Effect of flavonoid rich fraction of *Tephrosia purpurea* (Linn.) Pers. on complications associated with streptozotocin-induced type I diabetes mellitus

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Globally, diabetes is a serious health issue affecting one in 11 adults and consumes 12% of global health expenditure. Prevalence of dyslipidemia in diabetes is not uncommon since decades. Further, patients with type II diabetes have 2-4 folds more risk for cardiovascular disease (CVD). Plants with antioxidant potential are known to have beneficial effects in diabetes and its complications. Natural compounds, flavonoids particularly, ameliorate hyperglycemia as well as CVD. Here, we evaluated common wasteland weed *Tephrosia purpurea*, used traditionally as folk medicine to treat many disorders including diabetes. We studied the effect of 8-wk treatment of flavonoid-rich fraction of *T. purpurea* (FFTp) (40 mg/kg/day/p.o.) on various biochemical, cardiovascular and lenticular parameters on streptozotocin (STZ) (45 mg/kg, i.v.) induced type I diabetic rats. STZ administration produced significant hyperglycemia, dyslipidemia, and altered cardiac biomarkers like lactate dehydrogenase, creatinine kinase and reduced antioxidants in lenticular tissues of rats. Treatment with FFTp significantly prevented STZ-induced hyperglycemia, dyslipidemia as well as cardiovascular markers. We observed decreased rate of pressure development (+dp/dt) and decay (-dp/dt) in STZ diabetic hearts which was prevented by FFTp. Further, the soluble protein levels and the antioxidants were also elevated in the diabetic rats by the treatment. In conclusion, our data suggest that FFTp produces beneficial effects on diabetes induced cardiovascular complications and cataract. Such beneficial actions may be attributed to the antioxidant property of flavonoids, quercetin or rutin, present in *T. purpurea*.

Keywords: Ayurveda, Cardiovascular complications, Cataract, CVD, Dyslipidemia, Herbal, Hyperglycemia, Quercetin, Rutin, Wild indigo.

Diabetes mellitus and its associated complications are a significant health burden all over the world. Prevalence of dyslipidemia in diabetes has been a issue of concern since decades. Patients with type II diabetes face 2-4 folds higher risk of cardiovascular disease (CVD). According to International Diabetes Federation, 415 million people have diabetes in 2015 and it is expected to rise to 642 million by 2040. India, ranks next only to China with 69.2 million cases now, is projected to reach 123.5 million by then. Both, type I & II diabetes (TIDM & TIIDM) are associated with destruction of pancreatic β-cells. TIDM, an autoimmune defect wherein insulin producing β-cells are killed by body’s own defense system, though rare compared to TIIDM (7-12% in high income countries), is mostly observed in children and young adults aged <15 years. However, cases of TIDM are reported to be increasing possibly due to environmental risk factors and/or viral infections. More than half a million children are known to be affected across the globe with annual increase of 3%; USA ranks highest with 84100 cases followed by India, Brazil and China with 70200, 30900 and 30500 cases, respectively. Currently, diabetes consumes about 12% of global health expenditure.

Oxidative stress has been implicated in the development and progression of diabetic complications. Plants or plant extracts with antioxidant potential have been shown to have beneficial effects in diabetes and its complications. The search for antidiabetic agents have been fascinated towards plants and plant derived products partly because of large number of leads provided by traditional medicines. There is need to develop a drug which is not only effective in treating diabetes but also prevents the progression of diabetic complications.
**Tephrosia purpurea** (Linn.) Pers. (Leguminosae), commonly known as wild indigo (Sharapunkha in Sanskrit) is a highly branched, sub-erect, herbaceous perennial. It is reported to be used as digestive, anthelmintic, alexiteric, antipyretic, astringent, thermogenic, acrid and also to cure diseases of liver, spleen, heart, blood, tumors, ulcers, leprosy and asthma according to Ayurveda. Different parts of the plant have been extensively studied for various pharmacological actions including antihyperglycemic and antihyperlipidemic. However, there is hardly any report on the effects on long-term complications of diabetes. Further, there is no standard therapy available for treatment of diabetic complications, and hence, no reference compound has been used in the study. Thus, here we investigated the effect of the flavonoid rich fraction of *Tephrosia purpurea* on streptozotocin-induced diabetes and its related complications such as cardiovascular complications and cataract.

**Materials and Methods**

**Preparation and standardization of plant extract**

The whole plant of *T. purpurea* was collected from the medicinal plant garden of Institute of Pharmacy, Nirma University and botanically authenticated. Voucher specimen (No. PL08SVBRKGtp001) was deposited in herbarium of the Institute of Pharmacy, Nirma University. The shade-dried plant was powdered. The 200 g of coarse air-dried powder was soxhlet-extracted with 95% ethanol. After concentration under vacuum, the extract was suspended in distilled water and partitioned into ethyl acetate. The residue obtained after evaporation was dissolved in ethanol and treated with neutral lead acetate. The precipitate obtained after centrifugation, re-suspended in ethanol, treated with hydrogen sulfide and filtered. The filtrate was evaporated under vacuum to yield the flavonoid rich fraction (3.07% w/w).

Total phenolic content of the flavonoid rich fraction was determined following the method described by Singleton and Rossi and calculated from the calibration curve of gallic acid standard solution. Results were expressed as % w/w of gallic acid equivalent in dry extract. Total flavonoid content was measured using quercetin as the standard for calibration curve. Results were expressed as % w/w of quercetin equivalent in dry extract.

The flavonoid rich fraction was analyzed by thin layer chromatography for the presence of rutin using ethyl acetate: n-butanol:formic acid:water (5:3:1:1 v/v) and quercetin using toluene:ethyl acetate:formic acid (5:4:1 v/v) as solvent systems. The resulting chromatogram was scanned and quantified using CAMAG TLC scanner III at 254 and 374 nm, respectively.

**In vitro free radical-scavenging activity on 1,1-diphenyl-2-picrylhydrazyl (DPPH)**

The antioxidant activity of FFTp was measured in terms of electron transfer/hydrogen donating ability using a stable radical DPPH using the method described by Molyneux.

**Animals**

The protocol of the experiment was approved by our institutional animal ethical committee as per the guidance of the Committee for the Purpose of Control and Supervision of Experiments on Animals, Ministry of Social Justice and Empowerment, Government of India (Protocol no. IPS/PCOL/FAC 10-11/2001 dated 12th January, 2011).

Healthy Sprague Dawley rats of either gender (200–250 g) were made diabetic by single tail vein injection of streptozotocin (STZ) (45 mg/kg) dissolved in 0.1 M (pH 4.5) of citrate buffer. Control rats were injected with 0.1 M (pH 4.5) of citrate buffer alone. The induction of diabetes was checked 48 h after the STZ injection by measuring the extent of glycosuria with Diastix (Bayer Diagnostics, Ltd). Rats displaying glycosuria more than 2% were considered as diabetic. The rats were then randomly divided into 4 groups: control (CON), control treated with FFTp (COT), diabetic control (DIA) and diabetic treated with FFTp (DT). FFTp was suspended in distilled water and administered at a dose of 40 mg/kg/day, p.o. for 8 weeks. The food and water were given ad libitum.

**Blood sample collection and serum analysis**

At the end of 8 wk of treatment, animals were fasted for 12 h and blood samples were collected from the retro-orbital plexuses of each rat under light ether anesthesia and serum was separated. A small amount of blood was collected with heparin (20 USP Units/ml of blood collected) and analyzed for glycosylated hemoglobin using diagnostic kit (Accucare Diagnostics, Ltd, India). The serum samples were analyzed for glucose, total cholesterol, low-density lipoprotein (LDL)-cholesterol, high-density lipoprotein (HDL)-cholesterol, triglycerides, creatinine, urea, lactate dehydrogenase (LDH), creatinine kinase (CK) spectrophotometrically (Shimadzu UV, Japan) using
available biochemical diagnostic kits (Accucare Diagnostics, Ltd, India). Serum insulin was estimated by radioimmunoassay technique using kits obtained from Board of Radiation and Isotope Technology, Mumbai, India in gamma counter (Packard, USA).

**Measurement of cardiovascular parameters**

Blood pressure, heart rate, rate of pressure development and decay were recorded at the end of 8 wk by carotid artery cannulation. Briefly, the animals were anaesthetized by ketamine (20 mg/kg, i.p.) + xylazine (10 mg/kg, i.m.). The body temperature was maintained at 37±1°C during the experiment. The carotid artery behind the trachea was exposed and cannulated for the measurement of hemodynamic parameters using a transducer (BP 100) and Labscribe Systems (I-worx-118, USA). The hemodynamic parameters observed were mean arterial blood pressure, rate of pressure development (dp/dt\text{max}) and rate of pressure decay (dp/dt\text{min}). All the data were analyzed using Labscribe software (Version 2.0). After withdrawal of blood samples and recording of hemodynamic parameters, animals were sacrificed, hearts were excised, extraneous tissues were separated and wet weight of the entire heart, left ventricle, right ventricle along with femur length was noted down to calculate the index of cardiac hypertrophy and left ventricular (LV) hypertrophy index. Further, LV weight to right ventricular weight ratio (LVW/RVW) and the thickness of LV wall was measured using screw gauge micrometer. Quantification of left ventricular myocardial hydroxyproline concentrations in LV tissue was performed according to the method of Prockop and Udenfriend\textsuperscript{12} as an indicative of collagen deposition.

**Oxidative stress in lens**

At the end of the experimental period 8 weeks, after the measurement of cardiovascular parameters, the animals were sacrificed and the lenses were dissected out by posterior approach. The homogenate was prepared in 50 mM phosphate buffer. The oxidative stress inflicted to the lens was assessed by measurement of glutathione (GSH) depletion\textsuperscript{13}, superoxide dismutase\textsuperscript{14} as well as the level of lipid peroxidation in the form of malonaldehyde (MDA)\textsuperscript{15}. Moreover, the soluble protein levels were assessed in the lens homogenate following Lowry et al.\textsuperscript{16}.

**Statistical analysis**

Results are presented as mean ± standard error of the mean (SEM). Statistical differences between the means of the various groups were evaluated using 1-way analysis of variance followed by Tukey’s test. Data with \(P\) value <0.05 were considered statistically significant.

**Results**

**Phytochemical studies**

*Tephrosia purpurea* was found to have rich content of flavonoids and some amount of phenolics. The level of total flavonoids and phenolics in the FFTp was found to be 26.94% w/w and 3.83% w/w, respectively. On performing HPTLC analysis of the FFTp, rutin (2.37% w/w) and quercetin (1.75% w/w) were found to be present in the extract (Fig. 1).

**In vitro free radical scavenging activity by DPPH**

FFTp resulted in the reduction of DPPH free radical concentration. The IC\textsubscript{50} value of FFTp was 48.51 \(\mu\)g/mL. FFTp at the concentration of 100 \(\mu\)g/mL showed 70.65% reduction in DPPH free radical concentration.

Fig. 1—Spectral overlay of flavonoid rich fraction of *Tephrosia purpurea* with (a) standard rutin (b) standard quercetin.
Pharmacological studies

General features of experimental rats

Intravenous injection of STZ (45 mg/kg) in rats produced cardinal signs of type 1 diabetes i.e. loss of body weight, polyphagia and polydipsia. Glycosuria (>2%) and polyuria observed in these animals persisted throughout the period of 8 weeks. Treatment with FFTp (40 mg/kg/p.o./day) prevented STZ-induced loss of body weight (Table 1). In addition, there was significant reduction in the food intake and water intake with the treatment (Table 1). This significantly prevented polydipsia and polyphagia.

Biochemical parameters

The STZ-diabetic rats were found to exhibit significant hyperglycemia, hypoinsulinemia and significant increase in % glycosylated hemoglobin as compared to the control rats. Chronic treatment with FFTp (40 mg/kg/p.o./day) produced significant decrease in the elevated serum glucose levels (Table 1) and glycosylated hemoglobin (Fig. 2a) accompanied by significant increase in serum insulin levels (Fig. 2b).

There was a significant increase in total cholesterol, very-low-density lipoprotein (VLDL)-cholesterol, LDL-cholesterol and triglyceride levels and significant decrease in HDL-cholesterol levels in STZ-diabetic rats as compared with control rats. Treatment with FFTp (40 mg/kg/day) significantly reduced the elevated total cholesterol, VLDL, LDL-cholesterol and triglyceride levels in diabetic rats and increased the HDL-cholesterol levels (Table 2).

STZ also produced a significant increase in serum creatinine and urea levels as compared with control rats. Chronic treatment with FFTp (40 mg/kg/day) significantly reduced the elevated creatinine and urea levels of diabetic rats (Table 2).

Table 1—Effect of chronic treatment of flavonoid rich fraction of Tephrosia purpurea (40 mg/kg/day) on general features and glucose levels of experimental rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CON</th>
<th>COT</th>
<th>DIA</th>
<th>DIT</th>
</tr>
</thead>
<tbody>
<tr>
<td>% change in body wt.</td>
<td>13.10±3.71</td>
<td>6.02±1.51</td>
<td>-8.34±2.68*</td>
<td>2.45±1.84*</td>
</tr>
<tr>
<td>Food intake (g/animal/day)</td>
<td>27.78±0.95</td>
<td>26.11±2.61</td>
<td>46.67±2.75*</td>
<td>21.11±3.31*</td>
</tr>
<tr>
<td>Water intake (mL/animal/day)</td>
<td>38.89±0.95</td>
<td>36.11±1.12</td>
<td>128.89±0.97*</td>
<td>94.44±0.84*</td>
</tr>
<tr>
<td>Serum Glucose (mg/dL)</td>
<td>75.10±3.90</td>
<td>85.30±4.85</td>
<td>317.0±8.98*</td>
<td>135±12.80*</td>
</tr>
</tbody>
</table>

*Significantly different from control (P <0.05); #Significantly different from diabetic control (P <0.05). [CON, Control animals; COT, Control animals treated with flavonoid rich fraction of T. purpurea (40 mg/kg/p.o/day); DIA, Diabetic control animals; and DIT, Diabetic animals treated with flavonoid rich fraction of T. purpurea (40 mg/kg/p.o/day)]

Table 2—Effect of chronic treatment of flavonoid rich fraction of Tephrosia purpurea (40 mg/kg/day) on lipid profile, creatinine and urea levels.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CON</th>
<th>COT</th>
<th>DIA</th>
<th>DIT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cholesterol (mg/dL)</td>
<td>71.3±3.00</td>
<td>73.1±4.85</td>
<td>92.4±2.49*</td>
<td>78.4±0.84*</td>
</tr>
<tr>
<td>LDL – Cholesterol (mg/dL)</td>
<td>24.7±1.36</td>
<td>26.8±2.55</td>
<td>38.5±1.92*</td>
<td>25.7±1.43*</td>
</tr>
<tr>
<td>VLDL – Cholesterol (mg/dL)</td>
<td>19.6±0.33</td>
<td>19.9±0.47</td>
<td>35.5±2.12*</td>
<td>24.5±2.03*</td>
</tr>
<tr>
<td>HDL – Cholesterol (mg/dL)</td>
<td>29.8±3.07</td>
<td>27±2.00</td>
<td>13.2±1.26*</td>
<td>30.3±3.00*</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>98.0±1.67</td>
<td>99.5±2.33</td>
<td>176.8±8.82*</td>
<td>122±10.2*</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.59±0.03</td>
<td>0.60±0.02</td>
<td>0.85±0.06*</td>
<td>0.64±0.04*</td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
<td>45.9±3.73</td>
<td>47.3±3.07</td>
<td>65.6±1.41*</td>
<td>51.5±2.54*</td>
</tr>
</tbody>
</table>

*Significantly different from control (P <0.05); #Significantly different from diabetic control (P <0.05). [CON, Control animals; COT, Control animals treated with flavonoid rich fraction of T. purpurea (40 mg/kg/p.o/day); DIA, Diabetic control animals; and DIT, Diabetic animals treated with flavonoid rich fraction of T. purpurea (40 mg/kg/p.o/day)]
Cardiac parameters

STZ produced a significant increase in serum LDH and CK-MB levels as compared with control rats. Chronic treatment with FFTp (40 mg/kg/day) significantly reduced the elevated serum LDH and CK-MB levels as compared to the STZ-diabetic rats (Table 3).

Hypertrophic parameters

The LV weight to heart weight ratio, which is a measure of LV hypertrophy index and ratio of wet heart weight to femur length which is a measure of cardiac hypertrophy index was significantly high in diabetic control animals as compared to non-diabetic control animals. Chronic treatment with FFTp (40 mg/kg/day) significantly reduced the elevated LV hypertrophy index (Fig. 3a) and cardiac hypertrophy index (Fig. 3b) of diabetic rats. Further, LVW/RVW ratio and LV wall thickness were also significantly high in STZ-diabetic animals as compared to control animals. Chronic treatment with FFTp (40 mg/kg/day) significantly decreased the elevated LVW/RVW ratio and LV wall thickness in diabetic rats (Fig. 4a). Additionally, left ventricular collagen level was found to be significantly high in STZ-diabetic rats as compared to control animals. Chronic treatment with FFTp (40 mg/kg/day) significantly reduced the elevated LV collagen levels of STZ-diabetic rats (Table 3).

Hemodynamic parameters

The STZ-diabetic rats showed significantly elevated mean blood pressure and significantly lower heart rate as compared to control animals. Chronic treatment with FFTp (40 mg/kg/day) decreased the blood pressure and increased the heart rate in STZ-diabetic rats (Table 3). Functional cardiac performance was assessed by measuring the left ventricular responses in terms of maximum dp/dt and minimum dp/dt. Both the rate of

**Table 3**— Effect of chronic treatment of flavonoid rich fraction of *Tephrosia purpurea* (40 mg/kg/day) on cardiac, collagen and hemodynamic parameters.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CON</th>
<th>COT</th>
<th>DIA</th>
<th>DIT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatine kinase (U/L)</td>
<td>16.22±1.64</td>
<td>17.90±1.36</td>
<td>35.91±1.94*</td>
<td>21.85±3.62*</td>
</tr>
<tr>
<td>Lactate dehydrogenase (U/L)</td>
<td>725±61.3</td>
<td>751±56.0</td>
<td>1125±77.7*</td>
<td>802±84.4*</td>
</tr>
<tr>
<td>Collagen levels (mg/gm LV tissue)</td>
<td>1.16±0.21</td>
<td>1.31±0.09</td>
<td>2.9±0.23*</td>
<td>1.81±0.12*</td>
</tr>
<tr>
<td>Blood pressure (mmHg)</td>
<td>131±4.07</td>
<td>132±3.47</td>
<td>155±3.28*</td>
<td>135±3.14*</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>381±10.1</td>
<td>392±8.69</td>
<td>298±12.8*</td>
<td>354±12.0*</td>
</tr>
</tbody>
</table>

*Significantly different from control (P <0.05); *Significantly different from diabetic control (P <0.05). [CON, Control animals; COT, Control animals treated with flavonoid rich fraction of *T. purpurea* (40 mg/kg/p.o/day); DIA, Diabetic control animals; and DIT, Diabetic animals treated with flavonoid rich fraction of *T. purpurea* (40 mg/kg/p.o/day)]
pressure development (max dp/dt) as well as decay (min dp/dt) was severely reduced in the diabetic control rats. Chronic treatment with FFTp (40 mg/kg/day) showed a significant improvement in them (Fig. 4b).

**Antioxidant parameters in left ventricular tissue**

The STZ-diabetic rats exhibited significantly reduced glutathione and superoxide dismutase (SOD) levels in LV tissue as compared to the control animals. Treatment with FFTp (40 mg/kg/day) significantly increased reduced glutathione and SOD levels in LV tissue of diabetic rats (Table 4). Also, MDA levels indicative of lipid peroxidation was observed to be significantly elevated in diabetic rats which was found to be reduced with the treatment of FFTp (Table 4).

**Cataract parameters in lens**

No visual cataract was seen in diabetic lens. Anti-cataract activity was checked by estimating various biochemical parameters in the lens of the animals. STZ-diabetic rats exhibited significantly decreased soluble protein, reduced glutathione and superoxide dismutase (SOD) levels and significantly elevated lipid peroxidation levels in lens as compared to those of control animals while treatment with FFTp (40 mg/kg/day) significantly prevented the loss of soluble proteins (Table 4), reduced glutathione and SOD levels in lens of diabetic rats (Table 4). Also, chronic treatment with FFTp (40 mg/kg/day) showed a significant reduction in the lipid peroxidation levels (Table 4).

**Histopathological studies**

The transverse section of left ventricle of control animals showed normal cardiac fibers with nuclei and intercalations (Fig. 5a). STZ administration caused degenerative changes in cardiac muscle fiber. Hypertrophy of myocardial fibers was seen, nuclei were clustered, intercalations disrupted and vacuoles were formed (Fig. 5b). Left ventricular tissues from diabetic animals treated with FFTp (40 mg/kg/day) showed a lesser degree of these degenerative changes. A significant reduction in the distortion of cardiac muscle fibers and vacuole formation was observed (Fig. 5d). Further, the control animals treated with the extract showed normal cardiac fibers (Fig. 5c).

**Discussion**

*T. purpurea* has been reported to possess a large number of beneficial effects such as antidiabetic, antihyperlipidemic, antiulcer, antioxidant, anti-

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**Table 4—Effect of chronic treatment of flavonoid rich fraction of Tephrosia purpurea (40 mg/kg/day) on antioxidant parameters in left ventricle (LV) and lens.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CON</th>
<th>COT</th>
<th>DIA</th>
<th>DIT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reduced glutathione in LV (µg/mg protein)</td>
<td>2.17 ± 0.096</td>
<td>2.03 ± 0.078</td>
<td>0.97 ± 0.010*</td>
<td>1.75 ± 0.122</td>
</tr>
<tr>
<td>Superoxide dismutase in LV (U/min/mg protein)</td>
<td>4.11 ± 0.053</td>
<td>4.03 ± 0.035</td>
<td>2.53 ± 0.014*</td>
<td>3.76 ± 0.264</td>
</tr>
<tr>
<td>Lipid Peroxidation in LV (nmoles MDA/mg protein)</td>
<td>0.66 ± 0.044</td>
<td>0.71 ± 0.061</td>
<td>1.67 ± 0.113*</td>
<td>1.05 ± 0.140</td>
</tr>
<tr>
<td>Soluble Protein in lens (µg/mg wet tissue)</td>
<td>60.87 ± 3.29</td>
<td>59.91 ± 4.94</td>
<td>42.87 ± 1.73*</td>
<td>57.52 ± 1.70</td>
</tr>
<tr>
<td>Reduced glutathione in lens (µg/mg protein)</td>
<td>20.15 ± 2.54</td>
<td>18.92 ± 2.21</td>
<td>12.10 ± 1.71*</td>
<td>18.28 ± 1.96</td>
</tr>
<tr>
<td>Superoxide dismutase in lens (U/min/mg protein)</td>
<td>3.84 ± 0.09</td>
<td>3.69 ± 0.05</td>
<td>2.90 ± 0.09*</td>
<td>3.80 ± 0.28</td>
</tr>
<tr>
<td>Lipid Peroxidation in lens (nmoles MDA/mg protein)</td>
<td>2.32 ± 0.15</td>
<td>2.65 ± 0.18</td>
<td>3.86 ± 0.21*</td>
<td>2.96 ± 0.31</td>
</tr>
</tbody>
</table>

*Significantly different from control (P <0.05); #Significantly different from diabetic control (P <0.05). [CON, Control animals; COT, Control animals treated with flavonoid rich fraction of T. purpurea (40 mg/kg/p.o/day); DIA, Diabetic control animals; and DIT, Diabetic animals treated with flavonoid rich fraction of T. purpurea (40 mg/kg/p.o/day)]
inflammatory, wound healing, hepatoprotective, cytoprotective effect on mast cells etc. Studies on phytochemical analysis showed that flavonoid rich fraction contains significant amount of rutin as well as quercetin. Further, in the present study, FFTp showed significant free radical scavenging activity in vitro in left ventricular tissues as well as in lens.

In the present study, STZ produced cardinal signs and characteristics of diabetes, i.e., loss of body weight, polyphagia, polyuria, and polydipsia, which are consistent with those reported earlier. Chronic treatment with FFTp (40 mg/kg/day) prevented the reduction in body weight, polyphagia and polydipsia in STZ-diabetic rats.

Intravenous injection of STZ produces fragmentation of DNA of β-cells of pancreas leading to destruction of β-cells and hence resulting in hyperglycemia and hypoinsulinemia. Oxidative damage to the β-cells due to generation of reactive oxygen species (ROS) is observed in STZ-induced diabetes mellitus. Compounds with antioxidant properties have shown regeneration of β-cells and may protect pancreatic islets against the cytotoxic effects of STZ. In the present study, STZ produced a significant increase in glucose levels along with decrease in insulin levels in type 1 diabetic rats. Treatment with FFTp (40 mg/kg/day) significantly reduced the serum glucose levels and also produced elevation in the serum insulin levels of STZ-diabetic rats. Phytochemical investigations on T. purpurea have revealed the presence of glycosides, rotenoids, isoflavones, flavanones, chalcones, flavanols, flavones and sterols. The flavonoids are group of compounds possessing potential antioxidant properties. This antioxidative property of the plant might protect pancreatic islets and encourage regeneration of β-cells which leads to increased insulin levels, and thereby increased utilization of glucose by the peripheral tissues. Rutin is one of the major flavonols present in FFTp. Kamalakkannan and Prince have shown an expansion of the pancreatic islets in rutin-treated diabetic rats suggesting protective effect of rutin in diabetic rats.

The glycated haemoglobin levels in the present study were increased in diabetic rats. Chronic treatment with the FFTp (40 mg/kg/day) could effectively reduce the levels of glycated hemoglobin. A decrease in blood glucose levels might contribute to the decreased levels of glycated haemoglobin in the treated diabetic rats. Rutin with its free radical scavenging activity could effectively reduce the formation of glycated haemoglobin in diabetic rats. Nagasawa et al. 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 antioxidative property of FFTp was responsible for reduction in glycated hemoglobin levels in diabetic rats.

It has been reported that in STZ-diabetic rats, insulin deficiency is associated with dyslipidemia. Abnormal lipid levels lead to the development of coronary artery disease in diabetic patients. 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. ROS 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. The serum cholesterol and triglyceride levels of diabetic rats treated with FFTp (40 mg/kg/day) were found to be significantly decreased. Pavana et al. 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 metabolizing enzymes such as LCAT (Lecithin—cholesterol acyltransferase) and LPL (Lipoprotein Lipase). Flavonoids have been reported to suppress LDL oxidation and inflammatory progression in the arterial wall. Moreover, rutin is a potent inhibitor of HMG-CoA reductase, an enzyme responsible for cholesterol synthesis, and also beneficial for lowering serum cholesterol levels. This may suggest the mechanism of cholesterol lowering effect of FFTp. Besides, FFTp also significantly augmented serum HDL cholesterol in rats with STZ-induced diabetes. Rutin has been reported to prevent HDL-C from oxidative modification in vitro. Thus, the plant may possess the potential to prevent the formation of atherosclerosis and coronary heart disease.

In the present study, significant elevation in serum creatinine and urea levels indicating impaired renal function in diabetic animals was observed consistent with earlier reports. Treatment with FFTp (40 mg/kg/day) produced considerable lowering of elevated serum creatinine and urea levels in diabetic animals. The ethanolic extract of T. purpurea leaves has shown marked nephroprotective and curative
effect against gentamicin-induced acute renal injury in albino rats. This activity may be attributed to phenolic and flavonoidal compounds like rutin and quercetin. Hence, FFTp may be beneficial in providing some protection against diabetic nephropathy. However, further studies are required to prove its efficacy in diabetic nephropathy.

Increased serum CK and LDH levels in diabetic rats indicate cardiac muscular damage. Increased activity of LDH in diabetes mellitus has been reported. In our study, we found significant rise in LDH and CK levels in STZ-diabetic rats as compared to the control rats. Treatment with FFTp (40 mg/kg/day) was able to normalize the LDH activity and CK levels in the diabetic rats. 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. This may suggest that the treatment may be helpful in preventing cardiac damage.

Brady-cardia is frequently observed in STZ diabetic rats. The diabetic rats showed significantly reduced heart rate in the present study. Chronic treatment with FFTp (40 mg/kg/day) prevented STZ-induced bradycardia in the diabetic animals. Further, change in haemodynamics i.e. hypertension and decline in rate of pressure development and decay was found which was improved by the treatment. Rutin-treated rats have been shown to lower systolic blood pressure and improved endothelial function by scavenging free radicals. Furthermore, flavonoids have a potential to inhibit angiotensin 1-converting enzyme (ACE) in vitro and quercetin has been reported to be a potent inhibitor of ACE which may suggest a possible mechanism for lowering of blood pressure by FFTp.

In the present investigation, STZ diabetic rats expressed significantly high amount of collagen deposition in LV and chronic treatment with FFTp (40 mg/kg/day) significantly reduced collagen deposition in LV. Increase in LV cardiac collagen deposition also indicates the development of LV hypertrophy. Cardiac hypertrophy was 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, LVW/RVW ratio and LV wall thickness. The treatment with FFTp (40 mg/kg/day) significantly decreased hypertrophic parameters. Our histopathological findings also showed increased cardiac hypertrophy and decreased extracellular space in diabetic rats and hence resulting in high ECM accumulation. Treatment resulted in increased extracellular space compared to diabetic rats, which indicates regression in ECM accumulation. Sustained high blood pressure is one of the most powerful causes of the development of cardiac hypertrophy which was observed in our study also. The treatment may have antihypertrophic effect at least partly due to reduction in systolic load. ROS have been found to mediate cardiac hypertrophy induced by several stimuli. *T. purpurea* has been known to have potent antioxidant activity. Also, our studies have shown potent free radical scavenging activity of FFTp. Further, a significant elevation was found in the antioxidant levels as well as reduction in the lipid peroxidation levels in the LV tissue indicating potent antioxidant effect of FFTp. It is, thus possible that the antioxidant effect might play a role in the prevention of cardiac hypertrophy in FFTp treated rats. Further, rutin is proven to be effective in providing cardioprotection by improving LV dysfunction in STZ-diabetic rats which may further substantiate the role of *T. purpurea* in cardiac complications associated with diabetes.

In the present investigation, diabetic rats did not show visual cataract up to a period of 8 weeks. However, decrease in soluble protein levels was observed in the lens obtained from diabetic control animals. Under conditions of severe oxidative stress, free radical generation leads to protein modifications like advanced glycation/lipoxidation ultimately leading to insolubilization. FFTp treatment possessing potent antioxidant potential may prevent protein modification, and hence may be helpful in preventing the insolubilization of proteins. Reduced levels of GSH and SOD as well as increased lipid peroxidation were observed in diabetic lens in the present study. Chronic treatment with FFTp decreased the lipid peroxidation and restored the levels of GSH and antioxidant enzymes like SOD. This might be due to the potent antioxidant activity of the rich flavonoid content of the extract. Moreover, FFTp has shown significant free radical scavenging activity by producing reduction in DPPH radical in vitro. Reduction of glucose by aldose reductase is a major cause of diabetic cataract which involves both osmotic stress as well as oxidative stress. Quercetin has been reported to have potent aldose reductase inhibiting activity which may be one of the mechanisms of FFTp in preventing the development of cataract.
Conclusion
From the above mentioned data and discussions, it can be concluded that FFTp possess antidiabetic activity and prevented diabetes induced complications like dyslipidemia, cardiac dysfunction, nephropathy and development of cataract in rats. The possible mechanisms involved in antidiabetic, cardioprotection activity as well as prevention of cataract development appears to be antioxidant activity of the Tephrosia purpurea due to the presence of flavonoids such as rutin and quercetin. Further studies are required to prove its efficacy and the mechanism of action.

References


