Anti-Hyperglycemic Activity of an Aqueous Extract from Flower Buds of Cleistocalyx operculatus (Roxb.) Marr and Perry

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A screening of 5 plants used for making drinks in Vietnam revealed a Cleistocalyx operculatus (Roxb.) Marr and Perry flower bud extract to have the highest inhibitory activity against the α-glucosidase enzyme. The anti-hyperglycemic effects of an aqueous extract from flower buds of Cleistocalyx operculatus (CO), a commonly used material for drink preparation in Vietnam, were therefore investigated in vitro and in vivo. In vitro, the CO extract inhibited the rat-intestinal maltase and sucrase activities, with IC50 values of 0.70 and 0.47 mg/ml, respectively. These values are lower than those for a guava leaf extract (GE; IC50 0.97 and 1.28 mg/ml, respectively). Postprandial blood glucose testing of normal mice and STZ-induced diabetic rats by maltose loading (2 g/kg body weight (bw)) showed that the blood glucose reduction with CO (500 mg/kg bw) was slightly less than that with acarbose (25 mg/kg bw) but was more potent than that with GE (500 mg/kg bw). In an 8-week experiment, the blood glucose level of STZ diabetic rats treated with 500 mg of CO/kg bw/day was markedly decreased in comparison with that of non-treated diabetic rats. Consequently, CO is considered to be a promising material for preventing and treating diabetes.

Key words: Cleistocalyx operculatus; α-glucosidase inhibition; anti-hyperglycemic activity; diabetes

Diabetes mellitus is a serious, non-communicable disease characterized by hyperglycemia, with a rising incidence in both developed and developing countries.1-3 It is therefore necessary to find new approaches to managing this health challenge. One goal of dietary therapy for diabetic patients is the maintenance of normal blood glucose levels, including control of postprandial increases in blood glucose. Synthetic α-glucosidase inhibitors such as acarbose and miglitol are known to reduce postprandial hyperglycemia primarily by blocking the action of the α-glucosidase enzyme in the small intestine, thereby delaying glucose absorption.4 Stabilization of blood glucose is important for diabetic patients, because it prevents hyperglycemia and the complications associated with diabetes.5

In recent years, research on traditional medicinal plants for the management of diabetes has attracted the interest of scientists.6 More than 400 kinds of plant with blood glucose-lowering potential are known.7,8 A number of plants are known to exert their anti-hyperglycemic activity via the inhibition of carbohydrate-hydrolyzing enzymes in the small intestine. These include guava leaves,9 Commelina communis leaves,10 Punica granatum flowers,11 and Eugenia jambolana fruits.12 The polyphenols in plants reportedly play an important role in the mechanism for regulating the activities of carbohydrate-hydrolyzing enzymes.13-16 To date, there have been few studies on the polyphenolic contents of Vietnamese plants,17 and no scientific information about such plants which can inhibit carbohydrate-hydrolyzing enzymes is available. We therefore investigated 5 common plants used for making drinks in Vietnam for their α-glucosidase inhibitory activities in vitro as well as their polyphenolic contents. We found that an aqueous extract from flower buds of Cleistocalyx operculatus exhibited strong inhibitory activity against α-glucosidase.

Cleistocalyx operculatus (Roxb.) Marr and Perry (CO), also known by the scientific name of Eugenia operculata Roxb. (belonging to the Myrtaceae family), is commonly called Voi in Vietnam. CO is a large green tree which grows in rural areas of North Vietnam. Like green tea, the flower buds (Nu Voi) and leaves (La Voi) of CO have been used by Vietnamese to make a beverage since ancient times. However, there have been no studies reported on the anti-hyperglycemic activity of an aqueous extract of the flower buds of CO. Hence, the purpose of this study is to evaluate the in vivo anti-hyperglycemic activity of an aqueous extract of CO flower buds. We also investigated its mechanism of action in vitro to obtain scientific evidence for the development of CO as a potential beverage for the prevention and treatment of diabetes.

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Materials and Methods

Screening of plant materials. Five plants commonly used for making drinks, *Cleistocalyx operculatus* flower buds (Nu Voi), *Camellia sinensis* leaves (La Che xanh), *Psidium guajava* leaves (La Oi), *Nelumbo nucifera* leaves (La Sen) and *Sophora japonica* flowers (Hoa Hoe), were purchased from markets in Vietnam. After their collection, the raw materials were washed with water, lyophilized, and then ground into a fine powder. Aqueous extraction was conducted on 100 mg of each powder dissolved in 5 ml of distilled water and then boiled for 2 h. After centrifugation at 5,600 × g for 15 min at 4 °C, each aqueous extract was obtained. The sample was extracted three times and stored at −80 °C until needed.

Preparation of the CO extract for in vitro and in vivo experiments. The dried CO flower buds used in this experiment were collected from the Red River Delta market in Vietnam. One hundred grams of ground dried flower buds were extracted by boiling for 30 min in 2 liters of distilled water. After centrifugation, the extracted solution was lyophilized, and a yellow-brown powder was obtained that was stored at −80 °C until needed. The yield of the aqueous extract (w/w from the dried material) was 19.6%. The aqueous extract of guava leaves (GE) used as a positive control in this study was prepared under similar conditions.

Determination of the polyphenolic content. The polyphenol amount in each aqueous extract was determined according to the Folin-Ciocalteu colorimetric method, as modified by Thu et al. Briefly, to 50 μl of each sample, 250 μl of the Folin-Ciocalteu reagent and 750 μl of 10% Na₂CO₃ were added, before incubating at room temperature for 2 h. The absorbance of each sample was measured spectrophotometrically at 756 nm, results being expressed as milligrams of catechin equivalent per gram dry weight of the material (mg of catechin/g dw). Each sample was measured five times.

Assay of α-glucosidase inhibition in vitro. The assay procedure was performed according to the method described by Honda et al. with some modifications, rat intestinal acetone powder (Sigma Chemical Co., USA) being extracted as the source of α-glucosidase. The rat intestinal acetone powder was carefully homogenized with a 0.1 M maleate buffer at pH 6.9 (5 mg/ml) and then centrifuged at 5,600 × g for 15 min (4 °C). The supernatant was used as a crude enzyme solution and diluted at a ratio of 1:2 with the 0.1 M maleate buffer (pH 6.9) before the experiment. The enzyme solution (200 μl) was premixed with the sample extract (100 μl) for 15 min at 37 °C. Two hundred microliters of 2 mM p-nitrophenyl-α-D-glucoside (pNPG) as a substrate in a phosphate buffer at pH 6.9 was added to the mixture to start the reaction. The mixture was incubated at 37 °C for 30 min, and the reaction was stopped by boiling for 10 min before adding 1 ml of 0.1 M Na₂HPO₄. The α-glucosidase activity was determined by measuring the p-nitrophenol released from pNPG at 400 nm. All tests were done three times. The percentage of α-glucosidase enzyme inhibition by the sample was calculated by the following formula: \[ \% \text{ inhibition} = \frac{AC - AS}{AC} \times 100 \], where AC is the absorbance of the control and AS is the absorbance of the tested sample.

Assays of maltase and sucrase inhibitory activities by CO in vitro. The crude enzyme solutions of maltase and sucrase from rat small intestinal acetone powder were prepared as just described. Maltose and sucrose were purchased from Wako Chemical Co., Japan. The experimental procedures was according to the method of Matsui et al. with some modifications. Briefly, 50 μl of each enzyme solution was pre-incubated with 50 μl of a sample solution for 10 min at 37 °C. Fifty microliters of 2% maltose or 2% sucrose used as a substrate in a 0.1 M maleate buffer at pH 6.9 was added to the mixture to start the reaction. The enzymatic reaction was allowed to proceed at 37 °C for 30 min (maltase assay) or for 60 min (sucrase assay), before being stopped by heating at 100 °C for 5 min. The solution was then kept in an ice bath. The glucose generated was measured with a commercial assay kit (Glucose C II-test, Wako Pure Chemicals, Osaka, Japan). Each CO sample extract diluted with the 0.1 M maleate buffer (pH 6.9) was added in the final concentration range of 0.01–10 mg/ml. A guava leaf extract and the drug, acarbose (Wako Pure Chemicals, Osaka, Japan), were used as positive controls in equivalent concentrations. The final concentration of an inhibitor in the reaction mixture required to inhibit 50% of enzyme activity under the foregoing assay conditions is defined as the IC₅₀ value.

Animals. Twenty four male Wistar rats (7 weeks old, 150–170 g) and 24 male ICR mice (6 weeks old, 28–30 g) were purchased from Nippon Clea Co. The animals were fed on a standard diet (CE-2, Nippon Clea Co.) and tap water *ad libitum*. 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, all 24 rats, weighing now 280–300 grams, were injected with a single dose of streptozotocin (STZ; 50 mg/kg bw) dissolved in saline. Seven days after the STZ injection, those rats with a serum glucose level higher than 180 mg/dl were considered to be diabetic and were used for the subsequent experiment. Normal mice and the diabetic rats were each randomly assigned to 4 groups, each group consisting of 6 animals: control, CO, GE and acarbose. The GE and acarbose groups served as positive controls. 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 guidelines of the
Food and Nutrition Department at Japan Women’s University.

Effect on the postprandial blood glucose level in maltose-loaded normal mice and STZ-induced diabetic rats. All 4 groups of animals underwent an oral maltose tolerance test. After a 14-h overnight fast, the four groups of normal mice and the four groups of diabetic rats were respectively given saline only, or with CO (500 mg/kg bw), GE (500 mg/kg bw) or acarbose (25 mg/kg bw); 30 min later, they received maltose at 2 g/kg of body weight. Each test sample extract (suspended in 1 ml of saline) and substrate solution was orally administered to the animals via oral gavage. Blood samples were collected from the tail vein of each animal at 0-time, prior to sample, acarbose or saline oral loading, and 30, 60 and 120 min after the oral maltose loading. The blood glucose level was measured with a glucose test kit (Wako Pure Chemical Industries, Osaka, Japan).

Effects of a single CO dose (500 mg/kg bw) on the fasting blood glucose level in diabetic rats. To investigate the effect of the CO extract on the fasting blood glucose level, 2 groups of diabetic rats (control and CO-treated) were used for this experiment. Prior to the administration, a 0-min blood sample was taken from 14-h-fasted rats. The diabetic rats in the CO group each received a single oral dose of 1.0 ml of the CO extract (500 mg/kg bw in saline), and the control diabetic rats each received an equal amount of saline via oral gavage. Blood samples were collected from the tail vein 2 h, 4 h, 6 h and 8 h after the administration. The blood glucose level was determined with a glucose test kit from Wako.

Effects of administering CO for 8 weeks on the blood glucose level in normal mice and STZ-rats. Four groups, control normal mice, CO-treated mice, control diabetic rats and CO-treated diabetic rats, were used for this experiment. The control normal mice and control diabetic rats were each given 1.0 ml of saline, while the CO-treated normal mice and CO-treated diabetic rats received 1.0 ml of the CO extract (500 mg/kg bw in saline) once daily for 8 weeks via oral gavage. Blood was periodically taken from all 14-h-fasted animals (given tap water ad libitum only), before the treatment and 2, 4, 6 and 8 weeks after the treatment to assess the change in blood glucose level.

Analysis of CO polyphenols extracted by HPLC. A CO water extract was prepared to determine the polyphenol profile. One hundred milligrams of dried ground CO was extracted with 5 ml of water for 2 h at 80 °C. After centrifugation at 5,600 × g for 15 min, the supernatant was diluted 10 fold with distilled water and then filtered. This CO extract was analyzed by HPLC within 24 h with Shimadzu LC-10A apparatus. Distilled water and acetonitrile were used for the mobile phases. A YMC-pack ODS-AM (150 × 4.6 mm I.D.) column was eluted by linear gradient of 10% to 20% of acetoneitrile for 20 minutes. The flow rate was 1 ml/min, detection was set at 220 nm, and the injection volume was 20 μl. (+)-Catechin (Wako Pure Chem., Japan) was quantified by the external standard method.

Statistical analysis. Each value is expressed as the mean ± standard error (SEM). Differences among groups at various times of the experiment were subjected to a one-way analysis of variance (ANOVA) followed by Duncan’s multiple-range t-test. Statistical comparisons between the two groups were analyzed by using the Independent Samples t-test. A value of p < 0.05 is considered to represent a statistically significant difference.

Results

Alpha-glucosidase inhibitory activities of five plants and their polyphenolic contents

The inhibitory activities of the five aqueous sample extracts on α-glucosidase are shown in Table 1. These inhibitory activities ranged from 47.5% to 68.2% as compared to that of the control. The highest inhibitory activity was seen with the flower buds of CO (Nu Voi), followed by green tea leaves (La Che xanh), guava leaves (La Oi), lotus leaves (La Sen) and the flowers of the Japanese pagoda tree (Hoa Hoe). The polyphenol amounts in drinks made of these five plants ranged from 83.5 to 122.8 mg of catechin equivalent/g of dry material (Table 1). The polyphenolic contents were high in guava leaves and CO flower buds, at 122.8 and 122.5 mg catechin/g of the dried samples, respectively.
**CO extract inhibited maltase and sucrase activity in vitro**

As shown in Fig. 1A and B, the CO extract, GE and acarbose dose-dependently inhibited both maltase and sucrase. In the present study, guava leaves were used as a positive control and effectively inhibited the maltase and sucrase activity with IC$_{50}$ values of 0.97 mg/ml and 1.28 mg/ml, respectively. Interestingly, the CO extract inhibited both maltase and sucrase more effectively than GE with IC$_{50}$ values of 0.70 mg/ml and 0.47 mg/ml, respectively. These results indicate that the bioactive compounds in the CO extract were more potent than those in GE in terms of inhibiting the activities of these two enzymes. However, the inhibitory effect of the CO extract on these enzymes was lower than that of acarbose, which had IC$_{50}$ values for maltase and sucrase of 0.10 mg/ml and 0.08 mg/ml, respectively (Fig. 1).

**Effect of the CO extract on the postprandial blood glucose level in maltose-loaded normal mice and STZ-induced diabetic rats**

The in vitro results prompted us to examine the inhibitory effect of an aqueous extract of the CO flower buds (500 mg/kg bw) on the postprandial blood glucose level in normal mice and STZ-induced diabetic rats. Arcarbose (25 mg/kg bw) and GE (500 mg/kg bw) were the positive controls used in this experiment. Figure 2A and B show the effect of the CO extract on the postprandial blood glucose level in both normal mice and STZ-induced diabetic rats after the oral loading with 2 g/kg bw of maltose. The assay for normal mice showed that the blood glucose level of the CO-treated group was significantly (p < 0.028) lower than that of the control group after 30 min (Fig. 2A). The blood glucose level of the CO-treated normal mice after 30 min was slightly higher than that of the acarbose-treated normal mice. In comparison with the normal control mice, the postprandial blood sugar level of the normal mice treated with CO (p < 0.028) was more effectively suppressed than that of the normal mice treated with GE (p < 0.039). Similar results were obtained with the diabetic rats. Figure 2B shows that, in comparison with the diabetic control rats, the blood glucose level of the CO extract-treated diabetic rats was significantly lower 30 min (p < 0.05) and 60 min (p < 0.01) after maltose loading. The acarbose-treated diabetic rats also showed a tendency for the blood glucose level to be reduced after 30 min (p < 0.01) and 60 min (p < 0.05), while the GE-treated diabetic rats showed a smaller effect with p < 0.043 at 30 min and p < 0.26 at 60 min.

**Effect on fasting blood glucose level in diabetic rats given a single dose of the CO extract (500 mg/kg bw)**

Table 2 shows the effect on fasting blood glucose level in diabetic rats given a single dose of the CO extract (500 mg/kg bw). The maximum effect was seen within 4–6 h after the oral administration of the CO extract. In this experiment, the fasting blood glucose level of the CO-treated diabetic rats was significantly decreased 2 h (p < 0.05), 4 h (p < 0.01) and 6 h (p < 0.01) after a single dose of CO, as compared to their
After a 14-h fast, 4 groups of normal mice (or diabetic rats) were respectively given saline, the CO extract (500 mg/kg bw), GE (500 mg/kg bw) or acarbose (25 mg/kg bw). The blood glucose level was measured 0, 30, 60 and 120 min after an oral maltose loading of 2 g/kg of body weight. Each value is expressed as the mean ± SEM (n = 6). Statistically significant difference (\( p < 0.05 \); \( p < 0.01 \)) compared among the groups at each time-point (Duncan’s multiple-range t-test).

**Table 2.** Effect of the Aqueous Extract of **Cleistocalyx operculatus** Flower Buds (CO) (500 mg/kg bw) on the Fasting Blood Glucose Level of STZ-Induced Diabetic Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood glucose level (mg/dl) at different times after oral administration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0h</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>250.3 ± 15.5*</td>
</tr>
<tr>
<td>Diabetic + CO</td>
<td>244.7 ± 23.5*</td>
</tr>
</tbody>
</table>

Each value is expressed as the mean ± SEM (n = 6). Values in a column with different superscript letters are significantly different: *\( p < 0.05 \); **\( p < 0.01 \) compared to the control group at each time point (Independent Samples t-test). Significant difference from the 0-h value of the respective group at each time point: *\( p < 0.05 \); **\( p < 0.01 \) (Duncan’s multiple-range t-test).

**Table 3.** Effect of an 8-Week Administration of 500 mg/kg bw/day of the Aqueous Extract of **Cleistocalyx operculatus** (CO) on the Blood Glucose Level (mg/dl) in Normal Mice and STZ-Induced Diabetic Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial</th>
<th>2nd week</th>
<th>4th week</th>
<th>6th week</th>
<th>8th week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal mice</td>
<td>114.7 ± 15.5*</td>
<td>119.0 ± 12.1*</td>
<td>120.8 ± 10.4*</td>
<td>118.0 ± 13.7*</td>
<td>117.5 ± 12.3*</td>
</tr>
<tr>
<td>Normal mice + CO</td>
<td>116.7 ± 18.2*</td>
<td>114.0 ± 10.2*</td>
<td>114.4 ± 8.1*</td>
<td>113.0 ± 10.9*</td>
<td>113.5 ± 8.3*</td>
</tr>
<tr>
<td>Diabetic rat</td>
<td>268.5 ± 23.4*</td>
<td>273.8 ± 23.1*</td>
<td>277.3 ± 22.4*</td>
<td>283.9 ± 20.2*</td>
<td>283.7 ± 22.7*</td>
</tr>
<tr>
<td>Diabetic rat + CO</td>
<td>271.6 ± 18.9*</td>
<td>240.1 ± 25.6**</td>
<td>216.9 ± 32.5***</td>
<td>217.1 ± 27.2***</td>
<td>211.3 ± 20.4****</td>
</tr>
</tbody>
</table>

Each value is expressed as the mean ± SEM (n = 6). Means in the same column with different superscript letters are significantly different (\( p < 0.05 \); **\( p < 0.01 \); ***\( p < 0.001 \)) compared to the initial value of the respective group at each time point (Duncan’s multiple-range t-test).

In the saline-treated control group, the fasting blood glucose level did not change during the 8 hours of this experiment. On the contrary, in the CO-treated group, the fasting blood glucose level changed markedly from zero time to 2h, 4 h and 6 h after the oral administration.

**Effect of an 8-week treatment with the CO extract on the blood glucose level of normal mice and STZ rats**

The results obtained with STZ diabetic rats given a single dose of CO prompted us to perform an 8-week pilot study, with a prolonged CO extract treatment (500 mg of CO/kg bw/day), on normal mice and diabetic rats. Table 3 shows the effect of the CO extract on the blood glucose level in the STZ rats during the 8 weeks of treatment. Initially, the blood glucose level did not differ significantly between the diabetic rats with and without the CO treatment. However, the blood glucose level in the treated diabetic rats was significantly lower in the 2nd, 4th, 6th and 8th week of treatment (\( p < 0.05 \);
Thus, the CO extract continued to have an anti-hyperglycemic effect for the entire 8 weeks of the experiment. In this experiment, healthy mice were treated with 500 mg of CO/kg bw/day, and the blood glucose level was determined before CO administration and 2, 4, 6 and 8 weeks after starting the treatment. However, there was no significant change in the blood glucose level in either group of normal mice before and after 8 weeks of CO, showing that the CO extract did not influence the blood glucose level in normal mice.

Besides the blood glucose level change, the body weight and urine volume also changed in the experimental diabetic rats (Fig. 3A and B). The body weight of the CO-treated diabetic rats tended to remain stable, while that of the control group decreased. Treatment with the CO extract produced a slight weight gain, but the increase was not statistically significant. Similarly, we found that the volume of urine from the diabetic rats during 8 weeks of CO treatment was significantly lower after 4, 6 and 8 weeks (p < 0.05) than in the control diabetic rats.

**Discussion**

Inhibition of the activity of carbohydrate-hydrolyzing enzymes plays an important role in the prevention and treatment of diabetes. The result of screening 5 plants used for making drinks in Vietnam indicated that a CO flower bud extract had the highest inhibition activity against the α-glucosidase enzyme. In this study, the hypoglycemic activity of an aqueous extract of the flower bud of CO was investigated both in vitro and in vivo.

Our findings show that the aqueous extract of the flower bud of CO had an inhibitory effect on α-glucosidase, maltase and sucrase in vitro (Table 1 and Fig. 1), the efficacy being better than that of a guava leaf extract. Deguchi et al. (1998)\(^\text{20}\) have reported that a guava leaf extract inhibited the activities of α-amylase, maltase and sucrase in vitro, and that the inhibitory effect on maltase activity was stronger than that on sucrase activity. In this experiment, we compared the inhibition of carbohydrate-hydrolyzing enzymes between CO and GE and found the GE results to be in accordance with the results reported by Deguchi et al.\(^\text{20}\)

These in vitro results demonstrate the bioactive components of CO to inhibit the rat-intestinal α-glucosidase, maltase and sucrase activities to be stronger than those of GE. The polyphenolic fractions of plants have long been recognized to inhibit carbohydrate-hydrolyzing enzymes in mammals. The inhibition of α-amylase, maltase and sucrase by a polyphenolic extract of green tea has been reported.\(^\text{13,14}\) Polyphenolic compounds derived from red cabbage, strawberries and raspberries are also inhibitors of α-amylase and α-glucosidase.\(^\text{16}\)

Our present results suggest that polyphenolic compounds of CO have a potentially important role in managing diabetes via the inhibition of α-glucosidase enzyme activities. Moreover, the presence and quantity of catechin in the CO extract were demonstrated by an HPLC analysis (data not shown). The amount of catechin was approximately 1% of the total polyphenolic content. Other polyphenols, which were present in larger amounts, might have accounted for the inhibitory effect of this extract on the α-glucosidase and anti-hyperglycemic activities in diabetic rats. The identification of these polyphenols is in progress.

The in vivo results suggest that the CO extract improved diabetes, possibly by normalizing the postprandial blood glucose level, a mechanism similar to that of acarbose (Fig. 2). Suppression of the postprandial blood glucose level in normal mice and diabetic rats was also observed in this study. Thus, the CO extract showed
a significant inhibitory effect on the α-glucosidase enzymes both in vitro and in vivo. Acarbose is a synthetic drug designed to suppress the activities of α-glucosidase enzymes, thereby slowing the appearance of sugar in the blood after a meal. In this study, despite the polyphenolic content of the CO extract being similar to that of GE (equivalent to 122.5 mg and 122.8 mg catechin/gram dry weight, respectively), the carbohydrate-hydrolyzing enzyme inhibitory activity as well as the suppression of postprandial blood glucose of the CO extract was higher than that of GE (Fig. 2), thus indicating that the bioactive compounds in the CO extract were more active than in the GE extract both in vitro and in vivo. Furthermore, treatment of the fasting STZ diabetic rats with a single dose of CO showed that the fasting blood glucose was reduced (Table 2). In this experiment, the fasting blood glucose level did not change in the saline-treated control group, but in the CO-treated group, the fasting blood glucose level changed markedly from the initial time to 2 h, 4 h and 6 h after the oral administration. These results indicate that the CO extract reduced the glucose level in fasting diabetic rats, in the complete absence of carbohydrate compounds during 6 hours. However, 6 h after the oral administration, the blood glucose tended to rise to the initial level in the CO group. Therefore, the effectiveness of the CO extract in lowering blood glucose appeared to be maximal for 6 h after its administration. Richard et al. have reported that green tea polyphenols not only inhibited the activity of carbohydrate-hydrolyzing enzymes but also enhanced the insulin activity. Similarly, Maroo et al. have demonstrated a mechanism whereby Enicostemma littorale lowered blood sugar via the stimulation of insulin release from the pancreas in diabetic rats. In this study, the reduction in fasting blood glucose by CO raised the possibility that the CO extract might have stimulated the release of insulin in the pancreas or reduced in insulin resistance in peripheral tissues. However, more evidence is needed to explain this phenomenon.

The results obtained from an 8-week pilot study with prolonged a CO extract treatment in normal mice and diabetic rats clarified more the anti-hyperglycemic effect of the CO extract (Table 3). After 8 weeks of CO treatment, restoration of the weight gain, reduction of blood glucose and urine volume were observed in diabetic rats. The changes in the blood glucose level, weight gain and urine volume of the CO-treated diabetic rats demonstrated that the anti-hyperglycemic effect of CO had a positive influence on the state of the diabetic rats (Fig. 3). This result is in accordance with the recent findings of Yadav et al. (2002) who have studied the anti-hyperglycemic activity of Murraya koenigii leaves in STZ-induced rats. A significantly decreased urine volume has also been recognized by Grover et al. who treated diabetic rats with Murraya koenigii leaves. In addition, we administrated CO at 500 mg/kg bw/day to normal mice and diabetic rats to observe whether any sign of toxicity would appear. No abnormality was seen in either the healthy mice or diabetic rats. The dosage of the CO extract used in our study (500 mg/kg bw) is in accordance with that of Deguchi et al. who studied the lowering of blood glucose level by a guava leaf extract in mice. Since CO has long been a common material in drinks in Vietnam, like green tea, CO is likely to be sufficiently safe for daily use. Further clinical studies are in progress to evaluate the anti-hyperglycemic effects of CO in healthy humans and diabetic patients with a reasonable dosage.

To date, guava leaves and green tea have been used as raw materials for functional foods, namely those foods for specific health use. The results of the present study suggest CO to have a potential role in the management of the pre-diabetic state and in diabetes, based on its ability to control the blood glucose level. Thus, there is possibility for the development of Cleistocalyx operculatus as a beverage for preventing and treating diabetic patients in the future.

References


