Papers

Efficacy of an Aqueous Extract from Flower Buds of *Cleistocalyx Operculatus* on Type 2 Diabetic Patients in Vietnam

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The aim of the present study was to assess the ability of an aqueous extract of *Cleistocalyx operculatus* flower buds (COB) in type 2 diabetic patients to provide scientific evidence for development of COB tea as a potential natural oral hypoglycemic functional food. We assessed the improvement in glucose metabolism in diabetic patients upon drinking COB tea. The study was conducted in 65 diabetic patients, with a single ingestion of either COB extract or the placebo. The levels of blood glucose and HbA1c, decreased markedly in the COB group, compared to the initial time and to the placebo group. The concentration of serum insulin was improved significantly compared to the initial time and to the placebo group. We also determined the suppressibility of COB extract to the post prandial blood glucose level on diabetic patients. After they drank 150 ml of COB tea before and while eating 170 g of cooked rice, the increase of the postprandial glucose level in their blood was significantly suppressed. There was a significant effect of the COB extract on the areas under the curve (AUC) participants. Therefore, COB suppressed the increment of postprandial blood glucose and might be useful in preventing the development of diabetes.

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1.INTRODUCTION

Diabetes mellitus is a serious, non-communicable disease characterized by hyperglycemia, with a rising incidence in both developed and developing countries [1–3]. The World Health Organization has predicted that between 1997 and 2025, the number of diabetics will double from 143 million to about 300 million. According to a report of the Ministry of Health in Japan, it is estimated that 1 out of 6 Japanese will suffer from diabetes mel-

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litus in the near future. In developing countries including Vietnam, the number of people with diabetes is increasing rapidly in urban areas, due to industrialization and the shift of dietary habits to a high fat intake.

It is therefore necessary to find new approaches to manage 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 glu-
cose is important for diabetic patients because it prevents hyperglycemia and 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 plants with blood glucose-lowering potential are known [7-8]. A number of plants are known to exert their anti-hyperglycemic activity by inhibiting carbohydrate-hydrolyzing enzymes in the small intestine. The other common use of Psidium guajava Linn (guava) is as folk medicine. Interestingly, guava leaves have also attracted attention as a folk remedy for diabetes not only in Japan and East Asia [9-11] but also in Africa [12]. Green tea may also have a beneficial effect on glucose tolerance and the risk of developing diabetes. In a large cohort study on green tea, frequent consumption was found to be inversely associated with the risk of type 2 diabetes among Japanese women [13]. A cross-sectional study in Japan revealed an inverse correlation between daily consumption of green tea at a high concentration and fasting glucose levels in male subjects [14].

Cleistocalyx operculatus (Roxb.) Merr and Perry, also known by the scientific name Eugenia operculata Roxb. (belonging to the Myrtaceae family), is commonly known as Voi in Vietnam. Cleistocalyx operculatus (CO) is a large green tree which grows in rural areas of North Vietnam. Like green tea, the flower bud (Nu Voi) and leaves (La Voi) of CO have been used as a beverage by Vietnamese for a very long time. The water extracts from the flower bud of Cleistocalyx operculatus (COB) were used to examine its effect on hyperglycemia. In vitro assays, the inhibitory capacity of COB on α-glucosidase and sucrase activities were lower than the drug acarbose but higher than guava leaf extract. A fasting tolerance glucose test on normal mice and diabetic rats using 2 g/kg maltose loading showed that the reduction of the blood glucose level of COB extract (500 mg/kg) was similar to acarbose (25 mg/kg) and more effective than guava leaf extract [15]. We have found that the water extracted material from COB also had a high polyphenol content, which had anti-hyperglycemic, anti-oxidant, anti-hyperlipidemic effects in streptozotocin (STZ)-induced diabetic rats (500 mg of COB extract /kg/day for 8 weeks) [15-17]. Furthermore, we have confirmed that there was a higher polyphenol and flavonoid content, stronger antioxidant activities and higher pancreatic lipase inhibition of the COB extract in comparison with green tea and guava leaf extracts. From these results, it is estimated that COB might be useful for managing the pre-diabetic state and inhibiting the development of diabetic complications, and may be effective in preventing metabolic syndrome. It is also worth noting that CO seems to be safe for long-term use because it has been safely consumed through the ages by Vietnamese as a dietary constituent [18].

However, there are no studies on the effects of aqueous extract of bud CO for the management of diabetes and diabetic complications in humans. Hence, the purpose of this study is to evaluate the anti-diabetic effect of COB tea in diabetic patients in Vietnam. The present study has provided scientific evidence for the development of COB as a potential natural functional food in preventing diabetic and diabetic complications.

2. MATERIALS and METHODS

(1) Subjects

The trial was conducted from December 2008 to July 2009 at the National Institute of Nutrition, at Hanoi, Vietnam. We recruited 91 adults aged from 50 to 70 years with diabetes (male and female). Patients with type 2 diabetes with a fasting plasma glucose level from 7.0 to 8.3 mmol/L were recruited from the offices of general practitioners. The exclusion criteria were: presence of hepatic or renal disease, cigarette smoking, use of dietary antioxidant supplements and treatment with insulin, lipid lowering drugs, and hormone therapy during the preceding 3 months.

Subjects willingly agreed to participate in this study and completed a consent form that was approved by the Ethics Committee of the National Institute of Health.

The subjects participated in study A (a three-month intervention study) and study B (testing postprandial blood glucose).

(2) Study A (three-month intervention study)

• Study design

Ninety-one subjects took part in a case-controlled trial. The subjects were divided randomly into two groups and given either a placebo (control group) or 25 g of COB (COB group) for 12 weeks. They were instructed not to change their usual dietary and physical activity habits and not to use insulin therapy for the duration of the study. After the
subjects were recruited, they drank only water for 1–2 weeks prior to the start of the intervention period. Characteristic, hematology and biochemistry parameters were measured at interval points, at the start of the intervention period and at 12 weeks.

Subjects in the treatment group were taught how to make COB extract: 25 g of COB was added to 1,000 ml of water and then boiled for about 10 min, and used within 1 day. None of the subjects in the COB treatment group were allowed to drink any kind of tea other than COB tea throughout the study. In order to check the quality of the COB extract, some teabags were randomly tested for their total polyphenol content and organoleptic acceptability before the study began and during the study.

The subjects in the placebo group were not allowed to drink any herb tea, or change their dietary and physical activity habits.

All subjects recorded daily such information as how to make the tea, the amount of tea they consumed and any abnormal signs, if any. Every day, the monitoring staff conducted random checks and recorded the progress of the subjects. The subjects were advised to keep their body weight, physical activity, and diet under control during the entire study period.

• Biological parameters

Blood was collected after an overnight fast at the beginning of the study and after 3 months in heparinized tubes protected from light. The blood was then measured for the following biochemical parameters: Glucose (mmol/L), HbA1c (%), Total cholesterol (mmol/L), HDL-cholesterol (mmol/L), Triglyceride (mmol/L), GOT (U/L), GPT (U/L), Creatinine (μ mol/L), Uric Acid (μ mol/L), Insulin (pmol/L). The HbA1c level was measured by immunoassay, and other parameters were measured by routine laboratory methods at the Nutritional Biochemical Laboratory of the National Institute of Nutrition, Hanoi, Vietnam.

(3) Study B (Testing postprandial blood glucose in type 2 diabetic patients)

Of the 91 subjects, 20 were invited for the testing of postprandial blood glucose. The subjects were served two test breakfast meals, with and without COB tea, to assess the effect of COB extract on postprandial glycemia. On the first assay (control day), after an overnight fast, the subjects took 150 ml of water before and while eating 170 g of cooked rice. On the second day (COB day), 150 ml of COB tea replaced the 150 ml of water and all 20 subjects drank 150 ml of COB tea before and while eating 170 g of cooked rice. COB tea was prepared with a dosage of 25 g COB (ready roast-dryness) / 1,000 ml water, then boiled for 30 min at the Nutritional Biochemical Laboratory of the National Institute of Nutrition. The eating was carried out within 20 min. All subjects were instructed not to drink alcohol, green tea or coffee the evening before testing.

A baseline finger-prick blood sample was taken with an Accu-Chek lancet device, placed on a test strip, and read with an Accu-Chek Active blood glucose meter (Wako, Japan). Before subjects were served the meal (0 min), and at 15 min, 30 min, 60 min, and 120 min after eating cooked rice, finger-prick blood samples were taken and analyzed. The incremental area under the curve (AUC) was calculated for glucose for each subject.

(4) Statistical Analysis

All data were checked for missing data and outliers and cleaned before data analysis.

The results are given as means ± standard deviation (SD). SPSS version 12.0 (Statistical Package for Social Science, Inc.) was used for statistical analysis. An independent t-test was used to make comparisons between the water day and the COB day at each time (in study B). Due to the fact that the sample size of study A was small, we used nonparametric tests: statistical comparisons between the two groups (initial week baseline and treatment after 12 weeks) were analyzed using the Wilcoxon test (paired sample t-test), and statistical comparisons between different times in the same group using the Mann–Whitney test (two-Independent Samples t-test). A value of p < 0.05 is considered to represent a statistically significant difference.

a, p<0,05; c, p<0,001 compared to T0 in the same group, Wilcoxon test
*p<0,05; **p<0,01, ***p<0,001 compared to the control group, Mann–Whitney test

3. RESULTS

(1) Study A (three-month intervention study)

Clinical and biochemical data were obtained from 91 patients (placebo group: n=42, COB group: n=49) who
took part in the trial. However, 15 in the placebo group and 11 in the COB group did not meet the requirements of the 12-week follow-up period. Therefore, the remaining 65 patients were investigated.

Table 1 displays the measurements after 12 weeks of intervention. We analyzed the mean absolute inter- and intra-treatment differences of all clinically and biochemically described parameters. After 12 weeks, the COB group showed a significant reduction in glucose, HbA1c, cholesterol, triglyceride, and uric acid, and a marked increase in the levels of HDL-cholesterol and insulin. Table 1 also showed a significant reduction in the level of glucose, and HbA1c % in the COB group as compared with those of the placebo group.

(2) Study B (Testing postprandial blood glucose in type 2 diabetic patients)

At the initial stage, the mean blood glucose level after fasting was not statistically different between the water day and the COB day in 20 diabetic patients (Table 2 and Fig.1). The level of postprandial blood glucose did not increase on the COB day compared to the water day. The incremental area under the curve (AUC) was measured for glucose for each subject. The differences of blood glucose AUCs between each meal with and without COB tea were statistically significant.

4. DISCUSSION

Hyperglycemia is the principal cause of 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 in diabetic patients.

In our previous study, we demonstrated that administration of an aqueous extract from COB improved hyperglycemia, hyperlipidemia, oxidative stress, and sorbitol accumulation in STZ diabetic rats [15, 17]. We showed that COB had anti-hyperglycemic effects (reductions in plasma glucose and HbA1c levels), and protective and anti-cataract effects on β-cells in STZ-induced diabetic rats [17]. We also demonstrated that the aqueous extract of the COB had an inhibitory effect on α-glucosidase, maltase and sucrase in vitro, and was more effective than that of guava leaf extract [16]. We have suggested that the COB extract improved diabetes management, possibly by normalizing the postprandial blood glucose level, a mechanism similar to that of acarbose.

In the present study, we examined the effects of the aqueous extract from COB on type 2 diabetic patients. We showed that subjects who drank the COB extract for 12 weeks had significantly reduced blood glucose levels and HbA1c %, and increased plasma insulin levels. In addition, we showed that the increment of postprandial blood glu-
thus, each subject received a total of 945 mg of catechin equivalent in 150 ml of COB tea. The dosage of COB used in the present study was lower than the dosage of guava leaf extract in the study of Deguchi et al [20]. In Deguchi’s study, guava leaf extract with a dosage of 190 ml of 100 g dry guava leaf per 2,000 ml of hot water was administered to healthy subjects [20]. However, the COB extract was boiled for 30 min in our study, while the guava leaf extract was boiled for 10 min in Deguchi’s study [20]. Further long-term clinical studies are needed to evaluate the anti-hyperglycemic effects of COB in diabetic patients with a reasonable dosage.

So far, various ingredients contained in COB have been reported. Ye et al. reported that two flavonoids 3’-formyl-4’,6’-dihydroxy-2’-methoxy-5’-methylchalcone and (2S)-8-formyl-5-hydroxy-7-methoxy-6-methylflavanone together with five known compounds, were isolated from the dried COB [21]. Min et al. also reported that 3’-formyl-4,4’,6’-trihydroxy-2’-methoxy-5’-methylchalcone, 3’-formyl-4,6’-dihydroxy-2’-methoxy-5’-methylchalcone 4’-O-β-D-glucopyranoside, (2S)-8-formyl-6-methylnaringenin, and (2S)-8-formyl-6-methylnaringenin-7-O-β-D-glucopyranoside were isolated from EtOAc-soluble fraction of COB and their radical scavenging activities [22]. Sone et al. also reported that the malonaldehyde/gas chromatography assay revealed strong antioxidant activity in COB [23]. Moreover, Su et al. showed that 2’,4’-dihydroxy-6’-methoxy-3’,5’-dimethylchalcone (DMC), a chalcone isolated from COB, possessed cytoprotective activity in PC12 cells treated with H₂O₂. However, it is not clear which component was effective [24].

The results of the present study suggest that COB has a potential role in the management of the pre-diabetic state and of diabetes, based on its ability to control the blood glucose level. Since COB extract has long been a common

cose after eating cooked rice was significantly suppressed in diabetic patients when they were administrated COB tea. Thus, COB tea might be considered as an inhibitor for the control of postprandial blood glucose after meals. It is reported that administration of polyphenol-rich plant extracts like green tea increased HDL-cholesterol, and decreased Triglyceride and LDL-cholesterol [19]. However, in our observation, only HDL-cholesterol increased significantly in the COB group as compared with the placebo group.

In our previous study, we showed that despite the polyphenolic content of the COB extract being similar to that of guava leaf extract, the carbohydrate-hydrolyzing enzyme inhibitory activities as well as the suppression of postprandial blood glucose of the COB extract were more active than in the guava leaf extract both in vitro and in vivo [15]. In the present study, we administered a 150 ml dose of COB tea with 25 g dry COB/1,000 ml of hot water in this study. For this dosage, the polyphenol content in the COB extract was 6.3 mg of catechin equivalent/ml;
ingredient in drinks in Vietnam, like green tea, COB is likely to be sufficiently safe for daily use. It is suggested that COB extract is a useful and harmless ingredient for treating pre-diabetic and diabetic patients.

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6. REFERENCES


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ベトナムの2型糖尿病患者を対象としたCleistocalyx operculatusの花芽抽出液の効能

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ベトナムで飲料や食用に用いられているCleistocalyx operculatus花芽（COB）は非常に強い抗酸化活性、抗糖尿病活性などが認められている。そこで、本研究では実際にベトナムの2型糖尿病の患者を対象に、COB茶が糖尿病に効果があるのか評価した。対象者65名を、COB茶を飲用する群とプラセボ群に分けて、12週間後の血糖値等の変化を検討した。その結果、12週間後の空腹時血糖、HbA1cはCOB茶を飲用した群の方が有意に低かった。また血清インスリン濃度は、介入する前に比べ12週間後ではCOB茶を飲用した群の方が高かった。さらに食後の血糖値の上昇を検討するため、170gのご飯を食べる前および食事中に150mlのCOB茶もしくは水を飲用させ、食後2時間の血糖値の変化の違いをみた。その結果、COB茶を飲用した方が血糖値の上昇が抑えられていた。これらの結果から、COBは糖尿病の予防・改善に効果があると示唆された。

キーワード：糖尿病、Cleistocalyx operculatus、薬用植物、血糖、ベトナム