Research Article

An Evaluation of Anti Diabetic Potential and Physico-Phytochemical Properties of *Tephrosia purpurea* (Linn.) Pers in Streptozotocin Induced Diabetes Model in Albino Rats

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ABSTRACT

Tephrosia purpurea (Family: Fabaceae) is commonly used as medicine for treating many diseases in the native system of medicine. The hydro alcoholic and aqueous extract of leaf powder of this plant at dose level 400mg /kg b.w showed a good anti-diabetic potential as evidenced by changes in the biochemical parameters like glucose levels, phospholipids, total proteins, triglycerides, SGOT, and SGPT, after streptozotocin induced to rats. Histopathological examinations of the pancreatic tissue were done. The overall anti-diabetic potential exhibited by the extracts was found to be low compared to standard drug Glibenclamide at a dose level of 5mg / kg b.w. The hydro alcoholic extract was found to be more effective than alcoholic extract. Preliminary phytochemical analysis of this plant was also reported here. The results are highlighted and discussed.

Keywords: Anti-diabetic potential, Folk medicine, Physico-phytochemical parameters, Streptozotocin in rat model, *Tephrosia purpurea* (Linn) Pers.

INTRODUCTION

The diabetes mellitus is a growing health concern worldwide. The latest WHO publication (global burden of disease) estimates diabetes in adults to be around 173 million[1] and around two thirds of these live in developing countries.

It is a metabolic disease characterized by high-blood glucose levels resulting from defects in insulin secretion, insulin action or both.[2] Increase in sedentary lifestyle, consumption of energy-rich diet and obesity are some of the factors causing the rise in the number of diabetics.[3] Diabetes mellitus is currently is one of the most costly and burdensome chronic diseases and is a condition that is increasing in epidemic proportions throughout the world.[4] Renewed attention in recent decades to alternative medicines and natural therapies has stimulated a new wave of research interest in traditional practices. The plant kingdom has become a target for the search for new drugs and biologically active "lead" compounds.[5,6]

The plant *Tephrosia purpurea* has been used in different system of traditional medication for the treatment of diseases and ailments of human beings. *Tephrosia purpurea* (Linn) pers. (Family: Fabaceae) is known as wild indigo or fish poison in English, sharapunkha in Sanskrit and kolingi or kulluk-key-velai in Tamil. *Tephrosia purpurea* is a perennial herb found in dry gravelly or rockey and sandy soil. It is highly branched sub erect perennial herb.[7-9] The herb has white to purplish flowers and widely growing small plant found in the tropical regions.[10] Its aerial parts are used in bronchial asthma, hepatic ailments, cutaneous toxicities, pain and inflammation[11-13], anticarceogenic, antimicrobial activity, immunomodulation[14-16] and also few disease of kidney, liver, spleen, heart and blood.[17] Due to wide spread use of this plant by the folk people to treat several diseases and in the light of skimpy data on antidiabetic activity of above plant, the present study was undertaken to through more light on physico-chemical and pharmacological activities.

MATERIALS AND METHODS

Collection of Plant Material

Tephrosia purpurea leaves were collected during the months March and April from the fields of Poondi Thanjavur Dt (TN). The collected specimens were authenticated by a Director Dr.S.John Britto, S.J. The Rapinat Herbarium St. Joseph's College, Tiruchirapalli (T.N) and Botanist Dr.S.Rajan by comparing them with the herbium specimen of survey of Medicinal plants and collection unit (CCRH), Ooty. The voucher specimens were deposited in the herbarium of Department of Botany, A.V.V.M Sri Pushpam College (Autonomous), Poondi, Tamil Nadu, India for future reference. The work was carried out in the department of pharmacology, Periyar College of pharmaceutical sciences, Tiruchirappalli, Tamil Nadu, India. The clearance of Animal Ethical Committee has been obtained from the college. The sample was washed with distilled water and dried under shade, mechanically pounded to get coarse powder and passed through member 40 sieve mesh. The sample powders were processed in such a way that they are useful for carrying out powder studies and phyto chemical analysis.

Preparation of plant extracts

The coarse powder 100 grams of the given *T.purpurea* was extracted using 400ml of hydro alcohol (20:80) by

continuous hot percolation with help of soxhlet apparatus until the extraction procedure is complete. Similar extractions were done separately using each solvents namely water, ether, benzene, toluene and Chloroform following above procedure. The powder solvent ratio employed for the present study was 1:4. On completion, the extracts were filtered and the solvents were removed by distillation and dried under reduced pressure and controlled temperature 50-60°C and refrigerated the sample. The hydro alcoholic extract and other extract were subjected to various analysis such as organoleptic characters[18], fluorescence studies[19], physico-chemical parameters[20], and Preliminary photochemical screening.[21,22] The antidiabetic activity of the hydro alcoholic extract was evaluated as detailed below.

Evaluation of Toxicity

The LD_{50} study was carried out by Miller and Trainter method[23] for hydro alcoholic extract and result was reported.

Animal Studies

Albino rats of either sex weighing 150 to 200g belonging to Wistar strain were used in this study. The animals were procured from a registered animal dealer of the college (Sri Venkateshwara Enterprises, Bangalore). The rat pellet food was also supplied by the same firm (Hindustan Lever Company). The animals were acclimatized to the laboratory condition by subjecting them to dark and light cycles for 12hours period before commencement of work. All the animals were given food and water ad libitum.

Evaluation of Anti-diabetic activity

- Group I served as control 2 ml/kg b.w (Normal saline)
- Group II served as a diabetic control (Streptozotocin 120 mg/kg b.w induced)
- Group III received hydro alcoholic extract of *T.purpurea* 400 mg/kg body weight.
- Group IV received aqueous extract of *T.purpurea* 400 mg/kg body weight.
- Group V served as Reference standard drug Glibenclamide (5 mg/kg body weight)

Hyperglycemia was induced by a single intraperitonial injection of freshly prepared aqueous solution of Streptozotocin (SD Fine Chemicals Pvt Ltd.) at a dose of 120mg/kg body weight were given for Group II, Group III, Group IV and Group V, whereas Group I received, similar volume of vehicle (Normal saline) 2 mg/kg bw. The hydro alcoholic extract of 400mg/kg of b.w *T. purpurea* was administered for Group III, orally by using catheter after Streptozotocin induction. Similarly, for group IV 400kg/mg b.w of aqueous extract was given For Group V, reference standard Glibenclamide 5mg/kg b.w drug was given orally. Treatment continued for consecutive days. Before the treatment (0 day), and at the end of 2^{nd}, 4^{th}, 8^{th} and 16^{th} day plasma levels were estimated for glucose by glucose oxidase method[24] (Sharma 1997). The results were analyzed by student's t' test.

Histological studies

Diabetic animals were sacrificed at the sixteenth day after treatment and their pancreatic tissue were collected, washed with saline and placed in buffered formalin, followed by 70% alcohol solution for 24 hour. Tissue samples were fixed in Formalin and embedded in paraffin wax for light microscopic examination of HE sections. They were shown in Figure 1.

RESULTS AND DISCUSSION

Table 1-5 display the results of physico-chemical data's on the leaf of *T. purpurea*. The leaf powder appears green in colour and possesses astringent taste with raw smell odor and has rough texture. These powder when viewed under UV light at 365nm appear dark green in colour and under normal light, it appears in light green colour. After treating with various biochemical reagents, they displayed different ranging of colour variations.

The moisture content value is comparatively less when compare to total ash and water soluble ash values. The hydro alcoholic soluble extractive values and water soluble extractive value are higher when compare to non-polar solvents like benzene, toluene and chloroform. Preliminary phytochemical screening of *T. purpurea* leaf powder using various solvents is recorded. Majority of the constituents like flavonoids, glycosides, carbohydrates, proteins, alkaloids are present in hydro alcoholic and aqueous extract. The tannin is absent in all the extracts.

Table 6 shows the LD_{50} values of *T. purpurea* hydro alcoholic extract and aqueous extract which were found to be 2000 mg/kg bw for both extracts. $1/5^{th}$ of this dose was selected as initial dose 400mg/kg bw was choosen as initial dose in this work.

Table 7 summaries antidiabetic activity of *T.purpurea* leaf powder hydro alcoholic and aqueous extract on streptozotocin induced diabetic rats. The glucose level in mg/l are recorded on before the treatment (0 day), 2^{nd}, 4^{th}, 8^{th} and 16^{th} day. It is clear from the results that T.purpurea leaf powder of hydro alcoholic extract and aqueous extract showed antidiabetic potential as evidenced by the reduction of glucose levels $p<0.001$, but the reduction in glucose levels are comparatively lesser than standard drug Glibenclamide.

Table 8 summarizes the various biochemical parameters like phospholipids, total proteins, triglycerides, cholesterol, SGOT, and SGPT in albino rats. These parameters are elevated in diabetic control rats but they are reduced in *T.purpurea* extract, given rats.

Histopathological studies are shown on (figure 1). The regeneration of pancreatic tissue architecture is seen in the extract treated rat tissue with *T.purpurea* in those animals diabetes was induced by streptozotocin.

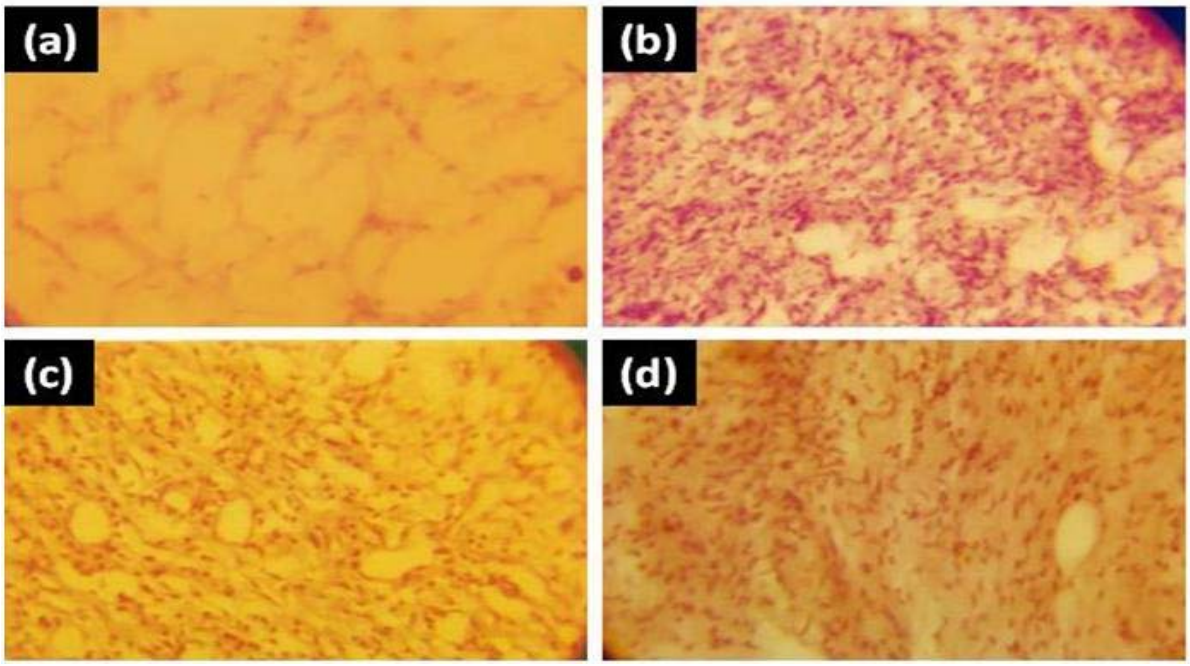

Figure 1 (a): Pancress structure of control**; (b):** Pancreatic structure after induction of diabetic control; **(c):** Showing the regeneration**; (d):** Showing the regeneration of pancreatic tissue by *T. purpurea* leaf the pancreatic tissues by the powder hydro alcoholic extract T.purpurea leaf aqueous extract.

Table 1: Data on organoleptic characters of *Tephrosia purpurea (Linn.) pers* (leaf powder)

Characters	*Tephrosia Purpurea*
Colour	Green
Odour	Raw Smell
Taste	Astringent
Texture	Rough

Table 2: Data on fluorescence Studies of *Tephrosia purpurea (Linn.)* pers (leaf powder)

Characters	Day light	UV light at 365nm
Sample as such	Light Green	Dark Green
Sample + 1N sodium hydroxide	Yellowish Green	Fluorescence Green
Sample + 1N Hydrochloric acid	Green	Dark Green
Sample +50% Sulphuric acid	Light Green	Greenish

Table 3: Physico-chemical characteristics of *Tephrosia purpurea (Linn.) pers* (leaf powder)

Characters	*Tephrosia purpurea(Linn.) pers*
Total ash	0.146
Water soluble ash	0.067
Acid insoluble ash	0.030
Sulphated ash	0.016
Moisture content	0.024

*Estimation values for 1g of the sample

Table 4: The plant drugs of *Tephrosia purpurea (Linn.) pers. leaf powder showing different extractive values in various solvents.*

Characters	*Tephrosia purpurea(Linn.) pers*
Hydroalcoholic soluble extractive	1.10
Water soluble extractive	0.987
Benzene soluble extractive	0.444
Toluene soluble extractive	0.242
Choloform soluble extractive	0.643

*Estimation values for 5g of the sample

Table 5: Qualitative phytochemical analysis of *Tephrosia purpurea* (Linn.) pers. Leaf powder

Characters	Benzene	Chloroform	Hydroalcohol	Aqueous
Alkaloids	+	+	+	+
Flavonoids	-	+	+	+
Terpenoids	-	-	-	-
Glycosides	+	-	+	+
Saponins	+	-	+	-
Tannins	-	-	-	-
Carbohydrate	+	+	+	+
Proteins	-	+	+	+

(+) indicates presence; (-) indicates absence

Table 6: LD_{50} value of hydro alcoholic extract of the plants (by oral route)

Test compounds	LD_{50} value (mg/kg)
Tephrosia purpurea (Linn.) pers. Leaf powder	2000

Table 7: Anti-Diabetic activity of Samples *Tephrosia purpurea(Linn.) pers.*on Streptozotocin diabetic rats (Glucose levels in mg/ l)

Sampling time	Control Normal Saline	Diabetic Control	Plant extract		Glibenclamide (5mg/Kg) b.w
			T.purpurea hydro alcoholic extract 400 mg/Kg b.w	*T. purpurea* aqueous extract 400 mg/Kg b.w	
0 day	86.4±1.18	304.0±12.4	316.4±12.4	322.2±9.8	306.8±13.0
2 day	86.22±1.24	310.8±12.7	298.0±13.6	308.4±12.8	260.8±P14.2
4 day	85.2±1.12	320.3±12.2	272.4±12.0	288.12±12.04	212.4±12.8
8th day	86.79±0.92	359.6±12.9	242.8*±10.8	252.4*±12.0	172.2*±12.0
16th day	86.66±1.48	358.2±11.9	210.80*±12.0	222.2*±10.42	140.4*±12.6

Data are expressed as mean ± S.E., n=6; P<0.001 Vs Standard drug by student's test

Table 8: Various biochemical parameters for the anti-diabetic activity in albino rats

Parameter/ Group	Phospholipids (mg/dl)	Total protein (g/dl)	Triglycerides (mg/dl)	Cholesterol (mg/dl)	SGOT (U/L)	SGPT (U/L)
Control	46.4±5.9	1.6±0.04	28.7±4.1	87.6±2.8	96.5±2.5	34.6±1.4
Diabetic Control	84.6±6.2	4.2±0.22	44.5±3.2	121.2±5.8	97.3±1.18	35.08±0.2
Tephrosia purpurea Hydroalcoholic Extract 400mg/Kg	52.2±0.8	1.8±0.02	39.4±1.8	96.08±2.8	82.4±1.4	32.2±0.08
Tephrosia purpurea aqueous extract 400mg/Kg	72.0±2.6	4.2±0.10	42.2±1.8	124.2±4.4	104.4±3.0	38.02±1.02

CONCLUSION

The results of the present study indicate the streptozotocin can act as a effective diabetogenic agent.[25] It is cytotoxic to beta cells of islets of langerhans and is capable of inducing chemical diabetes in wide variety of animal species through damage of insulin secreting cell.[26] Diabetes mellitus is associated with an increase risk of developing premature atherosclerosis due to increase in plasma triglycerides and cholesterol.[27]

Cholesterol enrichment in red cells resulting in loss in membrane fluidity has been reported.[28] Bhandaru et al[29] 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 consequence of increased glucose and insulin deficiency in plasma, hepatic regulation of lipid metabolism is greatly altered.[30] The liver is the major organ that can catabolize and excrete quantitatively important amounts of cholesterol. 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.[31]

The hydro alcoholic extract of *T.purpurea* leaf powder showed significantly the decrease in glucose level at 2nd, 4th, 8th, 16th day in hyperglycemic animals (P<0.001) than aqueous extract. However the reduction by both extracts is lesser than standard drug Glibenclamide.

The various biochemical parameters like phospholipids, total proteins, triglycerides, cholesterol, SGOT, SGPT levels were increased due to diabetic induction by streptozotocin and these levels were reduced by the *T.purpurea* extracts. The reduction is comparatively more in hydro alcoholic extract than aqueous extract and it was also confirmed through histopathological studies.

However further studies are necessary for the identification of bioactive principles responsible for antidiabetic property in the study plant. Those extracts are to be studied at still higher who levels to throw more light on their anti-diabetic activity.

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