1.1 DIABETES MELLITUS

Diabetes mellitus (DM) is one of the most severe and incurable metabolic disorders characterized by increased blood glucose level as a result of an absolute or relative lack of insulin and failure of insulin to act on its targets tissue [1]. It is one of the alarming worldwide health problems at present leading to micro vascular (retinopathy, neuropathy and nephropathy) and macro vascular (heart attack, stroke and peripheral vascular disease) complications [2]. It is characterized by hyperglycemia and is associated with disturbances in carbohydrate, protein and fat metabolism which occurs secondary to an absolute (type I) or relative (type II) lack of insulin [3].

1.1.1 Epidemiology

The International Diabetes Federation (IDF) released latest data showing that a staggering 387 million people worldwide suffer from diabetes. IDF predicts that, if the current rate of growth continues unchecked, the total number will exceed 592 million in 2035 [4]. In 2010, the prevalence of diagnosed diabetes in the U.S. in patients over age 20 was 11.8% for men and 10.8% for women [5]. The worldwide estimated prevalence rates for all types of diabetes are highest in India, China and the U.S [5].

In Africa, 76% of deaths due to diabetes are in people under the age of 60. Europe has the highest prevalence of type 1 diabetes in children. In the Middle East and North Africa, 1 in 10 adults have diabetes. In South and Central America, the number of people with diabetes will increase by 60% by 2035. In South-East Asia;
almost half of people with diabetes are undiagnosed. In the Western Pacific, 138 million adults have diabetes – the largest number of any region [4].

India has estimated to be ~40.9 million in the year 2007 and expected to increase to ~69.9 million by the year 2025. Type 2 diabetes in Asian Indians differs from that in Europeans in several aspects: the onset is at a younger age, obesity is less common and genetic factors appear to be more common [6]. The prevalence of type II diabetes ranged from 0.3 to 17.9% in Africa, 1.2 to 14.6% in Asia, 0.7 to 11.6% in Europe, 4.6 to 40% in the Middle East, 6.69 to 28.2% in North America and 2.01 to 17.4% in South America [7].

1.2 DIABETIC NEPHROPATHY

Most devastating complication of diabetes is nephropathy, which causes 14% of all deaths in diabetes patients and accounts for 40% of end-stage renal cases [8, 9]. Diabetic nephropathy (DN) is the common cause of chronic kidney failure and end stage of renal disease (ESRD) [10]. DN, a frequent and major micro vascular complication of DM, in many countries of the world [11]. Several factors, such as hyperglycemia, hyperlipidemia, oxidative stress and inflammatory cytokines, contribute to the progression of renal damage in DN. Both type 1 and type 2 diabetes mellitus can lead to DN, but is more common in individuals with type 2 diabetes. DN is characterized by glomerular and tubules hypertrophy, thickening of the basement membranes, accumulating of extracellular matrix components, glomerulosclerosis as well as tubulo-interstitial fibrosis in mesangium and interstitium [12, 13].

DN is characterized by a series of renal structure abnormality including basement membrane thickening, mesangial expansion, glomerulosclerosis and
tubulointerstitial fibrosis [14]. However, the pathogenesis of DN is still not fully understood. Although great care has been taken to patients with diabetes such as strict control of glycaemia and blood pressure, many patients still develop progressive renal failure [15]. DN affects approximately one-third of all diabetic patients. It usually accompanies albuminuria with glomerular hyper filtration and renal hypertrophy in the early stage and often shows a deteriorating course that may lead to end-stage renal failure.

1.2.1 **Risk factor of Diabetic nephropathy**

Diabetic nephropathy results from the combined effects of various genetic and environmental factors. Prolonged duration of diabetes, poor glycaemic control and hypertension are major risk factors for both DN and cardiovascular disease [16-18]. Hypertension, dyslipidemia, smoking, obesity, male gender and presence of retinopathy are well known risk factors for DN in type 1 diabetes [19]. There are several factors that can increase the risk of developing DN (Figure 1.1). These include:

- Having chronically elevated blood sugar levels [uptodate.com].
- Genetic susceptibility may be an important determinant of both the incidence and severity of DN[20, 21].
- Angiotensin-converting enzyme (ACE) gene genotype [22]. DD polymorphism [23].
- Angiotensin-II type 2 receptor gene (AT2) on the X-chromosome [24]. Aldose reductase gene [25]. PKCb1 gene [26].
Age: Among patients with type 2 diabetes, increasing age, along with increasing duration of diabetes, has been associated with an increased risk for developing albuminuria [27].

Overweight: High body mass index (BMI) increases the risk of development of chronic kidney disease in patients with DM [28]. Higher systemic pressure; Elevated glomerular filtration rate (GFR); Higher HbA1c levels and Oral contraceptives [29].

1.2.2 Epidemiology

Epidemiology studies of type 2 diabetic patients show that DN prevalence ranges from 7.6% to 55% [30], while in different international registries it varies between 11.5% in United Kingdom and 42.9% in Thailand [31, 32]. According to the most recent estimates published in the Diabetes Atlas 2006 [33], India has the largest number of diabetic patients in the world, estimated to be 40.9 million in the year 2007 and expected to increase to 69.9 million by the year 2025. African Americans, Native Americans, Asians, and Hispanics are more prone to developing both type 2 diabetes and DN than non-Hispanic whites.

Some of the most robust data relating to the development of DN in a population of predominantly white patients with type 2 was reported from the United Kingdom Prospective Diabetes Study [34]. In the United States, approximately 20.8 million people, or 7.0% of the population are estimated to have diabetes with a growing incidence. Roughly one third of this population, 6.2 million, is estimated to be undiagnosed with type 2 diabetes. The prevalence of diabetes is higher in certain racial and ethnic groups, affecting approximately 13% of African Americans, 9.5% of
Hispanics, and 15% of Native Americans, primarily with type 2 diabetes. Approximately 20% to 30% of all diabetics will develop evidence of nephropathy, although a higher percentage of type 1 patients progress to ESRD.

1.2.3 Signs and Symptoms

Early signs and symptoms of kidney disease in patients with diabetes are typically unusual. However, a vast array of signs and symptoms listed below may manifest when kidney disease has progressed [35].

- Albumin or protein in the urine; High blood pressure; Ankle and leg swelling, leg cramps; Going to the bathroom more often at night; High levels of blood urea nitrogen (BUN) and serum creatinine
- Less need for insulin or antidiabetic medications.
- Morning sickness, nausea, and vomiting; Weakness, paleness, anemia and Itching
Figure 1.1: A number of risk factors contribute to eventual manifestation of insulin resistance/hyperglycemia [36]
1.2.4 Diagnosis

1.2.4.1 Biochemical test

The first step in the screening and diagnosis of DN is to measure albumin in the first urine in the morning or at random. This method is accurate, easy to perform, and recommended by American Diabetes Association guidelines [37]. The early testing for glucose intolerance and diabetes to identify for developing microalbuminuria, particularly have other risks for type 2 diabetes, such as hypertension, lipid abnormalities, or central obesity. Once the diagnosis of diabetes has been made, then routinely check urinary protein levels only to guide therapy and prognosis. Further, a rise in urinary albumin excretion (UAE) and reduced renal function, as reflected by raised plasma creatinine concentration, reduced calculated creatinine clearance or decreased glomerular filtration rate (GFR) that indicates DN [38].

Two positive tests for albumin in the urine over several weeks indicate persistent albuminuria, a first sign of diabetic kidney disease. Other causes of albuminuria are high blood pressure, congestive heart failure, the metabolic syndrome, or kidney damage from nephrotic syndrome. Even without these diseases, having a higher than normal levels of albumin in the urine is a risk factor for cardiovascular disease.

1.2.4.2 Imaging Studies and kidney biopsy

A renal ultrasound is typically obtained to observe for kidney size. In the early stages of DN, kidney size may be enlarged from hyper filtration. With progressive kidney disease from diabetes, the kidneys diminish in size from glomerulosclerosis. In addition, a renal ultrasound can assess for hyperechogenicity that suggests chronic kidney disease and can assist in ruling out obstruction. Kidney biopsy is done in some
cases to check for a specific type of kidney disease, see how much kidney damage has occurred and help plan treatment. To do a biopsy, the doctor removes small pieces of kidney tissue and looks at them under a microscope.

1.2.5 Clinical features

The natural history of DN is characterized by five stages (table 1.1) [39].

**Stage I**: Hypertrophic hyperfiltration. In this stage, GFR is either normal or increased. Stage I lasts approximately five years from the onset of the disease. The size of the kidneys is increased by approximately 20% and renal plasma flow is increased by 10%-15%, while albuminuria and blood pressure remain within the normal range.

**Stage II**: The quiet stage. This stage starts approximately two years after the onset of the disease and is characterized by kidney damage with basement membrane thickening and mesangial proliferation. There are still no clinical signs of the disease. GFR returns to normal values. Many patients remain in this stage until the end of their life.

**Stage III**: The microalbuminuria stage (albumin 30-300 mg/dU) or initial nephropathy. This is the first clinically detectable sign of glomerular damage. It usually occurs five to ten years after the onset of the disease. Blood pressure may be increased or normal. Approximately 40% of patients reach this stage.

**Stage IV**: Chronic kidney failure (CKF) is the irreversible stage. Proteinuria develops (albumin > 300 mg/dU), GFR decreases below 60 mL/min/1.73 m², and blood pressure increases above normal values.

**Stage V**: Terminal kidney failure (TKF) (GFR < 15 mL/min/1.73 m²). Approximately 50% of the patients with TKF require kidney replacement therapy (peritoneal dialysis, hemodialysis, kidney transplantation).
Table 1.1: Stages of Chronic Kidney Disease [40].

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
<th>GFR (mL/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Kidney damage with normal or raised GFR</td>
<td>≥90</td>
</tr>
<tr>
<td>II</td>
<td>Kidney damage with mild decrease in GFR</td>
<td>60-89</td>
</tr>
<tr>
<td>III</td>
<td>Moderate decrease in GFR</td>
<td>30-59</td>
</tr>
<tr>
<td>IV</td>
<td>Severe decrease in GFR</td>
<td>15-29</td>
</tr>
<tr>
<td>V</td>
<td>Kidney Failure</td>
<td>&lt;15</td>
</tr>
</tbody>
</table>

The Clinical stage of DN is showed in table 1.2. In the initial stages of diabetic nephropathy, increased kidney size and changed Doppler indicators may be the early morphological signs of renal damage, while proteinuria and GFR are the best indicators of the degree of the damage [41].

Table 1.2: Clinical stages of Diabetic Nephropathy

<table>
<thead>
<tr>
<th>Stage</th>
<th>GFR</th>
<th>UAE</th>
<th>Blood Pressure</th>
<th>Year after Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Hyperfiltration</td>
<td>Supernormal</td>
<td>Less than 30mg/day</td>
<td>Normal</td>
<td>0</td>
</tr>
<tr>
<td>II. Microalbuminuria</td>
<td>High normal</td>
<td>30-300mg/day</td>
<td>Rising</td>
<td>5-15</td>
</tr>
<tr>
<td>III. Over proteinuria</td>
<td>Normal-decreasing</td>
<td>More than 300mg/day</td>
<td>Elevated</td>
<td>10-20</td>
</tr>
<tr>
<td>IV. Progressive nephropathy</td>
<td>Decreasing</td>
<td>Increasing</td>
<td>Elevated</td>
<td>15-25</td>
</tr>
<tr>
<td>V. ESRD</td>
<td>Less than 15mL/min</td>
<td>Massive</td>
<td>Elevated</td>
<td>20-30</td>
</tr>
</tbody>
</table>

1.2.6 Pathogenesis and clinical changes

Sustained hyperglycemia triggers various changes in the kidney tissues, such as glycation, induction of oxidative stress, glomerular hyperfiltration and activation of the polyol, protein kinase C (PKC) and hexosamine pathways [42]. These changes plays a central role in the development of DN, other factors such as hypertension, genetic predisposition, dyslipidemia and smoking also affect the onset and progression [43].
1.2.6.1 Glomerular Basement Membrane and Diabetic Nephropathy

Thickening of glomerular basement membrane (GBM) is a characteristic early change in DN, glomerulus shows both morphological and functional changes in all elements that constitute glomerular filtration barrier (GFB) of the kidney: endothelium, GBM and glomerular podocytes. Alterations in GFB results in reduced glomerular filtration rate with poor renal outcome ranging from microalbuminuria to overt proteinuria. The clinical manifestations of DN include thickening glomerular basement membrane, glomerular hypertrophy, mesangial cell expansion and loss of podocytes [44]. Advanced glycation end products (AGEs) modification of extracellular matrix (ECM) proteins results in alterations of their structure and function. The formation of inter- and intramolecular cross-links after the glycation of collagen leads to structural alterations, including changes in packing density and surface charge, manifested by increased stiffness, reduced thermal stability and resistance to proteolytic digestion [45].

1.2.6.2 Glycation and Diabetic Nephropathy

Sustained hyperglycemia leads to the production of AGEs through non-enzymatic binding between proteins and sugars [46]. The glycation of ECM and intracellular proteins may result in their dysfunction. Part of the excess glucose in chronic hyperglycemia binds to free amino acids of circulating or tissue proteins. This non-enzymatic process produces reversible early glycation products and later, irreversible AGEs, which accumulate in the tissues and contribute to the development of microvascular complications of DM [47].
AGEs result in the expression and activation of a number of transcription factors implicated in the development of DN, including nuclear factor κB (NFκB) and protein kinase C (PKC). This effect can be both direct (through AGE receptors) and indirect, via generation of reactive oxygen species (ROS) [48]. However, AGEs contribute to the release of proinflammatory cytokines and expression of growth factors, such as transforming growth factor-β1(TGF-β1) and Connective tissue growth factor(CTGF), by interacting via specific receptors with, for example, monocytes/macrophages and endothelial cells [49]. Although present at low levels in homeostasis, the multiligand receptor for AGEs (RAGE) expression is increased in diabetic kidney, especially in podocytes [50, 51]. AGEs and signaling via RAGE is strongly implicated in podocyte effacement, in the upregulation of podocyte expression of monocyte chemoattractant protein-1 (MCP-1) and podocyte apoptosis [50]. Podocyte RAGE activation also increases the expression/activation of vascular endothelial growth factor (VEGF), which, in turn, leads to both hyperpermeability and proteinuria (Figure 1.2) [51]. An activation of RAGE in endothelial cells is involved in the generation of ROS, which then promote endothelial dysfunction [52].

In mesangial cells (MC), RAGE activation inhibits cell proliferation and promotes MC hypertrophy as well as increasing the mesangial synthesis of ECM, such as fibronectin and collagen types I and IV and the mesangial secretion of MCP-1, which participates in the inflammatory process (Figure 1.2) [52]. Various mechanisms relevant to hyperglycemia - induced activation of TGF-β1 lead to tissue destruction in DN. The multifunctional cytokine TGF-β1, induced by advanced glycation end products, angiotensin II, proteinuria and reactive oxygen species, acts as the key mediator of tubulointerstitial and glomerular pathobiology in DN (Figure 1.3) [52].
1.2.6.3 Intracellular metabolism and Diabetic Nephropathy

In the liver, skeletal muscle, and fat cells, blood glucose is uptaken mainly via glucose transporter (GLUT-4), whose expression is upregulated by insulin. In contrast, renal cells uptake glucose through GLUT-1, independent of insulin. Therefore, the intracellular glucose concentration increases in renal cells when they are exposed to hyperglycemia. It has been demonstrated that intracellular glucose elevation leads to the activation of the polyol pathway, in which excessive glucose is converted to sorbitol and fructose via the function of an enzyme called aldose reductase (Figure 1.4) [53]. Accumulated sorbitol damages the glomerular cell function through elevation of the osmolality and nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) consumption. Increased intracellular glucose also results in the production of diacylglycerol (DAG), which is an activator of PKC [54]. PKC is known to mediate various cell functions, including growth, differentiation, contraction and ECM production. The activation of PKC is also involved in glomerular hyperfiltration and increases the expression of TGF-β, a potent stimulator of the ECM [55].

Diabetic nephropathy has traditionally been considered a nonimmune disease; however, recent evidence shows an increase in macrophage infiltration and overproduction of leukocyte adhesion molecules in kidneys from diabetic humans and in experimental animal models of diabetes [56, 57]. Leukocytes, monocytes, and macrophages have all been implicated in the process of DN [56, 57] and circulating inflammatory markers and proinflammatory cytokines are strongly associated with the risk of developing of diabetic complications [58-60]. Further support for inflammation to contribute to diabetes comes from studies where immunosuppressive
strategies reduce renal macrophage accumulation and attenuate the development of DN [60-62].

1.2.6.4 Cytokines and Diabetic Nephropathy

Inflammation in the diabetic kidney is characterized by increased synthesis of pro-inflammatory cytokines [e.g. interleukin (IL)-1, IL -6, and IL -18, tumor necrosis factor (TNF)], upregulation of adhesion molecules and enhanced expression of chemoattractant cytokines (e.g. MCP-1 and RANTES), with subsequent transmigration of monocytes, neutrophils and lymphocytes into renal tissue [63]. MCP-1 is an important chemokine for macrophages/monocytes and its expression in MCs is stimulated by high glucose, but also an increased tubular expression of MCP-1 with DN [64]. The expression of proinflammatory cytokines could be further stimulated in podocytes and tubular cells by proteinuria acting in concert with hyperglycemia and AGEs (Figure 1.5) [64].
Figure 1.2: Schematic diagram showing the proposed relationship between hyperglycemia, oxidative stress and inflammatory cytokines production in the pathogenesis and progression of diabetic nephropathy. [34]
Figure 1.3: Schematic view of the central role of transforming growth factor (TGF)-β1 in DN [65].

Figure 1.4: The polyol pathway and the pivotal role of aldose reductase [66].
One of the major elements implicated in this inflammatory reaction is NFκB, a ubiquitous transcription factor that is activated by many stimuli relevant to DN [63]. The accumulation of macrophages and neutrophils are features of the development of DN and these cells and their products (e.g. ROS, cytokines and proteases) exacerbate inflammation in the kidneys of patients with diabetes mellitus [63]. The diabetes-associated induction of proinflammatory and profibrotic cytokines, including TGF-β1, is partly dependent on Angiotensin II (Ang II) and contributes to the destruction of nephrons in DN [64].

1.2.6.5 Glomerular Hemodynamics and Diabetic Nephropathy

Glomerular hyperfiltration and increased glomerular pressure lead to glomerular endothelial dysfunction, infiltration of macrophages via the upregulation of endothelial adhesion molecules, platelet activation, and mesangial cell dysfunction (Figure 1.5) [67]. These alterations in cell functions give rise to the upregulation of various cytokines, such as TGF-β and further deteriorate the renal function.

Multiple factors contribute to the pathogenesis of DN. Via “crosstalking” the glomerular cell types (mesangial cells, glomerular endothelial cells, and podocytes) are involved in thickening of the glomerular basement membrane and mesangial expansion as well as via up regulation of various mediators, such as (TGF)-β1, VEGF, AngII, ROSand MCP-1, in glomerular inflammation, glomerulosclerosis, and vascular endothelial dysfunction. In addition to some of these glomerular changes and the pathology of podocytes, tubules also contribute to the development of albuminuria, a hallmark of DN. Increased ECM production by the renal interstitial cell types (tubular cells, resident fibroblasts, activated myofibroblasts, and mast cells) leads to tubulointerstitial fibrosis, another characteristic feature of DN (Figure 1.5) [50].
Many diverse factors, including prostanoids, nitric oxide (NO), growth hormone, insulin, insulin-like growth factors (IGFs), Ang II, VEGF and cytokines, have been implicated as agents causing hyperfiltration and hyperperfusion. The enhanced intraglomerular pressure leads subsequently to the development of MC matrix overexpression and podocyte injury, because the glomerular capillary basement membrane is in continuum with both podocytes and MCs (Figure 1.5) [66, 68]. Thus changes in capillary pressure cause abnormal shear stress or stretching of glomerular cells, which in turn stimulate the production of cytokines and growth factors in an autocrine and/or paracrine manner. For instance, exposure of glomerular cells (e.g. MCs, podocytes and glomerular endothelial cells) in vitro to stretching or shear stress can activate certain signal transduction systems, growth response, cytokine synthesis and increased production of ECM proteins [66, 68 & 69]. MCs, which are located in the intercapillary space, share many characteristics with vascular smooth muscle cells; they possess an abundant number of actin filaments and they contract and proliferate in response to numerous vasoactive substances and growth factors/cytokines [70,71].

After initial injury, the activated MCs may change phenotype, expressing fibroblast –like myosin [71]. Loss and disassembly of actin fibers in the mesangial area are found in diabetic glomeruli with an impaired contractility in response to vasoconstrictive agents [69]. MCs play an important role in the regulation of glomerular hemodynamics and are the principal glomerular cells involved in ECM deposition in the mesangium (Figure 1.5) [69].
1.2.6.6 Lipids and Diabetic Nephropathy

Dyslipidemia is common in patients with DM and is considered as a risk factor for the progression of DN [72, 43]. Diabetic patients often have multiple lipoprotein abnormalities such as, increased plasma levels of very low-density lipoprotein (VLDL), low-density lipoprotein (LDL) and triglycerides [73]. In addition to the abnormalities in amount of lipoprotein, the diameter of LDL particles is also reported to be smaller in patients with DN [74] compared to diabetic patients without nephropathy. Experimental studies in animal models demonstrate that lipid abnormalities contribute to glomerulosclerosis [75, 76]. The involvement of serum cholesterol in the development and progression of nephropathy in type 2 DM has also been well studied [77-79]. Dominguez et al [80] found a direct linkage between renal
injuries of rats with type 2 DM and elevated levels of blood LDL cholesterol. A study demonstrating that hyperlipidemia and hyperglycemia act synergistically to induce renal injury in LDL receptor deficient mice [81] has further indicated that lipid can exacerbate diabetic nephropathy.

1.2.6.7 Oxidative Stress and Diabetic Nephropathy

Oxidative stress also plays an important role in the etiology of diabetic complications [82, 83]. Oxidative stress is caused by the overproduction of reactive oxygen radicals that are highly toxic to all the components of the cells, particularly to the cellular membranes in which they interact with the lipid bilayer and produce lipid peroxides [84]. Hyperglycemia is suggested to promote oxidative stress through both non-enzymatic and enzymatic mechanisms [83].

Under normal physiological conditions, there is a balance in the generation of oxygen-free radicals and the antioxidant defense mechanisms used to deactivate free radical toxicity [85-87]. Impairment in the oxidant/antioxidant equilibrium results in oxidative stress in numerous pathological conditions including diabetes leading to cellular damage [85-87]. Increasing evidence in both experimental and clinical studies suggests that there is a close link between hyperglycemia, oxidative stress and diabetic complications [88, 89]. Increased oxidative stress in diabetes likely contributes to the pathogenesis of DN and its progression to end-stage renal disease [90, 64, 91]. Enhanced ROS production in experimental and clinical diabetes have been linked to vasoconstriction, vascular smooth muscle cell growth and migration, endothelial dysfunction, modification of ECM proteins and increased renal sodium reabsorption [92-94].
Although the ROS production may be influenced by numerous mechanisms, the most important role in their production is played by superoxide produced by glycolysis and oxidative phosphorylation in the mitochondria. ROS activate all important pathogenetic mechanisms, such as increased production of AGEs, increased glucose entry into the polyol pathway and PKC activation [95]. In addition, ROS directly damage endothelial glycocalyx, which leads to albuminuria without the concurrent damage to the GBM itself.

Moreover, numerous research studies indicated ROS cause direct and indirect damage on renal interstitium induced by oxidative stress under the long-term ongoing hyperglycemia condition: (i) renal vascular sclerosis; (ii) increased vascular permeability; (iii) structure and function damage; (iv) activating downstream mediators such as extracellular regulated protein kinases (ERK), p38 mitogen activated protein kinases (p38 MAPK), NF-kB and activator protein-1 (AP-1), triggering a series of cellular responses, which are thought to contribute to the development of DN. Activation of nicotinamide adenine dinucleotide phosphate oxidases (NOX) [96] and PKC [97], increased formation of AGEs[42] and the polyol pathway[98] are the major resources of ROS (Figure 1.6).
Figure 1.6: The pathogenesis of diabetic nephropathy [66].

ROS also damage cells indirectly by stimulating the expression of various transcriptional factors such as NF-κB that are involved in inflammatory pathways [91]. There is evidence that DM increases multiple pathways that lead to the increased generation of ROS; these pathways include PKC-dependent activation of NADPH oxidase, enhanced glucose oxidation, hypertension and AGEs[91,99]. NADPH oxidase is a major source of ROS in many nonphagocytic cells, including renal cells such as glomerular mesangial cells. Inhibiting NADPH oxidase activity can reduce the generation of ROS and produce renoprotection [99].

1.2.7 Treatment of Diabetic Nephropathy

As discussed above, DN is a multifactorial progressive disease involving many different cells, molecules and factors. The exact causes of DN are still not fully
elucidated, but various postulated mechanisms are: hyperglycemia (causing hyperfiltration and renal injury), oxidative stress, inflammation and fibrosis. Therefore, to control blood sugar level to alleviate the problem or to block the pathological process will be of significant value in DN.

1.2.7.1 Renin–angiotensin system (RAS) blockades

Aliskiren, an orally active direct renin inhibitor, has been intensively researched in drug combinations with angiotensin-converting enzyme inhibitors (ACEIs) and ARBs (angiotensin receptor blocker). It was co-developed by the Swiss pharmaceutical companies Novartis and Speedel [100, 101].

1.2.7.2 Dipeptidyl peptidase-4 (DPP-4) inhibitors

A small clinical trial among 36 type 2 diabetic patients showed that sitagliptin reduced albuminuria by 20% [102] Linagliptin further reduced albuminuria by 28% beside that from conventional therapy with a RAS blockade [103].

1.2.7.3 Mineralocorticoid receptor (MR) antagonists

Currently, with the identification of the MR [104], eplerenone, an aldosterone antagonist, has been investigated for the treatment of DN. However, monotherapy with ACEIs or ARBs prevents DN with a low efficiency and drug combinations enhance protective effects on kidney dysfunction with the drawback of increasing risk of severe cardiovascular disease [105,106]. Therefore, there is a pressing need for a new chemical entity and a novel strategy for DN treatment.

1.2.8 Naringenin

Herbal medicines are naturally occurring plant-derived substances with minimal or no industrial processing that have been used to treat illness within local or
regional healing practices. For a long time, herbal medicines or their extracts have been used to cure various diseases, because plant products are frequently considered to be less toxic and free from side effects than synthetic ones. Flavonoids are widely recognized as naturally occurring antioxidants that can inhibit lipid oxidation in biological membrane. They usually contain one or more aromatic hydroxyl groups, which is responsible for their antioxidant activity.

Naringenin (NARN) (Figure 1.7) is a flavanone compound found in citrus fruits, such as grapes and oranges, and also in tomato skin. Naringenin has been pharmacologically evaluated for antioxidant [107], anti-inflammatory [108], anticancerous [109], antiatherosclerotic [110], hepatoprotective [111], nephroprotective [112] and immunomodulatory [113] activities. Naringenin effectively quenches free radicals due to a 4’hydroxyl group in its B ring [114].

NARN is a biphenolic compound belonging the naturally occurring flavanone, widely distributed in tomatoes, cherries, and grapefruits and predominantly present in citrus fruits. It has been shown to possess potential health benefits as an anticarcinogenic, antimutagenic, anti-inflammatory and antioxidant [107]. However, to our knowledge no reports have recorded the precise biological action of NARN against on diabetic nephropathy in rats. Therefore, the present study was designed with an aim to determine the protective effects of NARN on diabetic nephropathy in rats.

![Figure 1.7: Structure of Naringenin.](image-url)