MRG4580002 รศ.สพ.ญ.ดร. ศิรินทร หยิบโชคอนันต์

สำนักงานคณะกรรมการการอุดมศึกษาและ
สำนักงานกองทุนสนับสนุนการวิจัย

รายงานวิจัยฉบับสมบูรณ์

โครงการการศึกษาฤทธิ์ของโปรตีนสกัดจากเนื้อของมะระขี้นกในประเทศไทยในด้านการกระตุ้นการหลั่งฮอร์โมนอินซูลินและการออกฤทธิ์เสมือนเป็นอินซูลิน

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24 ธันวาคม พ.ศ. 2547

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สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

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สัญญาเลขที่ MRG4580002

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สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

สนับสนุนโดยสำนักงานคณะกรรมการการอุดมศึกษาและ
สำนักงานกองทุนสนับสนุนการวิจัย

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วัตถุประสงค์ของการวิจัยครั้งนี้เพื่อศึกษาถึงผลของสารสกัดโปรตีนจากเนื้อของมะระขี้นกในรูปแบบที่ไม่มีซิงค์ต่อระดับน้ำตาล และฮอร์โมนอินซูลินในพลาสม่าของหนูขาวปกติและหนูที่ถูกเหนี่ยวนำให้เป็นเบาหวานด้วยสเตรปโตโซโตซิน นอกจากนี้ทางคณะผู้วิจัยยังได้ทำการศึกษาถึงผลโดยตรงของโปรตีนสกัดต่อการหลั่งอินซูลินและกลูคากอนจากตับอ่อน รวมทั้งการกระตุ้นการเข้าสู่เซลล์ของน้ำตาลกลูโคสในเซลล์กล้ามเนื้อ (C2C12) และเซลล์ไขมัน (3T3-L1) ของหนูขาว

การศึกษาฤทธิ์ลดน้ำตาลของสารสกัดโปรตีนจากมะระขี้นกในประเทศไทย โดยการฉีดเข้าใต้ผิวหนังในขนาด 5 และ 10 มก./กก. พบว่า สารสกัดโปรตีนสามารถลดระดับน้ำตาลในพลาสม่าได้อย่างมีนัยสำคัญทางสถิติทั้งในหนูปกติและหนูเบาหวาน โดยฤทธิ์ของสารสกัดจะแปรผันตามขนาดที่เพิ่มสูงขึ้น ระยะเวลาตั้งแต่เริ่มฉีดสารสกัดโปรตีนจนกระทั่งตรวจพบระดับน้ำตาลที่ลดลง คือ 4 และ 6 ชั่วโมงในหนูเบาหวานและหนูปกติตามลำดับ สารสกัดโปรตีนยังมีผลเพิ่มระดับฮอร์โมนอินซูลินในพลาสม่าประมาณ 2 เท่าใน 4 ชั่วโมงหลังจากฉีดเข้าใต้ผิวหนังด้วย ผลจากการทดลองผ่านสารสกัดโปรตีนขนาด 10 ไมโครกรัม/มล.เข้าไปภายในตับอ่อนโดยตรง พบว่ามีผลกระตุ้นการหลั่งฮอร์โมนอินซูลิน แต่ไม่มีผลต่อการหลั่งฮอร์โมนกลูคากอน ซึ่งผลดังกล่าวเกิดขึ้นภายใน 5 นาทีหลังจากได้รับสารสกัดและยังคงอยู่ตลอด 30 นาทีที่ได้รับสารสกัด นอกจากนี้สารสกัดโปรตีนยังมีฤทธิ์เพิ่มการเคลื่อนเข้าสู่เซลล์ของน้ำตาลกลูโคสในกล้ามเนื้อ (C2C12) และไขมัน (3T3-L1) ของหนูขาว ดังนั้นจากผลการทดลองทั้งหมดสรุปได้ว่า สารสกัดโปรตีนจากมะระขี้นก (*M.charantia*) สามารถลดระดับน้ำตาลในกระแสเลือดของหนูขาวได้โดยกลไกการกระตุ้นการหลั่งฮอร์โมนอินซูลินและการออกฤทธิ์เสมือนเป็นอินซูลิน

คำสำคัญ: มะระขี้นก ฤทธิ์ลดระดับน้ำตาล สารสกัดโปรตีน การผ่านสารเข้าตับอ่อน การกระตุ้นการหลั่งฮอร์โมนอินซูลิน การออกฤทธิ์เสมือนเป็นอินซูลิน

Abstract

Project Code : MRG4580002

Project Title : Protein extract from fruit pulp of Siamese *Momordica charantia* with insulin secretagogue and insulin-like activities

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Project period : 2 years (July,1st, 2002 – May, 31st, 2004)

The aim of this study was to investigate the effects of zinc-free protein extract from bitter gourd fruit pulp on plasma glucose concentration and insulin responses in normal and STZ-induced diabetic rats. Furthermore, we investigated the direct effect of the *MC* protein extract on insulin and glucagon secretions from the perfused rat pancreas as well as glucose uptake in C2C12 rat myocytes and 3T3-L1 rat adipocytes.

The protein from Thai bitter gourd (*Momordica charantia*) fruit pulp was extracted and studied for its hypoglycemic effect. Subcutaneous administration of the protein extract (5 and 10 mg/kg) significantly and markedly decreased plasma glucose concentrations in both normal and streptozotocin-induced diabetic rats in a dose-dependent manner. The onset of the protein extract-induced antihyperglycemia/hypoglycemia was observed at 4 and 6 h in diabetic and normal rats, respectively. This protein extract also raised plasma insulin concentrations by 2 fold 4 h following subcutaneous administration. In perfused rat pancreas, the protein extract (10 μg/ml) increased insulin secretion, but not glucagon secretion, which was apparent within 5 min of administration and was persistent during 30 min of administration. Furthermore, the protein extract enhanced glucose uptake into C2C12 myocytes and 3T3-L1 adipocytes. Thus, the *M. charantia* protein has both insulin secretagogue and insulin-like activities that could help explain its hypoglycemic effect in vivo.

Keywords: *Momordica charantia*; antihyperglycemia; protein extract; pancreatic perfusion; insulin secretagogue; insulinomimetic

สารบัญ

	หน้า
บทคัดย่อ	iii
รายการตารางประกอบ	vi
รายการภาพประกอบ	vii
Introduction	1
Materials and methods	2
Results	4
Discussion	6
Acknowledgement	8
References	8
Output	15

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

รายการตารางประกอบ

หน้า

Table 1 Plasma insulin concentrations of normal and streptozotocin (STZ)-induced diabetic rats receiving subcutaneous administration of10 mg/kg MC protein extract. The control rats received 0.15 M NaCl. Results are expressed as mean ± SE. (*n* = 8). **P*< *.05* vs. control group. 14

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

รายการภาพประกอบ

หน้า

Fig 1. A 8-h profile of plasma glucose concentration in normal rats (A), and diabetic rats (B). The dosage of the *MC* protein extract was 1 mg/kg (▽), 5 mg/kg (■) and 10 mg/kg (◇). 0.9 % NaCl was used in the control group (·). Data are expressed as mean ± SE. (n = 8) $*P < 0.05$, $** P < 0.01$ vs control group. **11**

Fig 2. Effects of the *MC* protein extract (10 μg/mL) on insulin release from perfused rat pancreas. Following the baseline period of 20 min, the *MC* protein extract was administered for 30 min. Values are mean ± SE; n =3., Basal control; ○, protein extract10 μg/mL. Range of baseline insulin concentration of effluent was 665 to 4,365 pg/mL. **12**

Fig 3. Effects of the MC protein extract (10 μg/mL) on glucose uptake assay in C_2C_{12} myocytes (A) and 3T3-L1 rat adipocyte (B) cells. These cells were incubated with the *MC* protein extract for 14-18 h and insulin for 30 min. Data are expressed as mean ± SE (n= 3-5). $* P < .05$ vs. control group. 2DG , 2-deoxyglucose; BG: bitter gourd extract. **13**

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

INTRODUCTION

Diabetes Mellitus is as a metabolic disease caused by an insufficiency of insulin secretion or insulin resistance. There are two types of diabetes: type 1 (insulin-dependent diabetes mellitus or IDDM) and type 2 (non-insulin-dependent diabetes mellitus). Type2 diabetes is continuously growing to be a major health problem in modern societies. There are many classes of pharmacological agents for type 2 diabetes such as sulfonylureas, biguanides, thiazolidinediones and alpha glucosidase inhibitors. However, these drugs have also shown adverse effects including lactic acidosis and diarrhea.[1] Numerous chemical compounds from traditional plants are considered for diabetic control in many countries. *Momordica Charantia* Linn.(*MC*) is commonly known as karela or bitter gourd. The extracts from fruit pulp, seed, leaves and whole plants of *MC* have shown antihyperglycemic effect in animal models such as alloxan-induced diabetes,[2] streptozotocin (STZ)-induced diabetes,[3] and glucose-loaded rats.[4] *MC* also displays antitumor,[5] anti-human immunodeficiency virus (HIV),[6] anti-ulcerogenic[7] and hypotriglyceridemic activities.[8]

Khanna et al. reported the first method for isolation of an active protein from the seeds, tissue, and tissue culture of seedling of *MC* by acid-ethanol extraction.[9] The active protein extract compound is called a protein extract-p or p-insulin, and consists of a methionine-containing protein with a minimum size of 11 KDa. Subcutaneous administration of the protein extract-p exerted the hypoglycemic activity in gerbils, langurs and diabetic patients.[9] Thai bitter gourd is commonly used as a traditional remedy for diabetes in Thailand. The extract also causes a decrease in blood glucose concentrations in STZ-induced diabetic rats.[10] In our *MC* protein extract preparation, we followed the Khanna's method except the step utilizing zinc solution to crystallize the protein extract. To date, there have been no reports regarding the direct stimulatory effect of the protein extract on insulin and glucagon secretions using the pancreatic perfusion technique.

The aim of this study was to investigate the effects of zinc-free protein extract from bitter gourd fruit pulp on plasma glucose concentration and insulin responses in normal and STZ-induced diabetic rats. Furthermore, we investigated the direct effect of the *MC* protein extract on insulin and glucagon secretions from the perfused rat pancreas as well as glucose uptake in C2C12 rat myocytes and 3T3-L1 rat adipocytes.

MATERIALS AND METHODS

Animals

Male Wistar rats procured from the National laboratory Animal Center (Salaya, Thailand) weighing 250-300 g were used in this experiment. The animals were acclimatized 1-2 weeks before being used in the experiment. They were maintained in the laboratory animal facility and fed with food pellet (CP, Bangkok, Thailand) and water ad libitum, following the Animal Care and Use Committee Guide of the Faculty of Veterinary Science, Chulalongkorn University.

Extraction of protein from Thai bitter gourd fruit

The fruit of Thai bitter gourd was purchased from a local market in Ang Thong province, Thailand and authenticated by the Department of Botany, Chulalongkorn University. The method of Khana[9] was followed with modification for extraction. Briefly, the fresh pulp was sliced and extracted at 4°C with ice-cold acid-ethanol (0.05 M H_2SO_4, 60 % ethanol). The mixture was filtered through a muslin cloth and centrifuged at 8,000 x *g* for 10 min. The supernatant was collected and pH adjusted to 3.0 using ammonia solution. Then, 4 volume of acetone was added to 1 volume of supernatant for precipitation, and kept at 4°C for 24 h. The precipitate was dialyzed with a dialysis membrane (molecular weight cut-off: 10KDa).

Induction of diabetes in rats

The rats were fasted overnight and received STZ (50 mg/kg, intraperitoneally). STZ was freshly prepared by dissolving it in citrate buffer (0.01 M, pH 4.5) and maintained on ice prior to use.[11] One week following STZ administration, diabetes was confirmed by measuring the fasting blood glucose concentration. The diabetic rats with blood glucose concentration of >200 mg/dL were used in the experiments. The experiments were performed 8-10 days after STZ administration.

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

Effect of MC extract on normal and diabetic rats

Normal and diabetic rats were fasted for 6 h and divided into 4 groups with 8 rats in each group. Group 1 received subcutaneous administration of 0.9% NaCl. Groups 2, 3 and 4 received 3 different doses of the *MC* protein extract (1, 5, 10 mg/kg), respectively. Blood samples were collected in chilled heparinized tubes from the tail vein before and 1, 2, 4, 6, 8 h after administration and centrifuged (2,000 x *g*) at 4°C for 5 min. The plasma was frozen for subsequent analysis of glucose and insulin. The plasma glucose concentrations were determined by glucose oxidase method (Sigma Chemical, St. Louis, MO) and the absorbance was measured with a spectrophotometer at the wavelength 450 nm. Plasma insulin concentrations were determined by using radioimmunoassay (RIA) kits (Diagnostic Products Corporation, Los Angeles, CA).

In situ pancreatic perfusion

The rats were fasted for 12 h before experiments with 3 rats in each group. The rats were anesthetized with pentobarbital sodium (60 mg/kg, intraperitoneally) and were maintained at 37°C on a hot plate during the experiment. The celiac artery and the hepatic portal vein were cannulated with a polyvinyl tubing. Then, the pancreas was immediately perfused with the Krebs-Ringer bicarbonate buffer (KRB) supplemented with 20 mmol/L HEPES, 5.5 mmol/L glucose, 1 % dextran, and 0.2% BSA as a basal medium. The KRB was continuously aerated with 95% O_2 - 5% CO_2 at pH 7.4. The perfusion rate was set at 1 ml/min. The rats were euthanized immediately after the placement of cannulas and the beginning of the flow. The perfusion was equilibrated for 20 min. Following the baseline period of 10 min, the perfusate containing 10 μg/ml *MC* protein extract was administered for 30 min, followed by a washout period of 10 min. The perfusate containing arginine (1 mM) was administered as a positive control for 5 min at the end of each experiment. The effluent fractions were kept at 4°C and subsequently assayed for insulin and glucagon using RIA as previously described.[12]

Cell culture

3T3-L1 adipocytes were maintained in Dulbecco's modified high glucose Eagle's medium (DMEM) and supplemented with 10% FBS at 37°C (5% CO_2 in air). Preadipocytes 3T3-L1 were grown in 24-well plates until 2 days postconfluence. The differentiation was induced by addition of 0.5 mmol/L isobutylmethylxanthine (IBMX), 1 μmol/L dexamethasone (DEX), and 167 nmol/L bovine insulin in DMEM with 10% FBS. Two days after induction, the IBMX and DEX were placed with 10% FBS and 167 nmol/L bovine insulin. The medium was subsequently replaced again with fresh medium for 2 more days. C_2C_{12} cells were grown in 24-well plates in high glucose DMEM and supplemented with 10% FBS, 1% antibiotic solution (penicillin G and

streptomycin) at 37°C (5% CO_2 in air) until 70% confluence. To induce differentiation into myocytes, confluent cells were exposed to DMEM supplemented with 2% horse serum for 4 more days.

Glucose uptake assay

After incubation with the *MC* protein extract for 14-18 h, C_2C_{12} myocytes and 3T3-L1 adipocytes were washed once with KRB containing no glucose or BSA, then incubated with the same KRB at 37 °C for 30 min. The 10 μg/ml of the *MC* protein extract or 10 nmol/L bovine insulin was added to the KRB and incubated for another 15 min. At 15 min, 0.2 μCi of 2 deoxy-[^{3}H]-glucose was added to each well and incubated for another 15 min. The reaction was terminated by washing cells twice with 1 ml of 100 mmol/L ice-cold PBS and solubilized in 0.1 N NaOH in a volume of 400 μl/well. The cell-associated radioactivity was measured by a liquid scintillation counter.

Test Agents

Streptozotocin and fetal bovine serum were purchased from Sigma Chemical (St. Louis, MO). Rat and bovine insulin and glucagon were donated by Eli Lilly laboratories (Indianapolis, IN). Insulin antibody was donated by Dr. V. Leclerq-Meyer of Free University of Brussels, Belgium. Glucagon antibody was donated by Dr. Joseph Dunbar of Wayne State University (Detroit, MI). ^{125}I-labeled glucagon was purchased from Linco Research (St Charles, MO). 2-Deoxy-[^{3}H]-glucose was purchased from PerkinElmer (Boston, MA).

Data Expression and Statistical Analysis

Data are expressed as mean ± SE. The effluent concentrations of insulin and glucagon are expressed as a percentage of baseline level. Data were analyzed using ANOVA followed by a Dunnett's multiple comparison test and Student's pair *t* test. The significance level was set at $P < .05$.

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

RESULTS

Effects on plasma glucose in normal and STZ-induced diabetic rats

Results in Figure 1 show the effect of the *MC* protein extract on plasma glucose concentration in normal and STZ-induced diabetic rats. The *MC* protein extract (5 and 10 mg/kg) significantly and markedly decreased plasma glucose concentrations in both normal (Fig 1A) and diabetic rats (Fig 1B) in a dose-dependent manner. In normal rats, the onset of the *MC* protein extract-induced hypoglycemia was observed at 6 h after subcutaneous administration. In STZ-induced diabetic rats with overnight fasting, the plasma glucose concentrations were 3 times higher than those of normal rats within 1 week after STZ

administration (normal control rats: 95 ± 3 mg/dL; STZ-induced diabetic rats: 263 ± 12 mg/dL). The onset of 5 mg/kg and 10 mg/kg protein extract-induced antihyperglycemia in diabetic rats was observed at 8 h and 4 h after subcutaneous administration, respectively. The highest concentration of the *MC* protein extract used in this experiment (10 mg/kg) exerted a 43 % maximal reduction in plasma glucose from 261 ± 8 mg/dL to 148 ± 14 mg/dL at 8 h. In contrast, the *MC* protein extract at 1 mg/kg, failed to decrease plasma glucose concentration in both normal and diabetic rats.

Effects on plasma insulin in normal and STZ-induced diabetic rats

As shown in Table 1, 10 mg/kg *MC* protein extract increased plasma insulin concentrations in normal and diabetic rats. In normal rats, the protein extract significantly increased plasma insulin concentration at 4 and 6 h after subcutaneous administration, when compared with the basal concentration at time 0. The plasma insulin concentrations were 1.7and 1.5 times of the concentration at time 0, respectively.

One week after diabetes induction, the STZ-induced diabetic rats had insulin insufficiency. The action of STZ in pancreatic β-cells is accompanied by alteration in blood insulin and glucose concentrations. It is taken up by pancreatic β-cells via GLUT2 ,[13] leading to impaired glucose oxidation[14] and decrease insulin biosynthesis and secretion.[15-16] Our results showed that the basal plasma insulin concentrations in these diabetic rats were about 7-fold lower than those of normal rats (normal rats: 837 ± 62 pg/mL; diabetic rats: 112 ± 14 pg/mL). Subcutaneous administration of 10 mg/kg *MC* protein extract significantly increased plasma insulin concentrations, which reached 200 ± 14 pg/mL at 4 h, and 313 ± 32 pg/mL at 6 h after subcutaneous administration. The plasma insulin concentrations were 1.7 and 2.6 times of the concentration at time 0, respectively.

Effects on insulin and glucagon secretions from perfused rat pancreas

Results in Fig 2 show the profile of insulin secretion remained constant during 50 min in the basal control group receiving KRB alone. The perfusate containing 10 µg/mL *MC* protein extract was administered for 30 min and it increased insulin secretion, which was 2.4-fold over the basal control group. In contrast, it did not change glucagon secretion (data not shown). The effluent concentration of insulin returned to baseline during the 10-min washout period in treated group. Administration of 1 mmol/L arginine increased insulin secretion to 7-fold of the baseline level at the end of the experiment.

Effects on glucose uptake in rat myocytes and adipocytes

The effects of the *MC* protein extract (10 µg/mL) and insulin (10 nmol/L) on a 2-deoxyglucose uptake assay in transformed C_2C_{12} (Fig 3A) and 3T3-L1 (Fig 3B) cells. The 2-

deoxyglucose uptakes in both kinds of cells were significantly increased by exposing them to the *MC* protein extract for 14-18 h and to insulin for 30 min. However, the *MC* protein extract did not potentiate the effect of insulin-induced glucose uptake in these cells. In C_2C_{12} myocytes, the *MC* protein extract and insulin increased 2-deoxyglucose uptake by 28 % and 37 % compared to the basal control group, respectively (Fig 3A). In 3T3-L1 adipocytes, the *MC* protein extract and insulin increased 2-deoxyglucose uptake by 2.4 fold and 6.3 fold compared to the basal control group, respectively (Fig 3 B).

DISCUSSION

The results of the present study showed that the *MC* protein extract had definite hypoglycemic/antihyperglycemic effect in both normal and STZ-induced diabetic rats via both insulin secretagogue and insulinomimetic pathways. Subcutaneous administration of the *MC* protein extract into both normal and STZ-induced diabetic rats produced a marked plasma glucose lowering effect in a dose-dependent manner. However, the onset of the *MC* protein extract-induced antihyperglycemia/hypoglycemia was observed at 4 and 6 h in diabetic and normal rats, respectively. In addition, this protein extract also raised plasma insulin concentrations ~2 fold 4 h following subcutaneous administration. This finding is novel since it is the first report on the *MC* protein extract-induced hypoglycemia with a delayed onset. Although, Khanna et al.[9] also reported an antihyperglycemic effect, which reached maximum between 4-8 h after subcutaneous polypeptide-P administration, the onset of action was observed within 30 min. This discrepancy may be explained by the fact that our protein extract preparation was not extracted using zinc acetate solution as used by Khanna et al.[9] Zinc has been shown to increase glucose uptake in adipocytes within 30 min of administration. Therefore, it is possible that, in the study by Khanna et al., zinc might have played the role in lowering plasma glucose rapidly.

The *MC-induced* hypoglycemia may be due to a mixture of steroidal saponins called charantins, insulin-like peptides and alkaloids.[22] Day et al.[4] has reported that these chemicals are divided into two categories with different time-dependent effects; one is present in the aqueous solution and the residue after alkaline chloroform extraction exerted a fast hypoglycemic activity; the other is present in acidic wash of chloroform extraction, which exerted a slow antihyperglycemic activity. However, they only studied the hypoglycemic effect of orally administered extracts of *MC*. Although, our extract also generated slow hypoglycemic effect, it should be different from the one that mentioned by Day et.al.,[4] since our extract is protein in nature, which should be destroyed by the enzymes in gastrointestinal tract.

In general, after distribution to target tissues, insulin activates its specific receptor, which leads to activation of tyrosine kinase, phosphatidylinositide-3 kinase, and other signals. This event decreases the plasma glucose concentrations by promoting glucose transport[23] and glycogen synthesis.[24] To determine which mechanisms underlie the *MC*-protein extract-induced hypoglycemia, we investigated its effect on insulin and glucagon secretion from the perfused rat pancreas and on glucose uptake in rat myocytes and adipocytes. To our knowledge, the present study is the first one to demonstrate the direct effect of the *MC* protein extract on insulin and glucagon secretions using pancreatic perfusion. These results provided the evidence of a direct pancreatic action on β-cells. The persistence of the insulinotropic activity in vivo for the *MC* protein extract was confirmed by the data obtained from the perfused rat pancreas. The *MC* protein extract (10 μg/mL) stimulated insulin secretion in the presence of 5.5 mmol/L glucose. This protein extract induced a transient insulin peak within 5 min of administration, which was 2.4-fold of the basal level. In contrast, it has no effect on glucagon release at the same concentration. The lack of activity on glucagon release, but the stimulatory effect of MC on insulin secretion would be a benefit to diabetic patients. Interestingly, the *MC* protein extract increased plasma insulin at 4 and 6 h after subcutaneous administration, but it immediately increased insulin secretion in pancreatic perfusion. This could be due to the much higher dosage of the protein extract (10 μg/mL) used in the perfusion study than *in vivo* study (5 and 10 mg/kg). The 10 mg/kg of the protein extract probably would not be able to yield the concentration close to 10 μg/mL in the pancreas.

The *MC* protein extract at 10 μg/ml increased glucose uptake in rat adipocytes and myocytes. In addition, the effect of the *MC* protein extract was not significantly different in the absence or presence of insulin. Furthermore, we investigated the time-course (1, 2, 4 and 8 h incubation period) effect of this protein on glucose uptake. We found that this protein increased glucose uptake after incubation for 4 and 8 h (data not shown). Therefore, this result confirms that our *MC* protein extract indeed exerts a slow hypoglycemic effect by increasing insulin secretion and glucose uptake. Our finding is consistent with the previous report that the protein extracts from bitter gourd fruits and seed as well as the fruit juice display glucose and amino acid uptakes.[10, 23, 25] However, it is different from the others in term of the delay in onset of action. Taken together, these results suggested that the *MC* protein extract exert both insulin secretagogue and insulin-like activities in lowering blood glucose in vivo. Further study is now in progress to isolate and characterize the *MC* protein that induces antihyerglycemia/hypoglycemia.

In summary, we demonstrated that the *MC* protein extract, a slow acting chemical, was able to decrease plasma glucose level, and increase plasma insulin secretion in both normal and diabetic rats. This activity was also observed in perfused rat pancreas, which has shown the stimulatory effect on insulin, but not glucagon secretions. In addition, the *MC* protein extract stimulated glucose uptake in rat myocytes and adipocytes just like insulin. Therefore, these results support the *MC* protein extract as a beneficial therapeutic agent for diabetes mellitus.

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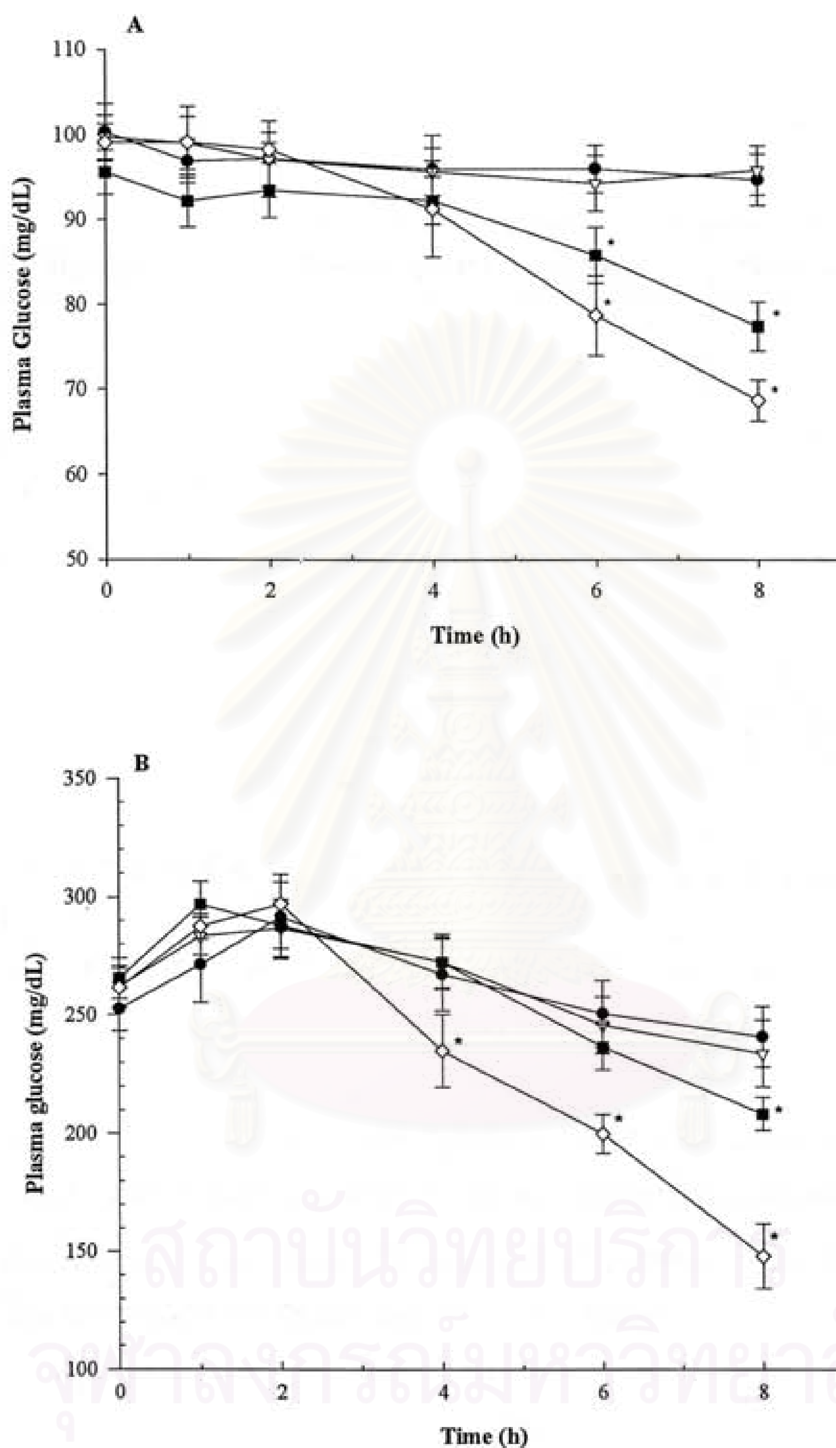

Fig 1. A 8-h profile of plasma glucose concentration in normal rats (A), and diabetic rats (B). The dosage of the *MC* protein extract was 1 mg/kg (▽), 5 mg/kg (■) and 10 mg/kg (◇). 0.9 % NaCl was used in the control group (·). Data are expressed as mean ± SE. (n = 8) **P< 0.05* , ** *P< 0.01* vs control group.

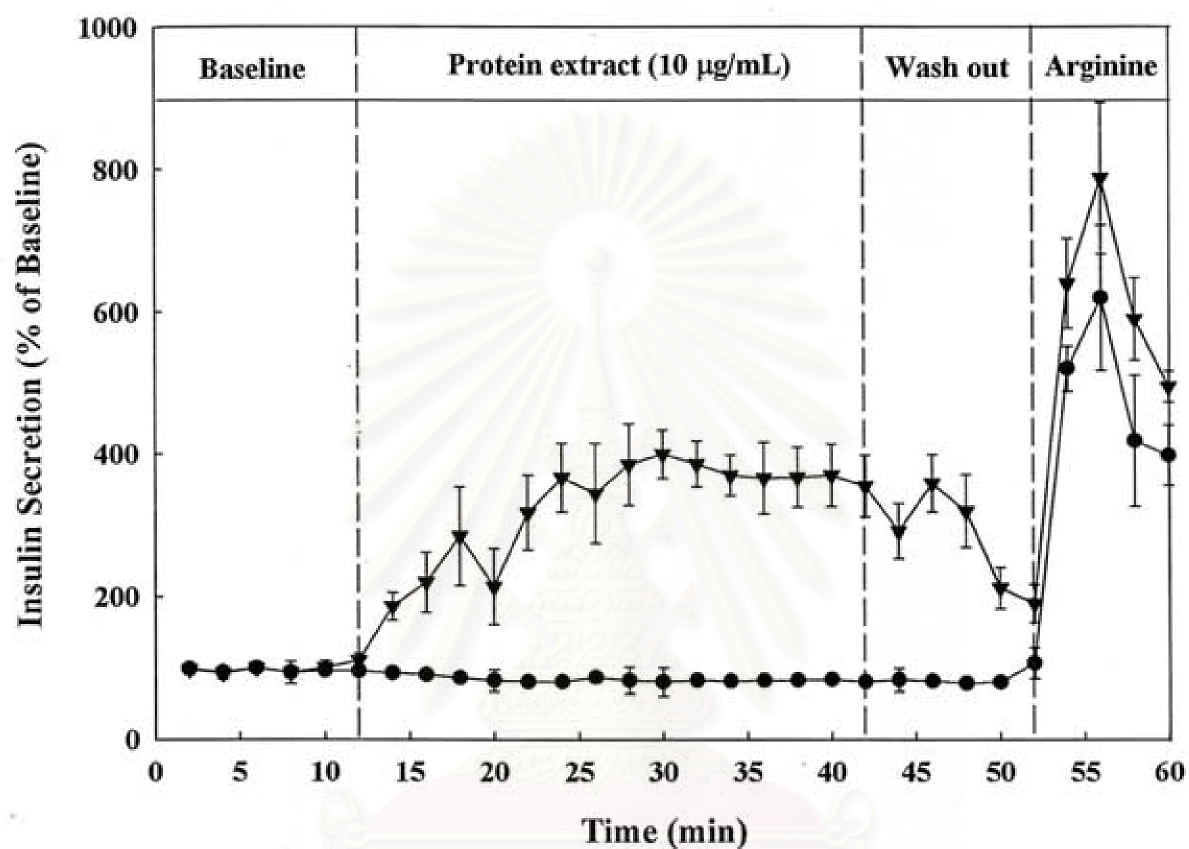

Fig 2. Effects of the *MC* protein extract (10 μg/mL) on insulin release from perfused rat pancreas. Following the baseline period of 20 min, the *MC* protein extract was administered for 30 min. Values are mean ± SE; n =3. ·, Basal control; ○, protein extract10 μg/mL. Range of baseline insulin concentration of effluent was 665 to 4,365 pg/mL.

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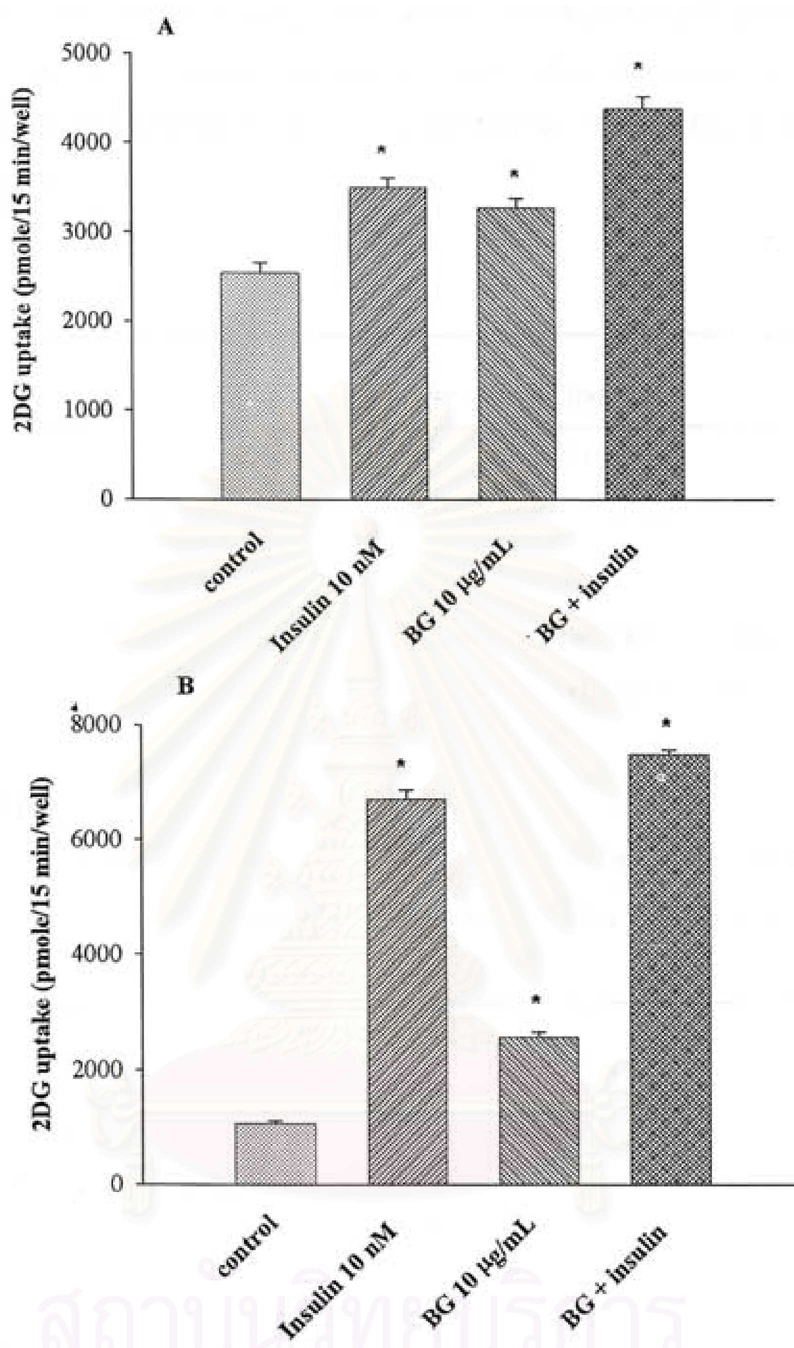

Fig 3. Effects of the MC protein extract (10 μg/mL) on glucose uptake assay in C_2C_{12} myocytes (A) and 3T3-L1 rat adipocyte (B) cells. These cells were incubated with the *MC* protein extract for 14-18 h and insulin for 30 min. Data are expressed as mean ± SE (n= 3-5). * $P < .05$ vs. control group. 2DG , 2-deoxyglucose; BG: bitter gourd extract.

Table 1. Plasma insulin concentrations of normal and streptozotocin (STZ)-induced diabetic rats receiving subcutaneous administration of10 mg/kg MC protein extract. The control rats received 0.15 M NaCl. Results are expressed as mean ± SE. (n = 8). *P< *.05* vs. control group.

Treatment	Plasma Insulin (pg/mL)					
	0 h	1 h	2 h	4 h	6 h	8 h
Normal rats						
Control	837 ± 62	835 ± 100	860 ± 148	792 ± 100	815 ± 109	849 ± 8
Extract (10mg/kg)	811 ± 199	761 ± 135	829 ± 148	1369 ± 196*	1202 ± 212*	779 ± 136
STZ-induced diabetic rats						
Control	112 ± 14	103 ± 11	109 ± 10	117 ± 5	101 ± 8	121 ± 13
Extract (10mg/kg)	120 ± 8	100 ± 9	99 ± 10	200 ± 14*	313 ± 32*	126 ± 14

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Output จากโครงการวิจัยที่ได้รับทุนจากสกอ.และสกว.

1. ส่งไปลงตีพิมพ์ในวารสารวิชาการนานาชาติ Metabolism (กำลังรอผลการพิจารณา)
2. จะนำไปเสนอผลงานในที่ประชุมวิชาการนานาชาติ "Experimental Biology 2005" ณ เมือง ซานดิเอโก มลรัฐ แคลิฟอร์เนีย ประเทศสหรัฐอเมริกา ในวันที่ 2-6 เมษายน พ.ศ. 2548

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