

Full Paper

Upregulation of glucose uptake in L8 myotubes by the extract from *Lagerstroemia speciosa*: a possible mechanism of action

Niwat Keawpradub¹ and Juntipa Purintrapiban^{2,*}

¹ Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat-Yai, Songkhla, 90112, Thailand

² Department of Biomedical Sciences, Faculty of Medicine, Prince of Songkla University, Hat-Yai, Songkhla, 90112, Thailand

* Corresponding author, e-mail: pjuntipa@medicine.psu.ac.th

Received: 18 May 2009 / Accepted: 14 December 2009 / Published: 18 December 2009

Abstract: The leaf of *Lagerstroemia speciosa* L. is used as an anti-diabetic herbal remedy in many countries. In an attempt to discover mechanisms of action of the *L. speciosa* extract that stimulate glucose uptake, a cell-based radioactive assay of glucose uptake was performed using L8 muscle cells. In this study, the methanol fraction of *L. speciosa* leaves (LSE) contained a high level of phenolic compounds and showed strong capability to stimulate glucose uptake in a dose-dependent manner. The LSE stimulation was slightly inhibited (8.8%) by SB203580. The inhibitory effect (23.6%) of wortmannin on LSE-stimulated glucose uptake was demonstrated, suggesting LSE action on glucose transporter translocation. LSE-induced glucose uptake was completely reversed by cycloheximide. In addition, an increased amount of total glucose-transporter-1 protein was observed indicating that new protein synthesis is necessary for elevated glucose transport. LSE also enhanced insulin-stimulated glucose transport. These results suggest that LSE action may be mediated primarily via the synthesis of new transporters and involve both insulin-dependent and independent pathways.

Keywords: *Lagerstroemia speciosa*, glucose uptake, GLUT1, polyphenols, L8 myotubes

Introduction

Diabetes Type II has become a predominant public health problem with increasing rate of affecting people worldwide [1]. About one third of Type II diabetic patients need insulin to reduce their blood glucose levels, and some 40% require oral agents for satisfactory blood glucose control. Currently, due

to lack of access to essential medicines developed through pharmaceutical research and as a part of local culture, a majority of the world's population still depends on traditional medicine for primary health care need. In addition, use of complementary and alternative medicine in the treatment of diabetes has increased steadily among the general public. In the U.S. alone, a 380% increase in the use of herbal supplements from 1990 to 1997 has been reported [2]. Thus, a provision of safe and effective traditional medical therapies could become a vital tool for increasing access to health care.

Lagerstroemia speciosa (L.) Pers. (Lythraceae) is a popular medicinal plant in South-East Asia, especially the Philippines. A decoction of *L. speciosa* leaves has been used for treatment of diabetes [3]. The extract of *L. speciosa* significantly lowered blood sugar in Type II diabetic mice and alloxan-induced diabetic rats [4-6], and stimulated glucose transport in adipocytes and Ehrlich ascites tumour cells [7-8]. Studies have shown an anti-obesity activity of extracts from this plant on KK-Ay mice and in 3T3-L1 cells [7, 9]. The hypoglycemic effect of the active component (corosolic acid) resulted from increased GLUT4 translocation in muscle of diabetic mice [10]. Recently, more active tannins, gallotannins and triterpenes were reported to enhance glucose uptake in 3T3-L1 cells [11-14]. It was also noted that the magnitude of the effect of *L. speciosa* extract was much larger than the effect of the individual active compound.

According to previous reports, glucose transport enhancement activity of the aqueous extract of *L. speciosa* leaves in 3T3-L1 adipocytes involved a rapid response (15-min) [7, 13]. However, no reports of the long-term effects or the mechanisms of action of the extract at cellular and molecular levels have been published despite the fact that the extract is commercially available [15]. This present study is designed to investigate the effects of the methanol fraction of the aqueous extract of *L. speciosa* leaves on the glucose transport in muscle cells, a physiological target cells of insulin, by employing L8 myotubes as cell model to assay for glucose uptake activities. The mechanisms by which the extract mediates the glucose transport activity in the presence of specific inhibitors are also investigated.

Materials and Methods

Materials

Rat L8 myoblasts were purchased from American Type Culture Collection (Rockville, MD). Cell culture media and supplements were acquired from Life Technologies, Inc. (Gaithersburg, MD). Bovine insulin, cytochalasin B (CB), wortmannin (WM), SB203580 (SB), cycloheximide (CHX), protease inhibitor cocktail, phloretin, α -actin antibody, enzyme-linked antibodies and standard chemicals were purchased from Sigma Chemicals (St. Louis, MO). 2-Deoxy-D-[^3H] glucose (2-dGlc) was obtained from Amersham Biosciences (Piscataway, NJ). A polyclonal antibody against glucose transporter 1 (GLUT1) was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). A monoclonal antibody against glucose transporter 4 (GLUT4) was purchased from R&D systems, Inc. (Minneapolis, MN). Electrophoresis and protein assay reagents were purchased from Bio-Rad (Hercules, CA). The CytoTox 96 non-radioactive cytotoxicity assay kit was purchased from Promega (Madison, WI).

Sample preparation

Lagerstroemia speciosa leaves were collected from the medicinal plants garden of the Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat-Yai, Thailand. They were cleaned by washing with running tap water and rinsing with distilled water, and placed in a 45°C oven until they were completely dried. Five grams of the powdered leaf sample were extracted with 200 ml water at 70°C for 30 min. After filtering, the solution obtained was centrifuged at 5,000 g for 20 min and the supernatant concentrated by freeze-drying to give a crude extract. A portion of the crude extract (1.45 g) was further separated by passage through a Sephadex LH-20 column with methanol as eluent to afford 32 fractions (20 ml each). The eluted fractions were subjected to thin-layer chromatographic analysis using silica gel 60 F₂₅₄ plates (0.20 mm thick) with ethyl acetate-chloroform-methanol (2:1:1) as developing solvent. Fractions 14-21, which showed similar chemical profiles, containing mainly 4 tailing phenolic compounds which gave greenish-blue colour upon spraying with 10% ferric chloride in absolute ethanol, were pooled and evaporated to give a dried residue of the methanol fraction (LSE: 986 mg). Upon phytochemical screening (see Table 1), besides phenolic compounds (including tannins), triterpenoids and carbohydrates were also detected in LSE. The residue (LSE) dissolved in water and sterilised with 0.45-µm filters was used for the glucose transport study.

Cell and culture conditions

L8 myoblasts were maintained and differentiated as described previously [16]. Briefly, the cells were seeded in complete Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum at 35,000 cells/cm² in 24-well plates for glucose uptake assay and 100-mm plates for protein analysis. When cells reached ~95% confluence, differentiation was initiated by culturing them in a medium with 2% horse serum (HS). In the present experiment, ~80-85% of the myoblasts fused into myotubes. The cells were rinsed with HEPES buffered saline, pH 7.4 (HBS). Thereafter, HBS (1x final concentration) containing 2% HS, 15 mM glucose and LSE at the desired concentration (0, 20, 40, 80, 100, 120 or 140 µg/ml) or 100 nM insulin were added to the cells, which were then incubated at 37°C for 16 h. In order to investigate the effect of insulin, the medium was changed to serum-free HBS (1x) containing 25 mM glucose, and incubated at 37°C for 5 h before the assay of glucose transport. For the inhibitor studies, 2 µg/ml CHX, 100 nM WM, 10 µM SB and 40 µM CB were added individually or in combination to the LSE-treated cells at a respective time (CHX, 16 h; WM, 20 min; SB, 30 min; and CB, 30 min) prior to the end of the 16-h incubation. Phloretin (10 mM), an inhibitor of a variety of membrane transporters including facilitated glucose transport [17], was added to the incubation mixture 20 min before the uptake assay to determine the effect of non-specific uptake. Using the CytoTox 96 non-radioactive cytotoxicity assay, an aliquot from each well was taken to determine the cytotoxic effect according to the manufacturer's instructions.

2-dGlc uptake assay

The L8 myotubes were rinsed twice with 1 ml HBS. After rinsing, glucose uptake was initiated by the addition of 0.3 ml of HBS (1x) containing 2-dGlc (1 µCi/ml) without or with 100 nM insulin as the

final concentration to the cells. After 10 min, the medium was aspirated and the plates were washed three times with ice-cold 0.9% NaCl to terminate the induced glucose uptake. The cells were lysed in 0.05 N NaOH and the radioactivity taken up by the cells was determined using a scintillation counter. Preliminary studies (data not shown) demonstrated that uptake of 2-dGlc (1 μ Ci/ml) was linear up to 15 min. Non-specific uptake measured in the presence of phloretin was less than 10% of the total uptake. Aliquots from each treatment were used to determine the protein concentration using the Bio-Rad protein assay.

Analysis of glucose transporter

A method described by Yu et al. [18] was used with modification. L8 myotubes grown in 100-mm plates and incubated with and without 100 μ g/ml LSE were washed twice with cold phosphate-buffered saline (PBS). The cells were treated with 20 strokes in homogenising buffer (25 mM sucrose, 20 mM HEPES, pH 7.4, 2 mM EGTA, 5 mM NaN₃, protease inhibitors). Cell lysates were centrifuged at 200 g for 5 min. The supernatant (whole cell lysate) was collected for total GLUT1 and GLUT4 protein analysis or further centrifuged at 16,000 g for 15 min to pellet the plasma membrane (PM) from the supernatant or soluble (S) fraction. Protein samples (150 μ g) were separated in 10% SDS-PAGE and transferred to PVDF for the Western blot analysis. Non-specific sites on the membrane were blocked with 5% non-fat dry milk. Antibodies against GLUT1 (1:700), GLUT4 (1:250) or α -actin (1:700) were added and maintained for 2 h at room temperature. Blots were then incubated with enzyme-linked second antibody followed by colourimetric or chemiluminescent detection. The immunoreactive protein was quantified using scanning densitometry.

Phytochemical screening and determination of total phenolics

Phytochemical screening of LSE was performed using the methods previously described by Farnsworth [19] and Harborne [20] with slight modification. In brief, several reagents were prepared to test for the presence of flavonoids, coumarins, anthraquinones, iridoids, cardiac glycosides, cyanogenetic glycosides, coumarins, saponins, alkaloids, tannins, carbohydrates, amino acids and peptides. The results were compared with the positive standards of each test. The amount of total phenolic compounds was determined spectrophotometrically using Folin-Ciocalteu reagent as described by Lee et al. [21] and was expressed in μ g of catechin equivalent (CE) based on a calibration curve for catechin.

Statistical analyses

Three to five separate experiments were conducted in all studies and all assay conditions were performed in triplicate. All data are expressed as means \pm SD. Statistical analyses were performed by a one-way analysis of variance followed by Dunnett t-tests. The level of $P < 0.05$ was considered significant.

Results

LSE-stimulated uptake of 2-dGlc by L8 myotubes in culture

Figure 1A shows the time course for the uptake of 2-dGlc in L8 myotubes induced by LSE. In contrast to previous studies in fat cells [7, 13], the response to LSE in muscle cells was found to be slow. The rate of 2-dGlc uptake increased about 1.35, 2.43 and 2.70-fold ($P<0.05$) of control levels at 8, 16 and 24 h respectively after exposure to 0.1 mg/ml LSE. The basal uptake activity response to prolonged (8-24 h) incubation was lower than that to short-term (0.5-2 h) exposure due to the effect of high glucose in the incubation medium [22-23]. When cells were maintained for 16 h with varying doses of LSE, a dose-dependent effect was observed (Figure 1B). The maximal uptake activity was achieved at 0.1 mg/ml LSE, and thereafter the rate decreased markedly due to the cytotoxic effect. LSE also caused a slight toxicity at dose of 0.1 mg/ml. LSE-induced uptake was blocked by cytochalasin B, which inhibits cytoskeletal reorganisation when added at 40 μ M prior to the uptake assay (data not shown). This indicates that LSE actively stimulates glucose uptake in the cells and could likely activate the movement of glucose across cell membranes.

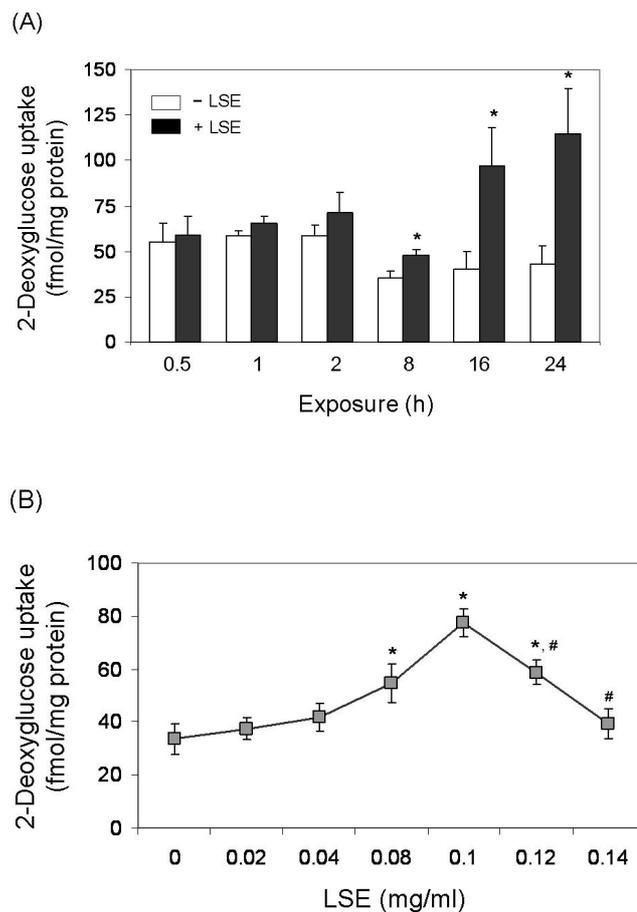


Figure 1. Time course and dose-dependent effects of LSE on 2-deoxyglucose uptake. Panel A: L8 myotubes were incubated with or without 0.1 mg/ml LSE for the time indicated; Panel B: L8 myotubes were incubated with increasing doses of LSE for 16 h prior to 10-min determination of 2-deoxyglucose uptake. Data were expressed as means \pm SD of the triplicates derived from three separate experiments.

* $P<0.05$ vs. control cells # toxicity observed

Figure 2 shows data from experiments in which the effects of the specific inhibitors on the uptake of 2-dGlc were examined in LSE-treated cells. CHX treatment reduced the 2-dGlc uptake rate in the control (LSE-untreated) cells by about 55%, indicating that active protein synthesis is necessary for maintaining cellular transport of glucose at the basal state. It is clear that CHX could reverse the effect of LSE. Approximately 71% reduction of LSE-stimulated 2-dGlc transport by CHX was observed. SB203580, which specifically inhibits the activity of p38 mitogen-activated protein kinase (p38 MAPK) through the interaction with its ATP-binding domain resulting in the inactivation of glucose transporter's intrinsic activity [24-25], slightly decreased the activity of LSE-induced 2-dGlc uptake ($8.87 \pm 3.69\%$, $n = 5$). Wortmannin, which has been shown to interact with the p110 activating subunit of phosphatidylinositol 3-kinase (PI3K) resulting in an irreversible inhibition of this kinase that leads to inhibition of GLUT4 translocation [26], only partially but significantly reduced the stimulation of LSE ($23.62 \pm 4.90\%$, $n = 5$). A higher degree of inhibition was observed when a combination of inhibitors was present.

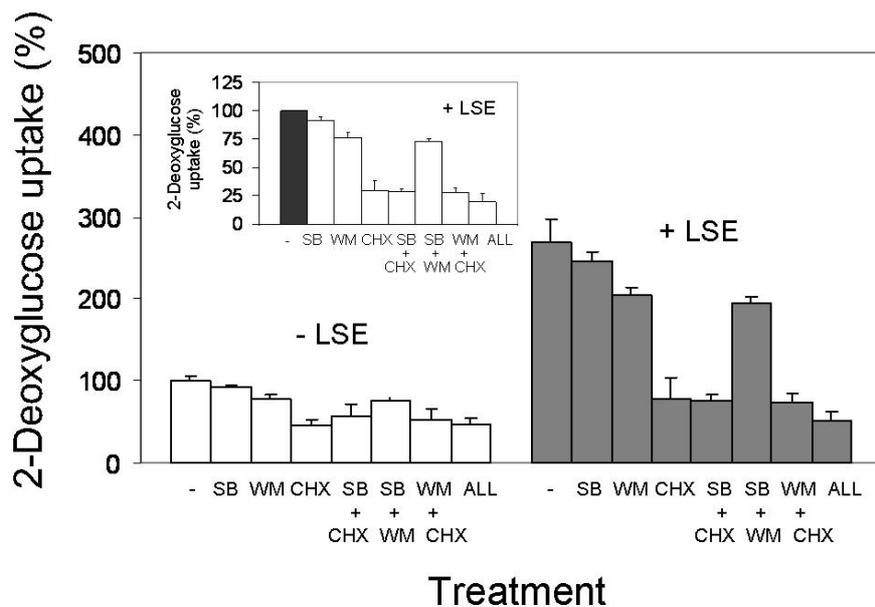


Figure 2. Inhibition of LSE-stimulated 2-deoxyglucose uptake activity by SB203580, wortmannin, and cycloheximide. L8 myotubes were pre-incubated with or without 0.1 mg/ml LSE for 16 h in the presence of SB203580 (SB), wortmannin (WM), cycloheximide (CHX) or all agents (ALL) as indicated prior to 10 min determination of 2-deoxyglucose uptake. Inset: The reduction in glucose uptake activity by inhibitors in LSE-treated cells was recalculated and expressed relative to the uptake activity of LSE alone. Data were expressed as means \pm SD of the triplicates derived from five separate experiments.

* $P < 0.05$ vs. values with LSE alone

Figure 3 shows the relationship between insulin and LSE effects on glucose uptake. The cells were maintained in serum-free media for 5 h to induce insulin stimulation response before the uptake assay was initiated [16, 27]. To enable the detection of additive effect, LSE at a concentration of 80 μ g/ml was used. The activation of glucose transport by insulin was enhanced by 39% in the presence of

LSE (for insulin, sugar uptake = 32.37 ± 5.92 pmol/mg protein; for LSE+insulin, sugar uptake = 45.23 ± 8.11 pmol/mg protein, $n = 5$). This indicates a partial additive effect of both agents.

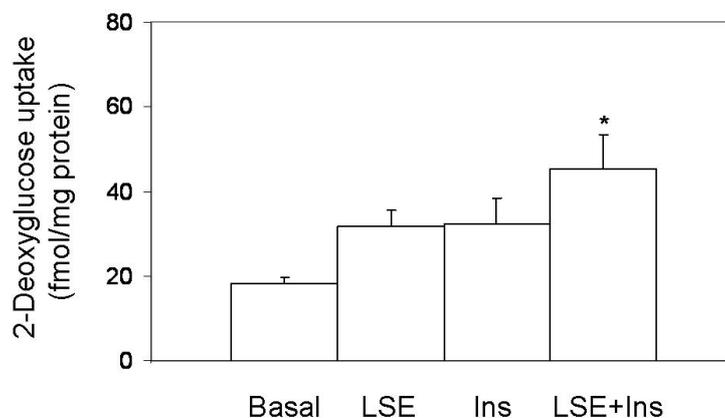


Figure 3. Additivity of insulin- and LSE-mediated uptake of 2-deoxyglucose in L8 cells. L8 myotubes were stimulated with or without 100 nM insulin and/or 0.08 mg/ml LSE for 16 h and serum-deprived for 5 h. Uptake of 2-deoxyglucose was measured over 10 min in the presence or absence of insulin. Data were expressed as means \pm SD of the triplicates derived from five separate experiments.

* $P < 0.05$ vs. values with either insulin or LSE alone

Figure 4 shows the results of a representative experiment: GLUT1 and GLUT4 content in cells exposed for 16 h or not exposed to LSE at 0.1 mg/ml. Western blot analyses of GLUT4 content from five separate experiments revealed no notable change in the amount of GLUT4 total protein in LSE cells (1.11 ± 0.27 -fold) compared to that in untreated cells. In contrast, there was an increase in total GLUT1 protein content (2.15 ± 0.96 -fold) in the LSE-treated cells and the level of GLUT1 protein present in the plasma membrane (PM) was much higher than that in the intracellular compartment (S). Increased GLUT1 protein content due to LSE was significantly lowered (0.74 ± 0.24 -fold, $n = 3$) to below the basal level when CHX was present (Figure 4C). These results imply that the effect of LSE is associated with significant increase in the content of GLUT1 and the magnitude of the gain in uptake activity by LSE may be due to enhanced GLUT1 protein synthesis.

Total phenols and active constituents of LSE

The amount of total phenolics in 1 mg LSE was 409.58 ± 39.57 μ g catechin equivalent (CE). Thus, polyphenols present in the maximal stimulation dose of LSE (0.1 mg/ml) was about 40.9 μ g CE. This implies that increasing glucose transport in L8 muscle cells resulted mainly from the additional effect of polyphenols. Table 1 shows the results of phytochemical screening of LSE for the presence of active constituents. As evident from the Table, LSE yielded a positive result with Liebermann-Burchard test, indicating the presence of triterpenoid compounds. It was also positive with Molisch's test, suggesting the presence of free carbohydrates and/or bound sugars in the form of glycosidic compounds. Furthermore, LSE gave positive results in reactions with 1% gelatin solution and 1% ferric chloride solution, suggesting the existence of tannins.

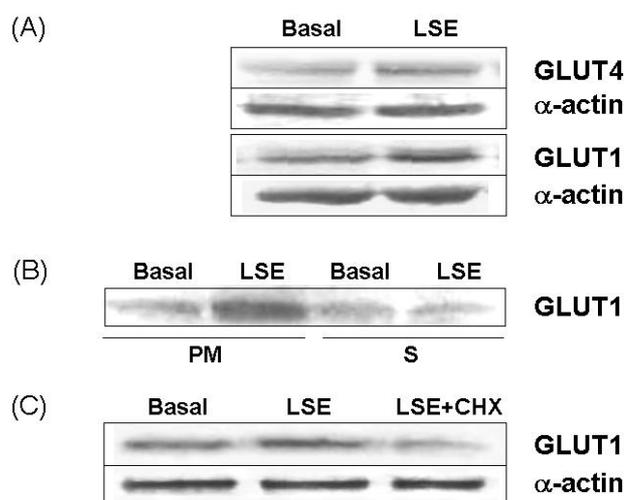


Figure 4. Determination of total GLUT4 and GLUT1 protein content (panel A), GLUT1 localisation (panel B), and the inhibitory effect of CHX on total GLUT1 level (panel C) in LSE-stimulated cells. L8 myotubes were treated with or without 0.1 mg/ml LSE and 2 μ g/ml CHX for 16 h. Cells were homogenised, and total lysate, plasma membrane (PM) and intracellular fraction (S) were prepared. Samples were Western blotted and probed for GLUT1, GLUT4 and α -actin with specific antibodies. Immunoblots are representative of 3-8 samples.

Discussion

Natural remedies are viable alternatives to oral medications that may cause undesirable side effects. Indeed, many plants with a glucose-lowering effect have been used successfully for the treatment of diabetes. Particularly, one of the various botanical health care products, *L. speciosa* extract, has been made available worldwide [28-29]. However, these health care products have not been evaluated by the US FDA and have little scientific analyses to confirm the effects and mechanisms of the plant extract and the active ingredients.

It is well established that the predominant GLUT4 isoform expressed in mature muscle cells and adipocytes is primarily responsible for the increase in glucose uptake in response to insulin stimulation [30]. Increased exocytosis and decreased endocytosis in the presence of insulin, which cause a shift in the steady-state distribution of GLUT4 favouring the plasma membrane, result in increase of the amount of GLUT4 at the plasma membrane [31]. The present study has shown that the *L. speciosa* extract (LSE) is a potent activator of glucose transport in L8 muscle cells. We have demonstrated that prolonged exposure to LSE stimulates glucose transport in the absence of insulin. However, analysis of blot density displayed no effect in total GLUT4 protein content. In addition, wortmannin, an inhibitor of PI3K, which blocks the translocation of insulin-sensitive GLUT4 from its intracellular pool to the plasma membrane [32-33], partially reversed the LSE-induced 2-dGlc uptake. The present results suggest that the translocation of the GLUT4 isoform mediated via insulin-regulated, PI3K-dependent signaling is essential for LSE-induced glucose transport.

Table 1. Phytochemical screening results of LSE

Test	LSE	Positive standard
Coumarins (NaOH paper, UV 365 nm)	(-)	Stem bark of <i>Alyxia reinwardtii</i> (+): blue colour under UV light
Anthraquinones (Borntrager test)	(-)	Aloe (<i>Aloe barbadensis</i>) (+): pink colour in the alkaline layer
Iridoids (formation of pseudoindicans)	(-)	Leaves of <i>Clinacanthus nutans</i> (+): green-blue solution
Cardiac glycosides Kedde's reagent test	(-)	Seed kernel of <i>Thevetia peruviana</i> (+): purple solution
Liebermann-Burchard test	(+): red colour (triterpenoids)	(+): green colour (steroids)
Keller-Kiliani test	(-)	(+): reddish-brown ring
Saponins Froth test	(-)	Fruit of <i>Sapindus rarak</i> (+): honeycomb froth (persisted for at least 30 min)
Liebermann-Burchard test	(+): red colour (triterpenoids)	(+): red colour (triterpenoids)
Flavonoids (Shinoda's test)	(-)	Stem of <i>Derris elliptica</i> (+): pink-red solution
Tannins Reaction with gelatin solution	(+): precipitate	Gambir (<i>Uncaria gambir</i>) (+): precipitate
Reaction with FeCl ₃ solution	(+): green-blue solution	(+): green-blue solution
Alkaloids Mayer's reagent	(-)	Stem of <i>Arcangelisia flava</i> (+): precipitate
Dragendorff's reagent	(-)	(+): precipitate
Carbohydrates (Molisch's test)	(+): purple ring	Honey (<i>Apis mellifera</i>) (+): purple ring
Amino acids and peptides Biuret test	(-)	Soy bean (<i>Glycine max</i>) (+): violet solution
Ninhydrin test	(-)	(+): grayish-blue solution

Note : (-) = negative result

(+) = positive result

It was reported by Liu et al [7, 13] that exposure to *L. speciosa* extract results in rapid activation of glucose uptake of serum-starved adipocytes. Employing serum deprivation that involves the depletion of GLUT1 prior to treatment of adipocyte and muscle cell cultures has been practiced in several studies, and this strategy allows the detection of strongly stimulated glucose transport in response to insulin and other stimulators which mediate GLUT4 translocation [17, 27, 34]. In addition, insulin, for example, has been shown in these studies to be capable of increasing the rate of glucose transport in adipocytes 15- to 20-fold, whereas about a 2-fold increase is achieved in muscle cells. In the present study, we have demonstrated that short-term exposure to LSE does not activate glucose transport in L8 cells. One possible reason is that the LSE effect on GLUT4 translocation is small (~23% of wortmannin-dependent inhibition), thus the increasing glucose uptake activity resulting from GLUT4 translocation is undetected under conditions in which the basal uptake is not down-regulated as previously reported by Diedrich [16] and Klip et al. [27].

GLUT1, which is plasma-membrane-localised with a relatively small amount found in intracellular membrane compartments in cells and tissues in the basal state [35], was reported to mediate a significant fraction of non-insulin-dependent transport of glucose [30]. GLUT1 gene expression is regulated by various stimuli including cell stress factors, mitochondria inhibitors, hypoglycemia tumour necrosis factor α and prolonged insulin exposure [36-41]. Studies in Clone 9 cells that express GLUT1 but not GLUT2 or GLUT4 have revealed that GLUT1 activation and translocation are not influenced by inhibitors of PI3K and p38 MAPK, which reportedly prevent GLUT4 translocation and/or activation by insulin [42], suggesting that GLUT1 expression may be mediated by a different mechanism. A lag period of more than 2 h preceding the increase in transport rate by LSE in the present study reflects the time required for the concentration of glucose transporters to increase above the threshold level whereby the stimulation of transport can be detected. Our results in L8 cells indicate that exposure to LSE increases both the number of GLUT1 at the cell surface and the GLUT1 total protein levels. Furthermore, cycloheximide blocks the activation of glucose uptake by LSE and diminishes LSE's effect on increasing GLUT1 protein level. These indicate that a component of glucose transport stimulation associated with LSE action is the number of GLUT1 up-regulated via GLUT1 protein expression. It is known that the mRNA half-life is linked to its translation and its stability also affects the rate of change of its abundance following an increase in transcription [43]. At the protein level, the half-life of GLUT1 could be increased or decreased by particular stimuli [39, 44]. Further studies on the actual mechanism by which LSE increases GLUT1 protein content that can be attributed to either altered GLUT1 gene expression or post-translation regulation of GLUT1 are necessary. Remarkably, cycloheximide interferes with LSE-induced glucose uptake, suggesting that the regulation is dependent on translation.

The p38 MAPK-dependent pathway has been implied in enhancing the intrinsic activity of GLUT1 and GLUT4 at the cell surface [24-25]. Inhibition of p38 MAPK with specific inhibitor SB203580 reduces insulin-induced glucose uptake without an effect on insulin-induced GLUT4 translocation [25]. In the present study, SB203580 has a small but significant inhibitory effect on LSE-induced glucose uptake, demonstrating that the activation of p38 MAPK is involved in LSE action. We have shown that LSE-mediated glucose uptake in L8 cells is apparently associated to the mechanisms by which insulin signals glucose transport. A further increase in insulin-stimulated glucose transport by LSE provides

evidence that LSE may also target diverse cascades, some of which lead to increased glucose transport. It has been reported that muscle contraction and hypoxia or metabolic stress cause a normal glucose transport response involving activation of 5'-AMP-activated kinase (AMPK) in the insulin-resistant skeletal muscles, in which insulin signalling of glucose transport and GLUT4 translocation are impaired [45]. Thus, taken together, these data suggest that LSE activates glucose transport in L8 muscle cells and involves the synthesis of GLUT1 and the activation of PI3K and p38 MAPK as well as the insulin-independent signalling, which may operate in tandem with the insulin-dependent pathway.

In this present study, a significant amount of phenolic compounds in LSE has also been detected. This is in good agreement with a previous study [46] that reported a high content (10.3%) of polyphenols in the leaf extract of *L. speciosa*. In addition, studies have shown that the most active components of *L. speciosa* leaves are polyphenols. These include ellagitannins (i.e. lagerstroemin, flosin B and reginin A) and corosolic acid, which have been shown to activate glucose transport in cell culture [8, 11]. Recent reports demonstrated the lowering effect of corosolic acid on postchallenge plasma glucose levels in human subjects and established its inhibitory effect on glycogen phosphorylase *a* [46, 47]. The antidiabetic activity of the leaf extract (standardised to contain 1% corosolic acid) in human has been described [15]. The presence of tannins and triterpenoids in LSE is consistent with recent studies, which reported more tannins and triterpenes in *L. speciosa* and their capacity to enhance glucose uptake in fat cells [11-13]. Furthermore, the activation of the insulin receptor by lagerstroemin (a polyphenol) has also been demonstrated in the Chinese hamster ovary cells [48]. Thus, it is apparent that LSE contains several active polyphenols that may act synergistically or through multiple mechanisms.

Conclusions

The present study has established that the extract of *L. speciosa* exerts a direct up-regulatory effect on GLUT1 protein expression and, in part, the GLUT4 translocation, which results in the gain of glucose transporters at the plasma membrane. The mechanisms of action contributing to increased glucose transport involve both the activation of insulin-dependent and insulin-independent signalling pathways.

Acknowledgement

This work was supported in part by grants from the Faculty of Medicine, Prince of Songkla University.

References

1. J. C. Seidell, "Obesity, insulin resistance and diabetes--a worldwide epidemic", *Br. J. Nutr.*, 2000, 83 Suppl.1, S5-S8.

2. D.M. Eisenberg, R. B. Davis, S. L. Ettner, S. Appel, S. Wilkey, M. Van Rompay and R. C. Kessler, "Trends in alternative medicine use in the United States, 1990-1997: Results of a follow-up national survey", *J. Am. Med. Assoc.*, **1998**, *280*, 1569-1575.
3. D. P. Carew and T. F. Chin, "Constituents of *Lagerstroemia speciosa* Flosreginae Retz", *Nature*, **1961**, *190*, 1108-1109.
4. T. Kakuda, I. Sakane, T. Takihara, Y. Ozaki, H. Takeuchi and M. Kuroyanagi, "Hypoglycemic effect of extracts from *Lagerstroemia speciosa* L. leaves in genetically diabetic KK-Ay mice", *Biosci. Biotechnol. Biochem.*, **1996**, *60*, 204-208.
5. H. Hong and W. J. Maeng, "Effects of malted barley extract and banaba extract on blood glucose levels in genetically diabetic mice", *J. Med. Food*, **2004**, *7*, 487-490.
6. Y. Mishra, M. S. Y. Khan, R. Zafar and S. S. Agarwal, "Hypoglycaemic activity of leaves of *Lagerstroemia speciosa* (L) Pers.", *Indian J. Pharmacol.*, **1990**, *22*, 174-176.
7. F. Liu, J. Kim, Y. Li, X. Liu, J. Li and X. Chen, "An extract of *Lagerstroemia speciosa* L. has insulin-like glucose uptake-stimulatory and adipocyte differentiation-inhibitory activity in 3T3-Li cells", *J. Nutr.*, **2001**, *131*, 2242-2247.
8. C. Murakami, K. Myoga, R. Kasai, K. Ohiani, T. Kurokawa, S. Ishibashi, F. Dayrit, W. G. Padolina and K. Yamasaki, "Screening of plant constituents for effect on glucose transport activity in Ehrlich ascites tumour cells", *Chem. Pharm. Bull.*, **1993**, *41*, 2129-2131.
9. Y. Suzuki, T. Unno, M. Ushitani, K. Hayashi and T. Kakuda, "Antiobesity activity of extracts from *Lagerstroemia speciosa* L. leaves on female KK-Ay mice" *J. Nutr. Sci. Vitaminol. (Tokyo)*, **1999**, *45*, 791-795.
10. T. Miura, Y. Itoh, T. Kaneko, N. Ueda, T. Ishida, M. Fukushima, F. Matsuyama and Y. Seino, "Corosolic acid induces GLUT4 translocation in genetically type 2 diabetic mice", *Biol. Pharm. Bull.*, **2004**, *27*, 1103-1105.
11. T. Hayashi, H. Maruyama, R. Kasai, K. Hattori, S. Takasuga, O. Hazeki, K. Yamasaki and T. Tanaka, "Ellagitannins from *Lagerstroemia speciosa* as activators of glucose transport in fat cells", *Planta. Med.*, **2002**, *68*, 173-175.
12. Y. Okada, A. Omae and T. Okuyama, "A new triterpenoid isolated from *Lagerstroemia speciosa* (L.) Pers.", *Chem. Pharm. Bull. (Tokyo)*, **2003**, *51*, 452-454.
13. X. Liu, J. K. Kim, Y. Li, J. Li, F. Liu and X. Chen, "Tannic acid stimulates glucose transport and inhibits adipocyte differentiation in 3T3-L1 cells", *J. Nutr.*, **2005**, *135*, 165-171.
14. G. Klein, J. Kim, K. Himmeldirk, Y. Kao and X. Chen, "Antidiabetes and anti-obesity activity of *Lagerstroemia speciosa*", *Evid.-Based Complement. Alternat. Med.*, **2007**, *4*, 401-407.
15. W. V. Judy, S. P. Hari, W. W. Stogsdill, J. S. Judy, Y. M. A. Naguib, and R. Passwater, "Antidiabetic activity of a standardized extract (Glucosol) from *Lagerstroemia speciosa* leaves in Type II diabetics. A dose-dependence study", *J. Ethnopharmacol.*, **2003**, *87*, 115-117.
16. D. F. Diedrich, "Photoaffinity-labeling analogs of phlorizin and phloretin: synthesis and effects on cell membranes", *Methods Enzymol.*, **1990**, *191*, 755-781.
17. J. Purintrapiban and S. Ratanachaiyavong, "The effects of insulin and metformin on glucose uptake in L8 myotubes", *ScienceAsia*, **2003**, *29*, 341-346.

18. B. Yu, L. A. Poirier and L. E. Nagy, "Mobilization of GLUT-4 from intracellular vesicles by insulin and K(+) depolarization in cultured H9c2 myotubes", *Am. J. Physiol.*, **1999**, 277, E259-E267.
19. N. R. Farnsworth, "Biological and phytochemical screening of plants", *J. Pharm. Sci.*, **1966**, 55, 225-276.
20. J. B. Harborne, "Phytochemical Methods", Chapman and Hall, London, **1973**.
21. K. Y. Lee, S. T. Weintraub and B. P. Yu, "Isolation and identification of a phenolic antioxidant from *Aloe barbadensis*", *Free Rad. Biol. Med.*, **2000**, 28, 261-265.
22. S. Sasson and E. Cerasi, "Substrate regulation of the glucose transport system in rat skeletal muscle", *J. Biol. Chem.*, **1986**, 261, 16827-16833.
23. S. Sasson, N. Kaiser, M. Dan-Goor, R. Oron, S. Koren, E. Wertheimer, K. Unluhizarci and E. Serasi, "Substrate autoregulation of glucose transport: Hexose 6-phosphate mediates the cellular distribution of glucose transports", *Diabetologia*, **1997**, 40, 30-39.
24. L. F. Barros, M. Young, J. Saklatvala and S. A. Baldwin, "Evidence of two mechanisms for the activation of the glucose transporter GLUT1 by anisomycin: p38 (MAP kinase) activation and protein synthesis inhibition in mammalian cells", *J. Physiol.*, **1997**, 504, 517-525.
25. G. Sweeney, R. Somwar, T. Ramlal, A. Volchuk, A. Ueyama and A. Klip, "An inhibitor of p38 mitogen-activated protein kinase prevents insulin-stimulated glucose transport but not glucose transporter translocation in 3T3-L1 adipocytes and L6 myotubes", *J. Biol. Chem.*, **1999**, 274, 10071-10078.
26. H. Yano, S. Nakanishi, K. Kimura, N. Hanai, Y. Saitoh, Y. Fukui, Y. Nonomura and Y. Matsuda, "Inhibition of histamine secretion by wortmannin through the blockade of phosphatidylinositol 3-kinase in RBL-2H3 cells", *J. Biol. Chem.*, **1993**, 268, 25846-25856.
27. A. Klip, G. Li and W. J. Logan, "Induction of sugar uptake response to insulin by serum deprivation in fusing L6 myoblasts", *Am. J. Physiol.*, **1984**, 247 [Endocrinol Metab 10], E291-E296.
28. R. Sahelian, "Banaba leaf extract", <http://www.raysahelian.com/banaba.html> (Retrieved: July 28, 2008).
29. "GlucoTrim-48™ for balancing blood sugar", <http://www.naturalways.com/glucosol.htm> (Retrieved: July 28, 2008).
30. J. E. Pessin and G. I. Bell, "Mammalian facilitative glucose transporter family: structure and molecular regulation", *Annu. Rev. Physiol.*, **1992**, 54, 911-930.
31. A. Rudich and A. Klip, "Push/pull mechanisms of GLUT4 traffic in muscle cells", *Acta. Physiol. Scand.*, **2003**, 178, 297-308.
32. T. Okada, Y. Kawano, T. Sakakibara, O. Hazeki and M. Ui, "Essential role of phosphatidylinositol 3-kinase in insulin-induced glucose transport and antilipolysis in rat adipocytes. Studies with a selective inhibitor wortmannin", *J. Biol. Chem.*, **1994**, 269, 3568-3573.
33. Y. Kaburagi, S. Satoh, H. Tamamoto, R. Yamamoto-Honda, K. Tobe, K. Veki, T. Yamauchi, E. Kono-Sugita, H. Sekihara, S. Aizawa, S. W. Cushman, Y. Akanuma, Y. Yazaki and T. Kadowaki, "Role of insulin receptor substrate-1 and pp60 in the regulation of insulin-induced glucose transport and GLUT4 translocation in primary adipocytes", *J. Biol. Chem.*, **1997**, 272, 25839-25844.
34. S. C. Frost and M. D. Lane, "Evidence for the involvement of vicinal sulfhydryl groups in insulin-activated hexose transport by 3T3-L1 adipocytes", *J. Biol. Chem.*, **1985**, 260, 2646-2652.

35. A. W. Hudson, M. Ruiz and M. J. Birnbaum, "Isoform-specific subcellular targeting of glucose transporters in mouse fibroblasts", *J. Cell Biol.*, **1992**, *116*, 785-797.
36. D. Y. Hwang and B. F. Ismail, "Stimulation of GLUT-1 glucose transporter expression in response to hyperosmolarity", *Am. J. Physiol. Cell Physiol.*, **2001**, *281*, C1365-C1372.
37. J. Z. Zhang, A. Behrooz and B. F. Ismail, "Regulation of glucose transporter by hypoxia", *Am. J. Kidney Dis.*, **1999**, *34*, 189-202.
38. N. Bashan, E. Burdett, A. Guma, R. Sargeant, L. Tumiaty, Z. Liu and A. Klip, "Mechanisms of adaptation of glucose transporters to changes in the oxidative chain of muscle and fat cells", *Am. J. Physiol.*, **1993**, *64*, C430-C440.
39. R. J. McMahan and S. C. Frost, "Nutrient control of GLUT1 processing and turnover in 3T3-L1 adipocytes", *J. Biol. Chem.*, **1995**, *270*, 12094-12099.
40. D. Szalkowski, S. White-Carrington, J. Berger and B. Zhang, "Antidiabetic thiazolidinediones block the inhibitory effect of tumor necrosis factor-alpha on differentiation, insulin-stimulated glucose uptake, and gene expression in 3T3-L1 cells", *Endocrinology*, **1995**, *136*, 1474-1481.
41. K. M. Tordjman, K. A. Leingang, D. E. James and M. M. Mueckler, "Differential regulation of two distinct glucose transporter species expressed in 3T3-L1 adipocytes: effect of chronic insulin and tolbutamide treatment", *Proc. Natl. Acad. Sci. U.S.A.*, **1989**, *86*, 7761-7765.
42. L. F. Barros, K. Barnes, J. C. Ingram, J. Castro, O. H. Porras and S. A. Baldwin, "Hyperosmotic shock induces both activation and translocation of glucose transporters in mammalian cells", *Pflügers Archiv.*, **2001**, *442*, 614-621.
43. J. Ross, "mRNA stability in mammalian cells", *Microbiol. Rev.*, **1995**, *59*, 423-450.
44. R. J. Sargeant and M. R. Paquet, "Effect of insulin on the rates of synthesis and degradation of GLUT1 and GLUT4 glucose transporters in 3T3-L1 adipocytes", *Biochem. J.*, **1993**, *290*, 913-919.
45. A. Krook, H. Wallberg-Henriksson and J. R. Zierath, "Sending the signal: Molecular mechanisms regulating glucose uptake", *Med. Sci. Sports Exercise*, **2004**, *36*, 1212-1217.
46. M. Fukushima, F. Matsuyama, N. Ueda, K. Egawa, J. Takemoto, Y. Kajimoto, N. Yonaha, T. Miura, T. Kaneko, Y. Nishi, R. Mitsui, Y. Fujita, Y. Yamada and Y. Seino, "Effect of corosolic acid on postchallenge plasma glucose levels", *Diabetes Res. Clin. Pract.*, **2006**, *73*, 174-177.
47. X. Wen, J. Xia, K. Cheng, L. Zhang, P. Zhang, J. Liu, L. Zhang, P. Ni and H. Sun, "Pentacyclic triterpenes. Part 5: Synthesis and SAR study of corosolic acid derivatives as inhibitors of glycogen phosphorylases", *Bioorg. Med. Chem. Lett.*, **2007**, *17*, 5777-5782.
48. K. Hattori, N. Sukenobu, T. Sasaki, S. Takasuga, T. Hayashi, R. Kasai, K. Yamasaki and O. Hazeki, "Activation of insulin receptors by lagerstroemin", *J. Pharmacol. Sci.*, **2003**, *93*, 69-73.