CHAPTER 1: INTRODUCTION

Diabetes is the world’s largest endocrine disease involving metabolic disorders of carbohydrate, fat and protein (King et al., 1998). Ancient records showed that physicians as Charak have described it as “madhumeha” or honey urine due to the phenomenon of attracting ants near the urine of a diabetic patient. Also in Egypt and Greece, knowledge about diabetes existed. The word “diabetes” is derived from the Greek word, ‘Diab- to pass through’ referring to the cycle of heavy thirst and frequent urination. ‘Mellitus’ is the Latin word for ‘sweetened with honey’ and refers to the presence of sugar in the urine.

In a survey conducted by National Health and Nutrition Examination, observed 40% of adults in U.S have diabetes or prediabetes (NHANES 2005-2006) (Li et al., 2009). A more rapid growth is seen in the Asian regions. IDF estimates for 2013 pointed out that globally 382 million people (8.3% of adult population) had diabetes in 2013, of these 175 million (44%) were undiagnosed. 17 % of all live births were associated with hyperglycaemia in pregnancy. Six Asian countries are among top 10 countries worldwide in prevalence of diabetes viz., United Arab Emirates (18.7%), Saudi Arabia (16.8%), Bahrain (15.4%), Kuwait (14.6%), Oman (13.4%) and Malaysia (11.6%). The top five Asian countries are: India (50.8 million), China (43.2 million), Pakistan (7.1 million), Japan (7.1 million) and Indonesia (7 million). Bangladesh is expected to replace Japan in 2030 and rank at the 8th (Yang, 2010; IDF, 2013).

According to the WHO estimates, India had 32 million diabetic subjects in the year 2000 and this number would increase to 80 million by the year 2030 (Wild et al., 2004). The IDF also reported that the total number of diabetic subjects in India is 41 million in 2006 and that this would rise to 70 million by the year 2025 (Sicree et al., 2006). The so called “Asian Indian Phenotype” refers to have shown that they have a higher predisposition to insulin resistance, type2 diabetes and coronary artery disease compared to other ethnic groups (McKeigue et al., 1991).
Figure 1.1: IDF Regions and global projections for the number of people with diabetes (20-79 years), 2010-2030 (Reproduced from IDF, 2013).

World Health Organization estimates, in the year 2000 India had 32 million diabetic subjects and by the year 2030 this number would increase to 80 million (Wild et al., 2004). The International Diabetes Federation also reported that in 2006 India had 41 million diabetic subjects and that would rise to 70 million by the year 2025 (Sicree et al., 2006). The “Asian Indian Phenotype” has been observed with a higher predisposition to coronary artery disease, insulin resistance and type 2 diabetes compared to other ethnic groups (McKeigue et al., 1991). In Asian Indians, a unique clinical and biochemical abnormality is one of the major factors for the increased prevalence of type 2 diabetes (Joshi, 2003; Deepa et al., 2006).

Diabetes is of two types, viz., diabetes insipidus and diabetes mellitus. Simply diabetes can be defined as a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Type 1 diabetes results due to cellular mediated autoimmune destruction of \( \beta \)-cells and its prevalence is 5-10%. Its markers include islet cell autoantibodies, autoantibodies to insulin, GAD (GAD65), and tyrosine phosphatases IA-2 and IA-2\( \beta \). Type 2 diabetes results due to a development of insulin resistance and its prevalence is 90% (American Diabetes Association, 2004). In India 70% patients with type 2 diabetes, are obese and a defects in secretion of insulin is observed in such patients (Lakhani et al., 2011).

Diabetes is a chronic disease that cannot be completely cured and many patients develop complications if not properly treated, the most devastating complication of diabetes is nephropathy. Diabetes mellitus has been interestingly changed from disease of kidney to kidney disease throughout its long history (Nagy and Eknoyan,
Ebers papyrus (about 1550 BC) was found to contain description, similar to diabetes mellitus indicating a polyuric state. Galen described diabetes is specific to kidneys and occurs due to weakness in their retentive faculties. In 1936, Kimmelstiel and Wilson first described diabetes as a cause of end stage kidney disease (Kimmelstiel and Wilson, 1936).

The most common cause of diabetes is chronic kidney disease (CKD). The prevalence of some degree of CKD among adults with type 2 diabetes is 40% (Pyrama et al., 2011). Diabetes is the leading cause of kidney failure, accounting for 44 percent of all new cases of kidney failure in 2008. 20.5% ESRD patients of India develops it due to diabetic nephropathy (USRDS, 2005). Around one third of diabetic patients develops diabetic nephropathy and such case are markedly increasing in the developing world, with the Asia-Pacific region being the most severely affected. According to survey, it is expected to develop 6.6 million case of diabetic nephropathy in Indian out of the 30 million patients with diabetes. A potential diabetic nephropathy epidemic is extremely worrying as diabetic nephropathy is the leading cause of ESRD world-wide and the most common cause of chronic renal failure (CRF) in eastern India. Further, Lee reports that in 9 of 10 Asian countries, diabetic nephropathy is the most common cause of end-stage renal disease (Lee, 2003).

Diabetic nephropathy is a chronic microvascular complication of diabetes characterized by pathophysiologic perturbations of increased glomerular filtration rate (GFR) termed hyperfiltration, and the excretion of albumin in smaller amounts called microalbuminuria followed by proteinuria, nephrosis, azotemia, and finally end stage renal disease (ESRD) (Friedman, 2010). There are two phases of DN, incipient nephropathy also called microalbuminuria phase where in urine albumin excretion (UAE) ranges between 20-199 µg/min (or 30-300 mg/24h.); and clinical nephropathy or proteinuria phase where UAE > 199µg/min (> 300mg/24h.) (Murussi et al., 2008).

Genetic predisposition, hyperglycemia and high blood pressure are the major factors contribute for the development of diabetic nephropathy. Hyperglycemia is responsible for development of microalbuminuria, both in type 1 and type 2 DM. Moreover, in type 1 DM patients the reversal of renal damage was demonstrated with mild to advanced DN lesions after renal transplantation. Arterial hypertension is probably the factor which is directly related to DN progression. In type 2 DM, a high serum cholesterol levels is responsible while in type 1 DM increased serum triglycerides (TG) and total and LDL cholesterol were associated with micro- and
macroalbuminuria (Zelmanovitz et al., 2009). Advanced glycation end products, and activation of cytokines such as transforming growth factor-β (TGF-β), vascular endothelial growth factor (VEGF), tumour necrosis factor-α (TNF-α) and interleukin-6 (IL-6) (Chiarelli et al., 2009; Choudhary and Ahlawat, 2008; Rask-Madsen and King, 2010; Navarro-Gonzalez et al., 2009). Hyperglycemia was found to increase the expression of matrix proteins in the glomeruli, stimulated by TGF-β and VEGF. This suggests the involvement of TGF-β in cellular hypertrophy, enhanced collagen synthesis, and vascular changes observed in patients with diabetic nephropathy (Chiarelli et al., 2009; Rask-Madsen and King, 2010). Experimental data suggested that IL-6 induces glomerular infiltration by leukocytes, influences glycosaminoglycans (components of vascular endothelium and GBM) metabolism, and also causes microalbuminuria. Moreover on the basis of clinical studies carried out in type 2 diabetics, (Choudhary and Ahlawat, 2008) hypothesized the participation of locally released IL-6 in the development of kidney damage. Hyperglycemia may also responsible for activation of protein kinase C (PKC), which contribute to development of DN (Ha and Kim, 1999). Ramana and Srivastava, (2010) demonstrated a novel role of aldose reductase, an enzyme responsible for conversion of glucose to sorbitol in the polyol pathway of glucose metabolism, in pathogenesis of DN.

Two typical patterns of glomerular changes have been described in diabetes. The nodular Kimelstiel-Wilson (K-W) lesion and diffuse mesangial lesion. The K-W lesion is characterized by nodular aggregates deposited in the glomerulus. In the diffuse diabetic glomerular lesion a widespread accumulation of extracellular matrix is observed throughout the glomerulus (Osterby et al., 1990; Mauer et al., 1981). However, tubulointerstitial lesions are also prominent in DM. Interstitial inflammation; tubulointerstitial fibrosis and tubular atrophy are, along with the glomerular changes, important features of the progression of diabetic nephropathy towards ESRD (Dalla Vestra et al., 2000). One common mechanism that leads to renal failure via tubulointerstitial injury is massive proteinuria. Accumulating evidence suggests critical effects of filtered macromolecules on tubular cells, including lysosomal rupture, energy depletion, and tubular injury directly induced by specific components such as complement components. Chronic hypoxia in the tubulointerstitium results in the loss of peritubular capillaries, impairing blood flow delivery. Interstitial fibrosis also impairs oxygen diffusion and supply to tubular cells
Evalution of bioflavonoids in diabetic nephropathy

(Nangaku, 2004). TGF-β has also been suggested to be involved in glomerular matrix accumulation (Park et al., 1997).

Figure 1.2: Natural history of diabetic glomerulosclerosis.
Podocyte integrity and numbers are related to diabetes-induced alterations in glomerular membrane permeability. However, hypertrophy of the glomerular capillaries might cause the podocytes to stretch. The hypertrophy of the glomerular capillaries would exceed the stretching ability of the podocytes; spaces would form between the podocytes and result in unwanted leakage. After the initial phase with renal hyperfiltration and hypertrophic alterations, the diabetic patients experience a quiet, non-symptomatic phase, with microscopic renal alterations. Further in the development of diabetic nephropathy follows a phase of incipient nephropathy with macroalbuminuria. The diabetic nephropathy is considered manifest when this phase aggravates, albuminur ia is persistent, blood pressure increases, and GFR declines (Brener, 2004). For some patients, this stage results in renal failure and the need for dialysis or transplantation. It has been reported that diabetic patients requiring hemodialysis have a decreased survival rate compared to other hemodialysed patients (Brener et al., 1993).

High-fat diet (HFD) causes increase in free fatty acids and triglycerides which decreases insulin sensitivity. This ultimately leads to an increased risk for developing type 2 diabetes mellitus. A suitable rat model of type 2 diabetes was developed by Srinivasan et al., (2005) for pharmacological screening. The HFD-fed rats exhibited significant increase in body weight, basal plasma glucose, insulin, triglycerides and total cholesterol levels and thereby produce glucose intolerance. Low kg body weight,
in those rats further provided push and uplifted the effect of dose of STZ 35 mg/HFD that led to diabetes which mimics human clinical type-2 diabetes. Increased triglycerides and total cholesterol due to HFD also increases oxidative stress and thus negatively affect the kidney making this model suitable for studying DN.

STZ-induced diabetic nephropathy is commonly induced in Sprague-Dawley (SD), Wistar-Kyoto (WKY) or spontaneously hypertensive (SHR) rats. STZ damages the DNA of pancreatic-β cells and triggers multiple pathways, including activation of protein kinase-C, poly (ADP-ribose) polymerase and NAD (P)H oxidase, with consequent generation of reactive oxygen species (ROS) and advanced glycation end products resulting in renal damage and nephropathy (Giacco and Brownlee, 2010; Zhang et al., 2011). In general, nephropathy was noted to occur in rats between 4–8 weeks after the administration of STZ as assessed in terms of significant increase in microalbuminuria, serum creatinine, blood urea nitrogen (BUN), extracellular matrix deposition and thickening of glomerular basement membrane (Michael, 2005; Singh et al., 2006).

Glycemic and blood pressure control is a key for management of DN. Antihypertensive therapy, irrespective of the agent used, slows the development of diabetic glomerulopathy. Mogensen, (1989) showed attenuation of rate of decline in renal function by antihypertensive treatment in type 1 DM patients with hypertension and proteinuria. Still there is no satisfactory therapy is available to treat patients with diabetic nephropathy except for fewer agents like angiotensin converting enzyme inhibitors and angiotensin AT1 receptor blockers. Though improved glycemic control, intensive insulin treatment and screening for microalbuminuria along with early intervention with antihypertensive like ACE inhibitors and AT1 antagonists can postpone the development of overt DN (Zelmanovitz et al., 2009). Moreover in a recent study by Rosolowsky et al., (2011) found that despite renoprotective treatment, together with transplantation and dialysis, patients with type1 diabetes and macroalbuminuria remain at high risk for ESRD.

From the turbid history of medicine, it is very difficult to reproduce absolute timing, from when the use of plants as medicine was started. Although, almost every literature regarding medicine have been found to contain description of plants, till now. Currently, for multiple reasons, nearly 80% of the World population is dependent on indigenous medicines for primary healthcare (Vedavathy, 2004). It is estimated that at least 25% of all modern medicines are derived, either directly or indirectly, from...
medicinal plants, primarily through the application of modern technology to traditional knowledge. Total global herbal market is of 83 billion dollars and in this India’s contribution is of only 1 billion dollar (Robinson and Zhang, 2011). About 800 plant species have been reported to possess antidiabetic properties (Pourmorad et al., 2006). The ancient use of botanical medicine is still the backbone of our pharmacopoeia. However more than 50% of drugs used in Western pharmacopoeia are isolated from herbs or derived from modification of chemicals first found in plants. Therefore, investigation on such agents from traditional medicinal plants has become more important (Peng et al., 2005).

Flavonoids are 15-carbon compounds generally distributed throughout the plant kingdom. Bioflavonoids are a group of phenolic secondary plant metabolites abundantly found in the nature. Flavonoids have well categorized structures are: flavans, flavanones, flavones, flavonols, flavanols, flavanonols, cetechins, anthocyanidins and isoflavones. Bio-flavonoids are scientifically reported for myarid pharmacological activity including anti-diabetic (Matsui et al., 2006; Brahmachari, 2006). Several studies have been conducted to confirm their role in the treatment of diabetes (Jung et al., 2006; Qi et al., 2010). A large number of clinical and preclinical trails have demonstrated the hypoglycemic effects of flavonoids which may be because of improving glucose tolerance. It has also been reported that flavonoids can act as insulin secretagogues or insulin mimetics to attenuate the diabetic complications; besides, the drug candidates have been found to stimulate glucose uptake in peripheral tissues, and regulate the activity and/or expression of the rate-limiting enzymes involved in carbohydrate metabolism pathway. Therefore bioflavonoids ar considered as promising candidate to enrich the current therapeutic options against diabetes.

Hesperidin is a flavanone glycoside (C28H34O15) abundantly found in citrus fruits. Its aglycone part is called hesperetin. Hesperidin plays an important role in plant defense and according to in-vitro studies it acts as an antioxidant (Hirata et al., 2005). In rats hesperidin has been reported to reduce cholesterol (Monforte et al., 1995) and blood pressure (Ohtsuki et al., 2003). In a mouse study a large dose of hesperidin decreases bone density loss (Chiba et al., 2003). Animal studies reoorted a protective effects of hesperidin as an anti-inflammatory (Emim et al., 1994; Galati et al., 1994) and act against sepsis (Kawaguchi et al., 2004). Hesperidin is also reported as a sedative, and act possibly through opioid or adenosine receptors (Loscalzo et al.,}
2008; Guzmán-Gutiérrez et al., 2009) and also penetrate the blood-brain barrier in an in vitro model (Youdim et al., 2003).

Hesperidin

Milk thistle (*Silybum marianum* L.) is a medicinal plant widely used in traditional European medicine (Morazzoni et al., 1996). Silymarin, a polyphenolic flavonoid isolated from milk thistle, primarily consists of four isomeric mixtures of active flavonolignans: silychristin, silydianin, and two groups of diastereoisomeric flavonolignans, silibinin, and isosilibinin (Lee et al., 2007). Silibinin, a flavanone, is the major and most active component constitutes about 60–70% in silymarin, (Saller et al., 2001), and has been proposed as an antidiabetic agent (Sotoa et al.; 2003) as well as in the prevention of alloxan induced diabetic kidney dysfunction (Sotoa et al., 2010). Various preclinical reports suggest the myriad pharmacological activities of silibinin. It has been reported for antioxidant and hepatoprotective activities in nonalcoholic steatohepatitis (Haddad et al., 2011). Silibinin markedly improves endothelial dysfunction in db/db mice by reducing circulating and vascular ADMA levels (Giovanni et al., 2011). Recently, Marrazzo et al., reported its neuroprotective effect due to DNA protection and antioxidant activity in diabetic mice (Marrazzo et al., 2011). In addition, several recent studies have shown the potential cancer preventive and therapeutic efficacy of silibinin in different animal models and cell culture systems (Raina et al., 2007; Singh et al., 2008).

Silibinin
Thus, there is an urgent need to develop or search a novel and promising interventions to hinder, halt or reverse the progression of this devastating complication of diabetes. Therefore tremendous efforts are being directed towards exploration of novel and promising therapeutic interventions. Yet, no systematic study has been carried out on these bioflavonoids in to study the safety and efficacy in the treatment of diabetes, and diabetic nephropathy. Hence, the present study was carried out to explore the detailed pharmacological evaluation of these bioflavonoids on diabetic nephropathy in experimental animal.