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Protective Role of *Tephrosia purpurea* Ethanolic Seed Extract on Glycoprotein Components in Streptozotocin Induced Diabetic Rats

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Abstract: The aim of the present study was to investigate the beneficial role of *Tephrosia purpurea* ethanolic seed extract on glycoprotein components in streptozotocin induced diabetic rats. Diabetes mellitus was induced in wistar rats by single intraperitoneal injection of streptozotocin (50 mg kg^{-1} b.wt.) dissolved in 0.1 M citrate buffer (pH 4.5) after overnight fasting for 12 h. Blood glucose and plasma insulin were measured and glycoprotein components (protein bound hexose, protein bound hexosamine, fucose and sialic acid) in plasma, erythrocyte membrane, liver and kidney were investigated in control and experimental animals in each group. Oral administration of TpESet at a dose of 300 mg kg^{-1} b.wt. to diabetic animals for 45 days revert back all the altered biochemical parameters in diabetic animals. The present study thus, indicates that TpESet has potent role in modifying altered glycoprotein components in streptozotocin induced diabetic rats.

Key words: Streptozotocin, diabetes mellitus, glycoprotein components

INTRODUCTION

Diabetes mellitus, a leading metabolic disorder worldwide, is characterized by hyperglycemia associated with impairment in insulin secretion and/or insulin action as well as alteration in intermediary metabolism of carbohydrate, protein and lipids. The number of peoples with type I and type II diabetes are dramatically increasing worldwide. Diabetes mellitus is one of the most prevalent metabolic disorders in both Western and developing countries. According to World Health Organization, 170 million peoples are currently affected by diabetes and this figure will expected to be double by the year 2025 (Boyle *et al*., 2001).

Glycoproteins are proteins that have carbohydrate moiety, attached covalently to their peptide backbone. They are found as enzymes, hormones and blood group substances and as constituents of extracellular membranes (Kumar *et al*., 2008). Glycoproteins exert multiple and complex functions in the cell surface which include cell-cell recognition, cellular adhesion and cell differentiation and as receptors for many hormones and viruses. Measurement of glycoproteins provides useful information about membrane structural integrity and function (Mittal *et al*., 1996).

Protein-bound hexose in the cell membrane provides hydrophobic nature, whereas protein-bound hexosamine provides cationic charges on the cell surface membrane and makes the membrane more polar (Gemayel *et al*., 2007; Maddux *et al*., 1995). Sialic acid is widely distributed in tissues of humans and in the circulation, it is chiefly present as the terminal sugar of oligosaccharide chains of glycoproteins. Plasma sialic acid is therefore used as a marker of NIDDM and/or diabetic micro-and macroangiopathy (Butun *et al*., 2007). Profound changes in the metabolism of glycoproteins and alterations in membrane glycosylation pattern have been well documented in diabetes mellitus (Prakasam *et al*., 2005; Pari and Ashokkumar, 2006). Altered glycoproteins have been implicated in the pathogenesis of liver and kidney diseases in diabetes mellitus (Yilmaz *et al*., 2007). Total sialic acid in the serum has received considerable attention as a possible marker for cardiovascular disease and mortality and has been claimed to be associated with cataract in diabetic subjects (Butun *et al*., 2007). The presence of sialic acid on the cell surface has been shown to be crucial for the survival of mammalian erythrocytes in circulation. Sialic acid varies greatly in a number of pathological conditions including diabetes (Crook *et al*., 2000). Administration of streptozotocin to animals induced altered glycoproteins synthesis and ultrastructural abnormalities in several tissues (Rossetti *et al*., 1993). Changes in the lysosomal enzymes involved in the degradation of glycoproteins may also play an important role in the pathophysiology of diabetes (Garry *et al*., 1980).

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Many medicinal plants are recommended by traditional practitioners of Siddha and Ayurvedic medicine for the treatment of various disorders including diabetes mellitus (Joshi, 2000). Prakasam *et al.* (2005) have reported the *Caseria esculenta* on glycoprotein metabolism in streptozotocin induced diabetic rats, in line this we have chosen *Tephrosia purpurea* since it is widely used as remedy for diabetes in rural areas and is reported to have multiple therapeutic properties such as inflammation, fever, bronchitis, kidney disorders and diabetes mellitus (Kritikar and Basu, 1956). Scientific studies have demonstrated its hepatoprotective and antiulcer effects (Ramamurthy and Srinivasan, 1993; Despande and Shah, 2003). The present communication describes the beneficial role of *T. purpurea* seed extract on glycoprotein components levels in streptozotocin induced diabetic rats.

MATERIALS AND METHODS

Chemicals: Streptozotocin was purchased from Sigma Aldrich Chemicals, Pvt., Ltd., Bangalore. All other chemicals and reagents used were of analytical grade. This study was conducted during the year of 2005.

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 h light and 12 h 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*.

Plant material: Seeds of *Tephrosia purpurea* were collected during the periods of August and September 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

Ethanol extract preparation: The ethanolic extract of *Tephrosia purpurea* seeds was prepared according to the method of Hossain *et al.* (1992). Five hundred gram of fresh seeds of *T. purpurea* were dried in shade, powdered and then soaked in 1500 mL of 95% ethanol overnight. After filtration, the residue obtained was again resuspended in equal volume of 95% ethanol for 48 h and filtered again. The above two filtrates were mixed and the solvents were evaporated in a rotavapour at 40-50°C under reduced pressure. An 11% semisolid light yellow material of *T. purpurea* seeds obtained was stored at 0-4°C until used. We have examined the ethanolic extract of *T. purpurea* seeds due to the fact that the ethanolic extract contains many bioactive constituents such as isoflavones, flavanones, flavanols, flavanoids and rutin (Soni *et al*., 2006).

Preparation of tissue homogenate: Tissue samples from animals were washed with ice cold saline and dried between folds of filter paper, weighed and homogenized using appropriate buffer of concerned parameter in an all glass homogenizer with Teflon pestle. The homogenate was centrifuged at 1000 g for 5 min and the supernatant was then used for the biochemical estimations.

Experimental protocol: The local institutional animal ethics committee, Annamalai university, Annamalai nagar India approved the experimental design.

In the experiment, 30 rats were randomized into 5 groups 6 animals in each. Group 1 served as untreated control rats. Group 2 served as diabetic control (50 mg kg^{-1} b.wt. i.p. streptozotocin). Diabetes mellitus was induced by single intraperitoneal injection of streptozotocin (50 mg kg^{-1} b.wt.) 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 h after injection of streptozotocin. The rats with blood glucose level above 250 mg dL^{-1} were selected for the experimental studies. Group 3 animals served as diabetic treated with TpESet (300 mg kg^{-1} b.wt.) for 45 days. Group 4 animals received oral administration of TpESet (300 mg kg^{-1} b.wt.) alone throughout the experimental period. Group 5 animals were diabetic and orally administered glibenclamide (600 μg kg^{-1} b.wt.) for 45 days. At the end of the experiment, all animals were sacrificed by cervical dislocation and biochemical studies were conducted on blood, plasma, erythrocyte membranes, liver and kidney 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. After plasma preparation erythrocyte

membrane was prepared by the method of Dodge *et al.* (1968) modified by Quist (1980). The defatted tissue obtained after treating liver and kidney with methanol and chloroform was used for the estimation of glycoprotein components. To this dry defatted tissue 0.1 N H_2SO_4 was added and hydrolyzed at 80°C for 1 h. It was cooled and aliquot was used for the estimation of total sialic acid. To the remaining solution 0.1 N sodium hydroxide was added and kept in an ice bath for 1 h from these aliquots protein bound hexose and fucose were estimated. Protein bound hexose, hexosamine, total sialic acid and fucose in plasma, erythrocyte membrane and tissues (liver and kidney) were estimated by the methods of Niebes (1972), Wagner (1979), Warren (1959) and Dische and Shettles (1948), respectively.

Statistical analysis: The data are expressed as means±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-value was less than 0.05.

RESULTS

The levels of blood glucose were significantly increased whereas the levels of plasma insulin were significantly decreased in streptozotocin induced diabetic animals as compared to control animals (Table 1). However, the above said parameters were significantly improved in diabetic rats treated with TpESet and glibenclamide. No statistical significance was observed between control animals and rats treated with ethanolic seed extract of *T. purpurea* alone.

Protein bound hexose, protein bound hexosamine, fucose and sialic acid contents were significantly increased in plasma whereas significantly decreased in erythrocyte membranes of diabetic animals as compared to control (Table 2, 3). The glycoprotein components levels were brought back to near normal range in diabetic animals treated with ethanolic seed extract of *T. purpurea* and glibenclamide. No statistical significance was observed between control animals and rats treated with ethanolic seed extract of *T. purpurea* alone.

Protein bound hexose, protein bound hexosamine and fucose contents were significantly increased whereas the sialic acid content was significantly decreased in liver and kidney of diabetic animals as compared to control (Table 4, 5). The glycoprotein components levels were brought back to near normal range in diabetic animals treated with "TpESet" and glibenclamide. No statistical significance was observed between control animals and rats treated with ethanolic seed extract of *T. purpurea* alone.

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

Parameters	Blood glucose ($mg\ dL^{-1}$)	Plasma insulin ($\mu U\ mL^{-1}$)
Control	78.5±6.80^a	14.18±1.10^a
Diabetic control	283.2±14.2^b	9.60±0.93^b
Diabetic+TpESet (300 mg kg^{-1} b.wt.)	105.6± 8.5^c	12.42±1.19^c
Control+TpESet (300 mg kg^{-1} b.wt.)	76.5±5.96^a	14.26±1.18^a
Diabetic+Glibenclamide (600 µg kg^{-1} b.wt.)	115.3±10.5^c	12.10±0.89^c

Values are expressed as mean±SD (n = 6), Values not sharing a common superscript letter(s) different significantly at p<0.05 (DMRT), TpESet: *Tephrosia purpurea* ethanolic seed extract

Table 2: Glycoprotein components in plasma of control and experimental animals in each group

Parameters	Protein bound hexose	Fucose	Protein bound hexosamine	Sialic acid
	($mg\ dL^{-1}$)			
Control	87.80±6.1^a	22.6±1.79^a	59.2±3.93^a	42.5±3.59^a
Diabetic control	135.2±15.3^b	42.5±5.80^b	92.2±10.0^b	65.2±7.30^b
Diabetic+TpESet (300 mg kg^{-1} b.wt.)	96.5±9.92^{ac}	29.6±2.75^{ac}	66.5±5.30^c	49.2±6.20^c
Control+TpESet (300 mg kg^{-1} b.wt.)	84.8±5.3^a	20.6±1.64^a	56.3±4.10^a	41.5±4.30^a
Diabetic+ Glibenclamide (600 µg kg^{-1} b.wt.)	101.2±8.7^c	31.4±2.52^c	71.2±6.26^c	52.2±5.90^c

Values are given as mean±SD (n = 6 rats), Values not sharing a common superscript letter(s) different significantly at p<0.05, TpESet: *Tephrosia purpurea* ethanolic seed extract

Table 3: Glycoproteins components in erythrocyte membranes of control and experimental animals in each group

Parameters	Protein bound hexose	Fucose	Protein bound hexosamine	Sialic acid
	($\mu g\ mg^{-1}$ protein)			
Control	114.50±9.91^a	29.20±3.3^a	94.2±8.12^a	37.61±3.1^a
Diabetic control	81.60±10.3^b	15.20±2.8^b	47.2±5.56^b	23.02±2.6^b
Diabetic+TpESet (300 mg kg^{-1} b.wt.)	109.40±8.50^{ac}	24.30±1.93^c	68.4±6.52^c	32.60±3.1^c
Control+TpESet (300 mg kg^{-1} b.wt.)	116.40±8.86^a	30.08±2.8^a	96.5±7.83^a	38.50±2.8^a
Diabetic+ Glibenclamide (600 µg kg^{-1} b.wt.)	102.50±6.95^c	22.40±1.84^c	64.2±5.90^c	29.80±2.5^c

Values are given as mean±SD (n = 6 rats), Values not sharing a common superscript letter(s) different significantly at p<0.05, TpESet: *Tephrosia purpurea* ethanolic seed extract

Table 4: Glycoproteins components in liver of control and experimental animals in each group

Parameters	Protein bound hexose	Fucose	Protein bound hexosamine	Sialic acid
	($mg\ g^{-1}$ defatted tissue)			
Control	25.0±2.1^a	17.2±1.51^a	9.08±0.74^a	7.02±0.64^a
Diabetic control	46.3±5.3^b	33.2±4.70^b	16.10±2.25^b	3.84±0.49^b
Diabetic+TpESet (300 mg kg^{-1} b.wt.)	29.2±3.6^{ac}	20.6±3.64^{ac}	10.60±1.85^{ac}	6.28±0.31^c
Control+TpESet (300 mg kg^{-1} b.wt.)	24.2±2.3^a	16.8±1.32^a	9.02±0.53^a	7.17±0.56^a
Diabetic+ Glibenclamide (600 µg kg^{-1} b.wt.)	31.2±4.1^c	23.0±2.94^c	11.80±1.73^c	6.14±0.34^c

Values are given as mean±SD (n = 6 rats), Values not sharing a common superscript letter(s) different significantly at p<0.05, TpESet: *Tephrosia purpurea* ethanolic seed extract

Table 5: Glycoproteins components in kidney of control and experimental animals in each group

Parameters	Protein bound hexose	Fucose	Protein bound hexosamine	Sialic acid
	(mg g^{-1} defatted tissue)			
Control	20.80 ± 1.63^{a}	12.90 ± 1.11^{a}	12.2 ± 0.95^{a}	6.80 ± 0.48^{a}
Diabetic control	40.08 ± 4.54^{b}	27.50 ± 3.87^{b}	29.2 ± 3.20^{b}	3.75 ± 0.39^{b}
Diabetic+TpESet (300 mg kg^{-1} b.wt.)	25.20 ± 2.83^{ac}	15.02 ± 2.54^{ac}	17.8 ± 2.60^{c}	6.10 ± 0.47^{ac}
Control+TpESet (300 mg kg^{-1} b.wt.)	19.80 ± 1.36^{a}	12.20 ± 1.07^{a}	11.9 ± 0.84^{a}	6.98 ± 0.42^{a}
Diabetic+ Glibenclamide (600 μg kg^{-1} b.wt.)	28.50 ± 2.79^{c}	20.30 ± 2.36^{c}	20.6 ± 2.40^{c}	5.95 ± 0.31^{c}

Values are given as mean±SD (n = 6 rats), Values not sharing a common superscript letter(s) different significantly at $p<0.05$, TpESet: *Tephrosia purpurea* ethanolic seed extract

DISCUSSION

Hexose, hexosamine, sialic acid and fucose contents are the basic components of the glycosaminoglycans and glycoproteins. It has been reported that hyperglycemia leads to increased synthesis of glycoproteins and glycosylated proteins due to the fact that excess glucose is redirected to insulin dependent pathway such as synthesis of glucosamine from glucose (Pari and Ashokkumar, 2006). The liver is primarily responsible for producing large amounts of glycoproteins present in blood. Guillot *et al*. (1998) have suggested that elevated levels of plasma glycoproteins in diabetic patients could be a consequence of abnormal carbohydrate metabolism. Many researchers have reported elevated levels of plasma protein bound carbohydrate compounds in diabetes (Sailaja *et al*., 2004; Rogers *et al*., 1992). An increase in plasma glycoprotein components has been reported to be related to the duration, severity and existence of degenerative vascular disease.

Decrease in erythrocyte membrane protein-bound hexose, hexosamine and sialic acid can be due to defective enzymatic glycation of protein and/or enhanced removal of carbohydrates moieties from the proteins under hyperglycemic conditions. The decrease in the hexosamine content in erythrocyte membranes under diabetic conditions indicates that a lesser amount of hexosamine is available for the synthesis of sialic acid. Desialylation alters the structure and function of glycoproteins in the membrane. Increased sialic acid concentration in plasma due to enhanced desialylation has also been reported in diabetes mellitus (Nayak and Bhaktha, 2005). The cleavage of sialic acid from membrane glycoproteins might be the cause of such high plasma sialic acid levels. Decreased sialic acid content in glycophorin A has been observed in erythrocyte membranes obtained from diabetic patients (Rogers *et al*., 1992). It is well established that serum TSA is elevated in patients with type-2 diabetes mellitus (NIDDM) as compared to non-diabetics (Faur *et al*., 2005). It has also been suggested that elevated plasma triglyceride levels have been supposed to be involved in the elevation of plasma sialic acid (Nayak and Bhaktha, 2005). Present results are in line with these findings.

The increased concentration of serum sialic acid is either due defect in the activity of sialytransferase, which transfers the sialic acid residues to the glycolipids and glycoproteins (Isaev *et al*., 1983). Decreased levels of sialic acid in the liver and kidney of diabetic rats were reported (Prakasam *et al*., 2005). Present results corroborate these observations. Although the exact reason for the decreased levels of liver and kidney sialic acid in diabetic condition is unclear, defect in the activities of enzyme associated with the synthesis of sialic acid play a possible role.

In the present study, oral administration of TpESet significantly lowered the blood glucose level as well as increased the plasma insulin levels and normalized the glycoprotein components levels in circulation and tissues. This indicates its potent role in carbohydrates and glycoproteins metabolism. The antihyperglycemic effect of TpESet is probably due to its role in elevating plasma insulin level by stimulating the secretion of insulin from remnant pancreatic β-cells or promote the utilization of glucose by the liver and extrahepatic tissues and the plant extracts can able to protect the abnormalities of glycoproteins during diabetes by stabilizing the structural integrity of the cellular membranes. The protective effects of *T. purpurea* seed extract are probably due to their inhibitory role in abnormal glycoprotein synthesis or on the activity of the enzymes (glycosyl transferases) involved in glycoprotein metabolism.

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