CHAPTER 2. Literature Review

2.1. Diabetes Mellitus

Diabetes mellitus is a disorder of carbohydrate, lipid and protein metabolism, characterized by high blood glucose levels and target organ damage at long term. However, as per research in recent decades, DM is much more than blood glucose level abnormalities. As glucose metabolism pertains to every cell of body, so does its abnormality. That means abnormalities of metabolism due to DM; affect each and every cell and organ of body.

2.1.1. Prevalence of DM

As per global report on diabetes released by WHO on 6th April, 2016, the prevalence of DM in adult population worldwide was 8.5% in 2014, which is nearly double of that (4.7%) in 1980 (World Health Organization, 2016). With associated risk factors like obesity, it was considered a disease of developed nations; on the contrary, however, its prevalence has increased faster in lower-middle income countries than in higher income countries. According to estimates of International Diabetes federation (IDF), global expenditure on DM has more than tripled in the duration of 2003-2013, the reasons being increase in number of diabetics and increased treatment cost per capita (Whiting et al., 2011). This rise in expenditure is expected to continue, more conspicuously in low to middle income countries. Hyperglycemia was responsible for 3.7 million deaths in 2014 worldwide, many of which were preventable. There were 51 million people with DM in India in 2010, which is estimated to increase to 87 million in 2030, a 58% rise (Snehalatha and Ramachnadaran, 2009).

ICMR, New Delhi conducted the first study reporting prevalence of T2DM in India from 1972 to 1975 in 35,000 adults (Ahuja, 1979). The urban prevalence of T2DM in people of 40 years and above was 5% while that in rural population was 2.8%.
After that several studies were done in different parts of India, showing an increasing prevalence year by year. In 1988 Ramachandran et al reported a prevalence of 5% in a study conducted in urban population of South India (Ramachandran et al., 1988). A national surveillance study in rural population was conducted between 1989 and 1991, reporting a prevalence of 2.8% (Ahuja, 2002). A survey conducted by Ramachandran et al in 1988 in Chennai showed 8.2% and 2.4% prevalence in urban and rural areas respectively. Another study conducted by Ramachandran et al. in 2000 indicated an urban prevalence of 12.1%. A nationwide study conducted in 2008 estimated a prevalence of 7.3% and 3.2% in urban and peri-urban areas respectively. A study in rural areas in Delhi showed 0.4-1.5% prevalence (Ahuja, 1991). A study conducted using WHO criteria for diabetes diagnosis demonstrated the rural prevalence to be 2.7% (Sadikot et al., 2004). Thus, the prevalence rates vary widely depending on the area, time and criteria for diabetes diagnosis used in the study. However, over a long period it is observable that the prevalence of DM is increasing constantly in India. A large community based study conducted by ICMR in 2011 indicated that the prevalence of DM was lower in Northern India as compared to southern India (Anjana et al., 2011). A possible justification for this was given by the difference in origin of the two populations, that the north Indians are migrated Asian populations, while south Indians are indigenous population of India (Arora et al., 2010). The rise in DM prevalence is apparent from the observations that prevalence of diabetes in the city of Chennai increased from 8.3% in 1989 to 18.6% in 2006. Similarly, a recent study conducted in Delhi, between 2010 and 2012 reported a prevalence of 23.0%, higher than what was observed to be 14.2% in 1994 (Tandon and Raizada, 2014).

Table 2.1. Studies showing prevalence of diabetes in India (urban and rural) in last five years (Tandon and Raizada, 2014)

<table>
<thead>
<tr>
<th>Year</th>
<th>Place</th>
<th>Prevalence (%) Urban</th>
<th>Prevalence (%) Rural</th>
</tr>
</thead>
<tbody>
<tr>
<td>2011</td>
<td>Chandigarh</td>
<td>14.2</td>
<td>8.3</td>
</tr>
<tr>
<td>2011</td>
<td>Tamil Nadu</td>
<td>13.7</td>
<td>7.8</td>
</tr>
<tr>
<td>2011</td>
<td>Maharashtra</td>
<td>10.9</td>
<td>6.5</td>
</tr>
<tr>
<td>2011</td>
<td>Jharkhand</td>
<td>13.5</td>
<td>3.0</td>
</tr>
<tr>
<td>2012</td>
<td>Orissa</td>
<td>15.7</td>
<td>-</td>
</tr>
<tr>
<td>2012</td>
<td>Haryana</td>
<td>-</td>
<td>13.3</td>
</tr>
</tbody>
</table>
2.1.2. Types of DM

American Diabetes Association classifies DM in 4 categories: Type 1 Diabetes mellitus, Type 2 Diabetes mellitus, other specific types of diabetes and Gestational diabetes associated with pregnancy (Ahuja, 2002).

Type 1 DM (T1DM) is characterized by severe deficiency of insulin due to beta cell damage. It is further classified in Type 1A and 1B depending on the mechanism of beta cell damage. Type 1A consists of the DM resulting from autoimmune destruction of beta cells, while in type 1B beta cell damage results due to unidentified factors therefore known as idiopathic in origin.

Type 2 DM (T2DM) is a multifactorial disease, with several possible risk factors leading to insulin resistance and relative deficiency of insulin.

2.1.3. Risk Factors of DM

Genetic factors

Heredity is an important factor for both types of DM. A family history of DM increases the chances of having DM 10 to 20 times. Human Leucocyte Antigen (HLA) located on chromosome 6 confers a 50% risk of T1DM. In contrast to this for T2DM there are several genes like, TCF7L2, KCNQ1, SLC30A8, FTO contributing towards the total risk of DM (Murea, Ma and Freedman, 2012).

Epigenetics

This phenomenon represents the effect of environment on gene function. This gives an explanation to the different occurrence of DM in individuals with similar genetic
risks (Ling and Groop, 2009). However, this phenomenon has also given an innovative way for prevention of DM using pharmacological or lifestyle interventions.

**Environmental factors and Life style**

Factors like stress, being overweight, alcohol, smoking, diet, sedentary life style, age and ethnicity play important role in development of T2DM (Lin et al., 2011). More recently, maternal nutritional status has been linked with development of DM in later stages of life (Yajnik, 2010).

### 2.1.4. Pathophysiology of Diabetes Mellitus

#### 2.1.4.1. Type 1 Diabetes Mellitus

T1DM generally occurs in genetically susceptible individuals as a result of autoimmune destruction of pancreatic beta cells. Autoantibodies against at least one of the three antigens namely, insulin, glutamic acid decarboxylase (GAD) and islet auto-antigen-2 (IA2), may be present in nearly 90% of all T1DM patients (Gillespie, 2006). Autoantibodies towards one of the pancreatic beta cell zinc transporter- ZnT8 are found in 25% of patients without one of the above mentioned three autoantibodies (Atkinson, 2012).
Figure 2.1: Time line of pathogenesis of Type 1 DM. In an individual with genetic susceptibility, environmental trigger causes beta cell damage and a chronic loss of Beta cell ensues (Van Belle, Coppieters and Von Herrath, 2011).

Attack by autoantibodies causes inflammation of beta cells i.e. insulitis. This condition continues for months or years before the symptoms of DM appear. Hyperglycemia develops only after 80-90% of beta cells are destroyed. Although insulin resistance plays no role in pathophysiology of T1DM, with increasing prevalence of obesity, it is possible that T1DM patient may also have insulin resistance in addition to insulin deficiency.

Figure 2.2 represents the detailed pathogenetic process involved in development of T1DM. A co-existence of genetic susceptibility and environmental factors starts the unfortunate series of changes in pancreatic beta cells. Beta cell upregulates IFN α and causes expression of MHC class I on cell surface. This exposes the beta cells to potential damage by autoreactive CD8 positive T cells. The environmental factor also causes a peripheral metabolomic change upregulating immunological processes in the periphery (Silveira, 2007). This along with CD4 T cells, causes proliferation of B cell into plasma cell, which can release antibodies against beta cell. In addition to this CD8 T cells are activated and targeted towards pancreas. This process kills some more beta cells releasing more beta cell antigens,
Figure 2.2 Pathogenesis of Type 1 DM representing decline in beta cell mass associated with various immunological mediators (Van Belle, Coppieters and Von Herrath, 2011)
resulting in heightened release of pancreatic autoantibodies. This destructive cycle continues till only 10-30% of beta cells are left, when clinical signs of DM appear. There is impaired utilization of glucose in peripheral tissues like, muscle and adipose tissue due to insulin deficiency. To counteract these effects, hormones like glucagon, cortisol, growth hormone and adrenaline are secreted. Glucagon increases gluconeogenesis and glycogenolysis increasing glucose output from the liver. Due to unavailability of glucose as a source of energy, fat is used, resulting in generation of ketone bodies as waste products. Such excessive generation of ketone bodies results in ketonuria and its accumulation in body may result in metabolic acidosis known as diabetic ketoacidosis (DK). DK is an acute emergency condition for the patient of T1DM and may prove to be fatal if not corrected.

2.1.4.2. Type 2 Diabetes Mellitus

Decreased insulin secretion, insulin resistance and disturbances in hepatic glucose metabolism are the important features of T2DM.

Beta cell dysfunction

Glucose entry in the β cells of diabetic patients through GLUT2 is impaired. Thus, insulin secretion control point in these patients is changed from glucokinase to transport of glucose (Leahy, 1990). In later stage of disease, the second phase of insulin release is also affected. This failure of β cells is attributable to several factors like accumulation of glycogen or sorbitol within β cells. Non-enzymatic glycation of beta cell protein is also one of the mechanism stemming from glucose toxicity within β cell (Robertson et al., 2003). Not only glucose dependent but insulin secretion in response to other secretagogues is also found to be impaired (Porte and Kahn, 1989). There is also impaired conversion of proinsulin to insulin (O'Rahilly, Turner and Matthews, 1988). Few patients of T2DM are found to have autoimmune destruction of pancreatic β cells (termed as LADA- Latent Autoimmune Diabetes in Adults). Few patients are also found with absence of glucokinase (referred to as Maturity Onset Diabetes of Young –MODY) (Groop and Bottazzo, 1994). However, in nearly 80 % of T2DM patients, the initial delay
of insulin secretion is followed by a hypersecretion of insulin in later phase. After many years of disease, β cell function is impaired, probably due to accumulation of intermediates of glucose metabolism (Kruszynska and Olefsky, 1996). This is the reason for secondary failures in management of T2DM with sulphonylureas (Jeng et al., 1989).

**Insulin resistance**

Insulin resistance is defined as reduced responsiveness of target cell towards insulin. In this condition, insulin fails to elicit glucose lowering response (Chawla, Nguyen, and Goh, 2011). Such decreased responsiveness to insulin is found in liver, skeletal muscle and adipose tissue cells in patients of T2DM, in especially those who are obese. Apart from DM IR has close associations with hypertension, obesity, polycystic ovary syndrome, non alcoholic fatty liver disease and metabolic syndrome (Liang and Koya, 2009; Laakso and Kuusisto, 2014). Insulin resistance originates from genetic or environmental factors. Several population based studies have demonstrated that the incidence of insulin resistance is higher in aboriginal populations migrated from different region of the world. Such migration has caused significant lifestyle, dietary and environmental changes leading to pathogenesis of DM. Extent of insulin resistance varies from patient to patient and in earlier phase of DM it may be masked by increased insulin secretion from beta cells. Insulin resistance is attributable to decreased insulin triggered glucose entry via GLUT 4. Several mechanisms of insulin resistance are reduced insulin receptor kinase activity even after insulin binding to the receptor, number of available phosphorylation sites may be decreased or the signalling molecule may be down regulated causing decrease in binding of downstream signalling molecules to the intracellular part of insulin receptor, defect in activation of downstream kinase cascade and second messenger signalling pathway, impaired GLUT4 fusion to the cell membrane. Accumulation of lipid metabolites like fatty acyl CoA, diacyl glycerol etc, in skeletal muscle or hepatocyte inhibits phosphorylation of IRS-1 (insulin receptor substrate 1) resulting in decreased phosphotidyl inositol 3 kinase activity downstream to IRS 1. In contrast to this, decrease in intracellular lipid metabolites secondary to weight loss in T2DM patients is accompanied by improved insulin sensitivity of the cell (Petersen and Shulman, 2006). Apart from,
lipid accumulation, inflammatory signalling molecules and genetic abnormalities of insulin receptor downstream proteins has also been implicated as reasons for insulin resistance (Sesti, 2006).

Obesity and insulin deficiency induced lipid accumulation in adipocytes play major role in inflammation contributing to IR. Such lipid accumulation in tissue causes activation of NF-κB and c-Jun N-terminal kinase (JNK) signaling pathways and subsequently upregulate the production of proinflammatory cytokines like TNF-α and IL-6 (Shoelson, 2006; Sharma et al., 2013). TNF α secreted by adipocytes activates JNK and IKKβ/ NFκB pathway. This increases serine-threonine site phosphorylation of IRS-1. IL-6 activates JAK-STAT signalling pathway, upregulates expression of SOCS3, thereby decreasing the expression of GLUT4, and IRS-1. Insulin resistance in skeletal muscle is mediated through activation of STAT3 and activation of NF-κB via expression of TLR-4. IL-6 also impairs insulin resistance by decreasing glycogen synthesis by upregulating the expression of FOG-2 and downregulating that of miR-200s.

Inflammasome pathway is another mechanism linking inflammation to insulin resistance (Grant and Dixit, 2013). Caspase-1, NOD-like receptor proteins (NLRPs), apoptosis associated speck-like protein (ASC), neutrophilic alkaline phosphatases (NALPs) etc are essential components of inflammasome complex. Inflammasome NLRP3 is activated by mitochondrial stress and is being studied extensively as decrease in NLRP3 in obesity has shown to be associated with better insulin sensitivity (Vandanmagsar et al., 2011). Caspase 1 is another such mediator that activates macrophage infiltration to the adipocytes and increase expression of proinflammatory cytokines. Decrease in ASC and caspase 1 levels were associated with reduction in plasma insulin, leptin, and resistin levels (Stienstra et al., 2010). Thus, inflammasomes are potential targets to treat insulin resistance especially associated with obesity (Stienstra et al., 2011).

Leptin, a white adipose tissue derived protein is also linked to development of IR (van der Wijden et al., 2014). Leptin suppresses hunger and increases energy expenditure. Further, a similar state of leptin resistance exists in obese patients, which reduces on weight loss (Wang et al., 2013). It was observed that leptin mediated PI3K signalling was essential for modulation of glucose metabolism and for β cell function (Yadav et al., 2013). Moreover, leptin is also a recommended biomarker for IR in utero and is a potential treatment for IR, as its administration
improves insulin sensitivity, glucose metabolism and lipid metabolism (Toyoshima et al., 2005). Another protein released by adipose tissue is adiponectin which acts as anti-inflammatory cytokine in conditions of obesity, IR and T2DM. It is deficient in these conditions. It is elevated in conditions of rheumatoid arthritis and T1DM, where it plays pro-inflammatory role (Stofkova, 2009). AdipoR1 and AdipoR2 are the adiponectin receptors that are highly expressed in skeletal muscle and liver cells. It increases glucose consumption in liver and reduces glycogenesis, thus having beneficial effect on insulin resistance (Crimmins and Martin, 2007).

Resistin is another important mediator released by macrophages in humans. Its levels are increased with rise in inflammatory mediators and IR (Reilly, 2005). It promotes insulin resistance mediated by TNF-α and IL-6 release via NF-κB-dependent pathway (Szulinska et al., 2014). TLR4 receptors and MAPK signalling pathway are also involved in mechanism inducing IR (Benomar et al., 2013). Although TNF-α plays key role in linking inflammation to IR, attempts to reduce IR by administration of monoclonal antibodies targeted against, TNF-α have remained inconclusive. On the other hand, thiazolidinediones (PPARγ agonists) have demonstrated significant improvements in IR attributable to their anti-inflammatory activity (Pascual et al., 2005). On the basis of linkage between inflammation and IR, the glucose lowering effects of salicylates in diabetic patients is now recognised to be at least in part due to its NF-κB inhibitory activity (Hundal et al., 2002; Shoelson, Lee and Yuan, 2003). Considering the fact that Aspirin exerts its anti-inflammatory action by inhibition of both COX 1 and COX 2, and may be associated with increased risk of bleeding, non acetylated salicylates like, sodium salicylate, salsalate, and Trilisate that directly inhibit inhibit NF-κB via IKKβ pathway were presumed to be safer alternatives. Clinical trials in patients of T2DM with salsalate demonstrated glucose lowering and HbA1C lowering effect. However, it could not cause long term reduction in AGE levels or endothelial dysfunction (Goldfine et al., 2014; Barzilay et al., 2013).

Insulin receptor substrate (IRS) molecules are important links between insulin receptor and its downstream second messengers. Four types of IRSs namely IRS1, IRS2, IRS3 and IRS4 have been identified which vary in their tissue distribution and molecular functioning. A defect in IRS genes may be central to inducing insulin resistance however, only the Gly→Arg substitution of IRS-1 has been found to be pathogenic in induction of IR (Sesti, 2001).
Protein Tyrosine Phosphatases are important mediators in insulin receptor signalling. These enzymes are sensitive to redox status of cells (Andersen et al., 2001). Insulin inactivates this enzyme by oxidation of a critical amino acid residue in the enzyme. Conversely, this oxidation is mediated via generation of ROS, indicating physiological role of ROS in cell (Goldstein et al., 2005; Mahadev et al., 2001). Inactivation of PTP prevents dephosphorylation of insulin receptor and IRS-1, thus enhancing insulin receptor signalling. In opposite to this, PTP1B expression is increased in muscle and adipose tissue cells in the state of insulin resistance (Wu et al., 2001). Inactivation of PTP1B by genetic modification makes the mouse resistant to development of insulin resistance. Such mouse is also resistant to TNF-α mediated IR (Nieto-Vazquez et al., 2007). Thus, PTP1B bears a great potential as a target to improve insulin sensitivity in diabetic patients. Several PTP1B inhibitors are under development as antidiabetic agents for treating T2DM (Koren and Fantus, 2007).

**Role of Liver**

Fat content of liver closely correlates with hepatic insulin resistance in T2DM patients, even those who are non-obese. Hepatic energy handling is disturbed in patients with under-utilization and overproduction of glucose. Hepatic glucose overproduction significantly contributes to hyperglycemia in T2DM. Recently kidney has also been shown to be involved in abnormal glucose production in T2DM. The effectiveness of metformin (via action on hepatic glucose metabolism) confirms the role of liver in hyperglycemia of T2DM (Yki-Järvinen, 2005).

**2.1.5. Current Treatment of DM**

Blood glucose level, Oral glucose tolerance test and HbA1C are considered for diagnosis of DM in addition to clinical features and family history of patient. However, it is important to consider age, race and anaemia or haemoglobinopathies in patient while interpreting the results. Table 2.2 describes the criteria for classification and diagnosis of diabetic state of patient. (Classification and Diagnosis of Diabetes, 2015).
Insulin is the mainstay of management of T1DM. Various insulin preparations are developed in order to overcome shortcomings of natural insulin like, short duration of action and to reduce frequency of administration. Several drugs are available for the treatment of DM. Table 2.3 describes various oral hypoglycemic agents for T2DM.

Table 2.3: Drugs approved by FDA for treatment of Type 2 DM alone or in Combination (Navale et al., 2013)

<table>
<thead>
<tr>
<th>Class</th>
<th>Mechanism of Action</th>
<th>Drug</th>
<th>Effect on Weight</th>
<th>Major Adverse effects</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulphonylurea: First generation</td>
<td>K⁺ channel blocker, Stimulate insulin secretion from β cells</td>
<td>Chlorpropamide, Tolazamide, Tolbutamide</td>
<td>Weight gain</td>
<td>Hypoglycemia</td>
<td>Most widely used and well known to clinician, cheap</td>
</tr>
<tr>
<td>Sulphonylurea: Second generation</td>
<td></td>
<td>Glimepiride, Glipizide, Gliburide</td>
<td></td>
<td></td>
<td>Received wide acceptance</td>
</tr>
<tr>
<td>Biguanides</td>
<td>Reduce hepatic glucose output</td>
<td>Metformin</td>
<td>Weight neutral</td>
<td>GI intolerance</td>
<td>First line agent for obese patients, contraindicated in patients with renal, hepatic impairment or cardiovascular disease due to risk of lactic acidosis</td>
</tr>
<tr>
<td>Thiazolidinediones</td>
<td>Agonist at PPAR-γ receptors, improves insulin sensitivity in peripheral tissues</td>
<td>Pioglitazone</td>
<td>Weight gain</td>
<td>Edema</td>
<td>Pioglitazone banned in June 2013 for the risk of bladder cancer, but the ban was revoked one month later making the drug</td>
</tr>
<tr>
<td>Non Sulphonylurea Secretagogues</td>
<td>Stimulate insulin secretion from β cells</td>
<td>Repaglinide, Nateglinide</td>
<td>Weight gain</td>
<td>Risk of Hypoglycemia</td>
<td>Taken with meal to control rapid onset</td>
</tr>
<tr>
<td>---------------------------------</td>
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</tr>
<tr>
<td>Alpha Glucosidase inhibitors</td>
<td>Dampen quick rise in post prandial blood sugar level by delaying complex carbohydrate absorption</td>
<td>Acarbose, Miglitol</td>
<td>Weight neutral</td>
<td>Gastrointestinal disturbances</td>
<td>Slow dose escalation to reduce gastrointestinal disturbances</td>
</tr>
<tr>
<td>Dopamine Agonist</td>
<td>Reduces insulin resistance mediated by central mechanism</td>
<td>Bromocriptine</td>
<td>Weight neutral</td>
<td>Nausea, Vomiting, Dizziness, Headache, Diarrhoea</td>
<td>Contraindicated in type I DM</td>
</tr>
<tr>
<td>DPP-4 Inhibitors</td>
<td>Inhibits enzyme Dipeptidyl peptidase reducing degradation of incretin hormone and increase in insulin secretion</td>
<td>Sitagliptin, Saxagliptin, Linagliptin</td>
<td>Weight neutral</td>
<td>Very few clinically significant side effects</td>
<td>Approved for use alone or in combination with metformin</td>
</tr>
<tr>
<td>GLP 1 Analogues</td>
<td>Stimulate insulin secretion, Slow gastric emptying, Induce satiety</td>
<td>Exenatide, Liraglutide</td>
<td>Weight loss</td>
<td>Nausea, Vomiting, Diarrhoea</td>
<td>Parenteral administration is required</td>
</tr>
</tbody>
</table>
2.2. Diabetic Complications

DM is a group of disease characterized by hyperglycemia. As glucose is ubiquitous entity for every cell of body, so are the effects of hyperglycemia. However, major tissues affected by hyperglycemia are nervous system, retina of eye, kidney, adipose tissue, liver and pancreatic β cells. Both types of DM result in long term complications which are broadly classified in two categories: Macrovascular and microvascular complications. Microvascular complications consist of diabetic retinopathy, neuropathy and nephropathy, while macrovascular complications include coronary artery disease, peripheral vascular disease and stroke.

2.2.1. Diabetic Neuropathy

Diabetic neuropathy (DN) is defined by the American Diabetes Association (ADA) as “the presence of symptoms and/or signs of peripheral nerve dysfunction in people with diabetes after the exclusion of other causes” (Standards of Medical Care in Diabetes-2007, 2006). The severity and progression of diabetic neuropathy is proportional to severity and duration of hyperglycemia, and occurs more commonly with T2DM, where it may also be present several years before the diagnosis of T2DM. It is also proposed that genetic predisposition in some individuals may promote faster progression of DN. The symptoms of neuropathy are the earliest and most common among other complications of DM (Boulton et al., 2004; Said, 2007) DN can be categorized in two types
a) Peripheral neuropathy (generally of symmetrical and distal pattern)

b) Autonomic neuropathy

Numbness in extremities of typical stalking-glove pattern and sensory disturbances are the initial signs of peripheral neuropathy. Lower extremities are more affected as compared to upper limbs. This can progress as numbness, loss of vibratory sensation and proprioception, diminution of knee jerk reflex as large myelinated nerve fibres damage. Whereas the loss of small myelinated fibres or unmyelinated fibres can lead to painful sensations like, heat, or pin prick sensations. Such loss of protective sensory function increases the risk of foot
injuries that progress to ulceration and foot amputations (Boulton, Kirsner and Vileikyte, 2004).

Autonomic neuropathy can affect gastro-intestinal system, cardiovascular system or reproductive system (Maser et al., 2003). Common signs of autonomic neuropathy are gastroparesis, constipation, diarrhoea, tachycardia, orthostatic hypotension and erectile dysfunction (Kempler et al., 2011).

2.2.1.1. **Pathophysiology of Diabetic Neuropathy**

Number of factors playing important role in development of neuropathy have been identified. Some of them are discussed here.

**Aldose reductase and production of polyols**

Glucose entry via nervous membrane is independent of carrier availability. Excess of glucose diffuses in neuron cell in the condition of hyperglycemia. This excess glucose causes several deleterious effects, the most prompt being its conversion to sorbitol by aldose reductase enzyme. Aldose reductase has low affinity for glucose in the condition of normoglycemia. However, in hyperglycemic state excess glucose is converted by aldose reductase (Oates, 2008). Sorbitol can not diffuse out of the cell and accumulates in the neuron. It causes an increase in osmotic pressure and reduces myoinositol content of cell. This inhibits protein kinase C and Na\(^+\)-K\(^+\) ATPase activities in peripheral nerves. Na\(^+\)-K\(^+\) ATPase is vital in maintaining the ionic movement across the axonal membrane and generation of action potential. Impaired Na\(^+\)-K\(^+\) ATPase activity impairs impulse conduction via nerve fibre and decreases nerve conduction velocity. Such decrease in nerve conduction velocity can be recorded in isolated sciatic nerve of diabetic animals. It is one of the reliable parameters for diabetes induced neuropathy.

**Protein kinase C activation**

Excessive glycolysis in hyperglycemia causes formation of glycerol-3-phosphate and then diacyl glycerol (DAG) (Xia et al., 1994). The PKC is upregulated by DAG and also by some oxidants like H\(_2\)O\(_2\). Such PKC activity
modifications results in disturbances in various key enzyme and protein functions, expression of cytokines and transcription factors as indicated in figure 2. These changes lead to altered nerve blood flow and conduction via the fibre. LY333531 an inhibitor of PKC has shown improvement of nerve function in animal studies (Cameron and Cotter, 2002). However, clinical studies with PKC inhibitors have failed to show significant improvement in neuropathy in contrast to other complications.

**Increase in oxidative stress**

Oxidative stress has been repeatedly incriminated to play an important role in development of neuropathy (van Dam, 2002). Figure 3 summarizes two important pathways which contribute to generation of oxidative stress in hyperglycemia. Non-enzymatic glycosylation reaction i.e. glycation is enhanced in the hyperglycemic state, leading to formation of intermediate amadori compounds. Further conversion of these compounds in advanced glycosylation end products (AGE) is associated with production of abundant ROS (Kaneto et al., 1996). Another pathway contributing to development of oxidative stress is the mitochondrial electron transfer system, where mitochondria overwhelmed with excessive glucose load lead to generation of lot of ROS as a byproduct of glucose metabolism. Several studies have demonstrated the effectiveness of antioxidants in preventing neuronal damage in clinical studies (Ametov et al., 2003). Oxidative stress has also been shown to reduce insulin synthesis and secretion (Rains and Jain, 2011) as shown in figure 4. Oxidative stress has also been shown to induce insulin resistance in 3T3-L1 adipocyte cell line (Rudich et al., 1998) and intact rat muscle (Dokken et al., 2008).
Figure 2.3: Mechanism of oxidative stress generation in hyperglycemia
TCA cycle: Tricarboxylic acid cycle; AGE: Advanced glycosylation end products; ROS: Reactive oxygen species (Kawahito, Kitahata and Oshita, 2009).

Figure 2.4: Effect of ROS on insulin sensitivity and secretion from pancreatic β cell
(Kawahito, Kitahata and Oshita, 2009). PDX-1: Pancreatic duodenal homeobox-1.

Inflammation

Axonal loss and fibre demyelination are two major pathologies associated with diabetic neuropathy. Local or cells infiltrating by chemotaxis release pro-inflammatory cytokines, which exert significant effects on fine metabolic balance of glial and neuronal cells. In condition of diabetes mellitus inflammatory effects of these cytokines are activated. Several studies have shown the association between increase in biochemical markers of inflammation and endothelial dysfunction in patients of peripheral diabetic neuropathy. Peripheral diabetic neuropathy is also associated with similar increase in markers of inflammation.
Endogenous production of a key inflammatory mediator TNF-α is found to be increased in vascular and neural tissues of patients with diabetic neuropathy, leading to hypercoagulability, increased microvascular permeability and nerve damage, initiating the pathogenesis of characteristic aspects of diabetic polyneuropathy (Satoh, Yagihashi and Toyota, 2003). Apart from this, hypercoagulability and inflammatory leucocyte infiltration may lead to occlusion of perineural blood vessels. Infiltration of endoneurium with mononuclear cells resumes low-grade endoneurial inflammatory process, culminating in mixed, axonal and demyelinative nerve lesions. Chronic inflammation might result in segmental demyelination and remyelination of nerve fibres and necrotizing inflammation of perineurial and endoneurial blood vessels. This results in ischemia and additional generation of reactive oxygen species which boosts the continuing inflammatory process (Said, 2006). Moreover, it's been shown that TNF-α promoter gene polymorphism, C(−857)T, is considerably related to prolonged F-wave latency within the median nerve, that's a sensitive marker of peripheral nerve pathology, in patients with type two diabetes. Michałowska-Wender G et al, didn't notice distinction within the expression of TNF-α in serum of patients of diabetic polyneuropathy and control patients. However, they discovered higher levels of GRO-α in patients with subgroup of diabetic polyneuropathy with concurrent demyelinating changes (Michałowska-Wender, Adamcewicz and Wender, 2006). GRO-α, the growth-regulated oncogene acting in some neoplastic and inflammatory processes, promotes neoplastic growth, metastasis and infiltration by leukocytes. Uceyler et al, also demonstrated the association of different proinflammatory cytokine levels with pain in diabetic neuropathy (Uceyler et al., 2007).

**Other factors**

A reduction in nerve blood supply and oxygen tension in sural nerve proportionate to reduction of nerve function has been reported in patients with diabetic neuropathy. Moreover, patients of chronic obstructive pulmonary disease develop a hypoxic neuropathy pathologically similar to diabetic neuropathy, indicating a possible involvement of nerve hypoxia. Therapies involving vasodilators and
angiogenesis promoters have been found to attenuate the development of diabetic neuropathy (Tesfaye, Malik and Ward, 1994). Diabetic neuropathy has also been associated with deficiency of nerve growth factors, however supplementation with brain-derived neurotrophic factor (BDNF) and Nerve growth factor (NGF) has been found to be ineffective in inhibiting the development of diabetic neuropathy (Tomlinson, Fernyhough and Diemel, 1997). A significant disturbance of essential fatty acid metabolism exists in diabetic patients, which results in defect in conversion of this to prostaglandin and other mediators necessary to maintain nerve blood flow. However, trials with γ-Linolenic acid were found to be ineffective (Jamal, 1994).

2.2.1.2. Current Management of Diabetic Neuropathy

Current management is mainly supportive in nature, especially after its development. Pain is the main symptom and simple analgesics suffice for mild pain. However, opiates may be required in severe condition. Amitriptyline, duloxetine, gabapentine and pregabaline are clinically used to control painful diabetic neuropathy and are found to be effective. The management of peripheral neuropathy requires a holistic approach and patient should be informed about the importance of foot care and regular checkups. Autonomic neuropathy manifesting in gastroparesis and erectile dysfunction has devastating effects on life style. Mild cases of gastroparesis can be treated with metoclopramide, domperidone, erythromycin and dietary changes, however, severe cases may require implantation of electrode to stimulate gastric contractions (Boulton et al., 2005).
Table 2.4: Treatment of diabetic neuropathy based on newer targets (Boulton et al., 2005)

<table>
<thead>
<tr>
<th>Pathology addressed</th>
<th>Mechanism of action</th>
<th>Drug</th>
<th>Status of drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyol pathway</td>
<td>Inhibit aldose reductase enzyme and prevent formation of polyols</td>
<td>Sorbinil, Tolrestat, Ponalrestat, Zopolrestat, Zenarestat, LidoRESTAT, AS-3201, Fidarestat, Epalrestat</td>
<td>Withdrawn (AE), Withdrawn (AE), Ineffective, Withdrawn, Withdrawn (AE), Withdrawn (AE), Effective in Clinical Trials, studies ongoing, Effective in Clinical Trials, studies ongoing, Marketed in Japan</td>
</tr>
<tr>
<td>Decreased nerve Myo-inositol</td>
<td>Replaces nerve myo-inositol</td>
<td>Myo-inositol</td>
<td>Equivocal results in trials</td>
</tr>
<tr>
<td>Increased oxidative stress</td>
<td>Reduces oxidative stress</td>
<td>α-Lipoic acid</td>
<td>Effective in Clinical Trials, studies ongoing</td>
</tr>
<tr>
<td>Nerve hypoxia</td>
<td>Increase nerve blood flow</td>
<td>Vasodilators ( \alpha )-inhibitors, PG analogs</td>
<td>Effect in one RCT Effective in one RCT</td>
</tr>
<tr>
<td></td>
<td>Increase angiogenesis</td>
<td>phVEGF(_{165}) gene transfer</td>
<td>RCTs ongoing</td>
</tr>
<tr>
<td>Increased activity of Protein kinase C</td>
<td>Increase nerve blood flow</td>
<td>Protein kinase C-β inhibitor (ruboxistaurin)</td>
<td>RCTs ongoing</td>
</tr>
<tr>
<td>Decrease in C-peptide</td>
<td>Increase nerve blood flow</td>
<td>C-peptide</td>
<td>Studies ongoing</td>
</tr>
<tr>
<td>Decrease in Neurotrophism</td>
<td>Nerve regeneration, growth</td>
<td>Brain-derived neurotrophic factor (BDNF), Nerve growth factor (NGF)</td>
<td>Ineffective</td>
</tr>
<tr>
<td>Decrease in long-chain fatty acid metabolism</td>
<td>Prevents long-chain fatty acid accumulation</td>
<td>Acetyl-L-carnitine</td>
<td>Ineffective</td>
</tr>
<tr>
<td>Decreased GLA synthesis</td>
<td>Increases essential fatty acid metabolism</td>
<td>γ-Linolenic acid (GLA)</td>
<td>Withdrawn</td>
</tr>
<tr>
<td>------------------------</td>
<td>----------------------------------------</td>
<td>-----------------------</td>
<td>----------</td>
</tr>
<tr>
<td>Increase in nonenzymatic glycation</td>
<td>Decreases advanced glycation end product (AGE) accumulation</td>
<td>Aminoguanidine</td>
<td>Withdrawn</td>
</tr>
</tbody>
</table>

AE: Adverse Effects, RCT: randomized clinical trial

### 2.2.2. Diabetic Nephropathy

#### 2.2.2.1. Pathophysiology of Diabetic Nephropathy

Diabetic nephropathy is typically defined as “macroalbuminuria or macroalbuminuria and abnormal renal function as represented by an abnormality in serum creatinine, calculated creatinine clearance, or glomerular filtration rate”. Progressive renal disease in diabetes is a multifactorial disease beginning initially via a process of glomerular hyperfiltration and increased GFR. Several histological changes observed in glomeruli of diabetic patients are, mesangial expansion, thickening of the glomerular basement membrane (GBM) and glomerulosclerosis. All these result in reduced filtration efficiency of the nephrons and extensive renal involvement in progression of diabetic complications. Nephropathy also serves as a risk factor for diabetic cardiomyopathy (Gilbert, 2006).

Hyperglycemia leads to AGE formation, which glycates the tissue matrix proteins that are resistant to degradation and are highly cross linked. Increased mesangial cell glucose concentration also leads to its proliferation and then hypertrophy. In addition to such reduced clearance of ECM, its excessive production driven by cytokines like, VEGF and TGF β results in glomerular basement membrane thickening and mesangial expansion. TGF β causes stimulation of collagen and fibronectin and supports mesangial expansion. Such ECM deposits are observable under light microscopy as Kimmelstiel-Wilson nodules.

Proteinuria is a classic marker and contributor to diabetic nephropathy. Increased glomerular permeability results in entry of protein in the renal tubules some of which are then taken up by proximal tubular cells triggering inflammatory
response there. This leads to interstitial scarring and fibrosis. This results in Tubulointerstitial fibrosis seen in advanced stages of diabetic nephropathy.

Activation of rennin angiotensin system occurs in mesangial cells, proximal tubular cells and podocytes. Angiotensin II (ATII) is an important contributor to diabetic renal damage and is present at high concentration in kidney. ATII causes constriction of glomerular efferent arteriole leading to increased intraglomerular pressure. ATII also upregulates renal growth factors and fibrosis through ATII type 1 receptors, which secondarily stimulate activity of TGF-β and other growth factors (Parving et al., 2001).

Multiple experimental and clinical studies have indicated the involvement of inflammatory mediators like, IL-1, IL-6, IL-18, TNF-α and TGF-β1 in development and progression of diabetic nephropathy. Various renal cells produce inflammatory cytokines in response to disturbed metabolic and vascular factors. IL-1 stimulates proliferation of mesangial cells and promotes matrix synthesis. It increases vascular permeability in the kidney leading to the development of intraglomerular haemodynamic abnormalities. It disturbs the expression of chemotactic factors, adhesion molecules and prostaglandin synthesis. IL-6 also increases fibronectin expression and glomerular permeability. IL-18 augments the pathogenic process by increasing release of other inflammatory cytokines, such as IL-1, interferon γ and tumor necrosis factor, and is found to be associated with endothelial cell apoptosis.

TNF α and TGF β also play an important role in pathogenesis of diabetic nephropathy.

Patients with diabetes associated microalbuminuria or nephropathy are at higher risk of cardiovascular mortality and morbidity. Significant interactions exist between heart and kidney, therefore integrated approach of treating the nephropathy is advised. Such condition of renal associated cardiac damage cardiac associated renal damage is denoted as cardio-renal syndrome (Ronco et al., 2009). There are many factors leading to cardio-renal damage, however, haemodynamic factors are found to play pivotal role. Both the organs are involved in maintenance of extracellular fluid volume and are sensitive to such alterations. Renin Angiotensin Aldosterone system is activated by juxtaglomerular apparatus of kidney in response to lowered perfusion pressure.
Macula densa of the kidney can detect lower sodium levels which may be associated with volume depletion. Activation of macula densa causes On the other hand, decrease in cardiac output can be restored by a mechanism via kidney. Kidney lead to rennin release and causes water and sodium retention. Thus, increases extra cellular fluid volume and venous return. Due to increased venous return cardiac output increases by Frank-Starling mechanism. This also corrects the renal perfusion pressure. Decreased cardiac output also activates RAAS due to activation of sympathetic nervous system. Thus, mechanism of functional restore of heart and kidney depends on each other. In this setting it is obvious that failure of function of one will adversely affect the other. Further, atherosclerosis or vascular damage complicates the scenario of either organ damage.

2.2.2.2. Current Management of Diabetic Nephropathy

Treatment of diabetic nephropathy is aimed at delaying the loss of renal function and prevention of its cardiovascular complications. The multiple factors addressed during treatment are microalbuminuria, hypertension, control of blood glucose, weight management, smoking cessation and dyslipidaemia. However, anaemia, acidosis, mineral and bone disease and malnutrition are some common conditions secondary renal disease, which also need to be treated.

Reduction in blood pressure is vital in prevention and treatment of diabetic nephropathy. The target blood pressure of 120-130 mm Hg is recommended for patients of T2DM to prevent loss of renal function and prevention of diabetic nephropathy (ACCORD Study Group, 2010). Albuminuria is another important risk factor for nephropathy in diabetic patients. Initiation of treatment is recommended for patients with urinary albumin excretion of >30 mg/day. Following interventions are suggested for control of BP and albuminuria in DM patients:

1. Limiting sodium intake to less than 6 gm salt per day.
2. Blockade of RAAS with ACE inhibitors or angiotensin receptor blocker drugs with maximum tolerable doses. Treatment with ACE inhibitors in clinical studies have demonstrated a reduction in progression of nephropathy in diabetic
patients (Lewis et al., 1993; Heart Outcomes Prevention Evaluation (HOPE) Study Investigators, 2000)

3. Diuretic therapy can be started with loop diuretic or thiazide diuretic respectively while taking in account for possible side effect of prescribed drugs.
4. If target blood pressure is not achieved add aldosterone receptor blocker to the therapy albeit with monitoring of potassium levels
5. If still target BP is not achieved add calcium channel blocker or beta blocker to the regimen to achieve the best possible BP control

Apart from this, best possible glycemic control should be achieved to prevent development and progression of renal damage. A target HbA1c of < 6.5% is advised for young otherwise healthy individuals. However, for older patients with significant renal damage or cardiovascular disease, 7-7.5% HbA1C can be targeted (Shurraw and Tonelli, 2013).

Dyslipidaemia is the important bridge between diabetic renal disease and cardiac disease. Control of lipid levels is an important corner stone of complication preventive therapy for DM. Statin therapy is recommended in nearly all diabetic patients as per current guidelines. Collaborative Atorvastin Diabetics Study (CARDS) demonstrated a 37% lower incidence of cardiovascular events in diabetic patients without any elevations in LDL levels treated with statin. A meta analysis considering different studies shows that a lipid lowering therapy is advisable in patients with estimated glomerular filtration rate (eGFR) of > 15 ml/min/1.73 m². While that in patients with eGFR<15ml/min/1.73 m² has shown conflicting result and therefore it is not advised (Upadhyyay et al., 2012).

Obesity or excessive weight increases the risk of diabetic renal damage and subsequent cardiac damage significantly. Therefore, weight reduction is important strategy for the diabetic kidney patients especially if the BMI is more than 25 kg/m². Other measures such as cessation of smoking and regular exercise of at least 30 minutes per day can improve the outcome.

Pancreatic β cell transplantation in type 1 diabetics resulted in dramatic improvement in various pathological parameters over time (Fioretto et al., 1998). However, in patient with established nephropathy renal replacement therapy may be needed.
2.2.3. Diabetic Retinopathy

2.2.3.2. Pathophysiology of Diabetic Retinopathy

Retinopathy is one of the commonest diabetic complication having grave consequences like blindness. The development and progression of DM is dependent on severity and duration of hyperglycemia and develops in both types of DM. However, its development may even proceed by several years in T2DM (Fong et al., 2004). Several factors have been identified for pathogenesis of retinopathy as presented in figure 2.5.

![Figure 2.5. Schematic diagram indicating the pathogenesis of diabetic retinopathy (Stitt et al., 2013)](image)


Aldose reductase is the enzyme involved in conversion of glucose to sorbitol (i.e. glucose alcohol). Glucose can diffuse through membranes without need of carrier.
in certain tissues, where it is later converted in sorbitol. Excessive intracellular glucose in retinal cells owing to hyperglycemia produces excessive sorbitol, which can not diffuse back out of the cell and accumulates causing osmotic stress. Several studies have demonstrated accumulation of sorbitol with formation of microaneurysm, basement membrane thickening and loss of pericytes. Pericytes surrounding retinal capillaries are important components of blood retinal barrier. Loss of pericytes secondary to osmotic or oxidative stress weakens blood retinal barrier and increases capillary permeability. This type of changes leads to formation of acellular capillaries with thick basement membrane. Studies with several aldose reductase inhibitors have shown promising results in preclinical studies, however, most of them have failed in clinical studies (Fong et al., 2004; Gabbay, 1975; Gabbay, 2004). High blood glucose level promotes non-enzymatic glycation of proteins and formation of glycoproteins i.e. Advanced Glycation End Products (AGEs). It was also observed that expression of AGE receptor (RAGE) is increased in diabetic patients (Hudson et al., 2007). AGEs cause cross-linking in basement membrane of retinal blood vessel proteins and also reduced their degradability. This reduces their elasticity and also causes death of pericytes and endothelial cells (Beltramo et al., 2002; Stitt et al., 2004). AGE inhibition is the potential therapy for prevention of diabetic retinopathy and several AGE inhibitors are under clinical development.

2.2.3.3. Management of Diabetic Retinopathy

At present clinical management of DR does not involve any medicines. Techniques like retinal photocoagulation and focal laser treatment are the mainstay of treatment. However, several new agents under development for treatment of DR are mentioned in Table 2.5.
### Table 2.5. Potential therapies in treatment of diabetic retinopathy (Nawaz et al., 2013)

<table>
<thead>
<tr>
<th>Targets</th>
<th>Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhibitor of AGE formation</td>
<td>Pyridoxamine, Aminoguanidine, OPB-9195, ALT-946, ALT-711, LR-90, N-Pheracyl/Thiazolium Bromide (PTB), Alagebrium</td>
</tr>
<tr>
<td>Protein Kinase C (PKCs) inhibitors</td>
<td>Ruboxistaurin (RBX), PKC412</td>
</tr>
<tr>
<td>Aldose Reductase Inhibitors (ARIs)</td>
<td>Sorbinil, Tolrestat, Epalrestat, Lidorestat, Zenarestat, Ranirestat, Ponalrestat, Zopolrestat, ARI-809, Fidarestat</td>
</tr>
<tr>
<td>Nonsteroidal Anti-Inflammatory Drugs (NSAIDs)</td>
<td>Aspirin, Nepafenac, Sodium salicylate, Sulfasalazine, Baicalein, Genistein, Nepafenac, Celecoxib</td>
</tr>
</tbody>
</table>

#### 2.2.4. Diabetic Cardiomyopathy

##### 2.2.4.1. Pathophysiology of Diabetic Cardiomyopathy

Diabetic cardiomyopathy is defined as structural and functional alterations in myocardium associated with diabetes and not directly with coronary artery disease (CAD). It is one of the reasons for heart failure in diabetic patients among other causes like coronary artery disease and myocardial ischemia. Structural changes observed in myocardium are discussed followed by the pathophysiological mechanisms.

**Left ventricular hypertrophy (LVH)**

LVH is an independent risk factor for heart failure and results in reduced myocardial compliance. Several studies have shown presence of increased LV thickness and mass in diabetic patients (Aneja et al., 2008; Galderisi et al., 1991). Leptin induced cardiomyocyte hypertrophy has been observed in obese diabetic patients (Xu et al., 2004). Similarly, resistin released from adipose tissue is found to cause cardiomyocyte hypertrophy *in vitro* through IRS-1 and MAPK signaling pathways (Kim et al., 2008). One set of observations indicates hyperinsulinaemia.
causing LVH, as deletion of insulin receptors has been found to reduce cardiac size (Belke et al., 2002).

**Myocardial lipotoxicity**

Increased myocardial lipid deposition has been found in LV of diabetic patients and this was found to be augmented by presence of obesity, insulin resistance and impaired glucose tolerance (McGavock et al., 2007). Increased fatty acid uptake and utilization has been found in diabetic patients of both types and also in animal models (Abel, Litwin and Sweeney, 2008; Boudina and Abel, 2007). The exact mechanism of fatty acid induced cell death is not clear however, involvement of ceramide biosynthesis, detachment of cytochrome C from the mitochondrial membrane and decreased mitochondrial cardiolipin level are identified as some of the mechanisms leading to myocardial apoptosis (Listenberger, Ory and Schaffer, 2001; Ostrander et al., 2011). Lipotoxicity is also accompanied by alterations in ER membrane phospholipids, leading to ER swelling and ER stress (Borradaile et al., 2006).

**Elevated myocardial oxidative stress**

Although many studies have proved a key role of oxidative stress in induction of diabetic cardiomyopathy, the exact mechanism of oxidative stress generation is not known. Experimental and human studies have suggested the involvement of lipid accumulation and increased FA metabolism in generation of ROS (Boudina et al., 2007). However, hearts of Akita mouse model of T1DM have not shown presence of elevated ROS in spite of increased FA metabolism (Bugger et al., 2008). This model however, lacks insulin resistance and exhibits normal insulin sensitivity. Concurrent with this was an observation that deletion of insulin receptors specific to myocardium resulted in increased hydrogen peroxide production and mitochondrial uncoupling (Boudina et al., 2009). This data indicates that insulin resistance may predispose myocardial mitochondria for ROS generation. ROS may also generate nitrosative stress by interacting with NO or alter gene expression in cardiomyocyte (Frustaci et al., 2000; Aragno et al., 2006).
Myocardial cell death

Both necrotic and apoptotic cell death was found to be increased in hearts of diabetic patients with heart failure. Necrotic cell death was even higher with concurrent hypertension (Frustaci et al., 2000). Various mechanisms like leptin deficiency, hyperglycemia and activation of RAS have been implicated in myocardial cell death (Frustaci et al., 2000; Barouch et al., 2006; Shenet al., 2009).

Interstitial and perivascular fibrosis

Several studies have shown an increased collagen deposition in myocardium surrounding blood vessels and between the muscle fibres. TGF β is supposed to play important role in development of fibrosis. Gene expression of TGF β is enhanced in condition of DM (Ban and Twigg, 2008). Insulin resistance is probably another important factor in inducing fibrosis as CIRKO mice lacking cardiac insulin receptor demonstrated similar features of fibrosis (McQueen et al., 2005). Cardiac fibrosis is a consistent feature of STZ induced models (Way et al., 2002) while it is absent in db/db mice (Van den Bergh et al., 2007).

Functional changes in myocardium

Cardiac dysfunction in DM is associated with three types of functional changes, Diastolic dysfunction (DD), Systolic dysfunction and diminished contractile reserve. DD is detectable very early in diabetic patients and found to be present in as high as 40–75% patients of T1DM and T2DM without CAD. Several mechanisms like TG accumulation (Christoffersen et al., 2003) and disturbed calcium homeostasis (Dong et al., 2006) have been implicated in inducing DD. Subtle changes in systolic function can be observed after development of DD in animal models as well as human subjects. Accumulation of glycation end products in cardiac tissue and insulin resistance are key mechanism responsible for systolic dysfunction (Joshi et al., 2009). DM patients may have cardiac dysfunction even if they are asymptomatic. However, exercise can induce cardiac dysfunction in such asymptomatic patients. This is secondary to diminished contractile reserve of heart due to impairment of myocardial sympathetic innervation (Scognamiglio et
al., 1998). This can serve as marker for early detection of cardiac dysfunction in diabetic patient after adjusting for age and gender specific parameters.

![Cardiac Dysfunction Diagram](image)

**Figure 2.6: Mechanisms of cardiac dysfunction** (Boudina and Abel, 2010)

**Changes in myocardial metabolic pattern**

Reduced GLUT4 content and defect in its translocation results in reduced rates of glucose oxidation. On the other hand, fatty acid oxidation via Randle cycle is increased. Activation of PPAR-alpha signaling pathways secondary to increase FA uptake in myocardium, further suppresses glucose oxidative enzymes. FAs are inefficient substrates and result in increased myocardial oxygen consumption and reduced cardiac efficiency (How et al., 2006). Mitochondrial dysfunction has been causally linked with cardiac dysfunction in diabetes based on various animals and human studies (Bugger and Abel, 2009). Changes in mitochondrial proteome, increased number of mitochondria, increased ROS generation and mitochondrial insulin resistance are some of the mechanisms implicated in development of cardiac dysfunction (Boudina and Abel, 2010).

**2.2.4.2. Management of Diabetic Cardiomyopathy**

Diabetic Cardiomyopathy may be asymptomatic in early stages, however, in later stages it may appear as overt heart failure. Patients develop symptoms related to forward heart failure or backward heart failure or both due to lack of pumping ability or impaired venous dumping due to congestion. Fatigue, weakness,
syncope, angina, dyspnoea, increased jugular vein pressure, edema in lower extremities and hepatomegaly may appear as symptoms of worsening cardiac function (Fang, Prins and Marwick, 2004). Perivascular and interstitial fibrosis is the hallmark of diabetic cardiomyopathy and the extent of fibrosis correlated fairly with the heart weight. Collagen crosslinking in addition to collagen deposition in diabetic hearts result in reduced myocardial compliance (Factor, Minase and Sonnenblick, 1980). Such interstitial fibrosis can be assessed by cardiac MRI (Picano et al., 1990). However, echocardiography can reveal diastolic dysfunction even in asymptomatic patients and those without any cardiac hypertrophic changes. Important aspects of treatment are, lifestyle modifications, regulation of blood glucose levels, and minimisation of cardiac risk factors and therapy of heart failure in patient with overt cardiac damage.

Lifestyle modification

This includes cessation of smoking, dietary changes, aerobic exercise and weight reduction are the pillars of the pillars of therapy. Several studies have proved lower incidence rate of diabetic cardiomyopathy in T2DM patients who reduced body weight and had regular aerobic exercise (Kodama et al., 2013; Stolen et al., 2009; Epp et al., 2013).

Tight Glycemic Control

Maintenance of euglycemia reduces the chances of diabetic cardiomyopathy and other cardiovascular adverse events (Sharma and Srinivasan, 2009). Although a variety of drugs are available to reduce blood glucose levels, they have limitations in their use in patient with cardiomyopathy. Metformin is contraindicated in patient with heart failure due to risk of lactic acidosis (Nichols et al., 2005). Insulin therapy in T2DM patients has been implicated to increase cardiovascular risk in several studies (Smooke, Horwich and Fonarow, 2005). However, as the studies were retrospective, this remains debatable. Pioglitazone increases body weight and causes fluid retention in 5-10% patients, thus increasing the burden on diabetic heart. It may worsen the heart failure and may increase hospitalization (Erdmann et al., 2007). However, incretin based therapies like DPP4 inhibitors...
and GLP-1 analogue have shown better outcomes in patients with cardiovascular complications (Poornima et al., 2008). These drugs have shown beneficial effects like weight loss, reduced lipid levels, anti atherogenic effect and cardioprotective activity (Bose et al., 2004). However, more data is required to accept this for the clinical practice. The most debatable question in management of diabetic cardiomyopathy is how low one should go in blood glucose levels to minimise the risk of cardiomyopathy. ACCORD, ADVANCE and VADT trials have shown no reduction in cardiovascular events or related mortality (Miki et al., 2012).

**Treatment of Heart failure**

ACE inhibitors, Angiotensin receptor blocker and aldosterone receptor antagonists are the main agents in treatment of heart failure and LV ejection fraction reduction. Diuretics can be added to treatment if needed (Vermes et al., 2003; Shindler et al., 1996). IV abradine therapy may be added to benefit the therapeutic outcomes. Abradine use has shown to reduce heart failure related hospitalizations in DM patients (Swedberg et al., 2010). Thus, therapeutic strategies for diabetic cardiomyopathy are based on general management of heart failure. Further studies are required to understand the pathophysiology and to lay confirmatory treatment of diabetic heart failure.
2.3 The Plant: *Anogeissus acuminata*

![Anogeissus acuminata tree with fruits](image)

**Figure 2.7: Anogeissus acuminata tree with fruits**

**Plant Name:** *Anogeissus acuminata*

**Family:** Combretaceae

**Distribution:** Common in dry deciduous forests of western India, in Gujarat, Rajasthan and Madhya pradesh

**Common Names:** Dhokra, Dhau, Dhok (Rajasthan)

Dhav, KaloDhavdo (North Gujarat)

Kardhai (Madhya Pradesh)

**Ethnomedicinal uses:**

- Aerial parts of AA are used in the treatment of Diabetes in India (Pullaiah and Naidu, 2003)
- Gum is taken by pregnant tribal ladies for their good health in Northern parts of Gujarat (Patel and Patel, 2011)
- Stem bark is used in dysentry, Gum is used in urinary problems in certain parts of Andhra Pradesh (Padal et al., 2010)
• In traditional and tribal medicine of Andhra Pradesh the plant is used to treat painful inflammatory conditions (Hemamalini et al., 2010)
• Poultice is applied on snakebite wound (Dahare and Jain, 2010)
• Stem and bark is used for toothache and dental caries in Tripura tribe of Bangladesh (Hossan et al., 2009)
• Bark juice is applied as antiseptic in Western Mizoram (Lalfakzuala, Lalramnghinglova and Kayang, 2007)
• Arial parts are used in cardiovascular diseases and as diuretic in tribal parts of Rajasthan (Jain et al., 2005)
• Decoction of bark is given for gastric disorders in Rajasthan (Jain et al., 2005)
• Tender shoots chewed and sap swallowed for treatment of dysentery and diarrhoea in Tribal parts of Andhra Pradesh (Raju and Reddy, 2005)

2.2.5. Chemical Constituents

The plant has tannins, flavonoids, alkaloids, glycosides and saponins. The plant is rich in complex tannins of flavono-ellagitanin type. Acutissimin A, Acutissimin C, Eugenigradin are the flavono-ellagitannins present in the plant (Lin et al., 1991). Apart from this plant has large amount of ellagitannins and ellagitannin dimers, like, Castamollinin, Anogeissusin A and B, Anogeissinin, castalagin, castamollinin, castalin, vescalagin and vescalagincarboxylic acid (Rimando et al., 1994a). Grandinin is the ellagitannin glycoside of castalagin and lyxose sugar present in the plant. Various lignans have been detected in aerial parts of AA, namely, pterostilbene, Anolignan A, B and C, secoisolariciresinol, leiocarpan A (Rimando et al., 1994b).

2.2.6. Pharmacological Studies

Hypoglycemic action

Manosroi and co-workers demonstrated the hypoglycemic effect of AA for the first time (Manosroi et al., 2011). In their study they tested five plants selected from database of Thai traditional medicine. The aqueous extract of bark of AA
was tested for its hypoglycemic action over a period of 4 hr in normal and alloxan induced mice. The effect was compared to standard treatments insulin and glibenclamide. In vitro free radical scavenging effect was tested by DPPH assay. AA demonstrated highest DPPH radical scavenging action with IC50 value of 11.00 g/mL, 4 times that of the standard ascorbic acid. AA did not show hypoglycemic activity in normal mice, however, shown a highest blood glucose reduction of 78.96% in diabetic mice at a dose of at 100 mg/kg bw. The hypoglycemic activity of AA was 1.1 folds of insulin and 1.76 folds of glibenclamide effect. They postulated improved glucose uptake by the tissue to be the possible mechanism for action of AA.

Hemamalini and Vijusha also demonstrated the hypoglycemic effect of methanolic extract of leaves in alloxan induced DM in rats over a period of 7 days (Hemamalini and Vijusha, 2012).

Zaruwa et al also assessed the hypoglycemic effect of methanolic extract of AA bark and its subfractions on alloxan induced DM in mice. Sub-fraction SF5 showed the highest blood glucose reduction at the dose of 400 mg/kg bw. They also evaluated the mechanism of the SF5 fraction by its combination with potassium channel opener and calcium channel blocker drugs. The effect of SF5 reduced when combined with K+ channel opener drug, indicating insulin release by K+ channel blockade to be the potential mechanism of SF5 (Zaruwa et al., 2012). Zaruwa et al in his recent study has examined different fractions of methanolic extract of AA for its hypoglycemic action. The researchers have isolated and structurally characterized a compound- castalagin responsible for the hypoglycemic effect of AA (Zaruwa et al., 2015).

**Anti-oxidant activity**

Moses et al also found the identical results when he tested methanolic extract of 11 different Thai plants in DPPH assay AA being one of them. AA demonstrated the highest free radical scavenging activity with EC50 of 51 ± 0.001 mg/ml, 7.3 folds of Vitamin E (Moses, Manosroi and Manosroi, 2009).
Neuroprotective activity

A polyphenolic compound, 5,7,3′,4′,5′-pentahydroxy dihydroflavanol-3-O-(2″-O-galloyl)-β-D-glucopyranoside (AP1) isolated from AA was evaluated for its effect on focal transient cerebral ischaemia in rats induced by middle cerebral artery occlusion (MCAO). It was also evaluated for in vitro antioxidant and COX inhibitory activity. AP1 treatment at a dose of 30 mg/kg i.p., before reperfusion injury could significantly reduce the cerebral edema, infarct volume, number of penumbral apoptotic cells. It also could decrease lipid peroxidation, protein carbonyl levels, total thiols in brain and corrected the abnormality of neurobehavioural score. Treatment with AP1 could also significantly bring down the catalase activity and NO levels in animals. AP1 demonstrated reducing and NO scavenging activities in in vitro studies. It also significantly inhibited cyclooxygenase activity (COX-1 and COX-2) and decreased lipid peroxidation. Researchers proposed the potential of using AP1 as a neuroprotective agent in stroke as it attenuated apoptosis and had a good antioxidant and anti-inflammatory activity (ArunaDevi et al., 2010).

Analgesic activity

Methanolic extract of AA has also been evaluated for its analgesic activity by Hemamalini et al. The methanolic extract of AA leaves were evaluated for their central and peripheral analgesic activity using acetic acid induced writhing model and tail flick method respectively at a dose of 300mg/kg P.O. The extract produced significant decrease in number of wriths and a significant increase in reaction time in tail flick test (Hemamalini et al., 2011).

Acute toxicity study

The extract was also subjected to acute toxicity study, in which it was found to produce no change in motor activity or gross behaviour of the animals during 24 h of observation post administration up to a dose of 3 g/kg (Hemamalini et al., 2011).
HIV–1 reverse transcriptase inhibitory activity and cytotoxic activity

Anolignan A and B have demonstrated potent HIV–1 reverse transcriptase inhibitory activity (Rimando et al., 1994a). Pterostilbene, conocarpan and dihydrodehydrodiconiferyl alcohol, isolated from AA demonstrated in vitro cytotoxicity on various cancer cell lines, including human breast cancer cell line and murine lymphoid neoplasma cells (Rimando et al., 1994b). Pterostilbene was found to possess potent antioxidant and cyclooxygenase enzyme inhibitory activity. It was also found to prevent neoplastic change in mouse mammary organ culture model (Rimando et al., 2002).