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Original Research Article

Anti-diabetic potential and safety profiles of *Tephrosia purpurea* on streptozotocin-nicotinamide induced diabetes in rats

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ABSTRACT

Background: *Tephrosia purpurea* was traditionally used for the management of diabetes mellitus. Since this claim has not been investigated scientifically, the aim of this study was to evaluate the antidiabetic activity of *Tephrosia purpurea* extracts against STZ-Nicotinamide induced diabetes in rat.
Methods: This preclinical study was done to evaluate the anti-hyperglycaemic activity of whole plant extract of *Tephrosia purpurea* in STZ- Nicotinamide induced diabetic rats, which produced a significant difference in blood glucose, lipid profile, renal and liver profile in comparison to untreated rats. In this study, the animals were divided into five groups and diabetes was induced by administering STZ-Nicotinamide and animals with blood sugar level >200 were enrolled in the study. Further the animals are grouped into Group I control (0.1% CMC), Group II, diabetic control and Group III reference control received glibenclamide. Group IV and V, diabetic rats treated with *Tephrosia purpurea* extract 200 mg/kg and 400 mg/kg respectively. All the test drugs were administered for 30 days.
Results: In diabetic rats, treated with 200 and 400 mg/kg of *Tephrosia purpurea* blood glucose level significantly lowered on 10th day ($p<0.05$) and 5th day ($p<0.01$) respectively as compared to untreated rats. At the end of 30th day there is significant reduction in blood glucose treated with TP 400 mg/kg ($p<0.001$). Safety assessment shows the protective effect of TP (400 mg/kg) on lipid profile TC, TG ($p<0.001$), HDL ($p<0.001$), LDL ($p<0.001$) and VLDL ($p<0.01$). It also shows protective activity against AST ($p<0.001$), ALT ($p<0.01$), ALP ($p<0.001$) and Renal functions BUN ($p<0.001$), Creatinine ($p<0.001$).
Conclusion: The anti-hyperglycaemic activity of *Tephrosia purpurea* is brought out in the study by its significant reduction in the blood glucose level. The safety and efficacy is established based on the protective effect of *Tephrosia purpurea* in lipid profile, renal and hepatic function of diabetic rats.

Keywords: *Tephrosia purpurea*, Streptozotocin, Blood sugar, Anti-diabetic, Glibenclamide

INTRODUCTION

Diabetes mellitus is one of the major public health burden leading to increased mortality. It is a metabolic disorder of multiple aetiologies characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism due to abnormalities in insulin production.[1] DM is also characterised by insulin resistance and pancreatic β cell dysfunction. Other factors predispose to type 2 Diabetes Mellitus are genetic variation, ageing, sedentary lifestyle and obesity. In 2000, there were around 171 million diabetes cases and it is estimated that the

number will double by 2030. A leading non communicable disease with multiple aetiologies, affects more than 100 million people worldwide and is considered as one of the five leading causes of death in the world. It is estimated that more than 62 million people are currently diagnosed with diabetes mellitus and is rapidly attaining the potential epidemic in India.[2] It has been reported that India (31.7 million) topped the world with the highest diabetic patients followed by China (20.8 million) and US (7.7 million).[3]

Ancient literature has explained the use of various herbs in the treatment of diabetes mellitus. Many investigations of oral anti-hyperglycaemic agents of plant origin used in traditional medicine have been conducted and many plants have been found to show positive activity. *Tephrosia purpurea* Linn is a pan tropical, polymorphic, perennial herb belongs to the family fabacae popularly known as Saraphunkha in Sanskrit, Purple *tephrosia* in English with medicinal properties. Chaudhari et al., 2012, demonstrated regarding the presence of glycosides, various phytochemical constituents especially flavonoids in *Tephrosia purpurea*.[4] Aqueous and ethanolic extract of seeds has anti-hyperglycaemic activity. *Tephrosia purpurea* was widely used as folklore medicine, commonly it is used for cough, Chest tightness, bilious febrile attacks, and obstructions of spleen, liver and kidney. The leaves of *Tephrosia purpurea* was used to purify the blood, and used to cure for boils and pimples. Roots of *Tephrosia purpurea* was used for dyspepsia and chronic diarrhoea by the traditional healers. Infusion of seeds used as cooling medicine and decoction of pounded leaves used for snake bites.[5] Literatures were also available to support the anti-hyperlipedemic, hepatoprotective, neuroprotective, anthelmintic, diuretic and anti-inflammatory activity of various parts of *Tephrosia purpurea*.[6-8] Though there are many studies available, lack of information on utility of whole plant extract of *Tephrosia purpurea* in treatment of diabetes and their safety in renal and hepatic profile. Hence the present study is designed to explore the anti-diabetic activity and its safety in STZ-Nicotinamide induced diabetic rats.

METHODS

Plant collection & authentication

The whole plant, *Tephrosia purpurea* Linn. was collected from the road side of Erode, Tamilnadu, during the month of October. It was authenticated by Prof. R. Duraisamy, pharmacognosist and the voucher specimen (NCP/Phcog/ 2016/0202) has been retained, for future reference in the herbarium of pharmacognosy department, Nandha college of pharmacy, Erode, India.

Extraction

The collected *Tephrosia purpurea*, was washed in running tap water to remove the soil debris, shade dried and grounded using mechanical blender to get coarse powder. The 200gm of coarsely powdered *Tephrosia purpurea* whole plant was soaked in one litre of ethanol (90%) in a tightly sealed flat bottom flask at room temperature, protected from sun light for 72 hrs with occasional shaking. After 72 hrs the mixture was filtered through muslin cloth and the solvent was evaporated by rotary evaporator at 40°C to get dry mass. The dried ethanolic extract of *Tephrosia purpurea* was stored in desiccators and used for further pharmacological studies.

Animals

Wistar albino rats of either sex weighing between 180-200 gms were used for this study. The animals were obtained from King's Institute, Guindy and was housed in animal house, Karpaga Vinayaga institute of medical sciences and research institute, Kancheepuram. On arrival, the animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2ºC and relative humidity of 30-70 %. A 12:12 light: day cycle was followed. All animals were allowed to free access to water and fed with standard commercial pelleted rat chaw (M/s. Hindustan Lever Ltd, Mumbai).

Induction of diabetes

Diabetes was induced experimentally in 12 hour fasted rats by a single intra-peritoneal injection of Streptozotocin (50mg/kg) dissolved in 0.1M of citrate buffer (pH 4.5), followed by intra peritoneal administration of Nicotinamide (120mg/kg) after 15 minutes[9]. Since STZ is capable of inducing fatal hypoglycaemia due to sudden marked release of insulin from the pancreas, the rats that had been administered STZ were provided after 6 hr with a 10% glucose solution orally for 24 hr continuously so as to prevent hypoglycaemia. After 72 hr, rats with a blood glucose concentration above 200 mg/dl were considered to be diabetic and were used for further diabetic studies.

Anti-diabetic screening

Group I, served as normal control, received 0.1% carboxyl methyl cellulose solution (1 mg/kg) as vehicle through oral route. Group II, served as diabetic control, in which animals were administered with STZ- Nicotinamide. Group III, STZ-Nicotinamide induced diabetic rats, and orally received Glibenclamide (5 mg/kg). Group IV and V were STZ-Nicotinamide induced diabetic rats, received 200 and 400 mg/kg of ethanolic extract of *Tephrosia purpurea* respectively. All the test drugs were administered for once daily for 30 days. Blood glucose levels were measured at different time intervals (0, 5th, 10th, 15th and 30th day) during the study. At the end of experimental period, the rats were fasted overnight, anaesthetized with Pentobarbitone sodium and the blood was collected by retro-orbital puncture in heparinized and non heparinized tubes. The blood was subjected for the determination of lipid profiles, liver function (AST, ALT, and ALP) and kidney function (urea and creatinine).

Statistical analysis

Results were represented as mean±SEM. The data were analysed by using one way analysis of variance (ANOVA) followed by Dunnett's'*t*' test using graphPad version 3, p values <0.05 were considered as significant.

RESULTS

Anti-diabetic activity

Effect of *Tephrosia purpurea* on blood sugar levels: The antidiabetic activity of ethanolic extract of *Tephrosia purpurea* plant was studied against the STZ-Nicotinamide induced diabetes in rats and the blood sugar levels of various time intervals were shown on (Table 1).

Table 1: Effect of *Tephrosia purpurea* on blood sugar levels in STZ-nicotinamide induced diabetic rats.

Drug treatment	Mean blood sugar levels (mg/dl)					
	Initial	0 Day	5th Day	10th Day	15th Day	30th Day
Control 1% CMC	98.00±3.91	100.33±4.47	101.17±4.67	99.67±4.66	97.17±3.03	102.00±5.39
Diabetic control STZ (50 mg/kg)-Nicotinamide (120 mg/kg)	213.00±4.07	220.50±5.83	228.83±3.17	216.33±5.83	226.50±2.57	222.17±5.67
Glibenclamide (5 mg/kg)	212.00±4.56	219.67±3.81	184.50±3.89**	143.17±2.73***	112.67±5.57***	103.33±5.34***
***Tephrosia purpurea* (200 mg/kg)**	211.33±4.49	219.00±3.45	210.00±4.07	157.17±5.55*	129.17±2.09***	114.17±3.28***
***Tephrosia purpurea* (400 mg/kg)**	208.83±6.09	217.50±4.86	189.00±5.05**	150.83±6.04**	119.67±4.36***	100.17±4.85***

Values are in mean±SEM (n=6); *p<0.05, **p<0.01 & ***p<0.001 vs. diabetic control

Table 2: Effect of *Tephrosia purpurea* on lipid profiles in STZ-Nicotinamide induced diabetic rats.

Drug treatment	Lipid profiles (mg/dl)				
	Total Cholesterol	Triglycerols	HDL-cholesterol	LDL-cholesterol	VLDL-cholesterol
Control 1% CMC	112.00±2.09	69.33±0.98	36.67±1.28	41.00±0.63	17.67±0.88
Diabetic control STZ (50 mg/kg)-Nicotinamide (120 mg/kg)	138.67±1.33	115.83±2.70	22.83±1.01	78.00±0.89	30.50±0.76
Glibenclamide (5 mg/kg)	113.50±1.80***	73.50±1.95***	31.17±1.09***	46.17±0.60***	20.17±0.70**
***Tephrosia purpurea* (200 mg/kg)**	125.67±2.20*	89.17±2.24**	27.83±0.91*	59.33±0.42**	24.50±0.62*
***Tephrosia purpurea* (400 mg/kg)**	116.00±1.41**	74.50±1.20***	32.67±0.49***	44.00±0.97***	21.67±0.71**

Values are in mean±SEM (n=6); *p<0.05, **p<0.01 & ***p<0.001 vs. diabetic control

The blood sugar levels of diabetic control rats were higher than those of normal rats on 0, 5th, 10th, 20th and 30th day. In diabetic rats, treated with 200 and 400 mg/kg of *Tephrosia purpurea* blood glucose level significantly lowered to 157.17±5.55 on 10th day (p<0.05) and to 189.00±5.05 on 5th day (p<0.01) respectively as compared to diabetic animals. The diabetic rats treated with reference control glibenclamide also significantly (p<0.001) lowered the blood sugar level to 184.50±3.89 on 5th day. On 15th day onwards until the end of the drug treatment, on 30th day 200 and 400 mg/kg of *Tephrosia purpurea* and glibenclamide significantly (p<0.001) lowered the blood glucose as compared to diabetic control animals.

Effect of Tephrosia purpurea on lipid profile

The effect of ethanolic extract of *Tephrosia purpurea*on lipid parameters in STZ-nicotinamide induced diabetes in rats were shown in table 2. In the animals of normal control

the total cholesterol was 112.00±2.09 mg/dl, whereas the total cholesterol was enhanced in diabetic control rats up to 138.67±1.3 3mg/dl. The 200 and 400mg/kg of ethanolic extract of *Tephrosia purpurea*significantly decreased the total cholesterol to 125.67±2.20 (p<0.05) and 116.00±1.41 mg/dl (p<0.01) respectively.

Table 3: Effect of *Tephrosia purpurea* on liver and kidney function profiles in STZ-Nicotinamide induced diabetic rats.

Drug treatment	Liver function profile			Kidney function profile	
	AST (IU/l)	ALT (IU/l)	ALP (IU/l)	BUN (mg/dl)	Creatinine (mg/dl)
Control 1% CMC	1.77±0.08	4.89±0.29	34.70±0.58	21.65±0.72	0.51±0.03
Diabetic control STZ (50 mg/kg)-Nicotinamide (120 mg/kg)	13.95±0.43	10.02±0.37	66.49±0.43	50.88±0.72	1.76 ±0.01
Glibenclamide (5 mg/kg)	5.37±0.25***	5.99±0.22***	38.54±0.60***	31.98±0.47***	0.65±0.02***
***Tephrosia purpurea* (200 mg/kg)**	8.12±0.48**	7.24±0.28*	45.34±0.61**	47.58±0.46*	1.18±0.04*
***Tephrosia purpurea* (400 mg/kg)**	5.59±.21***	6.38±0.20**	39.36±0.52***	32.67±0.44***	0.77±0.03***

Values are in mean±SEM (n=6); *p<0.05, **p<0.01 & ***p<0.001 vs. diabetic control

The reference control Glibenclamide also significantly (p<0.001) reduced the total cholesterol to 113.50±1.80 mg/dl. The effect produced by the ethanolic extract of *Tephrosia purpurea* is equipotent as that of the reference control. In the animals of normal control the triglyceride was 69.33±0.98 mg/dl, where as the triglyceride was increased in diabetic control up to 115.83±2.70 mg/dl, The 200mg/kg of ethanolic extract of *Tephrosia purpurea* significantly (p<0.01) decreased by reversed the elevated triglyceride to 89.17±2.24 mg/dl. *Tephrosia purpurea*, 400mg/kg and reference control glibenclamide, showed more significant (p<0.001) decrease in triglyceride level to 74.50±1.20 and 73.50±1.95 mg/dl respectively.

In the animals of normal control the HDL-Cholesterol was 36.67±1.28 mg/dl, where as it was decreased in hyperlipidemic control up to 22.83±1.01 mg/dl. The 200mg/kg of ethanolic extract of *Tephrosia purpurea* showed moderate increase in HDL-Cholesterol compared to diabetic control. The ethanolic extract of *Tephrosia purpurea* at 400mg/kg and the reference control glibencalmide significantly (p<0.001) enhanced the HDL-cholesterol to the level of 32.67±0.49 and 31.17±1.09 respectively. In the animals of normal control the LDL-cholesterol was 41.00±0.63 mg/dl, where as it was increased in diabetic control up to 78.00±0.89 mg/dl. The 200 mg/kg of ethanolic extract of *Tephrosia purpurea* showed significant (p<0.01) decrease in LDL-cholesterol compared to diabetic control. The ethanolic extract of *Tephrosia purpurea* at 400mg/kg and the reference control glibencalmide showed marked and significantly (p<0.001) decrease the LDL-cholesterol to the level of 44.00±0.97 and 46.17±0.60 respectively. VLDL-cholesterol levels in the normal animal was 17.67±0.88 mg/dl, where as in the diabetic control it was 30.50±0.76 mg/dl. VLDL-cholesterol (24.50±0.62 mg/dl) was significantly (p<0.05) decreased by 200mg/kg of ethanolic extract of *Tephrosia purpurea.* The levels of VLDL-cholesterol was more significantly (p<0.01) decreased by the treatment of 400mg/kg of *Tephrosia purpurea* and the reference control glibenclamide and the levels were 21.67±0.71 and 20.17±0.70 mg/dl.

Effect of Tephrosia purpurea on liver enzymes and kidney functions

The effects of ethanolic extract of *Tephrosia purpurea* on liver and kidney function profiles in STZ-Nicotinamide induced diabetes in rats were shown in table 3. The activity of AST, AST and ALT were significantly elevated in diabetic control rats compared to normal controls. Administration of ethanolic extract of *Tephrosia purpurea* at 200 mg/kg, significantly (p<0.01) reduced serum enzymes activity of ALT, and ALP to 8.12±0.48 and 45.34±0.61 IU/l respectively. It also significantly (p<0.05) the AST to 7.24±0.28 IU/l compared to diabetic control. *Tephrosia purpurea* at 400 mg/kg, significantly (p<0.001) reduced serum enzymes activity of ALT, and ALP to 5.59±0.21 and 39.36±0.52 IU/l respectively. It also significantly (p<0.01) the AST to 6.38±0.20 IU/l compared to diabetic control. The reference control glibenclamide, showed significant (p<0.001) decrease in all the three serum liver enzymes as compare to diabetic control. The Blood Urea Nitrogen and serum Creatinine were increased in the STZ-Nicotinamide induced diabetes animals as compared to normal control animals. Ethanolic extract of *Tephrosia purpurea* at 200 mg/kg, significantly (p<0.05) decrease the BUN (47.58±0.46) and serum Creatinine (1.18±0.04) as compared to diabetic control. *Tephrosia purpurea* at 400 mg/kg significantly (p<0.001) decrease both the BUN (32.67±0.44) and serum Creatinine (0.77±0.03) as compared to diabetes control. The effect produced by *Tephrosia purpurea* 400 mg/kg on BUN and serum creatinine was similar to that of glibenclamide.

DISCUSSION

The present study was planned to evaluate the antidiabetic activity of ethanolic extract of *Tephrosia purpurea* against STZ-nicotinamide induced diabetes in rats. Administration

of STZ and nicotinamide has been proposed to induce experimental diabetes in rats. STZ is well known to cause pancreatic B-cell damage, whereas nicotinamide is administered to rats to partially protect insulin-secreting cells against STZ. STZ is transported into B-cells via the glucose transporter GLUT2 and causes DNA damage leading to increased activity of poly (ADP-ribose) polymerase (PARP-1) to repair DNA. However, exaggerated activity of this enzyme results in depletion of intracellular NAD (+) and ATP, and the insulin-secreting cells undergo necrosis. The protective action of nicotinamide is due to the inhibition of PARP-1 activity. Nicotinamide inhibits this enzyme, preventing depletion of NAD (+) and ATP in cells exposed to STZ. Moreover, nicotinamide serves as a precursor of NAD (+) and thereby additionally increases intracellular NAD (+) levels. *In vitro* studies demonstrated that the insulin-secretory response to glucose is attenuated in STZ- nicotinamide induced diabetic rats compared with control animals. This is due to reduced B-cell mass as well as metabolic defects in the insulin-secreting cells.[9] The ethanolic extract of *Tephrosia purpurea* reduced blood glucose level in STZ-nicotinamide induced diabetic rats. The biochemical mechanism of actions of *Tephrosia purpurea* extract might be due to an insulin mimetic effect by either stimulating glucose uptake and metabolism, by inhibiting hepatic gluconeogenesis and glycogenolysis, by stimulation of regeneration process or increase pancreatic secretion of insulin from existing β-cells and/or inhibition activity against α-glucosidase enzymes in small intestine which convert disaccharides into monosaccharaides for sake of absorption.[10-14]

Tephrosia purpurea at the dose of 400 mg/kg exhibited significant decrease in blood glucose level, as compared to 200 mg/kg on 5th day of drug administration. The result was comparable with the standard drug glibenclamide which reduced fasting blood glucose level on same day. Moreover, both the doses of *Tephrosia purpurea,* showed significant blood glucose reduction in STZ-nicotinamide induced diabetic rats on day 10th, 15th and 30th days compared to diabetic control. Phytochemical investigations on *Tephrosia purpurea* have reported the presence of phytoconstituents such as, glycosides, carotenoids, isoflavones, flavanones, chalcones, flavanols, and sterols.[15] It has also been suggested that, aqueous seed extract of *Tephrosia purpurea* has potent antihyperglycemic and antioxidant effects in streptozotocin-induced diabetic rats.[16] Tremendous studies have found that flavonoids originated from foods could improve glucose metabolism, lipid profile, regulating the hormones and enzymes in human body, further protecting human being from diseases like obesity, diabetes and their complications.[17] In our findings, the antidiabetic activity exhibited by ethanolic extract of *Tephrosia purpurea* might be due to the presence of flavonoids in it.

Abnormalities in lipid profile are common complications in diabetes mellitus (Gibbons, 1986). Such abnormality represents the risk factors for coronary heart diseases.[18,19] Activation of hormone sensitive lipase during insulin deficiency causes an increase in free fatty acid mobilization from adipose tissue.[20] In addition, hyperglycemia is accompanied by a rise in TC, TG, LDL-C and a fall in HDL-C.[21] In the present study, serum total cholesterol, triglycerides, LDL-C and VLDL-C levels were decreased and at the same time HDL-C was increased in *Tephrosia purpurea* extracts treated diabetic rats.

The remarkable control of high serum triacylglycerol in ethanolic extract of *Tephrosia purpurea* treated diabetic rats could be due to inhibition of endogenous TG synthesis in liver or improvement in insulin level or the presence of active component(s) in *Tephrosia purpurea* that suppressed the activity of hormone sensitive lipase in adipose tissue or increased activity of hepatic lipase or lipoprotein lipase accountable for the hydrolysis of excess lipoprotein bound triacylglycerol into fatty acids.[22,23] Increased level of HDL-C in ethanolic extract of *Tephrosia purpurea* treated groups could be due to the enhancement of lecithin: cholesterol acyltransferase (LCAT) which plays a key role in incorporating the free cholesterol in to HDL which take back to the liver.[24] LDL-C reducing effect of *Tephrosia purpurea* presumably attributed to increased expression of low density lipoprotein receptor (LDLR), which enhance LDL particles uptake in liver from the circulation, through the depletion of intracellular cholesterol.[25]

Serum total cholesterol lowering property of ethanolic extract of *Tephrosia purpurea* could be attributed to the presence of hypocholesterolemic compounds in *Tephrosia purpurea* that may act as inhibitor for hepatic hydroxyl methyl glutaryl CoA (HMG CoA) reductase in liver, which take part in cholesterol synthesis.[26] The decrease in serum total cholesterol, triacylglycerol, LDL-C and VLDL-C and an increase in HDL-C after 30 days treatment showed a dose dependent trend, indicating that efficacy was proportional to the dose of ethanolic extract of *Tephrosia purpurea*. In general, *Tephrosia purpurea* was capable to reverse the values of TC, TG, HDL-C and LDL-C, VLDL - C near normal after 30 days of treatment; this could be due to antioxidant property of *Tephrosia purpurea.*[27] The activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) in serum are generally indicators for liver function. In diabetic rats, the levels of these enzymes are elevated due to the necrosis of liver cells by the injection of STZ-nicotinamide.[28] However, *Tephrosia purpurea* treated diabetic rats showed decreased in the activity of ALT, AST and ALP enzymes that might support its hepatoprotective effect and normalization capability of impaired liver metabolism in diabetic rats.[29] Negative nitrogen balance is manifested in diabetic rats associated with enhanced proteolysis in muscle and other tissues. Impaired balance of nitrogen coupled with lowered protein synthesis leads to increased concentrations of urea and creatinine in serum indicates progressive renal damage in diabetic rats.[30,31] Treatment with ethanolic extract of *Tephrosia purpurea* resulted in a considerable reduction to

near normal in BUN and creatinine level indicating the renoprotective role of *Tephrosia purpurea* or delay diabetic nephropathy development.

CONCLUSION

In the present study, administration of ethanolic whole plant extract of *Tephrosia purpurea* to STZ-nicotinamide induced diabetic rats have significant reduction in blood glucose level, just about normalization of serum biochemical parameters including lipid profile (total cholesterol, triglycerides, high density lipoprotein, low density lipoprotein and very low density lipoprotein), serum liver enzymes (alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase), blood urea nitrogen and serum creatinine compared to STZ-nicotinamide induced diabetic rats by similar mechanism as glibenclamide, which involves insulin sensitization effect.

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