IJHS

Original Article

In vivo and *in vitro* antidiabetic potentials of methanol extract of *Tephrosia pumila* against alloxan-induced diabetes in experimental animals

C. Ramesh[1], A. Prameela Rani[2]

[1]Department of Pharmacology, East West College of Pharmacy, Bengaluru, Karnataka, India, [2]Department of Pharmaceutics, ANU College of Pharmaceutical Sciences, Guntur, Andhra Pradesh, India

Address for correspondence:
C. Ramesh, Department of Pharmacology, East West College of Pharmacy, No-63, I Bharath Nagar, Off Magadi Road, Vishwaneedam (PO), Bengaluru - 560 091, Karnataka, India.
Phone: +91-9901514401.
E-mail: rameshcology80@gmail.com

WEBSITE: ijhs.org.sa
ISSN: 1658-3639
PUBLISHER: Qassim University

ABSTRACT

Objective: The objective present investigation was to determine the *in vivo* and *in vitro* antidiabetic potentials of the methanol extract of *Tephrosia pumila*.

Methods: The present study was designed to evaluate the acute and chronic effects of the methanol extract of *T. pumila* (TPME- Methanol extract of *Tephrosia pumila*) against alloxan-induced diabetes in rats. For acute study, oral glucose tolerance test (OGTT) was performed to check the ability of the TPME to utilize oral glucose load and the chronic study of 21 days was done to assess the effect of TPME for long time in diabetic rats and its effect was compared with glibenclamide (5 mg/kg i. p). The *in vitro* α-glucosidase and α-amylase inhibitory properties were performed and IC_{50} values were determined. The glucose uptake by rat hemidiaphragm model was also used to evaluate potentials of the TPME to enhance utilization of the blood glucose by peripheral tissues.

Results: In OGTT, blood glucose level was significantly reduced in glibenclamide and TPME at 200 and 400 mg/kg treated animals compared to diabetic control group. In chronic model, the TPME effectively reduced abnormalities of diabetes in therapeutic groups. Both α-glucosidase and α-amylase inhibitor assays have exhibited significant IC_{50} values and also increased utilization of glucose by the skeletal muscle.

Conclusion: The results of the present investigation recommend that TPME possess significant *in vivo* antidiabetic property. The data of the study also suggest that the enhancement of insulin secretion and reduction of insulin resistances are the mode of antidiabetic activity shown by TPME.

Keywords: Antidiabetic activity, *Tephrosia villosa,* α-glucosidase and α-amylase and IC_{50} values

Introduction

Diabetes mellitus develops due to disturbances of metabolism in the body as the result of absolute or relative absence insulin or insulin resistance which ultimately leads to alterations in metabolism of nutrients carbohydrates, amino acids, and fats.[1] According to the survey made by the World Health Organization, about 220 million people throughout the world will have diabetes mellitus and India would be diabetic capital of the world by 2020. Hence, there is always scope for the development of antidiabetic drugs due to its high prevalence and long-term complications of disease.[2]

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

In view of the toxicities, effects and adverse reactions associated with the therapy using presently available oral hypoglycemic drugs and insulin, searching for more potent and less toxic hypoglycemic drug from plant origin is under pipeline throughout the world since herbal medicine plays an essential role in this segment due to their minimum side effects.[4]

Since ancient period Ayurveda physicians, Charka and Sushruta had mentioned the usefulness of several medicinal plants for the effective management diabetes with fewer side effects in Ayurveda, the traditional medicinal system of India.

Herbal remedies for diabetes mellitus constituting of plant substances, either a single agent or in combination with other drugs, which are considerably safe and free from adverse reactions compared to synthetic agents.[5]

The *Tephrosia* is a genus of medicinal herb which is mainly originated from Indian. The various species of *Tephrosia* are biologically active and have been investigated and reported for their several therapeutic properties. About 24 types of *Tephrosia* were noted in India. The genus is well known for the presence of wealthy in prenylated flavonoids and is regarded to possess cytotoxic, insecticide, repellant, larvicidal, and antimicrobial potentials.[6-9] *Tephrosia pumila* belongs to the same genus and commonly known as Indigo Sauvage or small *Tephrosia* was essentially used for the management of diabetes, cancer, hyperlipidemia, hepatotoxicity, and renal problems in the folklore medicine but does not have the scientific evidence for the same.[10] Although the plant was extensively used in traditional medicine for the liver protection, there is a lack of scientific evidence for the same.[9,10] The study performed simultaneously for the evaluation of *in vivo* and *in vitro* antidiabetic potentials of the plant belongs to the same genus known as *Tephrosia calophylla* using the same set of normal and reference standard samples. The alcoholic extract of *T. pumila* has been reported for the antidepressant and anxiolytic,[11] antimicrobial,[12] anticancer,[13] and antiprotozoal[14] properties. Hence, the objective of the current investigation was to evaluate and provide the scientific data for both *in vitro* and *in vivo* antidiabetic potentials of *T. pumila* against alloxan produced experimental diabetes in rats. In the present study, methanol is used as solvent for the extraction of phytoconstituents since it is more polar than ethanol and other solvents.

Methods

Plant material

The aerial parts of plant *T. pumila* have been identified and obtained from the surrounding parts of Sri Venkateswara University, Tirupati, Andhra Pradesh, India, and plant substance was demoisturized under shade. The collected plant material was authenticated by Dr. Madhavachetty, Asst. Prof., Department of Botany, Sri Venkateswara University, Tirupati, and specimen herbarium sample was kept for future reference at the institute herbarium library. The aerial parts of plant *T. pumila* were separated from other unwanted parts, using pure water washed and cleaned and dried under shade for future investigation.

Preparation of methanol extract

The dried plant material was grounded into powder which then passed through sieve No. 22 mesh. The coarsely powdered drug material of about 350 g (approximate) was used for consecutive solvent extraction process using petroleum ether and methanol in Soxhlet apparatus.[15] As methanol is the best solvent for the extractions of phytochemicals from the plants with respect to its polarity and hydrophobic property, the methanol extract of *T. pumila* was used for the present study.

Preliminary phytochemical examination

The initial phytochemical examinations for the methanol extract of *T. pumila* had been performed according to methods described by Khandelwal.[16]

Drugs and chemicals

All reagents and chemicals employed in the present investigation were procured commercially and all were of analytical category. Alloxan was procured from Sigma Laboratory, India and glibenclamide was procured from Aventis Pharmaceutical Ltd., India.

Animals

The healthy albino Wistar rats of 180–220 g weight range and 9 months obtained from Sri Venkateswara Enterprises, Bangalore, accommodated under excellent laboratory conditions of temperature (22ºC ± 10ºC) and relative humidity (55% ± 10%) and given with standard pellet diet (supplied from Amrut, Pranav Agro Industries Ltd., Sangli, India) and water *ad libitum*. All animals used in experiment were randomly selected and classified into various groups before beginning of experimental investigation; all were adopted for the duration of 7 days under above said standard habitat conditions. The study protocol had been approved by the IAEC, IJAHSM, Bangalore (Ref. no. IJAHSM/IAEC/2014/03), which is registered under CPCSEA, New Delhi.

Acute oral toxicity studies

The OECD guidelines number 423 (up and down procedure) was referred to determine acute oral toxicity of the methanol extract of *T. pumila*. A starting dose of 2000mg/kg, B.W was administered to three albino rats orally and observed them for 14days. The same study was reiterated once more with the same dose range, 2000 mg/kg B. W p. o. of TPME for 3 days more, and noticed for 14 days.[17]

Evaluation of *in vivo* antidiabetic activity

Induction of diabetes in experimental animals

Alloxan monohydrate at 150 mg/kg was by injected intraperitoneal for the production of diabetes in experimental rats in both acute and chronic antidiabetic models. The dose alloxan was first calculated individually for each experimental rat based on their b. w and then dissolved using 0.2 ml saline (154 mM NaCl) just before administration. 2 days after alloxan injection, albino rats with blood glucose range of >140 mg/dl were selected for the investigation. Both the studies were started 48 h after alloxan injection.[18]

Group design

For both study models, oral glucose tolerance test (OGTT) and chronic antidiabetic study, the experimental animals were classified into six groups each containing of six and all the experimental rats except normal (Group I) were produced diabetes by injecting single dose of alloxan as described above. The animal grouping is as follows:

- Group I: Normal animals group given normal saline alone
- Group II: Diabetic alone group (vehicle control) administered p. o with alloxan and vehicle 3% of Tween 20
- Group III: Positive control (standard group) administered alloxan and glibenclamide 5 mg/kg
- Group IV: TPME (low dose) group administered alloxan and methanol extract of *T. pumila* 100 mg/kg, p. o
- Group V: TPME (medium dose) group administered alloxan and methanol extract of *T. pumila* 200 mg/kg, p. o
- Group VI: TPME (high dose) group administered alloxan and methanol extract of *T. pumila* 400 mg/kg, p. o.

OGTT

The suspensions of reference standard drug glibenclamide and TPME were formulated using Tween 20 as suspending substance and administered to specific group animals at the 3rd day after the induction of diabetes in experimental rats, using oral feeding tubes as described in the above protocol. 1 h after treatment with TPME and glibenclamide, the samples of blood were collected from all experimental animals and the basal blood glucose was estimated. All animals were given glucose solution (2 g/kg) orally and samples of blood from each animal were obtained at distinct intervals of time 30, 60, 90, and 120 min and quantified for plasma glucose using Glucometer (Accu-Chek).[19-21]

Chronic study model

In chronic antidiabetic study model also animals were divided into six groups as same as above. The reference standard glibenclamide and methanol extract of *T. pumila* were administered daily once in the early morning to respective group of animals as per their body weights from the 1st day to 21st day. Blood sampling from each experimental animal was obtained on day 1st, 7th, 14th, and 21st and determined for plasma glucose. On the 21st day of investigation, blood samples had been also determined for concentration of insulin, cholesterol, triglycerides, creatinine, urea, alanine aminotransferase, and aspartate transaminase (ALT and AST).[21-23]

Blood sampling method and determination of parameters

Blood sampling from all experimental animals was done by puncturing retro-orbital vein under mild ether anesthesia. The blood glucose was estimated using Glucometer (Accu-Chek). On the 21st day of study, serum layer was differentiated from blood samples and tested for concentration serum for triglycerides and cholesterol by enzymatic DHBS using colorimeter and ALT, AST, urea, creatinine, and insulin were also estimated using standard diagnostic reagent kits procured from Span Diagnostics (AUTOSPAN).

Evaluation of *in vitro* antidiabetic activity

α-Glucosidase inhibitory assay

The test was executed to explore *in vitro* inhibitory potentials of TPME on carbohydrate digestive enzyme α-glucosidase for sucrose and maltase in gastrointestinal tract (GIT). In spite of α-glucosidase enzyme separated from yeast is considerably used for the evaluation of α-glucosidase inhibitor drugs, the results may not always in accordance with those obtained from mammal enzymes.

Hence, in the present research study, small intestine homogenate of albino mice was used as solution of alpha-glucosidase enzyme since it postulated that it would better reflect the *in vivo* physiological state. The glucosidase and amylase inhibitory activity of TPME was measured by slightly modifying the methods used in the previous research studies.[24] The segment of the small intestine of experimental mouse duodenum and cecum was cut and removed after 20 h of fasting. The part of intestine collected was rinsed using ice-cold normal saline solution and subjected to homogenization with 12 mL of maleate buffer (100 mM, pH 6). The homogenate substance acquired was utilized as α-glucosidase solution for further investigation. The reaction mixture of assay composed of 100 mM maleate buffer (pH 6), 2% (w/v) of sucrose and maltose substrate solution (100 ml), and methanol extract of *T. pumila* (20–640 µg/mL). After the pre-incubation for 5 min at 37ºC reaction mixture, the reaction was started by adding raw α-glucosidase enzyme solution (50 ml), which is again incubated for 10 min at 37ºC. The quantity of glucose generated in the present reaction was estimated by a glucose assessment kits (Span Diagnostic Ltd., Mumbai, India). The amount of glucose released by the positive control (GCP- concentration of Glucose produced by the Positive control), glucose generation blank value (GCB- concentration of Glucose produced by the Blank), and quantity of glucose produced by the addition of TPME (GCT- concentration of Glucose produced by the Test) was noted.[24,25] The rate of carbohydrate degradation was assessed as a percentage ratio to the quantity of glucose generated when the carbohydrate was entirely degraded. The rate of inhibition was determined by the following formula:

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

α-Amylase inhibitory assay

The assay samples of the methanol extract of *T. pumila* at serial concentrations (6.25, 12.5, 25, 50, 100, and 200 mg/mL) and reference standard nojirimycin (6.25–200 µg/mL) of 500 ml were added to 500 ml of 0.02 M sodium phosphate

buffer (at pH 6.9 with 0.006 M sodium chloride) containing 0.5 mg/mL porcine pancreatic enzyme alpha-amylase solution and were kept for incubation for 10 min at temperature 25ºC. After the pre-incubation, 500 ml of solution of 1% starch in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) was incorporated to every assay at predetermined time intervals. The assay mixtures were then incubated for 10 min at 25ºC. The reaction of assay was concluded by incorporating 1 mL of 3,5-dinitrosalicylic acid color reagent. All test tubes were kept for incubation in a water bath at boiling condition for 5 min and cooled to normal room temperature under tap water. The assay was then diluted by incorporating 10 mL of distilled water and absorbance was recorded at 540 nm.[24,25]

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

Glucose uptake by isolated rat hemidiaphragm

The utilization of glucose by skeletal muscle of rat (hemidiaphragm) was assessed according to methods described in the previous investigations.[25] The study consisting of four categories, with each group containing six graduated test tubes, was regarded as follows:

- Category I: Consists of 2 mL of 2% glucose in Tyrode solution
- Category II: Consists of 2 mL of 2% glucose in Tyrode solution and regular insulin suspension
- Category III: Consists of 2 mL of 2% glucose in Tyrode solution and 1.38 mL of TPME (0.1% v/v)
- Category IV: Consists of mL of 2% glucose in Tyrode solution and regular insulin (0.62 mL of 0.4 U/mL) solution and 1.38 mL of TPME (0.1% v/v).

The quantities of all the assay tubes were made up to 4 mL individually by mixing distilled water to make up the total volume of the assay tubes. A total of healthy albino rats of Wistar species were kept fasting for whole night and sacrificed under light anesthesia. The diaphragms of experimental animals were quickly cutted with little damage and splitted into two equal halves. For the same set of study, two diaphragms from the same animal were not used. About xix diaphragms were utilized in every category of study. The collected skeletal muscles (diaphragm) were kept in assay tubes and incubated at 37ºC for about 30 min in an atmosphere constitutes 100% oxygen and were shuddered at a speed of 140 CPM. The amount of utilization of glucose per every gram of tissue was determined as the difference between the concentrations of starting and final glucose in the incubated medium.[25]

Statistical analysis

The data obtained from the present investigation were analyzed by ANOVA followed by *post hoc* Dunnett's *t*-test with the help of GraphPad Prism 5 software. All the values were shown as mean ± standard error of mean.

Results

Preliminary phytochemical study

The percentage yield of the methanol extract was observed to be 8.19 % w/w. The primary phytochemical examination performed in the study for the methanol extract of *T. pumila* has shown the presence of various constituent alkaloids, glycosides, polyphenols, flavonoids, tannins, steroids, and carbohydrates drug [Table 1].

Acute toxicity studies

The methanol extract of *T. pumila* was safe up to dose of 2 g/kg b. w. and produced neither mortality nor any indications of clinical complications in the TPME treated experimental animals during 14 days of survey period after administration of drugs. There was no significant modification in body weight of experimental animals before and after administration of extract and no signs of toxicity were seen. As per the results obtained from acute oral toxicity study, doses for further investigation considered as 100mg/kg (low dose), 200mg/kg (medium dose) and 400 mg/kg (high dose) on the ratio 1/20th, 1/10th and 1/5 respectively.

Evaluation of *in vivo* antidiabetic activity

Oral glucose tolerance test

In acute study of OGTT, diabetic control rats treated with vehicle have exhibited a significant increase in plasma glucose range throughout investigation period when collate to normal group of rats. However, administration of glibenclamide and TPME (at 200 and 400 mg/kg) could be capable to reduce blood glucose significantly ($P < 0.001$) in therapeutic animals by improving the utilization of oral glucose after 60 and 120 min. The results of OGTT are given in Table 2.

Assessment of chronic antidiabetic activity

In long-standing antidiabetic test, the blood glucose level was significantly ($P < 0.001$) elevated in disease (diabetic) alone rats when compared to normal group due to the induction of diabetes. But administration of glibenclamide and TPME (200mg/kg and 400mg/kg) in therapeutic animals could significantly (P<0.001) decreased blood glucose concentrations

Table 1: Results of preliminary phytochemical investigation on methanol extract of *Tephrosia pumila*

Phytoconstituent	Presence
Flavonoids	+++
Polyphenols	+++
Glycosides	+++
Alkaloids	+
Carbohydrates	++
Proteins	++

Table 2: Effect of the methanol extracts of *Tephrosia pumila* on blood glucose oral glucose tolerance test

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
TPME (100 mg/kg)	171.5±3.585	273.8±2.713	250.0±2.978	231.0±2.352	196.2±3.177
TPME (200 mg/kg)	174.7±3.639	270.8±5.338	220.7±3.461	194.2±2.242	152.7±6.081
TPME (400 mg/kg)	174.8±3.468	272.5±3.274	192.3**±1.856	159.2**±2.798	120.3**±3.938

Values are mean±SEM, n=6 symbols represent statistical significance. $^{ns}P>0.05$, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ versus diabetic control. $^{ns}P>0.05$, $^{+}P<0.05$, $^{++}P<0.01$, $^{+++}P<0.001$ normal control versus positive control. SEM: Standard error of mean, TPME: Methanol extract of *Tephrosia pumila*

Table 3: Effect of the methanol extracts of *Tephrosia pumila* 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 5 mg/kg)	234.2±6.290	211.8±4.143	171.5±3.374	145.2±1.740
TPME (100 mg/kg)	237.3±3.169	232.2±3.124	216.3±1.783	207.8±3.124
TPME (200 mg/kg)	235.7±6.270	218.8±3.462	203.8±2.522	171.8±1.778
TPME (400 mg/kg)	232.0±7.908	211.2±5.029	170.3±3.955	143.5±2.717

Values are mean±SEM, n=6 symbols represent statistical significance. $^{ns}P>0.05$, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ versus diabetic control. $^{ns}P>0.05$, $^{+}P<0.05$, $^{++}P<0.01$, $^{+++}P<0.001$ normal control versus positive control. SEM: Standard error of mean, TPME: Methanol extract of *Tephrosia pumila*

compare to diabetic control group at 14th and 21st day of the study [Table 3].

The significant ($P < 0.001$) decline of the concentration of serum insulin was found in vehicle control group compare to normal group due to the treatment of alloxan. In animals administered with glibenclamide and TPME (200 and 400 mg/kg), there was considerable ($P < 0.001$) elevation in plasma insulin quantities when compared to diabetic control group and the results were almost similar to that of normal animals [Table 4].

The total serum cholesterol, triglycerides, urea, and creatinine concentrations in the blood sample were significantly ($P < 0.01$) elevated in diabetic alone animals collate to normal group of animals. However, decline in the concentrations of serum total cholesterol, triglycerides, urea, and creatinine was found in glibenclamide and TPME (200–400 mg/kg) pre-treated rats when compare to disease control rats [Table 4].

It is found that there are no significant ($P > 0.01$) alterations in AST and ALT levels in diabetic alone compare to normal animals and also no significant change was found in therapeutic group given with reference standard and TPME when compare to rats of vehicle control group [Table 4].

Evaluation of *in vitro* antidiabetic activity

α-Glucosidase inhibitory activities

An *in vitro* α-glucosidase enzymes inhibitory test was performed to estimate the inhibitory potentials of the methanol extract of *T. pumila*. The half of maximal concentration

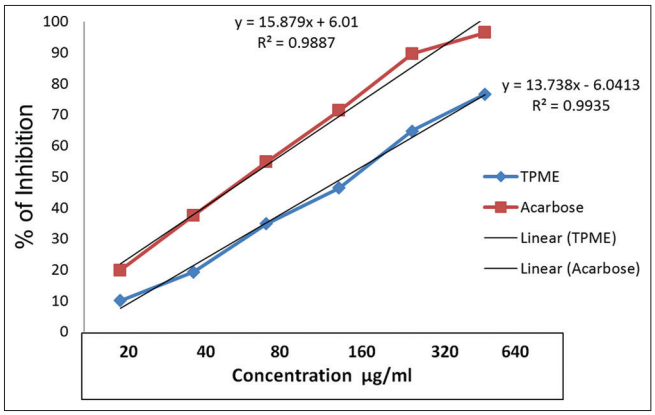

Figure 1: Effect of TPME on α-glucosidase (sucrase) and amylase inhibitory activity

requires to inhibit sucrose and maltase enzymes (IC_{50}) were 398.513 µg/mL and 78.412 µg/mL, respectively. The shows that TPME exhibited potent property which depends on dose and is thus considered to be a powerful α-glucosidase inhibitory drug [Table 4 and Figures 1, 2].

α-Amylase inhibitory activities

To evaluate the inhibitory activity of the methanol extract of *T. pumila on* postprandial glucose rise, an *in vitro* α-amylase inhibition test was performed. In this study, TPME have shown strong inhibitory action against α-amylase with IC_{50} of 133.483/mL, which was comparable with reference standard [Table 4 and Figure 3].

Effect on peripheral glucose uptake

Table 4: Effect of the methanol extracts of on serum parameters *Tephrosia pumila* 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
TPME (100 mg/kg)	80.00±1.807	107.8±1.733	131.3±1.323	1.204±0.01808	69.51±0.8286	63.93±1.057	135.0±2.907
TPME (200 mg/kg)	102.0±2.251	95.67±1.996	112.0±2.338	0.9867±0.03238	55.38±1.231	63.70±1.743	131.7±2.854
TPME (400 mg/kg)	133.3±3.263	89.58±3.070	105.9±2.682	0.5790±0.02607	38.34±1.050	62.78±1.079	130.3±2.011

Values are mean±SEM, *n*=6 symbols represent statistical significance. $^{ns}P>0.05$, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ versus diabetic control. $^{ns}P>0.05$, $^{+}P<0.05$, $^{++}P<0.01$, $^{+++}P<0.001$ normal control versus positive control. SEM: Standard error of mean, TPME: Methanol extract of *Tephrosia pumila*

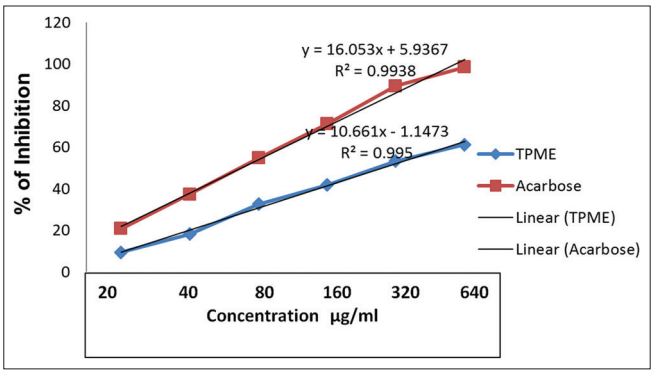

Figure 2: Effect of TPME on α-glucosidase (maltase) and amylase inhibitory activity

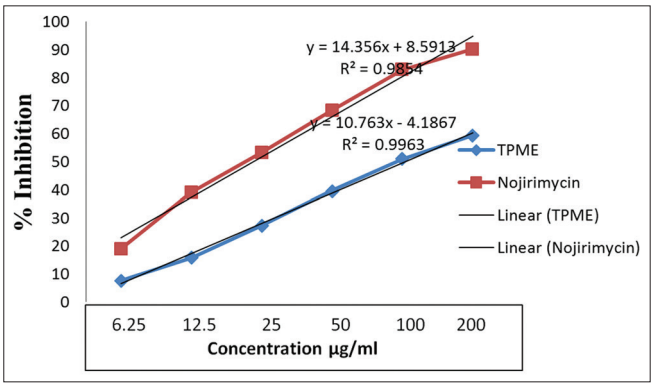

Figure 3: Effect of TPME on amylase inhibitory activity

In the present study, the methanol extract of *T. pumila A*significantly increased utilization of glucose by rat hemidiaphragm and the effect was comparable to standard agent insulin. The combination of TPME with insulin has shown synergistic property. The results clearly indicate that administration of insulin and TPME alone for 30 min caused a significant enhancement in glucose absorption by 3.37 and 2.80 times, respectively. Addition of both insulin and TPME to the incubation media exhibited the rate by 3.55 times, an elevation of the utilization of glucose hemidiaphragm of rat when compared with untreated control animals, but there was no much significant elevation compared insulin alone treated group [Table 6]. The glucose utilization by rat skeletal muscle was considerably large in all the categories examined when collate with the vehicle control.

Discussion

Diabetes mellitus is a metabolic, multifactorial, and devitalizing disease with increasing occurrence in the entire world[26] which may lead to various complications such as multiorgan failures, peripheral neuropathy, retinopathy, nephropathy, hyperlipidemia, and various cardiovascular disorders.[27,28] Alloxan potent diabetogenic chemically a cyclic urea analogue, which specifically kills β-cells of Langerhans of pancreas that generates insulin free radical mediated destruction when given to rats can produces diabetes mellitus. Hence, alloxan was

Table 5: Effect of the methanol extract of *Tephrosia pumila* on α-glucosidase (sucrase and maltase) and amylase inhibitory activity

Treatment	IC_{50} values (μg/ml)		
	α-glucosidase (sucrase)	α-glucosidase (maltase)	α-amylase
TPME	277.567	91.411	116.29
Acarbose	103.425	98.33	-
Nojirimycin	-	-	37.258

TPME: Methanol extract of *Tephrosia pumila*

Table 6: Effect of the methanol extract of *Tephrosia pumila* on glucose uptake by isolated rat hemidiaphragm

Serial number	Glucose uptake for 30 min (mg/g)
Control	78.234±1.66
Insulin	264.11±2.88**
TPME	219.69±1.23**
TPME+insulin	278.44±2.94**

Values are mean±SEM (n=6). **P<0.01 as compared with control. SEM: Standard error of mean, TPME: Methanol extract of *Tephrosia pumila*

reported as a potent diabetes causing agent[29] and has been extensively given to experimental rats for the production of diabetes laboratory.

In the *in vivo* study to evaluate the acute effect of TPME, OGTT was performed to test the ability of the body to utilize oral glucose load in the presence of the methanol extract of *T. pumila* (TPME) in diabetic animals. In diabetic control group of animals, the blood glucose was significantly elevated at all intervals, indicating the reduced ability of the system to utilize the glucose, whereas the blood glucose had fallen down <100 mg/dl in normal group of animals since the ability of the body was proper. Therapeutic groups treated with low and medium doses of TPME significantly decreased blood glucose concentration at 90 and 120 min interval indicates property of TPME to enhance the utilization of glucose by living system, and this effect was comparable to the reference standard drug glibenclamide.

The chronic *in vivo* study was designed to examine the long-term consequences of methanol extract against alloxan produced diabetes in albino Wistar rats. The blood glucose range in experimental animals was assessed at every 7-day interval of the investigation to test the ability of the TPME in removing glucose from blood in diabetic animals and, furthermore, insulin, total cholesterol, triglycerides, urea, creatinine, ALT, and AST in serum at the 21st day of the study.

In chronic study of 21 days, there was a significant rise in blood glucose range observed in disease control animals throughout study due to the destruction of β-cells of pancreas and impairment in insulin secretion. However, in animal groups treated with TPME (at 200 and 400 mg/kg), blood glucose significantly declined was at 14th and 21st days of the study which was witnessed by the enhancement in insulin secretion. This clearly indicates the potential of the TPME to reverse the pancreatic β-cell damage.

Along with other risk factors, secondary hyperlipidemia is one of the major causes of increased incidence of coronary atherosclerosis which is significantly observed in people with prolonged diabetes mellitus. Hyperlipidemia is impediment in metabolism which is characterized by the enhanced plasma cholesterol and triglycerides.[30-33] In the present study, diabetic control animals have shown significant elevation of serum cholesterol and triglycerides while in animals administered with glibenclamide and TPME (200 and 400 mg/kg), significant decrease was observed compared to diabetic control group.

The amount of serum creatinine and urea was significantly enhanced in diabetic alone animals due to renal malfunction caused by hyperglycemia, but their concentrations were significantly declined due to the administration of TPME (200 and 400 mg/kg) in therapeutic animals indicates the potential of the TPME to reverse renal complication in diabetes mellitus.

It is well known that there is clear connection between liver disease and diabetes, the general pervasiveness being considerably larger than that anticipated by a chance relation of two more general diseases.[34-36] However, in the present study, no significant changes or rise of the liver enzymes AST and ALT were observed in diabetic alone animals when compare to normal group of animals. The concentrations of ALT and AST in therapeutic groups were also normal.

One of the novel therapeutic approaches for the management of diabetes mellitus is inactivation of carbohydrate-hydrolyzing enzymes such as α-amylase and α-glucosidase enzyme to counter the absorption of glucose from GIT and thereby to reduce the postprandial hyperglycemia and its problems.[37,38] The α-glucosidase enzyme inhibition by TPME was examined by performing the α-glucosidase inactivity potential with 4-nitrophenyl-b-D-glucopyranosiduronic acid (pNPG) as the reaction precursor using small intestine of as a source of α-glucosidases, sucrase, and maltase.[39,40] In the present investigation, TPME have exhibited significant α-glucosidase and α-amylase inhibitory properties indicate its usefulness to reduce postprandial glucose, but still, it is not clearly understood whether the inactivation of α-amylase enzyme and α-glucosidase enzyme by TPME is due to competitive or non-competitive inhibition mechanisms. However, the rate of inactivation for α-glucosidase enzyme was near to that of acarbose a reference standard drug used in the study, but the inactivation rate for α-amylase was little than that of standard drug. This shows that TPME is a potent inhibitor of α-glucosidase with less potent inhibitory property versus α-amylase. The α-glucosidase along with α-amylase enzyme inhibitory properties of TPME can regard to be a productive approach for the prevention of diabetes mellitus by declining the uptake of glucose into blood. Significant post-meal

hyperglycemia very commonly experienced by patients with diabetes could be controlled if the rate of uptake glucose from the GIT into the blood circulation could be declined by inactivating hydrolysis of carbohydrate.[41]

Skeletal muscle comprises about 30%–40% of the total quantity of body, and hence, it can be one of the most major target tissues for the activity of insulin which enhances the utilization of glucose at the peripheral level. It is well understood that insulin and antidiabetic drugs stimulate glucose utilization by peripheral cells and tissues.[42] Another major finding of the present study is that TPME have significant action similar to insulin as witnessed by the stimulation of glucose utilization from the rat's hemidiaphragm, which constitutes muscle tissue that is essential tissues of insulin-regulated glucose discharge. TPME considerably enhanced the uptake of glucose by isolated rats muscle hemidiaphragm and is observed to be less potent than insulin. It seems that TPME has action on peripheral tissues and results of the normal group of glucose utilization by rat peripheral tissue corresponds with those of earlier findings.[43]

The similar study using same set of normal and standard was conducted simultaneously for *T. calophylla* belongs to the same genus *Tephrosia* but which possess different phytoconstituents.[44] The study revealed the significant antidiabetic activity in alloxan-induced diabetes in albino rats. The methanol extract was also significantly effective to inhibit carbohydrate digestive enzymes α-glycosidase and α-amylases and also significantly increased utilization of the glucose by skeletal muscle. The results obtained suggest that antidiabetic potentials of the methanol extract of *T. calophylla* were potent than that of methanol extract of *T. pumila*. The results of the study were communicated to a scientific journal.[45]

In spite of there is no clear specific mechanism of alloxan responsible for pancreatic damage understood, investigations propose that the alloxan destroys pancreatic β cells due to its free radical nature which followed by absolute insulin deficiency and diabetes mellitus.[46,47] The previous researches conducted have suggested that antioxidant activity can be one of the possible mechanisms of action for antidiabetic activity that protects pancreatic cells against oxidative damage.[46] Hence, further study can be performed to explore antioxidant activity of TPME to determine its ability to reduce reducing insulin resistance which is also an important mechanism required for antidiabetic activity.[48]

In the current *in vivo* assay, the methanol extract had been effective to stimulate insulin secretion and to regulate the normal glucose level in the therapeutic groups. The study should be conducted to determine the antioxidant properties of TPME which is possible mechanism of action in the present study that can defend pancreatic cells against alloxan-mediated damage and normalize the insulin release. In *in vitro* findings, TPME exhibited its potency to counter insulin resistance by increasing the utilization of glucose by peripheral tissues and extract also exhibited its potentials in inhibiting GIT digestive enzymes to prevent complications of postprandial hyperglycemia.

Conclusion

The methanol extract of aerial parts of *T. pumila* possesses significant *in vivo* antidiabetic activity against alloxan-induced diabetic animal model. The results acquired from the present study also propose that methanol extract of *T. pumila* could block enzymes α-glucosidase and α-amylase *in vitro* and also significantly increase utilization of glucose by skeletal muscle. However, further examination is necessary to isolate and estimate the specific components present in methanol extract of *T. pumila* that may be responsible for these beneficial properties to improve the health conditions connected with diabetes mellitus.

Acknowledgments

The authors of manuscript are thankful to the principal and management of East West College of Pharmacy, Bangalore and ANU College of Pharmaceutical Sciences, Guntur, for providing facilities to conduct this research work.

References

1. Valiathan MS. Healing plants. Curr Sci 1998;75:1122-27.
2. World Health and Organization. Prevalence Data of Diabetes Worldwide; 2009. Available from: http://www.who.int/mediacentre/factsheets/fs312/en/index.html. [Last cited on 2017 Sep 12].
3. Nicasio P, Aguilar-Santamaría L, Aranda E, Ortiz S, González M. Hypoglycemic effect and chlorogenic acid content in two *Cecropia* species. Phytother Res 2005;19:661-4.
4. Rao BK, Rao CH. Hypoglycemic and antihyperglycemic activity of *Syzygium alternifolium* (Wt.) Walp. Seed extracts in normal and diabetic rats. Phytomedicine 2001;8:88-93.
5. Sharma SB, Nasir A, Prabhu KM, Murthy PS. Antihyperglycemic effect of the fruit-pulp of Eugenia jambolana in experimental diabetes mellitus. J Ethnopharmacol 2006;104:367-73.
6. Jagat PS, Singh S, Garg H, Khanna NM, Dhar MM. A flavonol glycoside with anticancer activity from *Tephrosia candida*. Phytochemistry 1976;15:232-4.
7. Yuh-Lin C. New piscicidal flavonoids from *Tephrosia obovata*merr. Asian J Pharm 1978;3:18.
8. Bentley MD, Hassanali A, Lwande W, Njoroge PE, Yatagai M. Insect anti feed ants from *Tephrosia elata* Deflers. Insect Sci Appl 1987;8:85-8.
9. Baranwal A, Mazumder A, Chakraborthy GS, Gupta S. Phytopharmacological uses of *Tephrosia purpurea* a review. Pharmacophore 2014;5:658-65.
10. Lakshmi P, Ganapaty S, Lakshminarayana K. Phytochemical and biological examination of the aerial parts of *Tephrosia pumila*. J Pharm Res 2010;3:193-7.
11. Murthy CH, Sunil J, Kumar PS, Rajkumar G. Antidepressant and anxiolytic effects of alcoholic extract from *Tephrosia pumila* (l.) Pers. World J Pharm Sci 2017;6:1648-50.
12. Pethakamsetty L, Seru G, Kandula L. Antimicrobial activity of the root

extracts of *T. pumila* and *T. tinctoria* on clinical and phytopathogens. J Pharm Res 2009;2:1694-6.

13. Moshi MJ, Richard M, Mbwambo ZH, Nondoa RS, Masimbaa PJ, Masimbaa PJ, *et al*. Evaluation of ethnomedical claims and brine shrimp toxicity of some plants used in Tanzania as traditional medicines. Afr J Trad CAM 2006;3:48-58.
14. Ganapaty S, Pannakal ST, Srilakshmi GV, Lakshmi P, Waterman PG, Brun R. Pumilanol, an antiprotozoal isoflavanol from *Tephrosia pumila*. Phytochem Lett 2008;1:175-8.
15. Kokate CK. Practical Pharmacognosy. Vol. 4. New Delhi: Vallabh Prakashan; 1994. p. 110-1.
16. Khandelwal KR. Practical Pharmacognosy-Techniques and Experiments. Pune: Nirali Prakashan; 2000.
17. OECD. Acute Oral Toxicity-Acute Oral Toxic Class Method. Guideline No. 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: OECD; 2000.
18. Vogel G, Vogel H. The Text Book of Pharmacological Screening Methods and Drug Evaluation of anti-diabetics. Springer Publishers, Newyork, USA 2002.
19. Jain S, Bhatia G, Barik R, Kumar P, Jain A, Dixit VK, *et al*. Antidiabetic activity of *Paspalum scrobiculatum* Linn. In alloxan induced diabetic rats. J Ethnopharmacol 2010;127:325-8.
20. Balamurugan R, Duraipandiyan V, Ignacimuthu S. Antidiabetic activity of γ-sitosterol isolated from *Lippia nodiflora* L. In streptozotocin induced diabetic rats. Eur J Pharmacol 2011;667:410-8.
21. National Committee for Clinical Laboratory Standards. User Evaluation of Precision Performance of Clinical Chemistry Devices. NCCLS; 1984, NCCLS: Publication. Available from: http://www.thermo.com/eThermo/CMA/PDFs/Product/productPDF_50918.pdf. [Last Accessed on 2017 Nov 27].
22. Kennedy JW, Carey RN, Coolen RB. Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline (EP5 A). Wayne, PA: The National Committee for Clinical Laboratory Standards; 1999. Available from: http://www.qualtexlabs.org/pdf/ALT.pdf. [Last Accessed on 2017 Nov 27].
23. Dahlqvist A. Method for assay of intestinal disaccharidases. Anal Biochem 1964;7:18-25.
24. Kwon GJ, Choi DS, Wang MH. Biological activities of hot water extracts from *Euonymus alatus* leaf. J Korean Food Sci Technol 2007;39:569-74.
25. Walaas E, Walaas O. Effect of insulin on rat diaphragm under anaerobic conditions. J Biol Chem 1952;195:367-73.
26. Ajabnor MA, Tilmisany AK. Effects of *Trigonella feonumgraceum* on blood glucose levels in normal and alloxan diabetic mice. J Ethnopharmacol 1998;22:15-49.
27. Dunn JS, Sheehan HL, McLetchie NG. Necrosis of Langerhans produced experimentally. Lancet 1943;1:484-7.
28. Pamela CC, Richard AH, Denise RF. Type 1 Diabetes Mellitus. 1st ed. India: Lippincott's Wlliams and Wlikins; 1994. p. 336-7.
29. Chakrapani SU. Insulin, Glucose Homeostasis and Diabetes Mellitus. 3rd ed. Kolkata: Fundamentals of Biochemistry Kolkata; 2008. p. 678-80.
30. Mohan H. The Endocrine System: Textbook of Pathology. 4th ed. New Delhi: Jaypee Brothers Medical Publishers (P) Ltd.; 2002. p. 849-51.
31. Kumar V, Abul KA, Nelson F. Diabetes Mellitus: Pathologic Basis of Disease. Robbins and Cotran. 9th ed. India: Elsevier; 2004. p. 1189-226.
32. Abdul HZ, Wandro FA, Wadhwa MB, Lawaya BA, Masoodi SR, Shah NA. Serum Lipid profile in non-insulin-dependent diabetes mellitus associated with obesity. Int J Diabetes Dev Ctries 1995;15:9-14.
33. Moscatiello S, Manini R, Marchesini G. Diabetes and liver disease: An ominous association. Nutr Metab Cardiovasc Dis 2007;17:63-70.
34. Messeri S, Messerini L, Vizzutti F, Laffi G, Marra F. Glycogenic hepatopathy associated with Type 1 diabetes mellitus as a cause of recurrent liver damage. Ann Hepatol 2012;11:554-8.
35. Enyioma NO, Abdu A. Update in diabetic nephropathy. Int J Diabetes Metab 2005;13:1-9.
36. Bhandari MR, Anurakkun NJ, Hong G, Kawabata J. Alpha-glucosidase and a-amylase inhibitory activities of nepalese medicinal herb pakhanbhed (*Bergenia ciliata*, Haw.). Food Chem 2008;106:247-52.
37. 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;64:536-7.
38. Toeller M. Alpha-glucosidase inhibitors in diabetes: Efficacy in NIDDM subjects. Eur J Clin Invest 1994;24 Suppl 3:31-5.
39. Chiba S. Molecular mechanism in alpha-glucosidase and glucoamylase. Biosci Biotechnol Biochem 1997;61:1233-9.
40. Honda M, Hara Y. Inhibition of rat small intestinal sucrase and α-glucosidase activities by tea polyphenols. Biosci Biotechnol Biochem 1993;57:123-4.
41. DeFronzo RA, Jacot E, Jequier E, Maeder E, Wahren J, Felber JP, *et al*. The effect of insulin on the disposal of intravenous glucose. Results from indirect calorimetry and hepatic and femoral venous catheterization. Diabetes 1981;30:1000-7.
42. Melander A. Glucose uptake by hemidiaphragm. Diabetes Med 1996;13:143.
43. Cohen G, Heikkila RE. The generation of hydrogen peroxide, superoxide radical, and hydroxyl radical by 6-hydroxydopamine, dialuric acid, and related cytotoxic agents. J Biol Chem 1974;249:2447-52.
44. Saad T, Muhammad AS, Muhammad A. Review on the phytochemistry and pharmacology of genus *Tephrosia*. Phytopharmacology 2013;4:598-637.
45. Ramesh C, Rani AP. *In vivo* and *in vitro* evaluation of *Tephrosia calophylla* for anti-diabetic properties. Int J Pharm Pharm Sci 2018;10:138-44.
46. Okamoto H. Molecular basis of experimental diabetes: Degeneration, oncogenesis and regeneration of pancreatic β-cells. Bio Essays 1995;2:15-21.
47. Takasu N, Asawa T, Komiya I, Nagasawa Y, Yamada T. Alloxan-induced DNA strand breaks in pancreatic islets. Evidence for H_2O_2 as an intermediate. J Biol Chem 1991;266:2112-4.
48. Mishra MR, Mishra A, Pradhan DK, Panda AK, Behera RK, Jha S, *et al*. Antidiabetic and antioxidant activity of *Scoparia dulcis* Linn. Indian J Pharm Sci 2013;75:610-4.