Antihyperglycemic and antihyperlipidemic effects of *Tephrosia purpurea* leaf extract in streptozotocin induced diabetic rats

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Abstract: *Diabetes mellitus is a worldwide leading metabolic syndrome, associated with profound alterations in carbohydrate, lipids, lipoproteins and protein metabolisms. Worldwide, traditional practitioners for the treatment of diabetes and its complications use a wide variety of medicinal plants. In the present study, the aqueous extract of Tephrosia purpurea leaves (TpALet) was evaluated for its antihyperglycemic and antihyperlipidemic effects in streptozotocin induced diabetic rats. Profound alterations in the concentrations of blood glucose, lipids and lipoproteins were observed in diabetic rats. Oral administration of TpALet to diabetic rats at a dose of 600mg/kg body weight significantly reduced the level of blood glucose and increased the level of plasma insulin as well as normalized the lipids and lipoproteins profile. The present study thus demonstrated that TpALet has prominent antihyperglycemic and antihyperlipidemic effects in streptozotocin induced diabetic rats.*

Key words: *Diabetes mellitus, Tephrosia purpurea, Streptozotocin, Lipids*
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Introduction

Diabetes mellitus affects more than 10% of the population and is the fifth most common cause of death worldwide. Currently more than 170 million people worldwide are affected by this metabolic syndrome and this figure is expected to be double by the year 2025 (Boyle *et al*., 2001). Approximately 1.8 million new diabetic cases are diagnosed in people with the age of 20 and older in USA (Barcelo and Rajpathak, 2001). India is one of the leading countries for the number of people with diabetes mellitus and it is assumed that diabetes will affect more than 57 million people by the year 2025 in India (Aravind *et al*., 2002).

Diabetes mellitus is often linked with abnormal lipid metabolism and dyslipidemia and hyperlipidemia are recognized complications of diabetes mellitus characterized by increased levels of cholesterol, triglycerides and phospholipids and alterations in lipoprotein composition (Umesh *et al.,* 2004 ; Sophia and Manoharan, 2007). Reduced insulin secretion and defect in insulin function results in enhanced metabolism of lipids from adipose tissue to the plasma. Profound evidences link obesity to the development of diabetes and its complications (EL-Hazmi and Warsy*,* 1999; Frayn, 2002). It has been reported that abdominal obesity, impaired postprandial lipid metabolism and insulin resistance are all interrelated risk markers for coronary heart diseases (Frayn, 2002). Impairment in insulin sensitivity due to high concentration of lipids in the cells is responsible for the elevated cardiovascular risk in diabetes mellitus (Krishna Kumar *et al.,* 2000; Mironava *et al.,* 2000). Membrane fluidity is known to be dependent on the molar ratio of cholesterol to phospholipids (Kolanjiappan *et al.,* 2002). The liver participates in oxidation and metabolic conversion of free fatty acids, synthesis of cholesterol and phospholipids and in the secretion of specific classes of plasma lipoproteins. Erythrocyte membranes and liver cells showed marked alterations in the concentration of lipids during diabetes (Bhandaru *et al*., 1982; Pari and Latha, 2002).

Several medicinal plants are still recommended by traditional practitioners of Siddha and Ayurvedic medicine for the treatment of diabetes mellitus. Indian traditional practitioners treat various types of diseases using *Tephrosia purpurea* as folklore medicine. It is a perennial herb found throughout India and considered beneficial for the remedy of various disorders such as inflammation, fever, bronchitis, kidney disorders and diabetes mellitus (Joshi, 2000; Kritikar and Basu, 1956). A very few scientific studies have demonstrated its hepatoprotective and antiulcer effects (Despande and Shah, 2003; Ramamurthy and Srinivasan, 1993). However no scientific studies have documented the antihyperglycemic and antihyperlipidemic effects of "TpALet" in streptozotocin induced diabetic rats. Thus, the present study was designed to evaluate the antihyperglycemic and antihyprelipidemic effects of "TpALet" in streptozotocin induced diabetic rats.

Materials and Methods

Drugs and chemicals: Streptozotocin was purchased from Sigma Aldrich Chemicals Pvt. Ltd., Bangalore. All other chemicals and reagents used were of analytical grade.

Plant material: Leaves of *Tephrosia purpurea* were collected during the periods of August and September 2005 in and around Chidambaram, Tamilnadu and it was botanically authenticated by Dr.S.Paneerselvam, Professor and Head, Department of Botany, Annamalai University. A voucher specimen (AU05102) was deposited in the Department of Botany, Annamalai University, Annamalainagar, Tamilnadu.

Preparation of plant extract: 100 g of fresh leaves of *T.purpurea* were dried in shade, powdered and then suspended in 250 ml of water for 2 hr and then heated at 60-65°C for 30 min. The extract was preserved and the process was repeated for three times with the residual powder, each time collecting the extract. The collected extract was pooled and passed through the fine cotton cloth. The filtrate upon evaporation at 40°C yielded 14% semisolid extract. This was stored at 0-4°C until used.

Animals: Albino Wistar male rats 7 to 8 weeks old and weighing 150-200 g was used for the present study. The animals were obtained from central animal house, Rajah Muthiah Institute of Health Sciences, Annamalai University, India and were maintained under controlled environmental conditions of temperature 22±2°C and relative humidity 55±5% with 12 hr light and 12 hr dark cycles in the central animal house. The animals were randomized into control and experimental groups and housed 4 or 5 in polypropylene cages. Standard pellets obtained from Mysore Snack Feed Ltd., Mysore, India, were used as a basal diet during the experiment. The control and experimental animals were provided food and drinking water *ad libitum*.

Induction of diabetes mellitus: Diabetes mellitus was induced by single intraperitoneal injection of streptozotocin (50 mg/kg body weight) dissolved in 0.1 M-citrate buffer (pH 4.5) to overnight fasted Albino Wistar rats (Chang, 2000). The diabetes was assessed in streptozotocin-induced rats by determining the blood glucose concentration, 48 hr after injection of streptozotocin. The rats with blood glucose level above 250 mg/dl were selected for the experimental studies.

Study design: In the experiment, 30 rats (18 diabetic rats, 6 normal rats, 6 normal rats treated with TpALet alone) were used. The rats were divided into 5 groups of six each.

Group I : Served as untreated control rats

Group II : Served as diabetic control (50 mg/kg body weight i.p. streptozotocin)

Group III : Diabetic rats receiving TpALet (600 mg/kg body weight) daily for 45 days orally by intragastric tube

Group IV : Control rats receiving TpALet (600 mg/kg body weight) alone daily for 45 days orally by intragastric tube

Group V : Diabetic rats receiving glibenclamide (600 µg/kg body weight) daily for 45 days orally by intragastric tube

After the experimental period, all animals were sacrificed by cervical dislocation and biochemical studies were conducted on blood, plasma, erythrocyte membranes and liver of control and experimental animals in each group.

Biochemical analysis: Blood glucose and plasma insulin were estimated by the methods of Sasaki *et al.* (1972) and ELISA method (Enzyme Linked Immunosorbant Assay) using Boehringer

Mannheim kit (Anderson *et al.*, 1993) respectively. Total cholesterol and phospholipids were assayed according to the methods of Parekh and Jung (1970) and Zilversmit and Davis (1950) respectively. Free fatty acids and triglycerides were estimated by the methods of Falholf *et al.* (1973) and Foster and Dunn (1973) respectively. HDL cholesterol was estimated by the method of Gidez and Webb (1950). LDL cholesterol was calculated using the formula,

$$\text{LDL cholesterol} = \text{Total cholesterol} - \text{HDL} + \left(\frac{TG}{5}\right) \text{ and VLDL}$$

cholesterol was calculated using the formula $\left(\dfrac{TG}{5}\right)$.

Lecithin cholesterol acyl transferase (LCAT) activity was assayed by the method of Hitz *et al.* (1983). Lipoprotein lipase (LPL) activity was assayed by the method of Korn (1955).

Statistical analysis: The data are expressed as mean ± SD. Statistical comparisons were performed by one way analysis of variance (ANOVA) followed by Duncan's multiple comparisons test (DMRT). The results were considered statistically significant if the p values were 0.05 or less.

Results and Discussion

Table 1 shows the blood glucose and plasma insulin levels of control and experimental animals in each group. The level of blood glucose was significantly increased whereas the level of plasma insulin was significantly decreased in streptozotocin induced diabetic animals as compared to control animals. However, the above said parameters were significantly normalized in diabetic rats treated with "TpALet" and glibenclamide. "TpALet" showed antihyperglycemic effect in a manner similar to that of glibenclamide in streptozotocin induced diabetic rats.

Table 2 presents plasma lipids and lipoproteins pattern (total cholesterol, triglycerides, phospholipids, free fatty acids, HDL-cholesterol, LDL-cholesterol and VLDL-cholesterol) in control and experimental animals in each group. All the lipid parameters except HDL-cholesterol were significantly increased in streptozotocin

Table - 1: Blood glucose and plasma insulin levels of control and experimental animals in each group

	Blood glucose (mg/dl)	Plasma insulin (µU/ml)
Group I - Control	92.6±5.74[a]	16.1±0.81[a]
Group II - Diabetic control	285.3±12.8[b]	10.6±0.87[b]
Group III - Diabetic+TpALet (600 mg/kg body weight)	128.4±6.78[c]	14.1±1.03[c]
Group IV - Control+TpALet (600 mg/kg body weight)	87.5±4.72[a]	16.6±0.98[a]
Group V - Diabetic+ glibenclamide (600 µg/kg body weight)	112.6±9.2[c]	14.7±1.08[c]

Values are expressed as mean ± SD (n=6), Values not sharing a common superscript letter differ significantly at p<0.05 (DMRT)

Table - 2: Plasma lipids and lipoprotein patterns in control and experimental animals in each group

		Total cholesterol (mg/dl)	Phospholipids (mg/dl)	Triglycerides (mg/dl)	Free fatty acids (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)
Group I-	Control	80.16±6.5[a]	93.2±6.99[a]	75.5±6.04[a]	9.15±0.57[a]	35.33±1.47[a]	59.8±6.14[a]	15.2±1.2[a]
Group II-	Diabetic control	150.83±8.6[b]	146.3±9.83[b]	141.6±8.16[b]	16.8±0.86[b]	21.64±0.73[b]	157.5±8.86[b]	28.3±1.6[b]
Group III-	Diabetic+TpALet (600 mg/kg body weight)	120.9±7.3[c]	116.6±8.16[c]	114.2±9.72[c]	10.6±1.08[c]	28.36±1.08[c]	115.41±7.94[c]	22.8±1.9[c]
Group IV-	Control+TpALet (600 mg/kg body weight)	78.7±5.3[a]	91.3±7.75[a]	73.25±7.84[a]	9.08±0.61[a]	36.08±1.35[a]	56.31±6.6[a]	14.6±1.5[a]
Group V-	Diabetic + glibenclamide (600 µg/kg body weight)	114.6±7.5[c]	107.5±9.35[c]	108.5±9.35[c]	9.96±0.84[c]	29.1.09±0.94[c]	108.1±6.4[c]	21.5±1.8[c]

Values are expressed as mean ± SD (n=6), Values not sharing a common superscript letter differ significantly at p<0.05 (DMRT)

Table - 3: Activities of lecithin cholesterol acyltransferase (LCAT) and lipoprotein lipase (LPL) in plasma of control and experimental animals in each group

	LCAT (U[A]/l)	LPL (U[B]/l)
Group I - Control	60.25±4.05[a]	6.52±1.06[a]
Group II - Diabetic control	39.82±2.9[b]	3.63±0.77[b]
Group III - Diabetic+TpALet (600 mg/kg body weight)	48.23±3.8[c]	4.92±1.08[c]
Group IV - Control+TpALet (600 mg/kg body weight)	61.26±4.2[a]	6.86±1.36[a]
Group V - Diabetic+ glibenclamide (600 µg/kg body weight)	50.3±3.52[c]	5.02±0.92[c]

Values are given as mean ± SD (n= 6 rats), Values not sharing a common superscript letter differ significantly at p<0.05 (DMRT)
A= µmol cholesterol liberated /hr, B = µmol glycerol liberated /hr

Table - 4: Levels of cholesterol and phospholipids in erythrocyte membranes of control and experimental animals in each group

	Total cholesterol (µg/mg protein)	Phospholipids (µg/mg protein)
Group I - Control	126.3±4.6[a]	239.5±11.5[a]
Group II - Diabetic control	142.6±7.2[b]	258.0±12.2[b]
Group III - Diabetic+TpALet (600 mg/kg body weight)	129.7±6.5[a]	242.3±9.6[a]
Group IV - Control+TpALet (600 mg/kg body weight)	122.6±5.2[a]	235.2±9.8[a]
Group V - Diabetic+ glibenclamide (600 µg/kg body weight)	128.4±8.1[a]	241.6 ±8.5[a]

Values are given as mean ± SD (n= 6 rats), Values not sharing a common superscript letter differ significantly at p<0.05 (DMRT)

induced diabetic animals as compared to control animals. However, oral administration of TpALet to diabetic animals brought back all the values to near normal range.

Table 3 shows the activities of lipoprotein lipase (LPL) and lecithin cholesterol acyl transferase (LCAT) in plasma of control and experimental animals in each group. The activities of LPL and LCAT were significantly decreased in diabetic animals as compared to control animals. However, oral administration of TpALet to diabetic animals significantly improved the activities of LPL and LCAT.

Table 4 shows the levels of total cholesterol and phospholipids in erythrocyte membranes of control and experimental animals in each group. The levels of total cholesterol and phospholipids were moderately increased in diabetic animals as compared to control animals. However, the levels of total cholesterol and phospholipids were returned to near normal concentrations in diabetic rats treated with TpALet.

Table 5 depicts the lipid pattern in liver of control and experimental animals in each group. All the lipid parameters were significantly increased in diabetic animals as compared to control animals. The lipid pattern was brought back to near normal range in diabetic animals treated with TpALet.

No statistical significance was observed between control and rats treated with TpALet alone for the above said biochemical parameters.

Diabetes mellitus is mainly manifested by hyperglycemia and hyperlipidemia, which contribute directly to atherosclerosis at later stages (Garber, 2000). In the present study, we observed an increase in blood glucose concentration accompanied by reduction in plasma insulin and altered lipid and lipoprotein patterns in plasma, erythrocyte membranes and liver in streptozotocin induced diabetic rats. In recent years, considerable interest has been directed towards the investigation of plasma lipids (total cholesterol, triglycerides,

Table -5: Lipid profile in liver of control and experimental animals in each group

	Total cholesterol (mg/g tissue)	Triglycerides (mg/g tissue)	Phospholipids (mg/g tissue)	Free fatty acids (mg/g tissue)
Group I - Control	4.05±0.37[a]	3.89±0.25[a]	20.6±1.7[a]	7.58±1.24[a]
Group II - Diabetic control	8.46±0.59[b]	6.54±0.47[b]	42.1±2.89[b]	14.5±2.13[b]
Group III - Diabetic+TpALet (600 mg/kg body weight)	6.16±0.35[c]	4.96±0.20[c]	36.3±1.63[c]	11.4±1.31[c]
Group IV - Control+TpALet (600 mg/kg body weight)	3.98±0.31[a]	3.86±0.29[a]	19.2±1.78[a]	7.25±0.93[a]
Group V - Diabetic+ glibenclamide (600 µg/kg body weight)	6.02±0.44[c]	4.61±0.27[c]	32.83±1.42[c]	10.95±1.39[c]

Values are given as mean ± SD (n= 6 rats), Values not sharing a common superscript letter differ significantly at p<0.05

phospholipids) in diabetes mellitus due to the fact that abnormal lipid levels lead to the development of coronary artery disease in diabetic patients. Cholesterol and phospholipids constitute among two third of the total plasma lipids whereas free fatty acids (FFA) are metabolically more active. Increase in plasma and tissue cholesterol and phospholipids have been reported in diabetic rats (Pari and Latha, 2002). Several studies have demonstrated overeating may be an important factor for hypercholesterolemia in diabetic humans (Abrams *et al.,* 1982; Bennion and Grundy, 1977). Increased intestinal sterologenesis is a contributory factor to hypercholesterolemia seen in diabetes mellitus (Abrams *et al.,* 1982).

Diabetes mellitus is associated with an increased risk of developing premature atherosclerosis due to increase in plasma triglycerides and LDL levels and decrease in HDL levels. High concentration of fatty acids in plasma promotes the liver in the conversion of some fatty acids into phospholipids and cholesterol. Claudis *et al.* (2006) have shown three fold increment in lipoprotein in alloxan induced diabetic rats. Lowered HDL and elevated VLDL and LDL have been well documented in diabetes mellitus (Ruzaidi *et al.*, 2005). Our results corroborate these observations.

Alterations in the erythrocyte membranes lipid composition may be a reflection of alterations in the plasma lipid profile (Izabela *et al.,* 2006). Cholesterol enrichment in red cells resulting in loss of membrane fluidity has been reported (Cooper, 1977). Bhandaru *et al.* (1982), have suggested that rat erythrocyte membrane composition (total cholesterol, phospholipids) is altered both in hyperglycemic and hyperlipidemic conditions and may provide a useful model for evaluating lipid carbohydrate abnormalities of membrane structures in diabetes mellitus.

Liver has an important role in glucose metabolism and as a consequence of increased glucose and insulin deficiency in plasma, hepatic regulation of lipid metabolism is greatly altered. The liver is the major organ that can catabolize and excrete quantitatively important amounts of cholesterol. Insulin administration to diabetic rats normalized the lipid and lipoprotein patterns in diabetic rats (Pathak *et al.*, 1981). High concentration of cholesterol in plasma and liver in diabetes are mainly due to defect in insulin secretion and function. Accumulation of triglycerides in diabetic liver is due to increased synthesis or decreased output from liver as VLDL or combination of both (Mutlu *et al.*, 2003).

Insulin has a profound role in the regulation of key enzymes involved in the lipid and lipoprotein metabolism. Altered activities of lipid metabolizing enzymes may cause increase in the mobilization of free fatty acids from peripheral depots. Lowered activities of plasma lecithin cholesterol acyl transferase (LCAT) and lipoprotein lipase (LPL) have been reported in diabetes (Das, 2004). Our results lend credibility to these observations. In the present study, oral administration of TpALet at a dose of 600 mg/kg body weight to diabetic rats restored the levels of lipids, lipoproteins and LCAT and LPL activities in diabetic rats. The antihyperlipidemic effect of TpALet suggests that the extract stimulated the activities of lipid metabolizing enzymes LCAT and LPL.

In the present study, the plant extract has not only shown antihyperglycemic effect but also resulted in elevated plasma insulin concentration, probably by stimulating insulin secretion from remnant pancreatic β-cells, which might have corrected other metabolic alterations. The plant extract has also revealed antihyperlipidemic effect in a manner similar to that of the reference drug glibenclamide. We thus conclude that *Tephrosia purpurea* leaves can be used as an alternative herbal remedy for diabetes and lipids associated diabetic complications.

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