

Beneficial Effects of *Tephrosia purpurea* Ethanolic Seed Extract on Lipids and Membrane Bound Enzymes in Experimental Diabetic Rats

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ABSTRACT

Objective: Traditionally, *Tephrosia purpurea* Linn. (*Fabaceae*) is known to possess medicinal values in treating diabetes and other endocrine disorders. In this experiment, authors evaluated the beneficial role of *Tephrosia purpurea* Ethanolic seed extract [TpEt] standard (normal) versus streptozotocin [STZ] induced diabetic male albino wistar rats and studied the membrane stabilizing effects of TpEt in diabetic male albino rats. **Methods:** Male albino wistar rats (n=30) were used and divided into five groups, each having six animals. Diabetes mellitus was induced by giving streptozotocin intraperitoneally and blood glucose of above 250 mg/dl was considered diabetes. Blood glucose, glycosylated hemoglobin (HbA1c), plasma insulin, lipid profile and activities of membrane bound enzymes in erythrocytes and tissues were estimated. **Results:** Increased concentrations of blood glucose, glycosylated hemoglobin, and decreased plasma insulin, and accompanied by alterations in lipid profile and membrane bound enzymes activities in erythrocytes and tissues were noticed in experimental diabetic rats. Upon oral management of TpEt (300mg/kg body weight) for 45 days, the blood glucose and other alerted parameters in diabetic rats showed significant improvements. **Conclusion:** This study clearly showed *Tephrosia purpurea* seeds to possess membrane stabilizing effect in experimental diabetic rats probably due to the presence of flavonoids and isoflavonoids which could be responsible for its anti-lipid peroxidative and insulin stimula-

tory effects. Further studies are needed to separate and depict bioactive principle from the seeds of the same plant.

Key words: Blood glucose, Diabetes, Glycosylated hemoglobin (HbA1c), Lipid profile membrane bound enzymes, Plasma insulin, Streptozotocin (STZ), *Tephrosia purpurea* ethanolic seed extract (TpEt).

Key message: Oral administration of *Tephrosia purpurea* ethanolic seed extract for 45 days exhibited antihyperglycemic, antihyperlipidemic effects and improved membrane integrity by increasing membrane bound enzymes activities in streptozotocin induced diabetic rats. The positive improvements could be attributed to the presence of flavonoids and isoflavonoids in seeds responsible for positive effects.

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INTRODUCTION

Diabetes mellitus is a common disease affecting carbohydrate, protein and fat metabolisms and manifested by hyperglycemia due to lack of insulin secretion and resistance to insulin action on the target tissues secreted by pancreas. In diabetes, high concentration of blood glucose reacts with the membrane protein resulting in the formation of advanced glycosylated proteins, these proteins accumulate in tissues and organs leads to the development of diabetic complications like nephropathy, neuropathy and cardiovascular complications.¹ Currently diabetes is a life threatening problem worldwide and it is growing day by day, and the occurrence of diabetes mellitus is likely to touch up to 552 million people in the world by 2030.² Sedentary lifestyle, obesity, lack of physical exercise, changes in intake of food habits like fiber less diet and depression are the major contributing factors in increasing the diabetes incidence rate at a faster rate. Hyperglycemia induces free radical production due to autoxidation of glucose and glycation of protein results in decline antioxidant defense mechanism.¹ Chiefly cell membrane is made up of lipid bilayer it maintains the cell volume, cell to cell communications, membrane fluidity, cell signaling process and translocation of sodium, potassium, calcium ions. Three sodium ions pumped out and two potassium ions are pumped in across cell membrane against its concentration gradient for hydrolysis of each ATP. Reactive oxygen species reacts with membrane lipid

components and these oxidized lipids alter cellular functions and the activities of membrane bound enzymes.³

Management of diabetes with oral antidiabetic drugs, like sulfonylureas exerts its effects by various mechanisms, these drugs like any other drugs is not free from side effects and is known to cause, hypoglycemia, weight gain and resistance to long term treatment. To overcome these adverse effects, there is a constant search for newer medications with minimal side effects and hence a better safety profile as believed to be offered by blood glucose lowering components from medicinal plants. Ethnomedicinal information has been reported that around 800 medicinal plants are known to have antidiabetic effects.² Numerous experimental studies documented various herbs possessing antidiabetic properties and studies have been proved in experimental diabetic animal models.³⁻⁵ Streptozotocin is a diabetogenic agent to induce experimental diabetes research and is highly specific to insulin producing pancreatic β cells with free radicals, causes DNA alkylation and subsequent β cell death and is a perfect classic to educate for the favorable effects of exploring agents in STZ induced diabetes mellitus.⁶

In Indian traditional medicine, *Tephrosia purpurea* leaves, seeds, roots and the whole plant has great importance in treating diabetes. *Tephrosia*

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purpurea (Linn.) Pers belongs to Fabaceae family, commonly called as 'Sarapunkha' in Sanskrit and is widely distributed worldwide. Leaves of *Tephrosia purpurea* have shown its positive effects on liver and kidney problems were reported.⁷ It has been well reported the free radical scavenging activity of acetone, ethanol, and methanol extracts of leaves, seeds and complete plant in DPPH free radical assay.⁸ Previous studies have shown antihyperglycemic and antilipidperoxidative effects and protective role of glycoprotein components of *Tephrosia purpurea* leaves and seeds in streptozotocin induced diabetes mellitus in albino rats.^{9,10} In the present study, we like to evaluate the membrane functions by measuring membrane bound enzymes activities in experimental diabetic rats after treating with TpEt. However, there is no systematic study have been documented the positive effect of *Tephrosia purpurea* on cell membrane integrity in experimental models. Thus, we have designed the current study to investigate the membrane stabilizing effects of ethanolic seed extract of *Tephrosia purpurea* in experimental diabetic rats.

MATERIALS AND METHODS

Chemicals

Diabetogenic drug Streptozotocin was obtained from Sigma Aldrich Chemicals, Bangalore, India and analytical grades of additional compounds and chemicals were used.

Plant material

Tephrosia purpurea plant was collected from Chidambaram town situated in Northeast of Tamilnadu state in India. A voucher specimen (AU05102) was placed in the herbarium of Department of Botany, Annamalai University, located at Chidambaram, the same place from where it was collected.

Preparation of extract

Seeds of *Tephrosia purpurea* were air dried at the room temperature, powdered the seeds and the extract was prepared using ethanol,¹⁰ for this study we have chosen ethanolic seed extract due to the presence of bioactive compounds such as isoflavones, flavonoids, flavanones, flavanols and rutin.¹⁰

Animals

Albino wistar male rats, 9 weeks old and weighing 150-200g, were obtained from Central animal house, Rajah Muthiah Institute of Health Sciences, Annamalai University, India, for this study, and were kept in the central animal house with 12h. light and 12h. dark cycles. The animals were divided according to experimental work and retained in polypropylene cages. Feed pellets given as basal diet throughout the experiment, obtained from Mysore Snack Feed Ltd, Mysore, India, food and water *ad libitum* has been provided to all animals.

Experimental work

Experimentations were conducted as per the internationally accepted ethical guidelines for the care of laboratory animals (registration number 160/1999/CPCSEA). There were total 30 animals and were randomized into five groups and housed in polypropylene cages. The groups were Group I (control), Group II (control+TpEt extract), Group III (diabetic rats), Group IV (diabetic + TpEt extract), Group V (diabetic rat+glibenclamide). Each group had a total of six rats and thus making a total of 30. STZ (50mg/kg) was dissolved in 0.1M citrate buffer and it was injected intraperitoneally, after overnight fasting. Diabetes was confirmed by measuring the blood glucose levels within 48h of streptozotocin administration. A glucose concentration of above 250 mg/dl considered as diabetes.

Group I Served as Control rats alone. Control rats were given orally with "TpEt" at the dose of 300 mg/kg body weight using oral gavage for 45 days, were kept under Group II. Diabetic control rats used under Group III. Diabetic rats received "TpEt" with the dose of 300 mg/kg bw orally using oral gavage for 45 days were kept under Group IV. Group V served as Diabetic rats fed with "Glibenclamide" (600 µg/kg bw orally) using oral gavage for 45 days. At the end of experimental period, animals were sacrificed by cervical dislocation, after 12h of fasting. Biochemical parameters and membrane bound enzyme activities were measured on blood and tissues of all groups animals.

Sample collection

Blood was collected in EDTA tubes. Erythrocytes and tissues were collected for the measurement of Biochemical parameters.

Estimations of Blood glucose, plasma insulin and glycosylated hemoglobin

Blood glucose, plasma insulin and glycosylated hemoglobin were measured according to the methods of Sasaki, Anderson and Sudhakar Nayak and Pattabiraman.¹¹⁻¹³ respectively.

Measurement of Membrane bound enzymes

Total ATPase activity assayed by Evan method,¹⁴ (Na+/K+)-ATPase, Ca²⁺-ATPase, and Mg²⁺-ATPase were assayed by standard methods of Bonting, Hjerten and pan and Ohnishi's respectively.¹⁵⁻¹⁷ The liberated Phosphorous content was measured by Fiske and Subbarow method,¹⁸ Total protein was estimated by the method of Lowery's.¹⁹

Estimation of lipid profile

Serum total cholesterol, phospholipids, free fatty acids, Triglycerides, HDL were measured by the methods of parekh Jung,²⁰ Zilvermit and Davis,²¹ Falholf,²² Foster and dunn,²³ and Gidez and webb,²⁴ methods respectively. LDL cholesterol was calculated using this formula,

$$\text{LDL cholesterol} = \frac{\text{Total cholesterol} - \text{HDL} + \text{TG}}{5}$$

$$\text{VLDL cholesterol was calculated using the formula} = \frac{\text{TG}}{5}$$

Statistical analysis

Results were estimated, by one way analysis of variance (ANOVA), followed by Duncan's multiple comparisons test and values have shown as mean ± SEM. if the p value is less than 0.05, is considered as significant between the groups.

RESULTS

Effects of *Tephrosia purpurea* ethanolic seed extract on blood glucose, Glycosylated hemoglobin (HbA1C), Insulin concentrations in control and experimental animals

Table 1 presents the diabetic profile of control and experimental animals. Concentrations of blood glucose, Glycosylated hemoglobin (HbA1C), Insulin were significantly altered in diabetic control (Group III) rats as compared to control and TpEt treated control rats (Group I and II). After oral administration of "TpEt" with 300 mg/kg b. wt. and glibenclamide 600 µg/kg body weight to the diabetic rats, the concentrations of above said parameters were improved significantly. The results of "TpEt" treated animals were nearly like that of glibenclamide fed animals.

Influence of *Tephrosia purpurea* ethanolic seed extract on cholesterol, phospholipids, free fatty acids, triglycerides and HDL concentrations in control and experimental animals

Table 2 depicts the concentrations of serum lipids and lipoproteins of control and experimental animals. The levels of serum cholesterol, phospholipids, free fatty acids, and triglycerides were significantly increased whereas HDL was significantly reduced in diabetic control rats (Group III) as compared to control and TpEt treated control rats (Group I and II). However, the levels of lipids were significantly improved in "TpEt" with 300 mg/kg body weight and glibenclamide 600 µg/kg body weight fed diabetic rats. However, TpEt and glibenclamide both had shown similar effects in improving the lipids in diabetic rats.

Impacts of *Tephrosia purpurea* ethanolic seed extract on the total ATPases and Na⁺/K⁺-ATPase activities in control and experimental animals

Table 3 represents total ATPases and Na⁺/K⁺-ATPase concentrations in erythrocytes and liver and kidney of control and experimental animals. The activities of total ATPases and Na⁺/K⁺-ATPase were significantly reduced in erythrocytes, liver and kidney of diabetic control rats (Group III) as compared to control and TpEt treated control rats (Group I and II). The above enzyme activities were reverted in "TpEt" with 300 mg/kg body weight and glibenclamide with 600µg/kg body weight received diabetic rats. The results of "TpEt" treated animals were similar to that of glibenclamide fed animals.

Effect of *Tephrosia purpurea* ethanolic seed extract on the Ca²⁺-ATPase and Mg²⁺-ATPase activities in control and experimental animals

Table 4 shows Ca²⁺-ATPase and Mg²⁺-ATPase in erythrocytes and tissues of control and experimental animals. The concentrations of same enzymes were significantly decreased in erythrocytes, liver and kidney of diabetic control Group III when compared to control and TpEt treated control rats (Group I and II). However, the concentrations of same enzymes were significantly increased in diabetic rats treated with "TpEt" with 300 mg/kg body weight and showed similar effects to that of glibenclamide with 600 µg/kg body weight in diabetic rats.

DISCUSSION

Diabetes mellitus is a chronic disorder of macronutrients metabolisms, it affects the glucose utilization as fuel to the cells, in response to the low insulin concentration and insulin resistance. In hyperglycemia, blood glucose rejoins non-enzymatically with hemoglobin and accordingly increased glycosylated hemoglobin concentration. Hemoglobin is a protein which transports the oxygen and carbon dioxide to the tissues. Measuring of HbA1c is useful index parameter to know the uncontrolled glycemic status and to find the stage of complications of diabetes.²⁵ In the current study we have noticed markedly increased concentrations of glucose and glycosylated hemoglobin and significant reduction in plasma insulin level in streptozotocin induced diabetic rats, could be due to the decreased insulin levels in circulation results from the reactive oxygen species which damages the insulin producing pancreatic β cells. Previous authors have shown similar results in *Merremia emarginata* Burm. F. in

Table 1: Diabetic profile of control and experimental animals

Groups	Blood Glucose (mg/dl)	Glycosylated Haemoglobin (HbA1%)	Plasma Insulin (µU/ml)
Control	78.5±4.8 ^a	5.61±0.27 ^a	14.18±1.06 ^a
Control+TpEt (300mg/kg bwt)	76.3±5.46 ^a	4.11±0.32 ^a	14.26±1.13 ^a
Diabetic Control	283.2±14.2 ^b	13.08±0.97 ^a	9.60±0.52 ^b
Diabetes+TpEt (300mg/kg bwt)	105.6±8.5 ^c	8.03±0.52 ^a	12.4±0.79 ^c
Diabetes+glibenclamide (600µg/kg bwt)	115.3±9.5 ^c	9.18±0.63 ^c	11.56±0.88 ^c

Values are expressed as mean ± SD for six rats in each group

Values are not sharing a similar superscript letter, P<0.05 were considered as significant between the groups

Table 2: Serum lipid and lipoprotein levels of control and experimental animals

Groups	Total Cholesterol (mg/dl)	Triglycerides (mg/dl)	Phospholipids (mg/dl)	Free fatty acids (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Control	85.1±4.5 ^a	70.3±5.1 ^a	90.2±6.7 ^a	8.5±0.6 ^a	36.2±0.8 ^a	63.1±4.2 ^a
Control+TpEt (300mg/kg bwt)	79.5±4.8 ^a	66.4±4.8 ^a	83.1±5.5 ^a	8.1±0.5 ^a	37.4±1.05 ^a	56.3±5.1 ^a
Diabetic Control	150.83±8.6 ^b	143.6±9.6 ^b	147.4±9.3 ^b	19.4±0.9 ^b	16.3±0.5 ^b	162.5±10.4 ^b
Diabetes+TpEt (300mg/kg bwt)	114.9±7.3 ^c	104.2±6.7 ^c	112.6±8.2 ^c	10.6±0.8 ^a	28.5±1.03 ^c	115.4±7.2 ^c
Diabetes+glibenclamide(600µg/kg bwt)	121.6±8.2 ^c	119.7±7.2 ^c	118.5±9.5 ^c	11.4±0.7 ^a	23.1±0.97 ^c	124.3±6.8 ^c

Values are expressed as mean ± SD for 6 rats in each group

Values are not sharing a common superscript letter, P<0.05 were considered as significant between the groups.

Table 3: Total ATPase and Na⁺/K⁺- ATPase in erythrocytes and tissues of control and experimental animals

Groups	Total ATPase			Na ⁺ /K ⁺ - ATPase		
	Erythrocytes U ⁺ /mg protein	Liver U ⁺ /mg protein	Kidney U ⁺ /mg protein	Erythrocytes U ⁺ /mg protein	Liver U ⁺ /mg protein	Kidney U ⁺ /mg protein
Control	3.81±0.26 ^a	2.48±0.21 ^a	1.98±0.10 ^a	0.88±0.05 ^a	0.71±0.06 ^a	0.61±0.04 ^a
Control+TpEt (300mg/kg bwt)	3.91±0.18 ^a	3.08±0.19 ^a	2.11±0.13 ^a	0.94±0.06 ^a	0.79±0.04 ^a	0.69±0.05 ^a
Diabetic Control	0.92±0.07 ^b	1.16±0.12 ^b	0.56±0.04 ^b	0.48±0.04 ^b	0.36±0.01 ^b	0.27±0.03 ^b
Diabetes+TpEt (300mg/kg bwt)	3.11±0.18 ^c	2.62±0.15 ^c	1.17±0.09 ^c	0.74±0.05 ^c	0.62±0.05 ^c	0.52±0.04 ^c
Diabetes+glibenclamide (600µg/kg bwt)	2.98±0.22 ^c	2.38±0.19 ^c	0.95±0.06 ^c	0.67±0.04 ^c	0.58±0.06 ^c	0.46±0.03 ^c

Values are expressed as mean ± SD for 6 rats in each group

Values not sharing a common superscript letter, P<0.05 were considered as significant between the groups.

*-µmol of Pi liberated in h

Table 4: Ca⁺²- ATPase and Mg⁺²- ATPase in erythrocytes and tissues of control and experimental animals

Groups	Ca ⁺² - ATPase			Mg ⁺² - ATPase		
	Erythrocytes U ⁺ /mg protein	Liver U ⁺ /mg protein	Kidney U ⁺ /mg protein	Erythrocytes U ⁺ /mg protein	Liver U ⁺ /mg protein	Kidney U ⁺ /mg protein
Control	0.81±0.06 ^a	0.67±0.05 ^a	0.61±0.04 ^a	0.69±0.02 ^a	0.59±0.03 ^a	0.61±0.05 ^a
Control+TpEt (300mg/kg bwt)	0.87±0.07 ^a	0.71±0.04 ^a	0.68±0.03 ^a	0.71±0.05 ^a	0.63±0.04 ^a	0.68±0.07 ^a
Diabetic Control	0.37±0.01 ^b	0.26±0.01 ^b	0.31±0.02 ^b	0.28±0.01 ^b	0.24±0.02 ^b	0.31±0.04 ^b
Diabetes+TpEt (300mg/kg bwt)	0.65±0.07 ^c	0.57±0.04 ^c	0.51±0.04 ^c	0.58±0.03 ^c	0.50±0.04 ^c	0.52±0.02 ^c
Diabetes+glibenclamide (600µg/kg bwt)	0.59±0.06 ^c	0.51±0.06 ^c	0.46±0.02 ^c	0.51±0.02 ^c	0.43±0.02 ^c	0.49±0.03 ^c

Values are expressed as mean ± SD for 6 rats in each group

Values not sharing a common superscript letter, P<0.05 were considered as significant between the groups. * -µmol of Pi liberated in h

streptozotocin induced diabetic rats.²⁶ Oral treatment of TpEt significantly improved the above said parameters, with its insulin stimulatory effect on remnant pancreatic β-cells and its free radical scavenging activity, possibly by improving the glucose utilization by the muscles and peripheral tissues and decreasing the glucose production from the liver via gluconeogenesis pathway.

Hyperlipidemia is commonly associated with diabetes mellitus and is a chief contributor to the prevalence of macro vascular problems. Low concentration of insulin in circulation and hyperglycemia results in impairment of lipoprotein metabolism subsequently leads to hypertriglyceridemia in diabetes.²⁷ In diabetes induced rats, significantly increased levels cholesterol, phospholipids, free fatty acids, LDL, triglycerides and significant reduction of HDL were observed, our results are in agreements with earlier studies due to lipid peroxidation and insulin insufficiency.⁵⁻¹⁰ Lipid and lipoprotein metabolism have greatly influenced by insulin, surplus utilization of free fatty acids from fat deposits in diabetes since low insulin levels accelerates lipolysis and activates hormone sensitive lipase, some of free fatty acids are enter into the liver for conversion phospholipids and cholesterol. Altered cholesterol of membrane composition alters membrane fluidity and hyperlipidemia also influences the activities of membrane bound enzymes.⁴ Oral treatment of TpEt showed better improvement in lipid metabolism possibly by its insulin stimulatory effect.

Phospholipids are major contributor in cell membrane and it consists of hydrophilic head and hydrophobic fatty acyl chains. These unsaturated fatty acids are highly susceptible to lipid peroxidation result in the formation of oxidized lipids. Lipids plays a major role in integrity of membrane structure and function and transport of Na⁺, K⁺ and Ca⁺ ions across cell membrane. Oxidative stress, non-enzymatic glycosylation of membrane proteins, abnormal fatty acid metabolism and altered homeostasis of glucose metabolism are major factors for altering the

activities of membrane associated enzymes. Free fatty acids, diacylglycerols, eicosanoids, free radicals are liberated from oxidative stress altered phospholipids metabolism has been reported.²⁸ Na⁺/K⁺ ATPase is a ubiquitous enzyme it consists of α, β and γ subunits and it has major effect on the regulation of Sodium and potassium channels and ion transportation across membrane against concentration gradient and provides convenient driving force for the secondary transport of substrates such as glucose and amino acids.²⁹ Hyperglycemia promotes the formation of ployol sugars sorbitol and fructose via polyol pathway catalyzed by aldose reductase and sorbitol dehydrogenase, accumulation of sorbitol in cells results in reduced levels of myo-inositol and taurine which in turn reduces Na⁺/K⁺ ATPase activity and contribute to structural and functional changes.^{4,30} In the current study we have noticed the reduced levels of Na⁺/K⁺ ATPase in RBC membrane and tissues probably due to uncontrolled metabolism of glucose. Na⁺/K⁺ ATPase is rich in thiol groups and oxidized lipids and glycosylation of membrane proteins inhibits the enzyme activity by oxidation of thiol groups. However, the oxidized lipid alters membrane structure and function is not clear.²⁹ Ca⁺² ATPase is transport protein, and its function is to maintain the intracellular calcium concentration, and cell to cell signaling process. Mg⁺² ATPase provide energy for the biological activation of magnesium ions and is more susceptible to lipid peroxidation. Decreased Ca⁺² ATPase and Mg⁺² ATPase activity was observed in RBC membrane and tissues of diabetic rats, current experimental results on membrane bound ATPases are in agreement with earlier reports,^{4,30} could be due to altered membrane permeability due to insulin deficiency and declined antioxidant mechanism. Reduced activity of Ca⁺² ATPase increases the calcium concentration in cytosol responsible for the development cardiac dysfunctions. Insulin has shown to have regulatory effect on ATPases.³⁰ After treating with TpEt to streptozotocin induced diabetic rats have shown significant improvement in the activities of membrane bound ATPases.

CONCLUSION

Ethanollic seed extract of *Tephrosia purpurea* had improved glucose homeostasis and membrane lipid metabolism in streptozotocin induced diabetic rats after 45 days treatment. Thereby improving the permeability, transport functions, signal transduction process, enzyme activities associated with membrane structure and confirmation probably by the presence of flavonoids and isoflavonoids, and phytochemicals in the *Tephrosia purpurea* seeds. This might be responsible for its free radical scavenging activity and its positive effect on remnant pancreatic beta cells of pancreas in streptozotocin induced diabetic rats. Effects of *Tephrosia purpurea* seed extract have shown similar effects to that of antidiabetic drug glibenclamide in induced diabetic rats and additional studies are needed to separate and depict bioactive principle from the seeds of the same plant.

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