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GLUT4 as A Protein Target for Type 2-Diabetes Mellitus Therapy With Natural Compounds

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Abstract

WHO reported 1.9 million death cases of diabetes patients in 2019. Diabetes is caused by damage in the pancreas which resulted in a lack of insulin or insulin resistance. Medication for Type 2-Diabetes Mellitus (T2DM) mainly focuses on lowering blood glucose and treating affected organs. Current medications are still lacking; thus research is needed in finding novel medications to accommodate T2DM. This paper aims to present the current research on potential plant extract in increasing GLUT4 translocation in diabetes conditions. insulin resistance state affecting GLUT4 translocation which is important in affecting glucose uptake. Some research shows that plant extract proved to be potential in increasing the translocation of GLUT4 and helping lowering blood glucose levels.

Keywords: Diabetes Mellitus, T2DM, GLUT4, Extract

1. Introduction

Diabetes is a chronic disease either caused by damage to the pancreas which resulted in a decreased production of insulin or the body's unable to use available insulin. Diabetes is a public health problem[1]. There is an increase of mortality rate by 3% caused by diabetes between 2000 and 2019, while in lower-middle income countries the number increased to 13% mortality rate [1]. Type 2 diabetes (T2DM) is a condition characterized by the presence of insulin resistance and damage to the cell β pancreas. Insulin resistance is caused by several factors such as high lipid, increased inflammation signal, and activation of stress pathway in reticulum endoplasma. Damage in cells β pancreas prompt a decrease in pancreas function that caused dysfunction causing insulin deficiency [2].

Several oral medicines are regularly used by T2DM especially sulfonylurea and metformin, alongside some drugs which targeted the affected organ of diabetes. Sulfonylurea and thiazolidinedione each have roles in increasing insulin secretion and the latter increasing insulin sensitivity [3]. Nowadays, T2DM medication focuses on the use of metformin and a healthier lifestyle. The use of second-line and third-line drugs helps improve the condition of T2DM patients. However, current medications are still lacking because of the presence of side effects. Metformin in a contraindicative individual could increase the risk of lactic acidosis including liver disease, kidney disorder, and lung disease. Thus there is room for improvement in the research in finding novel medications for T2DM to overcome the shortcomings of current drugs [4]. Until recently, society use plants as a traditional medicine to help treat their illness [5].

GLUT (glucose transporter) is a transporter that plays roles in molecules exchanges through membrane cells with [6]each type has a unique and specific affinity towards its substrates, distribution, location, mechanisms, and physiological function [7]. GLUT4 is typically found in skeletal muscle and adipose tissue. Insulin-induced glucose

transport within fat and muscle tissue by stimulating GLUT4 increased glucose uptake [8,9]. In the Insulin resistance state, insulin is unable to increase the translocation of GLUT4 to the membrane and caused accumulation of GLUT4 in the membrane compartment. Thus insulin resistance involves a defect of GLUT4 traffic [10].

GLUT4 may show potential as a protein target for T2DM medication due to their roles in regulating glucose uptake by depending on insulin which is present in T2DM patients. Traditional medication may prove to be potential in targeting GLUT4 due to their nature of natural products thus safe to use. Several natural compounds derived from plants show their potential in targeting the activation of GLUT4. This paper's objective is to present the current research focusing on the potential of plant extract in increasing the activity of GLUT4 translocation to the membrane with the condition of diabetes.

2. Discussion

2.1. GLUT4

Glucose act as a source of energy for the most organism. Glucose characteristics are polar and have large molecule sizes so it can not pass cell membrane lipids through diffusion. Glucose molecules pass the membrane cell with the help of a structural transport protein family known as glucose transporter. Two main kinds of glucose transporter have already been identified which are sodium-glucose linked transporters (SGLTs) and facilitated diffusion glucose transporters (GLUTs). GLUT protein consists of 12 membrane-spanning regions with amino acid chains and carboxyl. Based on its amino acid sequences and several studies of its sequence alignment found three subclasses of facilitative transporter have been discovered [7].

GLUT4 fall in class 1 glucose transporter which is insulin-responsive transporter, found in the heart, skeletal muscle, adipose tissue, and brain. GLUT4 can be found in the vesicle inside the cytoplasm and would be translocated to the membrane after insulin binds to the insulin receptor. after binding, GLUT4 activation increased 10-20 folds [11].

GLUT4 structure is built with a unique sequence of N-terminal and COOH-terminal that play roles in responsibility towards insulin signal and membrane traffic. Glucose transfer along the membrane occurs via the GLUT4 mechanism through facilitated diffusion ATP-independent. After glucose influx into the cell, glucose would then be metabolized as energy or lipid synthesis or stored as glycogen [12].

The translocation of GLUT4 from the intracellular domain to the plasma membrane would occur after stimulation. During the unavailability of insulin or exercise, 90% of GLUT4 would be stored inside intracellular. If there is the availability of insulin or exercise hence the vesicle storing GLUT4 undergoes exocytosis toward the plasma membrane, sarcolemma, and T-tubules of skeletal muscle and plays its role in transporting glucose [13]. The increase of GLUT4 molecules on the surface of the membrane would accelerate the rate of glucose transport inside the cell. However, in the condition without stimulation from insulin, GLUT4 would undergo endocytosis back inside the cell through the process of budding of vesicles on the plasma membrane which contained clathrin and be stored back inside intracellular vesicle [11,13].

Activation of GLUT4 on skeletal muscle begins with insulin binding to the surface receptor which is a β subunit insulin receptor consisting of a tyrosine kinase domain that would autophosphorylate. Phosphorylated β subunit then trigger IRS and phosphorylate IRS. After IRS is phosphorylated then it would bind and activate PI3K and transfer toward the plasma membrane and convert PIP2 become PIP3. Then, after PIP3-dependent protein kinase has been activated, it would

phosphorylate and activate Akt (PKb). Akt activation leads to vesicle fusion which involves the translocation of vesicles containing GLUT4 from the intracellular compartment to the plasma membrane. GLUT4 elevation on the plasma membrane causes an increase in glucose uptake into cell [15].

2.2. Potential Plant Bioactive Compounds

Recent researches initiated investigations on plant extract in enhancing GLUT4 translocation in DM conditions. The table below presents the summary of each article.

Lipophilic extract from flowers of *Wisteria sinensis* is the potential as an antidiabetic compound, due to its activity in stimulating glucose uptake by activating protein Akt (Protein Kinase B) and increasing GLUT4 translocation on L6 cells *in vitro*. *In vivo*, the extract increases GLUT4 expression and helps ameliorate T2DM symptoms in T2DM mice from hyperglycemia to pancreatic islet destruction [14].

Cacao liquor procyanidin (CLPr) extract is the potential to increase GLUT4 translocation and glucose uptake in skeletal muscle, due to their content such as epicatechin, catechin, and other procyanidins. CLPr help suppressed hyperglycemia response in mice after carbohydrate ingestion while enhancing GLUT4 translocation in skeletal muscle, due to their beneficial potential CLPr help improves glucose tolerance [15].

Chloroform extract from the dandelion (DCE) plant may be another potential extract to help increase GLUT4 translocation. The research was performed *in vitro* using L6 cells and the protein was measured by western blot. The result shows that DCE upregulated GLUT4 expression which leads to an increase in GLUT4 translocation to the membrane, hence is instrumental to the enhancement of glucose uptake to the cells [16].

Anthocyanin-rich extract from black rice (*Oryza sativa* L.) extracted with ethanol from Hualien (HBRE) and Changhua (CBRE) region help promotes glucose uptake in C2C12 myotubes by increasing GLUT4 expression and phosphorylation of IRS-1. CBRE increased p-AMPK/AMPK while HBRE involves in PI3K/Akt and MAPK/ERK [17].

Phenolic compounds from Mulberry (*Morus alba* L.) leaf extract such as gallic acid help improves glucose uptake and enhanced GLUT4 translocation thus may be the potential as antihyperglycemic. Mulberry leaf extract help enhances GLUT4 translocation by mediating through the PI3K pathway due to glucose uptake getting inhibited by PI3K inhibitor (in this paper used wortmannin). [18].

Portulaca oleracea extract (POE) helps enhance glucose uptake by increasing GLUT4 expression. While enhancing GLUT4 translocation, POE helps the activation of IRS-1, PI3K, and Akt phosphorylation. Although POE did not involve in protein kinase C phosphorylation, POE involves in AMPK pathway activation [19].

Curcuma manga Val. rhizomes ethanol extract (EECM) can increase glucose uptake in lipid-laden 3T3-L1 cells, and increase GLUT4 mRNA expression [20]. Papaya seed extract (*Carica papaya* Linn.) had a significant influence on GLUT4 expression in the skeletal muscle of diabetic mice, measured by immunoreactive score and immunohistochemical staining [21].

Total anthraquinone from semen cassia (*Cassia obtusifolia* L.) extract (SCE) was evaluated for its action in improving glucose metabolism in diabetic rats. The result shows that SCE enhances GLUT4 translocation while increasing the expression of phosphorylated-AS160 (Thr642), phosphorylated-Akt (Ser473), and PI3K in skeletal muscle. SCE help promotes GLUT4 translocation due to the involvement in PI3K-Akt-AS160 signaling pathway activation [22].

Table 1. Summary of study in GLUT4 activation with various plant extract

Study	Plant extract	Contents	Length of follow up	Outcome
Yamashita et al., 2012 Japan	Cacao liquor procyanidin extract	Epicatechin, catechin, and other procyanidins	L6 myoblast ICR and C57BL/6 male mice (4 weeks old) 7 days of treatment Methods: Western Blot (WB)	Increased glucose uptake and promote GLUT4 translocation in L6 myotubes. Suppressed hyperglycaemic response after carbohydrate ingestion and enhanced GLUT4 in mice skeletal muscle.
Lv et al., 2020 China	Lipophilic extract from flowers of *Wisteria sinensis*	NA	L6 IRAP-mOrange and male C57BL/6j mice (8 weeks old) 4 weeks of treatment Methods: WB	Strongly increased glucose uptake to 2.0 fold compared to normal control and increased GLUT4 expression while stimulating Akt in L6 myotubes In mice, treatment ameliorated hyperglycemia, insulin resistance, and dyslipidemia. Also, increased GLUT4 expression by Akt activation in white adipose tissue and skeletal muscle.
Zhao et al., 2018 China	Dandelion chloroform extract (DCE)	NA	L6 IRAP-mOrange Methods: WB and real-time PCR	DCE increased GLUT4 translocation and expression through the AMPK pathway, GLUT4 fusion to the plasma membrane (PM) independent of Ca^{2+}.
Feng et al., 2022 Taiwan	Anthocyanin-rich extract from black rice (*Oryza sativa* L.)	anthocyanin	C2C12 myotubes Methods: WB	Hualien and Changhua black rice (HBRE and CBRE) promote glucose uptake. CBRE did not affect IRS-1 downstream but enhanced p-AMPK/AMPK. HBRE target PI3K/Akt and P38 MAPK/ERK which both

				promote GLUT4 glucose uptake.
Naowaboot et al., 2012 Thailand	Mulberry (*Morus alba* L.) leaf extract	1-deoxynojirimycin (DNJ), gallic acid, quercetin.	Male Sprague-Dawley rats 6 weeks of treatment. Methods: WB	Increased glucose uptake, and enhanced GLUT4 translocation while mediated via PI3K signalling pathway.
Park et al., 2018 Korea	*Portulaca oleracea* L. extract (POE)	Flavonoids and triterpenoids (data not shown)	Mouse 3T3-L1 preadipocyte cells Methods: WB	POE enhances glucose uptake, by increasing GLUT4 on PM. Increase of PM-GLUT4 associated with IRS-1 phosphorylation, PI3K activation, and Akt phosphorylation. PI3K inhibitor and AMPK inhibitor inhibit glucose uptake on POE.
Pujimulyani et al., 2020 Indonesia	White saffron rhizomes *Curcuma mangga* Val. ethanol extract (EECM)	Curcumin, gallic acid, catechin, epicatechin, epigallocatechin, epigallocatechin gallate, and gallocatechin gallate.	3T3-L1 fibroblasts-derived adipocyte cells Methods: RT-qPCR	EECM increased glucose uptake and GLUT4 mRNA expression.
Wulansari et al., 2017 Indonesia	Papaya seed extract (*Carica papaya* Linn.)	NA	White Wistar strain Rats 2 weeks of treatment Methods: IHC	Fasting blood glucose (FBG) decrease and increase of GLUT4 expression based on skeletal muscle staining with IHC after extract treatment
Zhang et al., 2018 China	Semen cassia (*Cassia obtusifolia* L.) extract (SCE)	anthraquinones	Male Sprague-Dawley rats 1. 30 days of treatment (SCE on normal rats) 2. 5 weeks of treatment (SCE on diabetic rats) Methods: WB	FBG and serum lipids decrease, while oral glucose tolerance (OGT), and fasting serum insulin (FSI) increase after SCE. SCE restored the low expression of GLUT4 translocation in diabetic rats. SCE increased Thr642, Ser473 and PI3K in skeletal muscle

3. Conclusion

GLUT4 plays a role in glucose uptake and affects blood glucose levels. T2DM patients' condition is mostly caused by insulin resistance, with the condition of hyperglycemia. GLUT4 may be a potential protein target to help cope with the symptom of hyperglycemia. Various beneficial plants show their ability in increasing GLUT4 expression on PM through activation either with PI3K or AMPK signaling pathway, which contributed to the increase of glucose uptake *in vivo* or *in vitro*. Based on the research mentioned above, research in finding precise extract and investigation to its exact mechanism pathway in promoting GLUT4 translocation is needed to discover the best potential plants to target the activation of GLUT4 in T2DM conditions.

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