DOI: 10.4149/BLL_2023_108

Bratisl Med J 2023; 124 (9)
707 – 717

CLINICAL STUDY

Molecular mechanisms of antidiabetic effect of betulinic acid in lotus rhizome

Damean De SILVA[1], Upul SENARATH[2], Pathirage Kamal PERERA[3]

Divisional Hospital, Halthota, Sri Lanka. **Damiandesilva2013@gmail.com**

ABSTRACT
OBJECTIVES: To explain the bio-physiological mechanisms of the antidiabetic effect of betulinic acid in Lotus rhizome.
BACKGROUND: Even though Sri Lankan native medicine uses Lotus rhizome as a medicinal food for diabetes mellitus, its antidiabetic property has not been scientifically explained yet. It is found to compose several medicinally active components with antidiabetic properties, including Betulinic acid.
METHODS: A narrator review was conducted with a literature search in PubMed and Google Scholar databases using the search terms “Nelumbo nucifera rhizome”, “Lotus rhizome”, “phytochemicals”, “antidiabetic effect”, “hypoglycaemic effect” “Betulinic acid”, and “molecular mechanism”.
RESULTS: The triterpenoid, Betulinic acid exerts its antidiabetic effect via seven bio-physiological mechanisms including, inhibiting α-glucosidase and α-amylase, upregulating the expression of peroxisome proliferator-activated receptor gamma coactivator-1 α, enhancing AS160 protein phosphorylation, stimulating adenosine monophosphate-activated protein kinase activation, stimulating Glucose transporter type 4 synthesis and translocation, inhibiting Protein Tyrosine Phosphatase 1 B activity preventing dephosphorylation of insulin receptor and stimulating Takeda-G-protein-receptor-5 resulting in an increased release of insulin from insulin-containing granules
CONCLUSION: The available scientific knowledge explains that betulinic acid in Lotus rhizome can improve glucose homeostasis contributing to the antidiabetic effect of this root (*Tab. 1, Fig. 6, Ref. 29)*. Text in PDF *www.elis.sk*
KEY WORDS: glucose homeostasis, hypoglycemic effect, bioactive compounds, phytochemical, medicinal food, functional food.

List of abbreviations: AMPK – Adenosine monophosphate-activated protein kinase, PGC-1 α – Peroxisome proliferator-activated receptor gamma coactivator-1 α, GLUT4 – Glucose transporter type 4, AS160 – AS160 protein, TGR5 – Takeda-G-protein-receptor-5, ATP – Adenosine triphosphate, AMP – Adenosine monophosphate, ADP – Adenosine diphosphate, PP2A – Protein phosphatase 2A, PPM1E – Mg^{2+}-/Mn^{2+}-dependant protein phosphatase 1E, PP2C – Protein phosphatase 2C, LKB1 – Liver kinase B1, CaMKK2 – Calcium/ Calmodulin – dependant kinase 2, TAK1 – TGFβ-activated kinase 1, CBS3 – Cystathionine-β-synthase 3, PKB – Protein kinase B, DAG – Diacylglycerol, PKC – Protein kinase C, GSV – GLUT4 storage vesicles, HNF4 – Hepatocyte nuclear factor 4, CRTC2 – CREB-regulated transcription coactivator 2, PPAR – Peroxisome proliferator-activated receptor, PGC1α – Peroxisome proliferator-activated receptor gamma coactivator-1 α, RXR – 9-cis retinoid X receptors, GTP – Guanosine triphosphate, GEF – Guanine nucleotide exchange factor, PTB – Protein Tyrosine Phosphatase, LPR1 – Low-density lipoprotein receptor-related protein 1, IRAP – Insulin-regulated aminopeptidase, SNARE – Trans-Soluble N-ethylmaleimide-sensitive factor attachment receptor, PI3K – Phosphoinositide 3-kinase, IRS – Insulin receptor substrate, PIP2 – Phosphatidylinositol 4,5-biphosphate, PDK – 3-phosphoinositide-dependant protein kinases, PKB – protein kinase B, PKC – protein kinase C, PTEN – Phosphatase and tensin homolog on chromosome 10, PTP-1B – Protein Tyrosine Phosphatase 1 B, TGR5 – Takeda-G-protein-receptor-5, GPBAR1 – G protein-coupled bile acid receptor 1, GLP – Glucagon-like peptide 1, BA – Bile acid, LCA – Lithocholic acid, DCA – Deoxycholic acid, GIP – Glucose-dependent insulinotropic polypeptide, GLP-1R – GLP-1 receptor

Introduction

Nelumbo nucifera rhizome has been used as a medicinal food for many communicable and non-communicable diseases in Sri Lankan traditional medical system (1, 2). Especially, the native Sri Lankan medical practice ‘Deshiya Chikithsa’, has been successfully using the medicinal effect of lotus rhizome for the management of Diabetes mellitus since very far in its history. For

[1]Faculty of Graduate Studies, University of Colombo, Sri Lanka, [2]Faculty of Medicine, University of Colombo, Sri Lanka, and [3]Institute of Indigenous Medicine, University of Colombo, Rajagiriya, Sri Lanka

Address for correspondence: Damean De SILVA, Dr, Divisional Hospital, Halthota, PO Code 12538 Sri Lanka.
Phone: +94717676982

Tab. 1. Summary of bio-physiological mechanisms of body glucose regulation by Betulinic acid.

Chemical component	Bio-physiological mechanism	Effect
Betulinic acid	Inhibitory effect on α-glucosidase and α-amylase	Reduction of intestinal glucose absorption
	Upregulation of PGC1-α	Increased insulin sensitivity
	AS160 phosphorylation	Enhanced GLUT4 expression
	AMPK activation	decreased endogenous glucose production
		increased insulin sensitivity
	Stimulatory effect on synthesis and translocation of GLUT4	increased cellular glucose uptake
	Inhibitory effect on the PTP-1B protein activity	enhanced insulin activity
		higher GLUT-4 translocation to the plasma membrane and increased cellular glucose uptake
	Stimulatory effect on TGR5 receptors	increased insulin sensitivity
		increased glucose homeostasis
		increased pancreatic function

the smooth management of diabetes mellitus, in addition to pharmaceutical management, the consumption of healthy food with medicinal properties in the routine diet is very important (3, 4). Even though the Sri Lankan traditional medical system explains the medicinal property of many foods for many noncommunicable diseases including diabetes mellitus, the lack of scientific evidence for them has restricted the western or allopathic medical practitioners using this traditional medicine knowledge in managing patients under western medical care.

This review is planned to compose a comprehensive document that explains the scientific basis of the antidiabetic effect of nelumbo nucifera rhizome, utilising the scattered scientific knowledge in PubMed and google scholar databases.

Materials and methods

The methodological approach for this narrative review on the antidiabetic effect of medicinally active components in lotus rhizome was conducted using google scholar and PubMed databases on the articles published until 30th September 2022. Initial search terms were "Nelumbo nucifera rhizome" AND "phytochemicals" AND "antidiabetic effect", "Nelumbo nucifera rhizome" AND "phytochemicals" AND "hypoglycaemic effect", "Lotus rhizome" AND "phytochemicals" AND "antidiabetic effect", "Lotus rhizome" AND "phytochemicals" AND "hypoglycaemic effect". After the initial scrutiny, with each identified medicinally active compound another search was conducted to identify the bio-physiological mechanisms of the antidiabetic effect. For this article, the search terms used were "Betulinic acid" AND "hypoglycaemic effect" AND "molecular mechanism", "Betulinic acid" AND "antidiabetic effect" AND "molecular mechanism". The reference list of each selected article was searched to ensure the inclusion of all important articles and all the relevant full-text articles in the English language were studied for this narrative review. Only the google scholar database was searched for identifying the articles to describe the seven identified molecular-level mechanisms of glucose homeostasis. The search terms used were "Regulation and function of AMPK in glucose metabolism", "regulation of glucose absorption by α-glucosidase and α-amylase", "regulation of glucose metabolism by PGC1-α", "molecular mechanism of AS160 mediated glucose metabolism", "regulation of synthesis and translocation of GLUT 4", "regulation of glucose metabolism by TGR5 receptors". Only the first two relevant full texts in the English language were referred for the review.

Results and discussion

Lotus rhizome is found to have nine bioactive compounds with an antidiabetic effect namely, Betulinic acid, Tryptophan, Nuciferin, Isoquercetin, Rutin, β-Sitosterol, Stigmasterol, Neferin, and Fibre. This article discusses only the bio-physiological mechanisms of the hypoglycaemic activity of Betulinic acid which belongs to the triterpenoid group. Betulinic acid is found to deliver its hypoglycaemic effect via seven molecular-level mechanisms (Tab. 1). For a comprehensive understanding of these mechanisms, it is mandatory to have a deep knowledge of some relevant glucose homeostasis mechanisms including α-glucosidase and α-amylase activity, molecular mechanisms of Adenosine monophosphate-activated protein kinase (AMPK)-regulated glucose homeostasis, blood glucose regulation via peroxisome proliferator-activated receptor gamma coactivator-1 α (PGC-1 α) activation, cellular glucose uptake by Glucose transporter type 4 (GLUT4) via AS160 protein (AS160) inactivation, mechanism of GLUT4 synthesis and translocation, cellular insulin signalling pathway, and insulin release by activation of Takeda-G-protein-receptor-5 (TGR5). These primary mechanisms are described in detail at the molecular level explaining the relevant stimulatory and inhibitory effects of Betulinic acid.

Inhibitory effect on α-glucosidase and α-amylase activity

In the human body, starches are digested into glucose and absorbed into the bloodstream via the enterocytes of the small intestine. Alpha amylase produced from the pancreas acts in the intestinal lumen and hydrolyses the α-(1,4) glycosidic bonds of polysaccharides and produces smaller oligosaccharides such as maltose, maltotriose, and dextrin. Alpha-glucosidase (Glucoamylase and Sucrose Isomaltose) acts on the digested products of α-amylase and further breaks them down to give glucose and fructose monosaccharides by hydrolysing the α-(1,4) and α-(1,6) glycosidic linkages.

Betulinic acid which is a pentacyclic triterpene has a fast affinity to α-glucosidase and binds tightly to the active site of the α-glucosidase enzyme and forms betulinic acid-α-glucosidase complex. Several amino acids in the binding pocket of the α-glucosidase enzyme, including PHE155, PHE298, PHE175, HIE277, GLU302, ASP347, and TRY311 strongly interact with

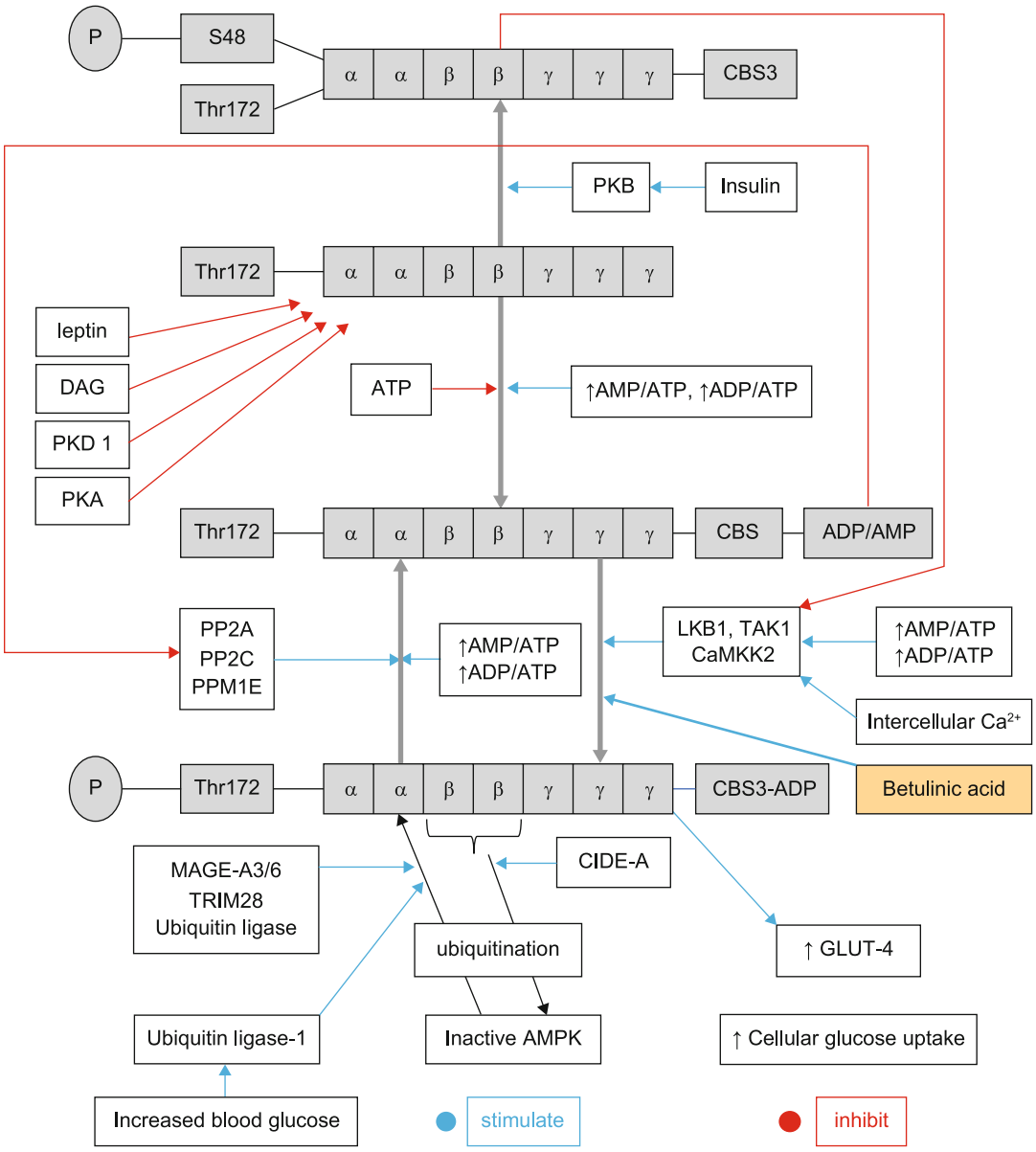

Fig. 1. Effect of Betulinic acid on AMPK-regulated glucose homeostasis. PKB (AKT), protein kinase B; ATP, adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine monophosphate; PP2A, Protein phosphatase 2A; PP2C, protein phosphatase 2C; PPM1E, Mg^{2+}-/Mn^{2+}-dependant protein phosphatase 1E; DAG, diacylglycerol; PKD1, protein kinase D 1 ;PKA, Protein kinase A; LKB1, Liver kinase B1 ; TAK1, TGFβ-activated kinase 1; CaMKK2, Calcium/calmodulin-dependent protein kinase 2 ;MAE-A3/6, ;TRIM28, tripartite motif containing 28; CIDE-A, Cell Death Inducing DFFA Like Effector A;GLUT-4, insulin-regulated glucose transporter 4; AMPK, Adenosine monophosphate activated protein kinase; CBS3, cystathionine-β-synthase 3.

betulinic acid and form a strong complex that dissociates slowly. This inhibition of the α-glucosidase activity leads to a reduction of postprandial hyperglycaemia (5, 6).

Activate AMPK

Molecular mechanisms of AMPK regulated Metabolic and physiological functions

AMPK is a principal enzyme that maintains cellular energy homeostasis. It is a serine/threonine protein kinase and depletion of phospho-compounds which are cellular-level high energy sources, activates it. AMPK, being the sensor of energy status within the cell, controls a wide range of metabolic and physiological functions of the body, and dysregulation of AMPK functions is one of the main pathological bases of many chronic diseases including obesity, diabetes mellitus, inflammation, and cancers. The main metabolic functions AMPK is involved in are glucose metabolism, protein synthesis, lipid metabolism, anti-inflammation, redox regulation, and anti-aging (7). When AMPK is activated, a

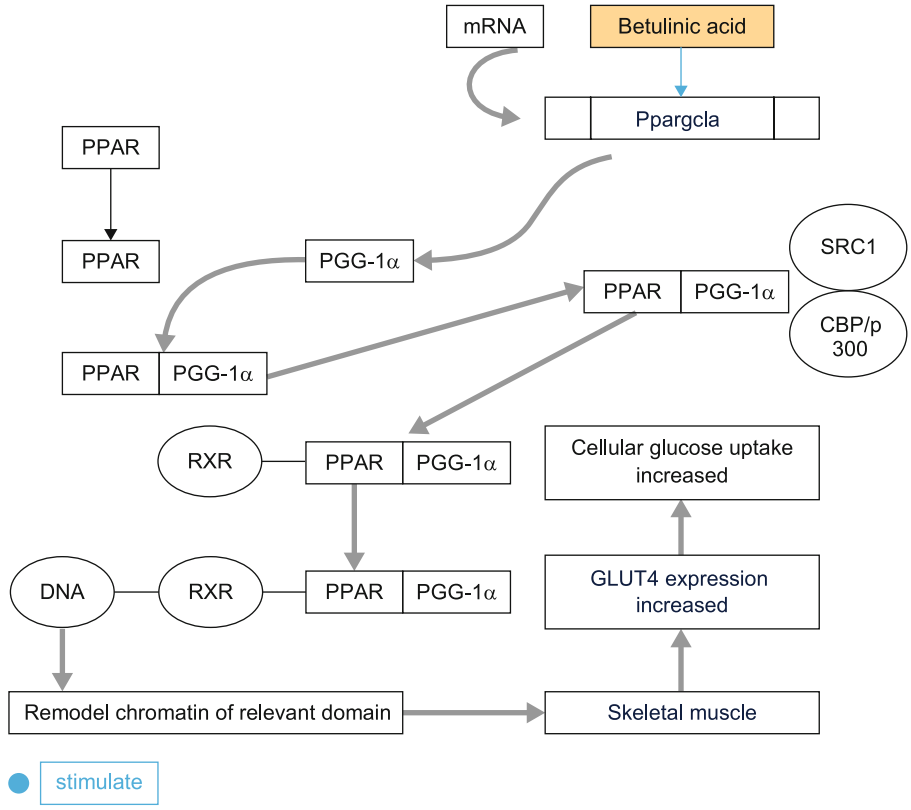

Fig. 2. Betulinic acid in blood glucose regulation via PGC-1 α activation. PPAR, peroxisome proliferator-activated receptor alpha; PGC-1 α, peroxisome proliferator-activated receptor gamma coactivator-1 α; SRC1, steroid receptor coactivator 1; CBP, CREB-binding protein; RXR, 9-*cis* retinoid X receptors; GLUT4, insulin-regulated glucose transporter 4; DNA, Deoxyribonucleic acid.

complex signaling process is initiated leading to energy production, by facilitating the substrate uptake and oxidation for adenosine triphosphate (ATP) synthesis and inhibiting ATP-consuming anabolic processes like glycogen, protein, and lipid synthesis (8).

AMPK is a heterometric enzyme composed of two α isomers, two β isomers, and three γ isomer subunits. Alpha subunits play the catalytic role while β and γ subunits play the regulatory role. Regulation of AMPK involves both allosteric and post-translational modification. The two best-defined AMPK activation mechanisms are:

In α subunit – by phosphorylation reaction at Thr172 position

In γ subunit – binding of adenosine monophosphate (AMP) and/or adenosine diphosphate (ADP) to γ unit.

Three phosphatases and three kinases regulate this phosphorylation reaction. Protein phosphatase 2A (PP2A), Mg^{2+}-/Mn^{2+}-dependant protein phosphatase 1E (PPM1E), protein phosphatase 2C (PP2C), Liver kinase B1 (LKB1), calcium/ calmodulin – dependant kinase 2 (CaMKK2) and TGFβ-activated kinase 1 (TAK1). In energy well-stocked conditions (low AMP/ATP and low ADP/ATP ratios) phosphatases easily reach the Thr172 of the AMPK α subunit and maintain the unit unphosphorylated. But, in energy depleted state the high AMP/ATP and high ADP/ATP ratios lead to the binding of AMP and ADP to CBS3 (cystathionine-β-synthase 3) of the AMPK γ-subunit preventing phosphatases from reaching Thr172 of the α subunit. This results in increased phosphorylation at Thr172 of the α subunit (7).

When AMP and ADP are bound to CBS3, it stimulates the phosphorylation which is mediated by LKB1 and this is a required step for the AMPK β subunit myristylation (9). Also, when the AMP is bound to CBS1, the allosteric activation (feedback from downstream products or feedforward from upstream substrates) of AMPK is induced and the intrinsic activity of AMPK is increased (7). Another activator for AMPK is the intracellular calcium which stimulates the phosphorylation of AMPK via CaMKK2. TAK1 too can phosphorylate AMPK and activate it (7).

Insulin induces AKT (also termed as PKB – protein kinase B) activity and stimulates direct phosphorylation of AMPK that inhibits its activity. The S487 of the α-1 subunit of the AMPK is phosphorylated by AKT, without being phosphorylated by the equivalent site of the α-2 subunit, S491. Therefore, phosphorylation of the Thr172 by upstream kinases is blocked.

Leptin, which stimulates satiety, can also inhibit AMPK. It can induce S491 phosphorylation of the α-2 subunit (7).

During hyperglycaemia and hyperlipidaemia, diacylglycerol (DAG) level is increased and DAG stimulates protein kinase C (PKC) which directly phosphorylates AMPK and inhibits it (7).

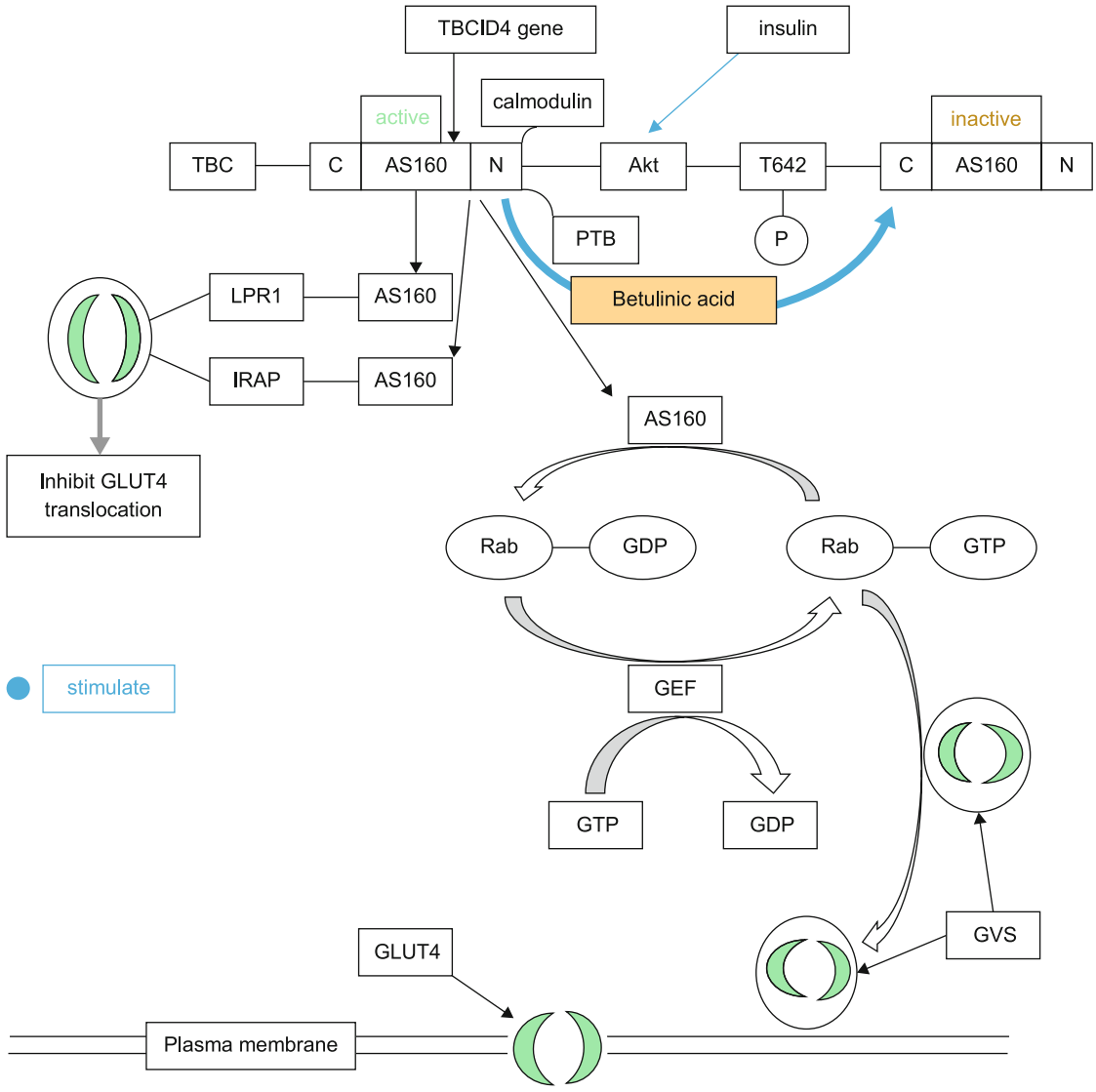

Fig. 3. Betulinic acid in cellular glucose uptake via AS160 inactivation. AS160, Akt substrate of 160 kDa; LPR1, LDL receptor-related protein (LRP)1; IRAP, Insulin-regulated aminopeptidase; PTB, Phosphotyrosine binding; Akt, is a serine/threonine protein kinase (also known as PKB); GDP, guanosine diphosphate; GTP, guanosine triphosphate; GEF, guanine nucleotide exchange factors; GVS, GLUT4 storage vesicles; GLUT4, insulin-regulated glucose transporter 4.

Protein kinase A (PKA) can phosphorylate S173 of the α-1 subunit and this results in blocking upstream kinases (LKB1 and CaMKK2) phosphorylating Thr172 of α-1 subunit, inhibiting AMPK (7).

Ubiquitination and sumoylation are two molecular-level mechanisms that induce AMPK degradation resulting in the inhibition of AMPK activity. CIDE-A is a cell death activator protein that interacts and ubiquitinates the β subunit of AMPK in brown adipose tissue. When the glucose levels are high, AMPK is degraded by ubiquitination of the α-2 subunit of AMPK, mediated by ubiquitin protein ligase 1. Scientific evidence shows that reactive oxygen species (ROS) play a role in the regulation of AMPK activity while protein-protein interaction and subcellular distribution of it also regulate AMPK activity (7).

AMPK inhibits the TBC1D1 which is the Rab-GTPase-activating protein by phosphorylating it. Rab-GTPase deactivates the active form of the phosphate-bound G protein of the Rab family. Fusion of GLUT4-containing intracellular vesicles – GLUT4 storage vesicles (GSVs) to the plasma membrane is mediated via Rab family G protein and AMPK enhances the GLUT4 expression by this molecular mechanism. AMPK is also found to degrade the TRX-interacting protein that stimulates glucose transporter 1 (GLUT1) internalisation and promotes GLUT1 expression. In addition, AMPK can increase GLUT4 by enhancing the expression

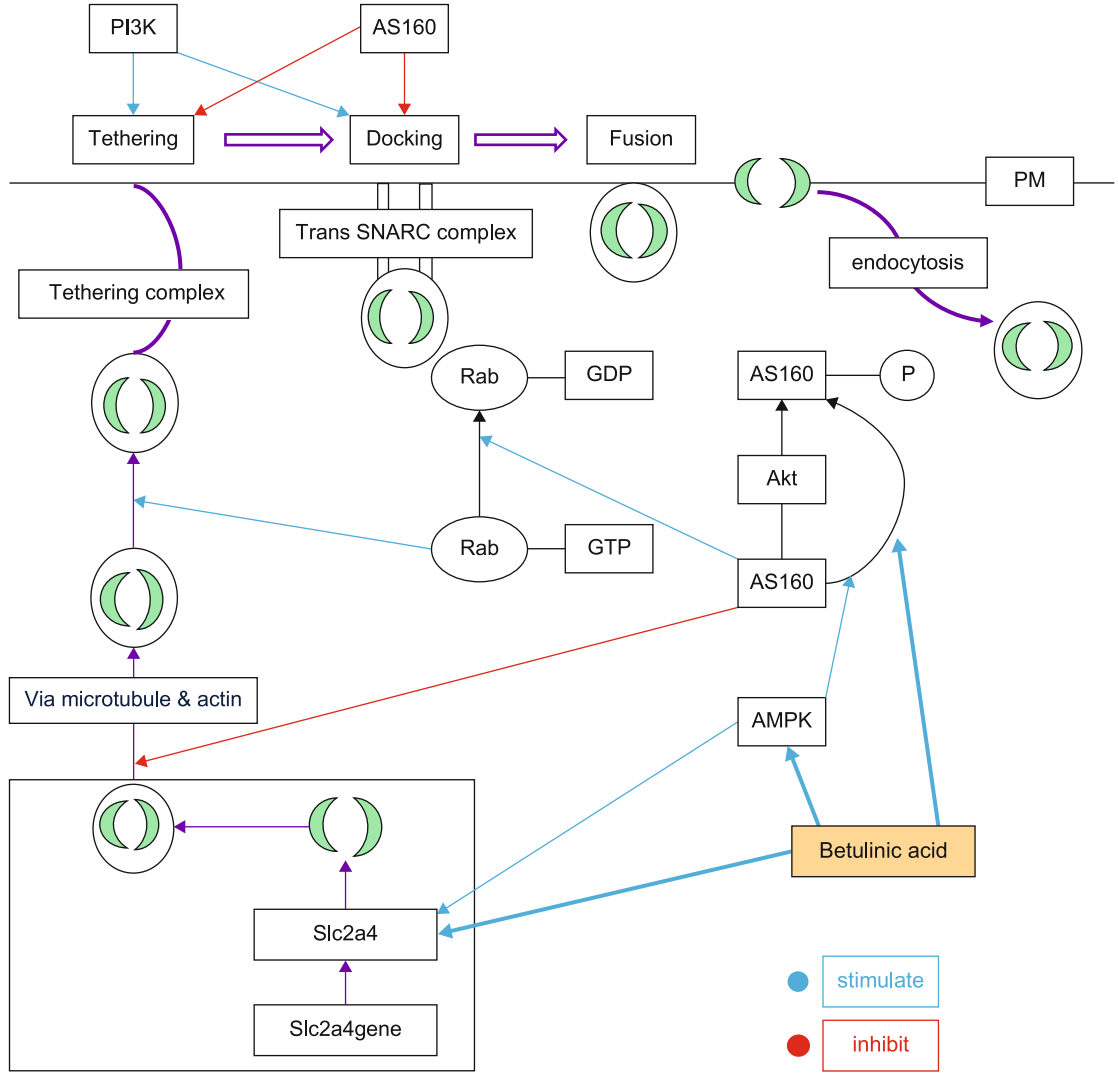

Fig. 4. Stimulatory effect of Betulinic acid on GLUT4 synthesis and translocation. PI3K, phosphoinositide 3-kinase; AS160; Akt substrate of 160 kDa; PM, plasma membrane; GDP, guanosine diphosphate; Akt, is a serine/threonine protein kinase (also known as PKB); GTP, guanosine triphosphate; AMPK, Adenosine monophosphate-activated protein kinase.

of mRNA which encodes GLUT4. AMPK stimulates glycolysis and inhibits glycogen synthesis inside the cells while it inhibits gluconeogenesis inside hepatocytes by inhibiting the transcription factors hepatocyte nuclear factor 4 (HNF4) and CREB-regulated transcription coactivator 2 (CRTC2) (7, 10).

Betulinic acid is found to activate AMPK by phosphorylating the Thr172 of the active site of the α subunit of the AMPK (Fig. 1). Treatment with betulinic acid in a dose of 100μg/ml results in peak phosphorylation one hour after administration of it (8).

Upregulating PGC1-α

The latest scientific evidence shows that mitochondrial capacity in oxidative metabolism is regulated at least partially at the gene transcription level. The control of gene transcription reprogrammes the enzyme expression responding to the raised physiological and pathological conditions. The peroxisome proliferator-activated receptor alpha (PPAR α) and its coactivator; peroxisome proliferator-activated receptor gamma coactivator-1α (PGC1α) are two important transcriptional regulators in the cells and they influence regulating several major metabolic processes including fatty acid beta-oxidation, the release of insulin from beta cells, adaptive thermogenesis, peripheral tissue glucose consumption and gluconeogenesis of liver. The three isomers of PPAR – PPARα, PPARβ, and PPARγ-need ligands for activation of the transcriptional regulation. Long-chain fatty acids and their metabolites may act as endogenous ligands. When a ligand is bound to PPAR, heterodimers are formed with 9-*cis* retinoid X receptors (RXR) which bind to the relevant gene promoter region of DNA.

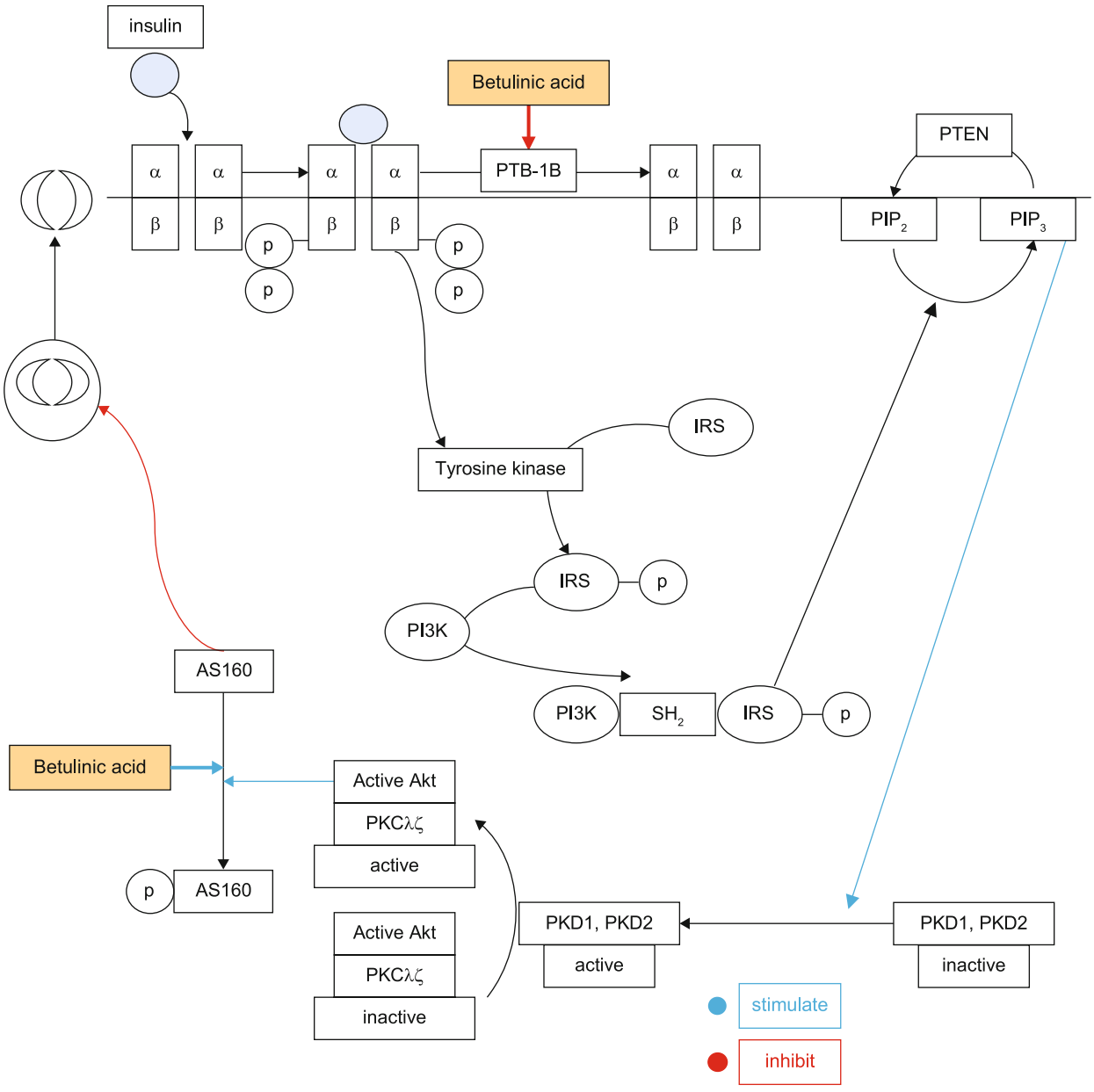

Fig. 5. Betulinic acid in regulating cellular insulin signalling pathway. PTB-1B, Protein Tyrosine Phosphatase 1 B; PTEN, phosphatase and tensin homolog on chromosome 10; PIP_2, phosphatidylinositol 4,5-biphosphate, PIP3, phosphatidylinositol 4,5-triphosphate; IRS, insulin receptor substrate; PI3K, phosphoinositide 3-kinase; AS160, Akt substrate of 160 kDa; PKC λ ξ, atypical protein kinase C λ and ξ; PKD1, 3-phosphoinositide-dependant protein kinase 1; PKD2, 3-phosphoinositide-dependant protein kinase 2.

As the PPARα has a large hydrophobic ligand-binding site, many pharmacologically active ligands can act their giving therapeutic effects. In addition to the endogenous ligands, transcriptional co-activators, and corepressors can activate PPAR. Out of them, the three PGC-1 isomers – PGC-1α, PGC-1β, and PGC-1γ-are salient metabolic regulators of mitochondria. PGC-1α mediates the binding of two other coactivators – SRC1 and CBP/p300 which contribute to remodelling chromatin (11, 12).

PGC1-α is the insulin sensitivity marker in skeletal muscles and is encoded in the *Ppargc1a* gene. Treatment with betulinic acid results in an increase of the mRNA assay of the *Ppargc1a* gene which shows that an increase in insulin sensitivity by betulinic acid is gained by upregulating the expression of PGC1-α (8) (Fig. 2).

AS 160 phosphorylation

The Rab proteins are small Guanosine triphosphatases (GTPases) inside the cells and they play the primary regulator role in vesicular trafficking pathways, regulating the transport of cellular cargo via membrane organelles. They are the molecular switches that regulate the transport of vesicle formation and transport them to the plasma membrane along microtubules. The transported vesicles are then docked and fused to the membrane under the regulation of these molecular switches. The regulatory power of these Rab proteins depends on their active and inactive status. Loading of GTP makes them active while hydrolysis of GTP from the Rab proteins turns them to be inactive. Loading and hydrolysis of GTP to Rab proteins are catalysed by guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs). (Binding of GTP activates these Rab proteins and gets membrane-bound while GDP inactivates them and keeps them in the cytosol).

AS 160 is a Rab GTPase-activating protein (GAP), regulated by insulin in adipocytes and skeletal muscles. AS160 is composed of two terminals – the C terminus and the N terminus. The C terminus composes a Tre-2/ Bub2/ Cdc16 (TBC) domain while the N terminus composes a calmodulin-binding domain and two phosphotyrosine-binding (PTB) domains. Phosphorylation of AS160 at Thr642 is stimulated by Akt when insulin is activated. With phosphorylation of AS160, the GTPase activating protein activity is inhibited leading to the accumulation of Rab proteins in GTP loaded state which in turn enhances the GLUT4 expression in the plasma membrane increasing GLUT4 activity (13). In addition to this AS160 in its unphosphorylated form has the ability to bind GVSs via low-density lipoprotein receptor-related protein 1 (LPR1) and insulin-regulated aminopeptidase (IRAP) inhibiting GLUT4 translocation and retaining GVS in the cytosol (14). Botulinic acid can phosphorylate AS160 by a separate mechanism than the PI3K/Akt signalling pathway and enhance GLUT4 activity delivering an antidiabetic effect (8, 15) (Fig. 3).

The stimulatory effect on synthesis and translocation of GLUT4

GLUT4 is a glucose transporter detected primarily inside the adipocytes and skeletal and cardiac muscle cells. About 50 % of GLUT4 is contained in GSVs in the intracellular compartment while the rest is placed in various other compartments such as trans-Golgi network, endosomes, and ubiquitous transport vesicles. The amount of plasma membrane located in GLUT-4 is only about 5 % of the total GLUT4 population. As GLUT4 is a very stable protein it does not need to be constantly replenished and only a small amount need to be biosynthesised. They are found intracellularly inside GSVs and with insulin stimulation, GSVs are attached to the plasma membrane (16, 17).

The storage vesicle of GLUT4, GSV is bio-generated either from endosomes or from the trans-Golgi network. Sometimes both may be involved in this. Bio-generated GSVs are then transported to the cell cortex by microtubules and actin. These GSVs are tethered to the plasma membrane via a low-grade interaction and this tethering is mediated by a tethering complex. Next, docking of these GSVs takes place by the trans-Soluble N-ethylmaleimide-sensitive factor attachment receptor (trans-SNARE) complex. PI3K and AS160 are found to be involved in regulating the tethering and docking of GSVs to the plasma membrane. At last, fusion takes place by rapidly fusing GSV lipid bi-layers with the plasma membrane (10).

GLUT4 is expressed by the *Slc2a4* gene and betulinic acid has the ability to selectively magnify *Slc2a4* mRNA expression and increase the GLUT4 activity resulting in an increase of glucose uptake into cells (8) (Fig. 4).

Inhibitory effect on the Protein Tyrosine Phosphatase 1 B (PTP-1B) activity

Molecular mechanism of Insulin signaling

When the insulin molecule which is an anabolic peptide hormone produced by beta cells of the pancreas, is bound to the cell surface glycoprotein, insulin receptor, a multistep signaling cascade inside the cell is initiated regulating many biological processes including carbohydrate, lipid, and protein metabolism, gene expression and growth, division, and survival of the cell. But the exact mechanisms of the reaction cascade following insulin-insulin receptor interaction are not yet completely understood.

The cell surface insulin receptor which is a tetramer glycoprotein is composed of four subunits – two alpha subunits, where the hormone binds, and two beta subunits, which are tyrosine-specific protein kinases. Each α subunit is composed of 1008 amino acids with cysteine while each β subunit is composed of 778 amino acids. Alpha subunits are placed utterly extracellularly on the plasma membrane while the beta subunits are placed in transmembrane space with an intracellular domain containing an amino acid sequence homologous to tyrosine kinase (18).

Insulin activity inside the body is regulated by multistep signalling cascades.

Insulin binds to the α subunit of the receptor → Autophosphorylation of the tyrosyl residues of the β subunit of the receptor → Tyrosine kinase of the β subunit of the receptor is activated → Phosphorylation of the tyrosine of insulin receptor substrate (IRS) – IRS1, IRS2, IRS3, IRS4, Gab1, Shc (IRSs are cytoplasmic proteins which are direct substrates of activated tyrosine kinase of β subunit of the insulin receptor) → IRS bind to the regulatory subunit of phosphoinositide 3-kinase (PI3K) via the Src homology 2 (SH2) domain → PI3K is activated → Membrane phospholipids and phosphatidylinositol 4,5-biphosphate (PIP2) on the 3' position are phosphorylated → 3-phosphoinositide-dependant protein kinases (PDK-1 and PDK-2) are activated → The serine/threonine kinase or the Akt or the protein kinase B (PKB) and atypical protein kinase C λ and ξ (PKC λ ξ) are activated (these are serine/threonine kinases).→ 160 kDa protein-substrate of Akt (designated AS160) is phosphorylated by activated Akt. AS 160 with activated PKC λ ξ stimulates GLUT4 storage vesicles (GSVs) → GLUT4 translocation from GSVs to the cell membrane is stimulated → PTP1B dephosphorylates insulin receptor (IR) and inactivates it – inhibitory effect on insulin action → phosphatase and tensin homolog on chromosome 10 (PTEN)-A lipid phosphatase hydrolyses phosphatidylinositol 3-4-5 triphosphate to PIP2 which antagonizes the PI3K pathway-the negative regulator of the PI3K pathway – inhibitory effect on insulin action.

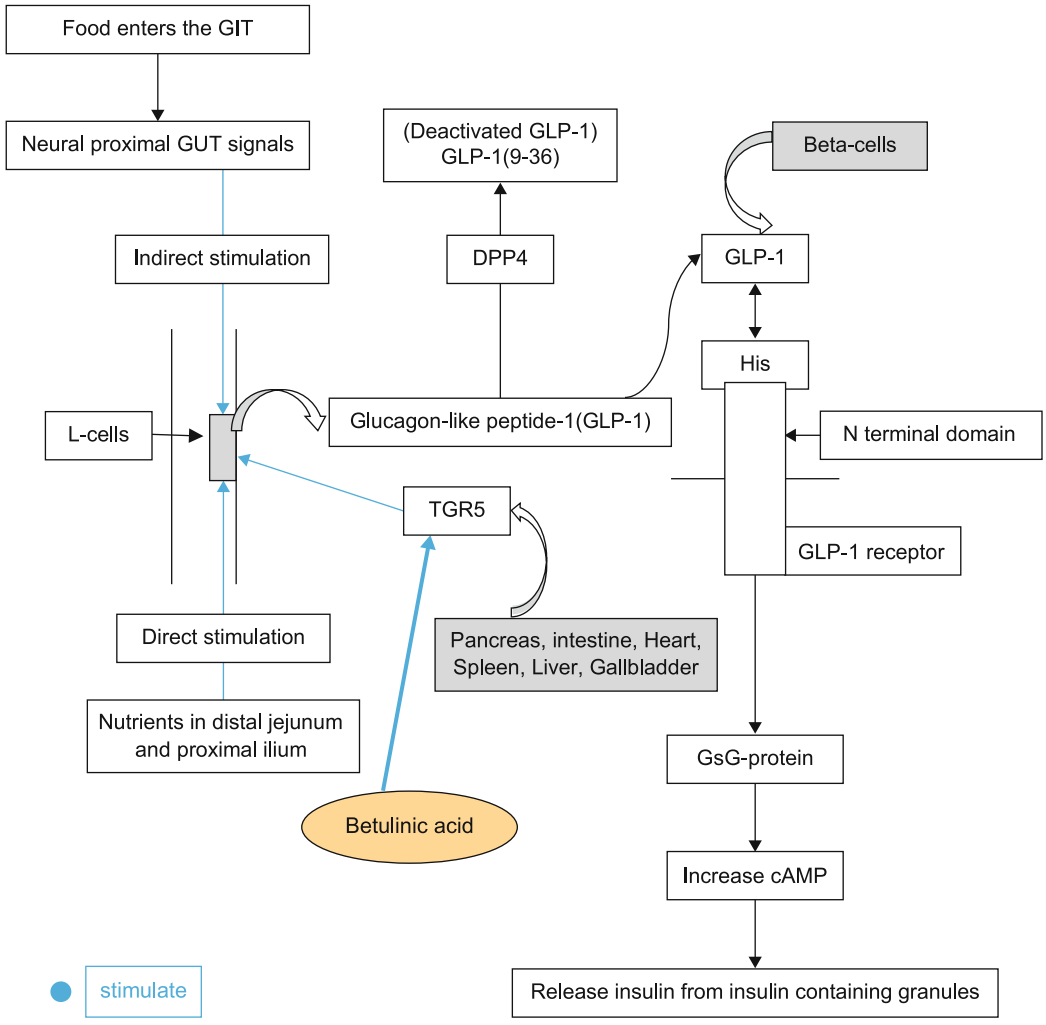

Fig. 6. Betulinic acid in stimulating insulin release by activation of TGR5 receptors. GLP-1, Glucagon-like peptide-1; DPP4, dipeptidyl peptidase-4; TGR5, Takeda-G-protein-receptor-5; cAMP, Cyclic adenosine monophosphate.

When insulin is bound to the α subunit of the insulin receptor, the tyrosine residues of the β subunit of the IR get phosphorylated and the insulin signalling cascade is initiated. But the phosphorylation of these tyrosine molecules is reversible and rapid dephosphorylation takes place even with the continuous availability of insulin. This is an important regulatory step in the insulin signalling cascade and Protein Tyrosine Phosphatase 1 B (PTP-1B) is the dephosphorylating enzyme here. Therefore PTP-1B delivers an inhibitory effect on insulin signalling and enhanced activity of PTP-1B leads to insulin resistance (19).

Betulinic acid can deliver an inhibitory effect on PTB-1B and prevent dephosphorylation of IR and maintain the active state of IR for a longer period (20) (Fig. 5).

The stimulatory effect on TGR5 receptors

Takeda-G-protein-receptor-5 (TGR5) which is also known as G protein-coupled bile acid receptor 1 (GPBAR1) belongs to the G-protein coupled receptors (GPRs) family and is composed of seven transmembrane domains. It is present in many tissues including the gall bladder, pancreas, intestine, stomach, liver, lung, and spleen. It is a salient regulator of many metabolic processes including glucose and energy metabolism. Secretion of Glucagon-like peptide 1 (GLP-1) from L cells of the intestine is stimulated by activation of TGR5 (21).

Bile acids (BA), specially lithocholic acid (LCA), conjugated and unconjugated deoxycholic acid (DCA), chenodeoxycholic acid (CDCA), and cholic acid, activate TGR5. But 6-ethyl-23 (S) methyl cholic acid (6EMCA/ INT-777) which is the specific BA for glucose homeostasis, specifically stimulates TGR5 inducing GLP-1 secretion from the L cells of the intestine, especially distal jejunum and proximal ileum (22).

The incretin effect which describes why higher insulin secretion occurs with oral glucose administration than with similar level intravenous glucose administration accounts for two gut-born

incretin hormones namely, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). These two hormones result in almost 60 % of insulin release after meals.

GLP-1 which is a peptide hormone with 30 residues plays a key role in the regulation of insulin secretion and increasing insulin sensitivity. Following consumption of nutrients, GLP-1 is secreted from the endocrine L cells of the intestine and two biologically active GLP-1 forms have been identified-GLP-1 (7–37) and GLP-1 (7–36) amide. These two are the two forms recognized by the pancreatic receptors. GLP-1 receptors are located in the Brain, pancreatic beta cells, lung, and cardiovascular system. GLP-1 which has a very short half-life in vivo is degraded by dipeptidyl peptidase-4 (DPP-4) to GLP-1 (9–36) amide soon after secretion and its biological activity is reduced. Therefore, an antihyperglycemic activity can be exerted by stimulating GLP-1 receptors and inhibiting DPP-4. The proximal-distal endocrine loop which is a complex neuroendocrine loop composed of the enteric nervous system, vagus nerve, and glucose-dependent insulinotropic peptide (GIP), regulates GLP-1 secretion. The factors involved in GLP-1 secretion include Nutrients such as saccharides, fibre, proteins, and glutamine, diseases such as type2 diabetes mellitus and non-alcoholic fatty liver disease (NAFLD), drugs such as acarbose and metformin, and bariatric surgery including Roux-en-Y and sleeve gastrectomy (23).

The GLP-1 receptor (GLP-1R) is a glycoprotein and comprises two domains, the extracellular N-terminal (NTD) and the transmembrane and intracellular C-terminal. GLP-1R is made up of seven transmembrane (7TM) bundles with seven α-helices (TM1-TM7). The seven α-helices are separated by three intracellular loops (ICL1-ICL3). The activated GLP-1R increases intracellular cAMP signalled through Gs G-protein and this is the main insulin secretion driver (24).

Increased cAMP levels in the β cells of the pancreas result in an increased cytochrome c oxidase (Cox) activity causing an increase in ATP/ADP ratio which leads to plasma membrane depolarization. Due to this membrane depolarization, calcium-gated voltage channels (Ca_v) are opened causing calcium influx. This increased intracellular calcium directly interacts with exocytotic proteins in the insulin-containing granule membrane and the plasma membrane of the pancreatic β cells resulting in insulin release (25).

Betulinic acid stimulates TGR5 and increases GLP-1 secretion and this increase occurs in a dose-dependent manner (26) (Fig. 6).

Conclusion

Nelumbo nucifera rhizome is composed of many bioactive components with antidiabetic effects namely, Betulinic acid, Tryptophan, Nuciferin, Isoquercetin, Rutin, β-Sitosterol, Stigmasterol, Neferin, and Fibre. Of these components, Betulinic acid that belongs to the triterpenoid group exerts its antidiabetic effect via seven bio-physiological mechanisms including, delivering an inhibitory effect on α-glucosidase and α-amylase enzymes, upregulating the expression of PGC1-α, enhancing AS160 phosphorylation, stimulating AMPK activation, stimulating synthesis, and translocation of GLUT4, delivering an inhibitory effect on the PTP-1B protein activity preventing dephosphorylation of IR and exerting a stimulatory effect on TGR5 receptors resulting an increased release of insulin from insulin-containing granules. Composing a single document with the existing scattered scientific knowledge on the antidiabetic effect of lotus rhizome allows the medical practitioners of various medical practices to understand the scientific basis of the medicinal value of lotus rhizome for diabetes mellitus and to conduct clinical trials to prove it scientifically. Even though the knowledge of the antidiabetic property of nelumbo nucifera rhizome is originally gained from Sri Lankan native medicine, as the current scientific knowledge can explain its antidiabetic property with scientific evidence, even the western medical practitioners can use this knowledge for the management of diabetic patients under their care.

Learning points

1. Lotus rhizome is found to have nine bioactive compounds with an antidiabetic effect namely, Betulinic acid, Tryptophan, Nuciferin, Isoquercetin, Rutin, β-Sitosterol, Stigmasterol, Neferin, and Fibre.

2. Betulinic acid is found to deliver its hypoglycaemic effect via seven molecular-level mechanisms including, inhibiting α-glucosidase and α-amylase, upregulating the expression of peroxisome proliferator-activated receptor gamma coactivator-1 α, enhancing AS160 protein phosphorylation, stimulating adenosine monophosphate-activated protein kinase activation, stimulating Glucose transporter type 4 synthesis and translocation, inhibiting Protein Tyrosine Phosphatase 1 B activity preventing dephosphorylation of insulin receptor and stimulating Takeda-G-protein-receptor-5 resulting in an increased release of insulin from insulin-containing granules

References

1. Samarakoon SM. Indigenous Medicine in Sri Lanka-History, Diversity and Current Developments. 2019.

2. Weragoda PB. The traditional system on medicine in Sri Lanka. J Ethnopharmacol 1980; 2 (1): 71–3. https: //pubmed.ncbi.nlm.nih.gov/7464187/.

3. Katulanda P, Constantine GR, Mahesh JG, Sheriff R, Seneviratne RDA, Wijeratne S et al. Prevalence and projections of diabetes and prediabetes in adults in Sri Lanka – Sri Lanka Diabetes, Cardiovascular Study (SLDCS). Diabet Med 2008; 25 (9): 1062–1069.

4. Illangasekera U, Nugegoda DB, Perera LS. Prevalence of diabetes mellitus and impaired glucose tolerance in a rural Sri Lankan community. Ceylon Med J 1993 Sp; 38 (3): 123–126. http: //europepmc.org/abstract/MED/7828231

5. Chen S, Lin B, Gu J, Yong T, Gao X, Xie Y et al. Binding Interaction of Betulinic Acid to α-Glucosidase and Its Alleviation on Postprandial Hyperglycemia. Molecules 2022; 27 (8).

6. Li X, Bai Y, Jin Z, Svensson B. Food-derived non-phenolic α-amylase and α-glucosidase inhibitors for controlling starch digestion rate and guiding diabetes-friendly recipes. LWT 2022; 153: 112455. https: //www.sciencedirect.com/science/article/pii/S002364382101608X.

7. Jeon SM. Regulation and function of AMPK in physiology and diseases. Exp Mol Med 2016; 48 (7): e245.

8. Song TJ, Park CH, In KR, Kim JB, Kim JH, Kim M et al. Antidiabetic effects of betulinic acid mediated by the activation of the AMP-activated protein kinase pathway. PLoS One 2021; 16 (4): e0249109.

9. Oakhill JS, Chen Z-P, Scott JW, Steel R, Castelli LA, Ling N et al. β-Subunit myristoylation is the gatekeeper for initiating metabolic stress sensing by AMP-activated protein kinase (AMPK). Proc Natl Acad Sci USA 2010; 107 (45): 19237–19241.

10. Larance M, Ramm G, James DE. The GLUT4 code. Mol Endocrinol 2008; 22 (2): 226–233.

11. Duncan JG. Peroxisome proliferator activated receptor-alpha (PPARα) and PPAR gamma coactivator-1alpha (PGC-1α) regulation of cardiac metabolism in diabetes. Pediatr Cardiol 2011; 32 (3): 323–328.

12. Arumugam R, Horowitz E, Lu D, Collier JJ, Ronnebaum S, Fleenor D et al. The Interplay of Prolactin and the Glucocorticoids in the Regulation of β-Cell Gene Expression, Fatty Acid Oxidation, and Glucose-Stimulated Insulin Secretion: Implications for Carbohydrate Metabolism in Pregnancy. Endocrinology 2008; 149 (11): 5401–5414. https: //doi.org/10.1210/en.2008-0051.

13. Treebak JT, Glund S, Deshmukh A, Klein DK, Long YC, Jensen TE et al. AMPK-Mediated AS160 Phosphorylation in Skeletal Muscle Is Dependent on AMPK Catalytic and Regulatory Subunits. Diabetes 2006; 55 (7): 2051–2058. https: //doi.org/10.2337/db06-0175.

14. Tan S-X, Ng Y, Burchfield JG, Ramm G, Lambright DG, Stöckli J et al. The Rab GTPase-activating protein TBC1D4/AS160 contains an atypical phosphotyrosine-binding domain that interacts with plasma membrane phospholipids to facilitate GLUT4 trafficking in adipocytes. Mol Cell Biol 2012; 32 (24): 4946–4959.

15. Mohankumar SK, Taylor CG, Zahradka P. Domain-dependent modulation of insulin-induced AS160 phosphorylation and glucose uptake by Ca2+/calmodulin-dependent protein kinase II in L6 myotubes. Cell Signal 2012; 24 (1): 302–308. https: //www.sciencedirect.com/science/article/pii/S0898656811002956.

16. Stöckli J, Fazakerley DJ, James DE. GLUT4 exocytosis. J Cell Sci 2011; 124 (Pt 24): 4147–4159.

17. Olson AL. Regulation of GLUT4 and Insulin-Dependent Glucose Flux. ISRN Mol Biol 2012; 2012: 1–12.

18. Rosen OM. Structure and Function of Insulin Receptors. Diabetes [Internet]. 1989; 38 (12): 1508–11. https: //doi.org/10.2337/diab.38.12.1508.

19. Choi K, Kim YB. Molecular Mechanism of Insulin Resistance in Obesity and Type 2 Diabetes. Korean J Intern Med 2010; 25: 119–129.

20. Behl T, Gupta A, Sehgal A, Albarrati A, Albratty M, Meraya AM et al. Exploring protein tyrosine phosphatases (PTP) and PTP-1B inhibitors in management of diabetes mellitus. Biomed Pharmacother 2022; 153: 113405. https: //www.sciencedirect.com/science/article/pii/S0753332222007946.

21. Guo C, Chen WD, Wang YD. TGR5, Not Only a Metabolic Regulator. Front Physiol 2016; 7: 646.

22. Pols TWH, Noriega LG, Nomura M, Auwerx J, Schoonjans K. The bile acid membrane receptor TGR5: a valuable metabolic target. Dig Dis 2011; 29 (1): 37–44.

23. Wang X, Liu H, Chen J, Li Y, Qu S. Multiple Factors Related to the Secretion of Glucagon-Like Peptide-1. Horowitz M, editor. Int J Endocrinol 2015; 2015: 651757. https: //doi.org/10.1155/2015/651757.

24. Donnelly D. The structure and function of the glucagon-like peptide-1 receptor and its ligands. Br J Pharmacol 2012; 166 (1): 27–41.

25. Thomas C, Gioiello A, Noriega L, Strehle A, Oury J, Rizzo G et al. TGR5-mediated bile acid sensing controls glucose homeostasis. Cell Metab 2009; 10 (3): 167–177.

26. Yun Y, Zhang C, Guo S, Liang X, Lan Y, Wang M et al. Identification of Betulinic Acid Derivatives as Potent TGR5 Agonists with Antidiabetic Effects via Humanized TGR5 (H88Y) Mutant Mice. J Med Chem 2021; 64 (16): 12181–12199.

27. Ríos JL, Máñez S. New Pharmacological Opportunities for Betulinic Acid. Planta Med 2018; 84 (1): 8–19.

28. Silva FSG, Oliveira PJ, Duarte MF. Oleanolic, Ursolic, and Betulinic Acids as Food Supplements or Pharmaceutical Agents for Type 2 Diabetes: Promise or Illusion? J Agric Food Chem 2016; 64 (15): 2991–3008.

29. Sato H, Genet C, Strehle A, Thomas C, Lobstein A, Wagner A et al. Anti-hyperglycemic activity of a TGR5 agonist isolated from Olea europaea. Biochem Biophys Res Commun 2007; 362 (4): 793–798.

Received March 1, 2023.
Accepted April 4, 2023.