Attenuation of Hyperglycemia and Hyperlipidemia in High Fat Diet and Streptozotocin Induced Diabetic Rats by Aqueous Extract of *Gynostemma pentaphyllum*

ฤทธิ์ลดระดับน้ำตาลและไขมันในเลือดของสารสกัดปัญจขันธ์ในหนูขาวที่ได้รับอาหารไขมันสูงร่วมกับ Streptozotocin เพื่อเหนี่ยวนำให้เกิดภาวะเบาหวาน

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ABSTRACT

This study was conducted to evaluate the antihyperglycemic and hypolipidemic effects of aqueous extract of *Gynostemma pentaphyllum* (GPE) in high fat diet and streptozotocin induced diabetic rats. After the supplement with 300 mg/kg BW of GPE for 20 weeks, the body weight, visceral fat, fasting plasma glucose, FFA, and cholesterol levels of diabetic rats were significantly lower than those of the diabetic control group. The GPE treatment profoundly affects the $TAUC_g$ from the OGTT compared to the diabetic control group but not the $TAUC_i$. The tissue triglyceride (TG) contents revealed that muscle TG content was reduced in the GPE-supplement diabetic group than in the diabetic control group, and this was correlated with improving of HOMA index and enhancing of total GLUT4 protein expression. These results suggest that the supplement of GPE may improve insulin sensitivity and together with the significant reduction of hyperglycemia and hyperlipidemia in diabetic rats.

บทคัดย่อ

การศึกษาเกี่ยวกับฤทธิ์ในการลดระดับน้ำตาลและไขมันในเลือดของสารสกัดปัญจขันธ์ในหนูที่ได้รับอาหารไขมันสูงร่วมกับ streptozotocin เพื่อเหนี่ยวนำให้เกิดภาวะเบาหวาน จากการศึกษาพบว่าหนูเบาหวานที่ได้รับสารสกัดปัญจขันธ์ในขนาด 300 mg/kg BW เป็นเวลา 20 สัปดาห์จะมีน้ำหนักตัว, ไขมันในช่องท้อง, ระดับน้ำตาล, กรดไขมันอิสระ และโคเลสเตอรอลในเลือดต่ำกว่าหนูเบาหวานกลุ่มควบคุม นอกจากนั้นการให้สารสกัดปัญจขันธ์ในหนูเบาหวานยังมีผลลดพื้นที่ใต้กราฟของกลูโคส จากการทดสอบความทนทานต่อน้ำตาลกลูโคสเมื่อเทียบกับหนูเบาหวานกลุ่มควบคุมโดยไม่มีผลต่อพื้นที่ใต้กราฟของอินซูลิน การศึกษาไตรกลีเซอไรด์ที่สะสมในกล้ามเนื้อพบว่าหนูเบาหวานที่ได้รับสารสกัดปัญจขันธ์จะมีการสะสมของไตรกลีเซอไรด์ในกล้ามเนื้อต่ำกว่าหนูเบาหวานกลุ่มควบคุม ซึ่งผลที่ได้สัมพันธ์กับการลดลงของค่า HOMA index และการเพิ่มขึ้นของการแสดงออกของตัวพากลูโคสชนิดที่ 4 (GLUT4) จากผลการศึกษานี้แสดงให้เห็นว่า การให้สารสกัดปัญจขันธ์จะช่วยเพิ่มความไวต่ออินซูลินรวมถึงมีผลลดระดับน้ำตาลและไขมันในเลือดในหนูขาวที่มีภาวะเบาหวาน

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Introduction

Type 2 diabetes mellitus (T2DM) is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both (American Diabetes Association, 2005). It has been recognized as one of the most common metabolic disease and the world largest growing metabolic disease (King et al., 1998). Hyperglycemia and hyperlipidemia are two important characteristics of T2DM, which contribute to many complications of diabetes. Sustained reductions in hyperglycemia decrease the risk of developing microvascular and macrovascular complications (Gaster et al., 1998). In modern medicine, no satisfactory effective therapy is still available to cure T2DM. Though pharmaceutical drugs which used for treatment of T2DM are either expensive or have undesirable side effects or contraindications. Nowadays, T2DM patients and healthcare professionals are considering complementary and alternative approaches, including the use of medicinal herbs with antihyperglycemic activities (Xie et al., 2002).

Gynostemma pentaphyllum (GP) is a perennial creeping herb of the genus Gynostemma. It has been widely used traditionally in Southeast Asian Folk medicine for its anti-inflammatory, antipyretic, diuretic and tonic properties (Blumert & Liu, 1999). Up to date, the GP is commonly consumption as hot tea in many countries in Asia including Thailand. The dammarane, saponins, also called gypenosides or gynosaponins, are believed to be the active ingredient responsible for its biological activity of the *Gynostemma pentaphyllum* extract (Razmoski-Naumovski et al., 2005). Previous study demonstrated that gypenosides have antihyperglycemic and antihyperlipidemic actions in the Zucker fatty rats (Megalli et al., 2006). However, effects of aqueous extracts of Thai strain GP in type 2 diabetic rat model has not been evaluate. The aim of this study is to explore the scientific basis of the utility of the aqueous extract of *Gynostemma pentaphyllum* for correction of hyperglycemia and hyperlipidemia in high fat diet and streptozotocin induced diabetic rats.

Materials and methods

Preparation of aqueous extract of *Gynostemma pentaphyllum*: *Gynostemma pentaphyllum* total plant extracts (Thai strain) was prepared by the Department of Medical Sciences, Ministry of Public Health. Briefly, dried herb was soaked in water, boiled for 60 minutes, and filter with Whatman filtered paper. The extract was then lyophilized and kept in a dessicator. A final purity of approximately 6% gypenosides was obtained.

Animals: Male adult Wistar rats, weighing 200-250 g, were obtained from the National Laboratory Animal Center, Mahidol University. All animals were housed under controlled temperature with a 12-hour light: dark cycle. The experimental protocol adhered to the Guide for the Care and Use of animals in compliance with the National Institutes of Health guideline for the care and treatment of animals and followed Faculty of Medicine, Chiang Mai University, for the standard operating procedures for animal care and research.

Developments of HFD-fed and STZ-treated type 2 diabetic rats: Rats were allocated into two dietary regimens by feeding normal diet (ND) (20% fat of

total energy) and high fat diet (HFD) (60% fat of total energy) ad libitum for the initial period of 2 weeks (Srinivasan et al., 2005). After the 2 weeks of dietary manipulation, the group of rats fed by HFD were injected intraperitoneally (i.p.) with streptozotocin (STZ) 35 mg/kg BW, while the respective control rats were given citrate buffer (pH 4.4). The fasting blood glucose level was measured at 1st week after the vehicle or STZ injection. Rats with fasting blood glucose level >250 mg/dL will be considered diabetes and used in this study.

Experimental design: In this study, a total of 50 rats were used and divided into five groups of 10 rats each. Group I: normal rats (NC); Group II: normal control administered with the aqueous extract of *Gynostemma pentaphyllum* (GPE) at 300 mg/kg BW (NCGPE); Group III: diabetic control (DMC); Group IV: diabetic rats administered with GPE at 300 mg/kg BW (DMGPE); Group V: diabetic rats administered with the reference drug pioglitazone, a PPAR-γ agonist, at a dose of 10 mg/kg BW (DMPGT). The extract and drug used in this study were administered by gavage feeding for 20 weeks. The effects of GPE administration were determined by measuring fasting plasma glucose, insulin and lipid levels, initial and final changes in body weight. At the end of study, the oral glucose tolerance test (OGTT) was performed. The rats were then sacrificed, blood and tissue samples were collected for further biochemical analysis.

Study of Oral Glucose Tolerance Test (OGTT): After overnight fasting, the animals were orally gavaged with 2 g/kg BW of glucose dissolved in water. Blood glucose and insulin levels were measured at 0, 15, 30, 60, and 120 minutes. The increment of plasma glucose and insulin following the glucose loading was expressed in term of the area under the curve (AUC) using the trapezoidal rule (Dokken & Henriksen, 2006).

Determination of Homeostasis Model Assessment (HOMA) Index: Insulin resistance was assessed by the homeostasis model assessment of insulin resistance (HOMA). HOMA is a mathematical model describing the degree of insulin resistance starting from fasting plasma insulin and glucose concentration. HOMA index is calculated as follows: HOMA index = [fasting plasma insulin level (μU/mL) x fasting plasma glucose level (mmol/L)]/22.5 (Matthews et al., 1985).

Plasma glucose, triglyceride, free fatty acid and insulin measurements: Plasma glucose and triglyceride levels were analyzed using a commercial enzymatic colorimetric kit (Biotech, Bangkok, Thailand). Plasma FFA level was analyzed using a commercial enzymatic kit (NEFA C, Wako pure chemical, Japan). The plasma insulin concentrations were determined using a sandwich ELIZA (Rat/Mouse Insulin ELIZA kit, LINCO Research, USA).

Determination of tissue triglyceride content: Liver, gastrocnemius and pancreas homogenates were prepared for triglyceride assay by a modification of the method of Frayn and Maycock (1980). The triglyceride concentrations were analyzed with a commercial colorimetric kit (Biotech, Bangkok, Thailand).

Determination of glucose transporter 4 (GLUT4): Soleus muscle was homogenated in 6 volume of ice-cold lysis buffer. Homogenate was incubated on ice for 20 minutes and then centrifuged at 1300×g for 20 min at 4ºC. Total protein concentration was determined with the Bradford protein assay reagent

kit (Bio-Rad Laboratories, USA), using BSA as a standard. Aliquots (35 μg) of muscle homogenate was subjected to SDS-PAGE (10% gel) and electrophoretically transfer to nitrocellulose membrane. Blotted protein was then probed with anti-GLUT4 (Chemicon International, USA) at 4ºC overnight. Membrane was then probed with HRD-conjugated secondary anti-rabbit antibodies (Chemicon International, USA). Probed proteins were visualized on Kodak Hyperfilm (Godak, Rochester, NY) using an enhanced ECL kit (GE Healthcare, Piscataway, NJ). The band intensities were quantified with a densitometer using Scion Image software. The concentrations of GLUT4 protein were expressed as arbitrary units of a reference muscle used for the comparison among groups.

Results

Effect of GPE on fasting plasma glucose concentrations

As shown in Figure 1, there was significant elevation in fasting plasma glucose concentrations of diabetic rats in respect to normal rats. There was also no significant different in fasting plasma glucose concentrations between diabetic groups at the beginning of experiment. Both DMGPE and DMPGT groups, fasting plasma glucose concentrations were gradually decreased after 4 weeks of administration and significantly lower than those in DMC group at 10 and 20 weeks ($p < 0.05$). Throughout the experimental period, there was no significant between NC and NCGPE groups in fasting plasma glucose concentrations.
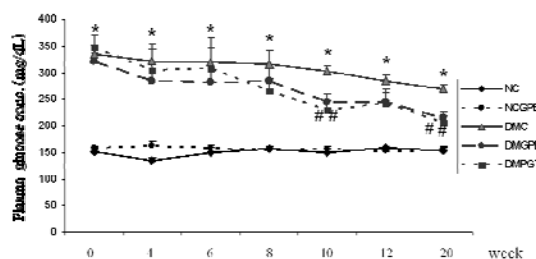

Fig. 1. Effect of GPE on fasting plasma glucose concentrations. Values are mean : SE. *$p < 0.05$ VS. ND, #$p < 0.05$ VS. DMC

Effects of GPE on body weight, visceral fat, fasting plasma glucose, insulin, triglyceride, cholesterol, HDL-cholesterol, FFA and HOMA index

The GPE had no effects on body weight, visceral fat, fasting plasma glucose, insulin, triglyceride, cholesterol, HDL-cholesterol, FFA concentrations and HOMA index when compared with NC and NCGPE groups (Table 1). As presented in Table 1, body weight, visceral fat, fasting plasma glucose, insulin, triglyceride, HDL-cholesterol and FFA concentrations were significantly increased in DMC group when compared with NC group. GPE administration for 20 weeks significantly decreased in visceral fat, fasting plasma glucose, FFA and cholesterol levels as compared to DMC group ($p < 0.05$). Likewise, PGT treatment significant lowered fasting plasma glucose, insulin, triglyceride and FFA levels in respect to the DMC group. There was also a decreased in fasting plasma insulin levels following GPE supplement for 20 weeks although no significant difference was noted. An increased HOMA index at the end of experiment in DMC group revealed that whole body insulin resistance was developed. In contrast, there was a significantly lowered HOMA index at week 20 in

Table 1. Effects of GPE on body weight, visceral fat, fasting plasma parameters, tissue TG content, area under the curve for glucose or insulin and HOMA index at the end of experiment.

	NC	NCGPE	DMC	DMGPE	DMPGT
BW (g)	552.38±17.45	610.17±25.11	710.50±25.86 *	643.67±19.74 *	752.50±19.63 *
Visceral fat (g)	35.44±3.02	39.79±3.08	80.80±6.09 *	63.54±6.33 *,#	58.86±4.12 *,#
Fasting plasma parameters					
Glucose (mg/dL)	152.89±5.32	156.49±4.93	289.00±77.34 *	214.32±13.26 *,#	205.22±13.83 *,#
Insulin (ng/dL)	1.75±0.35	1.81±0.46	3.58±0.23 *	3.12±0.63 *	1.70±0.9 #
Triglyceride (mg/dL)	118.50±6.59	119.67±23.67	221.6±33.83 *	182.20±20.08 *	109.20±15.16 #
FFA (mmol/dL)	0.30±0.10	0.33±0.07	0.84±0.10 *	0.38±0.05 #	0.32±0.08 #
Cholesterol (mg/dL)	89.40±2.94	76.00±5.45	89.00±3.36	71.80±4.98 #	99.40±2.68
HDL-cholesterol (mg/dL)	81.40±5.04	79.50±5.91	53.20±11.50 *	60.40±4.57	76.20±8.79
HOMA index	0.67±0.14	0.65±0.18	2.06±0.14 *	1.35±0.21 *,#	0.77±0.06 #
TAUC$_g$ (mg/dL×min×10^3)	24.25±1.37	23.80±0.84	44.03±3.52*	36.44±2.92 *,#	36.14±2.08 *,#
TAUC$_i$ (mg/dL×min)	330.34±74.83	294.82±41.47	324.20±41.79	329.41±42.46	194.21±35.87
Hepatic TG (mg/g tissue)	9.63±1.89	9.28±3.72	240.18±25.2 *	162.15±35.14 *,#	84.34±20.87 *,#
Muscle TG (mg/g tissue)	5.51±1.25	4.99±1.55	9.45±1.34 *	5.13±0.65 #	9.59±1.32 *
Pancreatic TG (mg/mg protein)	10.44±1.32	11.96±1.35	14.89±2.20 *	10.45±1.13	11.67±1.12

Values are mean ± SE. FFA: free fatty acid, HOMA index: Homeostasis Model Assessment of Insulin Resistance index, TAUC$_g$: total area under the curve for glucose, TAUC$_i$: total area under the curve for insulin. * $p < 0.05$ vs. NC, # $p < 0.05$ vs. DMC.

DMGPE and DMPGT groups compared with the DMC group ($p<0.05$).

Effect of GPE on an oral glucose tolerance test (OGTT)

To determine the whole body insulin-sensitizing capacity of GPE, the OGTT was performed at the last week of supplementation. As shown in Table 1, the OGTT results demonstrated that TAUC$_g$ in the DMC group was significantly higher than in the NC group ($p<0.05$). Although, the TAUC$_i$ was not significantly different among the NC and DMC groups. Compared with the DMC group, the TAUC$_g$ in both DMGPE and DMPGT groups

were significant decreased ($p<0.05$). Likewise, there was no significant difference in TAUC$_i$ between two treatment groups and the DMC group.

Effect of GPE on tissue triglyceride content

As demonstrated in Table 1, there was no significant difference between the NC and NCGPE groups in hepatic, gastrocnemius muscle and pancreatic triglyceride contents ($p>0.05$). The triglyceride content within liver, gastrocnemius muscle and pancreas in the DMC group significantly increased compared with those in the NC group ($p<0.05$). Interestingly, the GPE supplement in diabetic rats significantly decreased the hepatic and

gastrocnemius muscle triglyceride contents ($p<0.05$). While, the PGT treatment significantly lowered only hepatic triglyceride content compared with the DMC group ($p<0.05$).

Effect of GPE on total GLUT4 protein expression

There was a significantly decreased in total expression of GLUT4 protein (~25.93%) in the DMC group compared with the NC group ($p<0.05$, Fig 2). PGE supplement almost restored the total GLUT4 protein expression to the normal rats ($p>0.05$), while the PGT treatment caused a slightly increased of total GLUT4 protein expression by 13.64% compared to normal rats (Fig. 2). There was no effect of GPE supplement on total GLUT4 protein expression in normal rats.
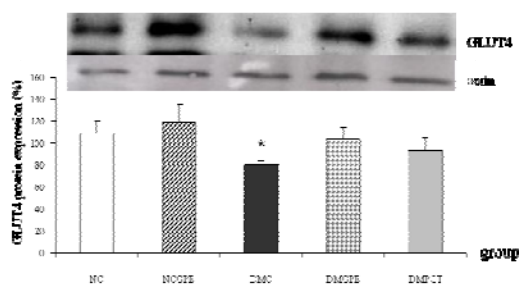

Fig.2. Effect of GPE on GLUT4 protein concentrations. Values are expressed as a percentage of GLUT4 protein concentration compared with internal control and presented as mean · SE. *$p < 0.05$ VS. NC

Discussions

In this study, the antihyperglycemic and antihyperlipidemic effects of aqueous extract of *Gynostemma pentaphyllum* was investigated using the obese-diabetic rat model induced by high fat diet and steptozotocin (STZ) injection. Although the high dose of STZ severely impairs insulin secretion mimicking type 1 diabetes, low-dose STZ has been known to induce a mild impairment of type 2 diabetes

(Reed et al., 2000). There was a significant enhancement of plasma insulin and glucose concentrations in diabetic rats as compared to normal controls, it suggesting that insulin resistance has been developed in these animals. Therefore, this rat model exhibits obesity, hyperglycemia, hyperlipidemia and insulin resistance that would closely reflect the natural history and metabolic characteristics of humans, and needed for further investigation of pharmacological testing.

Gynostemma pentaphyllum (GP) has been used traditionally in Southeast Asian medicine. It contains more than 90 of saponins, including gypenosides or gynosaponins that have been isolated and identified and are suggested to have a variety of pharmacological properties (Zhou, 1998). Antihyperglycemic potency of the aqueous extract of *Gynostemma pentaphyllum* (GPE) in type 2 diabetic rats has been indicated here by the study of fasting plasma glucose levels as it is an important basal parameter for monitoring of diabetes. The 300 mg/kg BW of GPE exerted the effective antihyperglycemic activity. This effective dose is similar to that report in previous studies (Megalli et al., 2005: Megalli et al., 2006) and supported by the results of those previous in vivo studies (Megalli et al., 2006: Yeo et al., 2008). In addition, GPE supplement (300 mg/kg BW) for 20 weeks also improved glucose tolerance as evidence by the decreased $TAUC_g$ from the OGTT in diabetic rats. Because of an OGTT was used to indicate the response of insulin receptors to the elevation of exogenous glucose, thus it was served as a marker for testing the sensitivity of insulin receptors. In these diabetic rats, the sensitivity of insulin receptors to glucose was significantly reduced (Reed et al., 2000). Following glucose loading to

diabetic rats, glucose levels were increased by approximately 81.5%. Interestingly, GPE was found to be effectively reducing glucose levels by 17.88% compared to non-treated diabetic rats at the 120 min interval. This finding also suggested that GPE may improve the sensitivity of insulin receptors in type 2 diabetic rats. It is supported by the significant decrease of HOMA index in GPE treated diabetic rats. The lack effect of fasting plasma glucose levels and glucose response from OGTT in normal rats indicate that GPE may not produce a hypoglycemic effect in situation of normal blood glucose regulation. These antihyperglycemic effects might have been due to the increased release of insulin from remnant β-cells and/or regenerated β-cells. However, GPE has no effect on fasting plasma insulin levels and the $TAUC_i$ from the OGTT in both normal and diabetic rats. This result suggests a lack of pancreatic mechanism through the stimulation of insulin secretion from pancreatic β-cells. Although, Norberg and coworkers (2004) recently demonstrated that Phanoside, from *Gynostemma pentaphyllum*, can stimulate insulin secretion.

For further explanation of the mechanism by which GPE decreased fasting plasma glucose levels and improved the sensitivity of insulin, the GLUT4 protein expression was investigated. Results showed that the total GLUT4 protein expression significantly reduced in type 2 diabetic rats. Administration with GPE significantly restored the total GLUT4 protein expression in type 2 diabetic rats. This observation is strengthened by the finding of decrease in muscle TG content in DMGPE group. Elevated intramyocellular TG is strongly associated with insulin resistance (Stannard & Johnson, 2004). However, further investigation is needed to characterize the pathway by which GPE enhanced the total GLUT4 protein expression.

Dyslipidemia in type 2 diabetic rats is characterized by increased levels of plasma TG, cholesterol, FFA and HDL-cholesterol levels. This study indicated that the GPE significantly decreased plasma levels of FFA and cholesterol of type 2 diabetic rats. Meanwhile, both plasma TG and HDL-cholesterol levels were restored toward the control level, indicating that possesses both protective and treatment properties. These findings were also observed the treatment of pioglitazone, a PPAR-γ agonist. Numerous studies have confirmed antihyperglycemic and antihyperlipidemic properties of purified gypenoside extract in vivo (Megalli et al., 2005: Megalli et al., 2006). However, the mechanism of GPE on antihyperglycemia in these studies is different which might depend on type of animal model. This hypolipidemic effect may be due to the elevation of HDL-cholesterol level or an improvement of fatty acid oxidation (Kelly et al., 2002).

Conclusions

This present study demonstrated that the aqueous extract of *Gynostemma pentaphyllum* beside its antihyperlipidemic activity, can improve hyperglycemia and insulin resistance in high fat diet and streptozotocin induced type 2 diabetic rats, where it possibly acts by increasing total GLUT4 protein expression. This study was also able to exert a potential new effect of GPE in diabetes. Further investigations, however, are in progress to elucidate the detailed mechanism of antihyperglycemic and hypolipidemic effects.

Acknowledgements

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