Chapter 3

REVIEW OF LITERATURE
3.1 Diabetes and Its Complications: An Introduction

Diabetes mellitus is a metabolic disorder of impaired carbohydrates, fat and protein metabolism, characterized by hyperglycemia, polyuria, polydipsia and weight loss in spite of polyphagia due to insulin deficiency or insulin resistance which results in decrease utilization of carbohydrate; excessive glycogenolysis and gluconeogenesis from amino acid by fatty acids (Pontiroli et al., 1994; Gruden et al., 2005). The prevalence of diabetes is 6.4%, affecting 285 million adults in 2010 and will increase to 7.7% affecting 439 million adults by 2030 (Shaw et al., 2010). Type I diabetes (insulin dependent) is caused by insulin insufficiency due to lack of functional pancreatic beta cells. Patients suffering from Type I diabetes are totally dependent on exogenous insulin while patients suffering from Type II diabetes (insulin independent) are unable to respond insulin and can be treated with dietary changes, exercise and medication (Fowler, 2008). Type II diabetes is the most common form of diabetes constituting 90% of the diabetic population. Symptoms for both diabetic conditions may includes: (i) high levels of sugar in blood; (ii) unusual thirst; (iii) frequent urination; (iv) hunger and loss of weight; (v) blurred vision; (vi) nausea and vomiting; (vii) extreme weakness and tiredness; (viii) irritability, mood changes etc. (Andrew, 2000).

Persistent hyperglycemia lead to various microvascular complications like nephropathy, retinopathy, neuropathy and macrovascular complications includes coronary artery disease, leading to myocardial infarction (heart attack) or angina, stroke (mainly ischemic type), peripheral vascular disease, which contributes to intermittent claudication (exertion-related foot pain) as well as diabetic foot and these complications contributes to significant morbidity and mortality in diabetic patients (Altan, 2003; Halder et al., 2003; Thomas et al., 2005).
3.2 Types of Nephropathy

Nephropathy is characterized by nodular glomerulosclerosis, glomerular basement membrane thickness and mesangial expansion, leading to a decline in glomerular filtration rate, persistent elevated albuminuria, elevated arterial blood pressure and fluid retention. Contrast-induced nephropathy (CIN), Immunoglobulin A (IgA) nephropathy, reflux nephropathy, membranous nephropathy, ischemic nephropathy, diabetic nephropathy are the most common type of nephropathy resulting in end stage renal disease.

CIN is one of the most leading causes of renal impairment in the United States (Rich and Crecelius, 1990; Stevens et al., 1999). Radio contrast agents have a direct toxic effect on renal tubular cells (MacNeill et al., 2003) and renal hemodynamics, leading to a reduction in the outer medullary blood flow (Heyman et al., 1991; Russo et al., 1995; Liss et al., 1996; Flemming et al., 2000). Infusion of the radiographic contrast agent increases the osmotic load and viscosity of blood which in turn increases oxygen demand towards the renal medulla causing hypoxemia and production of free radicals via post ischemic oxidative stress (Brezis and Rosen, 1995; Katholi et al., 1998, Chandel et al., 2000; Dada et al., 2003). Moreover, hyperosmolar stress triggers cellular generation of reactive oxygen species which leads to renal damage (Qin et al., 1999; Loitsch et al., 2000). Antioxidants such as Superoxide dismutase, allopurinol and vitamin C help in reducing CIN occurrence by decreasing the oxidative stress on kidney (Bakris et al., 1990; Spargias et al., 2004). N-acetyl cysteine (NAC) has been found to be the most trusted oxygen radical scavenger mediates its protective function through increased nitric oxide production and direct vasodilatory effect. Tumor necrosis factor (TNF) has been implicated in acute renal injury and NAC appears to inhibit TNF activity via the glutathione pathway (Toborek
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et al., 1995; Cunningham et al., 2002). NAC reduced the risk of CIN up to 90% in patients undergoing contrast-enhanced computed tomography (Tepel et al., 2000).

IgA nephropathy is the most common form of primary glomerulonephritis throughout the world (Julian et al., 1988; Levy and Berger, 1988). It is defined immunohistologically by the presence of glomerular IgA deposits accompanied by a variety of histopathologic lesions (Emancipator, 1994; Habib et al., 1994). The decreased IgA response to mucosal antigens may promote the increased production of polymeric IgA1 from the bone marrow, leading to increased serum levels of IgA1. Defective galactosylation of IgA1, perhaps due to decreased β1, 3-galactosyl transferase activity, may decrease the hepatic clearance of IgA1 and promote the binding of IgA1 complexes to glomerular mesangial cells. IgA1 deposits in the kidney trigger the production of a variety of cytokines, growth factors and other inflammatory cells by the renal cells, leading to characteristic histopathological features of mesangial cell proliferation and extracellular-matrix deposition. Three randomized clinical trials (Maschio et al., 1994; Bannister et al., 1995; Cheng et al., 1998) and large retrospective cohort study (Catran et al., 1994) found that angiotensin converting enzyme inhibitors moderately lowers urinary protein excretion without an accompanying improvement in the renal function.

Proteinuria is uniquely prominent in membranous nephropathy (MN), a common “classic” autoimmune disease that is produced by the formation of subepithelial immune deposits in the glomerular basement membrane (GBM). MN is the most frequent cause of glomerular scarring and chronic renal failure in patients with glomerulonephritis.

Analgesic nephropathy occurred due to continuous use of non steroidal anti-inflammatory drugs, an important cause of chronic kidney disease characterized by renal papillary necrosis particularly in Australia, parts of Europe and the United States
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(Buckalew and Schey, 1986; De Broe and Elseviers, 2009). Phenacetin was removed from over-the-counter analgesics and before legislation was enacted making combined analgesics only available by prescription in Sweden, Canada, Belgium, and Australia, suggested that analgesic nephropathy was responsible for 1-3 percent of cases of end-stage renal disease in the United States as a whole, up to 10 percent in areas of North Carolina; 13- 20 percent in Australia and some countries in Europe such as Belgium and Switzerland. The decrease in availability of phenacetin-containing analgesic mixtures and other combined analgesics led to a marked reduction in number of new cases of analgesic nephropathy.

The term ‘Reflux Nephropathy’ introduced in 1973, also known as chronic atrophic pyelonephritis; Vesicoureteric reflux; Nephropathy-reflux; Ureteral reflux, is a condition in which the kidneys are damaged by the backward flow of urine into the kidney occurs almost during the first five years of life resulting in hypertension, proteinuria, CRF and eventually ESRD.

Ischemic nephropathy (IN) can be defined as a significant reduction in glomerular filtration rate (GFR) in patients with hemodynamically significant renovascular occlusive disease (RVD) affecting the entire functional renal parenchyma (Pinter et al., 2004). When renal artery stenosis (RAS) presents as ischemic nephropathy, the most common clinical syndromes are:

(1) Unexplained renal insufficiency

(2) Progressive azotemia

(3) Angiotensin-converting enzyme inhibitor-induced acute renal failure.

Ischemic nephropathy secondary to renovascular disease is common. The renal dysfunction and worsening GFR are observed in patients result from RAS and distal parenchymal disease. The prevalence of ischemic nephropathy seems to be increasing in the aging population. The natural history of RAS and the accurate
measures to detect the progression of kidney disease are not certain. Identifying patients who are at risk for progression of CKD as well as those who will benefit from intervention remain elusive goals that should be addressed by prospective trials.

3.3 Pathophysiology of Diabetic Nephropathy

Diabetic nephropathy is microvascular complication affecting 25–40% of people of Type I or Type II diabetes and it increases by 6% per year (MacIsaac and Jerums, 2003), characterized by thickening of basement membranes and mesangial expansion with progression into glomerulosclerosis, tubular necrosis and interstitial fibrosis, which ultimately result in renal failure (Pauksakon et al., 2002). Hyperglycemia may lead to end stage renal damage through both metabolic and non metabolic pathways. The non-enzymatic glycation of proteins with irreversible formation and deposition of reactive advanced glycation end products (AGE) have been noted to play a major role in the pathogenesis of diabetic nephropathy (Brownlee, 1992). Further, diabetic nephropathy is associated with hyperactivity of sorbitol-aldose reductase pathway (Cooper, 1998), hyperactivity of hexosamine biosynthetic pathway, activation of protein kinase C (Gruden et al., 2005) or MAPK (Komers et al., 2007) and overexpression of growth factors and cytokines i.e. transforming growth factor-β, vascular endothelial growth factor, platelet-derived growth factor and insulin-like growth factor (Gojo et al., 2007). Moreover, high glucose concentration in diabetes has been noted to induce oxidative and nitrosative stress (Allen et al., 2005), activate intracellular Renin-Angiotensin Aldosterone System (RAAS), release endothelin-1 and prostaglandins (Okumura et al., 1999) to deteriorate the function of kidney. In addition, up-regulation of transforming growth factor-β (TGF-β) and consequent overproduction of extracellular matrix molecules have been implicated in the progression of diabetic nephropathy (Figure 1).
Antioxidant defences and cellular redox status should be considered as key player in diabetes and its complications (West, 2000). Increased oxidative stress and depleted antioxidant defence in diabetes and its complications are well established (Evans et al., 2002; Choi et al., 2008). Hyperglycemia and increased production of reactive oxygen species (ROS) resulting in increased oxidative stress with over activation of NADPH oxidase are important components of metabolic syndrome (Demircan et al., 2008). Moreover, insulin resistance is also positively associated with systemic oxidative stress. Oxidative stress leads to the development of diabetes mellitus by activating stress-signaling pathways such as NF-κB (Davi et al., 1999). Contribution of oxidative stress to diabetic complications may be tissue specific, mainly in microvascular diseases which occur only in diabetic patients. Thus antioxidant treatment coupled with other treatments for diabetic complications would most likely be effective in ameliorating these complications (Scott and King, 2004). Hence, there is a clear need for additional interventions to decrease the impact of high glucose and oxidative stress among those subjects who do not manage to reach normoglycemia. From the ancient time, plants are used as an essential component of traditional medicine systems (Fang et al., 2005). Many of these medicinal plants and herbs had been priced for their medicinal, flavoring and aromatic qualities for centuries. Plants are rich source of secondary metabolites like flavonoids, alkaloids, terpenoids, tannins and that have been implicated in several therapeutic approaches.

3.3.1 Stages of Diabetic Nephropathy

Stage-1 (Hyperfiltration), related to renal hypertrophy and hyperfiltration. The earliest clinical evidence of nephropathy is the appearance of low but abnormal levels (≥30mg/day or 20 µg /min) of albumin in the urine, referred to as microalbuminuria. The GFR is elevated by 20-40% and renal plasma flow is also elevated by 9-14% (Molitch et al., 2004).
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Stage-2 (Silent Phase), during this phase, the GFR remains elevated with normal urinary albumin excretion rate and blood pressure levels (Thomas and Viberti, 2003).

Stage-3 (Incipient Nephropathy), significant structural glomerular disease is present even at this early phase and patients with microalbuminuria are referred to as having incipient nephropathy (Ibrahim and Vora, 1999).

Stage-4 (Overt Nephropathy), without specific interventions, 80% of subjects with Type-I diabetes who develop sustained microalbuminuria have their urinary albumin excretion increase at a rate of 10-20% per year to this stage of overt nephropathy or clinical albuminuria (≥300 mg/day or ≥200 μg/min) over a period of 10-15 years, with hypertension also developing along the way (Ibrahim and Vora, 1999). The GFR starts to decrease during the phase of microalbuminuria, though it remains within the normal range until the albumin excretion rate approaches 200μg/min or 300mg/day (Thomas and Viberti, 2003). Once overt nephropathy occurs, the GFR gradually falls over a period of several years at a rate that is highly variable from individual to individual (2-20 ml/min/year).

Stage-5 (End Stage Renal Failure), ESRF develops in 50% of Type-I diabetic individuals with overt nephropathy or macroproteinuria within 10 years and in >75% by 20 years. It requires dialysis or transplant to maintain life (Thomas and Viberti, 2003).
Figure 1: Pathological Mechanism Involved in Diabetic Nephropathy

3.3.2 Risk factors for developing diabetic nephropathy

- Poor control of blood glucose
- Chronic duration of diabetes
- Co-morbid microvascular complications
- Pre-existing hypertension
- Family history of diabetic nephropathy
- Family history of hypertension (Ayodele et al., 2004)
3.4 Drug Therapy and Target of Diabetic Nephropathy

3.4.1 Glycemic Control

Improved glycemic control has been shown to prevent the development of microalbuminuria in both Type I and Type II diabetes (Reichard et al., 1993) and it can normalise the established microalbuminuria. Glycemic level is also important for to delay the rate of progression of overt diabetic nephropathy. Literature suggested a glycemic threshold for the development of diabetic nephropathy. However, no evidence for such threshold was found in the Diabetes Control and Complications Trial (DCCT), although the absolute risk reduction is greatest with reductions in Glycated Haemoglobin (HbA1c). A clinical observation in patients with diabetic nephropathy is that glycemic control tends to deteriorate the progression of nephropathy. Some studies have suggested that rapid acting insulin analogues may be beneficial in patients with overt nephropathy (Rave et al., 2001) and in the patients on haemodialysis treatment (Aisenpreis et al., 1999).

3.4.2 Treatment of Hypertension and Microalbuminuria

In the early 1980’s the progression of diabetic nephropathy was shown to be delayed by treatment with antihypertensive agents (Mongerson, 1982). A close correlation between arterial blood pressure and the rate of decline GFR has been shown in overt diabetic nephropathy. Statistical analyses did not reveal a lowest threshold for the adverse effect of high systemic blood pressure on the fall in GFR (Rossing et al., 2002). A meta-analysis trials with ACE inhibitor in diabetic nephropathy showed that these agents lead to a reduction in the risk of progression from microalbuminuria to overt nephropathy and they seem to be superior, in terms of renal protection to other anti-hypertensive agents. Treatment with ACE inhibitor also seemed to have long-term effects with preservation of diabetic nephropathy parameter, i.e. GFR, over at least eight years (Mathiesen et al., 1999). Early
intervention with ACE-I and blood pressure reduction in normotensive patients with microalbuminuria has been shown to be beneficial both in Type I and Type II diabetes (Ravid et al., 1993). Several clinical trials of angiotensin receptor blockers (ARB) in patients with diabetes have shown similar renoprotective effects as ACE-I, both in Type I and in particular Type II diabetes (Lewis et al., 1993). The use of ACE inhibitor and ARB can lead to renal protection in diabetic nephropathy but still nephropathy may continue to progress but at a slower rate. A more effective blockade effect of angiotensin-II by reducing both synthesis and its binding to the angiotensin type I receptor, i.e. a combination therapy with ACE-I and ARB has shown an additive effect on blood pressure and markers of renal function such as albuminuria (Mongensen et al., 2000). Clinical trials have also indicated that ACE-I and ARB may be more effective than traditional antihypertensive treatment in reducing the progression towards ESRD (Defferrari et al., 2002). It is clear that treatment of hypertension is an important task in preventing and postponing the development of diabetic nephropathy. In the UKPDS trial more than 2/3 of the patients with Type II diabetes and hypertension needed at least two antihypertensive agents to achieve a mean blood pressure of 144/82. A combination of antihypertensive agents will be required in most hypertensive patients with Type II diabetes. In the Swedish national guidelines for diabetes care, ACE-I has been recommended as a first choice in patients with Type I diabetes and signs of incipient or overt diabetic nephropathy. A blood pressure target ≤130/80, or perhaps even lower, is recommended in these patients (Molitch et al., 2004).

The rate of synthesis for angiotensin-II seems to play an important role in initiation and progression of diabetic nephropathy by affecting hemodynamic and non-hemodynamic mechanisms. In the future perhaps determination of I/D polymorphisms of the ACE gene or other candidate gene polymorphisms within the
RAS may improve the evaluation of the individual risk profile and help us to individualise antihypertensive treatment.

3.4.3 AGE Inhibitors

Aminoguanidine, the first targeted AGE product therapy, is a hydrazine derivative that prevents AGE product formation by binding reactive carbonyl intermediates. Aminoguanidine affects was evaluated in 690 Type I diabetic patients in a randomized double blinded placebo controlled trials for nephropathy and retinopathy that shows progression of glomerular filtration rate declined, proteinuria and retinopathy was significantly improved. Although, three patients treated with high dose of aminoguanidine developed glomerulonephritis. A similar follow up study was halted due to safety concern and an apparent lack of efficacy (Freedman et al., 1999). A newer agent alagebrium chloride (ALT 711) cleaves AGE product and protein cross links, thereby facilitating AGE product clearance. Two clinical trials in mixed diabetic populations with atherosclerosis and heart failure reveals that ALT 711 can improve vascular and left ventricular compliance with an adverse effect similar to placebo (Little and Brucks, 2005). In addition an experimental study shows that ALT 711 was beneficial in treating diabetic renal complications. Moreover, AGE inhibitors like aminoguanidine (Soulis et al., 1995), OPB-9195 (Tsuchida et al., 1999), ALT-946 (Forbes et al., 2003) and a RAGE-specific neutralizing antibody (Flyvbjerg et al., 2004) attenuated the development of diabetic nephropathy.

3.4.4 Aldose Reductase Inhibitors

Aldose reductase inhibitor (ARI) an ideal target for reducing the deleterious effects associated with polyol pathway activation. However, clinical trials with ARIs have shown lack of efficacy and adverse effects. In 1980, sorbinil became the first ARI to undergo clinical trials after promising preclinical results which is evaluated for treating retinopathy and nephropathy but in early 1990s it again showed a lack of
efficacy. Results from several studies on neuropathy were mixed, but the majority suggested a lack of significant effects (Martyn et al., 1987; Guy et al., 1988). Tolrestat or lidorestat were halted due to toxicities before their efficacy could be evaluated. Ponalrestat and zopolrestat were ineffective despite of having more side effects (Sundkvist et al., 1992) suggesting the utility of epalrestat in ameliorating autonomic neuropathies related to cardiac function, gastric and esophageal motility (Nakayama et al., 2001; Okamoto et al., 2003; Kinekawa et al., 2005). Epalrestat affects on nephropathy were evaluated via a placebo-controlled randomized trial over 5 years in which thirty five patient of Type II diabetics with baseline microalbuminuria were studied, significantly increased in the placebo controlled group suggesting a benefit in treating nephropathy and microalbuminuria was found to be unchanged treatment group, (Iso et al., 2001).

3.4.5 Lipid Lowering Therapy

Hypercholesterolemia plays an important role in aggravating renal damage of experimental diabetic animals and this can be prevented by lipid-lowering therapies (Kasiske et al., 1990). An independent association between hypercholesterolemia and rapid loss of renal function in diabetic nephropathy has been demonstrated in Type I diabetic patients (Mulec et al., 1990). Moreover, a similar result has also been observed in patients with Type II diabetes (Ravid et al., 1998). Intervention with the cholesterol-lowering agents like statins over a long period in Type II diabetes delayed the decline in GFR and reduced albumin excretion (Lam et al., 1995 and Tonolo et al., 1997). Moreover, lipid-lowering treatment have a beneficial effect on microalbuminuria has been demonstrated in Type I diabetes patient by a trial (Fried et al., 2001).
3.4.6 Erythropoietin Treatment

Anaemia due to erythropoietin deficiency may occur early in some patients with diabetic nephropathy. The mechanisms for this have not been elucidated but an association with autonomic neuropathy (Bosman et al., 2001) or early renal interstitial damage has been suggested. Anaemia in chronic renal failure is normally not observed until GFR drops to 20-40 ml/min. Many of these patients are also treated with ACE inhibitors, which may cause a small decrease in serum erythropoietin level as a side-effect (Kamper and Nielsen 1990). Early EPO substitution is probably beneficial in anaemia may contribute to insulin resistance and left ventricular hypertrophy (Levin et al., 1999), both of which are strong risk factors for cardiovascular disease, and may be lead to the progression of renal disease (Rossert et al., 2002).

3.4.7 Vitamins

No alterations in vitamin D metabolism have been shown in diabetic patients and normal renal function (Storm et al., 1983) even though an early loss of bone density has been observed in Type I diabetes (Levin et al., 1976). A more pronounced bone loss is seen in uremic patients with diabetes compared to non-diabetic patients (Andress et al., 1987). In chronic renal failure an early elevation of parathyroid hormone, partly due to hyperphosphatemia and hypocalcaemia is seen (Reichel et al., 1991). The activation of 1-α hydroxylation of 25(OH) D-vitamin is reduced in renal failure, and hence, early substitution with active D-vitamin and dietary phosphate restriction in early chronic renal failure may be recommended, particularly in patients with diabetes. Vitamin E has been suggested to prevent microvascular complications patients with Type I diabetes (Bursell et al., 1999). On the other hand, no beneficial effect of Vitamin E supplementation on cardiovascular events could be confirmed in a large clinical trial, the HOPE study (Lonn et al., 2002). Elevated levels of
homocysteine are observed in more than 90% of patients with ESRD (Bostom and Culleton, 1999), but they do not seem to be altered by diabetes (Wollesen et al., 1999) or microalbuminuria (Agardh et al., 1996). High levels of homocysteine have been linked to atherosclerosis, but not to the rate of progression of renal impairment (Samuelsson et al., 1999). It is speculated that a lowering of the homocysteine levels, using a folic acid and B-vitamin regimen in renal disease, could reduce the excess incidence of cardiovascular disease and this is explored in ongoing trials.

3.4.8 Growth Factors and Cytokines Modulator

Mesangial cell proliferation inhibitors like LTP4 antagonist, PDGF inhibitors MMP inhibitors, CDK inhibitor etc. are the potent targets, used in the treatment of diabetic nephropathy (Twigg et al., 2002). Various growth factors like HGF and BMP-7 has been reported to have protective effect in the progression of nephropathy where as other factors like TGF, PDGF, EGF and VEGF inhibitors shown to have protective effect in the prevention of diabetic nephropathy (Liu, 2004).

3.4.9 mTOR Pathway Inhibitor

mTOR has been shown to be a central regulator of cell growth by involving in protein synthesis and cellular hypertrophy in various types of cells and organs and it is regulated by a large number of signals including nutrients such as glucose, amino acids, growth factors such as insulin and IGF-1. mTOR activation also plays an important role in insulin resistance in insulin targeted tissues such as fat, muscle and liver (Leibowitz et al., 2008). Moreover, renal cortical homogenates from diabetic db/db mice showed decrease in eEF2 phosphorylation and increment in eEF2 kinase phosphorylation probably due to mTOR activation (Satranatarajan et al., 2007), p70S6-kinase, a downstream of mTOR, was highly activated in mesangial cells in diabetic obese db/db mice. Furthermore, systemic administration of rapamycin, a specific and potent inhibitor of mTOR markedly ameliorated pathological changes
and renal dysfunctions (Mori et al., 2009). Rapamycin is an FDA approved drug specifically inhibits the mammalian target of rapamycin (mTOR), a protein kinase (Leibowitz et al., 2008) and has been used clinically to inhibit both host rejection following organ transplantation, such as kidney and islets and the re-stenosis of coronary arteries post angioplasty (Tsang et al., 2007). Recent studies also found that rapamycin has potent growth inhibitory activity against the development of various types of tumors and has potential as an anti-cancer treatment.

3.4.10 Vascular Endothelial Growth Factor (VEGF) Inhibitors

VEGF also has a role in the pathogenesis of diabetic nephropathy. Increased production of VEGF from podocytes causes glomerular hypertrophy and is associated with proteinuria (Liu et al., 2007). SU5416 is a selective small-molecule inhibitor that blocks all VEGF receptors at the level of tyrosine kinase (Mendel et al., 2000). Inhibition of VEGF by SU5416 ameliorated albuminuria in an experimental model of diabetic nephropathy (Sung et al., 2006).

3.4.11 TGF-β Inhibitors

Administration of isoform-specific recombinant monoclonal anti-TGF-β2 IgG4 (termed CAT-152) antibody suppresses renal fibrosis and reduce albuminuria by maintaining the procollagen-I C-propeptide to normal level in diabetic rats with nephropathy (Hill et al., 2001). Further, administration of murine (1D11) or human (CAT-192) anti-TGF-β monoclonal antibodies alone or in combination with lisinopril have renoprotective effect by reducing proteinuria in diabetic rats with nephropathy (Benigni et al., 2006). Moreover, administration of circular antisense TGF-β oligodeoxynucleotides has renoprotective potential by inhibiting the over expression of TGF-β in the kidney of diabetic rats (Jeong et al., 2005).
3.4.12 PKC Inhibitors

PKC isoforms i.e. PKC-α and PKC-β appear to be the most relevant and most likely targets of new therapeutic agents for treatment of diabetic nephropathy. Ruboxistaurin (RBX), a highly selective PKC-β inhibitor, shows promising effects in the prevention of diabetic vascular complications (Gilbert et al., 2007) by inhibiting the activation of the PKC-β1 isoform without affecting PKC-α isoform activation and prevents the mRNA expression of TGF-β1 and extracellular matrix components, such as fibronectin and alpha1 (IV) collagen in the glomeruli (Koya et al., 2000). RBX also improves the glomerular filtration and albumin excretion rates (Ishii et al., 1996). RBX can abrogate the glucose-induced oxidative stress in the glomeruli of streptozotocin-induced diabetic rats through a decreased activation of critical NADPH subunits (Kitada et al., 2003). It can also regulate eNOS activity in glomerular endothelial cells that is decreased in response to glucose induced PKC activation (Chu and Bohlen, 2004) furthermore, eNOS provide renal protection by maintaining glomerular function through the inhibition of thrombosis, leukocyte adhesion/activation, apoptosis and oxidative stress.

3.4.13 Aldosterone Antagonists

Aldosterone accelerates renal damage by inducing the production of growth factors, ROS and interfering the process of extra cellular matrix degradation (Remuzzi et al., 2008). Treatment with spironolactone, an aldosterone receptor antagonist, down regulates renal expression of matrix regulating genes such as TGF-β, matrix metalloproteinase, VEGF and insulin growth factor and thus decreases albuminuria and mitigates glomerulosclerosis in experimental diabetic nephropathy and provide renoprotective effect by reducing the oxidative stress and attenuating the overexpression of monocyte chemoattractant protein-1 (MCP-1) in patients with diabetic nephropathy (Takebayashi et al., 2006). In a recent study, eplerenone, an
aldosterone receptor antagonist has been noted to improve GFR and inhibit glomerulosclerosis in Otsuka Long-Evans Tokushima Fatty (OLETF) rats. Moreover, the combined treatment of eplerenone with enalapril markedly decreased the renal expression of TGF-β, type IV collagen and PAI-1 in OLETF rats (Kang et al., 2006).

3.4.14 Glycosaminoglycans

Glycosaminoglycans important components of plasma membranes and are believed to be important determinants of glomerular basement membrane permeability. It is assumed that critical loss of glycosaminoglycans has a significant role in the pathophysiologic process of albuminuria and proteinuria (Gambaro and Vander, 2000). Sulodexide (80% low-molecular-weight heparin, 20% dermatan sulfate), an oral formulation of the natural polysaccharide glycosaminoglycan that preserves the ionic charge of the glomerular barrier and decreases proliferation and fibrosis in the diabetic kidney but the mechanism of its nephroprotective action is still unknown (Wiess et al., 2007).

3.4.15 Endothelin Receptor Antagonists

Endothelins comprise 3-member family of 21–amino acid peptides, regulates water and sodium excretion, acid-base balance and maintain normal renal cell proliferation with tonic vasoconstriction in kidney (Kohan, 2006). Diabetic nephropathy is associated with enhanced renal synthesis of endothelins which leads to kidney damage. Avosentan (SPP301) is a new orally available endothelin 1 antagonist which provides nephroprotective action in diabetic nephropathy (Sorokin and Kohan, 2003).

3.4.16 Calcium Channel Blockers

Nondihydropyridine Calcium Channel Blockers (CCBs) have been shown to be renoprotective by decreasing urinary albumin excretion (UAE) and improving glomerular barrier size selectivity in patients with Type II diabetes mellitus and overt
nephropathy (Smith et al., 1998). Nondihydropyridine CCBs combined with an ACE inhibitor may produce greater reductions in UAE than either agent alone. Diltiazem and lisinopril significantly reduced UAE compared with baseline (P < 0.05). The results of this study suggest that diltiazem may be an appropriate alternative agent for patients who cannot tolerate an ACE inhibitor due to adverse effects. The use of lisinopril and diltiazem over a loop diuretic plus beta-adrenergic antagonist for attenuating the progression of diabetic renal disease was also demonstrated (Slataper et al., 1993). The nondihydropyridine CCB, diltiazem shows better renoprotective action as compared to dihydropyridine CCB, nifedipine by reducing proteinuria and improving glomerular permselectivity, thus slowing nephropathy progression. The nondihydropyridine CCBs have been found to be as effective as ACE inhibitors in clinical trials and should be considered an alternative if there is a contraindication to the use of an ACE inhibitor or ARB or if adequate blood pressure control is not obtained with ACE inhibitor or ARB monotherapy (Crepaldi et al., 1998).

3.4.17 PPAR Ligands

Peroxisome proliferator activated receptors (PPARs) are ligand-activated transcription factors of nuclear hormone receptor superfamily, which comprises of three members such as PPAR α, PPAR γ and PPAR β/δ (Buse, 2003). PPAR ligands are promising agents to prevent the progression of diabetic nephropathy. Activation of PPARα induces gene expressions that promote lipid metabolism and oxidation of fatty acids (Balakumar et al., 2007). Fibrates class of interventions are PPARα agonist such as bezafibrate, fenofibrate and gemfibrozil are well-known hypolipidemic agents (Rose et al., 2007) and provide a new therapeutic option in managing diabetic nephropathy. Bezafibrate provide renoprotection by reducing albuminuria and circulating lipid levels in diabetic patients with nephropathy (Nagai et al., 2000). Treatment with fenofibrate affords renoprotection by reducing the occurrence of
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albuminuria and glomerular lesions in experimental diabetic mice (Park et al., 2006). Further, fenofibrate provide renoprotection by decreasing renal COX-2 expression and reducing nitrosative stress in the kidney of diabetic rats in early stages of nephropathy. The administration of gemfibrozil reduces albuminuria, glomerulosclerosis and tubulointerstitial fibrosis in diabetic mice with nephropathy (Calkin et al., 2006). Activation of PPARγ regulates gene expressions that promote insulin sensitization and glucose metabolism. Thiazolidinedione class of interventions such as troglitazone, rosiglitazone, ciglitazone and pioglitazone are well-known PPARγ agonists employed as insulin sensitizing antidiabetic agents. Chronic diabetes mellitus reduces the mRNA levels of PPARγ in the glomeruli (Zheng et al., 2002). Troglitazone have renoprotective effect by reducing urinary albumin/creatinine ratio and downregulating angiotensin-II-induced expression of plasminogen activator inhibitor-1 (PAI-1) in mesangial cells of diabetic rats with nephropathy (Nicholas et al., 2001) and also decreases TGF-β mediated upregulation of type-1 collagen in mouse mesangial cells. Treatment with rosiglitazone possess renoprotective effect as it reduces albuminuria and prevents renal endothelial dysfunction in diabetic patients with nephropathy (Pitrosch et al., 2005) and markedly reduced high glucose mediated over expression of intracellular adhesion molecule-1 (ICAM-1) and β-integrin in glomerular mesangial cells of rats. Treatment with pioglitazone markedly reduced the occurrence of albuminuria and prevented the development of glomerulosclerosis and glomerular hypertrophy by suppressing the expression of TGF-β, VEGF, PAI-1, type-IV collagen and ICAM-1 in the kidney of diabetic rats with nephropathy.

3.4.18 Herbal Drugs

Sun ginseng at dose of 50 or 100 mg/kg/day for 15 days attenuated water intake and urine excretion induced by diabetes. In addition, the diabetic rats given Sun ginseng at a dose of 100 mg/kg body weight showed significant decreases in serum
glucose, serum glycosylated protein and urinary protein levels. Furthermore, *Sun
ginseng* significantly reduces the AGE product formation and thiobarbituric acid-
reactive substance levels elevated in the kidneys of diabetic rats. Green tea is a useful
agent to protect against protein oxidation and glycation associated diseases
(Nakagawa and Yokozawa, 2002). It also indicated as beneficial agents to manage the
development of diabetic nephropathy induced by subtotal nephrectomy plus
streptozotocin injection (Yokozawa *et al.*, 2005). Corni Fructus (*Cornus officinalis*),
had an effect on STZ-induced diabetic rats as compared aminoguanidine Treatment
with Corni Fructus for 10 days suppressed hyperglycemia, proteinuria, renal AGE
formation, and related protein expressions, *i.e.*, receptor for AGEs, nuclear factor-kB,
TGF-β and *N*-(*N*-carboxymethyl) lysine, in the same way as with aminoguanidine
(Yamabe *et al.*, 2007). *E. jambolana* preparations employed by practitioner of natural
health for treatment of diabetes and related complications, antioxidant, anti-
inflammatory and antifertility agents. *Eugenia jambolana* plant serves varies purposes
in diabetic patients such as lowering blood glucose level, delaying diabetic
complications such as neuropathy and cataract (Kharya *et al.*, 2006). Moreover 1.5%
*C. aromatica*-containing diet for 1 wk before and 8 wk after administration of
streptozotocin improves all the events induced by streptozotocin except for
hyperglycemia decreased markedly. Thus, *C. aromatica* may have therapeutic
potential for the prevention of hyperglycemia-associated diabetic complications (Jung
*et al.*, 2006).

**3.5 MEDICINAL PLANTS USED IN DIABETIC COMPLICATIONS**

In the last few years there has been an exponential growth in the field of herbal
medicine and these drugs are gaining popularity both in developing and developed
countries because of their natural origin and less side effects (Rajasekaran *et al*.,
2001). Many traditional medicines in use are derived from medicinal plants, minerals
and organic matter. The World Health Organization (WHO) has listed 21,000 plants, which are used for medicinal purposes around the world. Among these 2500 species are in India, out of which 150 species are used commercially on a fairly large scale. India is the largest producer of medicinal herbs and is called as botanical garden of the world. The plant contains a large number of constituents such as flavonoids, alkaloids, steroids, triterpenoids, lipids, lignins, carbohydrates, proteins, and glycoproteins which are responsible for their activity. Herbal medicines are the medicinal products that contain plant materials as their pharmacologically active components (Schulz et al., 1998). Medicinal plants are important for pharmacological research and drug development, not only when plant constituents are used directly as therapeutic agents, but also as starting materials for the synthesis of drugs or as models for pharmacologically active compounds. Phytochemical screening has been carried out to identify these phytoconstituents from the various extract. Further, these extract are subjected to pharmacological evaluation to screen their therapeutically potential. It is estimated that about 25% of the drugs prescribed worldwide are derived from plants and 121 such active compounds are in use. Of the total 252 drugs in WHO essential medicine list, 11% is exclusively of plant origin (Rates, 2001). Nearly 80% of African and Asian population depends on traditional medicines for their primary healthcare (WHO, 2008). In India, about 80% of the rural population uses medicinal herbs or indigenous systems of medicine (Mukherjee and Wahile, 2006). Generally, in traditional medicine, many herbal drugs are combined in the form of a multi-herbal formula to enhance their functions. The herbal constituents are selected to emphasize the therapeutic actions or to reduce the toxicity or side effects of compounds from other herbal species in the mixture (Bansky and Barolet, 1990). Oral hypoglycaemic agents like sulphonylureas and biguanides are still the major players in management of the disease but there is growing interest in herbal remedies due to
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	side effects associated with the oral hypoglycaemic agents (Patel and Srinivasan, 1997). Nowadays, the use of complementary alternative medicine and especially the consumption of botanicals have been increasing rapidly worldwide (Borchers et al., 2000), mostly because of the less frequent side effects when compared to modern western medicine. Therefore, there is a need to search more effective and safe herbal drugs for diabetes and its complications (Pari and Maheshwari, 1999).

Polyphenolic compounds, flavonoids, terpenoids, saponins, polysaccharides and alkaloids are the major chemical moieties present in the plant species in these families and these major secondary metabolites tend to reverse or delay the diabetic complications by decreasing the persistent hyperglycemia, decreasing the formation of ROS, by increasing the secretion of insulin from β-cells and by inhibiting the formation of AGEs (Patel et al., 2012).

The flavonoids are the polyphenolic compounds which has been reported to increases the insulin release in vitro from pancreatic islets and decrease the levels of LDL, triglycerides and increases HDL level by dual upregulation of both peroxisome proliferators-activated receptors (PPARα and PPARγ) up to 3–4 folds leads to hypoglycemic and hypolipidemic effects in the management of diabetes (Sharma et al., 2008). Flavonoids mainly act by inhibiting free radical formation and propagation of free radical reactions through hydrogen donation and aromatic hydroxylation (Hanasaki et al., 1994). Flavonoids reduce oxidative stress leading to less degradation of GSH or either increases the biosynthesis of GSH. In addition, flavonoids also regenerate the pancreatic β-cells, reduces necrosis and degeneration and thus, effective in treating hyperglycemia thereby preventing diabetic complications (Sefi et al., 2010).

Alkaloids produce antihyperglycemic action by potentiating pancreatic secretion of insulin from β-cell of islets or by enhancing transport of blood glucose to
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peripheral tissue (Gulfraz et al., 2011), it modulates enzymes responsible for glucose metabolism, reducing oxidative stress and thus helps in restoring antioxidant status (Singh and Kakkar, 2009). Moreover, aqueous extract of the leaves of Murraya koenigii significantly improved renal function and antioxidant status in STZ-induced diabetic rats (Yankuzo et al., 2011).

Phenolic compounds were found to lower blood glucose in STZ induced diabetic rats by enhanced insulin secretion with regeneration of β-cells reduces oxidative stress and modulates enzymes responsible for glucose metabolism (Gandhi et al., 2011). Phenolic compounds increase the levels of GSH and reverses increased levels of lipid peroxidation in diabetic rats, thus contribute in the effective management of diabetes and associated complications (Dewanjee et al., 2009). Hyperglycemia generates ROS, which in turn cause lipid peroxidation and membrane damage (Hunt et al., 1988). Plants rich in phenolic content have been reported to possess higher antioxidant activities than vitamins and synthetic antioxidants. The phenolic compounds show a significant increase in antioxidant enzymes including glutathione peroxidase, glutathione reductase and glutathione S-transferase in the diabetic and moreover, increased GSH level and decreased malonaldehyde levels and oxidative stress indicating their ability to reduce blood glucose concentration, and subsequent oxidation.

Saponins isolated from medicinal plants are found to be renoprotective as they reduce fasting blood glucose and albuminuria, reverses the glomerular hyperfiltration state and ameliorates proliferative glomerular pathological changes during the early stages of diabetic nephropathy in rat models (Zhang et al., 2009). Saponins produce a significant reduction in blood glucose and lipid profile by stimulate remnant β-cells to produce insulin (Meliani et al., 2011). Panax quinquefolius L. has preventive effects
on diabetic nephropathy and it works through a combination of mechanisms such as antihyperglycemic and antioxidant activities (Sen et al., 2012).

Phytosterols play an important role in the prevention of diabetic complications by ameliorating oxidative stress and altering antioxidant enzyme levels (Kumar and Padhy, 2011). Antihyperglycemic and antioxidant effect of steroidal components of plants help in preventing renal complications associated with diabetes (Kumar and Padhy, 2011).

Tannins play an important role in preventing diabetic complications by reducing the formation of AGEs and oxidative stress (Soman et al., 2010, 2013; Omara et al., 2012). Amino acid like S-allyl cysteine decreased plasma glucose level, TBARS, hydroperoxide and GSSG in diabetic rats. In addition, the levels of plasma insulin, superoxide dismutase, catalase, GPx and reduced GSH level were also increased. Amino acid reduces oxidative damage, inhibits lipid peroxidation and enhances cellular antioxidant defense. Therefore amino acids can be useful in management of diabetes and the related complications (Saravanana and Ponmurugana, 2011).

Antioxidant defences and cellular redox status is key player in diabetes and its complications (West, 2000). Increased oxidative stress and depleted antioxidant defense in diabetes and its complications are well established (Evans et al., 2002; Choi et al., 2008). Hyperglycemia and increased production of reactive oxygen species (ROS) resulting in increased oxidative stress with over activation of NADPH oxidase are important components of metabolic syndrome (Demircan et al., 2008). Moreover, insulin resistance is also positively associated with systemic oxidative stress. Oxidative stress leads to the development of diabetes mellitus by activating stress-signaling pathways such as NF-κB (Davi et al., 1999). Contribution of oxidative stress to diabetic complications may be tissue specific, mainly in
microvascular diseases which occur only in diabetic patients. Thus antioxidant treatment coupled with other treatments for diabetic complications would most likely be effective in ameliorating these complications (Scott and King, 2004). Plants are used as an essential component of traditional medicine systems (Fang et al., 2005) and recent literature reveals that leaves are the most favorable storage sites for active ingredients (Chan et al., 2012) which have been maximally utilized for management of diabetic complications. Among various parts of plants used in the study are leaves (29%), roots (14%), whole plant (10%), fruits (9%), seeds (6%), flowers (5%), aerial parts (2%), stem (1%), and root barks, rhizomes, latex, etc. in small proportion. Literature reveals that flavonoids (30%), terpenoids (17%) and polyphenolic compounds (6%) were found to be effective in attenuation of diabetic complications by decreasing the persistent hyperglycemia, decreasing the formation of ROS, by increasing the secretion of insulin from β-cells and by inhibiting the formation of AGEs (Chan et al., 2012). On the basis of literature survey this may be speculated that antioxidant, antidiabetic provides the nephroprotective action due to the presence of phytochemicals like flavonoids, sterols triterpenoids and saponin as the active constituents which may be the possible mechanism of used extracts.

3.5.1 Artemisia dracunculus

Artemisia dracunculus or Russian Tarragon is a perennial herb that belongs to the Asteraceae family. Many edible and medicinal uses have been attributed to this species and it is commonly used for flavoring food in many traditional recipes (Phillips and Foy, 1990). An ethanolic extract of Artemisia dracunculus L. having antidiabetic activity was examined as a possible aldose reductase (ALR2) inhibitor, a key enzyme involved in diabetic complications. The compounds such as 4, 5-di-O-caffeoylquinic acid, davidigenin, 6-demethoxycapillarisin and 20, 40-dihydroxy-4-methoxydihydrochalcone was isolated from the Artemisia dracunculus that inhibit the
activity of aldose reductase (ALR2). At the dose of 3.75g/mL the ethanolic extract of
*A. DRACUNCULUS* shoots inhibited the human recombinant ALR2 enzyme activity
by the enhanced activation of the polyol pathway during hyperglycemia and therefore
evaluates its potential for treatment of diabetic complications (Longendra *et al.*, 2006).

### 3.5.2 *Bacopa monnieri*

*Bacopa monnieri* Linn belongs to family Scrophulariaceae (vernacular;
*Brahmi*), is an annual creeping plant and it is found throughout India, Nepal, Srilanka,
China and Taiwan. Oral administration of aqueous ethanolic extract of whole plant of
*Bacopa monnieri* to diabetic rats at a dose of 50,125 and 250mg/kg body weight daily
for 15 days showed significant reversal of disturbed antioxidant status and
peroxidative damage and hence reveals its potential to play a crucial role in defense
against free radicals. Significant increase in SOD (Superoxide dismutase activity),
CAT (Catalase activity), GPx (Glutathione peroxidase) activity and levels of GSH
(Reduced glutathione) and reduction in the level of MDA (Malondialdehyde) was
observed in extract treated diabetic rats. Thus, *Bacopa Monneiri* appears to have a
potential to inhibit the neuronal and renal complications due to diabetes (Kapoor *et
al.*, 2009).

### 3.5.3 *Enicostemma littorale* Blume

*Enicostemma Littorale* Blume is a glabrous perennial herb belonging to the
family Gentianaceae. The plant is used in folk medicine to treat the diabetes mellitus,
rheumatism, abdominal ulcers, hernia, swelling, itching and insect poisoning (Kirtikar
and Basu, 1999). Methanolic extract of whole plant of *Enicostemma littorale* Blume
was studied to assess its protective effects in alloxan-induced diabetic neuropathy in
male Charles foster rats. An intragastrically administration of extract at a dose of
2.5g/kg body weight for 45 days were significantly restored the changes in lipid
peroxidation status and anti-oxidant enzymes (super-oxide dismutase, glutathione peroxidase and catalase) levels in diabetic rats. Decrease in Na-K\(^+\) ATPase activity was also significantly restored by *Enicostemma litorale* Blume therefore; protective effect of *Enicostemma litorale* against neuropathy could be due to controlling hyperglycemia and reducing oxidative stress (Bhatt *et al.*, 2009).

**3.5.4 *Picrorhiza scrophulariiflora***

*Picrorhiza scrophulariiflora* is a perennial herb belonging to family Scrophulariaceae. The dried rhizomes of *Picrorhiza* have long been used to treat inflammatory diseases such as arthritis and asthma in southeast Asia (Yang *et al.*, 2003). Oral administration of ethanol extract of dried rhizomes of *Picrorhiza scrophulariiflora* at a dose of 400 mg/kg per day for 5 or 10 weeks in STZ-induced diabetic rats significantly attenuated oxidative stress in the diabetic kidney by a reduction in NADPH oxidase-dependent superoxide generation and decreased expression of malondialdehyde and advanced oxidation protein products in renal tissue. This was accompanied by improvement in renal inflammation, including decreased macrophage influx and downregulated expression of chemokines such as CCL2 and TGF-β1. These data suggest that *Picrorhiza Scrophulariiflora* might improve diabetic nephropathy, probably through inhibition of redox-sensitive inflammation (He *et al.*, 2009).

**3.5.5 *Propolis***

*Propolis* is a resinous hive product collected by honeybees from many plant sources (Tan-No *et al.*, 2006). Oral administration of ethanolic extract of *Propolis* at doses of 100, 200 & 300 mg/kg body weight for 40 days in streptozotocin induced diabetic rats improved the body and kidney weights, serum glucose, lipid profile, Malondialdehyde and renal function in a dose dependent manner. Significant increase in Superoxide dismutase activity, Catalase activity and levels of Reduced glutathione
and reduction in the level of Malondialdehyde was observed in extract treated diabetic rats. These results may suggest a strong antioxidant effect of Propolis which can ameliorate oxidative stress and delay the occurrence of diabetic nephropathy in diabetic patient (Abo-Salem et al., 2009).

3.5.6 Carum Carvi (Black Zeera)

Caraway commonly known as black zeera, is a member of aromatic umbelliferous plants. In ancient time, Caraway has been used for the treatment of digestive disorders. The major constituents of its seeds are carvone, flavonoids and limonone, whereas it’s minor constituents are myrcene, beta-caryophyllene, thujone, anethole and pinene. Co-treatment of Carvi aqueous seed extract (30 and 60mg/kg bwt) for 60 days prevents the streptozotocin induced diabetic nephropathy by reducing the level of serum glucose, urea, creatinine, total urinary protein and total albumin excretion in diabetic rats. The overall renoprotective of Carum Carvi is probably due to its antioxidant effect (Sadiq et al., 2010).

3.5.7 Ginger

Ginger (Zingiber officinale Roscoe, Zingiberaceae) is widely used around the world in foods as a spice. For centuries, it has been an important ingredient in Chinese, Ayurvedic and Unani herbal medicines for the treatment of catarrh, rheumatism, nervous diseases, gingivitis, toothache, asthma, stroke, constipation and diabetes (Wang and Wang, 2005). Diabetic rats received Ginger powder 5% of their consumed food daily in streptozotocin induced diabetic nephropathy for 8 weeks significantly reduces the extent of lipid peroxidation, which is measured in terms of malondialdehyde levels and improves plasma antioxidant capacity, therefore, Ginger causes a decrease in lipid peroxidation, increase of plasma antioxidant capacity and a reduction in renal nephropathy (Afshari et al., 2007).
3.5.8 *Andrographis paniculata*

The administration of chloroform extract of *A. paniculata* of family *Acanthaceae* to alloxan-induced diabetic rats for four weeks produced significant blood glucose reduction and significantly inhibited the induction of albuminuria, proteinemia and uremia. The studies clearly indicated a significant anti-diabetic activity with the chloroform extract of *A. paniculata* roots and supports the traditional usage of the plant also useful in preventing the incidence of long-term complication of diabetic nephropathy (Rao, 2006).

3.5.9 *Astragalus propinquus*

Administration of Astralagus propinquus of family Fabaceae improves the pathogenesis and development of diabetic nephropathy by affecting the plasma Endothelin I (ET-I) levels and platelet function (Liu *et al.*, 2006).

3.5.10 *Cinnamomum zeylanicum*

It belongs to family Lauraceae and commonly known as dalchini at dose 20 mg/kg prevents early stage diabetic nephropathy due to its antioxidant and antidiabetic effect in alloxan-induced diabetic nephropathy by reducing the glomerular expansion, eradicating hyaline casts and decreasing the tubular dilatations (Mishra *et al.*, 2010).

3.5.11 *Curcuma longa*

Chronic treatment with curcumin obtained from *Curcuma longa* of family Zingiberaceae significantly attenuates both renal dysfunction and oxidative stress in streptozotocin-induced diabetic rats and certainly provide nephroprotective action (Sharma *et al.*, 2006).

3.5.12 *Ganoderma lucidum*

*Ganoderma lucidum* of family ganodermataceae polysaccharide reduces the serum glucose, triglyceride levels, serum creatinine, blood urea nitrogen levels and
urinary albumin excretion in diabetic mice in a dose dependent manner and delay the progression of diabetic renal complications (He et al., 2006).

3.5.13 Ginkgo biloba

The effect of Ginkgo biloba leaf on renal lesions of early diabetic nephropathy was studied on sixty eight patients by oral administration for three months. Nephropathy indexes such as urinary micro-albumin, alpha 1 micro globulin, immunoglobulin, transferring, retinal binding protein protein and N-acetyl beta D glucosaminidase before and after treatment were compared. The elevated level significantly improved in the treated group. However, there was no significant decrease in the above mentioned indexes in the control group (Zhu et al., 2005).

3.5.14 Glycine max

Soyabean decreases the progression of diabetic nephropathy (Iritani et al., 1997) by preventing morphological destruction of the kidney associated with diabetes mellitus. They contain carbohydrates, fat, protein, vitamins, minerals like calcium, folic acid and iron (Lavigne et al., 2000). Soyabean feeding is known to enhance the conversion of polyunsaturated fatty acids to docosahexaenoic acid. A soyabean diet improves serum glucose and insulin levels, as well as insulin sensitivity in diabetes. Although the exact mechanism has yet to be elucidated, it is possible that the soluble fiber component of soyabean may be the most important factor (Anderson et al., 1998). Approximately 15% of the soyabean is composed of insoluble carbohydrates and over 30% of the fiber in soyabean is of the soluble variety. Moreover, soyabean are slowly digested and have a low glycaemic index.

3.5.15 Gymnema moutanum

It is an endemic plant species of India used traditionally for diabetes and its management. The ethanolic extract of Gymnema moutanum at a dose of 200mg/kg body weight significantly normalized the elevated blood glucose, renal markers and
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lipid peroxidation markers and increased antioxidant levels in diabetic kidney. The diabetic rats excreted large amount of proteins than untreated rats which was normalized during the treatment with the ethanol extract. The ethanol extract has been found to be effective in reducing oxidative stress and protecting diabetes and its complications (Ramkumar et al., 2009).

3.5.16 Indigofera tinctoria Leaves

The extract from leaves improved renal creatinine clearance and reduced renal total protein loss demonstrating nephroprotective properties. The organ to body weight ratio studies carried out showed pancreas and liver specific effects of Indigofera tinctoria leaves. These results were also supported by histopathological studies. It was concluded from the studies that alcoholic extract of leaves in long-term treatment may be beneficial in the management of Type-I, Type-II diabetes (Bangar et al., 2011).

3.6 Reviews on Jasminum Species

3.6.1 Jasminum grandiflorum Linn (Spanish jasmine, Common jasmine, Chameli)

Jasminum grandiflorum is a scrambling sub-erect twining evergreen shrub (Anonymous, 1987 and Anonymous, 2004), native to India, France, Italy, China, Japan, Morocco and Egypt (Chopra et al., 1958; Kirtikar and Basu, 1987; Chopra et al., 2002; Sharma et al., 2005). The leaves are opposite, entire ovate to somewhat elliptic in shape with acuminate mucronate apex, whereas flowers are terminal and axillary cymes, calyx lobes are long, linear (Cooke, 1967; Nadkarni and Nadkarni, 1967). Roots are useful in cephalalgia, mental debility, chronic constipation, flatulence, strangury, sterility, dysmenorrhoea, amenorrhoea, ringworm, leprosy, skin diseases and giddiness. Leaves are useful in odontalgia, fixing loose teeth, ulcerative stomatitis, leprosy, skin diseases, otterhœca, otalgia, strangury, dysmenorrhœa, ulcers, wound, corns and flowers are useful in stomatopathy, cephalopathy, odontopathy,
ophthalmopathy, leprosy, skin diseases, pruritis, strangury, dysmenorrhea, ulcers, as refrigerant, ophthalmic and vitiated conditions of pitta (Warrier et al., 2004).

Phytochemical studies revealed that leaves contains 2’”-epifraxamnoside, demethyl-2”-epifraxamside, jasminanhydride (Sadhu et al., 2007), oleacein, 2-(3, 4-dihydroxy phenyl)-ethanol, isoquercitrin, ursolic acid (Somanadhan et al., 1998), resin, salicylic acid, jasmine, indole oxygenase (Divakar et al., 1979), 3, 4-dihydroxy benzoic acid, 2-hydroxy-30, 40-dihydroxyacetophenone and oleanolic acid (Sadhu et al., 2007), flower contains Cis-3-hexenol, 2-vinyl pyridine, indole, myrcene, linalool, geranyl linalool, α-terpineol, geraniol, linalyl acetate, nerolidol, phytol, isophytol, farnesol, eugenol, benzyl alcohol, p-cresol, methyl benzoate, benzyl cyanide, benzyl acetate, methyl dihydrojasmonate, methyl anthranilate, jasnone, methyl- N-methyl anthranilate, vanillin, cis-3-hexenyl benzoate, benzyl benzoate, methyl palmitate, methyl linoleate (Rastogi and Mehrotra, 1999), jasgranoside, jaspolyoside, 8-epi-kingiside, 10-hydroxy-oleuropein, 10-hydroxyligistroside, oleoside-7,11-dimethylester (Zhao et al., 2008), 3-O-α-L-rhamnopyranosyl(1→2)-β-D-xylopyranosyl-hederagenin-28-O-β-D-galactopyranosyl(1→6)-β-D-galactopyranosylester, hederahederagenin-3-O-β-D-glucopyranosyl (1→3)-α-L-arabinopyranoside, 2-O,3β,23-trihydroxyolean-12-en-28-oic-O-β-D-glucopyranosyl ester, hederagenin-3-O-β-Dxylopyranosyl (1→3)-α-L-rhamnopyranosyl (1→2)-α-L-arabinopyranoside, 2α, 3β, 23-trihydroxyolean-12-en-28-oic-O-α-L-rhamnopyranosyl (1→4)-β-D-glucopyranosyl(1→6)-β-D-glucopyranosyl ester, hederagenin-3-O-α-L-rhamnopyranosyl (1→2)-α-Larabinopyranoside (Zhao and Dong, 2008), kaempferol-3-O-α-L-rhamnopyranosyl (1→3)-[α-L-rhamnopyranosyl (1→6)-β-D-galactopyranoside, kaempferol-3-O-rutinoside, 7-ketologanin, oleoside-11-methyl ester, 7-glucosyl-11-methyloleoside, ligistroside and oleuropein (Zhao and Dong, 2008). Moreover, jasmine oil consist of methyl jasmonate (Rastogi and

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Mehrotra, 1999), benzyl benzoate, linalool, linalyl acetate, benzyl alcohol, indole, jasnone, methyl anthranilate, P-cresol, geraniol, racemic (5-pent-2-enyl)-5, 1-pentanolide, benzyl benzoate, nerol, 1-α-terpineol, d and dl-linalool, γ-jasmolactone, farnesol, nerolidol and eugenol (Rastogi and Mehrotra, 2001). Hydro alcoholic extract of leaves of Jasminum grandiflorum L. showed anti ulcer activity in aspirin and pylorus ligation-induced acute gastric ulcer models with reduction in gastric fluid volume, free acid, total acid and an increase in the pH of gastric fluid (Mahajan et al., 2009). Petroleum ether, chloroform, acetone, methanol and aqueous extracts of leaves of Jasminum grandiflorum Linn were screened for their in vitro antibacterial activity against Staphylococcus aureus, Bacillus subtilis, Escherichia coli and Pseudomonas aeruginosa by using agar diffusion method. Out of all extracts tested, petroleum ether, methanol and aqueous extracts were effective against all four microorganisms. Chloroform extract was only effective against Bacillus subtilis and Pseudomonas aeruginosa. Acetone extract was effective against Pseudomonas aeruginosa and Escherichia coli (Paarakh and Gavani, 2009). Ethanolic flower extract of Jasminum grandiflorum have been shown wound healing activity by reduction in wound area, increased wet and dry granulation tissue weight and hydroxyproline content in excision and dead space wound models (Nayak and Mohan, 2007). Oleacein extracted from aerial parts of J. grandiflorum exhibited ACE inhibitor activity with IC₅₀ values 26-66 mM (Somanadhan et al., 1998). Oleuropein extracted from the flowers of J. grandiflorum demonstrated indubitable anti-HBV (hepatitis B virus) activity in HepG2 2.2.15 cells test in vitro and duck hepatitis B virus (DHBV) infected ducklings test in vivo (Zhao et al., 2009).

Oral administration of ethanol extract of J. grandiflorum flowers to 7,12-dimethylbenzanthracene (DMBA) injected animals prevented the formation of tumors in the pre-initiation period and exerted significant anti-lipid peroxidative effect and
improved the antioxidant defense system in DMBA-treated rats (Kolanjiappan and Manoharan, 2005). The antioxidant activity of ethanolic extract of leaves of *Jasminum grandiflorum* L. (JGLE) has been assayed by using in vitro methods like 2, 2-diphenyl-1-picyrlhydrazylhydride (DPPH) assay, reductive ability, superoxide anion scavenging activity, nitric oxide scavenging activity and it showed antioxidant activity in a dose dependent manner (Uمامaheswari *et al.*, 2007). Flowers of *J. grandiflorum* are useful to women when brewed as a tonic as it aids in preventing breast cancer and stopping uterine bleeding (Joshi, 2000). Ethanollic and aqueous extract of *J. grandiflorum* flowers and leaves in DMBA treated rats showed reduction of micronucleated polychromatic erythrocytes in bone marrow (Shanmugam *et al.*, 2006).

### 3.6.2 *Jasminum sambac* (Arabian jasmine, Indian jasmine, Sampaguita, Mogra)

*Jasminum sambac* is a member of Oleaceae family, known as sampaguita in the Philippines, where it is national flower, gunda mallige in India, mo li in China, pikake in Hawaii and Arabian jasmine in the mainland USA. It is commercially grown in India, Thailand, China and Philippines. It is an evergreen vine or shrub reaching up to 1-3 m. The leaves are ovate; phyllotaxy is opposite or in whorls of three. The flowers blooms throughout the year and are produced in clusters of 3-12 together. They are strongly scented and open at night, close in morning. The plant traditionally used as an analgesic, antidepressant, anti-inflammatory, antiseptic, aphrodisiac, sedative, expectorant and tonic (uterine) effects. Roots are used to treat wounds and snake bites where as the leaves and flowers have antipyretic and decongestant properties. The essential oil and methanol extract of flowers of *J. sambac* were evaluated for its antimicrobial activity against *E. faecalis* CIP103907, *E. coli* CIP 105182, *S. enterica* CIP105150 and *S. pyogenes.*, *B. Cereus* LMG 13569 by using disc diffusion and micro dilution methods (Latif *et al.*, 2010) and also subjected
for their antioxidant activity by DPPH free radical scavenging and β-carotene-linoleic acid assays. In the DPPH test system, the IC$_{50}$ value of essential oil and methanol extract were respectively 7.43 and 2.30µg/ml. In the β-carotene-linoleic acid system, oxidation was effectively inhibited by *J. Sambac* and the Relative antioxidant activity value of essential oil and methanol extract were respectively 96.6% and 93.9% (Latif *et al.*, 2010). The flowers are used for treatment of diarrhoea, abdominal pain, conjunctivitis and dermatitis. The leaves and roots are used for treating diarrhoea, fever, pain and as an anaesthetic. Phytochemical studies shown that the roots contains dotriacontanoic acid, dotriacontanol, oleanolic acid, daucosterol and hesperidin (Zhang *et al.*, 2004) and leaves contain sambacosides A, E and F (Tanahashi *et al.*, 1988), flower contains molihuaside A-E, sambaeoside A (Zhang *et al.*, 1995). Ethyl acetate (EAE) and water extract (WTE) of leaves of *Jasminum sambac* showed reduction in plasma glucose level, lipid profile and serum urea in diabetic rats (Upaganlawar *et al.*, 2009). The efficacy of jasmine flowers applied to the breasts to suppress puerperal lactation was compared that of Bromocriptine by reduction in serum prolactin level (Shrivastav *et al.*, 1988).

### 3.6.3 *Jasminum mesnyi* Hance (Primrose jasmine, Japanese jasmine, Japani chameli)

*Jasminum mesnyi* Hance (*Jasminum primulinum Hemsley*) also known as “Primrose Jasmine” or “Japanese Jasmine” found in tropical, sub-tropical and warm temperate regions of Asia. It is an open evergreen, rambling shrub, leaves are opposite, trifoliolate and attached to base of branchlets. Flowers are usually solitary, axillary or rarely terminal, yellow coloured, having 6-10 petals (Dicky, 1949; Chang *et al.*, 1996). The glossy dark green leaves are opposite and divided into three leaflets. The trumpet shaped flowers are borne in early spring and sporadically into summer (Flowers of India). *Jasminum mesnyi* leaves contains secoiridoids glucosides such as jasmoside and jasmesoside, 9”-hydroxyjasmesoside, 9”-hydroxyjasmesosidic acid,
jasminin 10”-O-β-d-glucoside, 2”-hydroxyjasminin, isojasminin, jasminin, 4”-hydroxyisojasminin, jasmosidic acid and phenolic glucoside like syringin or rutin (Inoue et al., 1985; He and Yang, 1989; Tanahashi et al., 1989; Inoue et al., 1991). Methanolic leaf extract and its n-butanol, ethyl acetate fractions of jasminum mesnyi have been shown to reduce fasting serum glucose level (Onozato and Tojo, 2005) and also showed in-vitro antioxidant activity in a dose dependent manner by DPPH radical scavenging and nitric oxide radical scavenging assays (Borar et al., 2011).

3.6.4 Jasminum angustifolium Linn. (Wild Jasmine, Banmallika)

Jasminum angustifolium Linn. belonging to the family Oleaceae, distributed in south India (kerala, Karnataka) on the hills of lower elevation (Deni, 1995). Leaves are simple ovate-lanceolate, acute, glabrous (Ayurvedic medicinal plant) and flowers are either solitary or more usually in three. Petals are linear, obtuse and acute (Flowers of India). Ethanolic and aqueous extracts of whole plant of Jasminum angustifolium Linn. have been shown antitumor activity by increasing the survival time (life span) and decrease in peritoneal cancer cell count and body weight against Dalton’s ascitic lymphoma (DAL) model (Raju et al., 2010). Hepatoprotective effect of ethanolic and chloroform extract of Jasminum angustifolium Linn were evaluated against carbon tetrachloride (CCl₄) (1ml/kg) induced hepatic damage and was evidenced by reduction in level of alkaline phosphatase (ALP), alkanine amino transferase (ALT), aspartate amino transferase (AST), cholesterol, glucose, total protein and bilirubin concentration in blood (Joshi et al., 2008).

3.6.5 Jasminum auriculatum (Needle flower jasmine, Juhi, Juyi)

Jasminum auriculatum Vahl (Oleaceae) commonly known as Juhi, Needle flower jasmine, Yutika, grows almost throughout South India, on dry slopes of the Western Ghats (Vaidyaratnam, 2003). Flowers are white, sweet scented and trifoliolate with two lower leaflets broadly ovate, acuminate or rounded (Ghosh, 1984). The roots
are useful in skin diseases especially for ringworm and flowers are fragrant, bitter, acrid, sweet, refrigerant, astringent, cardiotonic, diuretic and depurative in nature. They are useful in burning sensation, hyperdesia, ulcers, odontalgia, stomatopathy, ophthalmopathy, cardiopathy, urolithiasis, nephrolithiasis, strangury and dermatopathy (Ghosh, 1984). *Jasminum auriculatum* leaves has been reported to contain lupeol and jasminol (Deshpande and Upadyaya, 1967). Alcoholic and aqueous extracts of flowers of *Jasminum auriculatum* showed diuretic activity by increasing the total volume of urine and concentrations of potassium and sodium salts in urine (Bahuguna et al., 2009) and antiurolithiatic activity by reducing the elevated urinary oxalate synthesis (Bahuguna et al., 2009).

3.6.6 *Jasminum arborescens* Roxb. (Tree Jasmine)

*Jasminum arborescens* Roxb. belonging to family Oleaceae and distributed in Sub-Himalayan tract, Bengal, Central and South India. It is known as Nava-mallikaa in Ayurveda and Nagamalli in Siddha (Bhagath et al., 2010). Leaves are opposite, simple, ovate, acute or acuminate and are astringent, stomachic. Juice of leaves, with pepper, garlic and other stimulants, is used as an emetic in obstruction of bronchial tubes due to viscid phlegm (Bhagath et al., 2010). Ethanol, chloroform and petroleum ether extracts of leaves of *Jasminum arborescens* Roxb has been shown in-vitro antioxidant activity in a dose dependent manner by DPPH free radical scavenging and Fe³⁺ reducing power assays (Bhagath et al., 2010) and also anthelmintic activity of these extracts was performed on adult Indian earthworm Phoretima pasthuma in which time taken for paralysis and death of worms was found lesser in case of ethanol extract followed by chloroform and petroleum ether extract (Bhagath et al., 2010).

3.6.7 *Jasminum amplexicaule* Buch.-Ham.

*Jasminum amplexicaule* Buch.-Ham. belonging to the family Oleaceae, distributed in Sikkim, Bhutan, Khasia, South India to Hongkon. Leaves are opposite,
simple, ovate-lanceolate, acuminate and flowers are scentless, calyx is pubescent, corolla is white, tinged with red outside. This plant used as a traditional medicine in dysentery, diarrhoea and bellyache in China (Jia et al., 2008). It contained some di and trimeric iridoids like jasamplexoside A, B and verbascoside (Tanahashi et al., 1992) and leaves contained jaslanecosides B, E, jasminoside, isojasminoside. Methanol extract of twigs and leaves of *Jasminum amplexicaule* and different fractions of this extract showed anti-diarrhoea, analgesic activity in castor oil-induced and magnesium sulphate-induced diarrhoea models, gastrointestinal motility models and analgesic activities were investigated using hot-plate, writhing and formalin models (Shen et al., 1999).

3.6.8 *Jasminum lanceolarium* (Jasminum lanceolaria, Jasminum lanceolarium Roxb.)

*Jasminum lanceolarium* is a climbing shrub belongs to family Oleaceae, distributed in China, India, Myanmar and Taiwan. Leaves are opposite, alternate, simple or trifoliate. Leaves and stems revealed the presence of 5, 7, 3', 5'-tetrahydroxyflavanone, (2S)-5,7,3', 4'-tetrahydroxyflavan-5-O-beta-D-glucopyranosie, mannitol, nonacosane, trans-p-coumaric acid, cis-p-coumaric acid, ferulic acid and trans-cinnamic acid (Sun et al., 2008), trans-P-coumaroyl and trans-feruloyl esters of 10-hydroxyoleoside, jaslanecosides A–E (Shen and Lin, 1996; Sun et al., 2009) and (2S)-5,7,3',5'-tetrahydroxy-flavanone 7-O-beta-D-allopyranoside, Betulinaldehyde, betulinic acid, betulin, syringing, liriodendrin and compound (2S)-5,7,3',5'-tetrahydroxy-flavanone 7-O-beta-D-glucopyranosie exhibited significant radical scavenging activity through DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay (Sun et al., 2007).
3.6.9 *Jasminum nudiflorum* (winter jasmine, hardy jasmine)

*Jasminum nudiflorum* Lindl. belonging to family Oleaceae, is rambling, diffuse shrub with slender, arching stems and four-angled green branchlets that bear opposite compound leaves with three leaflets and distributed in South England, China. Leaflets are dark green, oblong and flowers are bright yellow, unscented, funnel shaped. In China, flowers and leaves are used in treatment of inflammatory swelling, purulent eruptions, bruises or traumatic bleeding (Chiang, 1978). Phytochemical studies revealed that leaves and stems contained jasnudiflosides A-C (Tanahashi *et al.*, 1999), leaves also contained jasnudiflosides F-L, nudifloside D, isooloeacteoside (Takenaka *et al.*, 2002), and stems contained jasnudiflosides D- E, nudiflosides A-C (Tanahashi *et al.*, 2000). Leave extract of *Jasminum nudiflorum* Lindl. has been shown inhibitory effect on the corrosion of cold rolled steel in 1.0 M hydrochloric acid by weight loss, potent iodynamic polarization and electrochemical impedance spectroscopy methods in a dose dependent manner (Li *et al.*, 2010).

3.7 *Jasminum grandiflorum*

3.7.1 Classical names

*Sanskrit*: Jati, Malati; *English*: Spanish jasmine, Common jasmine Catalanian jasmine; *Hindi*: Jati, Cameli; *Telugu*: Jati,Malati; *Tamil*: Jatimalli, Kotimalligai, Picci; *Kannada*: Mallige; *Bangla*: Cameli; *Urdu*: Cameli,Yasmeen; *Punjabi*: Chamelee; *Marathi*: Cameli; *Gujarati*: Cameli, Cambeli; *Malayalam*: Piccakam, Piccakamulla, Pichi; *Assam*: Yasmeen (Sharma *et al.*, 2005; Edwin and Edwin, 2006; Bedi *et al.*, 2008).
Fig 2: *Jasminum grandiflorum* leaves

### 3.7.2 Taxonomical classification

**Kingdom:** Plantae  
**Subkingdom:** Tracheobionta – Vascular plants  
**Super division:** Spermatophyta – Seed plants  
**Division:** Magnoliophyta – Flowering plants  
**Class:** Magnoliopsida – Dicotyledons  
**Sub class:** Asteridae  
**Order:** Scrophulariales  
**Family:** Oleaceae – Olive family  
**Genus:** *Jasminum*– jasmine  
**Species:** *grandiflorum* Linn
3.7.3 Marketed Product

Jatyadi taila, Jatyadi ghrita, Jatyadi varti, Gandharaja taila, Sinduradya taila, Visha taila, Vajraka taila, Vicharchikari taila, Pichiyila Koottu

3.7.4 Clinical Trial

A clinical trial was conducted to assess the effect of Jatyadi taila of which J. grandiflorum is one of the main ingredients. Group A (8 patients of eczema) were treated with Raktashodhaka vati (2 tab t.d.s) Surakta strong syrup (2 tsf b.d.), Panchatikta ghrita guggulu (15 mg b.d.) and external application of Marichyadi taila and group B (n=8) were administered the same schedule as group A except that Jatyadi taila was applied externally instead of Marchyadi taila. The duration of treatment was continued for 3 months and reviewed after 4 weeks. In group A, only 37.5% patients got cured whereas groups B, 62.5% patients got cured which suggests that Jatyadi taila is more effective in eczema patients.

3.8 Saraca asoca

*Saraca asoca* Roxb. De Wilde, sacred tree belonging to family caesalpinaceae, found almost throughout India, except North-Western India, upto 750m also found in the Andaman Islands. The leaves are parpinnate with 6 to 12 leaflets, oblong and rigidly sum-coriaceous. The fruit is a pod flat, leathery with 4 to 8 ellipsoid- oblong and compressed seeds (Biswa and Debnath, 1972; Warrier et al., 2000). The whole plant is claimed to possess medicinal properties and used for various ailments like diabetes, emollient, stomachic, blood disease, ulcers, menorrhagia, dyspepsia, inflammation, dysentery, haemorrhoids etc. (Khory and Katrak, 1999; Khare, 2004). Leaves extracts has been reported for antidepressant, analgesic, antipyretic, anti-inflammatory, antibacterial, antihelminthic, antimicrobial and antidiabetic activity (Satyavati et al., 1970; Pal et al., 1985; Sadhu et al., 2007). In present scenario many
reviewers explored and identified various antidiabetic traditional medicinal plants but a large number of medicinal plants are yet to be investigated. *Saraca asoca* is enriched with Carbohydrates, Proteins, Amino Acids, Flavonoids, Tannin, Saponin, Steroids (Pradhan *et al.*, 2010).

![Saraca asoca leaves](image)

**Fig 3: Saraca asoca leaves**

### 3.8.1 Classical Names

Kankeli (Sanskrit), Ashoka (Assamese), Ashoka (Bengali), Ashoka (Gujrati), Ashoka (Hindi), Ashokadamara (Kannada), Ashok (Kashmiri), Asokam (Malayalam), Ashok (Marathi), Ashoka (Oriya), Ashok (Punjabi), Asogam (Tamil), Ashokapatta (Telugu)

### 3.8.2 Taxonomical Classification (Biswa and Debnath, 1972)

**Kingdom:** Plantae

**Division:** Magnoliophyta

**Class:** Magnoliopsida

**Order:** Fabales

**Family:** Caesalpinaceae

**Genus:** Saraca
Species: asoca

3.8.3 Chemical Constituent

The Phytochemical study show in the bark of plant presence of (-) epicatechin, procyanidin p2,11'-deoxyprocyanidin B, (+) catechin, (24, £)- 24- methyl-cholesta-5-en-3p-ol (22 E, 21£)-24-ethylcholesta-5,22 dien-33-ol,(24 £)-24- ethylcholesta-5-en-3p-ol,leucopelargonidin-3-O-p-Dglucoside, leucopelargonidin and leucocyanidin. The flower part of plant contain Oleic, linoleic, palmitic and stearic acids,P-sitosterol, quercetin, kaempferol- 3-0-P-D- glucoside, quercetin- 3-0-P-D-glucoside, apigenin-7-0-p-D-glucoside, pelargonidin- 3, 5- diglucoside, cyanidin-3, 5- diglucoside, palmitic, stearic, linolenic, linoleic, p and y sitosterols, leucocyanidin and gallic acid. Seed and Pod contains oleic, linoleic, palmitic and stearic acids, catechol, (-) epicatechol and leucocyanidin (Rastogi, 2003; Jain, 1968; Sadhu et al., 2007). Five lignan glycosides, lyoniside, nudiposide, 5-methoxy-9-β-xylopyranosyl(-)-isolarciresinol, icariside E3, and schizandriside, and three flavonoids, (-)-epicatechin, epiafzelechin-(4β→8)-epicatechin and procyanidin B2, together with β-sitosterol glucoside, were isolated from dried bark (Dhawan et al., 1977).

3.8.4 Ayurvedic actions (Pradhan et al., 2009)

Vedana sthapanam- Useful in management of all painful conditions.

Varnya- Ashoka improves complexion of the body.

Grahi- Ashoka improves digestion and assimilation.

Trishanashnam- Ashoka alleviates excessive thirst.

Daha shamanam- Ashoka alleviates burning sensation.

Krimigha- Ashoka kills all infectious agents.

Shothajit- Ashoka is useful in management of all edematous conditions.

Vish asrajit- Ashoka is useful in toxicities and all blood disease.

Apachijit- It is useful in management of inflammation of lymph nodes.
3.9 SELECTION OF ANIMAL MODEL

Nephropathy is a leading cause of morbidity and mortality and its prevalence is continuously increasing in industrialized nations. Nephropathy is characterized to varying degrees by nodular glomerulosclerosis, glomerular basement membrane thickness and mesangial expansion, leading to a decline in glomerular filtration rate, persistent elevated albuminuria, elevated arterial blood pressure and fluid retention. Hyperglycemia, hyperlipidaemia and hypertension are considered to be the major risk factors implicated in the progression of nephropathy. Various signaling systems, such as vasoconstrictor peptides, inflammatory mediators, growth factors and adhesion molecules, are involved in the pathogenesis of nephropathy. Animal model are being developed to better understand the disease pathogenesis and develop drugs for nephropathy. Animal model of nephropathy resemble human nephropathy as a common feature includes proteinuria, glomerulosclerosis and reduced glomerular filtration rate. Various models employed to induce nephropathy are streptozotocin-induced diabetic nephropathy, cyclosporin-induced nephropathy, anthracyclin-induced nephropathy, aminoglycoside-induced nephropathy, cisplatin-induced nephropathy, electrolyte-nephropathy, vomitoxin-induced nephropathy, cadmium-induced nephropathy, carbon tetrachloride-induced nephropathy, maleic acid-induced nephropathy, ethylene glycol-induced nephropathy, mercury chloride-induced nephropathy and geranium dioxide-induced nephropathy (Balakumar et al., 2008).

Streptozotocin-induced diabetic nephropathy is a well accepted model for induction of diabetic nephropathy within 4-8 weeks after single injection of STZ has been reported. Streptozotocin (STZ) is a glucosamine-nitrosourea compound, chemical name of 2-deoxy-2-(3-methyl-3-nitrosoureido)-D-glucopyranose (C_{18}H_{18}N_{3}O_{7}) (Szkudelski, 2001) that show selective cytotoxicity to pancreatic β cells that’s why it
is used as an agent to induce experimental diabetes in animals. STZ, derived from a fermentation broth of *Streptomyces achromogenes*, was first isolated as a new antibiotic in 1956, which had a significant antimicrobial action for a wide spectrum of organisms. STZ is a therapeutic agent in treatment of metastatic insulin-producing islet-cell tumor. STZ has been determined to be the nitrosoamide methyl nitrosourea linked to the C2 position of D-glucose (Bennett and Pegg, 1981). The nitrosoamide contributes to its alkylation properties and glucose moiety directs it to the β-cell specifically. STZ enters beta cells via GLUT 2, glucose transporter responsible for specific vulnerability of beta cells towards STZ. Inside beta cells, STZ is metabolized to cut apart between the 2’-carbon and methyl nitrogen to form carbamoylating and alkylating species (Elsner et al., 2000; Schnedl et al., 1994; Thulesen et al., 1997).

The mechanisms of STZ induced hyperglycemia are as follows: (1) STZ causes DNA strand breaks in pancreatic islets and stimulates nuclear poly (ADP-ribose) synthetase and depletes the intracellular NAD+ and ATP levels, which inhibit proinsulin synthesis and induces diabetes; (2) activated oxygen species, such as superoxide (O₂⁻), hydrogen peroxide, hydroxyl radicals and singlet oxygen have been implicated to play important roles in diabetes. (3) STZ is a nitric oxide (NO) donor and NO was found to bring about the destruction of pancreatic islet cells and partially mediates restriction of mitochondrial ATP generation. Furthermore, NO bind to the iron-containing aconitase and inhibit enzyme activity (4) Augmented ATP dephosphorylation increases supply of substrate for xanthine oxidase and enhances the production of uric acid, the final product of ATP degradation. Xanthine oxidase catalyses the reaction in which superoxide anion is formed (Nukatsuka et al., 1990).

Administration of STZ at a dose of 40 mg/kg, 50 mg/kg, 55 mg/kg, 60 mg/kg and 65 mg/kg i.p. induces hyperglycemia in rats (Casey et al., 2004, Shah and Singh, 2004, Singh et al., 2005 and Haidara et al., 2008). STZ induces hyperglycemia which
activates PKC, aldose reductase, NADPH oxidase, formation of AGEs and increases Ang II levels (Brownlee, 2001; Chung et al., 2003; Onozato and Tojo, 2005). Hyperglycemia leads to development of diabetic nephropathy in 4-8 weeks, assessed in terms of serum creatinine, BUN, proteinuria, creatinine clearance, extracellular matrix deposition, dyslipidemia and consequent development of glomerulosclerosis and tubulointerstitial fibrosis (Budhiraja et al., 2006; Alqattan et al., 2008; Gojo et al., 2007).