Chapter 6

DISCUSSION
6.1 Discussion

Albino Wistar rats of either sex were employed in present study because of their small size, low cost and easy availability, Moreover, other species like Sprague Dawley rats resistant to renal lipid accumulation in high-fat diet model (Bobulescu et al., 2008). Streptozotocin-induced diabetic nephropathy is a well accepted model as successful induction of diabetic nephropathy within 4-8 weeks after single injection of STZ is reported (Szkudelski et al., 2001). 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$_7$) that show selective cytotoxicity to pancreatic β-cells. In contrast to alloxan, STZ has no detectable renal injury that’s why STZ have greater utility than alloxan (Evan et al., 1984). 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., 2005; Shah and Singh, 2006; Singh et al., 2005; Haidara et al., 2009). STZ-induced hyperglycemia activates PKC, aldose-reductase, NADPH oxidase, formation of AGEs and increases Angiotensin-II levels (Chung et al., 2003; Onozato and Tojo, 2005; Brownlee, 2001) which 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; Gojo et al., 2007; Alqattan et al., 2008). Estimation of blood glucose has been used as marker of hyperglycemia. ]’][Persistent elevation of blood glucose level leads to diabetes which is observed in our study i.e.353.01 ± 3.52. The results (Table-7, 8) observed in the present study demonstrate the hypoglycemic action of various SA and JG extracts in dose dependent manner. However, at the dose of 400 mg/kg JGE, SAE and SAAq extract shows blood glucose level 126.31 ± 2.98, 106.31 ± 4.11 and 115.432
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respectively which is significant as compare to standard drug i.e. glibenclamide, a hypoglycemic drug which increase release of insulin from the existing pancreatic β-cells and used in moderate STZ induced-diabetes (Proks et al., 2002). Administration of various medicinal plant extract in moderate STZ-diabetic rats resulted in the activation of β-cells and granulation bring glucose level to showing an insulinogenic effect (Kedar and Chakrabarti, 1982). The hypoglycemic activity of SA and JG extracts was associated with an increase in plasma insulin (Table-7, 8), suggesting that the antihyperglycaemic activity of SA and JG extracts could be due to an insulinogenic activity of the extract. The significant increased levels of insulin at the dose of 400 mg/kg JGE, SAE and SAAq extracts i.e. 11.9 ± 2.1, 14.4 ± 2.1 and 12.2 ± 1.1 µg/ml was observed in the present study indicate that the SA and JG extracts stimulates insulin secretion from the remnant β-cells and/or from regenerated β-cells which is consonant with previous reports (Pari and Latha, 2002; Gireesh et al., 2009). Treatment with SA and JG extracts not only reduced the fasting blood glucose level but also improved the glucose tolerance (Fig. 11, 12) in STZ rats. The effectiveness of SA and JG extracts in chronic hyperglycemia may be due to its extra pancreatic action and this is supported by improved glucose tolerance. Elevated glycosylated hemoglobin (HbA1c) was observed in the diabetic control group (13.8 ± 1.4% of Hb) when compared to normal rats (5.2 ± 0.67 % of Hb) which was similar to earlier reports (Adaramoye, 2012). High glycosylated hemoglobin level indicates poor glycemic control and responsible for the development of microvascular macrovascular complications like retinopathy, neuropathy and diabetic nephropathy. Persistent and chronic diabetes lead to glycosylation of various proteins like hemoglobin which is monitored as a reliable index of glucose level in diabetes (Ghacha et al., 2001). Treatment with SAE, SAAq and JGE extracts, the HbA1c value
was brought down to 5.5 ± 0.48 % of Hb, 8.4 ± 0.79 % of Hb and 6.1 ± 0.43% of Hb to almost normal which is consistent the previous reports (Fuji and Nomoto, 1984). A significant reduction in average weight was observed in STZ induced diabetic animal (Fig. 9, 19). The decrease in weight in diabetes was due to continuous excretion of glucose and decrease in peripheral uptake of glucose and glycogen synthesis (al-Shamaony et al., 1994). In the present study increase in body weight and decrease in blood glucose might be due to improving the glycemic control mechanisms and insulin secretions from remnant pancreatic-cells in diabetic animals. Hypoinsulinemia during diabetes activates HMG-COA reductase to stimulate the synthesis of cholesterol (Sevak and Goyal, 1996). Moreover, the occurrence of proteinuria has been suggested to upregulate HMG-COA reductase thereby produce hypercholesterolemia (Vaziri et al., 2003; Trivesan et al., 2006). Thus, STZ-induced diabetes is often associated with hypercholesterolemia and hypertriglycerideridemia. Moreover, lipids get deposited in the kidney of diabetic patients in presence of fibrotic tissue leading to glomerulosclerosis, tubulointerstitial fibrosis, and increased collagen expression subsequently leading to proteinuria (Sun et al., 2002; Li et al., 2008). This contention is supported by the results obtained in the present study in STZ induced diabetes i.e. increased serum triglycerides, renal cholesterol and renal triglycerides and increased collagen content leading to consequent development of diabetic nephropathy. Sun et al. showed increased renal lipid synthesis was responsible for the elevated level of renal lipid content. They showed a marked increase in sterol regulatory element-binding protein (SREBP)-1 and fatty acid synthase expression in STZ-diabetic rats, resulting in increased renal accumulation and glomerulosclerosis (Sun et al., 2002). Dyslipidaemia is associated with elevated total cholesterol, triglycerides and low level of high density lipoprotein (HDL) in diabetic animal.
Therefore, estimation of total cholesterol, triglycerides, and HDL has been used as the marker of dyslipidaemia. In addition, factors like oxidative stress and advanced glycation end-products, abnormal lipid metabolism and the renal accumulation of lipids play a key role in the pathogenesis of diabetic nephropathy (Sun et al., 2002). Several studies have shown the presence of lipid deposits in the kidney of diabetic human and experimental animals and have proposed that these deposits may play an important role in the pathogenesis of diabetic kidney disease (Guijarro et al., 1995). The elevated levels of renal lipid contents observed in diabetic rat of present study are consistent with previous reports. Estimation of total cholesterol, triglycerides and HDL has been used as a marker of dyslipidemia (Yoshino et al., 1996). Lipoprotein lipase (LPL) located in the vascular endothelium is involved in the breakdown of triglycerides into free fatty acids. It is well reported that decreased release of lipoprotein lipase is associated with vascular endothelium dysfunction leading to hypertriglycerideremia and decreased HDL levels without affecting serum cholesterol levels, which contributes to diabetic nephropathy (Kashiwazaki et al., 1998). A strong correlation between dyslipidemia and diabetic nephropathy has been reported (Keane, 2000). Further, insulin has an inhibitory action on 3-hydroxy-3-methyl-glutaryl-Co-A (HMG-COA) reductase, a key rate-limiting enzyme involved in the synthesis of cholesterol. Fenofibrate is a selective PPAR-α agonist which is reported to lower the total cholesterol, triglyceride level by increasing lipoprotein lipase activity (Staels et al., 1998). PPAR-α predominantly expressed in proximal tubules, medullary thick ascending limb and in mesangial cells. Further, it has been reported that exendin-4 has attenuated diabetic nephropathy by reducing renal lipid accumulation mediated through the PPAR-α (Cho et al., 2002). In the present study, treatment with fenofibrate has improved serum lipid profile and consequently reducing lipid
peroxidation in renal tissue and show improvement in diabetic nephropathy, Therefore, fenofibrate used as standard drug for lipid alteration evaluation in the present study. The ethanolic and aqueous extract of SA and JG extracts was able to significantly decrease the concentration of these lipids in treated diabetic rats compared with untreated diabetic rats and fenofibrate. This reduction could be beneficial in preventing diabetic complications, as well as in improving lipid metabolism in diabetic kidneys (Gruden et al., 2005).

Diabetic nephropathy has been documented to be associated with morphological changes in glomeruli (Gojo et al., 2007) associated with mesangial expansion due to increased mesangial matrix deposition and hypertrophy of mesangial cells (Wolf et al., 2003) numbers of glomeruli with sclerotic lesions were increased, interstitial fibrosis and mononuclear cell infiltration observed, which is similar to morphological changes observed in histopathological slides of diabetic rat in present investigation. Treatment with SA and JG extracts significantly improves these morphological changes (Fig. 13) and certainly provides the nephroprotective in pre clinical study and further study are warranted to assess its clinical applicability.

Elevated level of proteinurea, serum creatinine and BUN has been reported as an index of nephropathy thus the same were assessed as in present investigation. The elevated level of serum creatinine, proteinurea, BUN and reduction in creatinine clearance has been observed in diabetic rat Moreover, the percentage of Kidney weight to body weight and collagen deposition has been seen significantly increased which is a marker of renal hypertrophy and fibrosis in diabetic nephropathy (Cohen and Klein, 1979; Grover et al., 2002; Fujisawa et al., 2004; Sinuani et al., 2006), which is consonant with the observation of our investigation in diabetic rat. Treatments with the SAE, SaAq, JGE and JGAq extracts was able to significantly
attenuated BUN, serum creatinine and urinary protein excretion and improve creatinine clearance, reduce the collagen content and certainly provide the nephroprotective action. Clinically, renoprotective effect of lisinopril (ACE inhibitor) has been well reported to decrease serum creatinine, serum lipid levels and also reduction in mean arterial blood pressure in diabetic patients (Tarnow et al., 2000). Therefore, lisinopril has been employed as a standard drug in the present study (Mongenson et al., 2000).

Hyperglycemia induced-oxidative stress caused by free radical generation and decrease antioxidant defense system (Liu, 2004) which, has been assessed for estimate the degree of oxidative stress. Oxidative stress occurs due to an imbalance between reactive oxygen species (ROS) and intracellular antioxidants. Antioxidant systems either prevent reactive species from being formed, or remove them before they can damage vital components of the cell (Davies, 1995; Sies, 1997). However, reactive oxygen species also have useful cellular functions, such as redox signaling. Thus, the function of antioxidant systems is not to remove oxidants entirely, but to maintain at an optimum level (Rhee, 2006). Overproduction of peroxides and free radicals damage to components of cell, including proteins, lipids and DNA results in cancer, myocardial infarction and neurodegenerative disorders etc. Free radicals are known to be associated with natural metabolism of aerobic cells. Oxygen consumption in cell growth leads to the formation of oxygen free radicals that further react with molecules of lipid nature to form new radical peroxides. These radicals thus formed interact with biological systems in a cytotoxic manner; higher the amount of free radicals formed more will be the damage to cells and tissues leading to several diseases (Pryor et al., 1982; Torel et al., 1986). Thus there is a need of antioxidants of natural origin that can protect the human body from the diseases caused by free
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radicals (Harman, 1998). In the present study, diabetes has been noted to increase the tissue TBARS and decrease reduced form of glutathione (GSH) demonstrating the development of diabetes-induced oxidative stress. The oxidative stress has been documented to play a major role in the progression of nephropathy. The result (Table-11, 12) of present study demonstrate that *Jasminum grandiflorum* and *Saraca asoca* extracts has shown antioxidant activity in dose dependent manner, However the JGE extract at dose of and 400 mg/kg and JGAq at 200 and 400mg/ kg show maximum effect antioxidant activity by reducing the TBARS and improving the GSH level in the renal tissue. Moreover in In-vitro antioxidant evaluation using by percentage anti radical activity of all extracts summarized in (Fig. 7, 8) exhibited good but varying levels of antioxidant activity in both DPPH and H$_2$O$_2$ radical scavenging assay.

*Saraca asoca* was found to be effective as antibacterial, antimicrobial, antioxidant, antidiabetic, anticancer, antioxidant and in gynecological disorders due to the presence of (-) epicatechin, procyanidin p2,11'-deoxyprocyanidin B, (+) catechin, (24, £)- 24- methyl-cholesta-5-en-3p-ol (22 E, 21£)-24- ethylocholesta-5,22 dien-33-ol,(24 £)-24-ethylcholesta-5-en-3-p-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. Five lignan glycosides, lyonoside, nudiposide, 5-methoxy-9-β- xylopyranosyl(-)-isolariciresinol, 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). The leaf of Saraca asoca also contains gallic acid, flavonoids like epicatechol, leucocyanidin, quercitin.

Jasminum grandiflorum was used as diuretic, uterine tonic, antioxidant due to the presence of 2”-epifraxamides, demethyl-2”-epifraxamoids, jasminanhydride (Sadhu et al., 2007), oleacein, 2-(3, 4- dihydroxy phenyl)-ethanol, isoquercitrin, ursolic acid (Brinda et al., 1998), resin, salicylic acid, jasmine, indole oxygenase (Divakar et al., 1979), 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, jasmone, methyl- N-methyl anthranilate, vanillin, cis-3-hexenyl benzoate, benzyl benzoate, methyl palmitate, methyl linoleate (Rastogi and Mehrotra, 1999), jasgranoside, jaspolyoside, 8-epi-kingsiside, 10-hydroxy-oleuropein, 10-hydroxyligstroside, oleoside-7,11-dimethylster (Zhao et al., 2008), Oleacein extracted from aerial parts of J. grandiflorum exhibited ACE inhibitor activity with IC₅₀ values 26-66 mM (Somanadhan et al., 1998).

On the basis of result observed and the literature survey 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).

The flavonoids 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 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. 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). The phytochemical screening shows the presence of phytoconstituents like flavonoids, sterols triterpenoids and saponin (Table 2, 4) in the various extracts of Saraca asoca and Jasminum grandiflorum. On the basis of above discussion this may be speculated that antioxidant, antidiabetic and nephroprotective action may be due to the presence of phytochemicals like flavonoids, sterols triterpenoids and saponin as the active constituents.