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6. DISCUSSION

*T.purpurea* has been reported to possess a large number of beneficial effects such as anti-diabetic, anti-hyperlipidemic, anti-ulcer, anti-oxidant, anti-inflammatory, wound healing, hepatoprotective etc (Joshi et al, 2008; Chinniah et al, 2009; Jain and Singhai, 2009; Pavana et al, 2009; Lodhi et al, 2010; Akhtar et al, 2011; Kashaw et al, 2011; Velli et al, 2011a; Hussain et al, 2012). The pharmacognostic features have been mentioned in various books (Kirtikar and Basu, 1975; Warrier et al, 2004; Billore et al, 2004). The macroscopy and microscopy studies carried out in the present investigation authenticated the characteristics of various parts of plant material as in literature (Billore et al, Year). The phloem fibres in the transverse section of root were found to contain prismatic crystals. The transverse section of stem contained starch grains, strands of pericyclic fibres within phelloderm, vessels with bordered pits and xylem rays were heterogenous with parenchymatous pith at the centre. The transverse section of leaf showed midrib with single layered epidermis with thick cuticle, large vascular bundle surrounded by crystals of calcium oxalate and externally by strand of fibres. These characteristics are identical to those reported earlier by Billore et al (2004).

Preliminary phytochemical analysis in our investigation further authenticated the plant. It showed the presence of alkaloids, glycosides, flavonoids and phenols. This is consistent with the phytochemical analysis reported earlier by other investigators. The presence of flavonoids was shown by various workers (Gupta et al, 1980; Pelter et al, 1981; Sinha et al, 1982; Ventakata et al, 1984; Saxena and Choubey, 1997; Chang et al, 2000; Hegazy et al, 2009). Among various flavonoids, two biologically active flavonoidal compounds, quercetin and rutin have been reported for various pharmacological effects (Hubbard et al, 2003; Vessal et al, 2003; Prince and Kamalakkannan, 2006; Karthick and Prince, 2006). We carried out chemoprofiling using HPTLC for the qualitative, semi-quantitative and quantitative analysis of these.
compounds in various extracts used. This includes developing TLC fingerprint profiles and estimation of chemical markers and biomarkers. In the present study for all the extracts, HPTLC finger printing was developed and the amount of rutin and quercetin were estimated quantitatively. Rutin was 3.14% in aqueous extract, 5.37% in alcoholic extract and 2.37% in flavonoidal fraction. Quercetin was 0.18% in aqueous extract, 1.05% in alcoholic extract and 1.75% in flavonoidal fraction. Thus studies on phytochemical analysis of the plant *T. purpurea* showed that alcoholic extract and flavonoid fraction contain significant amount of rutin as well as quercetin whereas quercetin was found in a very low quantity in aqueous extract. In further studies rutin and quercetin were used for the biological activity of *T.purpurea*.

In the present investigation we found that STZ produced cardinal signs and characteristics of diabetes viz polyphagia, polyuria, polydipsia, hyperglycemia, hypoinsulinemia, dyslipidemia and cardiovascular alterations like bradycardia, hypertension and hypertrophy of heart. These results are consistent with those reported earlier (Umrani and Goyal, 2002). Chronic treatment with aqueous extract (300 and 500 mg/kg/p.o./day) of *T.purpurea* did not prevent the loss of body weight, polyuria arid polydipsia in STZ-diabetic rats. Chronic treatment with alcoholic extract (300 and 500 mg/kg/p.o./day) of *T.purpurea* did not prevent the loss of body weight but was able to slightly improve polyuria and polydipsia in STZ-diabetic rats. However, flavonoid fraction (40 mg/kg/p.o./day) of *T.purpurea* was able to prevent some reduction in body weight and significantly prevented polyuria and polydipsia in STZ diabetic rats.

In the present study, STZ produced a significant increase in glucose levels associated with decrease in insulin levels in type 1 diabetic rats. Treatment with aqueous extract (300 and 500 mg/kg/day), alcoholic extract (300 and 500 mg/kg/day) and flavonoid fraction (40 mg/kg/p.o./day) significantly reduced the serum glucose levels. All the treatment also produced elevation in the serum insulin levels of STZ-diabetic rats. However, flavonoid fraction was more effective in reducing the glucose levels as well as elevating the insulin levels. Intravenous
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Injection of STZ produces fragmentation of DNA of β-cells of pancreas that stimulates poly (ADP-ribose) and depletes NAD ultimately leading to destruction of β-cells and it is evidenced by clinical symptoms of hyperglycemia and hypoinsulinaemia (Rodrigues et al. 1986; Goyal, 1999). STZ-induced diabetes mellitus is associated with the generation of reactive oxygen species causing oxidative damage (Szkudelski, 2001), which thereby depletes the activity of antioxidative defense system and thus promotes de novo free radicals generation (Baynes and Thorpe, 1999). Chemicals with antioxidant properties and free radical scavengers may help in the regeneration of β-cells and protect pancreatic islets against the cytotoxic effects of STZ (Alvarez et al, 2004; Coskun et al, 2005).

Phytochemical investigations on T. purpurea have revealed the presence of glycosides, rotenoids, isoflavones, flavanones, chalcones, flavanols, flavones and sterols (Pelter et al, 1981). Rutin is a flavonol type of flavonoid present in about 4.65% in the leaves of T.purpurea (Prashanth Kumar et al, 2003). It is possible that the rich flavonoid content of T. purpurea may be the main antidiabetic principles of the plant. The antioxidative property of the plant may protect pancreatic islets and help in regeneration of β-cells which leads to increased insulin levels and hence increased utilization of the glucose by the peripheral tissues. In addition, flavonoids exert their effect either by promoting the entry of glucose into cells, thus stimulating glycolytic enzymes and glycogenic enzymes and reducing glycogen breakdown (Sarkhail et al, 2007) and depressing gluconeogenic enzymes or by inhibiting the glucose-6-phosphatase in the liver, consequently reducing the release of glucose in the blood (Naik et al, 1999). Rutin has been reported to decrease plasma glucose and increase insulin levels along with the restoration of glycogen content and the activities of carbohydrate metabolic enzymes in streptozotocin-induced diabetic rats (Prince and Kamalakkannan, 2006). Increased insulin levels could also be due to the stimulatory effect of rutin thereby potentiating the existing β-cells of the islets of Langerhans in diabetic rats (Kamalakkannan and Prince, 2006). The protective
effect of rutin on \( \beta \)-cells may also be because of blocking insulitis process due to its anti-inflammatory action (Srinivasan et al, 2005). Prince and Kamalakkannan (2006) have shown an expansion of the pancreatic islets and decreased fatty infiltrate of the pancreatic islets in rutin-treated diabetic rats suggesting protective effect of rutin in diabetic rats. Moreover, the aglycone of rutin i.e. quercetin as an antioxidant and a free radical scavenger prevents autopoly (ADP-ribosyl)-ation of PARP, thereby stabilizing Reg gene transcriptional complex and resulting in the regeneration of \( \beta \)-cells and protection of pancreatic islets against STZ and thus probably increase the insulin release in STZ-induced diabetic rats (Vessal et al, 2003). Coskun et al (2005) have reported that quercetin protected pancreatic \( \beta \)-cells by decreasing oxidative stress and preserving pancreatic \( \beta \)-cell integrity. These studies suggest that improvement in glycemic condition of the STZ-diabetic rats by treatment with \textit{T.purpurea} may be due to presence of both rutin and quercetin.

Measurement of glycated haemoglobin has proven to be particularly useful in monitoring the effectiveness of therapy in diabetes (Goldstein 1995). The glycated haemoglobin levels were found to be increased in diabetic rats. Chronic treatment with the flavonoidal fraction of \textit{T.purpurea} could effectively reduce the levels of glycated hemoglobin. Agents with antioxidant or free radical scavenging power may inhibit oxidative reactions associated with glycation (Elgawish et al, 1996). Rutin with its free radical scavenging activity could effectively reduce the formation of glycated haemoglobin in diabetic rats. A decrease in blood glucose levels might also contribute to decreased levels of glycated haemoglobin in the treated diabetic rats. Moreover, Grinberg et al (1994) have reported the protective effect of rutin against haemoglobin oxidation. Nagasawa et al (2003) have also shown that G-rutin (a water soluble rutin analogue) suppressed the accumulation of glycation products in serum and tissue (kidney) protein sources, attributing these to the antioxidant capacity of rutin. Thus, the anti-oxidative property of the flavonoid fraction may be responsible for the reduction in glycated hemoglobin levels in diabetic rats. All
these results indicate that both rutin and quercetin are required for anti-diabetic effect.

It has been reported that in STZ-diabetic rats, insulin deficiency is associated with hypercholesterolemia and hypertriglyceridemia (Rodrigues et al, 1986). A low level of plasma high-density lipoprotein cholesterol (HDL-C) is one component of a cluster of coronary disease risk factors that also includes abdominal obesity, hypertension, hyperinsulinemia, and insulin resistance (Kaplan 1989). Abnormal lipid levels lead to the development of coronary artery disease in diabetic patients. Increased intestinal sterol genesis is also a contributory factor to hypercholesterolemia seen in diabetes mellitus (Abrams et al, 1982). In the present investigation, the rise in serum triglycerides, total cholesterol, LDL-cholesterol and VLDL-cholesterol levels indicate derangement of lipid metabolism and increased incidence of cardiac dysfunction in diabetic rats. Elevation of serum lipids indicates either the defective removal or overproduction (or both) of one or more lipoproteins (Akula et al, 2003). An elevated plasma LDL concentration is a primary risk factor for the development of atherosclerosis and coronary artery disease (Inkeles and Eisenberg, 1981). LDL levels may decrease due to the reduction of very low-density lipoproteins (VLDL) and the increase of hepatic depuration of LDL precursors (Knekt et al, 2002). VLDLs are produced in the liver primarily for the transport of newly synthesized triglyceride to peripheral tissue. In the capillary beds, plasma VLDL is converted to cholesteryl ester–enriched triglyceride-poor LDL. Reactive oxygen species generated through lipid peroxidation can oxidatively modify the amino acid residues of LDL, and LDL oxidation in the arterial intima can initiate the atherosclerotic process (Glass and Witztum, 2001). The serum cholesterol and triglyceride levels of diabetic rats treated with aqueous extract (300 and 500 mg/kg/day), alcoholic extract (300 and 500 mg/kg/day) and flavonoid fraction (40 mg/kg/p.o./day) were found to be significantly decreased. Pavana et al (2007b) have reported that aqueous extract of *T.purpurea* have antihyperlipidemic effect in STZ diabetic rats which may be due to stimulation of activities of lipid

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metabolizing enzymes such as LCAT and LPL. Flavonoids are reported to be the major constituents of *T. purpurea* that are shown to decrease blood levels of triglycerides and total cholesterol (Imai and Nakachi, 1995). Flavonoids may suppress LDL oxidation and inflammatory progression in the artery wall (Peluso, 2006). Moreover, rutin is a potent inhibitor of HMG-CoA reductase, an enzyme responsible for cholesterol synthesis, and also beneficial for lowering serum cholesterol levels (Bok et al, 1999). This may suggest the mechanism of cholesterol lowering effect of the extracts. Quercetin like flavonoids have been shown to inhibit the oxidative modification of LDL by macrophages partly by conserving the α-tocopherol content of the LDL particles (de Whalley et al, 1990). Quercetin has been shown to scavenge lipid alkoyl and peroxy radicals and thereby repair neutral tryptophan and tyrosine radicals (Filipe et al, 2002). Flavonoids act as electron donor and there is formation of a semioxidized flavonoid radical. Inhibition of LDL oxidation by flavonoids is strictly dependent on binding of the flavonoid molecule to the LDL particle. For example, the presence of albumin, which transports flavonoids in the blood, was shown to significantly attenuate the effectiveness of quercetin by binding and stabilizing the flavonoid in a negatively charged state (Zsila et al, 2003). Moreover, total flavonoid and quercetin intakes were shown to be inversely correlated with plasma total and LDL cholesterol concentrations in a study on Japanese women (Arai et al, 2000). Quercetin has also been reported to reduce cholesterol and triglycerides as well as augment HDL-cholesterol in triton induced hyperlipidemic rats (Ricardo et al, 2001). Further, it has been suggested that dietary flavonoids may be anti-atherogenic agents. In this context, Raanan et al, (2008) have shown the beneficial effect of metabolic control on serum lipids and oxidative stress in patients with type 1 diabetes, indicating that such control reduces cardiovascular risk in these patients. Besides this, the extract was also able to significantly augment serum HDL cholesterol in rats with STZ-induced diabetes. This finding is advantageous since HDL-cholesterol is responsible for the transportation of cholesterol from peripheral tissues to the liver for metabolization. Further, rutin
has been reported to prevent HDL-C from oxidative modification in-vitro (Li et al, 2004). However, *all the three extracts were found to have similar effect on lipid profile*. It is possible that rutin or quercetin may be effective individually in *producing lipid lowering effect*. Thus, the plant may possess the potential to prevent the formation of atherosclerosis and coronary heart disease.

STZ produced changes in renal function including altered renal hemodynamics and structural changes that can be attributed to the development of diabetes. In the present study, significant elevation in serum creatinine and urea levels indicating impaired renal function in diabetic animals was observed. These observations are consistent with those reported earlier (Bleasel and Yong, 1982; Cam et al, 1993; Dai et al, 1993). Treatment with *T. purpurea* extracts produced considerable lowering of elevated serum creatinine and urea levels in diabetic animals. However, flavonoid fraction was found to be more effective than the aqueous or alcoholic extract in lowering the serum creatinine and urea levels in diabetic animals. Khan et al (2001) have reported that prophylactic treatment of rats with *T. purpurea* prevented N-diethylnitrosamine-initiated and potassium bromate promoted renal oxidative stress and toxicity by increasing the activities of antioxidant enzymes as well as reducing the levels of serum creatinine and blood urea nitrogen. The ethanolic extract of *T. purpurea* leaves has also shown marked nephroprotective and curative effect against gentamicin-induced acute renal injury in albino rats (Jain and Singhai, 2009). They have proposed antioxidant activity and inhibition of overproduction of NO and Cox-2 expression as the mechanisms involved in nephroprotection. These activities may be attributed to phenolic and flavonoidal compounds like rutin and quercetin. In our study, we found that the nephroprotective effect was greater in the flavonoid fraction as compared to aqueous or alcoholic extract. Hence *T.purpurea* extracts especially the flavonoidal fraction may be beneficial in providing some protection against diabetic nephropathy. However, further studies are required to prove the efficacy of the *T.purpurea* extract in diabetic nephropathy.
Within 12–24 hours after a myocardial infarction, LDH and CK levels increase (Howard-Alpe et al, 2006). Increased serum CK and LDH levels in diabetic rats indicate cardiac muscular damage (Hagar, 2002; Amin and Nagy, 2009). Once the cardiac cells are metabolically damaged, it releases its intracellular contents into the extracellular fluid and hence the serum levels of these marker enzymes indicate the alterations in membrane integrity, or permeability (Suchalatha and Devi, 2004). LDH is the enzyme involved in the final step of anaerobic glycolysis. Increased activity of LDH in diabetes mellitus has been reported. The LDH system reflects the NAD+/NADH ratio, indicated by the lactate/pyruvate ratio of hepatocyte cytosol. Normal LDH activity is indicative of improved channeling of (pyruvate) glucose by mitochondrial oxidation (Sekar et al, 2005). In our study, we also found significant rise in LDH and CK levels in STZ-diabetic rats as compared with control rats. Treatment with the aqueous extract (300 and 500 mg/kg/day) was able to normalize the LDH activity in the diabetic rats while CK levels were reduced only with the treatment at the dose of 500mg/kg/day. Alcoholic extract at both the doses and flavonoidal fraction was able to significantly lower the LDH as well as CK levels. Rutin has been reported to normalize the LDH and CK levels in isoproterenol induced myocardial infarction in rats indicating its cardioprotective effect which may be due to antioxidant property (Karthick and Prince, 2006; Punithavathi et al, 2010). Creatinine kinase system is in part responsible for the contractile dysfunction in diabetic cardiomyopathy (Matsumoto et al, 1995). Rutin pretreatment has been shown to lower the serum CK-MB levels in isoproterenol induced cardio toxic rats (Prince and Shanmuga, 2010) as well as in doxorubicin induced cardiotoxicity (Walla et al, 2011) due to its free radical scavenging or membrane stabilizing properties. Lowering the CK levels with the treatment may help prevent the progression of diabetic cardiomyopathy. Since, the reduction of CK-MB levels was more with the alcoholic extract and that of LDH was more with aqueous extract, it could be hypothesized that cardioprotection may not be due to anti-oxidant properties only. Other mechanisms may also be involved and further studies are required.
Diabetic animals also demonstrate reduced rate of cardiac contractility and relaxation in the absence of atherosclerosis or frank abnormalities of the microcirculation (Schaffer et al, 1989). Bradycardia is frequently observed in STZ diabetic rats (Savarese and Berkowitz, 1979). Our results are consistent with these reports. The diabetic rats showed significantly reduced heart rate. Chronic treatment with all the extracts of *T. purpurea* prevented STZ-induced bradycardia in the diabetic animals but the effect was more with flavonoid fraction. People with type I diabetes mellitus are frequently exposed to acute hypoglycemia, which, in humans, is associated with profound hemodynamic changes (Hayat et al., 2004). Changes in peripheral blood pressure include an increase in systolic and a decrease in diastolic pressure and it is an extremely common comorbid condition in diabetes, affecting ~20–60% of patients with diabetes (Fisher et al, 1987; ADA, 2004; Sommerfield et al, 2007). Moreover, the functional cardiac performance assessed by the rate of pressure development and decay in STZ treated rats are reported to be decreased (Borges et al, 2006). In present study, we found change in haemodynamics i.e. hypertension and decline in rate of pressure development and decay which was found to be improved by the treatment with alcoholic extract and flavonoid fraction. However, treatment with the aqueous extract was not able to improve the hemodynamics whereas the flavonoid fraction was more effective than the alcoholic extract. Studies have reported that the sorbitol pathway is significantly activated in the hearts of the streptozotocin-induced diabetic rats, which resulted in the marked cardiac accumulation of fructose (Kashiwagi et al, 1992). Increased fructose levels cause the non-enzymatic glycation of various intracellular components in the diabetic myocardium, which result in the formation of advanced glycation end-products. These advanced glycation end-products impair the intracellular Ca^{2+} homeostasis (Petrova et al, 2012) which affects the cardiac contractility. Flavonoids are reported to be effective aldose reductase inhibitors (Varma et al, 1975) and hence the *T.purpurea* extract may inhibit the sorbitol pathway, thus providing cardioprotection. Rutin, a reported flavonoid in *T.purpurea* is proven to

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be effective in providing cardioprotection by improving left ventricular dysfunction in STZ-diabetic rats (Krishna et al, 2005) which may further substantiate the role of *T.purpurea* in cardiac complications associated with diabetes. High blood pressure and endothelial dysfunction is correlated with oxidative stress and subsequently reduced NO bioavailability (Thomas et al, 2003; Gkaliagkousi et al, 2009; Kobayasi et al, 2010). A variety of flavonoids and polyphenols have shown the capacity to dilate blood vessels (Achike and Kwan, 2003; Sanchez et al, 2006; Engler and Engler, 2006) in endothelium dependent or independent manner which may be mediated by nitric oxide (Akhhlaghi and Bandy, 2009). Rutin-treated rats have been shown to lower systolic blood pressure and improved endothelial function by scavenging free radicals may increase the bioavailability of NO (Panchal et al, 2011). Various previous studies have suggested that polyphenolic compounds like quercetin are efficacious in reducing blood pressure in spontaneously hypertensive rats (Duarte et al, 2001; Machha and Mustafa, 2005), rats with surgically induced renovascular hypertension (Garcia-Saura et al, 2005), rats with chronic nitric oxide synthase inhibition (Duarte et al, 2002), deoxycorticosterone acetate-salt hypertensive rats (Galisteo et al, 2004) and rats with abdominal aortic constriction (Jalili et al, 2006). Furthermore, flavonoids have a potential to inhibit angiotensin 1-converting enzyme (ACE) in vitro (Balasuriya and Rupasinghe, 2011). Quercetin, aglycone of rutin has been reported to be a potent inhibitor of ACE in vitro and in vivo (Cyrino et al, 2002; Hackl et al, 2002; Nicolau et al, 2003; Oh et al, 2004) which may suggest a possible mechanism for lowering of blood pressure by the flavonoid fraction of *T.purpurea*. It is evident from these results that both rutin and quercetin may be required for cardioprotection in diabetic rats.

Collagen in the normal adult heart serves several important functions; however, a disproportionate increase in collagen accretion or collagen resorption from normal levels can cause defects in the function and supporting structural lattice of the heart. Diabetes is associated with alterations in extracellular matrix (ECM) turnover and regulation (Williamson and Kilo, 1976). Pathologic...
remodeling characterized by ECM deposition might contribute to cardiovascular complications that are the leading cause of morbidity and mortality in diabetic patients (Kannel and McGee, 1979a; Kannel and McGee, 1979b). Several studies of diabetic cardiomyopathy have demonstrated an accumulation of myocardial collagen including types I, III, and VI, leading to interstitial and perivascular fibrosis which has been correlated with left ventricle (LV) early diastolic and systolic dysfunction (Shimizu et al, 1993; Spiro and Crowley, 1993; Tschope et al, 2004). Alteration in diastolic filling of the LV associated with reciprocal changes in the LV collagen gene and accumulation of cardiac collagen in diabetic rats (Kitayama et al, 1994; Mizushige et al, 2000) suggest that increased interstitial cardiac collagen might cause cardiac fibrosis in diabetes. Depending upon its location and magnitude, collagen fiber crosslinking, fibrosis can adversely increase LV stiffness and decreased LV wall compliance leading to diastolic heart failure or heart failure with preserved ejection fraction (Zile and Brutsaert 2002a; Zile and Brutsaert 2002b). In the present investigation, STZ diabetic rats expressed significantly high amount of collagen deposition in LV and chronic treatment with alcoholic extract as well as flavonoid fraction significantly reduced collagen deposition in LV. Panchal et al (2011) have reported that rutin prevents cardiac fibrosis in high fat diet fed rats. Also, the left ventricle of these rats showed significantly lower collagen deposition. Collagen is the most thrombogenic component of subendothelium. Collagen-induced platelet aggregation is associated with a burst of hydrogen peroxide that, in turn, contributes to the activation of platelet function through calcium mobilization and inositol pathway activation (Pignatelli et al, 1998). Pignatelli et al (2000) showed that flavonoids such as quercetin and catechin significantly inhibited the release of platelet hydrogen peroxide elicited by collagen. Hydrogen peroxide contributes to activating phosphoinositol pathway. The flavonoids inhibited calcium mobilization and IP$_3$ formation because of their ability to quench hydrogen peroxide and thus may inhibit platelet function (Pignatelli et al, 1999). Flavonoids are known to inhibit the interaction of platelets on collagen coated surfaces.
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(Middleton et al, 2000). Further, Hubbard et al (2003) have reported that quercetin inhibits collagen-stimulated whole cell protein tyrosine phosphorylation and intracellular mobilization of calcium. Quercetin was also found to inhibit various events in signaling generated by the collagen receptor glycoprotein VI. This includes collagen-stimulated tyrosine phosphorylation of the Fc receptor gamma-chain, Syk, LAT and phospholipase Cgamma2. Inhibition of phosphorylation of the Fc receptor gamma-chain suggests that quercetin inhibits early signaling events following stimulation of platelets with collagen (Hubbard et al, 2003, Hubbard et al, 2004). These studies may indicate that flavonoids may help in inhibiting platelet cell signaling indicated by reduced collagen levels and thus reduce thrombus formation. Thus, T.purpurea extracts may be helpful in delaying the development of atherosclerosis.

Increase in left ventricular cardiac collagen deposition also indicates the development of left ventricular hypertrophy (LVH). Data from Framingham study indicate that, LVH is not a benign compensatory process but an independent risk factor for congestive heart failure, coronary artery disease, and sudden death (Kannel and Gorden, 1969; Gorden and Kannel, 1971). 72% of the diabetic patients are found to have LVH, whereas only 32% of the nondiabetic patients show LVH (Grossman et al, 1992). Pathophysiology of diabetes mellitus includes hypoinsulinemia, hyperglycemia, cardiac hypertrophy and a cardiomyopathy that is characterized by the presence of diastolic and/or systolic contractile dysfunction. Prolonged cardiac hypertrophy has been reported to be associated with the decompensation of heart function, the development of heart failure and sudden death in humans (Mann, 1999; Towbin and Bowles, 2002). Cardiac hypertrophy was also observed in the diabetic rats evident from the increased heart weight to femur length ratio as well as left ventricular hypertrophy characterized by increased LV weight to heart weight ratio, left ventricular weight to right ventricular weight ratio and LV wall thickness. The treatment with the aqueous, alcoholic as well as flavonoid fraction significantly decreased hypertrophic parameters. Epidemiological studies suggest that increased dietary
intake of flavonoids is associated with a reduced risk of cardiovascular diseases. A very wide range of biological actions of flavonoids, including antioxidant (Rice-Evans and Packer, 1998), antiaggregant (Gryglewski et al, 1987) and vasodilator effects (Duarte et al, 1993a, 1993b) support the protective effects in cardiovascular diseases. Our histopathological findings also showed increased cardiac hypertrophy and also decreased extracellular space and hence results in high ECM accumulation. Treatment resulted in more increased extracellular space compared to diabetic rats, which indicates regression in ECM accumulation. Rutin was shown to improve cardiovascular structure and function by preventing the eccentric hypertrophy, inflammation, and fibrosis in the high fat diet fed rats (Panchal et al, 2011). Sustained high blood pressure is one of the most powerful causes of the development of cardiac hypertrophy which was observed in our study also. Flavonoids especially rutin and quercetin have shown vasodilatory effects and have been effective in lowering the blood pressure. The treatment with the extracts may have antihypertrophic effect at least partly due to reduction in systolic load, which is a very potent stimulus for cardiac growth. Also, quercetin has been found to possess potent antihypertensive action in various models (Perez-Vizcaino et al, 2009) as well as inhibit angiotensin 2-induced hypertrophy in vitro in cultured neonatal rat cardiomyocytes (Qin et al, 2001). Jalili et al (2006) suggests that the effect of quercetin on ventricular weight may be attributed to attenuation of signal transduction pathways that regulate cardiac hypertrophy. Reactive Oxygen Species (ROS) have been shown to act as intracellular signaling molecules in stress response in a variety of cell types. In cardiomyocytes, ROS have been found to mediate cardiac hypertrophy induced by several stimuli, such as mechanical stretch, angiotensin II, and tumor necrosis factor-α (TNF-α). T.purpurea has been known to have potent antioxidant activity and free radical scavenging activity (Johri et al, 2010; Nile et al, 2011). It is thus possible that the antioxidant effect might play a role in the prevention of cardiac hypertrophy in T.purpurea treated rats.
Oxidative stress stimulates cardiac damage, endothelial dysfunction, apoptosis, and collagen synthesis in the heart and contributes to the pathogenesis of myocardial remodeling and failure (Cesselli et al, 2001, Lefer and Granger, 2000, Siwik et al, 2001). In alloxan induced diabetes, the glutathione activity decreases after 12 weeks in rabbit hearts (Gumieniczek et al, 2002). Yadav et al. (1997) reported that GSH content and glutathione reductase (GSSG-R) activity in the heart tissue were markedly lowered in diabetic hearts. In the study of Doi et al. (2001) the GSH level was significantly reduced in diabetic animals. In present study, there was a significant increase in left ventricular MDA levels and a significant decrease in SOD and glutathione levels left ventricular tissue of STZ-diabetic rats as compared to control rats. Chronic treatment with flavonoid fraction of *T.purpurea* reduced elevated level of MDA and increased the level of SOD and glutathione in the left ventricular tissue of diabetic rats. Rutin has shown potential anti-oxidant activity in various diabetic tissues such as liver, kidney and brain (Kamalakannan and Prince, 2006). This anti-oxidant activity of *T.purpurea* may be responsible for the beneficial effects observed on the cardiovascular complications in STZ-diabetic rats.

Overall, it may be suggested that quercetin may be important for the improvement in glucose, insulin, cardiac and renal parameters. However, both rutin and quercetin appear to produce equivalent effect on lipid profile. Also, it has been shown that in the lower intestine, the rutin is cleaved under the influence of bacterial microflora (Bokkenheuser et al, 1987; Hollman et al, 1999) into quercetin which results in increased local concentrations of quercetin. Since, the flavonoid fraction was found to be more effective in improving the cardiac and renal parameters, it is possible that effects of rutin and quercetin are additive.

From the above mentioned discussion it can be concluded that *T. purpurea* possess anti-diabetic activity and prevents diabetes induced complications like dyslipidemia, cardiac dysfunction and nephropathy in rats. The possible mechanisms involved in anti-diabetic and
cardioprotection activity appears to be anti-oxidant activity of the *T. purpurea*. Additional mechanisms cannot be ruled out because rutin rich aqueous extract or quercetin rich alcoholic extract individually failed to produce beneficial effects as compared to flavonoid fraction of *T. purpurea* in most of the parameters except lipid dysfunction and cardiac hypertrophy. The flavonoid rich fraction was found to produce beneficial effects in all the parameters studied suggesting that both rutin and quercetin are required for the beneficial effects.

Cataract is one of the major causes of visual impairment in diabetic patients. Chronic elevation of blood glucose in diabetes plays a critical role in the development and progression of major diabetic complications. Prolonged exposure to elevated glucose causes both acute reversible changes in cellular metabolism and long-term irreversible changes in stable macromolecules. The injurious effects of hyperglycemia are characteristically observed in tissues that are not dependent on insulin for glucose entry into the cell like eye lens and kidneys. These tissues cannot produce down-regulation of glucose transport with the increase of extracellular sugar concentrations (Kyselova et al, 2004).

In the present investigation, diabetic rats did not show visual cataract upto a period of 8 weeks. However, there was decrease in soluble protein levels in the lens obtained from diabetic animals. All the three extracts of *T. purpurea* were found to increase the levels of soluble proteins in diabetic lenses. This may be due to prevention of cross-linking/aggregation and distribution of soluble proteins. The decrease in soluble protein content in diabetic lenses compared with those in control lenses in the present study could be due to leakage of proteins and insolubilization. Under conditions of severe oxidative stress, free radical generation leads to protein modification. Proteins in the lenses undergo advanced glycation/ lipoxidation by specific interactions of free radicals with amino acids or reactive carbonyl compounds formed by the auto-oxidation of carbohydrates and lipids (Gumieniczek 2005). *T. purpurea* treatment possessing
potent anti-oxidant potential may prevent protein modification and hence may be helpful in preventing the insolubilization of proteins. This may prevent or delay the development of opacity of the lens.

Three mechanisms have been proposed in the development of diabetic cataract. *First*, excess glucose is converted by aldose reductase to sorbitol, which accumulates in the lens fibres and causes osmotic stress leading to aggregation of protein known as polyol pathway (Varma et al, 1977). *Second*, the elevated glucose levels that causes auto-oxidation of glucose leading to formation of $\text{H}_2\text{O}_2$ and oxygen free radical which damages the lens protein (Altomare et al, 1997). *Third*, excess glucose chemically attaches to the free amino group of protein of lens without aid of enzyme and undergo slow rearrangement to form irreversible advanced glycosylation end products (AGEs) (Turk et al, 1997; Stitt, 2005). By interaction of AGE with cell surface receptors such as receptor for AGE’s in the epithelium of the lens further generates superoxide anion and $\text{H}_2\text{O}_2$ (Hong et al, 2000) and may enhance cataract formation. Although there is cross talk between these mechanisms, results in several studies suggest that oxidative stress is a major determinant in diabetic complications (Brownlee, 2001; Chung et al, 2003; Suryanarayana et al, 2003). Cataract requires not just a surgical solution, but a chemical and pharmacological complement as well. Since oxidative stress is a common initiator of many diabetic complications, including cataract, chemical approach to delay the onset or retard the progression of cataract is valuable. Therefore, agents or compounds that exert multiple actions, such as antioxidant, hypoglycemic, and aldose reductase (AR) inhibitory, activity could be more effective. In addition to increased levels of free radicals, diabetic lenses also show an impaired antioxidant capacity increasing their susceptibility to oxidative stress. The loss of antioxidants is exacerbated by glycation and inactivation of lens antioxidant enzymes like superoxide dismutases (Ookawara et al, 1992).
Oxidative stress may be a predominant mechanism in STZ-induced hyperglycemia. Oxidative stress may cause direct modification of the inner lens proteins, such as cross-linking, aggregation, and precipitation (Reddy et al, 1988). The increased TBARS (MDA levels) along with the decreased GSH and altered activities of antioxidant enzymes like SOD in the present study suggest increased oxidative stress in diabetic conditions. The role of increased oxidative stress is also widely accepted in the development and progression of diabetes and its complications (Baynes and Thorpe, 1999; Ceriello, 2000). Recent reports indicate that diabetic complications are associated with overproduction of free radicals and accumulation of lipid peroxidation by-products (Palanduz et al, 2001). However, many non-enzymatic antioxidants like glutathione (GSH) and enzymatic antioxidants like superoxide dismutase (SOD) defense mechanism are involved in the protection of free radicals induced oxidative damage. Oxygen free radicals are formed disproportionately in diabetes by glucose oxidation, non-enzymatic glycation of proteins and the subsequent oxidative degradation of glycated proteins (Maritim et al, 2003). Chronic treatment with aqueous extract, alcoholic extract as well as flavonoid fraction of *T.purpurea* decreased the lipid peroxidation and restored the levels of GSH and antioxidant enzymes like SOD. This is consistent with various reports which have shown that *T.purpurea* possess potent anti-oxidant activity (Khan et al, 2001; Prashanth kumar et al, 2001; Soni et al, 2003; Soni et al, 2006). Moreover, alcoholic extract and flavonoid fraction of *T.purpurea* showed significant free radical scavenging activity by producing reduction in DPPH radical *in-vitro*. It is possible that the delay in the development of STZ-induced diabetic complications observed in the present study is predominantly due to its antioxidant activity.

Aldose reductase (AR) is a small monomeric protein belonging to the aldo-keto reductase superfamily. High levels of AR activity is found to be present in rat lens and during diabetic cataractogenesis, AR-derived polyols like sorbitol accumulate in the ocular lens (Srivastava et al, 2005) which causes osmotic...
swelling resulting in ionic imbalance and protein insolubilization leading to cataractogenesis. Reduction of glucose by AR is a major cause of diabetic cataract which involves both osmotic stress as well as oxidative stress. Reduced levels of GSH and SOD as well as increased lipid peroxidation is observed in diabetic lens. It has been suggested that diabetes-associated GSH depletion may be secondary to the osmotic stress caused by sorbitol accumulation. Osmotic swelling of diabetic lens may render the cells leaky (Reddy et al, 1976), enhancing the loss of GSH accumulated in the lens (Lou et al, 1988). Disrupted cell membrane by osmotic stress may also interfere with amino acid transport into the lens (Kinoshita et al, 1965), and hence the biosynthesis of GSH (Lou et al, 1988). Moreover, AR reduction of glucose to sorbitol probably contributes to oxidative stress by depleting its cofactor NADPH, which is also required for the regeneration of GSH (Lee and Chung, 1999). Studies have suggested that persistent high intracellular glucose concentration-induced superoxide generation inhibits GAPDH activity (Du et al, 2003). Inhibition of GAPDH increases the levels of all the glycolytic intermediates located upstream of GAPDH finally increasing the first glycolytic metabolite, glucose (Naudi et al, 2012). This increases flux through the polyol pathway, where the enzyme aldose reductase reduces it, consuming NADPH in the process and reducing available GSH. Moreover, inhibition of GAPDH is responsible for an increased formation of the AGE-forming compound methylglyoxal (Baynes and Thorpe, 1999; Nishikawa et al, 2000a). As recently shown by Chang et al (2002), methylglyoxal is also responsible for substrate-induced upregulation of AR, which may further facilitate development of diabetic cataract. AR inhibiting activity of the investigating plant *T.purpurea* was evaluated *in-vitro* using rat lens homogenate. The alcoholic extract as well as flavonoid fraction of the plant was found to produce significant inhibition of AR activity. This may also be a probable mechanism of *T.purpurea* in delaying the development of diabetic complications like cataract.

From the above-mentioned discussion, it is clear that *T.purpurea* is effective in delaying the progression of cataract development in diabetic rats. The
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oxidative stress and inhibition of AR may be the possible mechanisms involved in the delay of development of cataract. To substantiate further, we studied the effect of *T.purpurea* in selenite induced cataract model. Injection of sodium selenite (s.c) to nine-day old rat pups produced bilateral nuclear cataract after a week. The development of cataract was significantly reduced by the treatment with quercetin, alcoholic extract and flavonoid fraction of *T.purpurea* as observed visually with help of opthalmoscope. Treatment with quercetin produced greater effect as compared to alcoholic or flavonoid fraction of *T.purpurea*. In fact, one of the rat treated with quercetin did not developed the cataract at all.

Selenite-overdose cataract is an extremely rapid and convenient model of nuclear cataracts (Shearer et al, 1987). This is a useful model for studies on cataractogenesis owing to its ease of induction and reproducibility. Moreover, it incorporates many of the features present in the senile cataract and diabetic cataract (Ostadalova et al, 1978). The cataract was produced in suckling rat pups by an overdose of the essential trace mineral selenium. Selenium has been recognized both, as an essential and hazardous element. It is a micronutrient for many animals and human beings. It is important for many cellular processes being specifically incorporated into the active sites of several known proteins or enzymes as the amino acid selenocysteine (Letavayova et al, 2006). It can prevent the development of many cancers, thus showing carcinostatic activities (Rayman, 2005). Ironically, selenium has however been shown to induce widespread oxidative stress in biological systems (Manikandan et al, 2009). Selenite is strong oxidizing agent. It catalyzes the oxidation of critical sulfhydryl groups on proteins and on glutathione that results in the formation of protein aggregates which causes progression of cataract. The biochemical processes occurring during production of cataract by selenite includes ROS generation, oxidative damage of the critical sulfhydryl groups of membrane Ca$^{2+}$-ATPase, calcium accumulation, calpain mediated proteolysis, precipitation of fragmented lens crystallins and loss of cytoskeletal proteins (Shearer et al, 1997).
Normal lens and erythrocytes contain an unusually high concentration of GSH and the concentration of this peptide decreases in almost all types of cataract. This antioxidant, GSH maintains protein thiol groups in the reduced state and prevents the cross linking of soluble crystallins in the lens (Reddy, 1990). The mechanism of GSH loss in selenite model is by a non-enzymatic reaction of GSH with selenite, which results in the formation of the selenium derivative, GS–Se– SG. Oxidation of GS–Se–SG by a single electron transfer to oxygen results in formation of superoxide anion as an intermediate (Seko et al, 1989). Supplementation of GSH or maintenance of its level in lens may help to maintain its protective ability against oxidative stress and lead to slower age-related loss of antioxidant activity of lens and eventually to delay the onset of cataract (Harding, 2001). We found that alcoholic extract as well as the flavonoid fraction of *T.purpurea* were able to restore the levels of GSH and thus showing potential anti-oxidant effect. Similar effects were also observed in the diabetic cataract model studied in our lab.

The antioxidant enzyme, SOD plays a critical role in protecting cells from oxidative stress. SOD is reported to inhibit hydroxyl radical production (Fridovich, 1995). SOD reacts with superoxide radicals and converts them to $\text{H}_2\text{O}_2$; excessive amounts of these metabolites can start a lethal chain reaction, which oxidizes and disables structures that are required for cellular integrity and survival (Ray and Husain, 2002). Selenite administration has been reported to decrease the activity of SOD in the lens (Shearer et al, 1997), accompanied by the generation of free radical species in the aqueous humor and significant reduction in nicotinamide adenine dinucleotide phosphate (reduced form) (Harding, 1970). Restoration of activity of the antioxidant enzymes in quercetin, alcoholic extract and flavonoid fraction treated group could be attributed to their antioxidant effect. Similar results were reported by others research group (Padmaja and Raju, 2004; Gupta et al, 2005; Lija et al, 2006). Free radical induced lipid peroxidation is one of the basic mechanisms of lens opacity (Awasthi et al, 1996) in selenite model which was seen as higher levels of MDA.
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in selenite induced group. Lower levels of MDA in the alcoholic as well as flavonoid fraction treated group are an indication to the prevention of lipid peroxidation. This was comparable to that quercetin indicating potent free radical scavenging activity. *T. purpurea* has been shown to possess potent free radical scavenging activity *in vitro* by various researches (Patel et al, 2010; Nile et al, 2011) which support our observation *in-vivo*. Many studies have shown that flavonoids, as a group of natural compounds with antioxidant properties, can prevent oxidative damage and experimental cataract progression (Kilic et al, 1996; Durukan et al, 2006; Lija et al, 2006). Further, rutin, a major flavonoid present in *T.purpurea*, has been also reported to retard the progression of cataract in selenite-induced cataract (Isai et al, 2009). One of the pathological events leading to protein precipitation in selenite model is mediated through disulfide cross-linking of protein via sulfhydryl oxidation, leading to higher molecular weight aggregate formation, protein precipitation and lens opacification (Dong et al, 2000). Selenite induced group had significantly lower level of protein sulfhydryl content over control. In the treated groups, protein sulfhydryl content was found to be increased, again confirming its protective effect against oxidative damage. This may be due to its restoration of the glutathione reductase activity.

Selenite cataract is characterized by marked decline in water soluble protein through protein insolubilization (Shearer et al, 1997). Similar changes were also observed in human cataract (Srivastava and Srivastava, 2003). The crystalline lattice of the lens, mainly made up of proteins, is responsible for lenticular transparency and for the proper focusing of light. Dense opacification results when the proteins form large insoluble aggregates which results in light scattering. There was significant change observed in the soluble as well as the insoluble protein in the selenite-induced group compared to that of the age matched control rats. This may be due formation of the insoluble high molecular weight protein aggregates which represent an intermediate stage in conversion of water soluble to water-insoluble proteins in rabbit lenses (Liem-The and Hoenders, 1974). The insoluble protein levels were found to be reduced in the
groups treated with quercetin, flavonoid fraction and the alcoholic extract. Pre-treatment with these extracts may delay the oxidation of these proteins as well as formation of protein aggregates and hence may delay the progression of cataract.

Oxidative damage of the critical sulfhydryl groups of proteins could lead to the inactivation of membrane proteins like Ca\(^{2+}\)ATPase as well as inhibition of selective calcium permeability. Previous studies show that Ca\(^{2+}\)ATPase is particularly sensitive to oxidative stress and results in a loss of their activity (Ahuja et al, 1999). Selenite cataract shows significant decrease in Ca\(^{2+}\)ATPase activity and increase in lenticular calcium levels. Decrease in the activity of Ca\(^{2+}\)ATPase and accumulation of Ca\(^{2+}\) are considered as essential features in selenite cataract formation (Wang et al, 1993). Increased lens calcium could be due to inhibition of outwardly directed Ca\(^{2+}\)ATPase pumps or inhibition of Na/Ca exchange. Moreover, in rodent lenses, the calcium influx was observed to cause activation of calcium dependent proeases, which partially degrade the crystallins and thereby resulting in the protein insolubilization (Shearer et al, 1997). In the treated groups, lower levels of calcium and higher levels of Ca\(^{2+}\)ATPase activity were observed attributing to their protective effect on cataract. Disulfiram and Verapamil hydrochloride also reported similar effects (Nabekura et al, 2003; Ettl et al, 2004). Babizhayev (2004) reported that lipid peroxidation causes the oxidative inhibition of Ca\(^{2+}\)ATPase in several tissues including the lens, and induces the increase in membrane permeability of Ca\(^{2+}\). Since the plant shows potential reduction in the lipid peroxidation, as well as increases the protein sulfhydryl content, it may be responsible for the restoration of the Ca\(^{2+}\)ATPase activity as well as the Ca\(^{2+}\) levels.

Nitric Oxide (NO) reacts readily with the oxygen free radical, superoxide anion, to form a strong oxidant, peroxynitrite, whose cytotoxic potential is greater than that of NO (Beckman et al, 1990; Beckman and Crow, 1993). This reaction is highly favourable under reduced concentrations of SOD which is found in the selenite induced group. Peroxynitrite, which is known to oxidize sulfhydryls and...
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to yield products indicative of hydroxyl radical reactions with deoxyribose and
dimethylsulfoxide, induces membrane lipid peroxidation (Radi et al, 1991). The
resulting nitrosative stress can oxidize cytosolic free thiols leading to disulfide
linkage formation between proteins and affecting their function (Ghafourifar et al,
2001). Moreover, NO can affect the levels of both free protein thiols and total
glutathione levels in cells (Onoda and Inano, 2000) causing opacification. Nitrite
levels, an indirect measurement of NO was found to be significantly higher in the
selenite induced group. These levels of nitrite were found to be decreased with
treatment which may be responsible for increasing Ca^{2+}ATPase activity and
hence decreasing the Ca^{2+} accumulation. Thus, suggesting the protective effect
of the extracts in delaying the cataract formation. Thus, the results of the present
study indicates that treatment with \textit{T.purpurea} is effective in delaying the
progression of cataract by maintaining the antioxidant status and preventing
protein oxidation and lipid peroxidation in lens. This protective effect was also
manifested morphologically as a decreased frequency and intensity of lenticular
opacification.

The results of the data on the study of the effects of \textit{T. purpurea} on
cataract suggest potential beneficial effects in preventing not only diabetes
induced development of cataract in rats but also in prevention of selenite
induced cataract. The possible mechanism appears to be anti-oxidant
activity. However, aldose reductase inhibition may be additional
mechanism involved in the prevention of selenite induced cataract.