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Original Article

Curculigoside and polyphenol-rich ethyl acetate fraction of *Molineria latifolia* rhizome improved glucose uptake via potential mTOR/AKT activated GLUT4 translocation

Der Jiun Ooi [a], *Nur Hanisah Azmi* [a], *Mustapha Umar Imam* [a,1], *Noorjahan Banu Alitheen* [b], *Maznah Ismail* [a,*]

[a] *Nutri-Cosmeceuticals, Nutrigenomics & Nanodelivery Programme, Laboratory of Molecular Biomedicine, Institute of Bioscience, Universiti Putra Malaysia, 43400, Serdang, Selangor, Malaysia*

[b] *Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400, Serdang, Selangor, Malaysia*

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ABSTRACT

Adipose tissue is one of the major organs responsible for rapid restoration of postprandial glucose fluxes. Being the major isoform of glucose transporter in adipose tissue, regulations of insulin-dependent GLUT4 trafficking have always been of research interest. The present study aimed to examine the molecular mechanisms underlying the efficacy of curculigoside and polyphenol-rich ethyl acetate fraction (EAF) of *Molineria latifolia* rhizome in triggering glucose uptake. We assessed the adipogenic potential and glucose uptake stimulatory activity of curculigoside and EAF by employing a murine 3T3-L1 adipocyte model. The transcriptional and translational expressions of selected intermediates in the insulin signalling pathway were evaluated. While curculigoside neither promoted adipogenesis nor activated peroxisome proliferator activated receptor gamma, treatment with polyphenol-rich EAF resulted otherwise. However, both treatments enhanced insulin-stimulated uptake of glucose. This was coupled with increased availability of GLUT4 at the plasma membrane of the differentiated adipocytes although the total GLUT4 protein level was unaffected. In addition, the treatment increased the phosphorylation of both AKT and mTOR, which have been reported to be associated with GLUT4 translocation. The present findings proposed that curculigoside and EAF increased glucose transport activity of 3T3-L1 adipocytes via GLUT4 translocation as a result of potential mTOR/AKT activation. The more potent efficacy observed with EAF suggested potential synergistic and multi-targeted action.

Abbreviations: EAF, ethyl acetate fraction.

* *Corresponding author*. Fax: +603 8947 2116.
E-mail addresses: myhome.e@gmail.com, maznahis@upm.edu.my (M. Ismail).
[1] Present address. Department of Medical Biochemistry, Usmanu Danfodiyo University, P.M.B. 2346, Sokoto, Nigeria.

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1. Introduction

Solute carrier family 2, facilitated glucose transporter member 4 (SLC2A4) or commonly known as GLUT4, is a member of the sugar transporter proteins which catalyses the ATP-independent trans-membrane transport of hexose sugar. It is also the major isoform of glucose transporter expressed in adipose tissue. At the basal state, GLUT4 predominantly resides in the intracellular vesicular compartment. In the presence of insulin, the exocytic rate of GLUT4 vesicle increases, leading to translocation of the protein to the plasma membrane [1]. An increased availability of the transporter protein on the cell surface thus enhances uptake of glucose.

Insulin resistance is a condition whereby normal insulin level is ineffective in triggering desired response in glucose disposal organs such as adipose and skeletal muscle tissues. Diminished glucose uptake by these cells leads to impaired glucose homeostasis, elevated blood glucose level and ultimately, diabetes mellitus [2]. The use of insulin sensitising agents such as thiazolidinediones to antagonise insulin resistance has thus become one of the treatment options for type 2 diabetes. Thiazolidinediones function as agonists for peroxisome proliferator activated receptor gamma (PPARγ) and promote adipocyte differentiation [3]. Despite their potent anti-hyperglycaemic action, thiazolidinediones have restricted usage in the clinical management of diabetes owing to the occurrence of several side effects, including weight gain, bone fracture, congestive heart failure and potential bladder cancer [4,5].

Therefore, identification of novel bioactive compounds as a safer alternative to increase insulin sensitivity has always been of research interest. Curculigoside, a type of phenolic glycoside, has been reported to exhibit anti-oxidative, neuroprotective and anti-osteoporotic capabilities [6–9]. The compound, previously isolated from the rhizome of *Curculigo orchioides* Gaertn. [10], is also identified in *Molineria latifolia* (Dryand. ex W.T.Aiton) Herb. ex Kurz [11]. Both plants are highly related species and belong to the Hypoxidaceae family. The extract of *M. latifolia* had been reported to exhibit antidiabetic and hypolipidemic activities *in vivo* [12], potentially due to the presence of curculigoside.

Owing to particular interest in the use of polyphenols as pharmaceutical alternatives, the development and formulation of polyphenol-rich ethyl acetate fraction (EAF) from *M. latifolia* constitutes effort to maximise the potential of polyphenols and to improve quality assurance [13]. In the present study, both polyphenol-rich EAF and curculigoside (one of the major compounds present in EAF) were evaluated for their cytotoxic effects and abilities to induce the process of adipogenesis in 3T3-L1 preadipocytes. Additionally, the efficacies of curculigoside and EAF in triggering glucose uptake in differentiated 3T3-L1 cells were also examined using 2-NBDG glucose analogue. Transcriptional and translational levels of selected intermediates participating in the insulin signalling pathway were also studied to understand the underlying molecular mechanism of action.

2. Materials and methods

2.1. Chemicals

3T3-L1 mouse fibroblast was obtained from American Type Culture Collection (Manassas, VA, USA). Curculigoside was purchased from Biopurify Phytochemicals (Sichuan, China). Dulbecco's modified eagle medium (DMEM), methylthiazolyldiphenyl-tetrazolium bromide (MTT), protease and phosphatase inhibitor cocktails, NP-40, BCA protein assay kit and Chemi-Lumi One L were purchased from Nacalai Tesque (Kyoto, Japan). Cinnamic acid, foetal bovine serum, bovine calf serum, gentamicin, dexamethasone, 3-isobutyl-1-methylxanthine, rosiglitazone, bovine serum albumin, bovine insulin and oil-red-o were purchased from Sigma (St. Louis, MO, USA). 2-NBDG (2-(N-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)Amino)-2-Deoxyglucose) and trizol reagent were purchased from Life Technologies Ltd. (Paisley, UK). GenomeLab™ GeXP Start Kit was purchased from Beckman Coulter Inc. (Miami, FL, USA), while 0.45 μm Immobilon-FL polyvinylidene fluoride (PVDF) membrane and rabbit anti-GLUT4 primary antibody were purchased from Millipore (Bedford, MA, USA). Rabbit anti-insulin receptor beta, anti-α tubulin, anti-protein kinase B (PKB/AKT), anti-phospho AKT (Ser473), anti-mechanistic target of rapamycin (mTOR) and anti-phospho mTOR (Ser2448) primary antibodies and horseradish peroxidase-conjugated goat anti-rabbit secondary antibody were purchased from Cell Signaling Technology (Danvers, MA, USA). All the other chemicals were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA).

2.2. Preparation of EAF

M. latifolia rhizomes were collected from Beranang, Selangor, Malaysia (Geographical Coordinates: 2.8833° N, 101.8667° E). A voucher specimen of the plant (SK 1709/09) was confirmed and deposited in the Biodiversity Unit, Institute of Bioscience, Universiti Putra Malaysia, Serdang, Selangor, Malaysia. The plant name was cross-checked for accuracy according to The Plant List [14]. Briefly, the collected rhizomes were oven dried at 40 °C, ground into fine particles and passed through a 30-mesh sieve. The *M. latifolia* rhizome powder was subsequently extracted thrice with 80% methanol. After filtration and removal of residual solvent, a 7.95% (w/w) yield for the crude extract was obtained. Part of the crude extract was dissolved in water, followed by a bio-guided fractionation using n-hexane, ethyl acetate and n-butanol respectively. Among the derived fractions, ethyl acetate fraction (EAF) showed the highest total phenolic content [11] and was therefore subjected to further analysis in the present study. The EAF was prepared in 0.1% (v/v) dimethyl sulfoxide (DMSO) and stored at −20 °C until use.

2.3. Cell culture

3T3-L1 mouse fibroblast cells were maintained in DMEM supplemented with 4.5 g/L D-glucose, 4 mM glutamine, 10%

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