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EXPERIMENTAL EVALUATION OF ORAL ANTIDIABETIC ACTIVITY OF WHOLE PLANT EXTRACT OF TEPHROSIA PURPUREA IN STZ – NICOTINAMIDE INDUCED DIABETIC RATS

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ABSTRACT

INTRODUCTION: Diabetes mellitus is one of the world's major public health burdens. In 2000, there were around 171 million diabetes causes and it is estimated that the number will double by 2030. Many herbal medicines as single agents or in different oral formulations have been recommended for diabetes mellitus because they are less toxic than oral hypoglycemic agents such as sulfonylureas, metformin etc. Tephrosia purpurea Linn. (Fabaceae), is a pantropical, polymorphic, branches, sub-erect, perennial herb popularly known as Sarapunkha in Sanskrit, Purple Tephrosia in English. Various parts of T. purpura have been reported to produce antihyperglycemic activity in various animal models. The present study is designed to explore the anti-hyperglycaemic activity of whole plant extract of Tephrosia purpurea in laboratory animals.

AIM To study the antidiabetic activity of ethanolic extract of Tephrosia purpurea whole plant against streptozotocin – nicotinamide induced diabetes in Wistar rats and to assess the efficacy in comparison to the standard oral antidiabetic agent.

MATERIALS AND METHODS: In this study, 70% alcoholic extract of the whole plant Tephrosia purpurea will be evaluated for its antidiabetic activity in STZ and Nicotinamide induced Wistar Albino Rats. All the 30 animals will be divided into five groups and each group carries 6 animals. The first group will be treated as normal control rats (uninduced, untreated). In the remaining four groups, STZ will be injected (i.p) 50mg/kg in normal saline in a volume of 1ml/kg after the i.p. administration of 120mg/kg body weight Nicotinamide. Two weeks after STZ administration, rats with blood glucose concentrations> 140mg/dl are considered diabetic and will be included in the study. The second group will be diabetic induced untreated rats. Simultaneously, the third group will be treated with glibenclamide orally (5mg/kg). The fourth and fifth groups will be treated with the 70% alcoholic extract of whole plant Tephrosia purpurea at 200mg/kg& 400mg/kg orally. Blood glucose will be measured at different time intervals (0, 5th, 10th, 15th and 30th day). On the 31st day, blood is collected through sinus puncture under phenobarbitone anaesthesia into a heparinized tube and serum was separated by centrifugation for various biochemical parameters.

RESULTS Results are expressed as mean ± SEM. Data is analysed by using one way of analysis of variance(ANOVA) followed by Dunnet's t-test. P values < 0.05 are considered as significant. In diabetic rats, treated with 200 and 400 mg/kg of Tephrosia purpurea blood glucose level significantly lowered to 157.17±5.55 on the 10th day (P<0.05) and to 189.00±5.05 on the 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.

CONCLUSION Modern pharmacological and clinical investigation of Tephrosia purpurea is a valuable herbal therapy that has anti-diabetic, anti-oxidant, anti-microbial, anti-inflammatory and anti-viral properties. The phytochemical investigation on TP has revealed the presence of glycosides, carotenoids, isoflavones, flavanones and flavanoids. The present study is to analyse the effect of whole plant extract of Tephrosia Purpurea in the reduction of blood glucose levels.

KEYWORDS : Tephrosia purpurea, Blood glucose, Anti-hyperglycemic, STZ-Nicotinamide

INTRODUCTION:

Diabetes mellitus is one of the major public health burdens 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]In 2000, there were around 171 million diabetes cases and it is estimated that the number will be doubled by 2030. It has been reported that India (31.7 million) [2] topped the world with the highest number of diabetic patients followed by China (20.8 million) and the US(7.7 million).[3,4]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.[5] Various animal models like genetic models, fat-fed diet models, and chemical-induced diabetes models are available with their advantages. STZ/Alloxan induced diabetic model is the one that is commonly used in experiments.[7] The emerging ideal model for anti-diabetic activity is STZ-Nicotinamide induced model since has the advantage of the reduction in mortality.[6] *Tephrosia purpurea* Linn is a pantropical, polymorphic, perennial herb that belongs to the family Fabaceae popularly known as Saraphunkha in Sanskrit, Purple tephrosia in English with medicinal properties.[8] Aqueous and ethanolic extract of seeds have anti-hyperglycemic activity. Though there are many studies available, lack of information on the utility of whole plant extract of *Tephrosia purpurea* in the treatment of diabetes and their safety in renal and hepatic profiles. Hence the present study is designed to explore the anti-diabetic activity and its safety in STZ- Nicotinamide induced diabetic rats.

MATERIALS AND METHODS:

Plant Materials

Collection & Identification

The whole plant, *Tephrosia purpurea Linn.* was collected from the roadside of Erode, Tamilnadu, in October 2016. 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.

Figure 1 ***Tephrosia purpurea Linn***

Extraction of Plant Material

The collected *Tephrosia purpurea* was washed in running tap water to remove the soil debris, shade dried and grounded using a mechanical blender to get a 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 sunlight 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 were housed in the 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±2°C and relative humidity of 30 – 70 %. A 12:12 light: day cycle was followed. All animals were allowed free access to water and fed with standard commercial pelleted rat chaw (M/s. Hindustan Lever Ltd, Mumbai). All the experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Ethics Committee (1818/GO/Ere/S/15/CPCSEA) and in accordance with the Institutional ethical guidelines.

Experimental Induction of Diabetes in Rats

Diabetes was induced experimentally in 12 hour fasted rats by a single intraperitoneal injection of Streptozotocin (50mg/kg) dissolved in 0.1M of citrate buffer (pH 4.5), followed by intraperitoneal administration of Nicotinamide (120 mg/kg) after 15 minutes. Since STZ is capable of inducing fatal hypoglycaemia due to the 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 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.

Experimental Design

After the successful induction of experimental diabetes, the rats were divided into five groups each comprising a minimum of six rats (Table 1).

Table 1 – Study animal groups

Group I	Normal rats received 0.1 % Carboxyl Methyl Cellulose Solution (1mg/kg) as a vehicle through the oral route.
Group II	Rats with STZ – Nicotinamide induced diabetes that was left untreated.
Group III	Rats with STZ – Nicotinamide induced diabetes that was treated for 30 days with orally administered Glibenclamide (5mg/kg)
Group IV	Rats with STZ – Nicotinamide induced diabetes were treated for 30 days with orally administered ethanolic extract of *Tephrosia purpurea* (200mg/kg).
Group V	Rats with STZ – Nicotinamide induced diabetes were treated for 30 days with orally administered ethanolic extract of *Tephrosia purpurea* (400mg/kg).

During the study, blood glucose levels were measured at different time intervals (0, 5th, 10th, 15th and 30th day) with strict aseptic precautions. At the end of the experimental period, the rats were fasted overnight, anaesthetized with pentobarbitone sodium and the blood was collected by retro-orbital puncture in non-heparinized tubes.

STATISTICALANALYSIS

Results were represented as mean ± SEM. The data were analysed by using one-way analysis of variance (ANOVA) followed by Dunnet's't' test using graph Pad version 3. P values < 0.05 were considered as significant.

RESULTS:

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 in table 2. The blood sugar levels of diabetic control rats were higher than those of normal rats on the 0, 5th, 10th, 20th and 30th days. In diabetic rats, treated with 200 and 400 mg/kg of *Tephrosia purpurea* blood glucose level significantly lowered to 157.17±5.55 on the 10th day (P<0.05) and to 189.00±5.05 on the 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 the 5th day. From the 15th day onwards until the end of the drug treatment, on the 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.

Table No 2. Effect of Ethanolic Extract of *Tephrosia purpurea* on Blood Sugar Levels of STZ-Nicotinamide induced Diabetes in Rats

Drug Treatment	Mean Blood Sugar Level (mg/dl)						
	Before STZ + Nicotinamide	After STZ+ Nicotinamide	0 Day	5th Day	10th Day	15th Day	30th Day
Control 1% CMC	105.83 ± 3.22	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	101.50 ± 4.59	213.00± 4.07	220.50 ± 5.83	228.83 ± 3.17	216.33 ± 5.83	226.50 ± 2.57	222.17 ± 5.67
Reference Control	105.17 ± 5.41	212.00± 4.56	219.67 ± 3.81	184.50 ±3.89**	143.17± 2.73***	112.67± 5.57***	103.33± 5.34***
TP 200	100.67 ± 4.26	211.33± 4.49	219.00 ± 3.45	210.00 ± 4.07	157.17 ± 5.55*	129.17± 2.09***	114.17± 3.28***
TP 400	98.67± 3.45	208.83± 6.09	217.50 ± 4.86	189.00± 5.05**	150.83± 6.04**	119.67± 4.36***	100.17± 4.85***

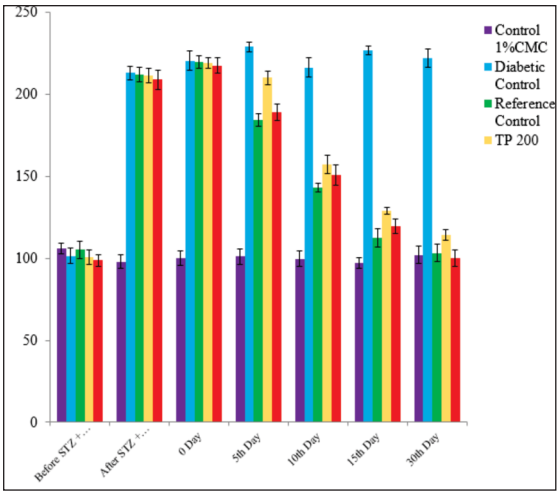

Figure No.2 Effect of Ethanolic Extract of *Tephrosia purpurea* on Blood Sugar Levels

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 β-cell damage, whereas Nicotinamide is administered to rats to partially protect insulin-secreting cells against STZ.[5] STZ is transported into β-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, the exaggerated activity of this enzyme results in depletion of intracellular NAD (+) and ATP, and the insulin-secreting cells undergo necrosis.[7] 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.[9] *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.[5]

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,[9] by stimulation of regeneration process or increase pancreatic secretion of insulin from existing β-cells[8] and/ or inhibition activity against α-glucosidase enzymes in the small intestine. *Tephrosia purpurea* at the dose of 400mg/kg exhibited a significant decrease in blood glucose level, as compared to 200mg/kg on the 5th day of drug administration. The result was comparable with the standard drug glibenclamide which reduced fasting blood glucose levels on the same day. Moreover, both the doses of *Tephrosia*

purpurea showed significant blood glucose reduction in STZ – nicotinamide induced diabetic rats on day 10^{th}, 15^{th} and 30^{th} days compared to diabetic control.

Tremendous studies have found that flavonoids originating from foods could improve glucose metabolism, lipid profile, regulating the hormones and enzymes in the human body. In our findings, the antidiabetic activity exhibited by ethanolic extract of Tephrosia *purpurea* might be due to the presence of flavonoids in it.

CONCLUSION

In the present study, administration of ethanolic whole plant extract of *Tephrosia purpurea* to STZ – nicotinamide induced diabetic rats have a significant reduction in blood glucose level. Consequently, it can be concluded that *Tephrosia purpurea* exhibited antidiabetic activity in a dose-dependent manner against STZ – nicotinamide induced diabetes in Wistar albino rats thereby authenticating its ethnomedicinal practice. From the observed antidiabetic activity of *Tephrosia purpurea* whole plant extract against STZ – nicotinamide induced diabetic rats, the study may support the use of it for the management of diabetes mellitus.

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