## 2.1 Insulin resistance and T2DM

Diabetes mellitus is a multifactorial disease that has of late, plagued the world to such an extent that the World Health Organisation (WHO) has already declared it as an epidemic disease. It occurs due to the imbalance of glucose homeostasis in the body. The pathogenesis of T2DM is complex which involves the development of insulin resistance leading to a number of conditions such as lowered insulin secretion from the β- cells, excess production of glucose from the liver and finally, glucose tolerance and other metabolic disorders[1,2].

Insulin resistance precedes the onset of T2DM. Some non diabetic individuals also exhibit insulin resistance[3]. It occurs mainly due to the disruption of the normal insulin signaling pathway. Lipids or saturated free fatty acids are a major factor for the development of insulin resistance and T2DM. It is worth mentioning that the elevated levels of the circulatory free fatty acid induce severe insulin signaling defects which ultimately leads to insulin resistance. Previous reports suggest that the excessive accumulation of lipid is associated with development of T2DM[4]. The biochemical and molecular mechanism of FFA induced insulin resistance is still only partly resolved. Lee, et al., 2006 reported that diets containing high concentrations of saturated fatty acids increase the contents of the skeletal muscle diacylglycerol (DAG) and ceramide which finally lead to insulin resistance. In contrast to this, a diet that is rich in polyunsaturated fattyacid has no impact in insulin sensitivity[5]. Obese patients are more prone to insulin resistance due to the impaired action of insulin in inhibiting hepatic glucose production, and uptake of glucose in the muscle and the adipose tissue[6,7]. Lipid deposition in liver leads to benign steatosis, which is closely associated with insulin resistance and T2DM. In the muscle cells, insulin resistance alters the distribution of energy storage leading to dislipidemia and other metabolic syndromes. Patients with T2DM often display signs of abnormal lipid metabolism. This is characterised by excess of plasma FFA level, which in turn reduces the insulin stimulated glucose uptake and thus, impairs insulin sensitivity[8,9,10,11]. It has been reported that fatty acids inhibit insulin stimulated glucose uptake in multiple cell types. Saini, 2001 reported that the elevated levelsof intracellular fatty acid metabolites activate

the serine kinase cascade which ultimately downregulates the insulin signaling pathway and leads to the development of insulin resistance and T2DM[12].

## 2.2 Insulin signaling pathway

Upon the binding of insulin to the insulin receptor (IR), activation of insulin receptor occurs which sequentially activates the tyrosine (Tyr) residues of its β subunit by the action of tyrosine kinase through autophosphorylation followed by phosphorylation of the Insulin Receptor Substrate (IRS) proteins. The activated IRS proteins generate downstream signals by the direct binding to the SH2 domains of various signaling proteins[13]. There are four types of IRS proteins. But in this signaling cascade only IRS1 and IRS2 phosphorylate phosphatidylinositol 4, 5 bisphosphate (PIP2) and form a three phosphate complex phosphatidylinositol 3, 4, 5 triphosphate (PIP3) with the help of PI3 kinase activity. This leads to the activation of phosphoinositide-dependent kinase-1 (PDK1), a ser/thr kinase. The activated PDK1 activates ser/thr kinase AKT/PKB through phosphorylation. AKT also contains a pleckstrin homology (PH) domain which helps AKT in direct interaction with PIP3. Akt plays an important role by linking GLUT4, the insulin-dependent glucose transporter protein, to the insulin signaling pathway. GLUT4 activated by Akt moves to the cell surface and helps in glucose infusion into the cell[14]. The overall insulin signaling pathway is shown in the Fig 2.1

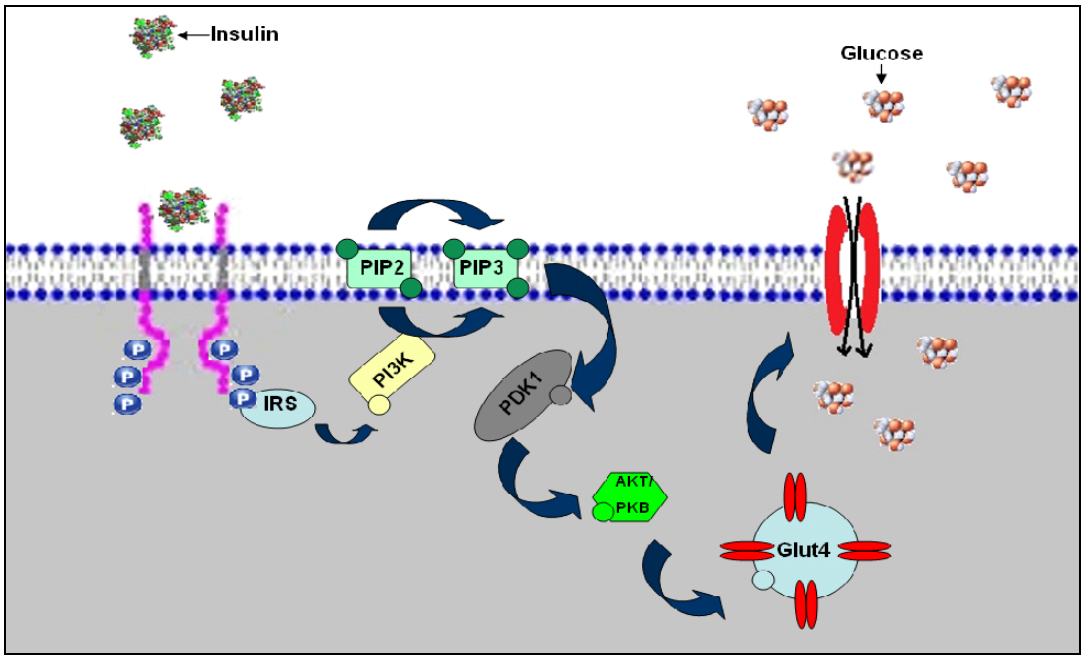


**Figure 2.1:The insulin signaling pathway**

## 2.3 Molecules involved in insulin signaling pathway

### 2.3.1 Insulin receptor

The insulin receptor (IR) is a heterotetrameric glycoprotein present across the plasma membrane. It contains two extracellular α subunits and two intracellular β subunits. The α subunits are the sites of insulin binding and β subunits are responsible for kinase activity. The binding of insulin to α subunits of IR leads to the trans-phosphorylation of tyrosine residues present in the β subunits. The tyrosine phosphorylation on the activation loop of the β subunits induces the catalytic activity of the kinase[15]. This transmembrane receptor is present virtually in all types of cells with different concentrations[16]. IR is an important regulator of cell differentiation, growth and metabolism[17]. It is a type of receptor tyrosine kinase which plays an important role in metabolic regulation. For their activity, receptor tyrosine kinases are allosterically regulated by their related ligands and they perform their function by forming dimers. Unlike the insulin receptor, these dimers are non-covalent. Only the insulin receptor tyrosine kinase is bonded covalently forming a disulfide bond between it's α subunits. It also activates a large substrate of protein through phosphorylation to engage the effector molecules.

### 2.3.2 Insulin Receptor Substrate

Insulin receptor substrate (IRS) is one of the most important proteins involved in the insulin signaling pathway. Four types of IRS have been identified with different patterns of expressions in different tissues[18]. The activated insulin receptor upon binding withinsulin induces the phosphorylation of IRS proteins on multiple tyrosine residues. The activation of IRS serves as a docking site for other downstream signaling mediators involved in the insulin signaling pathway. For e.g., activated IRS1 activates phosphatidylinositol- 3 kinase (PI3K)[19]. The phosphoryaltion of IRS at different residues also facilitates the different metabolic functions inside the body. For e.g., phosphorylation of IRS1 at serine residues is associated with obesity induced insulin resistance[20]. Several reports demonstrate that the elevation of some metabolites like free fatty acid, diacylglycerol (DAG), ceramide, etc. are responsible for serine phosphorylation on IRS1 which induces the activation of inhibitor κβ kinase (IKK) for the initiation of some other metabolic signaling cascades[21].

### 2.3.3 PI3K

The phosphoinositide 3-kinase (PI3K) is a group of proteins that is responsible for cell survival, growth, metabolism and glucose homeostasis[22]. This group of kinases phosphorylate the 3$^{rd}$ carbon of the inositol group in the phosphoinositides (PI), a membrane phospholipid. On the basis of its structure and function, PI3K can be classified into three classes[23]. The class 1 PI3K has been found to be more important than the others two classes. The Pleckstrin Homology (PH) domain of PI3K allows that protein to bind to PIP2 or PIP3. This interaction regulates the protein's activity by controlling and localization of the protein-membrane association. The formation of PIP3 helps in bringing phosphoinositide dependent kinase1 (PDK1) to the plasma membrane for binding. The binding of PDK1 with PIP3 helps PIP3 in bringing itself towards the close proximity of certain proteins including AKT with the help of PH domain.

### 2.3.4 AKT/ PKB:

The protein kinase B (AKT) belongs to the second messenger sub-family of protein kinases[24]. There are three isoforms of PKB, *viz.*, PKBα, PKBβ, and PKBγ that have been identified to possess three different domains: an amino terminal PH domain, a central domain and a carboxy terminal regulatory domain. These 3 isoforms of PKB are also known as AKT1, AKT2 and AKT3 respectively[26, 25]. Among them, AKT1 shows no compromise in insulin sensitivity and AKT2 is sensitive to higher concentration of insulin and responds normally[26]. The PKB (AKT) serves as the major protein of receptor tyrosine kinase (RTK) present downstream to RTK in the signaling pathway. The activated PI3K leads to the recruitment of PKB to the membrane and it is activated by phosphorylation at two different residues present in two different sites. The two phosphorylation sites of PKB are Thr308/309 present in the A-loop in the kinase domain and Ser473/474 at the carboxy terminal region. The kinase that activates AKT at Thr308/309 is termed as 'PI dependent protein' and the kinase that phosphorylates AKT at Ser 473/474 is termed as 'integrin linked kinase'. The activation of AKT/PKB plays an important role in glucose metabolism, transcriptional control and also at apoptotic regulation in different types of cells[27].

### 2.3.5 Glucose transporters

Glucose enters into the cells through a specific transporter, an ATP independent protein called glucose transporter (GLUT). Depending upon the different characteristics such as *Km* for maximal glucose transport and insulin dependency, GLUT proteins are subdivided into many classes. They are also present in different cell types according to their functional specificity. For example, most brain cells, having GLUT1 as the principal transporter protein, are able to move glucose intracellularly at very low blood glucose concentrations without the need for insulin. On the other hand, in the muscle and the adipose cells GLUT4 is the major glucose transporter protein, which requires insulin for its action having higher *Km* for glucose.

### 2.3.5.1GLUT1

GLUT1 is a major glucose transporter of human erythrocyte. It was earlier believed to be the glucose transporter expressed in brain, but it is virtually present in all cell types[28,29,30]. It is also found to be expressed highly in blood tissue barriers like blood-brain barrier, retina, placenta, etc[31,32,33]. On the basis of GLUT1 regulation by glucose, it is placed into a family of glucose-regulated proteins responsible for various cellular stresses[34,35]. The regulation of GLUT1 is found both in transcriptional and translational levels[36,37]. In several cell types, the GLUT1 protein expression and its activity is more in the glucose deprived condition, as can be seen in the case of bovine microvessel endothelial cells[38]. In whole brain preparation, GLUT1 is detected in two distinct isoforms of molecular weight 55 kDa and 45 kDa. Both the isoforms are transcribed from the same gene, but due to the post translational modification, it exists in two different isoforms[30].The distribution pattern of these two isoforms is different in different cell types.

### 2.3.5.2 GLUT2

The GLUT2 is the primary bidirectional transporter in mammalian liver. It is also found in the surface of small intestine, kidneys and pancreatic β cells[39,40]. In some selected population of astrocytes, GLUT2 serves the function of glucose sensing[41]. It is also detected in rat retina[42].

### 2.3.5.3 GLUT3

The function of GLUT3 is to mobilise the glucose molecules in neuron cells and the gene has been successfully cloned from the brain of mouse, rat, chicken and dog[43,44,45,46]. It is also expressed in non neuronal sites like placenta, sperm and human platelets. The sequence of GLUT3 is species specific. There is significant diversity in the GLUT3 sequence across species. Aberrant GLUT3 expression occurs in two situations, namely, in human gliomas and in HIV infected lymphocytes[47].

### 2.3.5.4 GLUT4

It is the only insulin dependent glucose transporter found mainly in the insulin responsive tissues like muscle, heart and adipocyte. In these tissues, binding of insulin to its target cell receptors elicits the translocation of cytoplasmic GLUT4 to plasma membrane[48,49]. Although the central role of GLUT4 is to maintain the glucose homeostasis, it is also involved in hormonal, nutritional and metabolic regulation of the body[34,37]. There are several reports on the expression of GLUT4 in brain including hypothalamus, pituitary cerebellum, olfactory bulb, etc., of rat and mouse[50,51,52,53].

### 2.3.5.5 GLUT5

GLUT5 is considered primarily as the fructose transporter and not glucose transporters. In the small intestine, testis and human sperm, the concentration of fructose is relatively high and GLUT5 acts as a primary transporter in these cells. From the data obtained from the study in *Xenopus* oocyte and transfected CHO cells, glucose is not effectively transported through GLUT5[54,55,56,57,58]. GLUT5 is also found in the human neutrophils and macrophages as well as both to human and rat brains at lower levels.

### 2.3.5.6 GLUT6

GLUT6 has sequence homology with other GLUTs, is not likely to be a functional proteins. It's gene is considered as a pseudogene with several stop codons and frame shift in it[56].

### 2.3.5.7 GLUT7

GLUT7 is the glucose transporter in the liver endoplasmic reticulum. For its function it forms complex with glucose-6-phosphatase. This complex is also found in a discrete population of human astrocytes[59,60].

Depending upon the distribution and their function, there are 5 more GLUT isoforms present in cells. The summary of the glucose transporters along with their sites of expression and major functions are tabulated below (Table 2.1).

**Table 2.1: Summary of the properties of facilitative Glucose Transporters[61]**

| Protein | Major isoform (aa) | Major sites of expression | Proposed function |
|---|---|---|---|
| GLUT1 | 492 | Ubiquitous distribution in tissues and culture cells | Basal glucose uptake; transport across blood tissue barriers |
| GLUT2 | 524 | Liver, islets, kidney, small intestine | High-capacity low-affinity transport |
| GLUT3 | 496 | Brain and nerves cells | Neuronal transport |
| GLUT4 | 509 | Muscle, fat, heart | Insulin-regulated transport in muscle and fat |
| GLUT5 | 501 | Intestine, kidney, testis | Transport of fructose |
| GLUT6 | 507 | Spleen, leukocytes, brain | Not clearly defined |
| GLUT7 | 524 | Small intestine, colon, testis | Transport of fructose |
| GLUT8 | 477 | Testis, blastocyst, brain, muscle, adipocytes | Fuel supply of mature spermatozoa; Insulin-responsive transport in blastocyst |
| GLUT9 | 511/540 | Liver, kidney | Urat homeostasis[62] |
| GLUT10 | 541 | Liver, pancreas | Mitochondrial DHA transporter[63] |
| GLUT11 | 496 | Heart, muscle | Muscle-specific; fructose transporter |
| GLUT12 | 617 | Heart, prostate, mammary gland | Basal and insulin-independent glucose transporter in the heart[64] |
| HMIT | 618/629 | Brain | H+/myo-inositol co-transporter |

## 2.4 Insulin resistance in major glucose storage cells

### 2.4.1 Insulin resistance in skeletal muscle

Since skeletal muscle cells accounts for 70-75% of glucose storage, defects in these cells impact heavily on the maintenance of glucose homeostasis[65]. An individual affected with T2DM shows a decrease in the peripheral glucose uptake, predominantly in the skeletal muscles, up to nearly 90% lesser than the non-obese subjects[66]. In the skeletal muscle of T2DM subjects, the insulin stimulated Tyr phosphorylation is impaired in IRS1 and PI3K while it is not seen in normal or non-obese T2DM subjects[67]. Apart from that, the AKT activation is also impaired in the T2DM patients. The mechanism of this impairment may include kinase dependent ser/thr phosphorylation, proteosome mediated degradation of IRS1 along with the dephosphorylation by the action of phosphatase[68,69,70,71]. This ultimately induces the abnormalities in the insulin signaling pathway and impairs insulin dependent glucose uptake in the skeletal muscle cells[67]. Randle et al., 1963 proposed the relationship between elevated free fatty acid and insulin resistance in the muscle tissue[72]. In 1987, Storlien et al., suggested that the increased level of lipid storage in the muscle elicited by the fat rich diet is one of the responsible factors for muscle insulin resistance[73]. It is also worth mentioning that, patients with T2DM have decreased fat oxidative capacity and have elevated level of circulatory free fatty acid in their blood plasma[74,75,76]. This causes the impairment of insulin stimulated glucose uptake *via* accumulation of lipid inside the muscle cells[77,78]. So, reduced fat oxidation and metabolic inflexibility plays an important role in developing insulin resistancein the skeletal muscle cells.

### 2.4.2 Insulin resistance in adipose tissue

Initially the adipose tissue was considered as an inert tissue. The only known function of the adipose tissue was thought of as an energy storage depot[79,80]. But recently, the adipose cells have been established as dynamic endocrine cells as these cells produce various bioactive adipokines and adipocytokines some of which target the metabolic and immune systems[79,80]. Adipokines like adiponectin, leptin, visfatin, resistin affect the insulin sensitivity in other insulin target cells and develop obesity induced insulin resistance[81,82,83]. Several cytokines including

TNFα, IL6, IL1 and MCP1 are associated with the impairment of insulin action leading to insulin resistance and T2DM[84,85,86]. The expression of GLUT4 is downregulated in adipocyte of T2DM patients, thereby lowering the glucose uptake by adipose tissue. Nevertheless, GLUT4 knockout from adipocyte in mice develops insulin insensitivity which is similar to the mice with knockout GLUT4 from the muscle tissue[87,88,89]. On the other hand, exposure of the cells to TNFα and elevated FFA induce the serine phosphorylation of IRS1 instead of the insulin stimulated IRS1 phosphorylation at the tyrosine residue. This causes the inhibition of the downstream signaling in the insulin signaling cascade[90,91,92]. It has been reported that both adipocyte as well as adipose tissue macrophage (ATM) develop insulin resistance by promoting inflammation in both endocrine and paracrine fashion[83].

### 2.4.3 Insulin resistance in liver cells

Liver is the major organ involved in the production, consumption and storage of glucose and lipid. Liver helps in glucose metabolism by the formation of glycogen or glucose from the non-sugar carbon substrates[93]. In physiological conditions, several metabolic pathways are regulated to maintain glucose as well as lipid homeostasis, like *de novo* synthesis of fatty acids, cholesterol and oxidation of fatty acids[94]. The hepatic glucose production is mainly regulated by insulin. Therefore, impaired insulin sensitivity and its activity in the liver contribute to the development of T2DM[95]. The regulation of insulin signaling in liver cells, IRS1 and IRS2 play an important role. These two proteins are also involved in the expression of genes of gluconeogenesis, glycogen synthesis along with lipid metabolism[95]. So, dysregulation of IRS1 and IRS2 contribute to the development of postprandial hyperglycemia, dysregulated lipid metabolism, increased glucose production from hepatocyte and ultimately leads to the development of T2DM.

## 2.5 Molecular basis of free fatty acid induced insulin resistance

### 2.5.1 Phosphorylation of IRS1

Though FFA is known to be one of the major players in insulin resistance**,** the actual mechanism of its action is still not clear. The elevated concentration of intracellular FFA activates various serine kinase cascades and impairs the insulin

signaling pathway downstream to the insulin receptor. Multiple proteins are involved in the insulin signaling pathway and they are activated by the action of various kinases.The deactivation of these proteins in insulin signaling pathway leads to insulin resistance. In the insulin signaling pathway the most important protein is the insulin receptor substrate 1 (IRS1) and phosphorylation of IRS1 at tyrosine residue leads to the activation of the insulin signaling pathway. However, the phosphorylation of the serine residues of IRS1 by the action of serine kinase, disables it to attract PI3 kinase and blocks the insulin signaling pathway, leading to the destruction of IRS1 protein[96]. This serine phosphorylation of IRS1 reduces the tyrosine phosphorylation impairing the downstream effectors. It is reported that the hyper serine phosphorylation of IRS1 is found in the insulin resistant rodents as well as young lean offsprings from T2DM parents. Elevated FFA also induces proinflammatory cytokines that are responsible for serine phosphorylation of IRS1 protein and thereby causes insulin resistance.

### 2.5.2 Activation of PKCs

The molecular mechanism underlying insulin resistance also depends on the activity of protein kinase Cs (PKC). There are several PKC isoforms present and are classified into three groups, (a) classical PKC (cPKC) which are activated by $Ca^{+2}$ and diacylglycerol (DAG); (b) novel PKC (nPKC) that is activated by DAG only, and (c) atypical PKC (aPKC), which neither depends on $Ca^{+2}$ nor DAG[97]. Among all the PKCs, nPKCs plays major role in the development of insulin resistance. The PKC isoforms that consists of nPKC are- nPKCθ, ε, δ and η[98]. Several reports suggest that elevated FFA and lipid infusion impairs insulin stimulated glucose uptake in the muscle by activating PKCθ and PKCδ. PKCε is responsible for free fatty acid induced impaired glucose disposal[97]. It has been observed that, PDK1 directly phosphorylates all the nPKCs except PKCε. PKCε can be activated by PDK1 independent palmitoylation induced by FFA. PKCε activation leads to its translocation to the nucleus and suppresses the insulin receptor (IR) gene expression[99]. The activated PKCε also induces the expression of NFκB and as a result it induces the expression of the proinflammatory genes which contribute to the development of insulin resistance[100,101].

### 2.5.3 Transcriptionalinhibition of Insulin Receptor (IR)

It has been reported that the promoter region of the human IR gene contains two unique AT rich sequences and is positively regulated by the transcription factor, HMGA1 (High Mobility Group of Proteins A1). HMGA1 binds to the AT rich regions of IR promoter and changes its conformation to recruit other transcriptional factors, i.e., SP1 and cEBPβ to the transcription start site. But in presence of elevated FFA, the activated PKCε enters into the nucleus and activates HMGA1 which in turn inhibits the binding of HMGA1 to the IR promoter region. Thus, no additional transcription factors are recruited to the promoter region resulting in reduced expression of the IR gene and subsequentlylead to insulin resistance[99].

### 2.5.4 Mitochondrial dysfunction

Mitochondrial dysfunction is also one of the important factors for insulin resistance. Elevated levels of glucose and FFA reduce mitochondrial biogenesis by increasing the rate of mitochondrial dysfunction. The mitochondrial dysfunction in the muscles of obese and insulin resistant patients is found to be more than the lean control subjects. The oxidative enzyme activities are also found to be decreased in the obese and insulin resistant patients[102,103,104]. A microarray study reported that the mitochondrial biogenesis and oxidative phosphorylation are downregulated in the obese and T2DM patients in comparison to the healthy control[105,106]. Decreased level of mitochondrial mRNA at both the genes and protein levels in the T2DM patients has been reported[107,108,109,110]. It is also reported that in presence of high level of FFA, the size of the mitochondria becomes smaller and compact in nature, which in turn attenuates the expression of some genes involved in metabolic activities. In the muscle cells, reduced expression of PGC1α (PPARγ co-activator 1α) and UCP1 (uncoupler protein 1) effect the normal mitochondrial functions. The elevated level of FFA also alters the expression of oxidative phosphorylation subunits and Mfn-2. In the adipocytes also, higher levels of FFA and glucose reduce the level of Mfn-1 protein along with the downregulation of NRF1 thereby affecting the mitochondrial function. PGC1α plays an important role in glucose homeostasis by regulating the GLUT4 gene along with the regulation of the genes involved in

oxidative phosphorylation which is also found to be reduced in the muscles of diabetic patients.

### 2.5.5 Inflammatory pathways

Growing evidences link obesity induced inflammatory state to the development of insulin resistance and T2DM. Sustained low grade inflammation induces the elevation of proinflammatory cytokines which precedes the development of T2DM[111]. Lipid accumulation in adipocyte activates the c-Jun N terminal kinase (JNK) and nuclear factor κ B (NFκB) pathways which induces the production of proinflammatory cytokines such as tumor necrosis factor (TNFα), interleukin 6 (IL6) and others. TNFα causes insulin resistance by enhancing adipocyte lipolysis and modulates ser/thr phosphorylation of IRS1[112,113,114]. It also activates JNK1/2 and causes insulin resistance in the visceral adipocyte. IL6 plays an important role in reducing the expression of GLUT4 and also reduces the expression of IRS1[115]. It occurs through the JAK-STAT pathway by increasing the expression of SOCS3 (suppressor of cytokine signaling 3). It is reported that it reduces the glucose uptake by blocking the PI3K pathway. It downregulates the expression of microRNA200s (miR-200s) resulting the suppression of glycogen synthesis[116]. In the human skeletal muscle cells, IL6 activates STAT3 which increases the gene expression of TLR4 [117]. The inflammasome activated proinflammatory cytokine interleukin-1β (IL-1β) impairs insulin signaling in the peripheral tissue and in the macrophages resulting in insulin resistance. It also plays its role in initiating and maintaining inflammation induced organ dysfunction in T2DM[118].

These cytokines induce insulin resistance following NFκB and JNK pathway mainly. Elevated levels of FFA induce the expression of Fetuin A (FetA) in the adipocyte and liver cells which in turn bind tothe TLR4 receptor and activate the NFκB pathway[119,120]. After stimulation of this pathway by various pathogenic stimuli and FFA binding Fetuin A, the IKK complex is activated which triggers the phosphorylation of IkBα on Ser32 and Ser36. This activation of IkBα leads to the autodegradation activity and triggers NFκB to the nucleus and induces the expression of the proinflammatory genes. In the JNK pathway, FFA leads to the

serine phosphorylation of IRS-1 by stimulating through endoplasmic reticulum (ES) stress[121]. Several reports suggest that by inhibiting the JNK activity, the release of proinflammatory cytokines can be suppressed.

Adipocytes and macrophages have been found to be the primary sources of inflammatory cytokines. Monocyte chemoattractant protein-1 (MCP-1) plays a crucial rolein the pathogenesis of insulin resistance[122]. The expression of MCP-1 in adipocyte induces the recruitment of macrophage called ATM (adipose tissue macrophage) and dendritic cells which in turn exacerbates inflammation induced insulin resistance[123]. MCP-1/CCR2 axis contributes in polarization of macrophages from M2 to M1 state leading to the production of proinflammatory cytokines[124].

### 2.5.6 Macrophage infiltration and their polarization

Macrophages are heterogenic innate immune cells found in most tissue types. These cells are involved in immuno surveillance for early signs of infection or tissue damage. A defining character of the cells of the macrophage-monocyte lineage is their considerable diversity and plasticity which is not lineage specific. Depending on the stimuli of the tissue microenvironment, the circulation of monocytes with low phagocytic and inflammatory activities upon homing into tissue spaces may undergo either of the two distinct and mutually exclusive activation programs: (i) classical activation and (ii) alternative activation giving rise to M1 and M2 macrophage phenotypes, respectively. Classical activation is mediated by the products derived from bacterial infection like bacterial LPS and IFNγ. M1 macrophages are proinflammatory, highly phagocytic and bactericidal. They are involved in progression of inflammatory diseases like rheumatoid arthritis, inflammatory bowel disease, insulin resistance and atherosclerosis owing to their pro-inflammatory nature[125]. Alternative activation is mediated by products of parasitic infection and cytokines such as IL-4 and IL-13. The M2 macrophages are involved in the resolution of inflammation and are responsible for tissue remodelling and repair. The difference between M1 and M2 type macrophages is best understood by the differences in their arginine metabolism. The M1 cells, catabolize arginine to NO and citrulline *via.* iNOS whereas the M2 cells upregulate arginase 1, which produces urea and ornithine that are necessary for collagen

synthesis and cellular proliferation, respectively[126,127].Obesity-associated chronic low-grade inflammation in the adipose tissue is a major focal point in the pathogenesis of insulin resistance and type 2 diabetes[83]. Obesity is associated with an increased infiltration of macrophages into the white adipose tissue (WAT). This increase in the representation of macrophages from 10% to 50% (or greater) of total cell number, when accompanied by a fivefold or-greater increase in overall adipose tissue mass, dictates that nearly all adipose tissue macrophages (ATMs) in obese individuals are recruited after the onset of weight gain[128,129]. The population of macrophages within lean and obese adipose tissue appear to be very dissimilar in their activation. The adipose tissue macrophages (ATM) of lean mice were found to express many characteristic of the genes of M2 or "alternatively activated" macrophages[130].Therefore obesity is implicated with the phenotypic switch from an anti-inflammatory M2 polarization state to the proinflammatory M1 polarization in the adipose tissue which at the same time corresponds to the development of insulin resistance[131]. Subsequent studies have demonstrated that the prevention of the macrophage M1 polarization state improved insulin sensitivity. This provides a potential link between inflammation and insulin resistance[132].

The milieu of proinflammatory cytokines (IL-1, IL-12, IL-6 and TNF-α), ER stress, elevated levels of saturated fatty acids and necrosis of adipocytes contribute to the classical activation of macrophages. These events are congruent with the chronic activation of the central regulators of inflammation, nuclear factor-κB (NF-κB) and Jun N-terminal kinase 1 (JNK1), in the adipose tissue from the obese and the insulin-resistant subjects[83,133,134]. JNK1 and inhibitor of NF-κB–kinase ß, IKKß, (NF-κB activating kinase) directly phosphorylate serine residues on the insulin receptor substrate 1, which is an abnormal inhibitory phosphorylation thereby mediating insulin resistance[135,136,137]. It was demonstrated by Karin and colleagues that JNK1 deletion from the non hematopoietic cells sufficiently protected the mice from the diet-induced obesity, while deletion of JNK1 from the hematopoietic compartment decreased hepatic and adipose tissue inflammation and improved insulin sensitivity without affecting adiposity. This suggests that diet-induced inflammation, not obesity, is directly responsible for insulin resistance and is mediated primarily by bone marrow–derived cells[138]. Myeloid-specific deletion of IKKß and loss was found to reduce inflammation.

Similarly loss of JNK-1 from the hematopoietic compartment achieved similar lowering of inflammation[139]. Given the fact that macrophages out number all other cells of myeloid lineage in the adipose tissue, they can be easily placed in the nexus of diet induced inflammation.

The population of macrophages within the lean and the obese adipose tissue appear to be very dissimilar in their activation. ATMs of the lean mice were found to express many genes characteristic of the M2 or the "alternatively activated" macrophages. These M2 macrophages have the ability to restrain their classically activated counterparts and inhibit classical activation. Metabolism plays both an instructive and provisional role in the activation of macrophages. Classically activated macrophages need to execute their functions over hours and days of activation while alternatively activated macrophages have a functional lifetime of months. Therefore the M1 cells induce a glycolytic program while the M2 cells induce fatty acid oxidation and oxidative metabolism. Overexpression of the PGC-1ß (peroxisome proliferator–activated receptor γ (PPARγ) coactivator 1ß) induces alternative activation while preventing classical activation of macrophages[140]. Instructive cues for alternative activation are provided by IL-4/STAT6 signaling while the maintenance is carried out by PPARs. The transcription factors PPARγ and PPARδ are fatty acid sensors and are responsible for sustaining the alternative activation in an overlapping but nonredundant manner. PPARγ is involved in the regulation of the metabolic program while PPARδ regulates the M2 immunologic effector functions and suppression of classical activation[141,142]. Importantly, the unsaturated dietary fatty acids such as oleic acid synergize with IL-4 to drive the alternative response by acting as metabolic substrates and by transcriptionally activating PPARδ. Elevated saturated fatty acid concentrations inhibit PPARδ transcriptional activity even as they activate antagonistic, proinflammatory pathways via ligation of TLR4[142].

## 2.6 Type2 Diabetes Mellitus and Medications

Antidiabetic drugs may be subdivided into the following groups:

- insulin (Humulin, Novolin),
- sulfonylureas (Glimepiride),
- alpha-glucosidase inhibitors (Acarbose, Voglibose),

- biguanides,
- meglitinides (Repaglinide and nateglitinide),
- thiazolidinediones (rosiglitazone, pioglitazone)
- GLP -1 analogues (liraGLUTide)
- DPP (IV) inhibitors (vildagliptin, sitagliptin, saxagliptin)

### 2.6.1 Insulin

Insulin is the hormone responsible for regulating glucose utilization. It is effective in both types of diabetes, since, even in insulin resistance, some sensitivity remains and the condition can be treated with larger doses of insulin. Most insulin's are now produced by the recombinant DNA techniques, and are chemically identical to natural human insulin. Isophane insulin suspension, insulin zinc suspension, and other formulations are intended to extend the duration of insulin action, and permit glucose control over longer periods of time.

**Precaution and side effects**

The greatest short term risk of insulin is hypoglycaemia, which may be the result of either a direct overdose or an imbalance between the amount injected and the extent of exercise and kind of diet. This also may occur in the presence of other conditions such as illness with vomiting and diarrhea which reduce the glucose load. Allergic reactions and skin reactions may also occur. Insulin is classified as category B in pregnancy, and is considered the drug of choice for glucose control during pregnancy. Insulin is not recommended during breast feeding because the low or the high doses of insulin may inhibit milk production.

### 2.6.2 Sulfonylureas

The primary mechanism of action of the sulfonylureasis enhancement of insulin secretion. These agents reduce glucose by increasing insulin secretion through direct action on the $K_{ATP}$ channel of the pancreatic β cells in patients with residual β cells function. Binding of these agents to $K_{ATP}$, result in more positive electric potential over the β cell membrane by hyperpolarisation efflux of $K^+$ ion. This depolarisation helps in opening of the voltage gated $Ca^+$ channel and induces the secretion of insulin. This group of drugs include chlorpropamaid, tolazamile,

acetohexamide and tolbutamide (first generation sulfonylureas); and glyburide, glypizide and glymepiride (second generation sulfonylureas). In the newly diagnosed T2DM patients, good results have been achieved with mild to moderate fasting hyperglycemia, good β cell functioning as reflected by a higher fasting C-peptide concentration.

**Precautions and side effects**

No sulfonylureas are currently approved for the use of children. The major adverse affect associated with sulfonylureas is hypoglycaemia. This may potentiate the hypoglycaemia associated with alcohol use. So, the consumption of alcohol is contraindicated when a person is taking sulfonylureas class of drugs. Other adverse effects including nausea, vomiting and skin reaction including rashes, purpurea and pruritus are also seen in the persons taking this class of drugs. The occurrences of leukopania, thrombocytopenia, haemolytic anaemia and cholestasis have also been reported. Weight gain is common to those taking these drugs.

### 2.6.3 Meglitinides

Meglitinides, the derivatives of benzoic acid helps in stimulating insulin release. This class of drugs comprises of two different members, Repaglimide (prandin) and Nateglitimide (starlix). Repaglitimide is the first non-sulfonylureas introduced in the early 1998s. Like sulfonylureas, it also increases insulin secretion from the β cells by binding to the $K_{ATP}$, but the response is quicker and of shorter duration than that of sulfonylureas. It is a suitable option for patients with severe sulfa allergy who are not the candidates for sulfonylureas therapy. This drug is used as monotherapy and in combination with metformin.

**Precautions and side effects**

Similar to the sulfonylureas, it causes weight gain and hypoglycaemia. These drugs are not suitable during pregnancy as it shows fetal abnormalities in rabbits at the similar doses prescribed for human subjects. It is also not recommended for nursing mother.

### 2.6.4 Biguanides (Metformin)

Biguanides lower blood glucose level primarily by decreasing hepatic glucose output and reducing insulin resistance[143,144].Metformin, a biguanide, has become the most commonly used agent for T2DM in children and teenagers. It is the only widely used oral drug that doesn't cause weight gain. It is used as monotherapy or in combination with sulfonylureas for the management of T2DM. When used as monotherapy, Metformin doesn't cause hypoglycaemia. The use of metformin decreases intestinal absorption of glucose and increases peripheral glucose uptake.

**Precautions and side effects**

The use of metformin increases the chance of causing gastrointestinal reactions in the patients. Sometimes it causes the reactions of lactic acidosis also[145].The risk of metformin can be reduced by careful renal monitoring and dose adjustment.

### 2.6.5 Thiazolidinediones

The thiozolidinediones are a unique drug class of "insulin sensitizers" that promote insulin stimulated skeletal muscle cell and adipose tissue glucose uptake with a lesser effect on the hepatic glucose uptake. They bind to Peroxisome Proliferated Activated Receptor γ (PPAR-γ), a type of nuclear regulatory protein involved in transcription of glucose regulatory genes and gene of fat metabolism. These PPARs act on Peroxisome Proliferator Responsive Element (PPRE). The PPREs influence insulin sensitive genes which enhance mRNA production of the insulin dependent enzymes. Thiozolidinediones lower the plasma insulin level and are used for the treatment of T2DM. This class of drugs is composed of two members, Rosiglitazone (Avandia) and Pioglitazone (Actos)[146]. For the action of these drugs, insulin is required, and hence thiozolidinedione is not suitable for the treatment of T1DM. This class of drugs raise the HDL cholesterol and lower the triglycerides.

**Precautions and side effects**

The drug Rosiglitazone was banned by the Food and Drug Administration (FDA) in 2011 due to its side effects. It causes myocardial infarction and heart disease related deaths. It has been reported that after the treatment for 1-16 months with Pioglitazone and Rosiglitazone, some patients develop edema and congestive heart failure[147]. Therefore it is important to monitor regularly the liver function when patients are prescribed these two drugs.

### 2.6.6 α-Glucosidase Inhibitors

Alpha-glucosidase inhibitors, such as acarbose (Precose) and Miglitol (Glyset) are not involved in insulin secretion. Instead, they inhibit the breakdown of disaccharides and complex carbohydrates to glucose and delay the absorption of the monosaccharides from the gastrointestinal (GI) tract. These agents are used as monotherapy or in combination with sulfonylureas for the management of Type 2 diabetes.

**Precautions and side effects**

The best part of this class of drugs is they do not cause hypoglycaemia. But the most common problems of these drugs are the GI problem, like diarrhoea, abdominal pain and flatulence. Also, α-glucosidase inhibitors are contraindicated in patients with inflammatory bowel disease, partial intestinal obstruction, colonic ulceration, etc. Dose dependent hepatotoxicity is associated with these drugs, so liver function test should be carefully monitored in the patients receiving higher doses of these medications.

### 2.6.7 Incretinmimetics

Incretinmimetics mimic glucose dependent insulin secretion, suppress elevated level of glucagon secretion and delay gastric emptying. These include the glucagon like peptide (GLP) analog Exenatide (Byetta). It improves glycemic control in patients with T2DM by enhancing glucose dependent insulin secretion from the pancreatic β cells, suppresses inappropriately elevated glucagon secretion and slows gastric emptying. The drug's 39 amino acid sequence partially overlap

that of the human incretin glucagon like peptide 1. It is indicated as adjunctive therapy to improve glycemic control in patients with T2DM, who are taking Metformin or Sulfonylureas drug without glycemic control.

**Precautions and side effects**

The common adverse affects of this drug include upper respirating tract infection, nasopharyngitis and headache.

### 2.6.8 DPP 4 (Dipeptidyl Peptidase 4) inhibitor

Dipeptidyl Peptidase 4 (DPP 4) inhibitors block the action of DPP 4, which is known to degrade incretin. Sitagliptin (Januvia) is the first drug that comes under DPP 4 inhibitors. It blocks the enzyme DPP 4, which is known to degrade incretin hormones. It increases the concentration of active intact incretin hormone (GLP-1 and GIP). The hormones stimulate insulin secretion in response to increased blood glucose levels following meals. This action enhances the glycemic control. It is used as monotherapy or in combination with Metformin or a PPAR-γ agonist i.e. thiozolidinediones.

**Precautions and side effects**

The main adverse effects of this drug include upper respiratory tract infection, nasopharyngitis and headache.

### 2.6.9 Amylin analogue

This class of drugs have endogenous amylin effects by delaying gastric emptying, decreasing post prandial glucagon release, and modulating appetite. They have all the incretin actions except stimulation of insulin release. Pramlintide is the only clinically available amylin analog. Like insulin, it is administered by subcutaneous injection.

**Precautions and side effects**

The most frequent and severe adverse affect of pramlintide is nausea, which occurs mostly at the beginning of treatment which gradually gets reduced.

**Table 2.2.** Anti-diabetic drugs and their molecular targets.

| Drug Class | Molecular Target | Action |
|---|---|---|
| Insulin | Insulin Receptor | Hypoglycaemia |
| Sulphonylureas (glibenclamide-nateglinide ) | Pancreatic -cell | Increase insulin secretion |
| Metformin | Exact target not known, acts on Liver | Reduce hepatic glucose production |
| Sitagliptin (Januvia) Vildagliptin (Jalvus) | Dipeptidyl peptidase-4 (DPP-4) in pancreatic islet's | Inhibits DPP-4 to enhance insulin secretion |
| Thiazolidinediones (Pioglitazone, Rosiglitazone) | PPAR | Enhance insulin action on muscle, liver and fat |
| Acarbose | Intestine ( glucosidase ) | Inhibit glucose absorption through gut |

## 2.7 New antidiabetic drug in the pipeline

Though there are several drugs available for the treatment of T2DM, majority of these drugs cannot maintain the normal blood glucose level at chronic state. Many new drugs are in the pipeline for the treatment of T2DM including SGLT-2 (sodium glucose co-transporter-2) inhibitor, glycogen phosphorylase inhibitor, GPR-119 (G-protein coupled receptor 119) agonists, 11β-HSD (11 β-hydroxysteroid dehydrogenase) inhibitor, PTP1B (protein tyrosine phosphatise 1B) inhibitor, glucokinase activator and glucagon receptor antagonists.

### 2.7.1 Sodium glucose co-transporter-2 inhibitor

These types of antidiabetic agents work in the kidney to balance glucose homeostasis through an insulin dependent manner. Two major glucose co-transporters are SGLT-1 and SGLT-2 where SGLT-2 is responsible for >90% renal

glucose reabsorption[148,149,150]. Inhibition of this type of glucose co-transporters decrease the reabsorption of filtered glucose and maintain glycemic control by lowering the plasma glucose concentration.

Dopagliflozin is the most advanced agent of SGLT-2 inhibitor class of drugs which is in phase 3 clinical trial. This agent can be used as monotherapy or with Metformin or Glimepiride, or with Insulin. But the use of this drug, small increase in blood urea nitrogen and hematocrit were observed[151,152,153].

### 2.7.2 Glycogen phosphorylase inhibitor

The endogenous glucose production inside the body is accounted by liver where it produces 90% of the total glucose. During insulin resistance and T2DM, elevated level of hepatic glucose is produced. So glycogen phosphorylase is one of the major targets for this class of drugs to inhibit the proteolytic cleavage of glycogen into glucose-1- phosphate[154]. CP-91149 is one of the drugs of this class inhibiting glycogen phosphorylase by binding to the inhibitory site of this target and hence it reduces the plasma glucose level[155,156]. Another inhibitor of glycogen phosphorylase is CP-320626 which inhibits the activity of glycogen phosphorylase by binding to its allosteric site instead of inhibitor site[157].

### 2.7.3 Agonist of G-protein coupled receptor 119

G-protein coupled receptor 119 (GPR-119) is a long chain fatty acid receptor mainly expressed in the pancreatic β cells[158]. By binding to its agonist ligand, GPR-119 increases the level of cAMP and induces the release of insulin. Activated GPR-119 also induces the release of GLP-1 and gastric inhibitory peptide and hence it could be a potential target for T2DM drugs. One promising GPR-119 agonist is AR231453. It activates GPR-119 facilitating binding to $G_{\alpha s}$ and the complex increases the release of cAMP in the cultured HEK293 cells. Oral treatment with AR231453 gives the better glycemic control than intravenous treatment. AR231453 controls glucose in T2DM patients in several complementary pathways[158].

### 2.7.4 11 β- hydroxysteroid dehydrogenase inhibitor

This is an enzyme that converts cortisone to cortisol in the target tissue resulting in insulin resistance[159]. It is found to be unregulated in the adipose tissue of T2DM patients. So its inhibition exerts beneficial effect for the treatment of T2DM. It improves the lipid profile and hepatic insulin sensitivity along with the fasting glucose level in human diabetic patients[160]. The most suitable drug of this class is INCB13739[161]. It demonstrated a significant suppression of the fasting plasma glucose and HBA1c on treatment for 12 weeks. All the drugs of this class are in phase 2 clinical trial.

### 2.7.5 Glucokinase activator

Glucokinase is a monomer, present in the liver and the pancrease that determines the glucose metabolism in response to the glucose level present in the blood. In presence of the elevated level of glucose, the glucokinase level in pancrease increases and as a result it increases the insulin release from pancreatic the β cells[162]. Glucokinase has been found to be reduced in the liver of T2DM patients and the reduction is approximately 50% [163]. Therefore, to activate glucokinase for more insulin production, its activators play an important role. Several glucokinase activators are present ranging from glucokinase activator 1 to glucokonase activator 14. They increase the activity of glucokinase by binding to its allosteric site and increase its activity. But the major drawback of this class of drugs is they induce moderate hypoglycaemia due to the increased rate of insulin release[164].

### 2.7.6 Protein tyrosine phosphatase 1B inhibitor

Protein tyrosine phosphatase 1B (PTP1B) is a member of the Protein tyrosine phosphatise (PTP) family which plays its role in removing phosphate group from the tyrosine phosphate receptor. In the insulin signaling pathway, it removes several insulin receptor kinase substrates and provides negative regulation of the insulin signaling cascade[165]. It also downregulates leptin signaling pathway by dephosphorylating the Janus kinase 2 pathway. Hence, it has been found to be an important molecule for the insulin and leptin signaling pathway. Several PTP1B

inhibitors have been developed, but the major problem is the lower affinity and selectivity for the enzyme. Membrane permeability is one of the other drawbacks of PTP1B inhibitors[166].

## 2.8 Natural products for the treatment of diabetes

The importance accorded to the use of herbal medicine or phytomedicine is reflective of the diverse plethora of botanical resources at our disposal which dates back to the very early civilizations. In recent times, increasing use of herbal medicines and supplements has been observed. The global market value of such herbal medicines has grown exponentially and is estimated to be 5 trillion by 2050[167]. Due to the economic restraints and unavailability of the conventional drugs, about 80% populations in the middle and low income countries of Asia and Africa use traditional medicine for primary healthcare.Even in the high income countries, the use of this form of medicine is increasingly becoming popularwith about 65% of the population reportedly using these medicines[168]**.** India has been reported as the highest user of traditional medicine (TM) but on the contrary, contributes less than 2.5% to the global market. In the contrary, > 60% market share is being controlled by the European Union and North America while 16% is shared by Japan and rest 19% by the Asian countries[169,170].

Herbal medicines used for healthcare or as dietary supplements contain active chemical concoctions extracted from the underground or the aerial plant materials or in combinations thereof. Although the risk of using TM is relatively low, it comes with its own set of challenges, *viz.*, proper identification of the plant species, its safety and effectiveness and the risk inducing interactions when TM used in conjunction with conventional drugs. Another major drawback of these medicines is limited bioavailability, i.e., being poorly absorbed if taken orally[171,172]. Thus it is crucial to understand the mechanism of the mode action of the TM. Moreover, pre clinical and clinical trials are crucial prior to the adaptation of any herbal medicine.

Presently, herbal formulations have gained extensive acceptability as therapeutic agents for diabetics, liver diseases, arthritics, cough remedies, adoptogens and memory enhancers[173]. Nevertheless, there are only a few numbers

of standardized herbal drugs due to lack of implementation protocols and regulatory standards. The authentication of the plant product at its origin is crucial for standardization. This involves adoption of good agricultural practices [9], proper collection strategies from the wild and high quality manufacturing practices for extraction and related parameters[174]. In addition, concentration of the active principle and the defined quantities of the active components in poly herbal formulations are vital for a plant product prior to its acceptance as lead compound for future candidate drug[175, 176, 177, 178]. The regulatory approvals to ascertain consistent chemical profile and biological activity of future drug candidate includes a) quality assurance by determining adulterants, pesticides residue, aflatoxin content, bacterial/fungal growth and heavy metals contamination; b) prevention of adverse reactions by evaluating pharmacodynamics, pharmacokinetics, dosage, stability, self-life and toxicity (acute/ chronic); c) reproducibility by repetitive testing, using different batches to control batch-to-batch variation and development of standard assay markers and; d) chemi-informatic approaches to ensure that the pharmacological profile match with the activity profiles of the active constituents of the drug itself[179].

Traditionally known antidiabetic plants might be useful sources of novel oral hypoglycemic compounds or as easy dietary adjuncts to the existing treatment. In the non-insulin-dependent diabetes mellitus (NIDDM), Sulfonylureas and Metformin are important drugs. However in most cases, these drugs fail to reduce or reinstate the normal pattern of glucose homeostasis. The presently available therapies are limited by their accompanying side affects, secondary failure rates and most importantly, by their pharmacokinetic properties. The fundamental biochemical abrasion remains unchanged in a diabetic patient as even the most common insulin therapy only partially attempts to reimburse for the metabolic disturbances. On the other hand, an increased insulin treatment may elevate the risks of hypoglycaemia and atherogenesis[180,181,182] .

Although a herbal alternative to insulin seems dubious, new compounds to stimulate endogenous insulin biosynthesis and secretion (and to promote insulin action) are practical possibilities. There are extensive reviewsonthe present status

of medical and scientific research with the use of traditional plant based medicine for management of diabetes mellitus[183].

## 2.9 Use of polyphenols for diabetes treatments

Polyphenols are plant based natural compounds present in the plant based food, fruits, vegetables, whole grains, cereals, legumes, tea, coffee, etc. These compounds are the secondary metabolites of the plants and play their roles in plant defense mechanism[184]. Based on the number of phenol rings and the structural elements associated with the binding of these rings, polyphenolic compounds can be categorised into many groups[185].

Polyphenols are well-known for their medicinal properties/values as they show antioxidant, anti-inflammatory, antimicrobial, antiviral, antiallergic, anti-proliferative, anti-mutagenic properties along with the anti-carcinogenic activities. Moreover, some of the polyphenolic compounds modulate some important cell signaling pathways including NFκB, PI3K, MAPK, ERK, AKT and NRF2, etc[186].

Several polyphenols are reported to play important roles in glucose metabolism, β- cell functioning and also preventing insulin resistance to maintain glycemic control in the body. Due to their anti-hyperglycemic effect without any side-effect, polyphenols are found to be more popular among all the known natural bioactive compounds. Several reports suggest the potential efficacy of polyphenols on glucose homeostasis in *in vitro* and in *in vivo* experimental models supported by results of some clinical trials[187].

Polyphenolic compounds show their effect in reducing intestinal absorption of the dietary carbohydrates and induce the activities of ezymes involved in glucose metabolism to maintain glucose homeostasis in the body. Some polyphenolic compounds also stimulate insulin secretion by improving β-cell function. The antioxidative and anti-inflammatory properties of polyphenolic compounds are also known to reduce hyperglycemia[188,189,190].

Some polyphenols including flavonoid, phenolic acid, tannins inhibit the α-glucosidase and α-amylase activities and reduce the production of glucose from carbohydrates[188, 191]. It is also reported that some polyphenols reduce glucose

absorbance by inhibiting $Na^+$ dependent glucose co-transporters, SGLT1 and SGLT2[192,193]. Several polyphenolic compounds show their hypoglycaemic activity by elevating the action of glucokinase along with the over production of glycogen in the liver in the diabetic rat model[194]. Compounds like quercetin, resveratrol, EGCG induce the AMPK pathway to increase the insulin dependent movement of GLUT4 in the muscle and adipocyte cells[195,196] which was found 50-200 times more effective than the anti-diabetic drug Metfornin. The activation of PI3K signaling pathway by the polyphenolic compounds increases glucose uptake[197]. Several reports suggested the role of polyphenolic compounds in the lipid metabolism. Since insulin resistance is directly related to lipid metabolism,hence,by maintaining lipid metabolism, the polyphenolic compounds can reduce hyperglycaemic condition in the body. The intake of dietary polyphenols along with some bioactive components reduce diabetic complications through the regulation of lipid and lipoprotein metabolism and reduction of oxidative damage[198 , 199 , 200] . Polyphenolic compounds are responsible for the digestion and absorption of dietary lipids along with the absorption of triglycerides[200]. Some polyphenols, including anthocyanin induces hyperlipidemia, hyperinsulinemia, etc. One of the main functions of polyphenolsinlipid metabolism is its activity in preventing lipoprotein oxidation and induced production of advanced glycation end products. Some polyphenols reduce the receptor of advanced glycation end products (RAGE) in the diabetic rat models. They also reduce the expression of NFκB and TGF-β in the myocardial tissue[201].

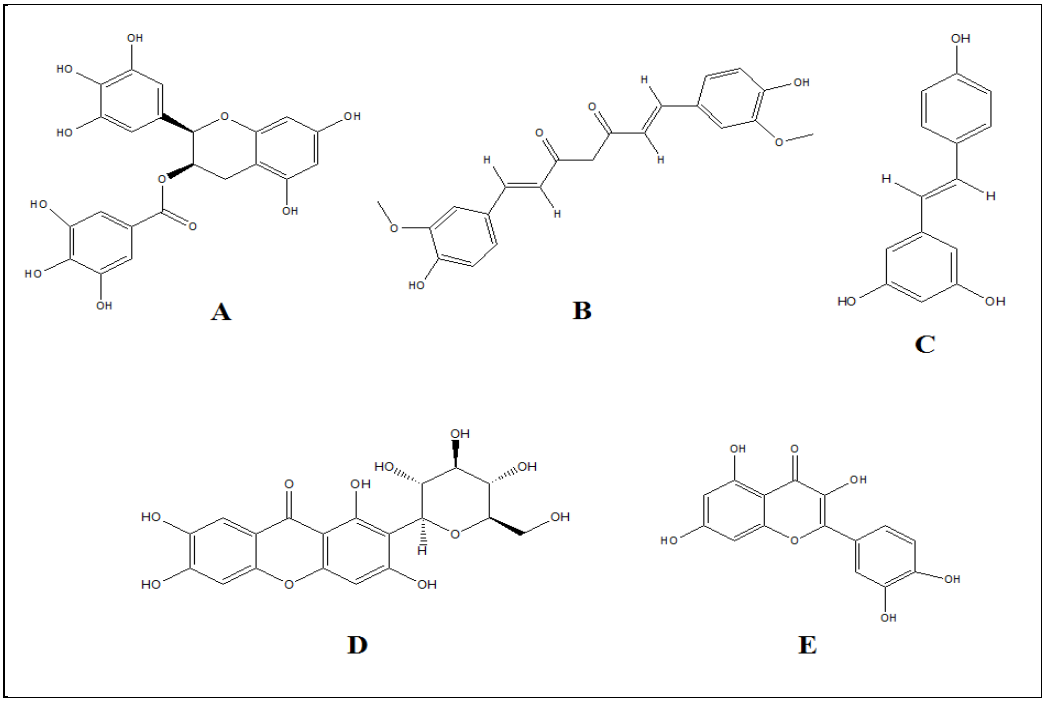


**Figure 2.2: 2D structure of some polyphenols having antidiabetic property**. A. Epigallocatechingallate (EGCG), B. Curcumin, C. Resveratrol, D. Magniferin, E. Quercetin

## 2.10 Flavonoids as antidiabetic agents

Flavonoides are polyphenolic plant secondary metabolites that are common in nature. The flavonoids play an important role in disease prevention. Depending upon their structural diversity they are classified into flavans, flavanones, flavones, flavonols, flavanols, flavanonols, catechin, anthocyanidines and isoflavons. Several reports suggest the presence of beneficial effects of dietary flavonoids on glucose homeostasis[202]. Flavonoids regulate carbohydrate digestion, insulin secretion, glucose uptake and insulin signaling through various siganaling pathways[203] showing the relationship between different flavonoids and T2DM[204]. Epigallocatechin gallate (EGCG) is a flavonol which improved insulin secretion and the viability of the β cells in glucotoxic rat pancreatic β cell line through the insulin receptor (IR)/ IRS-1/ AKT pathway. It also helps in maintaining normal mitochondrial function in the β cells. EGCG also helps in protecting β cells from pro-inflammatory cytokine induced cytotoxicity[205]. In the FFA induced insulin resistant cells, EGCG and EGE protect cells through various signaling pathways including protein kinase C (PKC), AMPK and AMPK/ACC[206,207,208,209]. Naringin

and hesperidin are the two major flavanones which have been reported to be involved in various activities associated with diabetes[210,211,212]. They induce the expression of GLUT 2 and GLUT 4 in liver and in white adipose tissue (WAT). In the liver and in the adipose tissue of db/db mice, naringin and hesperidin activates PPARγ and improves glucose homeostasis[210]. Oral administration of naringin to HFD mice coupled with low dose streptozotocin (STZ) reduces hyperglycemia, hyperinsulinemia and insulin resistance[213]. Naringin also induces phopsphorylation of IRS1 at Tyr162 in liver through increased expression of HSP and PPARγ[214]. Treatment of anthocyanin extract (cyanidine-3-glycoside, delphinidin-3-glucoside and petunidin-3-glucoside) by gavage was reported to increased serum insulin concetration and hence reduced insulin resistance[215]. Cyanidin-3- glucoside was also found to be the inducer of glucose uptake through PPARγ signaling pathway[216] and by reducing expression of inflammatory cytokines[217]. In the muscle cells, isolated quercetin derivatives quercetin-3-O-glucoside and quercetin-3-Ogalactoside from the berry extract induced glucose

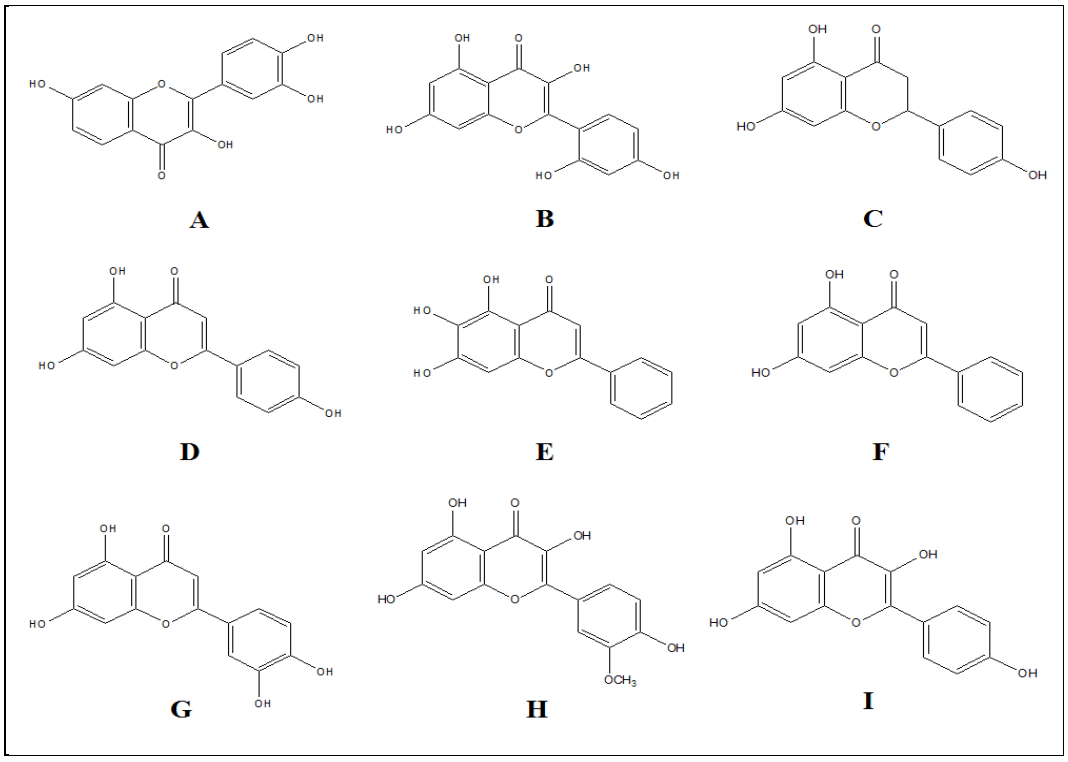


**Figure 2.3: 2D structure of some flavonoids having antidiabetic property.**A. Fisetin, B. Morin, C. Naringin, D. Apigenin, E. Baicalein, F. Chrysin, G. Luteolin, H. Kaemferol, I. Isorhamnetin

uptake in an insulin independent manner[218]. Dai X. et al. 2013 reported the inhibition of oxidative stress and suppression of nitric oxide accumulation by quercetin and quercitrin through the inhibition of NFκB translocation and reduced iNOS gene expression[219]. Apigenin, an another flavonoid has been reported to have anti-hyprtglycemic effect[220,221].*Myrcia multiflora* DC. (family: Myrtaceae) extract containing Myrciacitrins I, II, III, IV and V possessed significant inhibitory activity of aldose reductase [222] . Five 6-hydroxy-flavonoids *viz.* 6-hydroxyapigenin (scutellarein), 6-hydroxyluteolin-7-O-β-D-glucopyranoside, 6-hydroxyapigenin-7-O-βD-glucopyranoside,6hydroxyluteolin-7-O-(6-O-feruloyl)-β-D-glucopyranoside, 6-hydroxyapigenin-7-O-(6-O-feruloyl)-β-D-glucopyranoside from the leaves of *Origanum majorana* L. (family: Lamiaceae) showed inhibitory effect of α-glucosidase activity of rat intestine [223] . The genistein derivatives, 3',5'-Diprenylgenistein, 6',8'-Diprenylgenistein, Derrone and Alpinumisoflavone isolated from *Tetracera scandens* (family: Dilleniaceae) stimulated glucose uptake by inducing the expression of AMPK, GLUT1, GLUT4 and by inhibiting PTP1B in L6 myotubes [224] . Kaempferitrin (Kaempferol-3,7-O-(α)-dirhamnopyranoside), isolated from *Bauhinia forficata* (family: Leguminosae) leaves was reported to have hyperglycaemic effect in both normal and alloxan induced diabetic model[225]. The various cellular effects of dietary flavonoids to maintain glucose homeostasis are represented in the fig 2.4.

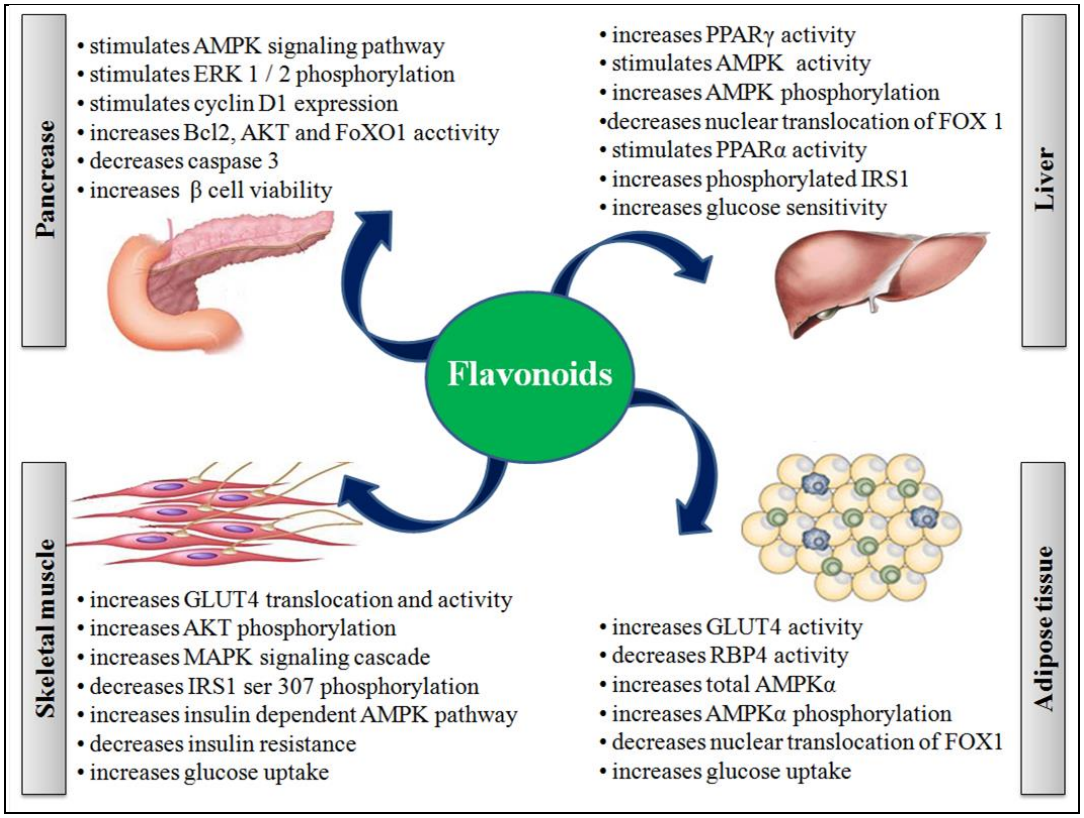


**Figure 2.4:** Dietary flavonoids exert their anti-diabetic effects by targeting various cellular signaling pathways in the pancreas, liver, skeletal muscle and white adipose tissues.

## 2.11 Network pharmacology in drug discovery

In order to discover a new drug for the treatment of the disease as well as for the identification of a new drug target of an existing drug, the identification of drug-target interaction plays an important role. But due to its expensive and time consuming nature, the process has become a challenging task. In this regard, *in silico* approach in predicting drug target interaction provides very efficient and useful information to carry out the experimental validation of such interactions. By the drug target interaction, it becomes easier to understand the disease mechanism. In this context, suitable target identification and validation are the more efficient methods that lead to computer aided drug discovery (CADD).

Several computational tools have been developed. For example, ligand based CADD, structure based CADD, QSAR, pharmacophore modelling and docking, proteochemometrices, polypharmacology modelling, etc. Among all, docking and pharmacophore modelling have become the most popular ones to identify novel compounds against the target through virtual screening studies.

Identification of inhibitors of the human Pin-1 kinase is one of the examples of this method. There are several such examples available in support of this method.

Moreover, a new network based approach has been developed to improve the drug discovery process for complex diseases by studying drug action. In this approach a new network based model can be generated by integrating the concepts of system biology, biochemistry and bioinformatics, etc. System biology is characterized by the involvement of biological components in a particular biological system and gives the idea of their association in functioning the normal metabolic processes and provides the information to understand the disease pathogenesis. This gives the clue to discover new lead molecules and identification of their targets.

Since most of the complex diseases occur due to the defective functioning of distinct gene groups, targeting multiple targets is one of theeffective ways for treatment of the disease. Drugs can impact the entire metabolic system by targeting the interactions of various components of the system. There are several reports on the use of network based polypharmacological approach to understand the disease pathogenesis and the discovery of bioactive components from the traditional medicinal formulations. For example, Yunxio et al, 2016 deciphered mechanism of traditional Chinese Medicine (TCM) formulation, danggui-shaoyao-san and their targets for the treatment of the neurodegenerative disease[226]. Another group of research Yu et al., 2015, identified the genes involved in the signaling pathway of cancer metastasis along with the identification of targets of Mifepristone, a synthetic steroid having anticancer property[227]. Many reports have been published which deal with the decoding of TCM used for the treatment of various diseases by identifying their respective targets. One drug- multiple targets or multiple drugs – multiple targets concept of network pharmacology provides an easier understanding of the disease pathogenesis as well as drug target interaction to develop a new drug entity.

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