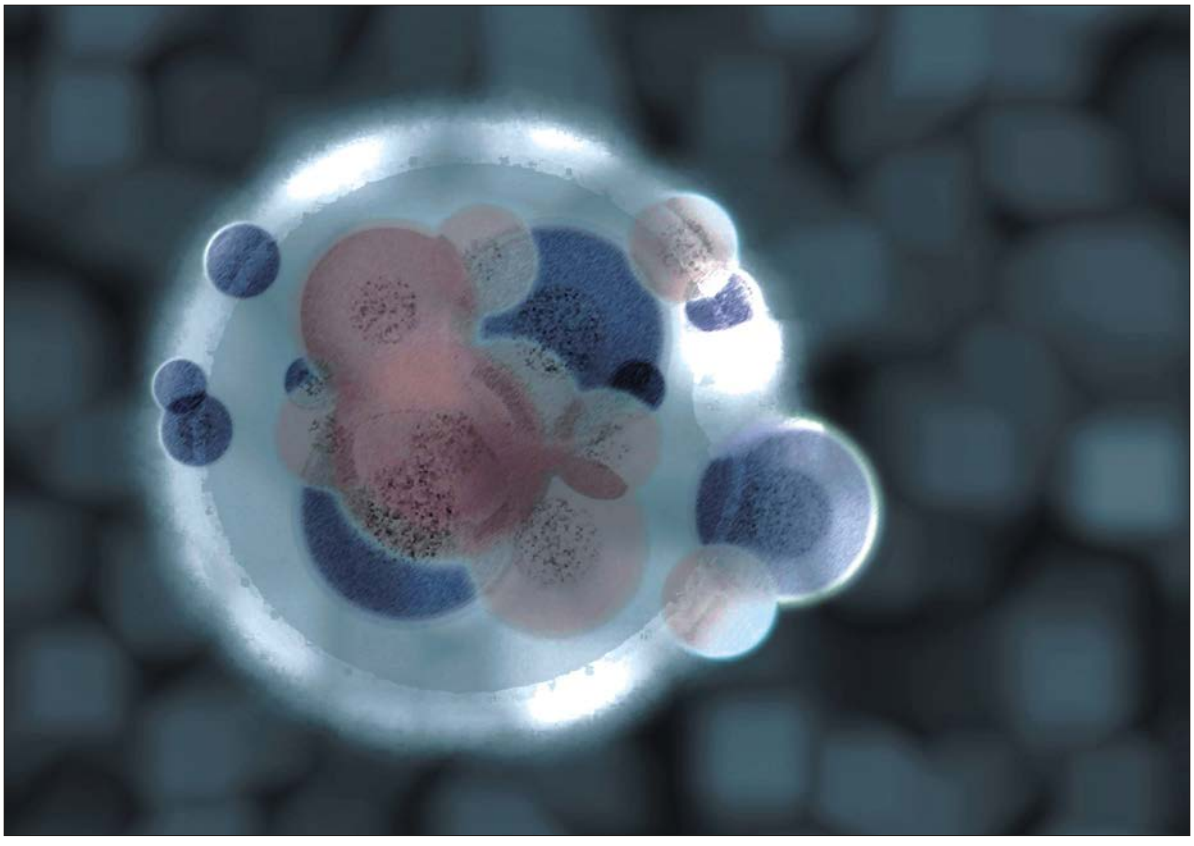

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Effect of *Ficus deltoidea* Extracts on Hepatic Basal and Insulin-Stimulated Glucose Uptake

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Abstract: The present study was designed to evaluate the potential of five extract and three fractions of *Ficus deltoidea* to enhance basal and insulin-stimulated glucose uptake into Chang liver cell line. The results showed that all *Ficus deltoidea* extracts and fractions except petroleum ether extract have the ability to enhance either basal or insulin-stimulated glucose uptake into liver cell line. Ethanolic and methanolic extracts as well as acidified chloroform and bacified chloroform fractions possess insulin-mimetic activity. Of all extracts and fractions, ethanolic extract possess the highest insulin-mimetic activity. Methanolic extract and n-butanolic fraction possess insulin-sensitizing activity, with the highest activity shown by methanolic extract. There is no synergistic effect between *Ficus deltoidea* extracts or fractions with 100 nM insulin. It can be suggested that antidiabetic action of *Ficus deltoidea* is partly associated with glucose disposal into liver cells.

Key words: *Ficus deltoidea*, antidiabetic, Chang liver cell line, glucose uptake activity

INTRODUCTION

Diabetes mellitus is a metabolic disease characterized by persistent hyperglycemia (high blood sugar level) with disturbances in carbohydrate, fat and protein metabolisms resulting from defects of insulin secretion or insulin action or combination of these two factors (World Health Organization, 1999). It remains the major global health problem in most countries even though there are plenty of antidiabetic agents available in the market.

Currently, treatment of diabetes involves the uses of biguanides, thiazolidinediones, sulphonyluurea, meglitinedes and α-glucosidase inhibitor (Vijayakumar *et al*., 2005). However, some of these synthetic antidiabetic agents are associated with side effects such as hypoglycaemic coma and hepatorenal disturbances (Suba *et al*., 2004). Therefore, combination of these dangerous side effects with the limited efficacy of the current antidiabetic drugs has fuelled the search for safer and more effective new antidiabetic agents.

The use of herbal remedies for diabetes treatment is well known since ancient times. *Ficus deltoidea*, from the family of Moraceae is one of the commonly used medicinal plants in Malaysia (Mat-Salleh and Latif, 2002). Based on ethnobothanical approaches, this plant has been traditionally claimed to have antidiabetic properties and has been used as remedy for diabetes treatment. However, the scientific evidence to confirm its efficacy is still lacking. This plant which is native to Southeast Asia and Philiphines (Forest *et al*., 2003) has also been used to treat other ailments such as headache and fever (Mat-Salleh and Latif, 2002). Recent study has reported on the antinociceptive activity of aqueous extract of *Ficus deltoidea* (Sulaiman *et al*., 2008). Hakiman and Maziah (2009) have found that aqueous extract of different *Ficus deltoidea* accessions possess non enzymatic and enzymatic antioxidant activities.

An antidiabetic plant or agent could benefit the diabetic condition by mimicking insulin action during glucose disposal into peripheral tissues and enhancing insulin secretion from pancreatic β-cells (Gray and Flatt, 1997). Glucose disposal into peripherals tissues is the most common mechanism by which high blood glucose in blood stream is reduced after meal. Beside adipocytes and muscle, liver is one of the target tissues for glucose disposal and storage. It may account for one third of an oral glucose load (Pagliassotti and Horton, 1994). Liver plays a major role in the regulation of glucose

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homoeostasis in the body, thus maintaining blood glucose to normoglycemic level (Nevado *et al*., 2006).

Previous study on *Ficus deltoidea* has reported the blood glucose lowering effect in animals (Aminudin *et al*., 2007; Adam *et al*., 2007). Study on the antidiabetic mechanism of *Ficus deltoidea* done by Hamid *et al*. (2008) has revealed that methanolic extract of this plant stimulated insulin secretion from BRIN BD11 cell lines by 1-3 folds as compared to 1.1 mM glucose alone. The present study which is the extension of the previous works was undertaken to elucidate the other possible antidiabetic mechanism of *Ficus deltoidea* extracts which is enhancing glucose uptake into liver by using Chang liver cell line as the model of hepatocytes cells.

MATERIALS AND METHODS

This study was conducted from December, 2007 to June, 2008 at Cell Culture Laboratory, Medical Technology Division, Malaysian Nuclear Agency.

Chemicals: Chang liver cell line was purchased from American Type Culture Collection (ATCC, USA). All cell culture supplements were purchased from Gibco® (Invitrogen, USA). Ethanol, methanol, petroleum ether and n-butanol were purchased from JT Baker Reagent Chemicals (Phillipsburg, NJ). Sulphuric acid (H_2SO_4) and chloroform were purchased from Merck (Germany). Sodium chloride (NaCl), potassium chloride (KCl), calcium chloride ($CaCl_2$), potassium dihydrogen phosphate (KH_2PO_4), magnesium sulphate ($MgSO_4$), sodium hydrogen carbonate ($NaHCO_3$), HEPES, Sodium Deodecyl Sulphate (SDS), 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), bovine insulin, ammonium hydroxide (NH_4OH), dimethylsulphoxide (DMSO) and D-glucose were purchased from Sigma Chemical Co. (St. Loiuse, USA). Ultima Gold™ LLT was purchased from PerkinElmer, USA. 2-Deoxy-[1-^{3}H]glucose was purchased from GE Healthcare. Rosiglitazone maleate (Avandia) was purchased from a local pharmacy.

Plant material: *Ficus deltoidea* plants were collected at Sungai Tengi Selatan, Selangor. The specimen was identified by taxonomist at Biodiversity Unit, Institute of Bioscience (IBS), Universiti Putra Malaysia. A voucher specimen was deposited at the institute herbarium with voucher number SK1467/07. The leaves of *Ficus deltoidea* were dried at 45°C and ground into fine powder.

The extraction procedure was done according to Harborne (1973) with some modifications. Hot and cold aqueous extract were prepared by boiling and soaking the powder respectively in distilled water for 3 h (100 g L^{-1}) by changing water every hour. The combined suspension was filtered using Whatman filter paper No. 54 and freeze dried. Petroleum ether and ethanolic extracts were prepared by soaking the sample powder (100 g L^{-1}) in 100% petroleum ether and 70% ethanol, respectively for 3 days at room temperature by changing solvent daily. The combined suspension was filtered using Whatman filter paper No. 54 and evaporated to dryness under pressure at 30°C. Methanolic extract was prepared by soaking the marc of petroleum ether extraction (100 g L^{-1}) in 90% methanol for 3 days at room temperature by changing solvent daily. The combined suspension was filtered using Whatman filter paper No. 54 and evaporated to dryness under pressure at 30°C.

For preparation of acidified chloroform fraction, third quarter of the total yield of the methanolic extract was acidified with 5% sulphuric acid (H_2SO_4) and extracted with chloroform (3×3:1). The acidified chloroform fraction was then separated from the aqueous portion using a separation funnel and evaporated to dryness under pressure at 30°C. The aqueous portion was bacified with concentrated ammonium hydroxide (NH_4OH) until pH 9 and extracted with chloroform (3×3:1). The bacified chloroform fraction was then separated from the aqueous portion using a separation funnel and evaporated to dryness under pressure at 30°C. The aqueous portion was extracted with n-buthanol (3×3:1). The n-butanolic fraction was then separated from the aqueous portion using a separation funnel and evaporated to dryness in the fume hood.

Cell culture and glucose uptake assay: Chang liver cells were maintained in RPMI medium supplemented with 10% (v/v) foetal bovine serum and 1% (v/v) antibiotic solution (10,000 units mL^{-1} penicillin and 10 mg mL^{-1} streptomycin) at 37°C humidified with 5% CO_2. Glucose uptake assay in Chang liver cell line was done according to the method of Liu *et al*. (2001) with some modifications. Briefly, cells were seeded onto sterile 12-wells plates at the concentration of 2×10^5 cells/well and then left overnight at 37°C humidified with 5% CO_2 to allow attachment prior to test. The next day, cells were washed thrice with Kreb's-Ringer bicarbonate buffer, KRB (115 mM NaCl, 4.7 mM KCl, 1.28 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 24 mM $NaHCO_3$, 10 mM Hepes-free acid, 1 g L^{-1} bovine serum albumin, 1.1 mM glucose; pH 7.4) and pre-incubated with KRB for 30 min at 37°C. Cells were further incubated with various concentrations (50-500 μg mL^{-1}) of *Ficus deltoidea* extracts either alone or in combination with 100 nM insulin for 30 min at 37°C. The potential *Ficus deltoidea* extracts enhancing basal and insulin-stimulated glucose uptake into Chang liver

cells were compared with untreated control (contains only KRB), insulin 100 nM alone and positive control, Rosiglitazone maleate (Avandia) 70 μM. To initiate glucose uptake reaction, 500 μL of 2-deoxy-[1-^{3}H]-glucose (1 μCi mL^{-1}) diluted in 0.1 mM D-glucose solution was added to each well and incubated further for 60 min at 37°C. After incubation, cells were washed thrice with ice-cold KRB buffer and solubilized with 0.1% Sodium Deodecyl Sulphate (SDS) dissolved in phosphate buffer, pH 7.4. The content of each well was transferred into scintillation vials and 15 mL of scintillation cocktail, Ultima Gold™ LLT was added. The radioactivity incorporated into the cells was measured using Liquid Scintillation Counter (Hewlett Packard, USA).

Cell viability study: The viability of Chang liver cell line in the presence of *Ficus deltoidea* extracts was conducted according to the method of Mosmann (1983) and Carmichael *et al.* (1987). Chang liver cells were maintained in RPMI medium supplemented with 10% (v/v) foetal bovine serum and 1% (v/v) antibiotic solution (10,000 units mL^{-1} penicillin and 10 mg mL^{-1} streptomycin) at 37°C humidified with 5% CO_2. Cells were seeded at a concentration of 1.5×10^5 cells well^{-1} onto sterile 96-well plate and incubated at 37°C overnight. Cells were further incubated at 37°C for 72 h in the absence or presence of various concentrations (50-500 μg mL^{-1}) of *Ficus deltoidea* extracts or fractions. Following the required incubation period, 20 μL of 5 mg mL^{-1} of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) was added to each well and incubated for 4 h. Subsequently, the media from each well was gently aspirated and 100 μL of dimethylsulphoxide (DMSO) was added to dissolve the formazan crystals. Plates were shaken for 5 sec and absorbance was measured at 570 nm using Anthos microplate reader (Beckman Coulter, USA).

Statistical analyses: Results were expressed as Mean±SD for a given number of observations. Data were analysed using one way Analysis of Variance (ANOVA). Mean of all group were considered significantly different at the level $p<0.05$. Calculations were performed using the SPSS statistical programme.

RESULTS

Table 1 shows the effect of *Ficus deltoidea* extracts and fractions on basal and insulin-stimulated glucose uptake into Chang liver cell line. Hot aqueous extract showed a significant enhancement of basal glucose uptake at concentration of 500 μg mL^{-1}. However, the extract has no effect on insulin-stimulated glucose uptake. In the presence of cold aqueous extract, all concentrations of extract evaluated exhibited a significant enhancement of glucose uptake under both basal and insulin-stimulated state relative to control. However, this uptake was not significantly different when compared to 100 nM insulin alone which evoked an enhancement of uptake by 1.38-fold ($p<0.01$).

In the presence of ethanolic extract, basal and insulin-stimulated glucose uptake was significantly enhanced a concentration of 500 μg mL^{-1} relative to control. Basal uptake at such concentration was significantly higher when compared to 100 nM insulin. Unlike other extracts, petroleum ether extract did not affect

Table 1: Effects of *Ficus deltoidea* extracts and fractions on basal and insulin-stimulated glucose uptake into Chang liver cell line

	Fold of glucose uptake relative to control and 100 nM insulin					
	Basal			Insulin-stimulated		
Ficus deltoidea extracts or fractions	50 μg mL^{-1}	100 μg mL^{-1}	500 μg mL^{-1}	50 μg mL^{-1}	100 μg mL^{-1}	500 μg mL^{-1}
Hot aqueous	-	-	1.60-fold***	-	-	-
Cold aqueous	1.38-fold **	1.58-fold***	1.40-fold**	1.49-fold***	1.50-fold***	1.47-fold***
Ethanolic	-	-	1.90-fold*** 1.4-*fold*$^{++}$	-	-	1.50-fold***
Petroleum ether	-	-	-	-	-	-
Methanolic	1.67-fold*** 1.21-*fold*$^{++}$	1.65-fold*** 1.20-*fold*$^{+}$	-	1.62-fold***	2.11-fold*** 1.49-*fold*$^{+++}$	2.11-fold*** 1.49-*fold*$^{+++}$
Acidified chloroform	-	-	1.80-fold*** 1.30-*fold*$^{+}$	-	-	1.71-fold***
Bacified chloroform	1.48-fold*** 1.28-*fold*$^{+++}$	1.37-fold*** 1.19-*fold*$^{++}$	0.69-fold**	-	1.44-fold***	-
n-butanolic	-	-	-	-	-	1.67-fold*** 1.23-*fold*$^{+}$
Rosiglitazone maleate (70 μM)		2.10-fold*** 1.51-*fold*$^{+++}$				

Cells were incubated for 1 h in the presence of various concentrations (50-500 μg mL^{-1}) of extracts and fractions with or without 100 nM insulin. Data are Means±SD, n = 4. Values in normal style indicate fold of glucose uptake relative to control, **$p<0.01$ and ***$p<0.01$. Values in italic style indicate fold of glucose uptake relative to 100 nM insulin, $^{+}p<0.05$, $^{++}p<0.01$ and $^{+++}p<0.01$

Table 2: Effects of *Ficus deltoidea* extracts on the viability of Chang liver cell line

Ficus deltoidea extracts or fractions	Cell viability relative to untreated (%)			
	0 μg mL^{-1}	50 μg mL^{-1}	100 μg mL^{-1}	500 μg mL^{-1}
Hot aqueous	100.00±4.09	95.85±10.67	92.68±8.94	81.76±8.26 (18.2%)**
Cold aqueous	100.00±3.51	85.12±3.40 (14.9%)**	80.40±3.53 (19.6%)**	65.95±1.88 (34.0%)**
Ethanolic	100.00±2.95	84.65±17.36 (15.4%)*	80.93±13.67 (19.1%)**	55.00±2.73 (45.0%)**
Petroleum ether	100.00±11.52	75.59±11.00 (24.4%)**	68.40±9.71 (31.6%)**	42.24±1.37 (57.8%)**
Methanolic	100.00±1.40	97.29±1.53	94.78±5.56	69.82±4.71 (30.2%)**
Acidified chloroform	100.00±2.03	81.54±6.39 (18.5%)**	60.60±5.68 (39.4%)**	46.44±1.00 (53.7%)**
Bacified chloroform	100.00±15.5	82.61±2.96 (17.4%)**	63.75±4.37 (36.3%)**	43.30±1.41 (56.7%)**
N-butanolic	100.00±6.53	98.77±10.25	94.83±6.05	87.47±3.04 (12.5%)**

Cells were incubated for 72 h in the presence of various concentrations of extracts (0-500 μg mL^{-1}). Values are Mean±SD of eight replicates from three independent assays. *$p<0.05$ and **$p<0.01$ compared with untreated control (0 μg mL^{-1}). Values in the bracket indicate percentage of cell viability reduction relative to untreated

both basal and insulin-stimulated glucose uptake. Methanolic extract significantly enhanced basal glucose uptake at concentrations of 50 and 100 μg mL^{-1} relative to control and this uptake was significantly higher when compared to 100 nM insulin alone. Under insulin-stimulated state, the glucose uptake was significantly enhanced at all concentrations relative to control and the uptake by high concentrations of extract was significantly higher compared to 100 nM insulin.

Acidified chloroform fraction demonstrated a significant enhancement of glucose uptake only at a concentration of 500 μg mL^{-1} under both basal and insulin-stimulated state. The basal uptake at such concentration was significantly higher compared to 100 nM insulin. In the presence of bacified chloroform fraction, basal glucose uptake was significantly enhanced at concentrations of 50 and 100 μg mL^{-1} relative to control and this uptake was higher compared to 100 nM insulin. In contrast, basal glucose uptake was decreased significantly at the highest concentration, 500 μg mL^{-1}. Under insulin-stimulated state, glucose uptake was significantly enhanced at a concentration 100 μg mL^{-1} relative to untreated control.

The n-butanolic fraction did not show any effect on basal glucose uptake at all concentrations evaluated. Under insulin-stimulated state, the fraction significantly enhanced glucose uptake at a concentration of 500 μg mL^{-1} relative to control and this uptake was also significantly higher when compared to 100 nM insulin which evoked a 1.4-fold ($p<0.05$) of uptake. Rosiglitazone maleate (70 μM), evoked an uptake of 2.10-fold ($p<0.001$) and 1.51-fold ($p<0.001$) as compared to control and 100 nM insulin respectively. Of all extracts evaluated, only methanolic extract at concentrations of 100 and 500 μg mL^{-1} under insulin-stimulated state, produce an uptake at a similar level to rosiglitazone maleate. The uptake by the remaining extracts under both states was less than that of rosiglitazone maleate, 70 μM.

The effect of five extracts and three fractions of *Ficus deltoidea* on the viability Chang liver cell line, as quantified by the MTT assay for 72 h exposure to various concentrations (0-500 μg mL^{-1}) of extracts or fractions are shown in Table 2. In this study, all extracts and fractions significantly reduced the viability of Chang liver cell line at high concentration (500 μg mL^{-1}) after 72 h treatment. It was shown that cold aqueous extract, ethanolic extract, petroleum ether extract, acidified chloroform fraction and bacified chloroform fraction reduced cell viability at all concentrations evaluated whereas hot aqueous, methanolic and n-butanolic extract reduced cell viability at the highest concentration only. Petroleum ether extract, acidified chloroform fraction and bacified chloroform fraction at the concentration 500 μg mL^{-1} significantly reduced cell viability more than 50% after 72 h treatment.

DISCUSSION

Ficus deltoidea has been used for a long time as traditional medicine to treat high blood glucose levels. However, scientific studies to evaluate its efficacy and possible mode of action are still lacking. Only two studies on *Ficus deltoidea* has been done which reports the glucose lowering effect of aqueous extract of this plant in normal rats (Aminudin *et al*., 2007) and mild diabetic rats (Adam *et al*., 2007). The present study reports the hepatic glucose uptake activity of five extracts and three fractions of *Ficus deltoidea* under basal and insulin-stimulated state using Chang liver cell line as the model of hepatocytes cells. Cell lines provide a good model for studying the effects of plant extracts on insulin action because they are more homogenous compared to isolated cells or tissue as these two models are mixed in nature of each preparation and have limited lifespan (Chandrakesan *et al*., 2009). In addition, the effect of extracts on insulin action can be examined direct without influences by endogenous insulin from animal system if test is done *in vivo* (Christopher *et al*., 1983).

In this study, significant enhancement of basal and insulin-stimulated glucose uptake by Chang liver cell line

in the presence of *Ficus deltoidea* extracts and fractions at certain concentrations suggests that there is a possibility of the presence of antidiabetic compounds in this plant which exert its antidiabetic mechanism through glucose disposal into liver. Further study is needed to be carried out to isolate and identify such bioactive compounds and to elucidate the possible molecular mechanism by which this disposal is mediated.

Liver is one of the targeted cells for glucose disposal from the blood stream after an oral glucose loaded (Christopher *et al*., 1983). Liver glucose uptake is facilitated by GLUT2, one of the proteins in glucose transporters family (Kemp *et al*., 1997). The transport of glucose into the liver is stimulated by insulin, an anabolic hormone that regulates the metabolism of carbohydrate, lipid and protein (Saltiel and Pessin, 2002). In basal state (without insulin), glucose uptake is very low whereas in the presence of insulin, hepatic glucose uptake is augmented (Cherrington *et al*., 1998). This report is parallel to the observation from the present study that insulin at a concentration of 100 nM significantly enhanced glucose uptake by approximately 1.4-fold. Thus, the same concentration of insulin was used to assess the ability of *Ficus deltoidea* extracts and fractions to potentiate insulin-stimulated glucose uptake into Chang liver cell line. In addition insulin at such concentration was widely used in insulin-stimulated glucose uptake studies (Daniel *et al*., 2002; Kenichi *et al*., 2004).

The results of the present study showed that under basal state, all extracts and fractions have the ability to enhance glucose uptake into Chang liver cell line except n-butanolic fraction and petroleum ether extract. Hot aqueous extract (500 μg mL^{-1}), cold aqueous extract (50, 100 and 500 μg mL^{-1}), ethanolic extract (500 μg mL^{-1}), methanolic extract (50 and 100 μg mL^{-1}), acidified chloroform fraction (500 μg mL^{-1}) and bacified chloroform fraction (50 and 100 μg mL^{-1}) have been shown to enhance basal glucose uptake relative to untreated control. Interestingly, the enhancement of basal glucose uptake by ethanolic extract, methanolic extract, acidified chloroform fraction and bacified chloroform fraction at such concentrations were significantly higher than 100 nM insulin alone. This observation suggests that the mentioned extracts and fractions mimic the action of insulin on glucose uptake into Chang liver cell line and possess insulin-mimetic activity (Martineau *et al*., 2006). However, in this study, molecular mechanism of the insulin mimetic activity of the extracts and fractions was not elucidated. Few antidiabetic plants have been reported to possess insulin-mimetic activity such as *Lagerstreoma speciosa* (Liu *et al*., 2001), *Agaricus campestris* (Gray and Flatt, 1998) and Fenugreek seed extract (Vijayakumar *et al*., 2005). The mechanism of insulin-mimetic activity of Fenugreek seed extract is through the induction of tyrosine phosphorylation of insulin receptor, Insulin Receptor Substrate 1 (IRS1) and p85 subunit of phosphatidylinositol 3-kinase (PI3K), thus lead to glucose uptake into adipocytes and liver cells (Vijayakumar *et al*., 2005). In this study, there is a possibility that *Ficus deltoidea* extracts and fractions mimic the insulin action through the mentioned mechanisms. However, further works are needed to be done to confirm this hypothesis.

Under insulin-stimulated state, cold aqueous extract (50, 100 and 500 μg mL^{-1}), ethanolic extract (500 μg mL^{-1}), methanolic extract (50, 100 and 500 μg mL^{-1}), acidified chloroform fraction (500 μg mL^{-1}), bacified chloroform fraction (100 μg mL^{-1}) and n-butanolic fraction (500 μg mL^{-1}) significantly enhanced glucose uptake relative to untreated control. Interestingly, the enhancement of insulin-stimulated glucose uptake produced by methanolic (100 and 500 μg mL^{-1}) extract and n-butanolic fraction was greater than 100 nM insulin alone. This result suggests that these extract and fraction have the ability to potentiate insulin action on glucose uptake into Chang liver cell line and possess insulin sensitizing activity (Martineau *et al*., 2006). However, in this study molecular mechanism of the insulin sensitizing activity of *Ficus deltoidea* extracts and fractions was not elucidated. Plants like *Salvia miltiorrihiza* Bunge (Jung *et al*., 2009) and *Campsis grandiflora* (Jung *et al*., 2005) exert insulin-sensitizing activity by acting as IR activator. They enhanced the tyrosine phosphorylation of IR and activation of downstream kinases activity. A Southeast Asian herb, *Cortidis rhizoma* acts as an insulin-sensitizer by enhancing IRS1-PI3K-Akt-GLUT4 translocation to the cells membrane and lead to glucose uptake (Ko *et al*., 2005). In this study, there is a possibility that *Ficus deltoidea* extracts and fractions act as an insulin-sensitizer through the mentioned mechanisms. To confirm this hypothesis, further evaluations are needed to be carried out.

Among all extracts and fractions evaluated, methanolic extract seem to be the most potent in enhancing glucose uptake into liver cells under both basal and insulin-stimulated state (Table 1). In addition, it showed insulin-mimetic and insulin sensitizing activity and interestingly, the insulin-sensitizing activity of methanolic extract was similar to that of rosiglitazone maleate 70 μM. Zunoliza *et al*. (2009) has reported that the methanolic extract of *Ficus deltoidea* var. *angustifolia* which was also used in this study contains high amount of flavonoids and tannins. There is possibility that those secondary metabolites responsible for insulin-mimetic and

insulin sensitizing activity of methnaolic extract of *Ficus deltoidea*. However, further evaluations are needed to be carried out to correlate the α-glucosidase inhibitory activity of *Ficus deltoidea* var. *angustifolia* extract with flavonoids and tannins presence in this extract.

Some antidiabetic plants possess synergistic effect with insulin in their action on glucose uptake activity. For example, the ethanolic extract of *Amomi semen* has been reported to potentiate insulin-stimulated glucose uptake synergistically with 10 nM insulin (Kang and Kim, 2004). In this study however, there is no synergistic effect occurs between *Ficus deltoidea* and 100 nM insulin on glucose uptake into Chang liver cell line as neither extracts nor fractions produced insulin-stimulated glucose uptake significantly higher than under basal state and 100 nM insulin alone.

In order to challenge the potential of *Ficus deltoidea* extracts in enhancing glucose uptake into hepatocytes cells, a conventional oral antidiabetic agent, rosiglitazone maleate was used as positive control. Rosiglitazone maleate at a concentration of 70 μM was used because this concentration produced the highest stimulation of glucose uptake according to a dose-response evaluation (data not shown). Rosiglitazone has been reported to increase hepatic glucose uptake in type II diabetes patients (Iozzo *et al*., 2003). It is a member of thiazolidinedione group and is a selective peroxisome proliferator-activated receptor-γ (PPARγ)-agonists. The antidiabetic mechanism of rosiglitazone is through the reduction of insulin resistance and improvement of insulin sensitivity in insulin responsive cells, resulting in a reduction of fasting plasma glucose (Boelsterli and Bedoucha, 2002). In this study, only methanolic extract at concentrations 100 and 500 μg mL^{-1} produced insulin-stimulated glucose uptake at a similar level of 70 μM rosiglitazone maleate. This indicates that combination of methanolic extract at these concentrations with 100 nM insulin possess glucose uptake activity as potent as rosiglitazone maleate at 70 μM. The remaining extracts and fractions, even though have the ability to produce significant enhancement of glucose uptake in both basal and insulin-stimulated state, but their effects were lesser than rosiglitazone maleate. This observation indicates that the effect of all *Ficus deltoidea* extracts and fractions except methanolic extract on glucose uptake into Chang liver cell line was not as potent as rosiglitazone. This may be due to the presence of other compounds in the extracts which do not have glucose uptake activity. In contrast, rosiglitazone which consists of a single compound, its glucose uptake activity into hepatocytes has been scientifically proven (Iozzo *et al*., 2003).

The viability of Chang liver cell in the presence of *Ficus deltoidea* extracts at various concentrations was evaluated using MTT assay (Mosmann, 1983). In this assay, the yellow tetrazolium salt, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) is reduced by the mitochondrial enzymes, succinate dehydrogenase to form insoluble purple formazan crystals which are solubilized by the addition of a detergent (Mosmann, 1983). The colour produced then can be measured spectrophotometrically at 570 nm (Carmichael *et al*., 1987). According to Mosmann (1983), the MTT reduction activity was proportional to cell viability. In this study, all extracts and fractions of *Ficus deltoidea* showed the tendency to reduce cell viability at either the highest concentration or at all concentrations evaluated. Acidified chloroform fraction (500 μg mL^{-1}), bacified chloroform fraction (500 μg mL^{-1}) and petroleum ether extract (500 μg mL^{-1}) significantly decreased cell viability more than 50% relative to untreated controls after 72 h treatment. Even though acidified chloroform and bacified chloroform fractions at respective concentrations were significantly effective in enhancing glucose uptake into liver cells, but since these fractions are toxic to Chang liver cell line, their efficacy in enhancing glucose uptake may not be taken into account. This is because the cytotoxic effect due to the presence of cytotoxic compounds in such fractions, can damage one or more sub-cellular organelles such as mitochondria and lead to cell death (Sabino *et al*., 1999). Therefore, to ensure this fractions is safe to be used as an antidiabetic agent, further works are needed to be carried out to separate the cytotoxic compounds from the fraction.

The results of the present study showed that *Ficus deltoidea* extracts have the ability to enhance both basal and insulin-stimulated glucose uptake into liver cells. The extracts also possess either insulin-mimetic activity or insulin-sensitizing activity or combination of both activities. Therefore, it is suggested that the blood glucose lowering effect of *Ficus deltoidea* in rats is mediated partly, by the augmentation of glucose uptake into liver cells.

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