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IN VIVO AND *IN VITRO* EVALUATION OF *TEPHROSIA VILLOSA* 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 villosa 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 test the acute effect of methanol extract of Tephrosia villosa (TVME) to reduce blood glucose in OGTT. The chronic study of 21 days 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-glucisidase inhibitory activity and α-amylase inhibitory activity were performed and IC_{50} values of extract was determined. The glucose up take by rat hemi-diaphragm model was also used test potentials of the extract to increase utilization of the glucose by tissues. Results: In OGTT, standard glibenclamide and TVME at 400mg/kg treated anomals have shown significant reduction in blood glucose at 90 mins but at 120 mins, blood glucose level was significantly reduced in glibenclamide and TVME at 200mg/kg and 400mg/kg treated animals compared to diabetic control group. In chronic model the methanol extract effectively, reduced blood glucose levels (P<0.001) at 14 th 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, cholesterol 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).*

KEY WORDS

Anticancer activity, Tephrosia villosa, IC_{50} value, MTT assay and antioxidant activity.

1. INTRODUCTION

Diabetes mellitus is associated with absolute or relative deficiency insulin or insulin resistance which ultimately leads to alterations in metabolism of carbohydrates, amino acids and fats[1]. According to estimation of World Health Organization (WHO), about 220 million individuals throughout world will have diabetes mellitus and India would be diabetic capital of the world. There is always scope for the development of anti-diabetic agents due it high prevalence and long term complications[2].

The insulin produced from recombinant DNA technology is used in insulin dependent diabetes mellitus (Type 1 or IDDM) and oral hypoglycemic agents are used in non-insulin dependent diabetes mellitus (Type 2 or NIDDM) to bring hyperglycemic to euglycemic condition[3]. Although there is availability of several pharmacological approaches for the management of NIIDM, still there is no ideal drug for its effective management with minimum side effects. Hence identification and development of newer therapeutic agents remains highly desirable.

In view of the adverse effects and toxicities associated with the treatment by presently available oral hypoglycemic agents and insulin, searching for effective and safer hypoglycemic agent from plant source is going on throughout the world since herbal drugs play an important role in this part due to their least side effects[4].

Since ancient time Charka and Sushruta had mentioned the use of several medicinal plants for the effective and safe management of Ayurveda, the traditional medicinal system of India. Herbal remedies for diabetes mellitus consist of plant constituents, either a single drug or in combination with others, which are considerably safe and free from adverse effects compared to synthetic agents[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* and 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 puropses. The *Tephrosia villosa* which belongs to same genus also a important component of traditional system of medicine Ayurveda for the treatment of diabetes but there is no scientific evidence the same[8,9]. Hence it was necessary to provide a clear scientific background for the beneficial property of plant *Tephrosia villosa* in diabetes and in this attempt, study had been designed to determine *in vitro* and *in vivo* potentials of methanol extract of areal parts of *Tephrosia villosa* for anti-diabetic activity.

2.0 MATERIALS AND METHODS

2.1 Plant material

The areal parts of plant *Tephrosia villosa* have been collected from the surroundings of Sri Venkateshwara university, Tirupati, India and dried under shade. The plant material was identified and authenticated by Dr. Madhava chetty Asst.Prof., Department of Botany Sri Venkateshwara university, Tirupati and specimen herbarium was preserved at institute herbarium library. The aerial parts of plant were separated from other parts, washed, cleaned and dried under shade for further use.

2.2 Preparation of extract

The shade dried plant material was pulverised into powder and passed through sieve No. 22 mesh. About 350 g (appx.) of coarse powder was subjected to successive solvent extraction using petroleum ether and methanol in soxhlet's apparatus[10].

2.3 Preliminary phytochemical investigation

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

2.4 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.

2.5 Animals

The healthy albino wistar male rats (180-220g) were procured from Sri Venkateswara Enterprises, Bangalore housed under standard conditions of temperature (22 ± 10C), relative humidity (55 ± 10%), 12 hr 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 experiment, the rats were acclimatized for a period of 7 days 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.

2.6 Acute Oral Toxicity Studies

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

2.7 Evaluation of *in vivo* anti-diabetic activity

2.6.1 Induction of Diabetes in Experimental Animals

In both acute and chronic models, rats were made diabetic by a single intra peritoneal injection of alloxan monohydrate (150 mg/kg). Alloxan was first weighed individually for each animal according to the body

weight and then solubilized with 0.2ml saline (154mM 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].

2.7.2 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 single dose of alloxan as explained above. The animal grouping is as follows.

- Group I: Normal control treated with normal saline (10ml p.o) alone,
- Group II: Diabetic control treated with alloxan and vehicle 3% of Tween20,
- Group III: Standard treated alloxan and Glibenclamide 5mg/kg,
- Group IV: TVME (Low dose) treated alloxan and methanol extract of Tephrosia villosa 100mg/kg, p.o
- Group V: TVME (Medium dose) treated alloxan and methanol extract of Tephrosia villosa 200mg/kg, p.o
- Group VI: TVME (High dose) treated alloxan and methanol extract of Tephrosia villosa 400mg/kg, p.o

2.7.2.1 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 mins, 60 mins, 90mins and 120 mins and estimated for blood glucose using Glucometer (Acucheck) [14,15,16].

2.7.2.2 Chronic study model

In chronic study also, animals were divided in to six groups as above. The standard drug glibenclamide and methanol extract of *Tephrosia villosa* were administered to respective animals according to their body weights from 1st day to 21st day. (Subramonium et al., 2003). 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[16,17,18].

2.7.2.3 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 colorimetric method and ALT, AST, urea, creatinine and insulin were estimated using standard kits.

2.8 Evaluation of in vitro anti-diabetic activity

2.8.1 α-Glucosidase inhibitory assay

This assay was carried out to investigate the in vitro inhibitory activity of TVME 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 a albino mouse was used as 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 hours 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 mM, pH 6). The homogenate was used as α-glucosidase solution. The assay mixture consisted of 100 mM maleate buffer (pH 6), 2% (w/v) of each sugar substrate solution (100 ml), and the sample extract (20-640 µg/mL). The mixture was preincubated for 5 minutes at 37^{O}C, and the reaction was initiated by adding crude α-glucosidase solution (50 ml), followed by incubating the mixture again for 10 minutes at 37^{O}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 blank (GCB) and amount of glucose produced by the addition of TVME (GCT) were recorded[19,20]. 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:

$$\text{Inhibition rate (\%)} = \frac{\text{GCP- GCT-GCT}}{\text{GCP}} \text{ X100}$$

2.8.2 α-Amylase inhibitory assay

Test samples of TVME (6.25, 12.5, 25, 50, 100, 200 mg/mL) and nojirimycin (6.25-200 µg/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^{O}C for 10 minutes. 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^{O}C for 10 minutes. 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 minutes 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[19,20].

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

2.8.3 Glucose uptake by isolated rat hemidiaphragm

Glucose uptake by rat hemidiaphragm was estimated according to earlier works[20], 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 TVME (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 TVME (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 hemidiaphragms were placed in test tubes and incubated for 30 minutes at 37^{O}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[20].

2.8 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 mean (S.E.M.).

3.0 RESULTS

3.1 Preliminary phytochemical study

The percentage yield of the TVME was found to be 8.19 % w/w. The preliminary phyto-chemical study performed in the study for the methanol extract of *Tephrosia villosa* exhibited the presence of alkaloids, glycosides, poly phenols, flavonoids, tannins, steroids, and carbohydrates dug.

3.2 Acute toxicity studies

The methanol extract was safe up to dose of 2000 mg kg^{-1} b.w. and caused neither mortality nor any signs of clinical abnormality in the TVME treated animals during the 14 days of observation period after administration. There was no considerable change in body weight before and after treatment of the experiment and no signs of toxicity were observed. As per the results obtained in acute oral toxicity study doses were selected as 100, 200 and 400mg/kg on the ratio 1/20th, 1/10th and 1/5th respectively.

3.3 Evaluation of *in vivo* anti-diabetic activity

3.3.1 Oral glucose tolerance test

In oral glucose tolerance test, animals of diabetic control group have shown significant elevation in blood glucose level through entire study when compare to normal animals. But treatment with standard drug glibenclamide and methanolic extract (200mg/kg and 400mg/kg) of *Tephrosia villosa* could able to improve utilization of oral glucose by animals and significantly ($P<0.001$) reduced blood glucose level in therapeutic groups after 60 mins and 120 mins. The results of OGTT have shown in [Table No 1].

3.3.2 Determination of chronic anti-diabetic activity

In chronic study, the blood glucose level was significantly ($P<0.001$) increased in diabetic control animals compare to normal animals due to the induction of glucose. While treatment with glibenclamide and TVME extract at 200mg/kg and 400mg/kg could significantly ($P<0.001$) reduced blood

glucose concentrations compare to diabetic control animals at 14th and 21st day of the study [Table No .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 TVME (200mg/kg and 400mg/kg) there was significant (P<0.001) increasing in blood insulin level compare to diabetic control animals and the results were comparable to normal animals [Table No .3].

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, triglycerides, urea and creatinine concentration was observed in glibenclamide and TVME (200mg/kg 400mg/kg) treated animals when compare to diabetic control [Table No .3].

It is found that there is no significant (p>0.01) change in AST and ALT levels in diabetic alone compare to normal animals and also no significant change was observed therapeutic animals treated with glibenclamide and TVME when compare to diabetic animals. [Table No: 3]

3.4 Evaluation of in vitro anti-diabetic activity

3.4.1 α-Glucosidase inhibitory activities

An *in vitro* α-glucosidase inhibition study was conducted to investigate the inhibitory effect of *Tephrosia villosa* extract. The half maximal inhibitory concentration (IC_{50}) values of sucrase and maltase inhibitory activities were 398.513µg/mL and 78.412µg/mL, respectively. The shows that TVME exhibited strong activity in a dose-dependent manner and is thus inferred to be an effective α-glucosidase inhibitor [Table No .4 and Figure No.1 and 2].

Figure No-1: Effect of TVME on α-Glucosidase (Sucrase) and amylase inhibitory activity.

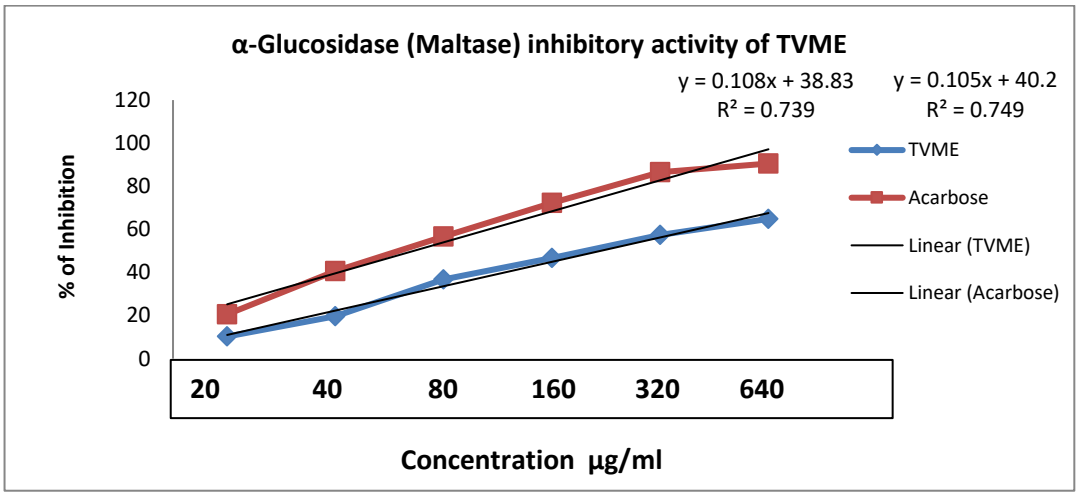


Figure No 2: Effect of TVME on α-Glucosidase (maltase) and amylase inhibitory activity

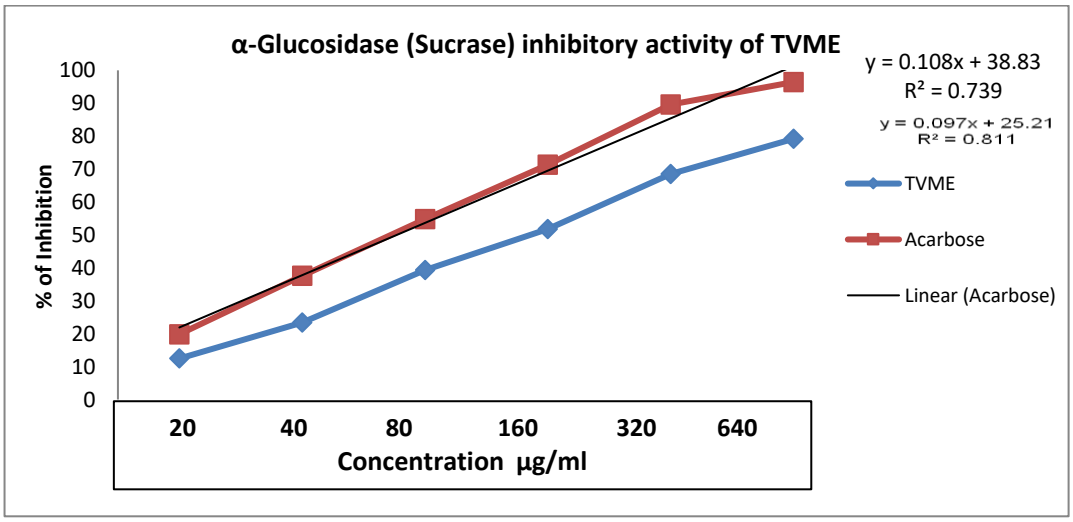
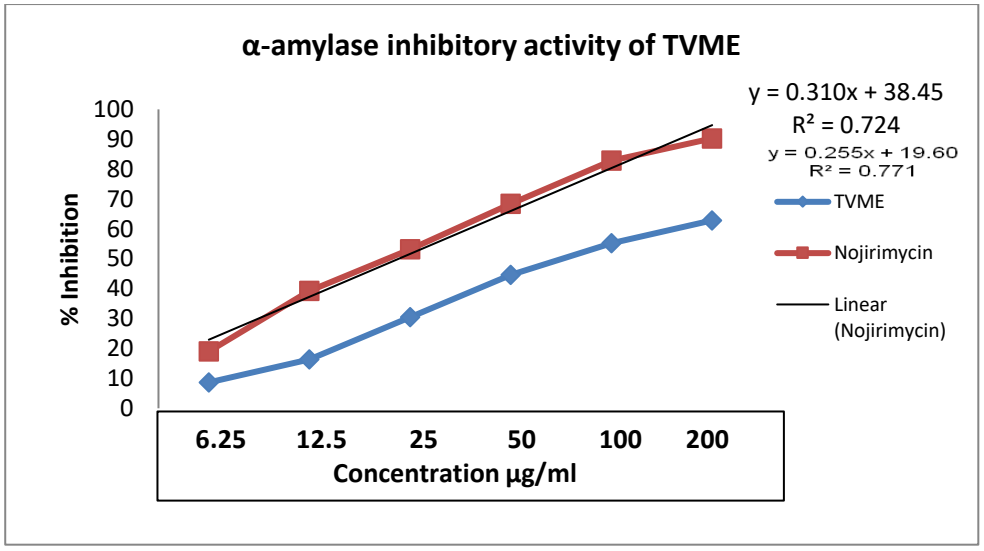


Figure No 3: Effect of TVME on amylase inhibitory activity



3.4.2 α-Amylase inhibitory activities

To investigate the inhibitory effect of methanol extract of *Tephrosia villosa* postprandial glucose, an in vitro α-Amylase inhibition test was done. In this study, TVME exhibited strong inhibitory activity against α-amylase with IC_{50} of 133.483/mL, which is comparable with positive controls [Table No .4 and Figure No.3].

3.4.3 Effect on peripheral glucose uptake

The estimation of glucose content in rat hemidiaphragm was used for the *in vitro* study of peripheral uptake of glucose. The extract shows glucose uptake (milligrams/gram tissue weight) in an isolated rat hemidiaphragm muscle in the presence of insulin (0.4 U/mL) and TVME (0.1% w/v). The results of this experiment indicate that the addition of TVME to the incubation media (Tyrode solution) caused significant increase in glucose uptake by the rat hemidiaphragm and was found to be less effective than insulin. Moreover, TVME seemed to be more effective in enhancing peripheral glucose uptake in rat hemidiaphragm in the absence of insulin. Treatment with FMcME (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 TVME alone for 30 minutes produced a significant increase in glucose uptake by 3.37- and 2.92- times, respectively. Addition of both insulin and TVME to the incubation media exerted the rate by 3.01-times, an increase of glucose uptake in rat hemidiaphragm compared with untreated control groups but there was no much significant increase compared insulin alone treated group [Table No 5]. The glucose uptake by rat hemidiaphragm was significantly more in all the groups tested when compared with the control group.

Table No 1: Effect of methanol extracts of *Tephrosia villosa* on blood glucose OGTT

Treatment	Concentration of Blood Glucose (mg/dl)				
	0 Mins	30 Mins	60 Mins	90 Mins	120 Mins
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 5mg/kg)	174.7 ±2.963	279.2 ±3.911	199.7 ±3.442	158.8 ±4.729	119.8 ±4.936
TVME 100 mg/kg	171.0 ±3.396	275.2 ±3.525	251.7 ±2.231	231.5 ±2.473	183.3 ±4.208
TVME 200 mg/kg	173.2 ±4.445	264.8 ±3.478	224.0 ±5.132	183.8***±1.973	131.3*** ±2.459
TVME 400 mg/kg	169.8 ±5.564	270.7±3.252	185.7**±2.37	158.8***±3.42	111.7***±3.451

Values are mean ± S.E.M, 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 No 2: Effect of methanol extracts of *Tephrosia villosa* on blood glucose in chronic study

Treatment	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 5mg/kg)	234.2± 6.290	211.8±4.143	171.5±3.374	145.2±1.740
TVME 100 mg/kg	244.5 ± 5.408	227.5 ±2.907	216.0±1.966	212.0±3.256
TVME 200 mg/kg	231.0 ± 5.145	218.0 ±2.029	197.8**±9.945	176.5***±1.765
TVME 400 mg/kg	241.54.006	204.0 ± 4.531	179.3***±1.856	144.5***±4.121

Values are mean ± S.E.M, 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 No 3: Effect of methanol extracts of on serum parameters *Tephrosia villosa* 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^{+++} ±1.91	108.4^{+++} ±2.72	131.8^{+++} ±2.43	1.464^{+++} ±0.0566	72.57^{+++} ±1.30	62.37 ± 1.372	134.0 ±2.385
Standard (Glibenclamide)	137.7** *±2.74	79.57*** ±3.1	100.9*** ±2.9	0.6318*** ±0.036	35.48*** ±1.85	63.09** ±0.76	126.7*** ±2.21
TVME 100 mg/kg	94.67 ± 2.060	98.28 ± 3.792	131.4 ± 1.470	1.216 ± 0.01243	65.53 ±0.9600	64.18± 3.339	131.8 ±1.937
TVME 200 mg/kg	111.8** ±2.212	86.13** ± 8.124	113.4** ± 2.202	0.9205** ± 0.02685	49.87** ±0.6924	63.19** ± 2.055	130.1** ±1.447
TVME 400 mg/kg	136.2*** ±4.143	208.1*** ±122.9	102.2*** ± 2.276	0.5742*** ±0.0202	32.22*** ±1.508	63.05*** ± 1.143	125.7*** ±3.349

Values are mean ± S.E.M, 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 No 4: Effect of TVME on α-Glucosidase (Sucrase and maltase) and amylase inhibitory activity.

Treatment	IC_{50} Values (µg/ml)		
	α-Glucosidase (Sucrase)	α-Glucosidase (Maltase)	α-Amylase
TVME	255.567	93.333	119.215
Acarbose	103.425	98.33	--
Nojirimycin	--	--	37.258

Table No 5: Effect of TVME on glucose uptake by isolated rat hemi-diaphragm

S.No	Glucose uptake for 30 mins (mg/g)
Control	78.234±1.66
Insulin	264.11±2.88**
TVME	235.18±1.81**
TVME + Insulin	285.68±3.21**

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

4.0 DISCUSSION

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 hemi-diaphragm method.

Diabetes mellitus is a debilitating and multifactorial disorder with increasing incidence throughout the world[21]. Alloxan, a cyclic urea derivative, which selectively destroys insulin-producing pancreatic cells by free radical mediated damage when administered to rats can cause diabetes mellitus. Hence it was reported as a potent diabetogenic agent[22] and has been widely used for the induction of experimental diabetes in animals. The serious complications of diabetes are organ failures, neuropathy, retinopathy, nephropathy, hyperlipidemia and various cardiovascular diseases[23,24].

In the *in vivo* acute study, OGTT was performed to test the ability of body to utilize oral glucose in presence of methanol extract of *Tephrosia calophylla* (TVME) against animals induced with diabetes using alloxan. The blood glucose was estimated 0mins, 30 mins, 60mins, 90 mins and 120 mins after administration of oral glucose. In diabetic control animals, the blood glucose was significantly increased at 30mins interval when compare to normal animals and the blood glucose was above 190mg/dl even at 120 mins interval due to the reduced capacity of the system to utilize the glucose whereas the blood glucose had fall down below 100mg/dl in normal animals since the ability of the body was proper. But in animals treated with low and medium doses of TVME significantly reduced blood glucose when compared to diabetic control animals at 90 mins and 120 mins interval shows its property to enhance the utilization of glucose in living system and the effect was comparable to the standard drug glibenclamide.

The chronic *in vivo* study was designed to evaluate the long-term effects of methanol against alloxan induced diabetes in albino rats. 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 diabetic animals and in additionally, insulin, total cholesterol, triglycerides, urea, creatinine, ALT and AST in serum at 21st day of the study.

In present chronic study, there was significant increase in blood glucose level was observed in animals of diabetic control compared to normal animals throughout study due to the loss of β cells and impairment in insulin secretion. But in therapeutic groups treated with standard drug glibenclamide, TVME (200mg/kg and 400mg/kg), significant decrease in blood glucose concentration was found when compared diabetic alone animals at 14th and 21st day of the study which was witnessed by the enhanced insulin secretion. This clearly shows that the potential of the TVME to reverse the pancreatic damage.

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 keto acidosis[25]. 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 condition which is due to the accumulation of nitrogenous waste products like urea and creatinine in blood and usually found during diabetic nephropathy[26,27].

Along with other risk factors such as hypertension, smoking, obesity etc., increasing importance has been given to secondary hyperlipidemia in the causation of accelerated coronary atherosclerosis which is one of the most common and serious chronic complications of long-term diabetes mellitus. Hyperlipidaemia is abnormality in metabolism of and is frequently associated with diabetes mellitus and characterized by the increased plasma cholesterol and triglycerides[28]. In the present study administration of alloxan caused significant elevation of serum cholesterol and triglycerides in diabetic alone animals compared to normal group while in animals treated with glibenclamide and TVME (200mg/kg and 400mg/kg), significant reduction was observed compared to diabetic control animals.

The blood urea and creatinine was significantly increased in diabetic control animals when compared to normal animals may be due to renal complication caused by hyperglycemia but there was significant reduction of blood creatinine and urea was found due to the administration of glibenclamide and TVME (200mg/kg and 400mg/kg) compared to diabetic control group indicates the ability of the extract to reverse renal complication in diabetes mellitus.

It is well known that there is clear association between liver disease and diabetes, the overall prevalence being

significantly higher than that expected by a chance association of two very common diseases[29,30,31]. But in the present study, any significant changes or elevation of the liver enzymes AST and ALT were not found diabetic control animals compare to normal animals. The ranges of ALT and AST in therapeutic groups were also normal.

One of the novel therapeutic approach for diabetes is inhibition of carbohydrate hydrolyzing enzymes such as α-amylase and α-glucosidase to prevent the absorption of glucose from GIT and there by decrease in the postprandial hyperglycemia and it complications[32,33]. The α-glucosidase inhibition by TVME was evaluated by determining the α-glucosidase inhibitory activity using 4-Nitrophenyl-b-D-glucopyranosiduronic 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[34]. In the present study the TVME has shown significant α-glucosidase and α-amylase inhibitory activity indicates its usefulness to manage postprandial glucose but still it is not clear whether the inhibition of α-amylase and α-glucosidase by methanol extract is due to 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 a reference standard agent, but the inhibition rate for α-amylase was lower than that of standard drug nojirimycine. This indicated that TVME was a strong inhibitor for α-glucosidase with mild inhibitory activity against α-amylase. The inhibition of α-glucosidase, together with α-amylase by TVME, is considered to be an effective strategy for the control of diabetes by diminishing the absorption of glucose 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[36].

Skeletal muscle constitutes about 30-40% of the total body weight and seems to be one of the most important target tissues for the action of insulin which enhances the uptake of glucose at the peripheral level. It is a well-known fact that insulin and anti-diabetic drugs promote glucose uptake and its utilization by peripheral cells and tissues[37]. Another important outcome of the present work is that TVME possesses considerable insulin-like activity, as evidenced by the enhancement of glucose uptake from the diaphragm, which represents muscle cells that are a major tissues of insulin medicated glucose disposal. The TVME significantly enhances the uptake of glucose by isolated hemidiaphragm and is found to be less effective than insulin. It appears that TVME has direct peripheral action. The control value of glucose uptake by rat hemidiaphragm corresponds with those of earlier findings[38].

Although the exact mechanism of action of alloxan is not fully understood, evidences indicate that the alloxan causes pancreatic β cell damage followed by insulin deficiency and diabetes mellitus[39,40].

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. The study should be conducted to find out the antioxidant properties of TVME which is possible mechanism in the present study that can protect pancreas against alloxan mediated damage and normalize the insulin secretion. In *in vitro* investigation the TVME has shown its potency to reduce insulin resistance by increasing the utilization of glucose by tissues and extract also exhibited its potentials in inhibiting GIT enzymes to prevent post-prandial hyperglycemia.

5.0 CONCLUSION

The methanol extract of *Tephrosia villosa* possess significant *in vivo* anti-diabetic activity in alloxan induced diabetic animal model. The results of the present study also suggest that methanol extract of *Tephrosia villosa* 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 villosa* that may be responsible for these beneficial effects to improve in health conditions associated with diabetes mellitus.

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6.0 REFERENCES

1. Valiathan MS. Healing plants. Curr. Sci. 1998; 75:1122–7.

2. World Health and Organization, 2009. Prevalence Data of Diabetes Worldwide. <http://www.who.int/mediacentre/factsheets/fs312/en/index.html>.
3. Nicasio P, Aguilar-Santamarı L, Aranda E, Ortiz S, and Gonza´ lez, M, Hypoglycemic effect and chlorogenic acid content in two Cecropia species. Phytother. Res. 2005;19, 661–664.
4. Rao BK and Rao CH. Hypoglycemic and antihyperglycemic activity of Syzygium alternifolium (Wt.) Walp. seed extracts in normal and diabetic rats. Phytomedicine 2001; 8:88–93.
5. Suman BS, Afreena N, Krishna MP and Pothapragada SM. Antihyperglycemic effect of the fruit-pulp of Eugenia jambolana in experimental diabetes mellitus. J Ethnopharmacol. 2006; 104:367–373.
6. Baranwal A, Mazumder A, Chakraborthy GS and Gupta S. Phytopharmacological uses of tephrosia purpurea - a review. Pharmacophore 2014;5(4): 658-665.
7. Saad T, Muhammad AS, Muhammad A. A Review on the Phytochemistry and Pharmacology of Genus Tephrosia. Phytopharmacol 2013;4(3): 598-637.
8. Hari Kishore P, Vijaya Bhaskar Reddy M, Gunasekar D, Marthanda Murthy M, Caux C, Bodo B. (2003). A new coumestan from *Tephrosia villosa*. Chemical and pharmaceutical bulletin 51, 194-196.
9. Reddy RV, Khalivulla SI, Reddy BA, Reddy MV, Gunasekar D, Deville A, Bodo B. (2009). Flavonoids from Tephrosia villosa. Natural product communications 4, 59-62.
10. Kokate CK. Practical Pharmacognosy. Vallabh Prakashan New Delhi: 1994; 4:110-1.
11. Khandelwal KR, Practical Pharmacognosy-techniques and experiments. Pune; Nirali Prakashan; 2000.
12. OECD, 2000. Acute Oral Toxicity-Acute Oral Toxic Class Method. Guideline 423 adopted 23.03.1996. In: Eleventh Addendum to the OECD Guidelines for the Testing of Chemicals. Organisation for Economic Co-operation and Development, Paris.
13. Vogel G and Vogel H. The text book of pharmacological screening methods and drug evaluation. Evaluation of anti-diabetics.
14. Sanjay J et al. Antidiabetic activity of Paspalum scrobiculatum Linn. In alloxan induced diabetic rats. J Ethnopharmacol 2010; 127:325–328.
15. Rangachari B, Veeramuthu D and Savarimuthu I. Antidiabetic activity of γ-sitosterol isolated from Lippia nodiflora L. in streptozotocin induced diabetic rats. European J Pharmacol 2011; 667:410–418.
16. National Committee for Clinical Laboratory Standards. User evaluation of Precision Performance of Clinical Chemistry Devices.NCCLS ;1984, NCCLS: Publication. http://www.thermo.com/eThermo/CMA/PDFs/Product/productPDF_50918.pdf.
17. Kennedy JW, Carey RN, Coolen RB, et al.Evaluation of Precision Performance of Clinical Chemistry Devices;Approved Guideline (EP5- A).Wayne, PA: The National Committee for Clinical Laboratory Standards, 1999.http://www.qualtexlabs.org/pdf/ALT.pdf.
18. Dahlqvist A. Method for assay of intestinal disaccharidases. Anal Biochem. 1964; 7:18-25.
19. Kwon GJ, Choi DS and Wang MH. Biological activities of hot water extracts from Euonymus alatus leaf. J Korean Food Sci Technol. 2007; 39:569-574.
20. Walaas E and Walaas O. Effect of insulin on rat diaphragm under anaerobic conditions. J Biol. Chem. 1952; 195:367-373.
21. Ajabnor MA and Tilmisany AK. Effects of Trigonella feonum graceum on blood glucose levels in normal and Alloxan-diabetic mice. J.Ethnopharmacol 1998; 22: 15-49.
22. Dunn JS, Sheehan HL, McLetchie NG. Necrosis of langerhans produced experimentally. Lancet 1943; 1:484-487.
23. Pamela CC, Richard AH, Denise RF. Type 1 Diabetes mellitus: Lippincott's Wlliams and wlikins, India, 1st edition 1994: 336-337.
24. Satyanarayana U, Chakrapani. Insulin, glucose homeostasis and diabetes mellitus. Fundamentals of biochemistry Kolkata; Third edition, 2008:678-680.
25. Harshmohan. The Endocrine system: Textbook of Pathology. New Delhi, Jaypee Brothers Medical Publishers (P) Ltd: 4th ed; 2002:849-851.
26. Vinay kumar Abul KA, Nelson F. Diabetes mellitus: Pathologic basis of Disease. Robbins and Cotran. &th edition, 2004, India. 1189-1226.
27. Abdul HZ et al. Serum Lipid Profile in Non-insulin-dependent Diabetes Mellitus Associated with Obesity. Int. J. Diab. Dev. Countries 1995; 15:9-14.
28. Simona M, Rita M and Giulio M. Diabetes and liver disease: An ominous association. Nutrition, Metabolism & Cardiovascular Diseases 2007; 17: 63-70.
29. Sara M, Luca M, Francesco V, Giacomo L and Fiabo M. Glycogenic hepatopathy associated with type1 diabetis mellitus as a cause of recurrent liver damage. Annals of hepatology 2012; 11:554-558.
30. Enyioma NO and Abdu A. Update in Diabetic Nephropathy. Int J Diabetes & Metabolism 2005; 13: 1-9.
31. Bhandari MR, Anurakkun NJ, Hong G, Kawabata J. a-Glucosidase and a-amylase inhibitory activities of Nepalese medicinal herb Pakhanbhed (Bergenia ciliata, Haw.). Food Chem. 2008; 106:247-252.
32. Puls W, Keup U, Krause HP, Thomas G, Hoffmeister F. Glucosidase inhibition. A new approach to the treatment of diabetes, obesity, and hyperlipoproteinaemia. Naturwissenschaften.1977;64:536-537.

33. Toeller M. a-Glucosidase inhibitors in diabetes: efficacy in NIDDM subjects. Eur J Clin Invest. 1994;24(Suppl. 3):31-35.
34. Chiba S. Molecular mechanism in a-glucosidase and glucoamylase. Biosci Biotechnol Biochem. 1997; 61:1233-1239.
35. Honda M, Hara Y. Inhibition of rat small intestinal sucrase and a-glucosidase activities by tea polyphenols. Biosci Biotechnol Biochem. 1993; 57:123-124.
36. DeFronzo RA, Jacot E, Jequier E, Maeder E,Wahren J, Felber JP.The effect of insulin on the disposal of intravenous glucose. Results fromindirect calorimetry and hepaticandfemoral venous catheterization. Diabetes. 1981; 30:1000-1007.

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37. Melander A. Glucose uptake by hemidiaphragm. Diabet Med. 1996; 13:143.
38. Cohen G, Heikkila KE. Generation of hydrogen peroxide, superoxide radical by 6-hydroxy dopamine, dialuric acid and related agents. J Biol and Chem 1974; 49:2447-2452.
39. Okamoto H. Molecular basis of experimental diabetes: Degeneration, oncogenesis and regeneration of pancreatic β-cells. BioEssays 1995; 2:15-21.
40. Takasu N, Asawa T, Komiya I, Nagaswa Y, Yamada T. Alloxan-induced DNA strand breaks in pancreatic islets: Evidence for H_2O_2 as an intermediate. J Biol and Chem 1991; 266:2112-2114.

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