1. ABSTRACT

Diabetes mellitus is a metabolic disorder of multiple etiology characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. Hyperglycemia is involved in the etiology of development of diabetic complications. Diabetic vascular complications cause acquired blindness due to retinopathy, end-stage renal failure, neuropathy and atherosclerosis in diabetic patients. Independent of vascular complications, diabetes also produces other complications such as cardiomyopathy and heart failure. Cataract is considered to be a major cause of visual impairment in diabetic patients and it can also occur independent of vascular complications.

Though development of modern medicine resulted in the advent of modern pharmacotherapeutics including insulin, biguanides, sulfonylureas and thiazolidinediones, there is still a need to look for newer drugs as no drug (except strict glycemic control with insulin) has been shown to modify the course of all the diabetic complications. Traditional plant medicines or herbal formulations might offer a natural key to unlock diabetic complications.

Tephrosia purpurea (Linn.) pers, popularly known as “Sarapunkha” (Family: Fabaceae, Leguminosae) has been reported to be useful as digestive, anthelmintics, alexiteric, antipyretic, astringent, thermogenic, acrid and also used to cure diseases of liver, spleen, heart, blood, tumours, ulcers, leprosy and asthma. Different parts of the plant have been extensively studied for various pharmacological actions. The extracts of seeds, roots and leaves has shown significant in vivo hypoglycaemic activity in diabetic animals. However, effect of the plant on the long term diabetic complications has not been studied so far. Phytochemical investigations of T. purpurea have revealed the presence of glycosides, rotenoids, isoflavones, flavanones, chalcones, flavanols, flavones and sterols. Rutin is a flavonol type of flavonoid present in about 4.65% in the
leaves of *T.purpurea*. The objective of this study was to investigate the effect of the various extracts of the *T.purpurea* on streptozotocin induced type 1 diabetes and its related complications such as cardiovascular complications and cataract. We also made an attempt to find out the mechanism of action in diabetic complications including formation of cataract.

Before undertaking pharmacological work, pharmacognostic and phytochemical standardization of *T. purpurea* was carried out. The aqueous extract was prepared by suspending 50 g of coarse air-dried powder in 250 ml of water for 2 h and heating at 60-65°C for 30 min. This aqueous extract was gave a yield of 14.51%w/w. Alcoholic extract was prepared by extracting 500 g of coarse air-dried powder exhaustively in a round bottom flask with alcohol for 48 hours. The flavonoid fraction was prepared by partitioning the alcoholic extract with ethyl acetate followed by dissolving the residue in alcohol and treating it with neutral lead acetate solution. The precipitates obtained were resuspended in alcohol and treated with hydrogen sulphide and filtered. The yield of alcoholic extract and flavonoid fraction was found to be 9.28%w/w and 3.07%w/w respectively. All the extracts were subjected to preliminary phytochemical analysis for the detection of various class of compounds like alkaloids, triterpenoids, anthraquinones, flavanoids, phenols, tannins and coumarins using specific reagents. We also estimated the total phenolic and flavonoid content of all the extracts. The phenolic content was found to be greater in alcoholic extract (22.67%w/w) as compared to aqueous extract (10.83%w/w). The flavonoid fraction had greater amount of flavnoids (26.94%w/w) as compared to other extracts. We also estimated rutin and quercetin qualitatively as well as quantitatively in all the extracts. Rutin was found to be 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.

The cardioprotective effects of *T.purpurea* were studied in streptozotocin (STZ)- diabetic rats. Healthy Sprague Dawley rats of either sex weighing 250-300
gm were made diabetic by single intravenous tail vein injection of STZ 45mg/kg dissolved in 0.1mol/lit citrate buffer. Control rats were injected with 0.1mol/lit citrate buffer alone. Animals showing glycosuria (>2%) were considered as diabetic. Animals were divided into four major groups namely control, control treated, diabetic control and diabetic treated (n = 6 in each group). The effects of aqueous, alcoholic extract and flavonoid fractions of *T.purpurea* was studied. Control treated and diabetic treated group were administered orally (p.o) aqueous extract in the dose of 300 and 500 mg/kg/day, alcoholic extract in the dose of 300 and 500 mg/kg/day, and flavonoid fraction in the dose of 40 mg/kg/day for eight weeks with food and water ad libitum.

STZ produced cardinal signs of diabetes-mellitus such as significant loss of body weight, polyuria and polydypsia in type 1 diabetic rats. Chronic treatment with aqueous extract did not prevent the loss of body weight, polyuria and polydipsia in STZ-diabetic rats. Chronic treatment with alcoholic extract did not prevent the loss of body weight but was able to slightly improve polyuria and polydipsia in STZ-diabetic rats. However, flavonoid fraction was able to slightly improve the reduction in body weight and significantly prevented polyuria and polydipsia in STZ diabetic rats.

Type 1 STZ-diabetic rats were found to exhibit significant hyperglycemia and hypoinsulinemia as compared to control rats. Treatment with aqueous, alcoholic extract and flavonoid fraction 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 as compared to other fractions. Moreover, the glycated haemoglobin levels were found to be increased in diabetic rats. Chronic treatment with the flavonoidal fraction of *T.purpurea* could effectively reduce the levels of glycated hemoglobin.

Type 1 STZ-diabetic rats exhibited significantly higher cholesterol, LDL-cholesterol, VLDL-cholesterol and triglycerides levels as compared to those of control rats. Treatment with all the three extracts was found to be equi-effective.
in decreasing these lipid levels. Besides this, there was a significantly lowered HDL-cholesterol levels in diabetic rats and the extracts were able to significantly increase serum HDL cholesterol in rats with STZ-induced diabetes.

Type 1 STZ-diabetic rats exhibited significantly higher serum creatinine and urea levels as compared to those of control rats. 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.

Increased serum CK (creatine kinase) and LDH (lactate dehydrogenase) levels in diabetic rats indicate cardiac muscular damage. In the present study, type 1 diabetic rats exhibited significantly higher serum LDH and CK levels as compared to those of 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 and flavonoidal fraction at both the doses significantly lowered the LDH as well as CK levels. The decrease in LDH and CK levels further substantiates the beneficial effects of the plant in reducing the cardiovascular complications in diabetes mellitus.

The STZ-diabetic rats showed significantly reduced heart rate. Chronic treatment with all the extracts of *T. purpurea* was able to prevent STZ-induced bradycardia in the diabetic animals but the effect was more with flavonoid fraction. Moreover, in present study, change in haemodynamics i.e. hypertension and decline in rate of pressure development and decay were found to be improved by the treatment with alcoholic extract and flavonoid fraction. However, the effect of flavonoid fraction was greater as compared to alcoholic extract. Treatment with the aqueous extract did not improve the hemodynamic parameters.

Cardiac hypertrophy was also observed in the diabetic rats evident from the increased heart weight to femur length ratio as well as left ventricular (LV)
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hypertrophy characterized by increased LV weight to heart weight ratio, LV weight to RV weight ratio and LV wall thickness. The treatment with the aqueous, alcoholic as well as flavonoid fraction significantly decreased these hypertrophic parameters. STZ diabetic rats expressed significantly high amount of collagen deposition in left ventricle. Chronic treatment with alcoholic extract and flavonoid fraction significantly reduced this collagen content. Histopathological findings further substantiated the results. There was increased cardiac hypertrophy and decreased extracellular space with high ECM accumulation in diabetic rat heart. Extracellular space was found to be increased in hearts obtained from T. purpurea treated rats as compared to diabetic rats, which indicates regression in ECM accumulation. These results further support the contention that T. purpurea is beneficial in STZ-induced cardiovascular dysfunction.

Oxidative stress stimulates cardiac damage, endothelial dysfunction, apoptosis, and collagen synthesis in the heart and contributes to the pathogenesis of myocardial remodeling and failure. Left ventricular tissue of STZ-diabetic rats showed significant increase in left ventricular MDA levels and a significant decrease in SOD and glutathione levels. 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. This anti-oxidant activity of T. purpurea may be responsible for the beneficial effects observed on the cardiovascular complications in STZ-diabetic rats.

Oxidative stress may cause direct modification of the inner lens proteins, such as cross-linking, aggregation, and precipitation. In the present study, the increased TBARS (MDA levels) along with the decreased GSH and altered activities of antioxidant enzymes like SOD in the lens suggest increased oxidative stress in diabetic conditions. Chronic treatment with aqueous, alcoholic extract and flavonoid fraction of T. purpurea decreased the lipid peroxidation as well as was effective in restoring the levels of GSH and antioxidant enzymes like SOD in the lenticular tissue.

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High levels of aldose reductase (AR) activity is found to be present in rat lens. Reduction of glucose by AR is a major cause of diabetic cataract which involves both osmotic stress as well as oxidative stress. 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 showed significant AR inhibiting activity which may propose the probable mechanism of the plant in delaying the development of diabetic complications like cataract.

Selenite-overdose cataract is an extremely rapid and convenient model of nuclear cataracts. The cataract was produced in nine days old suckling rat pups by a single subcutaneous injection of 4 mg/kg body weight of sodium selenite. The sodium selenite treated pups were divided into four groups as control and treated orally with quercetin (1 mg/kg/day), alcoholic extract (300 mg/kg/day) and flavonoid fraction (40 mg/kg/day). The cataract was categorized into various stages upon examination with an ophthalmoscope. Treatment with standard quercetin, alcoholic extract and flavonoid fraction of *T. purpurea* delayed the progression of nuclear cataract which was observed morphologically.

Selenite administration was found to decrease the levels of GSH and activity of SOD in the lens. Restoration of levels of GSH and SOD activity of the in quercetin, alcoholic extract and flavonoid fraction treated group could be attributed to the antioxidant effect of the plant. Higher levels of MDA in selenite induced group were lowered by alcoholic as well as flavonoid fraction treatment. Also 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. 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. 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.

Selenite cataract shows significant decrease in Ca\(^{2+}\)ATPase activity and increase in lenticular calcium levels. 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. 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.

In conclusion, our data suggest that

- *T. purpurea* possess anti-diabetic activity and prevents diabetes induced complications like dyslipidemia, cardiac dysfunction, nephropathy and development of cataract in rats. It was also effective in prevention of selenite induced cataract.

- The possible mechanisms involved in anti-diabetic and cardioprotection activity or delaying the development of cataract appears to be anti-oxidant activity. Aldose reductase inhibition may be additional mechanism involved in the prevention of selenite induced cataract.

- The anti-oxidant potential of flavonoids rutin and quercetin present in the *T. purpurea* appear to be responsible for various effects including prevention of diabetes-induced complications studied.

- 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
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most of the parameters except lipid dysfunction, cardiac hypertrophy and cataract formation.

- 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.