

Article

Effects of Sweet Potato Leaf Extracts and Chlorogenic Acid on Glucose Uptake in C2C12 Cells

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Abstract: Edible sweet potato leaf can be exploited in the management and treatment of insulin resistance. This study investigated the effects of three sweet potato leaf extracts (SPLEs) and chlorogenic acid (CGA) on glucose uptake (2-NBDG uptake and GLUT4 abundance) and expression of their related regulatory factors (such as IR, IRS-1, p-Akt1, and p-AMPK α 1 abundances) using Western blot analysis in insulin-treated insulin-resistant C2C12 cells. The results show that both purple and green SPLEs improved glucose (2-NBDG) uptake efficacy with insulin treatments, and both SPLEs also increased GLUT4 and IR abundances via activation of p-Akt in the PI3K/Akt pathway, whereas the IR abundance efficacy influence was the same as in the insulin-treated group. The yellow SPLE and CGA have higher protein abundances of IR and IRS-1, while CGA (20 μ g/mL) exhibits the highest abundance of p-Akt1 and p-AMPK α 1. These results suggest potential benefits of purple and green SPLEs in promoting glucose uptake, possibly through modulation of insulin signaling pathways.

Keywords: sweet potato leaf; insulin resistance; chlorogenic acid; 2-NBDG; GLUT4; PI3K/Akt pathway; type 2 diabetes



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1. Introduction

Sweet potato leaves (*Ipomoea batatas* L., SPLs) contain high nutritional value in minerals, vitamins, soluble fiber, and proteins [1,2]. Studies on the functional compounds of SPLs show that their health promotion is related to a high content of polyphenols, flavonoids, and carotenoids [3–7]. These compounds exhibit liver protection [8] and antioxidant [9,10], anticancer [11], and anti-mutagenic activity [12]. SPLs are also rich in chlorogenic acids (CGAs), which can be used for health promotion, including preventing cancer [13], high blood pressure [14], bacterial growth promotion [15], diabetes mellitus [16], and heart disease [17]. Furthermore, two components of SPLs (cyanidin and quercetin) have shown anti-inflammatory effects through reduced mitogen-activated protein kinase (MAPK), nuclear factor-kappa B (NF κ B), and extracellular signal-regulated kinase 1 and 2 (ERK1 and ERK2) expressions [18]. In type 2 diabetes, phosphorylation level of Akt1 is reduced due to impaired insulin signaling, resulting in a reduced cellular response to insulin, which prevents glucose from entering cells smoothly and causes a hyperglycemic state [19]. Phospho-protein kinase (p-Akt1) is an important step in initiating the transfer of the

glucose transporter 4 (GLUT4) glucose transporter to the cell membrane, promoting glucose entry into the cell. As p-Akt1 level decreases, GLUT4 function is inhibited and cellular glucose uptake is reduced, further exacerbating hyperglycemia. Independently of insulin, p-AMPK α 1 increases GLUT4 transport in muscle and other peripheral tissues to promote glucose uptake. Therefore, activation of AMP-activated protein kinase (AMPK) is often considered as a potential strategy to treat diabetes, especially when insulin signaling is impaired. Um et al. [20] observed that mice deficient in AMPK α 1 or α 2 display the metabolic effects of resveratrol (a polyphenolic compound) mediated by AMPK. In addition, Cho et al. [19] reported that mice deficient in Akt2 are impaired in the ability of insulin to lower blood glucose because of defects in the action of the hormone on liver and skeletal muscle; thereby, *Akt2* is an essential gene in the maintenance of normal glucose homeostasis.

Myoblast cell line C2C12 has been widely used to study the effects of various drugs on glucose transport [21]. Wong et al. [22] further reported that C2C12 is a well-documented model that can facilitate our understanding of glucose metabolism, insulin signaling, insulin resistance, oxidative stress, reactive oxygen species, and glucose transporters at cellular and molecular levels. Del Aguila et al. [23] described how TNF- α treatment of muscle cell C2C12 can impair the ability of insulin-stimulated insulin receptor substrates 1 and 2 (IRS-1 and IRS-2) in order to regulate phosphate idylinositol3-kinase (PI3K) activation, decrease insulin-regulated IRS-1 tyrosine phosphorylation, inhibit the phosphorylation of p42MAPK (ERK 2) and p44MAPK (ERK 1) tyrosines, and develop an *in vitro* cellular model of insulin resistance. Tzeng et al. [24] illustrated that interleukin-6 (IL-6) treatment of C2C12 can reduce insulin receptor (IR), phospho-insulin receptor (p-IR), IRS-1, phospho-insulin receptor substrate 1 (p-IRS-1), PI3K, p-Akt B, and GLUT4 expressions. Therefore, the C2C12 model can be used to explore new therapeutic compounds or natural herbal medicines in glucose transporters and target sites of genes and signaling pathways [22].

Several studies have shown that myricetin [25], anthocyanins [26], dietary fiber [27], caffeic acid derivatives [28], CGAs [29], and caffeoylquinic acid derivatives [30] have anti-diabetic effects. Those caffeic acid and quinic acid derivatives are the main phenolic components of SPLs [2,31–34]. In humans, CGAs in SPLs can reduce the release of glucose into the blood [29], lower the glycemic index of diabetic patients, and reduce the risk of type 2 diabetes [16]. In addition, polyphenolic contents of 116 SPL lines or genotypes cultivated in China have shown anti-diabetic activity [30]. Olowu et al. [35] demonstrated that sweet potato leaf extracts (SPLEs) can reduce the blood glucose concentration of healthy and diabetic rats by stimulating the secretion of glucagon-like peptide-1 (GLP-1) to combat hyperglycemic activity. Moreover, it has been found that caffeoylquinic acids (CQAs) and anthocyanins of SPLs are key to lowering blood sugar [26]. Zhang et al. [30] showed that phenethyl cinnamides and 3,4,5-tri-*O*-caffeoylquinic acid (3,4,5-tri-CQA) components in SPLs have strong inhibitory effects on α -glucosidase.

Previously, we reported that purple (CYY 98-59, nick-shaped leaves), yellow (Asian vegetable CN1927-16), and green (Tainong No. 64, heart-shaped leaves) SPLs significantly increased both glucose uptake and glucose transporter 2 (GLUT2) activity in insulin-resistant FL83B hepatocytes compared with the TNF- α treatment group [36]. In the present study, we utilized the myoblast cell line C2C12 as a study model to investigate the potential anti-diabetic biological activity of SPLs. Our analysis focused on their effects on glucose uptake, related protein expressions, including glucose transporter 4 (GLUT4), insulin receptor (IR), and insulin receptor substrate-1 (IRS-1), and signaling pathway molecules (p-Akt1 and p-AMPK α 1).

2. Materials and Methods

2.1. Materials and Reagents

Leaves of three varieties of sweet potato (*Ipomoea batatas* L.; purple CYY 98-59, green Taoyuan 2, and yellow CN 1927-16) were provided by Dr. Yung-Chang Lai, Head of the Department of Agronomy, Chiayi Agricultural Experiment Station, Chiayi, Taiwan. Mouse muscle myoblast C2C12 cell line (derived from ATCC; CRL-1772) was purchased from

the Bioresource Collection and Research Center (BCRC) of the Institute of Food Industry, Hsinchu City, Taiwan. Chlorogenic acid (CGA $\geq 5\%$ titration) was purchased from ChromaDex (Irvine, CA, USA). Dulbecco's modified Eagle's medium (DMEM), penicillin-streptomycin-glutamine, 0.25% trypsin-EDTA, fetal bovine serum (FBS), horse serum (HS; heat inactivated, New Zealand origin), and (2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxy-D-glucose; 2-NBDG) were obtained from Gibco-Thermo Fisher, Waltham, MA, USA). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and β -actin antibody were purchased from Supelco Sigma-Aldrich (St. Louis, MO, USA). The antibodies of anti-IR, anti-IRS1, anti-GLUT4, and mouse IgG HRP-linked whole antibody were purchased from MERCK (Rahway, NJ, USA). Anti-phosphorylated-Akt1/PKBa (Thr308), Anti-phosphorylated-AMPK α 1 (Thr172), and goat anti-mouse secondary antibody conjugated to HRP were purchased from Thermo Fisher (Waltham, MA, USA).

2.2. Sweet Potato Leaf Extract Preparation

After obtaining SPLs, samples were lyophilized using a Freeze Dryer (FD-5060, Panchum Scientific Corp., Taipei, Taiwan), ground, and then stored at $-80\text{ }^{\circ}\text{C}$ until further use. An extract was produced from 0.5 g of stored SPL powder with a 10-fold volume of methanol at room temperature and vortexed for 30 min, then filtered through Whatman #1 filter paper (Sigma-Aldrich, St. Louis, MO, USA). The remaining residue was re-extracted twice until colorless. The three extracts were combined, concentrated to a powder by Freeze Dryer, and stored at $-80\text{ }^{\circ}\text{C}$ until further use. SPLE powder was re-dissolved with 5 mL of ethanol and filtered through a $0.22\text{ }\mu\text{m}$ nylon filter, then diluted (25-fold) with HS-free DMEM to reach the prepared treatment concentrations (4 mg/mL), followed by adding 100 μL (final concentration 400 $\mu\text{g}/\text{mL}$) to the incubation medium to treat with C2C12 cells.

2.3. Cell Culture, Differentiation Culture, and MTT Assay

After thawing the C2C12 cell line, cells were fixed and sub-cultured every two to three days (d). Cells were cultured in high-glucose (25 mM) DMEM containing 10% FBS in an incubator at $37\text{ }^{\circ}\text{C}$ and 5% CO_2 . When cells reached about 80% confluence, the medium was removed, and the cells were washed twice with PBS. One-and-a-half mL of 0.25% trypsin-EDTA was added to the cells and incubated at $37\text{ }^{\circ}\text{C}$ for 2 min to remove the cells, and then 4.5 mL of DMEM containing 10% FBS was added to stop the trypsin reaction. The cell suspension was diluted with 10x Trypan blue solution for staining, and cells were counted under an inverted microscope (Eclipse TE2000-U, Tokyo, Japan) using a cell counting plate. Plates were divided according to the number of cells and seeded into new culture plates at a density of 1×10^5 cells/mL for sub-culturing. When the cell density reached about 80% confluence in 2 to 3 d, the medium was aspirated, and cells were washed twice with PBS. High-glucose (25 mM) DMEM containing 2% HS differentiation medium was changed in the differentiation medium every 2 d. After 4–6 d, differentiation was completed [37].

The number of cells to $1 \times 10^5/\text{mL}$ was adjusted in a 24-well plate after the differentiation culture was completed. After removing the culture solution, various concentrations of SPLE (200 and 400 $\mu\text{g}/\text{mL}$) and CGA (10, 20, 40, and 80 $\mu\text{g}/\text{mL}$) in HS-free DMEM were prepared and placed in an incubator at $37\text{ }^{\circ}\text{C}$ and 5% CO_2 for 24 h. The culture medium was removed from the incubator, and 0.4 mg/mL of MTT (Sigma-Aldrich) in HS-free DMEM medium was then added to each well, followed by a second incubation for 2 h at $37\text{ }^{\circ}\text{C}$ protected from light. After draining, cells were again washed with PBS, dissolved in 500 μL of dimethyl sulfoxide (DMSO) for 15 min, and monitored for absorbance at 570 nm using a Varioskan Flash Spectral Scanning Multimode Reader (Thermo Fisher) for cell viability. Cell viability (%) = $[(A_{570} \text{ of the extract treated group} - A_{570} \text{ of blank}) / (A_{570} \text{ of the control group} - A_{570} \text{ of blank})] \times 100\text{ }(\%)$ [38].

2.4. Cellular Glucose Uptake

The use of 2-NBDG, a fluorescent deoxy-glucose analog, was transported by a glucose transporter intracellularly. NBDG fluorescence typically shows maximum excitation/emission at approximately 465~540 nm, which can be visualized using filters specific for luciferin [39]. Differentiated C2C12 cells were cultured in DMEM containing 2% HS with high-glucose (25 mM) DMEM for 4–6 days [40], followed by washing twice with PBS. SPLEs at a final concentration of 400 µg/mL and CGA (10 and 20 µg/mL) in HS-free DMEM were prepared and then added to HS-free low-glucose (5.5 mM) DMEM (maintenance medium) supplemented with 1% BSA, treated overnight for 18 h, and washed twice with PBS. The medium was replaced with glucose-free DMEM without HS. Moreover, 150 nM insulin (Sigma-Aldrich) and 100 µM 2-NBDG were added to the medium, and cells were then incubated at 37 °C and 5% CO₂ for 30 min in the dark. After washing with cold PBS three times, a fluorescence inverted microscope was used for an exposure time of 57 µs/750 ms to obtain a fluorescent image using a SGHD-3600 HDMI high-pixel microscope system (SAGE VISION CO., LTD., Taipei, Taiwan), followed by analysis of fluorescence intensity [40].

2.5. Western Blot Analysis (WB) for GLUT4, IR, IRS-1, p-Akt1, and p-AMPKα1 Abundances

C2C12 cells were cultured for 2–3 days and then divided into 100 mm culture dishes at a cell density of 1×10^5 /mL. After culturing to about 80% confluence, the medium was changed to DMEM containing 2% HS for differentiation. The medium was changed every 2 d, and the cells cultured for 4–6 days. After differentiation, the medium was removed, and the cells were washed twice with PBS. SPLEs (at a final concentration of 400 µg/mL) and CGA (at a final concentration of 10 and 20 µg/mL) were prepared in HS-free DMEM and then added to HS-free low-glucose (5.5 mM) DMEM supplemented with 1% BSA. The cells were cultured at 37 °C with 5% CO₂ for 18 h. Afterward, cells were washed twice with PBS, and the latter was then replaced with a serum- and glucose-free medium to a final concentration of 150 nM insulin and cultured for 2 h [41,42].

Cells were treated as indicated, detached, thoroughly washed with PBS, and then lysed in ice-cold lysis buffer containing 20 mM Tris, 5 mM EDTA, 10 mM Na₃PO₄, 100 mM NaF, 1% Nonidet P-40, and 1 mM PMSF at pH 7.5. Following centrifugation at 13,000× g for 10 min at 4 °C, the supernatant (20–30 mg protein) was boiled with reducing sample buffer for 5 min, subjected to electrophoresis in sodium dodecyl sulfate (SDS)-polyacrylamide gels (Bio-Rad Laboratories, Hercules, CA, USA), and then transferred onto a polyvinylidene difluoride membrane (PVDF, Bio-Rad Laboratories) [43]. Membranes were blocked in TBST (10 mM Tris-HCl, 150 mM NaCl, and 0.1% Tween-20, at pH 7.4) containing 5% nonfat milk and then incubated with a specific primary antibody (GLUT4, IR, IRS-1, p-Akt1, and p-AMPKα1, obtained from MERCK and Thermo Fisher) and a secondary antibody. Finally, proteins were visualized using a goat anti-mouse secondary antibody conjugated to HRP (for GLUT4, IR, and IRS-1 from Thermo Fisher) and mouse IgG HRP-linked whole Ab (for p-Akt1 and p-AMPKα1 from MERCK) and detected by a chemiluminescence detection system (MultiGel-21-C2-1M, TOP BIO CO., New Taipei City, Taiwan).

2.6. Statistical Analysis

All the data are expressed as mean ± standard deviation, and statistical significance is analyzed using one-way analysis of variance (ANOVA) followed by Duncan's test at a 0.05 significance level using SAS version 9.4 (SAS Institute, Cary, NC, USA).

3. Results

3.1. Effect of SPLEs and CGA on C2C12 Cell Viability

The effects of SPLEs at two concentrations (200 and 400 µg/mL) on the viability of C2C12 treated with HS-free DMEM were studied, and >92.4% of cells remained viable at both treatment concentrations (Figure 1A). Cell viability of CGA at 10, 20, 40, and 80 µg/mL

ranged from 90.1% to 97.0% (Figure 1B); therefore, we chose both 10 and 20 $\mu\text{g/mL}$ of CGA and 400 $\mu\text{g/mL}$ of SPLEs for further study.

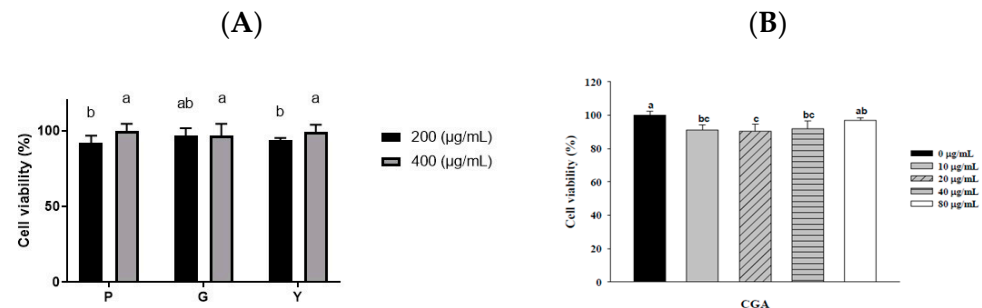


Figure 1. Percentage viability of C2C12 cells treated with different concentrations of (A) SPLEs and (B) CGA. Treatment groups in (A) were P, purple SPLE; G, green SPLE; Y, yellow SPLE, at 200 and 400 $\mu\text{g/mL}$ concentrations; in (B) were CGA at 0, 10, 20, 40, and 80 $\mu\text{g/mL}$ concentrations. Different letters denote statistically significant differences between results ($p < 0.05$). Bars indicate standard deviation with three repetitions.

3.2. Effects of SPLEs, CGA, and Insulin on 2-NBDG Uptake in C2C12 Cells

Differentiated mouse skeletal muscle C2C12 cells were used to assess 2-NBDG uptake in response to insulin (150 nM), SPLEs (400 $\mu\text{g/mL}$) and CGA (10 and 20 $\mu\text{g/mL}$), with all treatments compared to control group (C) without any treatment. Figure 2A displays the fluorescent image of 2-NBDG intake with insulin, SPLE, and CGA treatments. Figure 2B illustrates fluorescent intensity counts when adding 150 nM insulin (I) compared to the control (C) group. It also shows the effects of SPLEs and CGA on 2-NBDG uptake by C2C12 fluorescent intensity data showing that cells with 150 nM insulin treatment had better 2-NBDG uptake than cells without insulin treatment in the control group. Purple SPLE (P) exhibited significantly higher values ($p < 0.05$) compared to other treatment groups, with green SPLE (G) following closely behind. Yellow SPLE (Y) and CGA (CGA10 and CGA20) treatments showed no significant differences compared to the insulin-treated group. Remarkably, compared to the insulin-treated group, the intensity of 2-NBDG uptake significantly increased by 2.2 and 1.3 times in purple and green SPLEs, respectively. Consequently, both purple and green SPLEs exhibited superior abilities in 2-NBDG uptake.

3.3. Abundances of GLUT4, IR, IRS-1, p-Akt1, and p-AMPK α 1 in SPLEs and CGA Treatments on C2C12 Cells

The GLUT4 protein is highly expressed in C2C12 cells, which can be used in the investigation of natural-compound-inducible responses of insulin signaling pathway and glucose uptake mechanisms [22–24]. Figure 3 shows all SPLE treatments (P, G, and Y) had significantly higher GLUT4 abundances than the control (C), insulin (I), and CGA (10 & 20) groups.

The interaction between the insulin receptor (IR) and insulin receptor substrate 1 (IRS-1) constitutes a pivotal signaling cascade in insulin-mediated cellular responses. Figure 4A indicates that the IR abundances in C2C12 cells for all treatments were significantly higher than those in the control (C) group. Additionally, the IR abundances of the yellow SPLE (Y) and low-dose CGA (CGA10) treatment groups were significantly higher than those of the control, insulin, and green SPLE (G) treatments. Figure 4B also demonstrates that C2C12 cells treated with yellow SPLE (Y) and low-dose CGA exhibit consistent results in increasing the abundance of IRS1, similar to the abundance of IR, indicating yellow SPLE and CGA may promote glucose uptake by increasing the abundance of IR and IRS1, thereby influencing glucose metabolism and regulation.

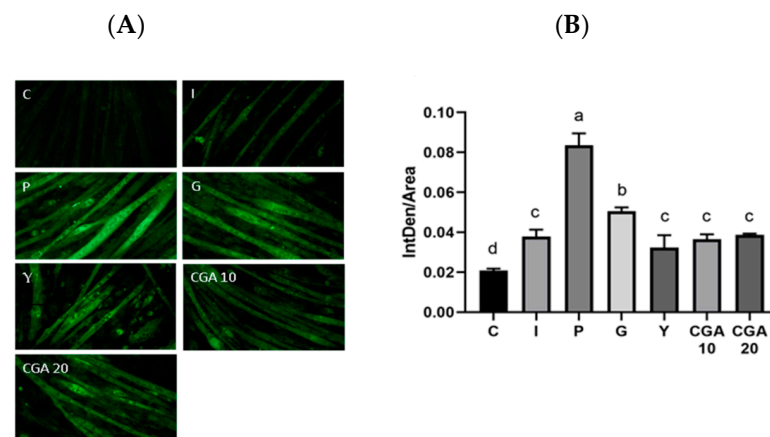


Figure 2. Effects of SPLEs, CGA, and the addition of 150 nM insulin on the uptake of 2-NBDG by C2C12. SPLEs (400 µg/mL) and CGA (10 and 20 µg/mL) were treatments. A fluorescence inverted microscope was used for an exposure time of 57 µs/750 ms to obtain a fluorescent image (A) (SAGE vision SGHD-3600 HDMI high-pixel microscope system), followed by analysis of fluorescence intensity (B). More detailed explanations of cellular glucose uptake are described in Section 3.2. C, control, cells without any treatment; I, containing 150 nM insulin; P, purple SPLE containing 150 nM insulin; G, green SPLE containing 150 nM insulin; Y, yellow SPLE containing 150 nM insulin; CGA10, 10 µg/mL CGA containing 150 nM insulin; CGA20, 20 µg/mL CGA containing 150 nM insulin. Data are expressed as mean ± SD with three repetitions. Different small letters in (B) indicate significant differences among treatments and the control ($p < 0.05$).

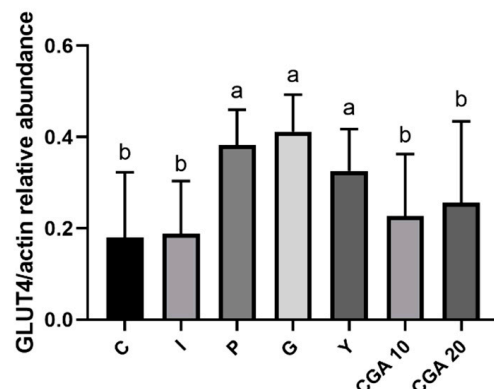


Figure 3. Analysis of GLUT4 abundance of SPLEs and CGA on C2C12 cells. SPLEs (400 µg/mL) and CGA (10 and 20 µg/mL). More detailed explanations of GLUT4 abundance in SPLEs and CGA treatments on C2C12 cells are described in Section 3.3. C, control, cells without any treatment; I, 150 nM insulin treated only; P, purple SPLE containing 150 nM insulin; G, green SPLE containing 150 nM insulin; Y, yellow SPLE containing 150 nM insulin; CGA10, 10 µg/mL CGA containing 150 nM insulin; CGA20, 20 µg/mL CGA containing 150 nM insulin. Data are expressed as mean ± SD with three repetitions. Different small letters indicate significant differences among treatments and the control ($p < 0.05$).

The Akt signaling pathway primarily participates in insulin-mediated metabolic regulation, while AMPK serves as a critical mechanism for adjusting energy balance and metabolic adaptation during cellular energy depletion, both of which are therapeutic targets for diabetes. In Figure 5A, the abundance of p-Akt1 in C2C12 cells for all sample treatments was significantly higher compared to both the control (C) and insulin (I) groups. Among them, the abundance of p-Akt1 in the CGA20 treatment was significantly the highest. In Figure 5B, the expressions of p-AMPKα1 in CGA20 and yellow SPLE (Y) treatments were significantly higher than in other treatments, followed by CGA10 and green SPLE (G).

However, no significant differences were observed among the purple SPLE (P), insulin-only (I), and control (C) groups.

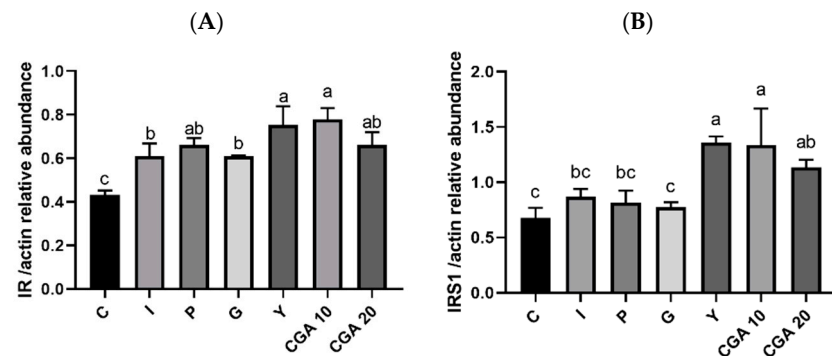


Figure 4. Analysis of (A) IR and (B) IRS1 abundances of SPLEs and CGA on C2C12 cells. SPLEs (400 µg/mL) and CGA (10 and 20 µg/mL). C, control, cells without any treatment; I, 150 nM insulin treated only; P, purple SPLE containing 150 nM insulin; G, green SPLE containing 150 nM insulin; Y, yellow SPLE containing 150 nM insulin; CGA10, 10 µg/mL CGA containing 150 nM insulin; CGA20, 20 µg/mL CGA containing 150 nM insulin. Data are expressed as mean ± SD with three repetitions. Different small letters indicate significant differences among treatments and the control ($p < 0.05$).

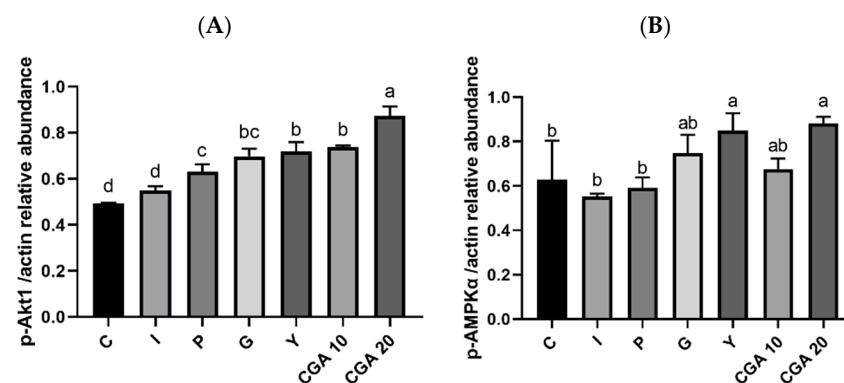


Figure 5. Analysis of p-Akt1 (A) and p-AMPK (B) abundances of SPLEs and CGA on C2C12 cells. SPLEs at a final concentration of 400 µg/mL and CGA (10 and 20 µg/mL). C, control, cells without any treatment; I, 150 nM insulin treated only; P, purple SPLE containing 150 nM insulin; G, green SPLE containing 150 nM insulin; Y, yellow SPLE containing 150 nM insulin; CGA10, 10 µg/mL CGA containing 150 nM insulin; CGA20, 20 µg/mL CGA containing 150 nM insulin. Data are expressed as mean ± SD with three repetitions. Different small letters indicate significant differences among treatments and the control ($p < 0.05$).

4. Discussion

Both purple and green SPLEs improved 2-NBDG uptake efficacy with insulin treatments and also increased GLUT4 and IR expressions via activation of p-Akt in the PI3K/Akt pathway. However, the IR expression efficacy influence was the same as in the insulin-treated group. The yellow SPLE and CGA have higher protein expressions of IR and IRS-1, while CGA (20 µg/mL) exhibits the highest expression of p-Akt1 and p-AMPKα1. GLUT4 is the insulin-regulated glucose transporter found primarily in adipose tissues and skeletal muscle, and the glucose uptake by GLUT4 involves the translocation of GLUT4-containing vesicles to the plasma membrane. Chen et al. [44] stated that HepG2 cells treated with CGA-containing extracts might enhance their glucose uptake by increasing GLUT4 expression and translocation. IR is a heterotetrameric membrane glycoprotein consisting of two α-subunits and two β-subunits. Insulin binds to the α-subunit to activate the β-subunits by the auto-phosphorylation of specific tyrosine residues [45]. Insulin-bound IR further promotes the binding and activation of the IRS, which plays an important role in faci-

tating insulin-induced GLUT translocation. Insulin signaling is also highly dependent on the PI3K/Akt pathways for insulin regulation. IRS-1 transmits insulin signaling through both metabolic and mitotic pathways. It becomes phosphorylated on serine and tyrosine residues, with these phosphorylated tyrosines serving as docking sites for PI3K via their SH2 domains, thereby activating IRS [46]. Glucose uptake involves GLUT-4 translocation through a mechanism that is likely to involve the upstream effectors of the PI3-K/Akt pathway [21]. Akt1, also known as protein kinase B (PKB) or RAS- α , plays an important role in a variety of biological reactions by phosphorylating a variety of proteins, such as regulating metabolism, cell survival, and growth. Kinase is activated by insulin, PI3K, and various other growth and survival factors [47]. As a member of the insulin signaling pathway, it is required to induce glucose transport [48].

Previously, we compared the glucose uptake capacity of insulin-resistant cells with SPLEs after different drying treatments [36]. SPLEs had an improvement effect on the amelioration ratio of insulin-resistant cells and respectively improved glucose uptake capacity based on data conversion of the cyto-fluorescence value. In our study, the intake of 2-NBDG resulted in remarkably high GLUT4 activity in purple (P) and green (G) SPLEs, while the protein expressions of IR were significantly higher compared to the control and similar to those observed with insulin (I) treatments. Shyur et al. [49] reported that the expression of the p-Akt1 protein was significantly reduced in C2C12 cells treated with TNF- α for 24 h. Conversely, the expression of p-Akt1 significantly increased dose-dependently when treated with various concentrations (12.5–200 $\mu\text{g/mL}$) of sweet potato tuber extracts. Furthermore, GLUT4 protein expression was up-regulated with increasing tuber extract concentrations from Simon No. 1 sweet potato, demonstrating that phenolic compounds in sweet potato activated the PI3K/Akt pathway and increased GLUT4 translocation. Luo et al. [50] showed sweet potato leaf polyphenols (SPLPs) decreased fasting blood glucose levels (by 64.78%) and improved oral glucose tolerance of diabetic mice fed with a high-fat diet compared with those of untreated diabetic mice. Furthermore, SPLP not only could up-regulate the PI3K/AKT/GSK-3 β signaling pathway to improve glucose metabolism, but also up-regulate the phosphatidylinositol PI3K/AKT/GLUT-4 signaling pathway in the skeletal muscle to enhance glucose transport. In our study, the uptake of 2-NBDG in purple (P) and green (G) SPLEs led to significantly increased protein abundance of p-Akt1 compared to both control and insulin treatment. This finding could potentially explain the up-regulation of GLUT4 abundance in C2C12, which was activated by insulin signaling pathways and may be regulated by these two varieties of SPLEs.

AMPK is a metabolic and stress-sensing kinase crucial for maintaining homeostasis and is a key therapeutic target in the treatment of type 2 diabetes and obesity. AMPK is phosphorylated by upstream kinases such as AMPK kinase (AMPKK) and liver kinase B1 (LKB1), which leads to its activation [51]. Hesham et al. [52] revealed that the extract of rosemary leaves (*Rosmarinus officinalis* L.) could improve the insulin-stimulated glucose uptake in L6 rat muscle cells and increase the acute insulin-stimulated tyrosine phosphorylation while reducing the high-glucose- and high-insulin-induced serine phosphorylation of IRS-1 and the phosphorylation of the mechanistic target of rapamycin and p70S6 kinase. In addition, rosemary leaf extracts could also increase the activation of AMPK, acetyl-CoA carboxylase, and plasma membrane GLUT4 levels. Ong et al. [53] reported that CGA has been shown to increase AMPK phosphorylation, promote glucose transport, and enhance AMPK expression in a time- and dose-dependent manner. They also demonstrated that in L6 skeletal muscle cells, glucose transport induced by CGA increased in a dose-dependent manner (0.5–10 mM), along with CGA-mediated promotion of AMPK phosphorylation and expression. Some studies have also demonstrated that phenolic compounds such as catechin, chlorogenic acid, and proanthocyanidin inhibit insulin resistance by activating AMPK signaling [54,55]. The ethanolic fraction of purple sweet potato leaf dose at 200 mg/Kg body weight of the alloxan-induced diabetic rats displayed a greater effect on the reduction of blood glucose levels and better pancreatic histopathological features than hexane and ethyl acetate fractions [56]. In our study, both yellow SPLE and CGA exhibited

only a similar enhancement of 2-NBDG uptake activity as the insulin-treated group. This indicates that yellow SPLE and CGA did not exhibit synergistic effects with insulin in regulating blood sugar homeostasis. The noteworthy point is that both yellow SPLE and CGA achieve the promotion of 2-NBDG uptake by increasing the abundance of IR or IRS-1 and by positively regulating the phosphorylation of Akt1 and AMPK α 1. Active AMPK regulates metabolism by phosphorylating enzymes involved in metabolic pathways and controlling gene expression that regulates blood sugar levels [50,53–55]. The high content of di-CQAs derivatives (di-CQAs and 3,4,5-tri-CQA) in yellow SPLE could explain its activity in improving 2-NBDG intake [57]. In particular, 3,4,5-tri-CQA (10 mM) stimulated GLP-1 (glucagon-like peptide-1) secretion in GLUTag cells, which was accompanied by an increase in insulin secretion in rats, thereby promoting the reduction of hyperglycemia [26]. However, this requires further verification.

5. Conclusions

This study concluded that edible sweet potato leaves (SPLEs) improve the uptake of 2-NBDG, with the improving ability being superior in purple leaves (P) compared to green leaves (G), and finally, yellow leaves (Y). Both purple leaf (P) and green leaf (G) SPLEs increased GLUT4 abundance via activation of IR and p-Akt in the PI3K/Akt pathway, whereas the IR abundance efficacy influence was higher than only the insulin-treated group. Alternatively, yellow-SPLE-affected GLUT4 abundance might be through regulation by upstream pathway factors, such as IR, IRS-1, p-Akt1, and p-AMPK α 1, without affecting 2-NBDG uptake. Both CGA10 and CGA20 affected upstream pathway factors, such as IR, IRS-1, and p-AMPK α 1, under the treatment of insulin without affecting GLUT4 abundance or 2-NBDG uptake. Further research could be performed based on the mechanisms of the cellular uptake of glucose, which could be used as a reference for reducing blood sugar in type 2 diabetes.

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Abbreviations

Chlorogenic acid, CGA; Mouse myoblast cell line, C2C12; 2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxy-D-glucose, 2-NBDG; glucose transporter 4, GLUT4; insulin receptor, IR; insulin receptor substrate 1, IRS-1; phospho-protein kinase B, p-Akt1; AMP-activated protein kinase, AMPK; phospho-AMP-activated protein kinase α 1, p-AMPK α 1; phosphatidylinositol 3-kinase, PI3K.

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