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Full Length Research Paper

Influence of the methanolic extract from *Abutilon indicum* leaves in normal and streptozotocin-induced diabetic rats

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The methanolic leaf extract of *Abutilon indicum (AI)* was investigated for hypoglycemic effect in normal and streptozotocin-induced diabetic rats. The chemical screening of the extract showed that phenolic compounds and flavonoid contents were 1.04 ± 0.01 mg/g and 59.92 ± 3.88 µg/g extract, respectively. A single oral administration of the extract at a dose of 500 mg/kg significantly decreased the blood glucose concentrations in both normal and diabetic rats after 2 h administration. Metformin was used as the reference drug and reduced the blood glucose only in diabetic rats. To clarify the involved mechanism, normal rats were orally administered with sucrose and maltose at a dose of 3 g/kg with or without *AI* extract. The postprandial elevation in the blood glucose concentrations at 30 min after the administration of sucrose with the extract was significantly suppressed when compared with the control group. No significant change in blood glucose concentrations was observed in maltose-loading rats. An *in vitro* study indicated that *AI* extract inhibited α-glucosidases, the disaccharide-digesting enzyme in the small intestine. The extract showed a potent sucrase inhibitory activity with IC_{50} of 2.45 ± 0.13 mg/ml while the extract was less potent on the maltase inhibition. The results suggested that the extract from *AI* extract would be effective for lowering and suppressing elevation of blood glucose.

Key words: *Abutilon indicum*, methanolic extract, α-glucosidase inhibition, diabetes.

INTRODUCTION

Diabetes mellitus is a metabolic disease of elevated blood glucose, a condition caused either by insufficient insulin or insulin resistance. The number of diabetic patients is rapidly rising in most parts of the world, especially in developing countries such as Thailand. The prevalence of diabetes in Thai adults is increasing steadily thus adding to the urgency for the need to improve management (Aekplakorn et al., 2003). Maintaining near normal blood glucose concentrations is mainly based on the use of oral hypoglycemic agents and insulin. However, the fact is that all of these treatments have limited efficacy and associated undesirable side effects (Harrower, 1994; Campbell et al., 1996; Reuser et al., 1994). This has lead to increasing interests in the use of medicinal plants for alternative management of diabetes mellitus. *Abutilon indicum* (L.) Sweet (Malvaceae) is widely distributed in Africa, Asia, Sri Lanka, India and the Indochina region especially in Laos, Vietnam and Thailand. Its leaves are traditionally used for the treatment of diabetes, diuretic infection, gingivitis and inflammatory disease. The aqueous extract of *A. indicum* has shown hepatoprotective activity in CCl_4- and paracetamol induced toxicity in rat liver (Porchezhian and Ansari, 2005). Previous studies of aqueous and alcoholic leaf ex-tracts (400 mg/kg, p.o.) have been reported to produce a significant lowering of plasma glucose in fasting normal rats 4 h after administration (Seetharam et

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al., 2002). To the best of our knowledge, there is no report on the hypoglycemic effect of *A. indicum* extract in diabetic rats. The aim of the present study is to evaluate the effect of methanolic extract of *A. indicum* leaves in normal and streptozotocin (STZ)-induced diabetic rats. Furthermore, the study focused on the investigation of *in vivo* and *in vitro* alpha glucosidase inhibition abilities of *A. indicum* extract.

MATERIALS AND METHODS

Plant material

A. indicum (AI) leaves were colleted from Prachuap Khiri Khan Province, Thailand and authenticated by a taxonomist of the Department of Botany, Chulalongkorn University. A voucher specimen (BCU0060) has been deposited in the herbarium of the Department of Botany, Chulalongkorn University, Bangkok, Thailand.

Preparation of extract

A. indicum leaves were cut into small pieces and marcerated in 99.5% methanol for 3 days at room temperature. The mixtures was filtered and then concentrated under reduced pressure at 50 - 60°C, weighed and the residue was used in experiments (yield 5.3%).

Determination of phenolic compounds and flavonoid contents

The amount of total phenolic compounds in the extract was determined using the Folin-Ciocalteu reagent according to a previously described method (Asami et al., 2003). Gallic acid was used as a standard. Flavonoid content in the extract was assayed spectrophotometrically according to the method of Chang et al. (2002).

Animals

Male Wistar rats (180 - 200 g) were obtained from the National Laboratory Animal Centre, Mahidol University, Thailand and were housed in an individual stainless steel cage with free access to water in a room maintained at 24 ± 1°C on a 12:12 h light-dark cycle. Animal facilities and protocol were approved by the Laboratory Animal Care and Use Committee at the Faculty of Veterinary Science, Chulalongkorn University, Thailand

Induction of diabetes

Animals were induced to diabetic with streptozotocin (STZ) (60 mg/kg, i.p). The streptozotocin was freshly dissolved in citrate buffer (0.01 M, pH 4.5) and maintained on ice prior to use. 1 week after STZ administration, rats with fasting blood glucose concentrations (>300 mg/dL) were considered to be diabetic and were used in the experiment.

Effects of the extract on normal and STZ-induced diabetic rats

The normal and diabetic rats were divided into 4 groups and each only 1% Tween solution. Groups 2 and 3 were administered with 250 and 500 mg/kg of the extract, respectively. Group 4 received 500 mg/kg metformin. Blood samples were collected from the tail vein before and after 30, 60 and 120 min after administration. After centrifugation (1,500 x *g*), plasma was removed and stored at -20°C. The plasma glucose concentrations were measured using the glucose oxidase method with absorbance at a wavelength of 450 nm.

Assay of oral maltose and sucrose tolerance test

The normal rats were fasted and divided into 3 groups, each group contained 6 rats. Group 1 was the control group receiving only 1% Tween solution. Group 2 received oral administration of the extract at 500 mg/kg. Group 3 received acarbose at 3 mg/kg. At 10 min after the extract administration, 3 g/kg substrate (maltose or sucrose) solution was administered to each rat. Blood samples were collected from the tail vein before and after 30, 60 and 120 min after administration. After centrifugation (1,500 x *g*), plasma was removed and stored at -20°C. The plasma glucose concentrations were measured using the glucose oxidase method.

α-Glucosidase inhibition assay

α-Glucosidase from rat intestinal acetone powder was purchased from Sigma Chemical Co. (St. Louis, MO). The α-glucosidase inhibitory activity was assayed according to the literature (Toda et al., 2000). Briefly, rat intestinal acetone powder (100 mg) was homogenized in 3 ml of 0.9% NaCl solution. After centrifugation (12,000 *g* x 30 min), 0.1 ml of the supernatant was incubated with 0.7 ml of substrate solution (37 mM maltose, 37 mM sucorse), and 0.2 ml of the extract at various concentrations in 0.1 M phosphate buffer pH 6.9 at 37°C for 30 min (maltase assay) and 60 min (sucrase assay). The mixtures were suspended in boiling water for 10 min to stop the reaction. The concentrations of glucose released from the reaction mixtures were determined by glucose oxidase method.

Statistical analyses

Data are expressed as means ± S.E.M. Statistical analysis was performed by one-way analysis of variance (ANOVA). The least significant difference test was used for mean comparisons and $P<0.05$ was considered to be statistically significant. IC_{50} values were determined from plots of concentration versus percent inhibition curves using Sigma Plot.

RESULTS AND DISCUSSION

Total phenolic compounds in 1 g of the extract contained 1.04 ± 0.01 mg, gallic acid and 59.92 ± 3.88 µg flavonoid. The plasma glucose concentration in normal and STZ-diabetic rats at various time intervals after oral administration of *AI* extract are shown Figure 1. In normal rats, the extract dose of 500 mg/kg significantly reduced plasma glucose at 60 and 120 ($P< 0.05$) min after oral administration. While the metformin treated group slightly decreased plasma glucose concentrations, but was insignificant when compared with the control group (Figure 1A). The diabetic rats treated with 500 mg/kg of the extract produced a significant reduction in plasma glucose concentration at 1 h after administration ($P<0.01$). Moreover, the percentage of glucose reduction after administration was 26.69% in diabetic rats. Metformin also caused a significant decrease in plasma

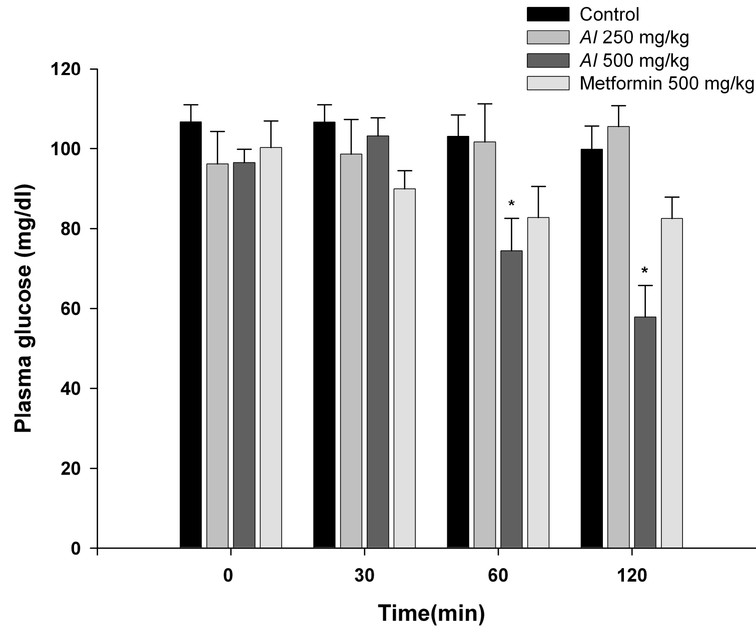

Figure 1A. Effect of *AI* leaves extract on blood glucose concentrations in normal rats. Data were expressed as mean ± S.E.M.; *n*= 6 rats per group;*P < 0.05.

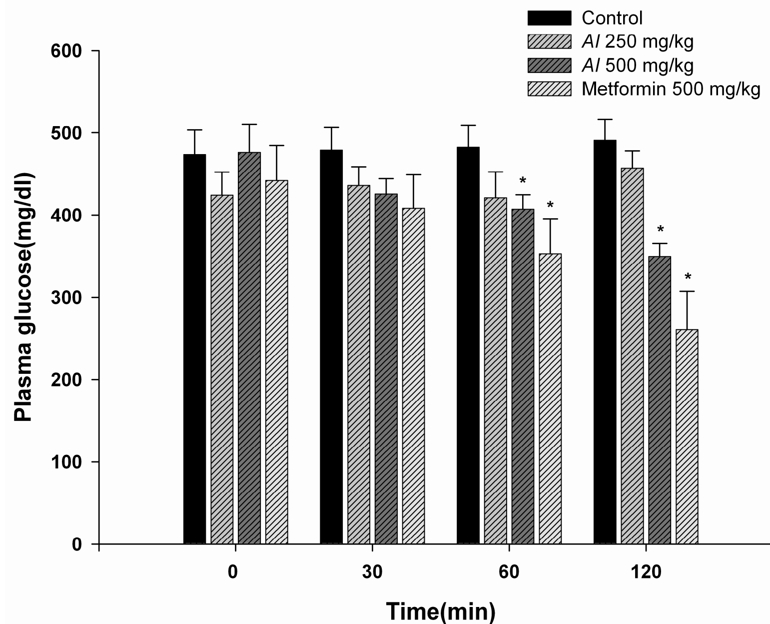

Figure 1B. Effect of *AI* leaves extract on blood glucose concentrations in STZ-induced diabetic rats. Data were expressed as mean ± S.E.M.; *n*= 6 rats per group; *P < 0.05.

glucose concentrations (P <0.01) (Figure 1B). In contrast, *AI* extract of 250 mg/kg did not have any significant effect on plasma glucose concentrations in both diabetic and normal rats. Furthermore, metformin reduced blood glucose concentration (46.86%) only in diabetic rats but not in normal rats which is consistent with previous reports (Jouad et al., 2002; Soon and Tan, 2002). Although metformin's exact mechanism of action is not completely understood, its main blood glucose lowering activity appears to be primarily through the suppression of gluconeogenesis and increasing the sensitivity of peripheral tissues without insulin action (Campbell et al.,

Table 1. Effect of oral administration of 500 mg/kg of *AI* extract on blood glucose concentrations in maltose-, sucrose-loaded rats and α-glucosidase inhibition.

Groups	Plasma glucose (mg/dL)				α-Glucosidase inhibition
	0 min	30 min	60 min	120 min	IC_{50} (mg/mL)
Maltose-loading					
Control	82.9 ± 7.5	169.3 ± 8.0	134.0 ± 5.7	103.7 ± 8.0	> 5
AI (500 mg/kg)	90.1 ± 11.7	141.9 ± 8.1	136.2 ± 10.8	124.4 ± 8.4	
Acarbose (3 mg/kg)	81.0 ± 4.3	121.2 ± 4.4**	116.1 ± 3.0*	94.9 ± 3.2	
Sucrose-loading					
Control	86.6 ± 7.8	170.1 ± 8.1	161.8 ± 6.1	129.0 ± 4.3	2.45 ± 0.13
AI (500 mg/kg)	79.9 ± 4.8	128.3 ± 17.6**	126.9 ± 7.6**	101.6 ± 12.0	
Acarbose (3 mg/kg)	83.0 ± 4.1	116.7 ± 2.6**	122.6 ± 3.5**	99.7 ± 1.9	

Data were expressed as mean ± S.E.M.; *n*= 6 rats per group; *P<0.05, **P<0.01.

1996; Radziuk et al., 2003). The results indicated that the extract was less potent than metformin on the reduction of blood glucose concentrations in diabetic rats. In contrast, the extract showed hypoglycemic effect, but metformin did not in normal rat.

The possible hypothesis is that the extract might be involved in other mechanisms of hypoglycemic activity such as stimulating insulin secretion and glucose uptake activity. These experiments are being investigated for further study. Several phenolic compounds and flavonoid possess marked anti-diabetic activity (Hsu et al., 2000). In addition, caffeic acid, a phenolic compound, has been reported to increase glucose uptake in rat myocytes (Cheng and Lui, 2000). In addition, quercetin-3-rutinoside was isolated from *A. indicum* leaves (Matlawska et al., 2007) and was reported to exhibit antihyperglycemic and antioxidant activities in normal and diabetic rats (Kamalakkannan and Prince, 2006). It is possible that the phenolic and/or flavonoid compounds in *AI* extract May play a role in the control of hyperglycemia. Further studies are needed to characterize the bioactive compounds of *A. indicum* with regards to the mechanism of antihyperglycemic actions such as insulinotropic and glucose uptake activities. The effects of *AI* extract on the suppression of plasma glucose concentrations in maltose and sucrose loaded rats were examined. As shown in Table 1, no significant change in plasma glucose concentration in the extract treated group was observed when compared with control rats administered maltose during the experiment period. In this study, acarbose was used as a positive control which showed a marked blood glucose reduction 30 min after administration (P<0.01). The extract significantly suppressed the rising plasma glucose 30 and 60 min after the administration of sucrose (P<0.01). The area under the curve in the extract treated group (AUC= 78.4 ± 16.5) during 2 h was significantly lower than that of the control group (AUC= 119.4 ± 14.4, P<0.05). Consequently, the AUC of the acarbose treated group was also significant when compared with control group (AUC= 48.1 ± 4.6, P<0.01). α-Glucosidase inhibitory activities againstmammalian enzyme are shown in Table 1. As the results show, *AI* extract had a sucrase inhibitory activity with an IC_{50} of 2.45 ± 0.13 mg/ml, while the extract was less potent on maltase inhibition (IC_{50} > 5 mg/ml). Acarbose was used as the positive control and the IC_{50} values against maltase and sucrase are 1.72 ± 0.13 µg/ml and 13.65 ± 1.97 µg/ml, respectively. As the results indicate, the extract had more potent α-glucosidase inhibitory activity against intestinal sucrase than maltase. However, the extract had less potent α-glucosidase inhibitory activity against maltase and sucrase than acarbose. The inhibition of α-glucosidase is one of therapeutic approach for reducing postprandial hyperglycemia. α-Glucosidase inhibitor, acarbose, has been effective in delaying the absorption of carbohydrates and the suppression of post-prandial hyperglycemia which contribute to a decrease in hemoglobin A_{1C} (HbA_{1c}). The decreasing in HbA_{1c} could reduce the incidence of chronic vascular complication in diabetic patients (Baron, 1998). It has been shown that flavonoid compounds are a potent α-glucosidase inhibitor which could suppress the rise of postprandial hyperglycemia. For example, theaflavins delays and inhibits glucose production in the intestine through maltase inhibition (Matsui et al., 2007). Therefore, α-glucosidase inhibition by flavonoid compounds is one of the mechanism of action of *AI* extract on blood glucose lowering effect.

In conclusion, the methanol extract of *A. indicum* was effective on lowering blood glucose of both normal and diabetic rats. The extract would be effective for suppressing postprandial hyperglycemia through α-glucosidase inhibition. To understand more of the mechanisms of action, the isolation and characterization of active compounds are in progress.

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