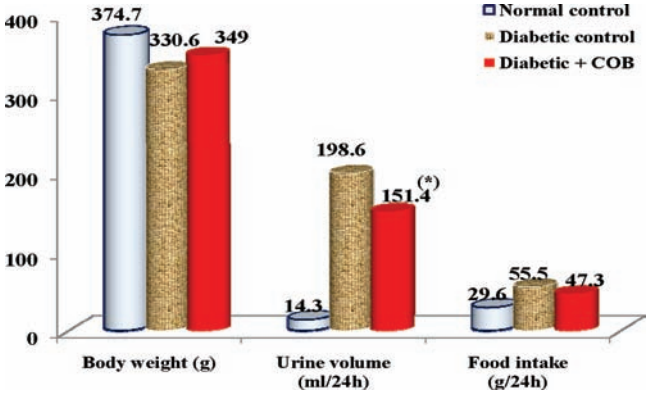

Figure 1. Changes in body weight (g), urine volume (mL/24 h), and food intake (g/24 h) of diabetic rats after 9 weeks of COB treatment. *$p < 0.05$ as compared with diabetic control.

Protective Effect of COB in β-Cells. In normal rats, most cells showed significant but slight hydropic degeneration and lymphoid cell infiltration in the peripheral parts of the islets of Langerhans (**Figure 2A**). In diabetic rats with no treatment, degenerative and necrotic changes were consistently found in the histological sections of pancreatic tissues stained with hematoxylin and eosin, in addition to shrinking of the islets of Langerhans (**Figure 2B**). The nuclei of necrotic cells showed either pyknosis or marginal hyperchromasia. There was hydropic degeneration and degranulation in the cytoplasm of the degenerative and necrotic cells, whereas some cells with pyknotic nuclei had a dark eosinophilic cytoplasm (coagulation necrosis) (**Figure 2B**). In diabetic rats treated with COB, the degenerative and necrotic changes in the islet of Langerhans parenchyma were less severe after 9 weeks of treatment than those in the diabetic group. However, a few cells showed significant but slight hydropic degeneration as compared to the islets of diabetic rats with no treatment, partly indicating degranulation of the islet cells (**Figure 2C**). There were more islets of Langerhans in

Table 3. Effect of Administration of 500 mg/kg bw/Day of the Aqueous Extract of COB for 9 Weeks on Serum Insulin Levels (ng/mL), Glucose Levels (mg/dL), and HbA1c (%) in STZ-Induced Diabetic Rats[a]

marker	week	normal rat	diabetic control	diabetic + COB
HbA1c (%)	initial	2.95 ± 0.25 a	8.02 ± 0.64 b	7.85 ± 0.41 b
	week 9	3.15 ± 0.09 a	11.12 ± 1.11 d	9.6 ± 0.41 c
	increase rate (%)		+3.10	+1.75
insulin (ng/mL)	initial	1.65 ± 0.81 a	0.80 ± 0.42 b	0.76 ± 0.67 b
	week 9	1.63 ± 0.62 a	0.51 ± 0.05 c	1.01 ± 0.46 b
	increase rate (μg/mL)		−0.29	+0.25
glucose (mg/dL)	initial	97.1 ± 9.2 a	253.1 ± 53.6 b	237.5 ± 55.9 b
	week 9	97.5 ± 9.9 a	416.2 ± 62.3 c	220.2 ± 108.0 b
	increase (mg/dL)		+163.1	−17.3

[a] Each value is expressed as the mean ± SD ($n = 7$). Means in the same column or row with different letters are significantly different ($p < 0.05$; Duncan's multiple range *t* test).

Table 1. Effect of Administration of 500 mg/kg bw/Day of the Aqueous Extract of COB for 9 Weeks on Serum Insulin Levels (ng/mL) of STZ-Induced Diabetic Rats[a]

group	before STZ	initial	week 3	week 6	week 9
normal rat	1.65 ± 0.97 a	1.65 ± 0.81 a	1.75 ± 1.11 a	1.61 ± 1.21 a	1.63 ± 0.62 a
diabetic rat	1.56 ± 1.25 a	0.80 ± 0.42 b	0.63 ± 0.38 b	0.48 ± 0.09 c	0.51 ± 0.05 c
diabetic rat + COB	1.78 ± 0.87 a	0.76 ± 0.67 b	0.89 ± 0.52 b	0.94 ± 0.46 b	1.01 ± 0.46 b

[a] Each value is expressed as the mean ± SD ($n = 7$). Means in the same column with different letters are significantly different ($p < 0.05$; Duncan's multiple range *t* test).

Table 2. Effect of Administration of 500 mg/kg bw/Day of the Aqueous Extract of COB for 9 Weeks on Blood Glucose Levels (mg/dL) of STZ-Induced Diabetic Rats[a]

group	before STZ	initial	week 3	week 6	week 9
normal rat	103.2 ± 10.2 a	97.1 ± 9.2 a	108.7 ± 12.1 a	97.5 ± 13.8 a	97.5 ± 9.9 a
diabetic rat	111.4 ± 13.4 a	253.1 ± 53.6 b	354.9 ± 68.1 c	425.9 ± 32.1 c	416.2 ± 62.3 c
diabetic rat + COB	98.6 ± 18.9 a	237.5 ± 55.9 b	189.0 ± 36.1 b	217.0 ± 89.5 b	220.2 ± 108.0 b

[a] Each value is expressed as the mean ± SD ($n = 7$). Means in the same column with different letters are significantly different ($p < 0.05$; Duncan's multiple range *t* test).

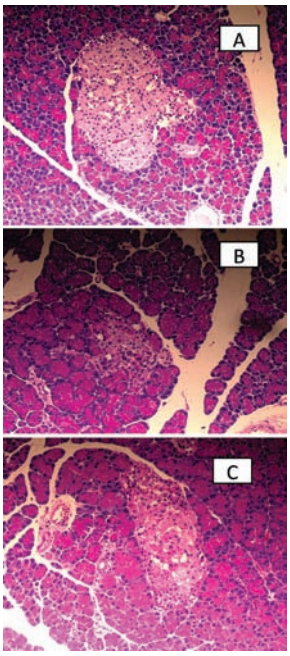

Figure 2. Histopathology of islets of Langerhans. (**A**) Islets of Langerhans of normal rats. (**B**) Shrunken islets of Langerhans displaying degenerative and necrotic changes in diabetic rats with no COB treatment. (**C**) Islets of Langerhans displaying an increase in size and slight degenerative changes in the majority of cells of diabetic rats treated with COB for 9 weeks. Observed with a microscope at 200× magnification.

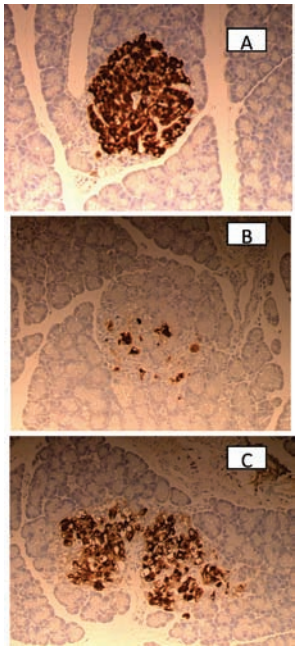

Figure 3. Immunohistochemical staining of pancreatic tissues. (**A**) Strong insulin antigen reactivity in β-cells of normal rats. (**B**) No insulin-immunoreactive β-cells observed in the islet of Langerhans of diabetic rats with no COB treatment. (**C**) Increased number of insulin-immunoreactive β-cells and granules of diabetic rats after 9 weeks of COB administration. Observed with a microscope at 200× magnification.

COB-treated rats than the STZ-treated rats. There was also lymphoid cell infiltration in the peripheral portions of the islets of Langerhans in diabetic rats with COB treatment.

In the immunohistochemical staining of the pancreatic tissues of normal rats, an insulin antigen positive area was strongly observed in the β-cells of the islets (**Figure 3A**). In diabetic rats with no treatment, there were no insulin-immunoreactive β-cells in the islet of Langerhans (**Figure 3B**). In diabetic rats treated with COB, the numbers of both insulin-immunoreactive β-cells and their granules were higher than those of untreated diabetic rats (**Figure 3C** and **Tables 4** and **Table 5**).

Table 4. Comparisons of the Areas of the Insulin Immunoreactive β-Cells in the Islets of Langerhans[a]

group	*N*	area (μM^2)	%	maximum area (μM^2)
normal rat	50	62.2 ± 71.9 a	86.4	261.9
diabetic control	50	17.4 ± 23.5 b	24.2	90.3
diabetic + COB	50	35.0 ± 30.6 c	48.7	125.0

[a] Values are expressed as the means ± SDs with $n = 50$ islets of Langerhans for the three groups. Values in each column with different letters are significantly different at $p < 0.05$ as analyzed by the Kruskal−Wallis test.

Table 5. Semiquantitative Analysis of Immunohistochemical Staining of Insulin in β-Cells in Pancreatic Islets[a]

		islet number		
group	islet number/section	weak	moderate	strong
normal rat	203.0 ± 88.9 a	10.7 ± 7.4 a	12.7 ± 9.0 a	179.9 ± 73.5 a
percentage (%)		**5.3**	**6.2**	**88.5**
diabetic control	81.4 ± 23.5 b	45.0 ± 13.6 b	6.1 ± 1.1 b	30.3 ± 12.3 b
percentage (%)		**55.3**	**7.5**	**37.2**
diabetic + COB	122.0 ± 29.9 c	47.1 ± 13.6 b	23.0 ± 11.9 a	51.9 ± 17.5 c
percentage (%)		**38.6**	**18.8**	**42.6**

[a] Values are expressed as the means ± SD with n = 7 animals for all groups. Values in each column with different letters are significantly different at p < 0.05 as analyzed by the Kruskal−Wallis test.

Table 6. Anticataract Effect of Administration of 500 mg/kg bw/Day of the Aqueous Extract of COB for 9 Weeks on Diabetic Rat Lenses

marker	normal rat	diabetic control	diabetic + COB
cataract formation (%)[a]	0	55	10
TBARS (nmol MDA/g lens)	59.3 ± 16.4 a	142.3 ± 29.5 b	89.5 ± 25.4 a
glucose (μmol/g lens)	0.16 ± 0.09 a	2.76 ± 0.35 b	1.02 ± 0.56 c
fructose (μmol/g lens)	0.19 ± 0.08 a	9.74 ± 1.82 b	6.32 ± 1.08 c
sorbitol (μmol/g lens)	1.34 ± 0.39 a	17.45 ± 3.23 b	12.78 ± 4.67 c

[a] Degree of cataract formation after 9 weeks, as compared to the normal group. Values in each row with different letters are significantly different at $p < 0.05$ as analyzed by Duncan's multiple range t test.

Table 7. Effects of COB Extract on Antioxidation of Diabetic Rats after 9 Weeks of Administration[a]

marker	normal rat	diabetic control	diabetic + COB
LPO (nmol MDA/L serum)	5.12 ± 0.67 a	14.42 ± 1.91 b	9.92 ± 1.63 c
8-OHdG (nmol/24 h urine)	2.56 ± 0.63 a	6.67 ± 2.64 b	4.42 ± 1.93 b
vitamin E (mg/dL serum)	1.72 ± 0.64 a	3.87 ± 1.06 b	2.99 ± 0.36 b
vitamin A (mg/dL serum)	0.22 ± 0.10 a	0.20 ± 0.02 a	0.23 ± 0.06 a
vitamin C (mg/dL serum)	1.13 ± 0.38 a	0.87 ± 0.12 b	1.03 ± 0.12 c

[a] Each value is expressed as the mean ± SD (n = 7). Values in each row with different letters are significantly different at p < 0.05 as analyzed by Duncan's multiple range t test.

Anticataract Effect of COB in STZ-Diabetic Rats. Table 6 shows the diabetic rats treated for 9 weeks with COB had significantly decreased levels of glucose, fructose, and sorbitol in the lens ($p < 0.05$). The observation of cataract formation showed that the degree of cataract formation of the COB-treated groups was significant lower than that of the control diabetic group. Moreover, a marked reduction in the LPO level in the lens was observed in COB-treated rats ($p < 0.05$).

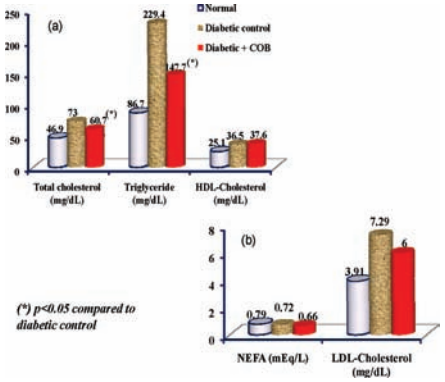

Figure 4. Effect of administration of 500 mg/kg bw/day of the COB extract for 9 weeks on the serum lipid profile. NEFA, nonesterified free fatty acids.

Table 8. Effects of COB on Liver and Kidney Function of Diabetic Rats after 9 Weeks of Administration[a]

marker (in blood)	normal rat	diabetic control	diabetic + COB
protein (g/dL)	4.76 ± 1.64 a	4.93 ± 0.47 a	5.34 ± 0.28 a
creatinine (mg/dL)	0.30 ± 0.11 a	0.25 ± 0.05 a	0.27 ± 0.04 a
BUN (mg/dL)	21.3 ± 9.7 a	35.6 ± 5.9 b	27.3 ± 6.7 c
GOT (IU/L)[b]	76.4 ± 30.6 a	145.0 ± 84.7 b	89.3 ± 15.4 b
GPT (IU/L)[b]	52.1 ± 26.7 a	93.3 ± 26.6 b	63.9 ± 15.9 c

[a] Each value is expressed as the mean ± SD (n = 7). Values in each row with different letters are significantly different at $p < 0.05$ as analyzed by Duncan's multiple range t test. [b] Using nonparametric analysis.

Antihyperlipidemic and Antioxidant Effects of COB. We observed significantly decreased levels of LPO in the serum from COB-treated diabetic rats after 9 weeks of administration (**Table 7**). In addition, there was a statistically significant increase of vitamin C and vitamin E levels in the serum of COB diabetic rats, but the changes in vitamin A levels in the serum were not significant. The urinary 8-OHdG levels of COB-treated diabetic rats showed a decreasing trend as compared with the control diabetic rats.

In this study, the administration of COB to the diabetic rats significantly decreased the total cholesterol ($p < 0.05$) and triglyceride levels ($p < 0.05$) in the plasma as compared with the diabetic control group, which is shown in **Figure 4**. Moreover, after 9 weeks of COB treatment, the total cholesterol level of the COB-treated diabetic rats was normal as compared with the normal rats ($p > 0.05$). In addition, the COB treatment caused slight decreases in the total fatty acid and LDL cholesterol levels as compared with the control diabetic group, but these changes were not statistically significant.

Table 8 shows the significant decreases in the BUN and GPT levels in COB-treated diabetic groups as compared with the control diabetic group. The GOT level of the COB-treated group was lower, but this difference was not statistically significant. The plasma protein and creatinine levels of the COB-treated diabetic group did not differ in comparison to the control diabetic rats.

DISCUSSION

Hyperglycemia is the principal cause of the life-threatening complications associated with diabetes mellitus. Effective blood glucose control is the key to preventing or reversing diabetic complications and improving the quality of life for diabetic patients. In our previous study, we demonstrated that administration of an aqueous extract of COB restored the hyperglycemia, hyperlipidemia, oxidative stress, and sorbitol accumulation phenotypes in STZ-diabetic rats. Moreover, a series of in vitro studies revealed that COB exerted antihyperglycemic, antihyperlipidemia, and antioxidant effects via inhibition of carbohydrate-hydrolyzing enzymes, pancreatic lipase, and LPO. However, in our previous animal experiment, treatment of fasting STZ-diabetic rats with a single dose of COB for 6 h (*19*) showed a reduction in fasting blood glucose, which uncovered the possibility that the COB extract could stimulate the release of insulin in the pancreas or reduce insulin resistance in peripheral tissues. The present study indicated that the antihyperglycemic action of COB could be partly due to amelioration in the β-cells of pancreatic islets, causing an increase in insulin secretion in addition to the α-glucosidase inhibitory activity of the COB extract that was found in the previous study. In addition, an anticataract effect of COB was also observed in the present study, which when combined with the evaluation of other biochemical parameters, shows that COB possesses antihyperglycemia, antihyperlipidemia, and antioxidant activities.

Although the cytotoxic action of STZ in β-cells is not fully understood, the STZ-induced breakage of DNA is probably associated with the alkylation activity of STZ (*14*) or nitric oxide production (*24*), which is thought to be mediated by the inhibition of free radical scavenging enzymes that enhance the production of the superoxide radical. The latter has been implicated in LPO, DNA damage, and sulfhydryl oxidation. In the present study, COB treatment caused a decrease in the elevation of plasma glucose levels and an increase in the depressed plasma insulin concentrations in STZ-diabetic rats. In histopathology and immunohistochemical staining of islets of Langerhans of COB-treated diabetic rats, almost all of the insulin-positive β-cells were degranulated, degenerated, or necrosed. It has been suggested that the active agents from plant sources might act by several mechanisms, such as stimulating insulin secretion, increasing repair/proliferation of β-cells, enhancing the effect of insulin and adrenaline, and increasing antioxidative capability (*25*). This study is the first to show a partial amelioration in the β-cells of pancreatic islets in diabetic rats treated with COB. As Yamamoto et al. have shown, injection of STZ into fetuses in utero caused β-cell destruction, but the β-cells regenerated after 48 h (*26*); therefore, young animals may recover from STZ damage. However, we used 7 week old rats in this experiment, so the rats recovered using the COB feeding.

It has been suggested that oxidative stress is involved in the onset and progression of diabetes (*27*). Reactive oxygen species have been reported to interfere with insulin signaling at various levels and to inhibit the translocation of glucose transporter IV in the plasma membrane (*27*). On the other hand, the oxidative stress induced by chronic hyperglycemia plays a key role in β-cell dysfunction and insulin resistance (*28*). Protective effects of nutrient antioxidants, including flavonoids, on diabetes have also been reported (*29*). The antioxidant activity of COB has been reported in vitro and in diabetic rats (*17*, *19*). In this study, COB was also found to decrease serum LPO in STZ-diabetic rats, in addition to maintaining plasma insulin levels and possessing antihyperglycemic activity. COB was also found to decrease 8-OHdG and vitamin E, which were increased by STZ treatment; it was shown that the vitamin E level was increased in human diabetes patients (*30*); therefore, COB will be effective for human diabetes. Our results indicated that decreased blood glucose levels together with stabilization of serum insulin levels by COB treatment might be due to partial regeneration/proliferation of the pancreatic β-cells or protection of the pancreatic β-cells from damage. The antioxidative effect of COB might be responsible for the reduction in the oxidative cytotoxic status of the β-cells, which leads to protection of the pancreatic β-cells and insulin deficiency. However, extra-pancreatic mechanisms, such as reduced insulin

resistance in peripheral tissues, enhanced glucose transport into cells, and improved formation of glycogen in the liver might be involved in the COB-induced decrease in the blood glucose concentration of STZ-diabetic rats.

High glucose levels are associated with nonenzymatic glycation, the accumulation of sorbitol via the aldose-reductase pathway, and the increase in LPO. The generation of reactive oxygen species may be a common downstream mechanism by which the multiple byproducts of glucose adversely affect blood vessels. Oxidative stress is thought to be a major factor in cataract formation in both diabetic and nondiabetic aging humans; therefore, dietary antioxidants may be a useful tool for assessing the efficacy of treatments that may slow or prevent cataract formation (*31*). In our previous study, we reported that the COB extract had a strong antioxidant activity in vitro and in vivo. In the present study, the antihyperglycemic effect of COB might also contribute to the reduction in oxidative stress in diabetic rats. In addition, reductions in LPO in the lens and serum, glucose levels, and sorbitol accumulation in the lenses of the COB-treated diabetic group were also shown in the present study. Consequently, the COB extract seems to prevent oxidative damage and was found to delay the development of cataracts.

Aldose reductase, the key enzyme of the polyol pathway, and oxidative stress are known to play important roles in the complications of diabetes. Matsuda et al. (*32*) have found a structure−activity correlation of various flavonoids, such as quercetin, luteolin, flavone, flavanone, etc., with the potency of inhibition of aldose reductase in rat lenses. Lim et al. (*33*) have reported that chalcones inhibited aldose reductase activity and reduced sorbitol accumulation in STZ-induced diabetic rat tissues. Ye et al. (*34*) reported that COB has a high amount of chalcone. Gp-1447, an aldose reductase inhibitor (*35*), the seed of *Nigella sativa* (*36*), and quercetin (*37*) prevented and protected against STZ-induced oxidative stress and β-cell damage. Therefore, more evidence is needed to clarify the effects of the COB extract and its bioactive flavonoids on the polyol pathway to characterize the anticataract effect of COB.

Tocopherol and ascorbic acid are nonenzymatic antioxidants. The α-tocopherol reduces lipid hydroperoxides generated during the process of peroxidation and protects cell structures against damage (*38*). The lipid oxidation level in serum increased in diabetes and was also found in this study. The higher vitamin E concentration in diabetic rats may be due to an increase in the demand using α-tocopherol as an antioxidant defense against increased LPO in blood. However, in this study, the decreased level of ascorbic acid observed in diabetic rats may be due either to increased utilization as an antioxidant defense against increased reactive oxygen species or to a decrease in glutathione level, since glutathione is required for the recycling of ascorbic acid (*39*). Vitamin C or ascorbic acid is an excellent hydrophilic antioxidant in plasma and disappears faster than other antioxidants on exposure to reactive oxygen species (*40*, *41*).

The hyperglycemia in diabetes that causes cellular injury and death is caused by a process known as oxidative stress and leads to diabetic complications, such as cataracts (*42*). The observed decreased levels of oxidative damage after COB treatment could simply be an indirect effect of its antihyperglycemic effect. In this animal model of type 1 diabetes, COB appears to be equally effective at improving the diabetic state by means of antihyperglycemic and antihyperlipidemic effects, which in turn inhibits the biochemical indicators of diabetes. In the present study, a series of biochemical parameters, such as Cr, BUN, GOT, and GPT, were also examined. We showed that COB-treated diabetic rats experienced a marked improvement in liver and kidney function as compared to the control diabetic rats after 9 weeks of COB treatment. The role of insulin in the regulation of lipid metabolism in diabetes has been reported by Reaven and Chen (*43*). The insulin-improving effect of COB might positively affect lipid metabolism of the diabetic rats in this study. Moreover, improvements in body weight, urine volume, and food intake of the COB-treated diabetic rats as compared to the control diabetic rats may be attributed to the ability of the COB extract to significantly improve glucose homeostasis. This observation is in agreement with our previous reports (*19*).

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