

IN VIVO AND IN VITRO EVALUATION OF *TEPHROSIA CALOPHYLLA* FOR ANTI-DIABETIC PROPERTIES

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ABSTRACT

Objective: The objective the present work was to investigate *in vivo* and *in vitro* anti-diabetic potentials of methanol extract of *Tephrosia calophylla* against alloxan-induced diabetes in albino rats.

Methods: For *in vivo* evaluation, diabetes was induced in albino rats by administering a single dose of alloxan. The study was designed to test the acute effect of methanol extract of *Tephrosia calophylla* (TCME) to reduce blood glucose in OGTT. The chronic study of 21 d was performed against diabetic rats and blood glucose was determined at 1st, 7th, 14th and 21st day. In chronic *in vivo* study, serum concentrations of insulin, urea, creatinine, total cholesterol, triglycerides, ALT and AST were also estimated at 21st day. The *in vitro* α -glucosidase inhibitory activity and α -amylase inhibitory activity were performed and IC₅₀ values of the extract was determined. The glucose uptake by rat hemidiaphragm model was also used test potentials of the extract to increase utilization of the glucose by tissues.

Results: In OGTT, standard glibenclamide and TCME at 400 mg/kg treated animals have shown significant reduction in blood glucose at 90 min but at 120 min, blood glucose level (BGL) was significantly reduced in glibenclamide and TCME at 200 mg/kg and 400 mg/kg treated animals compared to diabetic control group. In chronic model the methanol extract effectively reduced blood glucose levels (P<0.001) at 14th and 21st day of study in therapeutic groups and effect was comparable to that of standard. The extract could also significantly (P<0.001) reduce concentrations of SGOT, triglycerides (TGs), Total cholesterol (TC) and urea in serum and significantly (P<0.001) increased the insulin level in blood which proves beneficial effects of the extract in diabetes. The change in concentrations of SGPT and urea were less significant (P>0.01). *In vitro* studies, against both α -glucosidase and α -amylase inhibitory activities, extract has shown significant IC₅₀ values and it also enhanced glucose utilization by rat hemidiaphragm.

Conclusion: The results obtained from the present study suggest that, the methanol extract of *Tephrosia calophylla* possess significant *in vivo* anti-diabetic properties against alloxan induced diabetes in rats. The results also suggests that, TCME also possess the significant *in vitro* anti-dabetic potentials.

Keywords: Anticancer activity, *Tephrosia calophylla*, IC₅₀ value, MTT assay and antioxidant activity

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INTRODUCTION

Diabetes mellitus is a pathological condition characterized by metabolic alterations leads to hyperglycemia and other complications due to defects in secretion of insulin, action or both. There are two types of diabetes mellitus: Type I and Type II. Type I is known as insulin dependent diabetes mellitus often referred to as juvenile onset diabetes characterized by absolute deficiency of insulin due destruction of pancreatic cells and the Type II, which is non-insulin dependent, usually develops in adults over the age of 40. It has already been established that chronic hyperglycemia of diabetes is associated with long term damage due to insulin resistance or defect in release of insulin from pancreas [1, 2]. It has an adverse effect on carbohydrate, lipid and protein metabolism resulting in chronic hyperglycemia and abnormality of lipid profile. These lead to series of secondary complications including polyurea, polyphasia, ketosis, retinopathy as well as cardiovascular disorder [3]. The oral hypoglycemic and other synthetic drugs used in diabetes have their own serious adverse effects. Hence in spite of the introduction and extensive utilization of hypoglycemic agents, diabetes and the related complications continue to be a major health problem worldwide, which is affecting nearly 10% of the population all over the world [4]. In this regard medicinal plants continue to be an important therapeutic aid for alleviating ailments of humankind such as diabetes mellitus due to their less or no adverse effects. Over the last 2500 y, there have been very strong traditional systems of medicine such as Ayurvedic, Chinese, and the Unani, born and practiced, more in the eastern continent [5]. The *Tephrosia* is a genus of plant which is of Indian origin. The various

species of *Tephrosia* are medicinally important and have been proved for their several pharmacological activities [6,7]. The *Tephrosia purpurea* belongs to the same genus frequently used in traditional system, considered to be medicinally important and proved for many health benefits such as anti-diabetic, anti-cancer, anti-ulcer, antihyperlipidemic, anti-bacterial and many purposes. The *Tephrosia calophylla* belongs to same also a important component of traditional system of medicine ayurveda for the treatment of diabetes but has lack of scientific evidence for its antidiabetic potential [8, 9]. Hence it was necessary to provide a clear background proof for the beneficial property of the plant in diabetes. In this attempt, study had been conducted to determine *in vitro* and *in vivo* anti-diabetic potentials of methanol extract of areal parts of *Tephrosia calophylla*.

MATERIALS AND METHODS

Plant material

The areal parts of *Tephrosia calophylla* have been collected from Sri Venkateshwara University, Tirupati, India and dried under shade. The leaves were identified and authenticated by Dr. Madhavachetty Asst. Prof. Dept. of Botany and specimen (Herbarium number 977) were preserved at institute herbarium library. The leaves part were separated from other parts, washed, cleaned and dried for further use.

Preparation of extract

The shade dried leaves were pulverised into powder and sieved through No. 22 mesh. About 350 g (appx.) of coarse powder was

subjected to successive solvent extraction using petroleum ether, benzene, chloroform and methanol in soxhlet's apparatus [10].

Preliminary phytochemical investigation

The preliminary phytochemical investigation for the methanolic extract of *Tephrosia calophylla* had been conducted as per procedure prescribed by Khandelwal [11].

Drugs and chemicals

All reagents and chemicals used in the study were obtained commercially and were of analytical grade. Alloxan was obtained from Sigma Laboratory, India and Glibenclamide was procured from Aventis Pharma Ltd., India.

Animals

The healthy albino wistar male rats (180-220g) were procured from Sri Venkateswara Enterprises, Bangalore housed under standard conditions of temperature (22±10°C), relative humidity (55±10%), 12 h light/dark cycles and fed with standard pellet diet (Amrut, Pranav Agro Industries Ltd., Sangli, India) and water ad libitum. After randomization into various groups and before initiation of the experiment, the rats were acclimatized for a period of 7 d under above said environmental conditions. The experimental protocol has been approved by the Institutional Animals Ethics Committee, IJAHSM, Bangalore (Ref. no. IJAHSM/IAEC/2014/03) with the permission from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Acute oral toxicity studies

The OECD guidelines 423 (up and down procedure) were used to determine acute oral toxicity for methanol extract of *Tephrosia calophylla*. A starting dose used was 2000 mg/kg body weight p. o. of extract (TCME) was administered to 3 male rats, observed for 14 d. The experiments were repeated again with the same dose level, 2000 mg/kg body weight p. o. of extracts for 3 d more, and observed for 14 d [12].

Evaluation of *in vivo* anti-diabetic activity

Induction of diabetes in experimental animals

In both acute and chronic models, rats were made diabetic by a single intraperitoneal injection of alloxan monohydrate (150 mg/kg). Alloxan was first weighed individually for each animal according to the body weight and then solubilized with 0.2 ml saline (154 mmol NaCl) just prior to injection. Two days after alloxan injection, rats with plasma glucose levels of >140 mg/dl were included in the study. Treatment with plant extracts was started 48 h after alloxan injection [13-15].

Group design

For both OGTT and chronic study, the animals were divided into six groups consisting of six animals in each and all the animals except normal (Group I) were induced diabetes by administering a single dose of alloxan as explained above. The animal grouping is as follows.

- Group I: Normal control treated with normal saline (10 ml p. o) alone,
- Group II: Diabetic control treated with alloxan and vehicle Tween20,
- Group III: Standard treated alloxan and Glibenclamide 5 mg/kg,
- Group IV: TCME (Low dose) treated alloxan and methanol extract of *Tephrosiacalophylla* 100 mg/kg, p. o
- Group V: TCME (Medium dose) treated alloxan and methanol extract of *Tephrosiacalophylla* 200 mg/kg, p. o
- Group VI: TCME (High dose) treated alloxan and methanol extract of *Tephrosiacalophylla* 400 mg/kg, p. o

Oral glucose tolerance test [OGTT]

At third day after inducing diabetes in animals, the suspensions of standard drug glibenclamide and extract were prepared using Tween20 as suspending agent and administered to respective animals with help of oral feeding tubes according to below protocol (Koteeswara Rao *et al.*, 2006). One hour after administration of extract and glibenclamide, the blood samples were collected from all six group of animals and the basal blood glucose was determined. All rats were fed with oral glucose solution (2g/kg) and blood samples from each rat were collected at different intervals of 30 min, 60 min, 90 min and 120 min and estimated for blood glucose using Glucometer (Accucheck) [16-18].

Chronic study model

In a chronic study also animals were divided into six groups as above. The standard drug glibenclamide and methanol extract of *Tephrosia calophylla* were administered to respective animals according to their body weights from 1st day to 21st d.

Blood samples from each rat were collected on day 1st, 7th, 14th and 21st and estimated for blood glucose. On last day of study blood samples had been also estimated for insulin, cholesterol, Triglycerides, creatinine, urea, ALT and AST [19-20].

Collection of blood sample and estimation of parameters

Blood samples were collected from retro-orbital plexus under mild ether anesthesia from rats. The blood glucose estimated using Glucometer (Accucheck) On the 21st day, serum was separated from blood samples and analyzed serum for cholesterol, triglycerides by enzymatic DHBS colourimetric method and ALT, AST, urea, creatinine and insulin were estimated using standard kits.

Evaluation of *in vitro* anti-diabetic activity

α -Glucosidase inhibitory assay

This assay was carried out to investigate the *in vitro* inhibitory activity of TCME on sucrase and maltase (α -glucosidases). Although α -glucosidase isolated from yeast is extensively used as a screening material for α -glucosidase inhibitors, the results did not always agree with those obtained in mammals. Hence in present study small intestine homogenate of albino mouse was used as an α -glucosidase solution since it speculated that it would better reflect the *in vivo* state. The inhibitory effect was measured by slightly modifying the method used by "Dahlqvist" [19]. After 20 h of fasting, part of the animals' small intestine immediately below the duodenum and immediately above the cecum was cut, rinsed with ice-cold saline, and homogenized with 12 ml of maleate buffer (100 mmol, pH 6). The homogenate was used as an α -glucosidase solution. The assay mixture consisted of 100 mmol maleate buffer (pH 6), 2% (w/v) of each sugar substrate solution (100 ml), and the sample extract (20-640 μ g/ml). The mixture was preincubated for 5 min at 37 °C, and the reaction was initiated by adding crude α -glucosidase solution (50 ml), followed by incubating the mixture again for 10 min at 37 °C. The amount of glucose released in this reaction was determined by a commercially available glucose estimation kit (Span Diagnostic Ltd., Mumbai, India). The amount of glucose produced by the positive control (GCP), glucose production value in the blank (GCB) and amount of glucose produced by the addition of TCME (GCT) were recorded [21-23]. The rate of carbohydrate decomposition was calculated as a percentage ratio to the amount of glucose obtained when the carbohydrate was completely digested. The rate of prevention was calculated by the following formula:

$$\text{Inhibition rate (\%)} = \frac{\text{GCP} - \text{GCT} - \text{GCB}}{\text{GCP}} \times 100$$

α -amylase inhibitory assay

Test samples of TCME (6.25, 12.5, 25, 50, 100, 200 mg/ml) and nojirimycin (6.25-200 μ g/ml) of 500 ml concentration were added to 500 ml of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) containing 0.5 mg/ml porcine pancreatic α -amylase solution (Sigma Chemical Co., St. Louis, MO, USA) and were incubated at 25 °C for 10 min. After the preincubation, 500 ml of 1%

starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) was added to each tube at prespecified intervals. The reaction mixtures were then incubated at 25 °C for 10 min. The reaction was stopped by adding 1 ml of 3,5-dinitrosalicylic acid color reagent. The test tubes were then incubated in a boiling water bath for 5 min and cooled down to room temperature. The reaction mixture was then diluted after adding 10 ml of distilled water and absorbance was measured at 540 nm [21-23].

$$\% \text{ inhibition} = \frac{\text{Abs(Control)}(540) - \text{Abs(Extract)}(540)}{\text{Abs(Control)}(540)}$$

Glucose uptake by isolated rat hemidiaphragm

Glucose uptake by rat hemidiaphragm was estimated according to earlier works [27, 28], but with some modifications. Four groups, with each group containing six graduated test tubes (n=6), were considered as follows:

- Group 1: 2 ml of Tyrode solution with 2% glucose.
- Group 2: 2 ml of Tyrode solution with 2% glucose and regular insulin solution (Novo Nordisk; 0.62 ml of 0.4 U/ml).
- Group 3: 2 ml of Tyrode solution and 1.38 ml of TCME (0.1% v/v).
- Group 4: 2 ml of Tyrode solution with 2% glucose and regular insulin (0.62 ml of 0.4 U/ml) solution and 1.38 ml of TCME (0.1% v/v).

The volumes of all the test tubes were made up to 4 ml by adding distilled water to match the volume of the test tubes in Group 4. A total of 12 albino rats were fasted overnight and decapitated. The diaphragms were quickly dissected with minimal trauma and divided into two halves. Two diaphragms from the same animal were not used for the same set of experiments. Six diaphragms were used for each group. The hemi diaphragms were placed in test tubes and incubated for 30 min at 37 °C in an atmosphere of 100% oxygen and were shaken at a speed of 140 cycles/minute. Glucose uptake per gram of tissue was calculated as the difference between the initial and final glucose content in the incubated medium [24].

Table 1: Effect of methanol extracts of *Tephrosia calophylla* on blood glucose OGTT

Treatment	Concentration of blood glucose (mg/dl)				
	0 Min	30 Min	60 Min	90 Min	120 Min
Normal Control	81.17±1.352	133.5±1.839	129.2±1.740	105.8±1.956	79.67±2.789
Diabetic Control	171.8±3.877	281.0±3.578	254.2±1.641	229.0±2.620	214.8±2.088
Standard (Glibenclamide 5 mg/kg)	174.7±2.963	279.2±3.911	199.7±3.442	158.8±4.729	119.8±4.936
TCME 100 mg/kg	170.7±3.263	273.3±3.584	255.5±2.643	226.3±3.499	182.8±5.108
TCME 200 mg/kg	169.2±6.488	269.3±4.341	224.0±3.856	180.3±3.703	157.2±6.467
TCME 400 mg/kg	167.2±4.354	268.8±5.683	179.8±1.470	150.0±3.109	114.0±4.960

Values are mean±SEM n=6 symbols represent statistical significance, ^{ns}p>0.05, *p<0.05, **p<0.01, ***p<0.001 vs diabetic control, ^{ns}p>0.05, +p<0.05, ++p<0.01, +++p<0.001 normal control vs positive control.

Determination of chronic anti-diabetic activity

In chronic study, the blood glucose level was significantly (P<0.001) increased in diabetic control animals compared to normal animals due to the induction of glucose. While treatment with glibenclamide and TCME extract at 200 mg/kg and 400 mg/kg could significantly (P<0.001) reduced blood glucose concentrations compared to diabetic control animals at 14th and 21st day of the study [table 2].

The significant (P<0.001) decline of serum insulin was found in diabetic control animals compare to normal animals due to the administration of alloxan. In animals treated with glibenclamide and TCME (200 mg/kg and 400 mg/kg) there was significant (P<0.001) increasing in blood insulin level compared to diabetic control animals and the results were comparable to normal animals.

The total cholesterol, triglycerides, urea and creatinine levels in the blood were significantly (P<0.01) increased in diabetic compare to normal animals. But reduction in the serum cholesterol,

Statistical analysis

The data obtained in the study was analyzed by ANOVA and post hoc Dunnet's t-test using Graphpad prism5 software. All the values were expressed as mean±standard error of the mean (SEM).

RESULTS

Preliminary phytochemical study

The percentage yield of the TCME was found to be 7.84% w/w. The preliminary phytochemical investigation for the methanol extract of *Tephrosia calophylla* reveals the presence of polyphenols, flavonoids, tannins, steroids, alkaloids and carbohydrates in the leaves.

Acute toxicity studies

The methanol extract was safe up to a dose of 2000 mg kg⁻¹ b.w. and caused neither mortality nor any signs of clinical abnormality in the tested animals during the observation period of 14 d after administration of the highest dose. There was no considerable change in body weight before and after treatment of the experiment and no signs of toxicity were observed. When the experiments were repeated again with the same dose level, 2000 mg/kg body weight p. o. of extracts for 3 d more, no changes were observed for 14 d. As per the results obtained in an acute oral toxicity study doses were selected as 100, 200 and 400 mg/kg on the ratio 1/20th, 1/10th and 1/5th respectively.

Evaluation of *in vivo* anti-diabetic activity

Oral glucose tolerance test

In acute study (OGTT) animals in a diabetic alone group have shown significantly elevated their blood glucose level through entire study when compared to normal animals. But treatment with standard drug glibenclamide and methanolic extract (200 mg/kg and 400 mg/kg) of *Tephrosia calophylla* could able to improve utilization of oral glucose by animals and significantly (P<0.001) reduced blood glucose level in therapeutic groups after 60 min and 120 min. The results of OGTT have shown in [table 1].

triglycerides, urea and creatinine concentration was observed in glibenclamide and TCME (200 mg/kg 400 mg/kg) treated animals when compared to diabetic control. It is found that there is no significant (p>0.01) change in AST and AL T levels in diabetic alone compare to normal animals and also no significant change was observed therapeutic animals treated with glibenclamide and TCME when compare to diabetic animals [table 3].

Evaluation of *in vitro* anti-diabetic activity

α-Glucosidase inhibitory activities

An *in the vitro* α-Glucosidase inhibitors potential of methanol extract of *Tephrosia calophylla* was examined. The half maximal inhibitory concentration (IC₅₀) values of sucrose and maltase inhibitory activities were found to be 399.733 μg/ml and 78.412 μg/ml respectively. The results in that TCME have shown strong activity in a dose-dependent manner and is thus inferred to be an effective α-glucosidase inhibitor [table 4 and fig. 1 and 2].

Table 2: Effect of methanol extracts of *Tephrosia calophylla* in the chronic study

Treatment	The concentration of blood glucose (mg/dl)			
	DAY 1	DAY 7	DAY 14	DAY 21
Normal Control	147.8±2.301	135.3±1.476	136.2±2.442	133.2±3.506
Diabetic Control	241.6***±2.113	236.2***±2.664	231.0***±2.033	236.7***±3.159
Standard (Glibenclamide 5 mg/kg)	234.2±6.290	211.8±4.143	171.5±3.374	145.2±1.740
TCME 100 mg/kg	238.5±6.329	223.5±5.898	217.2*±2.937	205.2*±4.840
TCME 200 mg/kg	236.7±4.958	218.3±3.575	190.2**±4.324	177.7***±2.848
TCME 400 mg/kg	226.8±7.109	198.7±5.524	175.8***±2.330	140.2***±3.637

Values are mean±SEM, n=6 symbols represent statistical significance. ^{ns}p>0.05, * p<0.05, ** p<0.01, ***p<0.001 vs diabetic control, ^{ns}p>0.05, +p<0.05, ++p<0.01, +++p<0.001 normal control vs positive control.

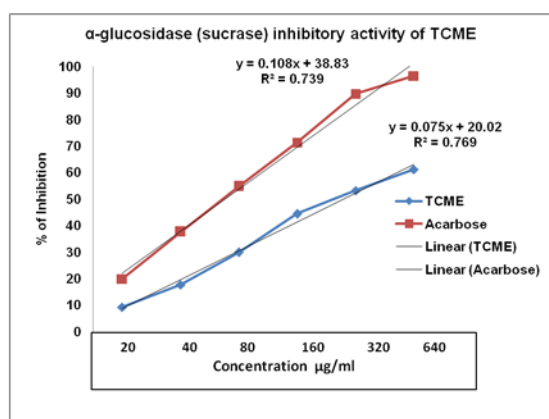
Table 3: Effect of methanol extracts of on serum parameters *Tephrosia calophylla* on blood glucose in chronic study

Treatment	Serum parameters						
	Insulin (IU/l)	Total Cholesterol (mg/dl)	Triglycerides (mg/dl)	Creatinine (mg/dl)	Urea (mg/dl)	ALT (IU/l)	AST (IU/l)
Normal Control	136.7±2.499	80.02±2.223	105.1±1.542	0.5417±0.01647	31.06±1.703	63.30±1.273	129.7±1.978
Toxic Control	66.50***±1.910	108.4***±2.724	131.8***±2.432	1.464***±0.05661	72.57***±1.302	62.37±1.372	134.0±2.385
Standard (Glibenclamide)	137.7***±2.741	79.57***±3.075	100.9***±2.994	0.6318***±0.03644	35.48***±1.855	63.09***±0.7677	126.7±2.212
TCME 100 mg/kg	93.50±3.631	106.2±3.444	126.4±5.142	1.181±0.03347	63.07±0.9838	62.34±2.356	132.4±2.832
TCME 200 mg/kg	107.2**±3.468	92.27**±2.214	110.0**±2.864	0.9072**±0.04888	45.52**±0.5945	62.65±1.392	132.6±1.525
TCME 400 mg/kg	138.7***±2.813	77.58***±2.355	105.1***±5.167	0.5757***±0.01777	32.33***±1.998	62.26±2.043	122.6±1.603

Values are mean±SEM, n=6 symbols represent statistical significance. ^{ns}p>0.05, * p<0.05, ** p<0.01, ***p<0.001 vs diabetic control, ^{ns}p>0.05, +p<0.05, ++p<0.01, +++p<0.001 normal control vs positive control.

Table 4: Effect of TCME on α -glucosidase (Sucrase and maltase) and amylase inhibitory activity

Treatment	IC ₅₀ values (μ g/ml)		
	α -Glucosidase (Sucrase)	α -Glucosidase (Maltase)	α -Amylase
TCME	399.733	78.412	135.042
Acarbose	103.425	98.33	--
Nojirimycin	--	--	37.258

Fig. 1: Effect of TCME on α -glucosidase (Sucrase) and amylase inhibitory activity

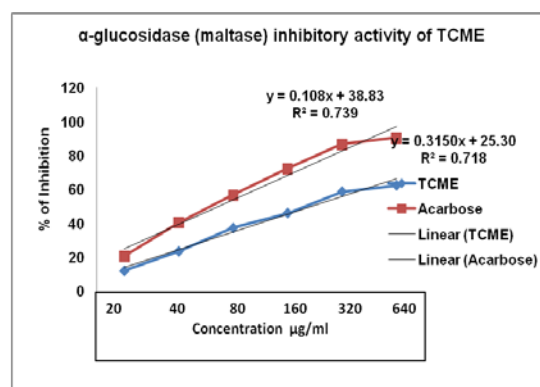
α -Amylase inhibitory activities

To amylase inhibitory effect of methanol extract of *Tephrosia calophylla* was tested to determine the ability of the drug to reduce postprandial glucose. In this study, TCME exhibited strong inhibitory activity against α -amylase with IC₅₀ of 135.042 μ g/ml, which is comparable with positive controls [table 4 and fig. 3].

Effect on peripheral glucose uptake

The results of the present study suggest glucose uptake (milligrams/gram tissue weight) in an isolated rat hemidiaphragm muscle in the presence of insulin (0.4 U/ml) and TCME (0.1% w/v). The results of this experiment indicate that the addition of TCME to

the incubation media (Tyrode solution) caused a significant increase in glucose uptake by the rat hemi diaphragm and was found to be less effective than insulin. Moreover, TCME seemed to be more effective in enhancing peripheral glucose uptake in rat hemi diaphragm in the absence of insulin. Treatment with TCME (0.1% w/v) also elicited a significant increase (p<0.001) in glucose uptake by the isolated rat hemidiaphragm when compared with the control groups. These results show that treatment with insulin or TCME alone for 30 min produced a significant increase in glucose uptake by 3.37- and 2.92- times, respectively. Addition of both insulin and TCME to the incubation media exerted the rate by 3.01- times, an increase of glucose uptake in rat hemi diaphragm compared with untreated control groups but there was no much significant increase compared insulin alone treated group. The glucose uptake by rat hemi diaphragm was significantly more in all the groups tested when compared with the control group.

Fig. 2: Effect of TCME on α -glucosidase (maltase) and amylase inhibitory activity

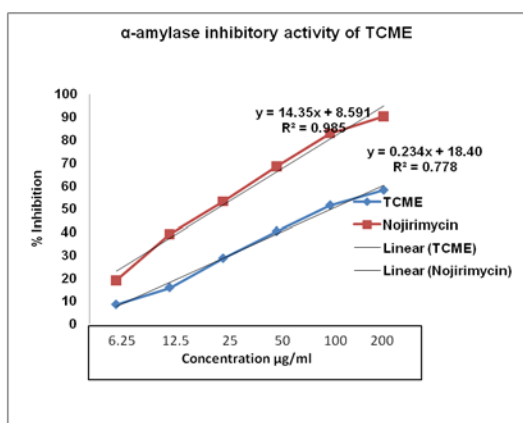


Fig. 3: Effect of TCME on amylase inhibitory activity

Table 5: Effect of TCME on glucose uptake by isolated rat hemidiaphragm

S. No.	Glucose uptake for 30 min (mg/g)
Control	75.812±0.911
Insulin	243.54±1.34**
TCME	218.66±1.41**
TCME+Insulin	274.59±1.68

Values are mean±SEM (n Z 6), ** p<0.01 as compared with control

DISCUSSION

Diabetes mellitus is a serious pathological condition due to disturbances in the metabolism of nutrients such as carbohydrates, amino acids and lipids. It is a multifactorial disorder with increasing incidence throughout the world [25]. The Alloxan a diabetogenic used in the present study, a cyclic urea derivative, which selectively destroys insulin-producing pancreatic cells by free radical-mediated damage and when administered to rodents cause an insulin-dependent diabetes mellitus [26] and has been widely used for the induction of experimental diabetes in animals. The organ toxicities are serious complications of diabetes such as neuropathy, retinopathy, nephropathy, hyperlipidemia and various cardiovascular diseases. The objective of the present research was to evaluate *in vivo* anti-diabetic potentials of *Tephrosia villosa* against alloxan induced diabetes in rats and also to evaluate *in vitro* anti-diabetic properties of *Tephrosia villosa* by α -amylase and α -glucosidase inhibitor activity and glucose uptake by rat hemidiaphragm method.

In the current study, 2 set of experiments were designed to explore the *in vivo* antidiabetic potential of methanolic extract of *Tephrosia calophylla* leaves against alloxan-induced diabetic rat model. The study was designed to evaluate the acute and chronic effects of extract in animals induced with diabetes and to compare the results with anti-diabetic potentials of reference drug glibenclamide. In the present study, the acute effect of the extract was studied by Oral Glucose Tolerance Test (OGTT). For the chronic study, we have made the animals diabetic by administering Alloxan and treated the animals with varying doses of extract for 21 d. The blood glucose level in animals was estimated at 1st, 7th, 14th and 21st day of the study to evaluate the potency of the extract in clearing blood glucose in animals induced with diabetes. In additionally, we evaluated methanolic extract of *Tephrosia calophylla* leaves for the enhancement of insulin secretion and hence ability to lower the blood glucose level in diabetic rats by estimating the concentration of insulin in the blood. We have also studied the potentials of the extract to lower the diabetic complications by assessing the lipid profile, liver and kidney function tests. For the evaluation of lipid profile, concentrations of total serum cholesterol and triglycerides were estimated. The liver enzymes alanine transferase and aspartate transferase were estimated to study the hepatic function and blood urea and creatinine were estimated to study the renal function. The

in vitro study was also conducted to evaluate the extract against α -glucosidase and α -amylase inhibitory effects and also by glucose utilization by skeletal muscle.

The oral glucose tolerance test is performed to study the acute effects of extract in diabetic animals and it based on the ability of the body to utilize or tolerate the glucose load administered orally [24, 25]. In OGTT, induction of diabetes in toxic control rats resulted in increased concentration of blood glucose due to the inability of the system to utilize glucose in the absence of insulin. But in TCME and glibenclamide treated therapeutics animals blood glucose level was significantly reduced than the control group which clearly shows the ability of the extract to increase the utilization of the glucose by cells and tissues.

In chronic study, diabetes was induced in all animals except normal group by administering alloxan before three days of study. Hence there was a significant decrease in insulin secretion in diabetic animals due to the destruction of pancreatic cells which resulted in decreased utilization of glucose and hence the blood glucose level was elevated. But in therapeutic groups treated with standard drug glibenclamide, TCME (200 mg/kg and 400 mg/kg), a significant increase in insulin release and subsequent decrease in blood glucose concentration was found.

The diabetes mellitus is a chronic metabolic disorder and it is also associated with several secondary complications such as hyperlipidemia, atherosclerosis, hypertension, diabetic nephropathy, diabetic neuropathy and diabetic ketoacidosis. Hyperlipidemia is one of such common complication of diabetes which is characterized by increase in serum total cholesterol (TC), triglycerides (TG), LDL and VLDL. The azotemia is a condition which is due to the accumulation of nitrogenous waste products like urea and creatinine in blood and usually found during diabetic nephropathy [27, 28].

Accelerated Coronary and peripheral vascular atherosclerosis is one of the most common and serious chronic complications of long-term diabetes mellitus. Along with other risk factors such as hypertension, smoking, obesity etc., increasing importance has been given to secondary hyperlipidemias in the causation of accelerated atherosclerosis. Hyperlipidaemia as a metabolic abnormality is frequently associated with diabetes mellitus. The most characteristic lipid abnormality in diabetics is hypertriglyceridemia, with or without an associated increase in plasma cholesterol [29].

In our present study administration of alloxan in control animals caused elevation of serum cholesterol, triglycerides, and urea as a consequence of secondary complications of diabetes. In animals of therapeutic groups treated with TCME (200 mg/kg and 400 mg/kg) have shown a significant reduction in above serum parameters.

The association between liver disease and diabetes mellitus is well known, the overall prevalence being significantly higher than that expected by a chance association of two very common diseases [20, 31]. But in the present study, there were no significant changes or elevation of the liver enzymes AST and ALT found in any group of animals including diabetic control animals.

The high blood sugar levels can damage to the fine blood vessel walls in the kidney filters, and these filters become leaky. The earliest sign of diabetic damage to kidneys is protein in the urine. [32]. In the present study, the concentration of blood urea was estimated to evaluate renal function in diabetic animals. In control animals treated with alloxan alone, there was a significant increase in blood urea and creatinine level whereas chronic treatment with our methanol extracts for 21 d could able to normalize blood urea and creatinine concentration in test animals and the results were comparable to the standard group treated with glibenclamide.

One of the therapeutic approaches for diabetes is inhibition of carbohydrate-hydrolyzing enzymes, such as α -amylase and α -glucosidase to prevent the absorption of glucose from GIT and thereby a decrease in the postprandial hyperglycemia [32, 33]. α -Glucosidases are enzymes that catalyze the absorption of digested glucose from dietary polysaccharides in the small intestine. The α -glucosidase inhibition by TCME was evaluated by determining the α -

glucosidase inhibitory activity using 4-Nitrophenyl-b-D-glucopyranoside-uronic acid (pNPG) as the reaction substrate. The crude enzyme solution prepared from a mouse's small intestine was used as a source of α -glucosidases, sucrose and maltase. The inhibition of α -Glucosidases can be the useful mechanism to block the absorption of dietary carbohydrates and suppresses postprandial hyperglycemia [33, 34]. In the present study the TCME shown significant α -glucosidase and α -amylase inhibitory activity but it is unclear whether the mechanism of inhibition of α -amylase and α -glucosidase by TCME is due to the competitive and noncompetitive method. The fact that α -amylase and α -glucosidase showed different inhibition kinetics seemed to be due to structural differences related to the origins of the enzymes [35]. However, the inhibition rate for α -glucosidase was close to that of acarbose, but the inhibition rate for α -amylase was obviously lower than that of nojirimycin. This indicated that TCME was a strong inhibitor for α -glucosidase with mild inhibitory activity against α -amylase. The inhibition of α -glucosidase, together with α -amylase by TCME, is considered to be an effective strategy for the control of diabetes by diminishing the absorption of glucose [36]. Severe postprandial hyperglycemia commonly experienced by patients with diabetes could be prevented if the rate of glucose uptake from the intestine into the circulation could be reduced by inhibiting carbohydrate digestion and absorption. Skeletal muscle represents 30-40% of the total body weight and seems to be one of the most important target tissues for the action of insulin and for the uptake of glucose at the peripheral level [37]. It is a well-known fact that insulin and anti-diabetic drugs promote glucose uptake by peripheral cells and tissues [35]. Another important finding of this work is that TCME possesses considerable insulin-like properties, as evidenced by the enhancement of glucose uptake in the diaphragm, which represents muscle cells that are a major site of insulin-mediated glucose disposal. Pieces of hemidiaphragm were incubated with different concentrations of TCME and insulin for 30 min. The estimation of glucose content in rat hemidiaphragm is a commonly used and a reliable method for the *in vitro* study of peripheral uptake of glucose. In addition, TCME significantly enhances the uptake of glucose by isolated hemidiaphragm and is found to be less effective than insulin. It appears that TCME has direct peripheral action. The control value of glucose uptake by rat hemidiaphragm corresponds with those of earlier findings [38-40].

Although the exact mechanism of action of alloxan is not fully understood, evidence indicates that the alloxan causes pancreatic β cell damage followed by insulin deficiency and diabetes mellitus [41-45].

In the present, *in vivo* study, the extract had been successful to increase insulin secretion and to maintain the normal glucose level in the therapeutic animals. In *in vitro* investigation, the TCME has shown its potency to reduce insulin resistance by increasing the utilization of glucose by tissues. The TCME also exhibited its potentials inhabiting GIT enzymes to prevent post-prandial hyperglycemia.

CONCLUSION

The methanol extract of *Tephrosia calophylla* possesses significant *in vivo* antidiabetic activity in the alloxan-induced diabetic animal model. The results of the present study also suggest that methanol extract of *Tephrosia calophylla* could inhibit *in vitro* α -glucosidase and α -amylase and also significantly increase glucose utilization by skeletal muscle. But further investigation is needed to isolate and determine the individual components present in *Tephrosia calophylla* that may be responsible for these beneficial effects to improve in health conditions associated with diabetes mellitus.

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AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICT OF INTERESTS

All authors are hereby declaring there is no conflict of interest with respect to the manuscript.

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