Synopsis

Glucose is the major energy source of the human body. Pancreas plays an important role in maintaining blood glucose homeostasis by secreting hormones like insulin and glucagon. Among these, insulin is the most important hormone that regulates blood glucose homeostasis by stimulating the uptake of excess glucose from blood to the peripheral tissues like muscles and adipocytes. Any defects in insulin regulated glucose uptake can increase blood glucose level leading to a complex metabolic disorder called Diabetes mellitus.

Diabetes in general has been classified into three types as type 1, type 2 and gestational diabetes. Although type 1 diabetes has an increase in its prevalence, the most common type of diabetes is type 2 diabetes mellitus (T2DM) that accounts for more than 90% of all the diabetic cases in the world. Unlike type 1 diabetes in which insulin production is affected mainly due to the autoimmune destruction of β cells, type 2 diabetes is characterized by insulin resistance, a phenomenon, where insulin fails to exert its effect in the peripheral tissues like adipocytes and muscles resulting in impaired glucose transport.

Glucose transport into cells is mediated by a family of 12 trans-membrane domain proteins known as glucose transporters (GLUTs). Among the 14 isoforms reported so far, the principal insulin sensitive glucose transporter isoform that mediate glucose uptake into adipocyte and muscle is GLUT4 (Gene name: SLC2A4; protein name: GLUT4). Insulin stimulates glucose transport by increasing the translocation of GLUT4 to the plasma membrane. Defects in insulin signaling result in impaired GLUT4 translocation and glucose uptake in target tissues and that leads to the patho-physiological condition called insulin resistance. As this is the major abnormality in type 2 diabetes, there has been considerable interest in identifying insulin mimetic and insulin sensitizing agents to counteract insulin resistance. Any compound that increases the exocytosis and/or decreases the endocytosis will result in increased GLUT4 expression on plasma membrane and ultimately enhances glucose absorption. The present research thesis focuses on identification and characterization of compounds from medicinal plants that can modulate GLUT4 translocation and elucidating the molecular mechanism underlying
the process. Revealing the molecular and biochemical mechanisms of GLUT4 translocation by such compounds will lead to the development of new therapeutic targets.

Identification and characterization of compounds that modulate GLUT4 trafficking.

For the present study, plants were selected in a very judicious manner including various criteria. One criterion was the selection of plants which are described as anti diabetic in medical practices like Ayurveda and plants shown to have scientifically proven hypoglycemic effects in either animal studies or in cell based assays. Several plants considered as ‘single plant remedies’ owing to the presence of large repertoire of chemicals in them having various biological activities were also included for the screening purpose. Based on a detailed literature survey, a total of 100 plants were used for the present study. Crude extracts were prepared from the selected plants and subjected to GLUT4 translocation and glucose uptake assay in adipocytes (3T3-L1) and myotubes (C2C12). In order to isolate the active compound, the crude extract was fractionated in a bioassay directed manner. Compounds were isolated in sufficient quantities for molecular characterization. After the isolation in pure state, the molecular structures were derived by classical and modern techniques like chemical reactions, ultra violet (UV), infra red (IR) and nuclear magnetic resonance (NMR) spectroscopy.

Kaempferitrin modulates GLUT4 translocation and glucose uptake in adipocyte and muscle

Kaempferitrin (kaempferol 3,7-dirhamnoside), a glycosylated flavonoid, was isolated from Bauhinia acuminata leaves and found to modulate GLUT4 translocation and glucose uptake in 3T3-L1 adipocytes and C2C12 myotubes. In the absence of insulin, addition of kaempferitrin did not affect GLUT4 translocation or glucose uptake in 3T3-L1 adipocytes whereas kaempferitrin acted as an inhibitor of insulin-stimulated GLUT4 translocation and glucose uptake in 3T3-L1 adipocytes by inhibiting Akt activation. Molecular docking studies using a homology model of GLUT4 showed that kaempferitrin binds directly to GLUT4 at the glucose transportation channel, suggesting the possibility of a competition between kaempferitrin and glucose during the transport. Taken together, this study demonstrates that kaempferitrin inhibits GLUT4 mediated glucose uptake at least by two different mechanisms, one by interfering with the insulin signaling pathway.
and the other by a possible competition with glucose during the transport. Although kaempferitrin in the absence of insulin did not show any modulation in glucose uptake in adipocyte, a stimulatory effect on GLUT4 translocation and glucose uptake was observed in muscle cells under the similar condition. Further studies revealed that kaempferitrin activate AMPK in muscle cells and was important for enhanced GLUT4 translocation. A similar activation of AMPK was not observed in 3T3-L1 adipocytes treated with kaempferitrin, suggests a differential effect mediated by kaempferitrin in these cells lines.

An enriched fraction from Bauhinia acuminata leaves containing aliphatic compounds exhibits GLUT4 translocation and glucose uptake activity.

*Bauhinia acuminata*, a species native to tropical southeastern Asia has been shown to have anti hyperglycemic activity and has been used as a traditional remedy for diabetes. Crude methanol extract of *Bauhinia acuminata* leaves and its fractions were studied in the GLUT4 translocation assay. Further purification resulted in the isolation of an active faction from the methanol extract. Various spectroscopic techniques such as $^1$H NMR, $^{13}$C NMR, MS and IR were used for further characterization and the enriched active fraction was found to be a mixture of small aliphatic compounds. In addition to GLUT4 translocation activity, the active fraction was also found to increase glucose uptake in differentiated 3T3-L1 cells. This enriched fraction was found to stimulate GLUT4 translocation in an Akt independent manner.

Gallic acid induces GLUT4 translocation and glucose uptake

Bioassay directed fractionation resulted in the isolation of gallic acid (GA) from seabuckthorn leaves (*Hippophae rhamnoides* L. Elaeagnaceae), as a compound that induces GLUT4 translocation and glucose uptake in 3T3-L1 adipocyte and C$_2$C$_{12}$ muscle cells. Seabuckthorn is widely distributed in the mountainous regions of Asia and Europe. This plant is an important resource of natural products with antioxidant, anti-tumor, hepatoprotective and immuno-modulatory properties. The present study identified and functionally characterized GA as the active principle from seabuckthorn leaf extract that increases GLUT4 translocation and glucose uptake in *in-vitro* cell based assays. GA treatment stimulated GLUT4 translocation and glucose uptake in a concentration dependent manner. Further to investigate the mechanism of stimulation of GLUT4
translocation and glucose uptake, the effect of GA on cellular signaling pathways known to modulate these processes was studied. The observations demonstrate that GA stimulates glucose uptake by inducing GLUT4 translocation in a PI3K dependent manner. This was confirmed by the inhibitory effect of GA mediated glucose uptake by wortmannin, an inhibitor of PI3K activation. In order to identify the downstream activators of PI3K, an analysis was carried out on the phosphorylation status of Akt (protein kinase B) and aPKCζ/λ (atypical protein kinase C), the two important protein kinases shown to regulate insulin mediated GLUT4 translocation. Unlike insulin, GA did not stimulate Akt phosphorylation in differentiated adipocytes. However, GA treatment resulted in the activation of aPKCζ/λ similar to insulin, suggesting GA stimulate GLUT4 translocation and glucose uptake in an Akt independent but aPKCζ/λ dependent manner.

In conclusion, the present study reports the identification and characterization of two small molecules viz. kaempferitrin and gallic acid, as modulators of protein traffic and therefore the glucose transporting activity of GLUT4 in adipocytes and muscle cells. Kaempferitrin isolated from Bauhinia acuminata found inhibit insulin stimulated GLUT4 translocation and glucose uptake in adipocytes and muscles, where as addition of kaempferitrin in the absence of insulin showed a stimulatory effect on GLUT4 translocation in muscle cells. Additionally, gallic acid isolated from Hippophae rhamnoides was found to stimulate GLUT4 translocation and glucose uptake in these cells. Detailed analysis on molecular mechanism revealed that these compounds used distinct signal transduction pathways for the modulation of GLUT4 translocation in different cell lines.