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Full Length Research Paper

Insulin secreting and α-glucosidase inhibitory activity of *Coscinium fenestratum* and postprandial hyperglycemia in normal and diabetic rats

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This work focused on the effect of *Coscinium fenestratum* ethanolic extract on plasma glucose concentrations in normal and streptozotocin (STZ)-induced diabetic rats, the stimulatory effect on insulin secretion from perfused rat pancreas and the inhibitory effects on rat intestinal α-glucosidase enzymes, maltase and sucrase. In oral glucose, maltose and sucrose loading tests, the extract (250 - 1,000 mg/kg) significantly decreased plasma glucose concentrations in a dose-dependent manner. The extract (1,000 mg/kg) was most effective in decreasing plasma glucose concentrations and the response was closed to those of glibenclamide (5 mg/kg) and acarbose (3 mg/kg). In perfused rat pancreas, the extract (10 µg/ml) stimulated insulin secretion in a biphasic pattern. However, the berberine at the same dose as the extract slightly increased insulin secretion by 1.33-fold over the basal control group. In addition, the extract inhibited the activities of both maltase and sucrase with the IC_{50} of 3.89 and 11.22 mg/ml, respectively. Our findings suggest that the *C. fenestratum* ethanolic extract exerted anti-hyperglycemic activity by stimulating insulin secretion and α-glucosidase inhibition.

Key words: *Coscinium fenestratum*, streptozotocin, α-glucosidase, insulin secretion, perfused rat pancreas.

INTRODUCTION

Diabetes mellitus is in the group of metabolic diseases characterized by hyperglycemia, dyslipidemia, and protein metabolism that results from defects in both insulin secretion and/or insulin action. The disease is associated with a reduced quality of life and increased risk factors for mortality and morbidity. Long-term hyperglycemia is an important factor in the development and progression of micro- and macro-vascular complications, which include neuropathy, nephropathy, cardiovascular and cerebrovascular diseases (Altan, 2003; Strojek, 2003). The prevalence of diabetes worldwide will increase from 171 million people in the year 2000 to 366 million people by the year 2030. In addition, the total number of people with diabetes from WHO South-East Asia region division, especially in Thailand, is projected to rise from 1.536 million in the year 2000 to 2.739 million by the year 2030. In general, the control of blood glucose concentrations to near normal range in patients is mainly based on the use of oral hypoglycemic agents and insulin. However, all of these treatments have limited efficacy and are associated with undesirable side effects (Harrower, 1994; Reuser et al., 1994; Campbell et al., 1996), which had led to an increasing interest in the use of medicinal plants as an alternative management for type 2 diabetes mellitus.

Coscinium fenestratum Colebr (Menispermaceae) or Hamm is widely distributed in Africa, Asia, Sri Lanka, India and Indochina region especially in Laos, Vietnam and Thailand. It is used in traditional medicines for treat-

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ment of diabetes (Varier, 1994). The stem of *C. fenestratum* contains berberine, which is the major active compound. The interest in the biological activities of this extract has accelerated in recent years. For example, it has been found that water extracted from *C. fenestratum* shows antibacterial activity against *Clostridium* species (Nair et al., 2005). Moreover, the stem of *C. fenestratum* extract has been reported to exert hypotensive (Singh et al., 1990), and hepatoprotective activities (Venukumar et al., 2004). Recent study has revealed that the alcoholic stem extract of *C. fenestratum* also produces a significant reduction blood glucose levels in normal and diabetic rats (Shirwaikar et al., 2005). However, the insulinotropic properties in perfused rat pancreas and the inhibition of α-glucosidase activity of the alcoholic extract has not been reported.

The aim of this study is to investigate the antihyperglycemic activity of *C. fenestratum* extract in normal and STZ-induced diabetic rats by oral glucose, maltose and sucrose loading tests. We also investigated the *in vitro* α-glucosidase inhibitory activity of the extract against maltase and sucrase. Furthermore, we investigated the direct effect of the extract on insulin secretions from the perfused rat pancreas.

MATERIALS AND METHODS

Chemicals

Streptozotocin, rat intestinal acetone powder and glucose oxidase test kits were purchased from Sigma Chemical (St. Louis, MO, U.S.A.). Pentobarbital sodium was purchased from Sanofi-Ceva (Bangkok, Thailand). The insulin radioimmunoassay (RIA) kit was purchased from Diagnostic Products Corporation (Los Angeles CA, USA). All other chemical reagents used in this study were of analytical grade.

Plant materials

A stem of *C. fenestratum* was collected from Nongkhay Province, Thailand and authenticated by taxonomist, Department of Botany at Chulalongkorn University. A voucher specimen has been deposited in the herbarium of the Department of Botany at Chulalongkorn University, Bangkok, Thailand.

Extraction and isolation

The dry stem of *C. fenestratum* was cut into thin pieces and was dissolved in 95% ethanol for 4 days. The mixture was filtered and then evaporated under reduced pressure, weighed and the residue was used in experiments (yield 17.9%). Berberine was isolated by chromatrographic methods (Pinho et al., 1992). The chemical structure of berberine was confirmed using ^{1}H-NMR, ^{13}C-NMR.

Animals

Male Sprague Dawley was obtained from the National Laboratory Animal Center, Mahidol University, Salaya, Thailand. Animal facilities and protocol were approved by the Laboratory Animal Care and Use Committee at Faculty of Veterinary Science at Chulalongkorn University. The animals were acclimatized for 1 - 2 weeks before being used in the experiment. Rats were housed in individual stainless steel cages with free access to water and feed in a room maintained at 24 ± 1°C on a 12 : 12 h light–dark cycle.

Induction of diabetes

Animals weighing 100 - 150 g were fasted overnight. Diabetes was induced by intravenous injection of streptozotocin (STZ) in a single dose of 50 mg/kg. STZ was dissolved in a cold citrate buffer solution (0.01 M, pH 4.5) immediately before use. The fasting blood glucose (FBG) concentration in the animals was measured at days 3 after STZ injection. The rats with a FBG level higher than 300 mg/dl were included in the study.

Effect of *C. fenestratum* on plasma glucose in normal and diabetic rats by the oral glucose and disaccharide tolerant test

The rats were divided into 5 groups, each group contained 8 animals. The control group was fed with distilled water. The other three groups were fed orally with three different doses of the *C. fenestratum* extract (250, 500 and 1000 mg/kg). For the last group, glibenclamide (5 mg/kg) or acarbose (3 mg/kg) was used as a positive control in rats receiving glucose or disaccharides, respectively. In the diabetic rats, the rats were divided into 3 groups, each group containing 8 animals. Group 1 was fed with only distilled water. Group 2 was fed orally with *C. fenestratum* extract (500 mg/kg) and group 3 was fed with glibenclamide (5 mg/kg). All treatments were administered to the rats 5 min before loading glucose, maltose, or sucrose (3 g/kg). Blood samples were collected from a tail vein at 0, 30, 60 and 120 min. Heparin-containing blood samples were immediately centrifuged (1,500 g), and the plasma was separated and frozen at – 20°C until being analyzed for glucose concentration. The plasma glucose concentrations were determined by glucose oxidase method.

In situ pancreatic perfusion

Male Sprague Dawley (380 - 450 g) was fasted for 12 h before performing the experiment. The rats were anesthetized with pentobarbital sodium (60 mg/kg, IP) and were maintained at 37°C on a heat pad during the experiment. *In situ* pancreatic perfusion experiments were performed as previously described (Yibchok-anun et al., 1999). Briefly, the perfused pancreas was equilibrated for 20 min before starting the experiment. After a baseline period of 10 min, the perfusate containing 10 µg/ml of *C. fenestratum* extract (n = 5) or berberine (n = 5) was administered for 20 min, followed by a washout period with the basal medium for 10 min. The perfusate containing glucose (15 mM) was administered as a positive control for 6 min at the end of the experiment. The insulin concentration was determined by Insulin Radioimmunoassay kits.

Assay for α-glucosidase inhibitory activity

The AGH inhibitory activity assay was done as previously described (Adisakwattana et al., 2009) with slight modifications. The substrate (maltose 37 mM or sucrose 37 mM) and the test compounds (*C. fenestratum* or acarbose) were dissolved in a 0.1 M phosphate buffer solution (pH 7.0). A crude enzyme solution 20 µl and the test compounds 40 µl were pre-incubated simultaneously for 10 min. After the pre-incubation period, the substrate 140 µl was added and

incubated at 37°C for 30 and 60 min, for maltose and sucrose, respectively. The assays tubes were immediately immersed in boiling water for 10 min, to stop the reaction. Glucose concentration was determined by glucose oxidase test.

Data expression and statistical analysis

Data were expressed as means ± S.E.M. Area under the curve (AUC) value was reported as total areas and performed by one-way analysis of variance (ANOVA). In the data from perfusion experiments, areas calculated using a modification of the trapezoidal rule. In animal models, statistical analysis was under the curve (AUCs) for the treatment period were calculated and expressed as a percentage of the area of the basal control group. The Least Significant Difference test (LSD) was used for mean comparisons; $p < 0.05$ was considered to be statistically significant.

RESULTS

Effect of *C. fenestratum* in normal rats by oral glucose and disaccharide tolerant test

The hypoglycemic effects of *C. fenestratum* extract in the glucose loaded normal rats are shown in Figure 1a. Three doses of *C. fenestratum* extract (250, 500 and 1,000 mg/kg) were evaluated in fasted normal rats along with the standard drug glibenclamide (5 mg/kg). The *C. fenestratum* extract significantly decreased plasma glucose levels in a dose-dependent manner. The AUC was significantly lower than those of normal rats by 49, 50 and 77%, respectively (normal control group = 178.4 ± 10.2 mg/dl.h, the normal treated group with extract 250 mg/kg = 88.5 ± 13.7 mg/dl.h; 500 mg/kg = 88.8 ± 6.9 mg/dl.h; 1000 mg/kg = 40.1±7.2 mg/dl.h). Glibenclamide showed a significant decrease in plasma glucose levels when compared with the control group (AUC for glibenclamide = 101.5 ± 29.5 mg/dl.h).

The effects of *C. fenestratum* in the maltose and sucrose loaded normal rats are shown in Figures 1b and 1c, respectively. *C. fenestratum* extract induced a significant decrease in plasma glucose concentrations after maltose loading. The AUC of normal rats treated with extract (250, 500 and 1000 mg/kg) were 97.5 ± 6.0, 69.1 ± 14.7 and 101.5 ± 11.5 mg/dl.h, respectively (AUC for normal control groups = 161.8 ± 27.3 mg/dl.h). In addition, the plasma glucose concentrations of normal rats treated with the extract at 500 and 1000 mg/kg were also significantly decreased after sucrose loading. The AUC was significantly lower than that of normal rats by 50 and 56%, respectively (AUC for normal control group = 95.5 ± 5.8 mg/dl.h; AUC for the normal treated group with extract 500 mg/kg = 47.1 ± 17.8 mg/dl.h; 1000 mg/kg = 42.0 ± 13.6 mg/dl.h). In maltose and sucrose loading, acarbose (3 mg/kg) markedly reduced the AUC of normal rats by 47 and 63% ($p < 0.05$), respectively (AUC for acarbose of maltose loading = 84.7 ± 9.0 mg/dl.h, maltose loading; AUC for acarbose of sucrose loading =34.9 ± 13.1 mg/dl.h).

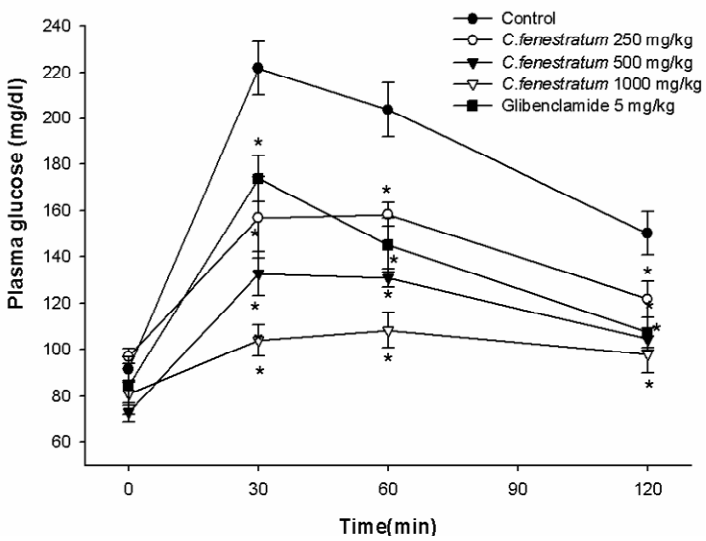

Figure 1a. Effect of *C. fenestratum* on plasma glucose in oral glucose-loaded normal rats. Results are expressed as means ± S.E.M., n = 8. *P < 0.05 compared with the control.

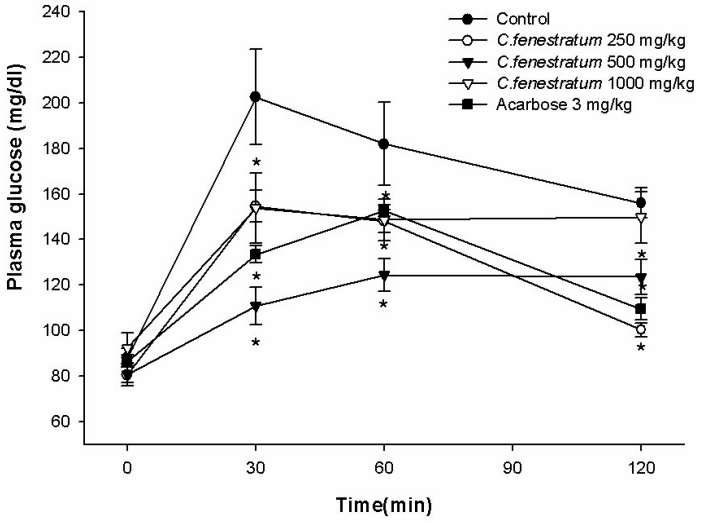

Figure 1b. Effect of *C. fenestratum* on plasma glucose in oral maltose-loaded normal rats. Results are expressed as means ± S.E.M., n = 8. *P < 0.05 compared with the control.

Effect of *C. fenestratum* in STZ-diabetic rats by oral glucose and disaccharide tolerant test

C. fenestratum extract (500 mg/kg) and glibenclamide (5 mg/kg) significantly decreased plasma glucose concentra-

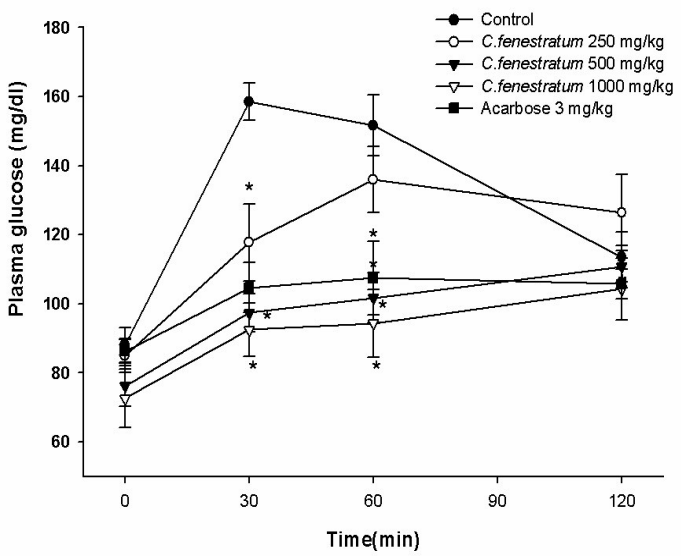

Figure 1c. Effect of *C. fenestratum* on plasma glucose in oral sucrose-loaded normal rats. Results are expressed as means ± S.E.M., n = 8. *P < 0.05 compared with the control.

tion by 20 and 15% at 30 min after glucose administration, respectively. Consequently, the extract suppressed plasma glucose concentrations at 60 and 120 min, after glucose administration ($p < 0.05$; Table 1). In addition, *C. fenestratum* extract (500 mg/kg) significantly reduced the postprandial hyperglycemia at 60 min after maltose loading whereas it did not suppress a rise in postprandial hyperglycemia by sucrose loading.

The stimulatory effects of *C. fenestratum* on insulin secretion from perfused rat pancreas

The effects of *C. fenestratum* extract and berberine on insulin secretion were performed by *in situ* pancreatic perfusion. The profile of insulin release was shown in Figure 2 together with basal control, which was obtained by perfusion with KRB alone for 40 min. The *C. fenestratum* extract increased insulin secretion in a biphasic pattern: a peak followed by a sustained phase. Within 20 min of administration, it stimulated three biphasic pattern profiles, in which the maximum insulin secretions were 2.98-, 3.85- and 3.55-fold, respectively, over the basal control group. However, the berberine at the same dose as *C. fenestratum* extract slightly and gradually increased insulin secretion from the rat pancreas with the maximum of 1.33-fold over the basal control group. The effluent insulin concentration returned to the baseline during 10 min washing period and increased to 4 to 13.02 fold of the baseline value on the administration of 15 mM glucose (positive control). The areas under the curve were calculated for 20 min of administration. *C. fenestratum* significantly stimulated insulin secretion, but not berberine, compared with the basal control group.

Effect of *C. fenestratum* on α-glucosidase inhibition

The inhibitory effects of *C. fenestratum* and acarbose on the intestinal α-glucosidase activity (maltase and sucrase) were shown in Table 2. The IC_{50} of *C. fenestratum* extract against maltase and sucrase was 3.89 and 11.22 mg/ml, respectively. The result showed that the inhibitory activities of *C. fenestratum* on maltase were higher than sucrase. Similar to the *C. fenestratum*, acarbose was more effective to inhibit the activities of maltase than sucrase with the IC_{50} of 0.66 and 6.76 µg/ml, respectively.

DISCUSSION

C. fenestratum has been mainly used for treating diabetes mellitus in the traditional medicine. Previous studies supports that oral administration alcoholic extract of *C. fenestratum* causes a significant increase hepatic antioxidant enzymes such as catalase, superoxide dismutase, glutathione synthetase, peroxidase, and glutathione peroxidase, resulting in the protection of the cell against free radical damage (Punitha et al., 2005). Moreover, *C. fenestratum* increases glycolysis by stimulating the activity of glycolytic enzymes whereas it decreases gluconeogenesis by suppressing the activity of gluconeogenic enzymes in diabetic rats (Punitha et al., 2005). The administration of the extract to diabetic rats also demonstrated the hypotriglyceridemia, hypocholesterolemia, reduction of body weight and hemoglobin A_{1C} (HbA_{1C}) (Shirwaikar et al., 2005).

The present investigation suggests that the alcoholic extract of *C. fenestratum* has the capacity to lower blood glucose levels in normal and diabetic rats by oral glucose tolerant test. At this point, it is possible that the antihyperglycemic mechanisms of *C. fenestratum* may be due to the stimulatory insulin secretion from pancreas. To investigate this hypothesis, the *in situ* pancreatic perfusion was performed in normal rats. Our investigation was the first report on the directly stimulatory effect of *C. fenestratum* on insulin secretion in normal pancreatic β-cells. As shown in the results, *C. fenestratum* extract was effective in stimulating insulin secretion but not in berberine. Berberine has been reported as a plant alkaloid that is widely distributed in *Coscinium* plants and it is the major chemical compound in *C. fenestratum* (Pinho et al., 1992). Our results were consistent with the previous reports by Yin et al. in which berberine had no a direct effect on an increase in insulin secretion in *β*TC3 cells, a *β*-cell line derived from transgenic mice expressing a hybrid insulin gene-oncogene (Efrat et al., 1988). Recent study has confirmed that berberine inhibits insulin secretion

Table 1. Effect of the *C. fenestratum* extract on plasma glucose concentrations in oral glucose-, maltose- and sucrose-loaded diabetic rats (n = 8).

	Plasma glucose (mg/dL)			
Groups	**0 min**	**30 min**	**60 min**	**120 min**
Glucose-loading				
Control	425.2 ± 23.2	603.3 ± 24.1	582.0 ± 27.4	557.3 ±15.3
C. fenestratum (500 mg/kg)	388.3 ± 14.8	517.6 ± 22.9*	531.1 ± 18.2*	487.1 ± 24.3*
Glibenclamide (5 mg/kg)	417.7 ± 38.5	560.8 ± 19.6*	598.8 ± 27.8	543.5 ± 37.8
Maltose-loading				
Control	444.32 ± 25.59	531.07 ± 22.86	581.66 ± 25.40	507.38 ± 32.14
C. fenestratum (500 mg/kg)	464.60 ± 22.17	522.36 ± 11.73	512.58 ± 12.22*	496.91 ± 21.54
Acarbose (3 mg/kg)	397.68 ± 29.62	496.48 ± 13.36	522.33 ± 21.50	473.85 ± 21.29
Sucrose-loading				
Control	410.60 ± 12.36	565.27 ± 14.67	567.95 ± 14.86	525.29 ± 17.38
C. fenestratum (500 mg/kg)	410.38 ± 41.69	500.60 ± 50.59	534.47 ± 52.95	486.05 ± 50.39
Acarbose (3 mg/kg)	397.79 ± 34.86	487.34 ± 27.36	481.93 ± 16.45	495.11 ± 21.18

*p < 0.05, compared with control group at the same time.

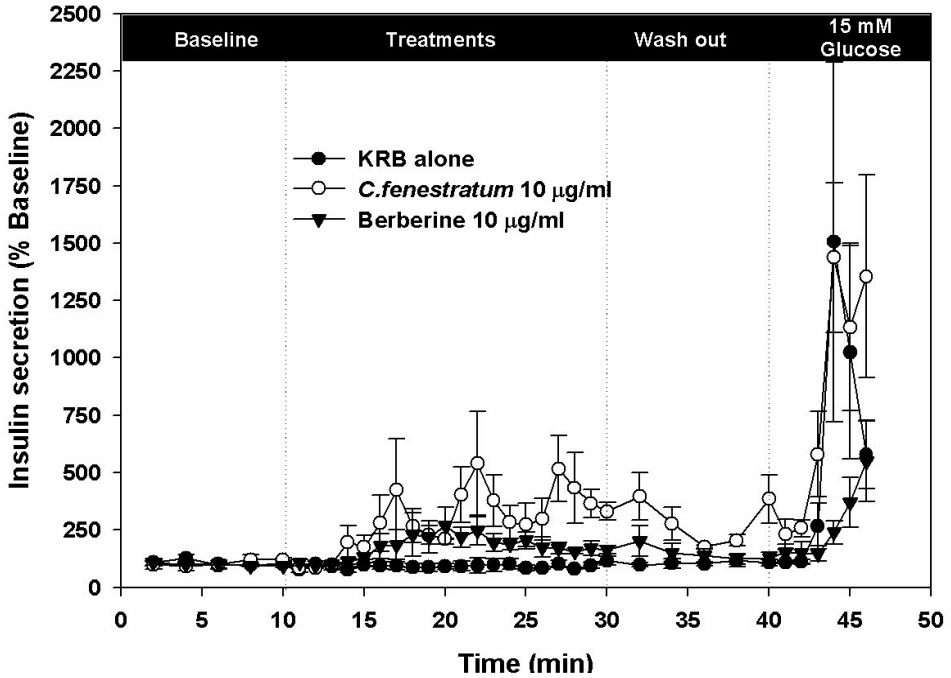

Figure 2. Effects of the *C. fenestratum* extract (10 µg/ml) and berberine (10 µg/ml) on insulin secretion from perfused rat pancreas. In these experiments, a 20 min equilibration period preceded time 0. Values are means ± S.E.M.; n = 5.

Table 2. The inhibitory effects of the *C. fenestratum* extract and acarbose on α-glucosidase activities (maltase and sucrase).

Enzyme (Substrate concentration)	**IC_{50} (Concentration)**	
	***C. fenestratum* (mg/ml)**	**Acarbose (µg/ml)**
Maltose (37 mM)	3.89	0.66
Sucrase (37 mM)	11.22	6.76

from beta-cells through 3', 5'-cyclic adenosine 5'-monophosphate signaling pathway and reduces the glucose-stimulated insulin secretion in rat islets (Zhou et al., 2008). Thus, we speculate that other compounds in the presence of *C. fenestratum* extract may exert the insulinotropic activity. Further studies are needed to characterize the bioactive compounds of *C. fenestratum* with regard to this mechanism.

The inhibition of α-glucosidase activity is one of therapeutic approaches for reducing postprandial hyperglycemia. α-Glucosidase inhibitor is effective in delaying absorption of carbohydrates and suppressing postprandial hyperglycemia which contribute to the decrease in hemoglobin A_{1C} (HbA_{1c}). The decreasing of HbA_{1c} could reduce the incidence of chronic vascular complication in diabetic patients (Baron, 1998). As shown in table 2, our findings indicate that *C. fenestratum* had more potent α-glucosidase inhibitory activity against intestinal maltase. It has recently been reported that the activity of sucrase and maltase were inhibited by berberine (Pan et al., 2003a). Berberine also decreased glucose transport through the intestinal epithelium by inhibiting the sodium-dependent glucose transporter (SGLT) which plays a crucial role in the process of glucose intake in the small intestine (Pan et al., 2003b). As the data mentioned above, it is possible that α-glucosidase inhibitory activity of *C. fenestratum* might be due to berberine which is a major composition in the extract.

In conclusion, our findings indicate that *C. fenestratum* markedly decreased plasma glucose level in diabetic rats and it exerted antihyperglycemic activity by stimulating insulin secretion and α-glucosidase inhibition. These findings suggest that the *C. fenestratum* extract may be useful in the control of diabetes mellitus.

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