Chapter 1
Introduction

1.1 Diabetes: Definition and disease pathology

Diabetes is a hyperglycaemic state of the body, characterized by the imbalance in glucose and lipid homeostasis caused due to the underlying defect in either insulin secretion or its action or both [1]. The global prevalence of diabetes is approximately 430 million thus designating it as an "epidemic" [2]. The slowly progressing prolonged consequences of diabetes are retinopathy, neuropathy and nephropathy [3]. The very early age onset of insulin deficiency due to the defect in pancreatic β-cell function and insulin secretion is defined as type 1 diabetes (T1D) whereas lifestyle-associated impairment of insulin action causing insulin resistance (IR) leads to type 2 diabetes (T2D). T2D has acquired the majority number of cases (90- 95%) in the global diabetic count and thus is the most common type [4]. Obesity is a prime factor in the development and progression of T2D. Clinically, diagnosis of diabetes is carried out by measuring (i) fasting and postprandial plasma glucose (FPG), (ii) oral glucose tolerant test (OGTT), (iii) random plasma glucose level and (iv) haemoglobin A1C level, respectively. Sedentary life style, high calorie food and physical inactivity cause the overweight or obese state which result in a high plasma free fatty acid (FFA) levels, endoplasmic reticulum (ER) stress, oxidative stress as well as imbalanced lipid and glucose homeostasis [5]. All these consequences result in the dysfunction of insulin action known as insulin resistance due to which attenuation of glucose uptake occurs in insulin target cells including skeletal muscle cells, adipocytes and hepatocytes [6].

1.2 Obesity mediated adipose tissue inflammation

Deregulation of fat metabolism and consumption of high fat diet result in overloading of the already existing mature adipocytes with excessive lipid which transforms it into fat laden enlarged adipocytes termed as adipocyte hypertrophy. These hypertrophic adipocytes cause enhanced secretion of pro-inflammatory adipokines (leptin, adiponectin, IL-6, TNF-α) and promotes macrophage infiltration into the pbese adipose tissue wherein both recruited and resident macrophages adopt a polarization state of M1 pro inflammatory phenotype that aggravate leading adipose tissue inflammation [7]. Obesity-associated low grade inflammation is a major cause for the development of T2D, thus, targeting the reduction in adipose tissue inflammation could improve the insulin sensitivity of the inflamed adipocytes. Toll-like receptor 4 (TLR4) signaling and Adenosine receptor (AR) signaling pathways have the ability to regulate this low grade

inflammation as the activation of TLR4 promotes the inflammation whereas stimulation of AR augments the resolution of inflammation.

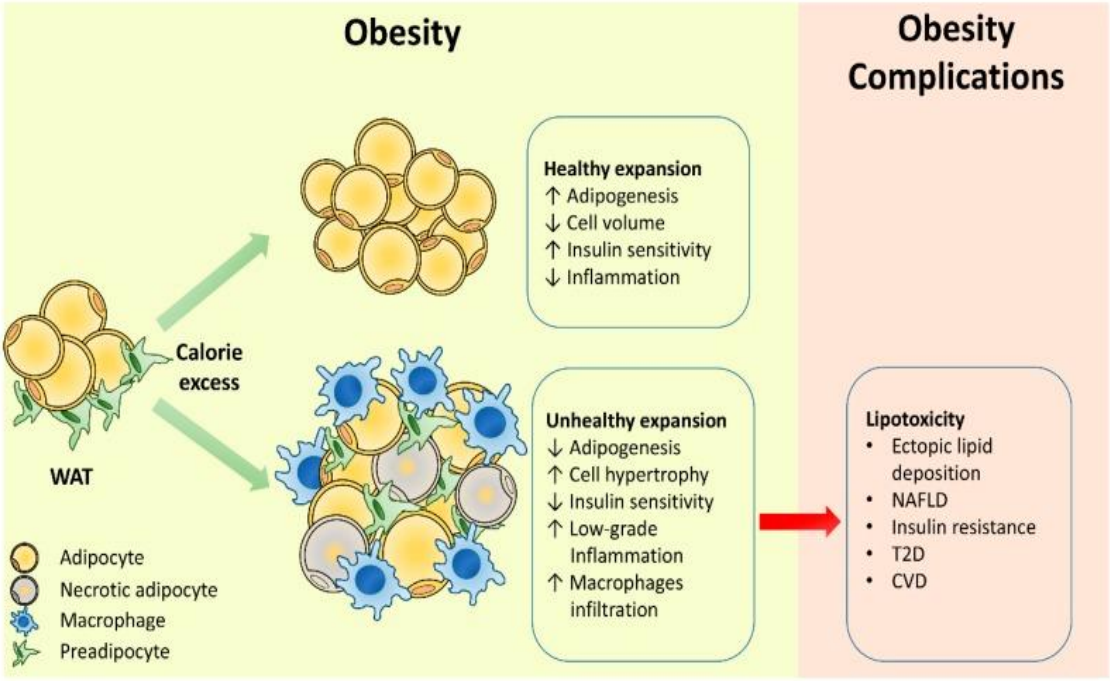


Figure 1.2: A schematic representation of the overview of obesity and related complications (Adopted from [7]).

1.3 Adenosine receptor signalling pathway: Role in inflammation and insulin sensitivity

Adenosine receptor activation is reported to display protective effect against diabetic nephropathy, diabetic neuropathy, improving insulin sensitivity and insulin hormone regulation mainly via its subtypes A_{2A} and A_{2B} [8]. Literature in this field showcases the anti-inflammatory and insulin sensitive role of adenosine receptor (A_{2A} and A_{2B}) signalling pathway [9]. The ligand adenosine which stimulates the activation of adenosine signaling pathway is also reported to improve obesity-mediated insulin resistance [10]. A_{2A}AR activation mediates PKA-mediated immunosuppression, predominantly in immune cells [11]. The anti-inflammatory effect imparted by A_{2A}AR activation via the down regulation of pro-inflammatory cytokine profile has been proven to protect the body from inflammation mediated organ damage in various diseases including diabetic nephropathy and diabetic retinopathy [12, 13]. ATL-146e is a reported A_{2A}AR agonist which prevented the progression of diabetic nephropathy through the blockage of macrophage infiltration and attenuation of pro-inflammatory

TNF-α, MCP-1 and IFN-γ secretion that eventually improved the inflammatory status of the urinary system [14,15]. Furthermore, $A_{2A}AR$ activation also known to attenuate insulin resistance by the enhacement of browning of white adipocytes and the reduction of gluconeogenesis in rat hepatocytes [16]. Although few $A_{2A}AR$ agonists derived from the 5′-N-ethylcarboxamine (NECA) that have anti-inflammatory role such as CGS-21680, ATL-146e, UK-432,097, YT-146 and MRE-0470, but very little is explored in the direction to find out A_{2A}-specific non-toxic agonist that could prevent IR by $A_{2A}AR$ activation [17]. This drives the need for exploring the small molecules phytochemicals on the stimulation of $A_{2A}AR$ signaling pathway and the impairment of IR.

1.4 TLR4 signaling pathway: Role in adipose tissue inflammation and insulin sensitivity

TLR4 signaling pathway one of the major inflammatory pathway that is involved in triggering obesity induced adipose tissue inflammation and IR. TLR4 mRNA expression was observed to be significantly high in the monocytes of glycemic control patients [18]. High glucose and lipid levels in the body strikingly up-regulates the TLR2 and TLR4 protein expression which triggers NF-κB activation-dependent downstream signaling cascade resulting in the enhanced expression and secretion of pro-inflammatory cytokines (IL-1β, TNF-α, IL-6), chemokines (MCP-1 and IL-8) and acute phase protein CRP from adipocytes and macrophages [19,20]. These pro-inflammatory molecules reduce insulin sensitivity by the inhibitory phosphorylation of the insulin receptor substrates 1 and 2 (IRS-1 and IRS-2) at serine/threonine residues thus abrogating insulin signaling pathway [21,22]. Activation of TLR4 also affects the expression of insulin gene in rodent islets of langerhans [23]. Increased abundance of plasma glucose also provokes oxidative stress generating massive amount of reactive oxygen species (ROS) which mediates apoptosis of pancreatic β-cells with loss in insulin secretion in vivo [24]. High level of ROS known to be recognized by TLR4 as damage-associated molecular pattern (DAMPs) which intensifies the inflammatory status and cause chronic inflammation in pancreatic islets [25]. Also, activation of NLRP3 inflammasome by ROS results in the secretion of IL-1β which is a inducer for the successive batch of cytokines and chemokines driving the infiltration of macrophages thus compromising the normal insulin secretion process of pancreatic islets [26, 27]. In adipocytes, FFA induces macrophage polarization marked by the high

ratio of TLR4 mediated pro-inflammatory M1 macrophages to anti-inflammatory M2 macrophages leading to chronic low grade inflammation which eventually progresses to T2D [28]. Higher abundance of TLR4 expression in macrophages than adipocytes indicates that obesity-mediated inflammation in adipose tissue could be due to the TLR4 activation in macrophages [29]. Macrophages with TLR4 ($TLR4^{-/-}$) or MyD88 ($MyD88^{-/-}$) gene deletion rescued the cells from the FFA-induced inflammation thus further supporting the fact that FFA triggers the up-regulation of pro-inflammatory molecules secretion via the activation TLR4 signaling pathway [30]. All these observations suggests that blocking or inhibiting the activation of TLR4 signaling could rescue and improve the inflammation and insulin resistance scenario. A very recent report on an in-silico approach screening the potent TLR4 inhibitor suggested that curcumin analogs have the ability to significantly inhibit TLR4 signaling [31]. Another inhibitor named as MAL/MyD88 inhibitory peptide 2 also shows inhibition to various TLRs including TLR4 [32]. Therefore, natural and plant derived small molecules that are able to block the TLR4 receptor or molecules downstream of TLR4 signaling pathway has become an attractive area of research for the management of various chronic inflammatory diseases including type 2 diabetes.

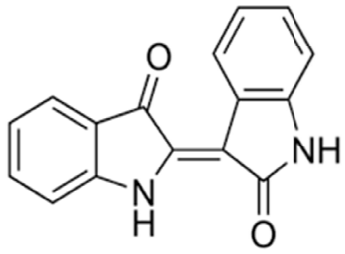
1.5 Natural products and small molecules

Traditional knowledge driven medicines belonging to Indian ayurveda, Chinese or African herbal medicine has been utilized due to their easy accessibility by people since time immemorial [33]. Natural products, particularly phytochemicals, extracted from different plants have been traditionally utilized for the management of various human diseases and to develop various derivatives with reduced toxic side effects, improved pharmacokinetics and enhanced efficacy [34-35]. World Health Organization (WHO) has designated approximately 11% of the 252 natural drug candidates was from plant sources [36]. Almost 5000 plant species are already explored as active drug sources considering valuable insights in modern drug discovery attire as a fair fraction of small molecules derived from the parent product have been reported to have drug-like properties [37]. Several small molecules belongs to the category of alkaloids, glycosides, sugars, lipids were investigated due to their potential as drug candidates [38-39]. Due to the cell membrane diffusibility of small molecules, they are actively able to bind to various cellular targets such as different protein kinases and receptors such as G-

protein coupled receptors (GPCRs), TLRs and fibroblast growth factor receptors [40]. The US FDA declared approximately 89 small molecules acting as protein kinase inhibitors serving as anti-tumour and anti cancer agents. Alongside, many are proved to have evident utilization as antibiotics, anti allergic, anti-mycobacterial, analgesic and anti-malarial [41].

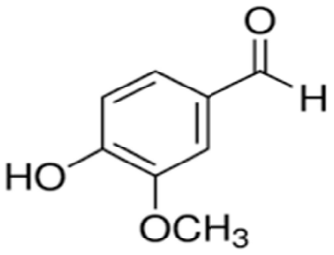
Indirubin, 3,2' bis-indole isomer of indigo is the main component of Indigo naturalis. Indirubin is the prime ingredient of the Chinese herbal medicine, Danggui Longhui Wan, which has been proven to show valuable bioactivity in treating various leukemias namely chronic myelocytic leukemia and inflammatory diseases [42-43]. The parent backbone of indirubin chemically modified and substituted by various functional groups result in the formation of indirubin derived compounds which consists of alkylated, halogenated, O- and N-substituted derivatives. These derivatives or analogs as compared to the parent backbone possess improved pharmacokinetic properties with enhanced bioavailability and solubility [44-45]. Indirubin and its analogs have been reported to show anti-cancer, anti-viral, anti-angiogenic and anti-inflammatory activities by specifically targeting the inhibition of cyclin-dependent kinases (CDKs), glycogen synthase kinase-3β (GSK-3β), JAKS/Src family kinase and nuclear factor kappa B (NF-κB) [46-49]. Indirubin-3'-monoxime (I3M) is one of the most studied synthetic indirubin derivatives exhibiting higher potency and bioavailability in comparison to its parent compound [42]. The structure of I3M is depicted in Figure 1.5(A).

Vanillin (4-hydroxy-3-methoxybenzaldehyde), a plant secondary metabolite and the main constituent of vanilla, is typically used as a flavouring agent in confectionery, beverages, foods and pharmaceuticals [50]. The structure is depicted in Figure 1.5 (B). Interestingly, relationship of vanillin with the phenylpropanoid pathway and the mechanisms of salicylic acid (2-hydroxybenzoic acid) formation [50], a potent anti-inflammatory molecule, made it an attractive choice for therapeutic use. Several studies implicated that vanillin has a profound beneficial effect on human health for daily consumption because of its anti-inflammatory [51-53], anti-oxidant [53, 54], anti-microbial [55], anti-mutagenic [56] and anti-tumor [57] efficacies. Vanillin exhibits anti-diabetic effect primarily by improving insulin sensitivity in adipocytes and hepatocytes [58-59]. The underlying mechanisms of many of these effects, however, remain largely unexplained.



Indirubin-3' monoxime (I3M)

Figure 1.4(A): Chemical structure of Indirubin-3`-monoxime



Vanillin (VNL)

Figure 1.4(B): Chemical structure of Vanillin

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