

FICUS DELTOIDEA ENHANCE GLUCOSE UPTAKE ACTIVITY IN CULTURED MUSCLE CELLS

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ABSTRACT

Ficus deltoidea or locally known as *Mas cotek* is one of the common medicinal plants used in Malaysia. Our previous studies showed that this plant have blood glucose lowering effect. Glucose uptake into muscle and adipocytes cells is one of the known mechanisms of blood glucose lowering effect. This study was performed to evaluate the effect of *Ficus deltoidea* on glucose uptake activity into muscle cells. The cells were incubated with *Ficus deltoidea* extracts either alone or combination with insulin. Amount of glucose uptake by L6 myotubes was determined using glucose tracer, 2-deoxy-[1-³H]-glucose. The results showed that *Ficus deltoidea* extracts at particular doses enhanced basal or insulin-mediated glucose uptake into muscle cells significantly. Hot aqueous extract enhanced glucose uptake at the low concentration (10 µg/ml) whereas methanolic extract enhanced basal glucose uptake at high concentrations (500 and 1000 µg/ml). Meanwhile, ethanolic extract enhanced glucose uptake at low and high concentrations. Methanolic extract also mimicked insulin activity during enhancing glucose uptake into L6 muscle cells. Glucose uptake activity of *Ficus deltoidea* could be attributed by the phenolic compounds presence in the plant. This study had shown that *Ficus deltoidea* has the ability to enhance glucose uptake into muscle cells which is partly contributed the antidiabetic activity of this plant.

ABSTRAK

Ficus deltoidea atau tempatan yang dikenali sebagai *Mas cotek* merupakan salah satu tumbuhan ubatan yang biasa digunakan di Malaysia. Kajian ini dijalankan sebelumnya menunjukkan bahawa tumbuhan ini mempunyai kesan merendahkan glukosa darah. Penyerapan glukosa ke dalam sel-sel otot dan adipocytes merupakan salah satu mekanisma yang dikenali glukosa darah mengurangkan kesan. Kajian ini telah dilaksanakan untuk menilai kesan *Ficus deltoidea* aktiviti penyerapan glukosa ke dalam sel-sel otot. Sel-sel yang telah incubated dengan *Ficus deltoidea* ekstrak sama ada bersendirian atau kombinasi dengan insulin. Jumlah penyerapan glukosa oleh L6 myotubes adalah ditentukan dengan menggunakan pengesanan glukosa, 2 - deoxy-[1-³H]-glukosa. Hasil kajian menunjukkan bahawa ekstrak *Ficus deltoidea* pada dos tertentu dipertingkatkan penyakit atau sel-sel penyerapan glukosa diantara insulin ke dalam otot dengan ketara. Ekstrak air panas meningkatkan penyerapan glukosa pada kepekatan rendah (10 µg/ml) manakala ekstrak methanolic meningkatkan penyerapan glukosa

penyakit pada kepekatan yang tinggi (500 dan 1000 µg/ml). Sementara itu, ekstrak ethanolic meningkatkan penyerapan glukosa rendah.

Keyword: *Ficus deltoidea*; glucose uptake; L6 myotubes; glucose tracer; phenolic compounds

INTRODUCTION

Diabetes mellitus is a metabolic disease characterized by persistent hyperglycaemia resulting from defects of insulin secretion or insulin action or combination of these two factors (Alberti and Zimmet, 1998). It is the first leading causes of death in developed country and has been an epidemic in many developing countries including Malaysia (Ooyub *et al.*, 2004). Globally, the prevalence of diabetes was estimated to be 2.8% in the year of 2000 and expected to rise to 4.4% in 2030. In Malaysia, it has been increased with an estimate number of diabetes patients was 6.3% in 1986 and 14.9% in 2006 (Zanariah *et al.*, 2008).

In Malaysia, there were 3.2 million (16.6%) of diabetes cases among adult in Malaysia in 2014. This prevalence is projected to rise to 4.2 million (21.6%) in the year of 2020 (Saari and Noraini, 2013). The rising trend in the prevalence of diabetes could possibly be due to the growth of population, aging, urbanization, and changes in dietary habits, obesity and sedentary lifestyle (Zanariah *et al.*, 2008). Diabetes mellitus can be controlled with the uses of oral antidiabetic drugs. Even though plenty of antidiabetic drugs available, the disease remains major global health problems. This could possibly be due to the drawback of the drugs such as adverse effects and lack of efficacy (Kirchheiner *et al.*, 2005). Therefore, searching for new antidiabetic agents should be continued.

Ficus deltoidea, from Moraceae family, is one of the common medicinal plants in Malaysia. Based on ethnobotanical approaches, this plant has been claimed to have antidiabetic properties (Mat-Salleh and Latif, 2002). Previous studies showed that this plant possess anti-hyperglycemic property (Adam *et al.*, 2007; Adam *et al.*, 2010b; Adam *et al.*, 2011a). Following 15-days treatment, hot aqueous extract of *F. deltoidea* stimulated insulin release and reduced fasting hyperglycemia (Adam *et al.*, 2011b). Elucidation of antihyperglycemic mechanisms demonstrated that this plant enhanced basal and insulin-stimulated glucose uptake into liver cells (Adam *et al.*, 2009) and reduced the rate of glucose absorption from small intestine by inhibiting intestine sucrase activity (Adam *et al.*, 2010a). The present study was performed to find other possible antidiabetic mechanisms of *F. deltoidea*, if any, by evaluating the potential of this plant to enhance glucose uptake into muscle cells. The effect of *F. deltoidea* on glucose uptake activity was evaluated either in the absence (basal) or presence (insulin-mediated) of 100 nM insulin.

MATERIALS AND METHODS

Chemicals and reagents

L6 cell line was purchased from American Type Cell Culture (ECACC, Salisbury UK). All cell culture supplements were purchased from Invitrogen, USA. Ethanol and methanol were purchased from J.T. Baker Chemical. Sodium chloride (NaCl), potassium chloride (KCl), calcium chloride (CaCl₂), potassium dihydrogen phosphate (KH₂PO₄), magnesium sulphate (MgSO₄), sodium hydrogen carbonate (NaHCO₃), HEPES, sodium deodecyl sulphate (SDS), 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), bovine insulin, ammonium hydroxide (NH₄OH), dimethylsulphoxide (DMSO), gallic acid, quercetin, 1,1-diphenyl-2-picrylhydrazyl (DPPH), Folin-Ciocalteu's phenol reagent, sodium carbonate (NaCO₃), aluminum chloride (AlCl₃), metformin and D-glucose were purchased from Sigma Chemical Co. (St. Louis, USA). Ultima Gold™

LLT was purchased from PerkinElmer (USA). 2-Deoxy-[1-³H]-glucose was purchased from GE Healthcare (USA).

Plant material and extraction procedure

Plants of *F. deltoidea* were collected at Sungai Tinggi Selatan, Selangor, Malaysia. The plants were identified by a taxonomist from the Biodiversity Unit of the Institute of Bioscience, Universiti Putra Malaysia (SK1467/07). The leaves of *F. deltoidea* were oven dried at 45°C and ground to a fine powder. Hot aqueous extracts were prepared by boiling the powdered sample in distilled water for 3 hours (100 g/L) by changing the water every hour. The combined suspension was filtered using Whatman filter paper No. 54 and freeze dried to give the powdered form. Ethanolic and methanolic extracts was prepared by soaking the powder in 95% ethanol and methanol, respectively for 3 days (100 g/L) 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 to give ethanolic and methanolic extracts. The yields of the extracts were 21 g, 17 g and 19 g for hot aqueous, ethanolic and methanolic extract, respectively.

Total phenolic content assay

The concentrations of phenolic compounds in the extracts of *F. deltoidea* were measured according to the Folin-Ciocalteu method as described elsewhere (Sim *et al.*, 2010). Briefly, 100 µl of 1mg/ml of *F. deltoidea* extracts was added to 4.5 ml distilled water and 100 µl of 2N Folin-ciocalteu reagent with shaking for 3 minutes. Then, 200 µl of 2% (w/v) of sodium carbonate solution was added to the mixture. The reaction mixtures were incubated in dark at room temperature for 3 hours. The absorbance was measured at 760 nm using EnSpire® Multimode Plate Reader (PerkinElmer, USA). All extracts were assayed in triplicate. Gallic acid (0-1500 µg/ml) was used for calibration. Total phenolic content in the extracts is expressed as mg gallic acid equivalence (GAE) per g extract.

Total flavonoids content assay

The concentrations of phenolic compounds in the extracts of *F. deltoidea* were measured according to Yang *et al.* (2011). Briefly, 150µl of 1mg/ml of *F. deltoidea* extracts was added to 150 µl of 2% (w/v) of AlCl₃ solution in 96-wells plate. The mixture was incubated in dark at room temperature for 15 minutes. The absorbance was measured at 435 nm using EnSpire® Multimode Plate Reader (PerkinElmer, USA). All extracts were assayed in triplicate. Quercetin (0-100µg/ml) was used for calibration. Total flavonoids content in the extracts is expressed as mg quercetin equivalence (QE) per g extract.

Cell lines

L6 myoblast was maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% (v/v) foetal bovine serum (FBS) and 1% (v/v) antibiotic solution (10,000 units/ml penicillin and 10 mg/ml streptomycin) at 37°C humidified with 5% CO₂. Differentiation into myotubes was induced by reducing the FBS in the complete culture medium from 10% to 2%. Cells were maintained in this medium for 4 to 6 days post-confluence. The extent of differentiation was established by observing multinucleation of cells. In the present experiment, about 85% - 90% of the myoblasts was fused into myotubes (Anandharajan *et al.*, 2005).

Cell viability assay

The cells were seeded at concentration of 1.5x10⁴ cells/well onto a sterile 96-wells plate and incubated at 37°C overnight. Cells were further incubated for 72 hours at 37°C in the absence or presence of *F. deltoidea* extracts (10-1000 µg/ml) and metformin (10-2000 µM). Endpoint measurement of viable cells was done according to widely established methods (Mosman, 1983; Carmichael *et al.* 1987). Following the required incubation period, 20 µl of 5 mg/ml of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) was added to each well and incubated for 4 hours. Subsequently, the media from each well was then gently aspirated and 100 µl of dimethylsulphoxide (DMSO) was added to dissolve the formazan crystals. Plates were shaken for 5 seconds and

absorbance was measured at 570 nm using Anthos microplate reader (Beckman Coulter, USA). The percentage of cell viability was calculated using the following formula:

$$\% \text{ of cell viability} = \frac{\text{Absorbance of samples}}{\text{Absorbance of control}} \times 100$$

Glucose uptake assay

Glucose uptake assay was done according to Liu *et al.* (2001) with some modifications. Briefly, confluent cells were seeded at a concentration of 2×10^5 cells/well in a 12-wells plate and left overnight at 37°C to allow attachment prior to test. The next day, cells were washed thrice with serum-free DMEM and pre-incubated with this medium for 5 hours at 37°C. After starvation period, cells were washed thrice with Kreb's-Ringer bicarbonate buffer (KRB). Cells were further incubated for 30 minutes at 37°C with various concentrations of *F. deltoidea* extracts (10-1000 µg/ml) either alone or in combination with 100 nM insulin. Metformin was used as positive control. To initiate glucose uptake reaction, 2-deoxy-[1-³H]-glucose (1 µCi/ml) diluted in 0.1 mM D-glucose solution was added to each well and incubated further for 60 minutes 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).

Statistical analyses

All results are expressed as mean ± standard deviation for a given number of observations. Statistical analyses were done using GraphPad Prism version 3 software. Data were analyzed using one way ANOVA followed by Tukey post hoc test. Significant level was set at $p < 0.05$.

RESULTS

Cell viability

In L6 myotubes, a significant reduction of cell viability occurs in the presence of hot aqueous extract at concentrations of 50 – 1000 µg/ml, ethanolic extract at concentrations of 50 – 1000 µg/ml, methanolic extract at concentrations of 100 – 1000 µg/ml and metformin at concentrations of 2000 and 5000 µM (Table 1). Reduction of L6 myotubes in the presence of ethanolic extract at concentrations of 500 and 1000 µg/ml was more than 50%.

The total phenolic and flavonoids content in *F. deltoidea* extracts are shown in Table 2. Methanolic and hot aqueous contain high amount of phenolic compounds, whereas the ethanolic contains considerable amount. Flavonoids also presence in the *F. deltoidea* extracts, however the amount of such metabolite is low.

Table 1: L6 myotubes viability in the presence of *F. deltoidea* and metformin

| Test agents | Cells viability (%) | | | | | |
|---------------------|---------------------|---------------------|------------------------------|------------------------------|------------------------------|------------------------------|
| | 0 µg/ml/ 0 µM | 10 µg/ml/ 100 µM | 50 µg/ml/ 500 µM | 100 µg/ml/ 1000 µM | 500 µg/ml/ 2000 µM | 1000 µg/ml/ 5000 µM |
| Hot aqueous extract | 100.00 ± 5.70 | 94.77 ± 6.57 | 83.49 ± 6.31 *** (16.51%) | 83.08 ± 2.99 *** (16.92%) | 75.67 ± 5.22 *** (24.33%) | 70.71 ± 7.95 *** (29.29%) |
| Ethanolic extract | 100.00 ± 2.14 | 101.95 ± 2.04 | 81.17 ± 9.83 *** (18.83%) | 77.51 ± 7.72 *** (22.49%) | 40.54 ± 1.55 *** (59.46%) | 46.17 ± 2.19 *** (53.83%) |
| Methanolic extract | 100.00 ± 1.58 | 99.31 ± 1.68 | 91.92 ± 8.90 | 84.41 ± 8.41 *** (15.59%) | 81.28 ± 10.31 ** (18.72%) | 52.77 ± 1.42 *** (47.23%) |
| Metformin | 100.00 ± 14.24 | 94.57 ± 18.72 | 99.65 ± 14.19 | 85.21 ± 19.54 | 79.92 ± 15.18 ** (20.08%) | 63.55 ± 6.51 *** (36.45%) |

Notes: Cells were incubated for 72-hour in the presence of various concentrations of *F. deltoidea* extracts (16-1000µg/ml) and metformin (100 – 5000 µM). Values expressed as percentage of Means ± Standard Deviations (n=8) of the cells viability from three independent assays. *p<0.05, **p<0.01 and ***p<0.001 compared with control. Values in the bracket indicate percentage of cell viability reduction.

Table 2: Total content results on *Ficus deltoidea* (Mas cotek)

| Extract | Total phenolic content (Gallic acid equivalence, µg/mg extract) | Total flavonoids content (Quercetin equivalence, µg/mg extract) |
|-------------|---|---|
| Methanolic | 159.58 ± 6.89 | 19.52 ± 0.34 |
| Ethanolic | 49.58 ± 3.96 | 16.58 ± 0.50 |
| Hot aqueous | 126.67 ± 3.98 | 9.08 ± 0.37 |

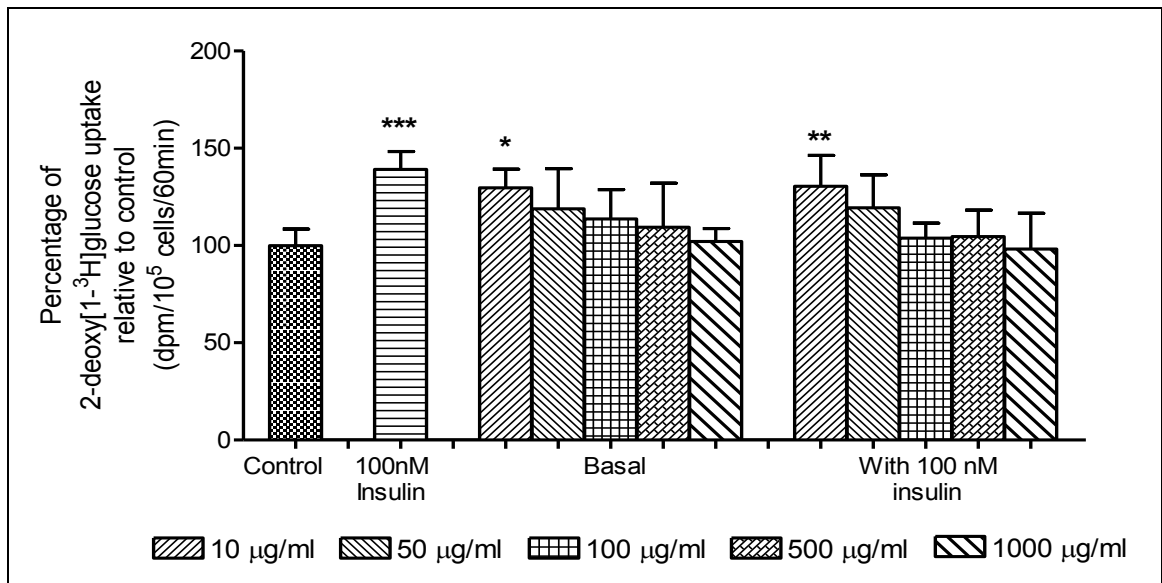


Figure 1: Glucose uptake activity of *F. deltoidea* hot aqueous extract

Note: Values represent the means \pm S.D from three independent experiments with four replicates in each experiment. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with control incubation.

Hot aqueous extract showed a concentration-dependent decrease of basal and insulin-mediated glucose uptake into muscle cells. Extract at concentration of 10 µg/ml significantly enhanced glucose uptake by 1.28-fold ($p < 0.05$) and 1.30 ($p < 0.01$) under basal and insulin-mediated state, respectively relative to control. 100 nM insulin enhanced glucose uptake by 1.39-fold ($p < 0.001$) relative to control.

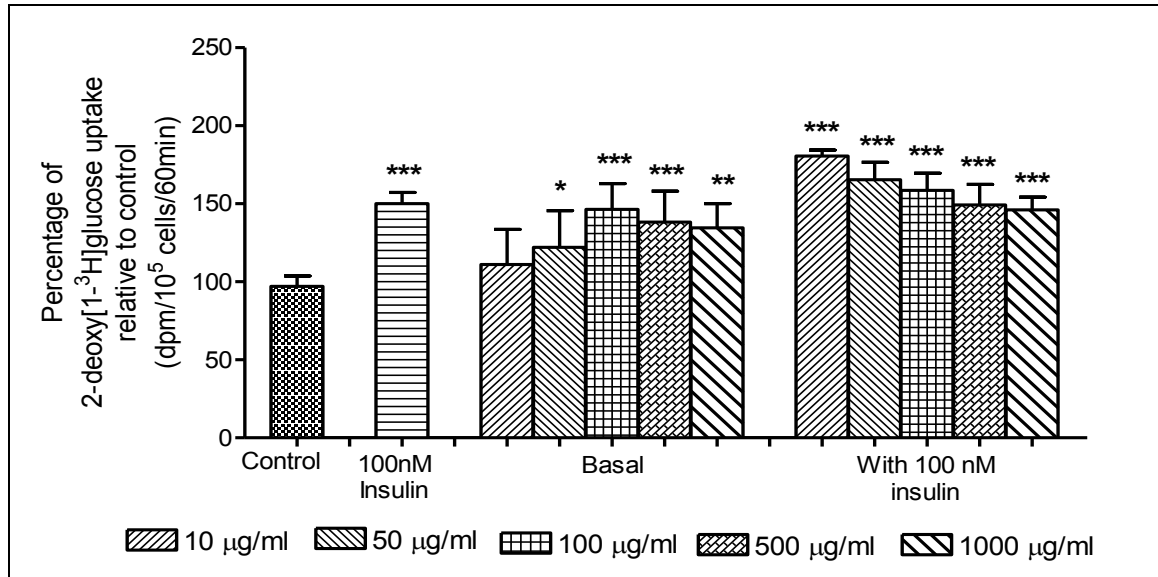


Figure 2: Glucose uptake activity of *F. deltoidea* ethanolic extract

Note: Values represent the means \pm S.D from three independent experiments with four replicates in each experiment. * $p < 0.05$; *** $p < 0.001$ compared with control incubation.

Ethanolic extract significantly enhanced basal glucose uptake by 1.26-fold ($p < 0.05$), 1.46-fold ($p < 0.001$), 1.38-fold ($p < 0.001$) and 1.35-fold ($p < 0.001$) at concentrations of 50, 100, 500 and 1000 µg/ml respectively, relative to control. Insulin-mediated glucose uptake was enhanced significantly at all concentrations evaluated with the magnitude of uptake were 1.80-fold ($p < 0.001$), 1.63-fold ($p < 0.001$), 1.59-fold ($p < 0.001$), 1.49-fold ($p < 0.001$)

and 1.46-fold ($p < 0.001$) at concentrations of 10, 50, 100, 500 and 1000 $\mu\text{g/ml}$, respectively compared to untreated control.

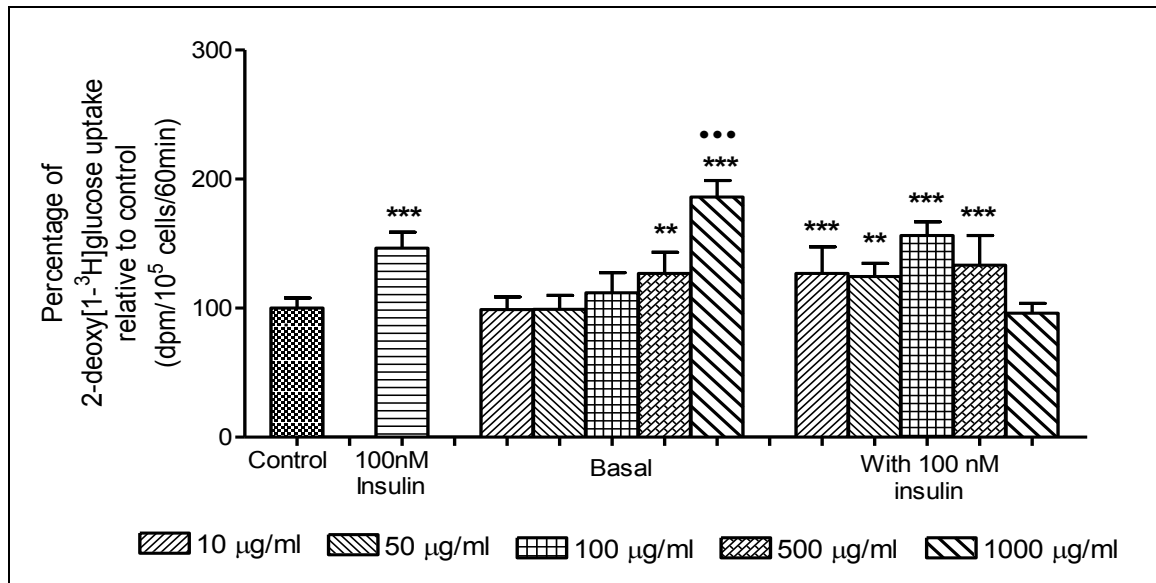


Figure 3: Glucose uptake activity of *F. deltoidea* methanolic extract

Note: Values represent the means \pm S.D from three independent experiments with four replicates in each experiment. ** $p < 0.01$, *** $p < 0.001$ compared with control incubation. ●●● $p < 0.001$ compared to 100 nM insulin.

Methanolic extract enhanced basal glucose uptake by L6 myotubes in a concentration-dependent manner. Extract at concentrations of 500 and 1000 $\mu\text{g/ml}$ exhibited a significant enhancement of glucose uptake by 1.27-fold ($p < 0.01$) and 1.86-fold ($p < 0.001$), respectively relative to control. The enhancement by the later concentration was 1.27-fold ($p < 0.001$) higher than that of 100 nM insulin which evoked an uptake of 1.46-fold ($p < 0.001$) relative to control. Insulin-mediated glucose uptake was significantly enhanced by 1.27-fold ($p < 0.001$), 1.25-fold ($p < 0.01$), 1.56-fold ($p < 0.001$) and 1.33-fold ($p < 0.001$) at concentrations of 10, 50, 100 and 500 $\mu\text{g/ml}$ respectively.

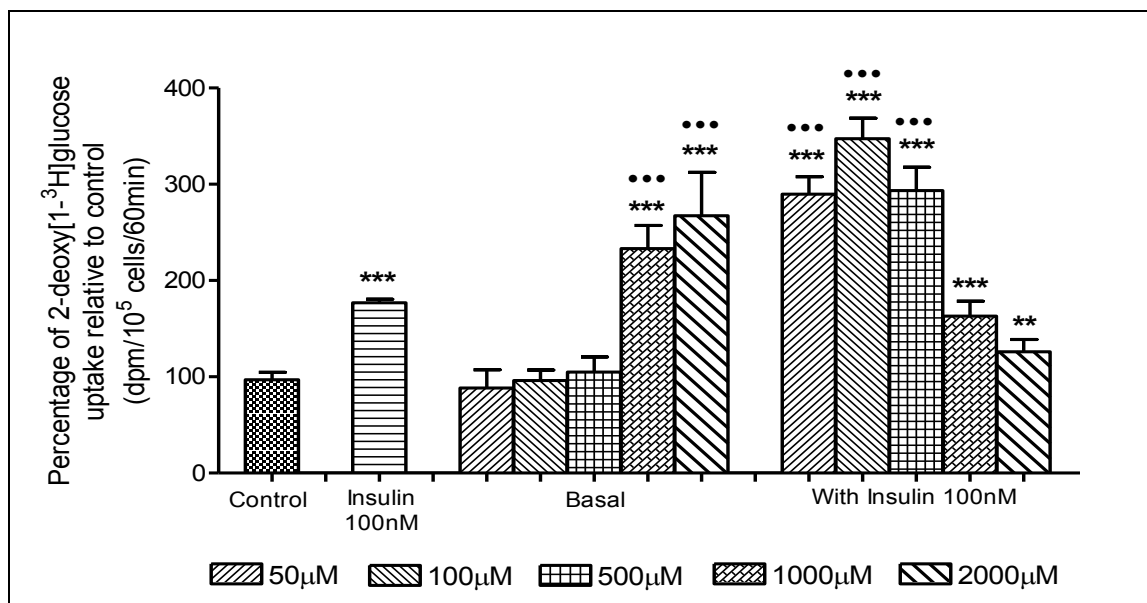


Figure 4: Glucose uptake activity of metformin

Note: Values represent the means \pm S.D from three independent experiments with four replicates in each experiment. ** $p < 0.01$, *** $p < 0.001$ compared with control incubation. ●●● $p < 0.001$ compared to 100 nM insulin.

Basal glucose uptake following metformin treatment was enhanced significantly by 2.41-fold ($p < 0.001$) and 2.76-fold ($p < 0.001$) at concentrations of 1000 and 2000 μM , respectively compared to control. The enhancement by these concentrations were 1.32-fold ($p < 0.001$) and 1.51-fold ($p < 0.001$) higher than that of 100 nM insulin which evoked a 1.83-fold ($p < 0.001$) of uptake relative to control. The later concentration exhibited the highest basal glucose uptake and was used as positive control to challenge the effect of *F. deltoidea* extracts on basal glucose uptake into L6 myotubes. Under insulin-mediated state, enhancement of glucose uptake were in the range of 1.68 – 3.58-fold ($p < 0.01$; $p < 0.001$) compared to control and 1.63 – 1.95-fold ($p < 0.001$) compared to 100 nM insulin. The highest insulin-mediated glucose uptake was found at concentration of 100 μM which evoked a 3.58-fold of enhancement. Therefore, metformin at this concentration was used as positive control to challenge insulin-mediated glucose uptake activity of *F. deltoidea* on L6 myotubes.

DISCUSSIONS

The present study reports the effect of hot aqueous, ethanolic and methanolic extracts of *F. deltoidea* on *in vitro* basal and insulin-mediated glucose uptake activity into skeletal muscle using L6 myotubes as the model of muscle cells. This cell has been widely used as the model system for studying glucose uptake activity into skeletal muscle (Patel and Mishra, 2008). This study revealed that *F. deltoidea* extracts at particular concentrations have the ability to enhance basal and insulin-mediated glucose uptake into L6 muscle cells. This suggests that there is a possibility of presence of antidiabetic compounds in *F. deltoidea* extracts which exert its antidiabetic mechanism through the enhancement of glucose disposal into muscle cells.

The viability of L6 myotubes in the presence of *F. deltoidea* extracts was evaluated using MTT assay. In this assay, the yellow tetrazolium salt, MTT is reduced by the mitochondrial enzymes, succinate dehydrogenase to form insoluble purple formazan crystals which are solubilized by the addition of a detergent. The color produced then can be measured spectrophotometrically at 570 nm. MTT reduction was proportional to cell viability (Mosman, 1983; Carmichael *et al.* 1987). In cytotoxicity evaluation, the highest concentration of a test agent should be 1000 $\mu\text{g}/\text{ml}$ or 1000 μM . If none of the concentrations of test agents exhibited cytotoxic effect in excess of 50% of cell populations, the test agent is considered non-toxic against the tested cell line (Elmore *et al.*, 2002). In the present study, ethanolic extract at concentrations of 500 and 1000 $\mu\text{g}/\text{ml}$ reduces the viability L6 myotubes to less than 50% after 72 hours exposure. Thus, the ethanolic extract was considered toxic against the L6 myotubes (Elmore *et al.*, 2002) and glucose uptake activity possess by these concentration of ethanolic extract should not be taken into account.

Glucose uptake by insulin-targeted cells (liver, adipocytes and muscle cells) becomes the initial step in the process of glucose disposal from blood circulation (Olson, 2012). Insulin, an anabolic hormone secreted by pancreatic β -cells was found to mediate glucose uptake into liver, adipocytes and muscle cells by binding to insulin receptor (IR) proteins at the surface of the cells, activating a series of proteins within the cells which leads to the translocation of glucose transporter 4 (GLUT4) protein to cell surface and permit the entrance of glucose into the cells (Watson and Pessin, 2006; Roffey *et al.*, 2007). It was reported that insulin enhanced basal glucose uptake into muscle cells (Mitsumoto *et al.*, 1991). The present study was in agreement with such reports that insulin at concentration of 100 nM significantly enhanced glucose uptake by 1.39 - 1.83-fold in L6 myotubes. Therefore, this concentration of insulin was used in this study to mediate glucose uptake activity into muscle cells by *F. deltoidea* extracts. This concentration of insulin (100 nM) was also been used to mediate glucose disposal into adipocytes cells (Konrad *et al.*, 2002; Sakurai *et al.*, 2004).

Basal glucose uptake was significantly enhanced in the presence of hot aqueous extract (10 $\mu\text{g}/\text{ml}$), ethanolic extract (50, 100, 500 and 1000 $\mu\text{g}/\text{ml}$), methanolic extract (500 and 1000 $\mu\text{g}/\text{ml}$) and metformin (1000 and 2000 μM). The enhancement of basal glucose uptake activity by methanolic extract at concentration of 1000 $\mu\text{g}/\text{ml}$ and metformin at concentrations of 1000 and 2000 μM were significantly higher than that of 100 nM

insulin. These results indicate that methanolic extract and metformin possess insulin-mimetic activity during enhancing glucose uptake into L6 myotubes cells. Several antidiabetic plants such as *Lagerstreoma speciosa*, *Agaricus campestris* and *Trigonella foenum-graecum* have been reported to possess insulin-mimetic activity (Liu *et al.*, 2001; Vijayakumar *et al.*, 2005). *Trigonella foenum-graecum* was reported to mediate glucose uptake into adipocytes and liver cells through the activation of tyrosine phosphorylation of β -subunit of insulin receptor (IR), subsequently enhancing tyrosine phosphorylation of insulin receptor substrate-1 (IRS-1) and p85 subunit of phosphatidylinositol 3-kinase (PI3-Kinase) and lead to glucose uptake (Vijayakumar *et al.*, 2005). There is a possibility that *F. deltoidea* extracts also mimic insulin mimetic activity through the same mechanisms. However, to confirm this suggestion, further evaluations are needed to be carried out.

The insulin-mimetic activity of some plants during enhancing glucose uptake into targeted cells has been reported to be associated with the phenolics compounds in the plants. For example, gallic acid, a type of phenolic acid was reported to increase GLUT4 translocation and induce glucose uptake into 3T3-L1 adipocytes (Prasad *et al.*, 2010). Patel *et al.*, (2012) reported that insulin-mimetic activity of many antidiabetic plants were attributed to the presence of phenolic, flavonoids, terpenoids, coumarin and other constituents. It was also reported that procyanidins, a flavonoid from grape seed possess insulin-mimetic activity during stimulating glucose uptake into 3T3-L1 adipocytes cells (Pinent *et al.*, 2004). In this study, it was shown that methanolic extract of *F. deltoidea* contains high amount of phenolic compounds. Methanolic extract also contain moderate amount of flavonoids. Thus, there is possibility that insulin-mimetic activity showed by methanolic extract is attributed by phenolic compounds, including flavonoids presence in the extract.

Insulin-mediated glucose uptake into L6 myotubes was significantly enhanced in the presence of hot aqueous extract (10 $\mu\text{g/ml}$), ethanolic extract (all concentrations), methanolic extract (10, 50, 100 and 500 $\mu\text{g/ml}$) and metformin (all concentrations). The enhancement of insulin-mediated glucose uptake into this cell by metformin at concentrations of 50, 100 and 500 μM was significantly higher than that of 100 nM insulin. This result suggests that metformin possess insulin-sensitizing activity during enhancing insulin-mediated glucose uptake into L6 myotubes (Ko *et al.*, 2005; Benhaddou-Andalousi *et al.*, 2008). This was in accordance with the previous studies which reported that metformin sensitize the action of insulin on glucose uptake into muscle cells (Klip and Leiter, 1990). However, all *F. deltoidea* extracts did not exerted insulin-sensitizing activity during enhancing glucose uptake into L6 muscle cells.

Metformin was used as positive control to challenge glucose uptake activity of *F. deltoidea* extracts into L6 myotubes. Metformin is an effective antihyperglycemic agent with the main antidiabetic mechanism is through the enhancement of insulin-mediated glucose uptake in muscle tissue and reduction of gluconeogenesis in the liver, resulted in reduction of hyperglycemia (Chehade and Mooradian, 2000). It was shown that, under both basal and insulin-mediated states, the magnitude of glucose uptake by all *F. deltoidea* extracts were less than metformin. This indicates that glucose uptake activity of *F. deltoidea* into L6 myotubes was not potent as metformin. This could possibly be due to that the plant extracts consist of mixture of compounds which are bioactive and non-bioactive. There is possibility that the non-bioactive compounds antagonized the action of the active compounds, hence decrease the glucose uptake activity of the extracts. Unlike the extracts, metformin is a single compound and its potential to stimulate glucose uptake into muscle cells has been scientifically proven and documented (Viljanen *et al.*, 2005; Amini *et al.*, 2005). Therefore, to ensure the effectiveness of *F. deltoidea* extracts in enhancing glucose uptake into the targeted cells, the bioactive compounds should be isolated and purified.

CONCLUSION

The results showed that aqueous, ethanolic and methanolic extracts of *F. deltoidea* have the ability to enhance basal and insulin-mediated glucose uptake into L6 muscles cells. The plant possesses insulin-mimetic activity during enhancing glucose uptake into such cells. From this study, it is suggested that the antihyperglycemic

action of *F. deltoidea* extracts is mediated partly, by enhancement of glucose uptake into the muscle cells. This study provide pre-clinical pharmacological data on the anti-diabetic activity of *F. deltoidea* and this information can be used to support the development of *F. deltoidea* -based phyto-medicine for diabetes management.

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