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Research Article

ANTI-DIABETIC ACTIVITY OF *TRICHODESMA INDICUM* (L.) LEAF EXTRACTS

K Narendra, M Satya Prasad, DSD Suman Joshi, KVN Rathnakar Reddi, J Swathi, KM Sowjanya, A Krishna Satya *
Department of Biotechnology, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur, Andhra Pradesh, India
*Corresponding Author Email: akrishnasatya78@gmail.com

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ABSTRACT

The objective of the study was aimed to investigate the anti-diabetic activity of *Trichodesma indicum* (L.) in both *in vitro* amylase assay and *in vivo* streptozotocin- nicotinamide induced type 2 diabetic rats. *T. indicum* leaves were extracted with four solvents Hexane, acetone, methanol and aqueous. They were tested for *in vitro* α amylase inhibitory activity and glucose uptake by yeast cells. The effect of four extracts at 200mg/kg and 400mg/kg is studied in normal and streptozotocin (STZ) – Nicotinamide induced type 2 diabetic rats. The results showed that methanolic leaf extract has moderate α –amylase inhibitory activity (IC_{50} value = 91.3 µg/ml) when compared to acarbose. Glucose uptake assay of four extracts showed significant activity. Methanolic extract showed effective inhibition (IC_{50} value = 46.72 µg/ml) of glucose uptake along with standard. The anti-diabetic activity of four extracts prominently reduces blood glucose levels in STZ-nicotinamide induced diabetic rats. Methanol extract has shown estimable decrease of blood glucose level ($P<0.01$) along with glibenclamide. These findings suggest that anti-diabetic property of *Trichodesma indicum* (L.) methanol extract in type 2 diabetes mellitus is potential.

KEY WORDS: *Trichodesma indicum* (L.), α - amylase, Yeast cells, Glucose, Streptozotocin, Nicotinamide, glibenclamide.

INTRODUCTION

In Ancient times people always depend upon plants for health, food and shelter. The relationship between plants and animals helped to heal diseases in olden days[1]. The world health organization relies on traditional medicine up to 80% for primary health care[2]. 25% of Modern medicines have been derived from medicinal plants[3]. According to world health organization 20,000 plant taxa were included for medicinal uses. In the folk medicine 70,000 plants were included for medicinal purpose, in that majority are found in the Asia-Pacific region[4]. Pharmaceutical industry depends on plants oil for ecofriendly and good source of drugs in the herbal remedies, flavonoids, perfumes and cosmetics[5-7].

Modern medicine gives faster relief than traditional medicine so people are attracted towards synthetic medicine but herbal medicine is cost effective, has fewer side effects and is more bioavailable[8].

Diabetes is the inability of the body to utilize glucose, due to the pancreas failure to secrete sufficient insulin[9]. Diabetes mellitus, one of the most common endocrine metabolic disorder has caused significant morbidity and mortality due to micro vascular (retinopathy, neuropathy, and nephropathy) and macro vascular (heart attack, stroke and peripheral vascular disease) complications. In Type I diabetes body fails to produce insulin (insulin-dependent diabetes mellitus, IDDM). Type II diabetes is a condition in which cells fail to use insulin properly, it also combines with an absolute insulin deficiency (non-insulin dependent diabetes mellitus, NIDDM, adult onset diabetes). Gestational diabetes is found in pregnant women or non-diabetic woman; they have a high blood glucose level during pregnancy[9]. According to World Health Organization the diabetic population is likely to increase up to 300 million or more by the year 2025. Recently, some medicinal plants have been reported to be useful in diabetes worldwide and have been used empirically in anti-diabetic and anti-hyper lipidemic remedies. Anti-hyper glycemic activity of the plants is mainly due to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or to the facilitation of metabolites in insulin dependent processes. More than 400 plant species having hypoglycemic activity have been available in literature. Most of plants contain glycosides, alkaloids, terpenoids, flavonoids, carotenoids, etc., that are frequently implicated as having anti-diabetic effect.

Trichodesma indicum is a perennial herb having effective medicinal properties belongs to the family Boraginaceae. *T. indicum* distributed in south Asia region and roots are used for diuretic, snake bite, diarrhea and dysentery. Previous studies also stated that chloroform root extract have shown anti-inflammatory activity in both acute and chronic models[10]. *T. indicum* whole plant have antitussive activity[11], anti-diarrheal activity[12,13], insecticidal activity[14], metal chelating activity[15] and corrosive inhibitor[16]. *T. indicum* aerial parts are effective against cancerous cell lines and showed highest cytotoxicity against human breast cell line MCF-7[17].

The *T. indicum* plant selection is based on its proven scientific activities like immuno modulatory activity, anti-inflammatory, free radical scavenging activities. These properties may produce pancreatic cellular and tissue protection by their antioxidant activity and avoiding infection and inhibiting the inflammatory damage to pancreas and avoiding pancreatitis.

MATERIALS AND METHODS

Plant material and extraction

T. indicum plant material was collected from seshachalam Hills, Tirupathi, Andhra Pradesh, India. Plant material was taxonomically identified and authenticated by the botanist. Leaves were shade dried and powdered with pulveriser.

Leaves were extracted with four solvents such as Hexane, Acetone, Methanol and Aqueous. The crude extracts were extracted with Soxhlet apparatus and condensed with rotary evaporator. The four crude extracts were condensed and lyophilized to obtain powder form for animal studies.
HETI: Hexane Extract
ACTI: Acetone Extract
METI: Methanol Extract
AQTI: Aqueous Extract

Chemicals and reagents

Potato starch-(1%w/v), Alpha amylase (1%w/v), Acetate Buffer (0.1M, 7.2pH), Iodine-Iodine indicator (635mg Iodine and 1gm potassium iodide in 250 ml distilled water), Acarbose, Yeast cells, Glucose Solution, Metronidazole, Streptozotocin (Sigma chemical co., U.S.A), glibenclamide (micro labs, India) and Glucose.
Other chemicals and reagents used for the study were of analytical grade and procured from approved organizations.

In-vitro Alpha-Amylase Inhibitory Assay:

Alpha amylase is an enzyme that hydrolyses alpha-bonds of large alpha linked polysaccharide such as glycogen and starch to yield glucose and maltose. Alpha amylase inhibitory activity was based on the starch iodine method that was originally developed by shekib[18]. In alpha amylase inhibition method 1ml substrate-potato starch (1% w/v), 1ml of extract solution (HETI, ACTI, METI & AQTI) of five different concentrations such as 100, 200, 300, 400 & 500 µg/ml, 1ml of alpha amylase enzyme (1% w/v) and 2ml of acetate buffer (0.1 M, 7.2 pH) was added. Potato starch solution, alpha amylase solution and drug solution was prepared in acetate buffer. The above mixture was incubated for 1 hr. Then 0.1 ml Iodine-iodide indicator was added in the mixture. Absorbance was taken at 565 nm in UV-Visible spectroscopy. The percentage inhibition was calculated by comparing slope of test substance with that of enzyme activity. All the tests were performed in triplicate.

$$\% \text{ of Inhibition} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} \times 100$$

A dose response curve was plotted to determine the IC_{50} values. IC_{50} is defined as the concentration sufficient to obtain 50% of a maximum scavenging capacity.

Glucose uptake in Yeast Cells Assay

Yeast cells were prepared according to the method of Yeast cell method[19] briefly, commercial baker's yeast was washed by repeated centrifugation (3,000×g; 5 min) in distilled water until the supernatant fluids were clear and a 10% (v/v) suspension was prepared in distilled water. Various concentrations of extracts (1–5 mg) were added to 1 mL of glucose solution (50 mM) and incubated together for 10 min at 37°C. Reaction was started by adding 100 µl of yeast suspension, vortex and further incubated at 37 °C for 60 min. After 60 min, the tubes were centrifuged (2,500 × g, 5 min) and glucose was estimated in the supernatant. Metronidazole was taken as standard drug. The percentage increase in glucose uptake by yeast cells was calculated by comparing slope of test substance with that of enzyme activity. All the tests were performed in triplicate[20].

$$\% \text{ of Inhibition} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} \times 100$$

A dose response curve was plotted to determine the IC_{50} values. IC_{50} is defined as the concentration sufficient to obtain 50% of a maximum scavenging capacity[21].

Experimental Animals

Male Sprague Dawley rats weighing between 150-200 g were obtained from Sainath Agency, Hyderabad, Andhra Pradesh, India. The animals were housed under standard environmental conditions (temperature of 25 ± 2 °C with an alternating 12h light-dark cycle and relative humidity of 50 ±15%), one week before the start and also during the experiment as per the rules and regulations of the Institutional Animal Ethics committee and of the Regulatory body of the government. They were fed with standard laboratory diet and water *ad libitum* during the experiment. The experimental protocol was approved by the institutional animal ethical committee (IAEC) No: ANUCPS/IAEC/AH/P/13/2015 dated 13/03/2015.

Acute toxicity studies

Oral toxicity test was performed according to OECD guide lines 423 single dosage of 2000 mg/kg body weight. The overnight fasting Rats were given oral dosage of 2000mg/kg body weight observed for 14 days for morbidity and mortality. The four extracts were analyzed for the toxicity study and behavioral changes.

In vivo Acute Anti-diabetic analysis
Oral glucose tolerance test (OGTT)

Oral glucose tolerance test was performed in overnight fasted normal rats. They were divided into four groups, each group consisting six animals. Second and third group treated orally with four extracts of *Trichodesma indicum* leaves at a dose of 200 and 400 mg/kg. Group four is the positive control treated with Glibenclamide (0.5mg/kg). Glucose (2g/kg) was fed after 30min of administration of extracts[22]. Control animals were administered with equal volumes of water. Blood was withdrawn from the retro orbital plexus at 0, 30, 60, 90, 120 min of glucose administration and glucose levels were estimated.

Induction of type 2 diabetes

Type II diabetes was induced in overnight fasted adult albino rats weighing 150-200g by a single intra peritoneal injection of 60 mg/kg of Streptozotocin (STZ) dissolved in a freshly prepared 0.1 M citrate buffer (pH 4.5), 15 min after 120 mg/kg of Nicotinamide (NA) dissolved in normal physiological saline[23]. Hyper glycemia was confirmed by elevated blood glucose levels at 72h and then on day 7 after injection and only animals with fasting blood glucose levels greater than 250 mg/dl were selected for antidiabetic study.

Experimental design

The animals were divided into five groups of three animals each as follows

Group I- Vehicle control, Normal saline (0.9 % w/v NaCl).
Group II- Diabetic control
Group III- Diabetes + Extract 200 mg/ kg, p.o. (HETI/ACTI/METI/AQTI of 200 mg/kg)
Group IV- Diabetes + Extract 400 mg/ kg, p.o. (HETI/ACTI/METI/AQTI of 400 mg/kg)
Group V- Diabetic standard treated, 0.5 mg/ kg of glibenclamide, p. o (micro labs)

The experiment was performed for the four extracts of HETI, ACTI, METI, AQTI with their two different concentrations of 200 and 400mg/kg bwt**.**

The four extracts were given at two different concentrations of 200 & 400 for each individual dose. The effective dosage calculated accordingly and changes in the blood glucose levels were noted in the subsequent hours for 24 h.

Statistical analysis

Values Expressed as Mean ±SEM (n=6) animals. The significance of various treatments and evaluation of data was calculated using One-way analysis of variance ANOVA method followed by Dennett's multiple comparison test and unpaired student's t test method in graph pad prism 5 analysis software and Microsoft Excel software; positive control group was calculated with reference to normal group; experimental groups were calculated with the positive control group & $P<0.05$ was accepted as significant.

RESULTS AND DISCUSSION

***In-vitro* Alpha Amylase Inhibitory Anti-diabetic Assay**

In vitro analysis for anti-diabetic activity mainly depends on inhibition of amylase. Suppression of glucose production or glucose adsorption from intestine has been increased by natural product treatment[24]. α amylase convert starch in to simple sugars. Inhibition of amylase enzyme leads to lowering of adsorption of glucose from starch[25].

The percentage of inhibitory response was calculated for four different extracts and their 50% inhibitory concentration was measured (Table 1 & Graph 1). The concentration of extract was directly proportional to polarity of solvents. The values of HETI 120.6, ACTI 164.08, METI 91.3 and AQTI extract 126.75 inhibitory concentrations. Methanol extract was having its own ability to inhibit amylase enzyme along with standard acarbose. Hexane and aquoues extract was closure to methanol inhibitory concentration, the order of activity follows as: ACTI < AQTI < HETI < METI

Glucose uptake by yeast cells

Anti-diabetic activity has two ways of expression regarding its enzyme and glucose uptake by the cell. Glucose uptake by yeast cells is another type of activity to evaluate anti diabetic assay. Yeast is a unicellular eukaryotic organism. It's easy to conduct experiment in a lab conditions. The uptake of glucose by yeast cell is a facilitated diffusion mechanism. It results lowering of glucose concentration called as hypoglycemic activity.

Four plant extracts were tested for activity (Table 2 & Graph 2). Five concentrations of 100 to 500 µg / ml were evaluated for Inhibitory concentration. METI was having best activity among four extracts when compared with standard metronidazole. Methanol Extract has dose dependent nature and has the ability to exhibit hypoglycemic activity. *T. indicum* has the capacity to inhibit amylase enzyme as well as lowering of glucose concentration. Among all extracts METI has better activity.

The IC_{50} values were 262.06 µg / ml for hexane, 124.4 µg / ml for acetone, 234.33 µg / ml for aqueous and 46.72 µg / ml for the methanol extract. Metronidazole was the standard drug for reference. *Trichodesma indicum* has the ability to inhibit the enzyme responsible for diabetes as well as to inhibit the glucose uptake by the cells.

T. indicum has its own significance towards multiple activities to enhance its nature extended for the additional activities. On comparison of four extracts METI has shown extendable and effective activity against all areas of biological activities.

***In vivo* Acute Anti - diabetic Assay**

Hyperglycemia fundamentally involves over production and decrease glucose utilization by the tissues[26]. Streptozotocin destroys β cells of the pancreas leads to pancreatic islet β cell cyto toxicity mediated through free radical release and oxidative stress has been created[27]. Nicotinamide restore some of the β cells and prevent the loss of β cells leads to partial diabetes. Diabetes induced in rats very close to type 2 model so that these rats were used for the study of type 2 diabetes mellitus in *in vivo* studies.

Four extracts prepared from leaves with two different concentrations were selected for acute anti diabetic activity based on acute toxicity study. Doses selected for the study based on their toxicity levels and literature available. Type 2 diabetes mellitus was induced by STZ-Nicotinamide induced rats selected for evaluation of anti-diabetic activity.

Preliminary oral glucose tolerances were calculated for sustainability of rats towards high glucose levels shown in table 3 to 6 & Graph 3. Glucose levels were measured for eight hours after administration of treatment doses. The results were obtained for each parameter (given in table 7 to 10 & Graph 4). Four extracts have shown significant effect on lowering of blood glucose levels, HETI, ACTI are less effective and METI and AQTI has significant anti-diabetic activity.

The results stated in table 7 to 10, hexane, acetone, methanol and aqueous extracts of *Trichodesma indicum* leaves, at single dose levels and double dose levels are tested for their antidiabetic activity on STZ-Nicotinamide induced model. The methanol extract has shown best diabetic control at a concentration of 400mg/kg body weight. The other three extracts also have significant anti diabetic activity. Hexane extract (HETI) and acetone extract (ACTI) has less activity due to soluble nature of phytochemical compounds. METI and AQTI activity is very much equivalent to the standard drug. The P value of each experiment was calculated and it was significant ($P<0.05$). Diabetic control P value was measured against normal control. The values varied in between groups and it was more significant ($0.001 < P > 0.05$).

The four extracts were selected for the study with two concentrations and each group is having six rats. All the values were expressed as mean ± standard error of mean (SEM). Statistical difference was evaluated by using one-way analysis of

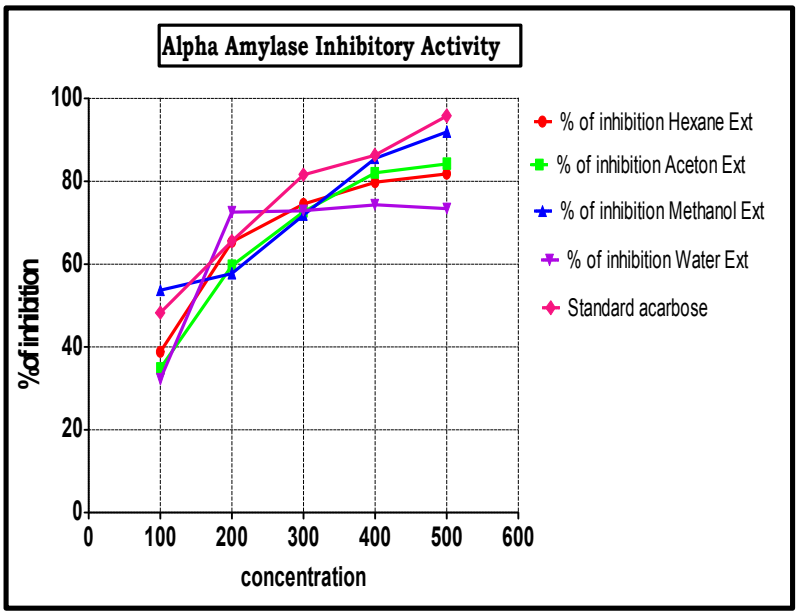
variance (ANOVA) followed by Dunnett's multiple comparison test. Data was considered statistically significant at P value ≤ 0.05. Statistical analysis was performed using Graph Pad prism and Microsoft Excel statistical software.

Table 1. Alpha Amylase Inhibitory, Anti-diabetic Assay of *Trichodesma indicum (L)*

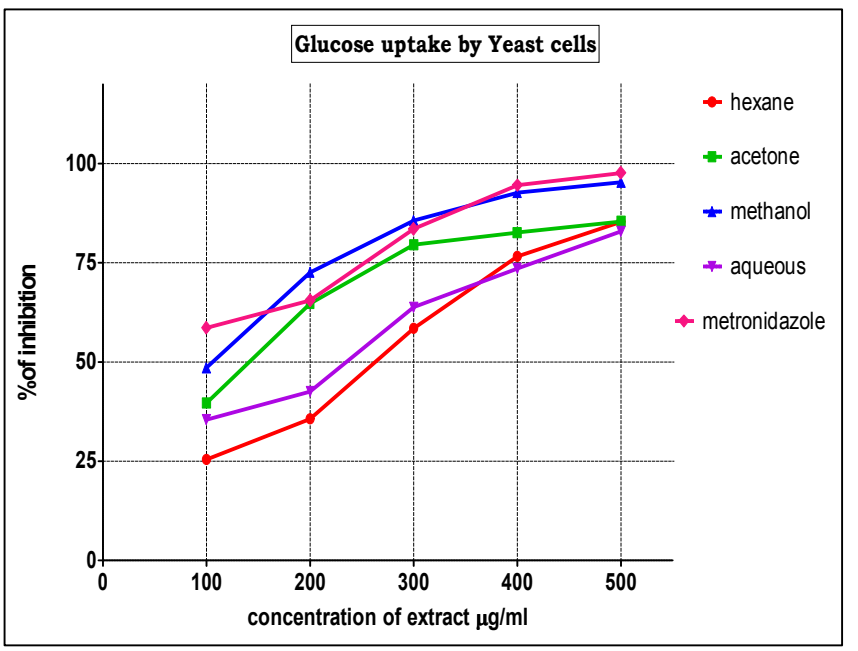
S. No	Name of the Extract	IC $_{50}$ Value (µg/ml)
01.	Hexane Extract	120.6
02.	Acetone Extract	164.08
03.	Methanol Extract	91.3
04.	Aqueous Extract	126.75
05.	Acarbose	84.27

Table 2 Glucose uptake by yeast cells, Anti-diabetic Assay of *Trichodesma indicum (L)*

S. No	Name of the Extract	IC $_{50}$ Value (µg/ml)
01.	Hexane Extract	262.06
02.	Acetone Extract	124.4
03.	Methanol Extract	46.72
04.	Aqueous Extract	234.33
05.	Metronidazole	21.4



Graph 1. Alpha Amylase Inhibitory, Anti-diabetic Assay of *Trichodesma indicum (L)*



Graph 2 Glucose uptake by yeast cells, Anti-diabetic Assay of *Trichodesma indicum (L)*

Table 3 Oral glucose tolerance test on rats HETI Extract

Group	0 min	30 min	60 min	90 min	120 min
Gr I Normal Control	82.26 ± 1.25	153.24 ± 1.50	131.25 ± 2.55	120.54 ± 1.55	104.85 ± 1.60
Gr II HETI 200	83.65 ± 0.64	160.54 ± 1.26	130.56 ± 1.52	121.85 ± 1.45	105.32 ± 2.40
Gr III HETI 400	85.25 ± 0.50	146.32 ± 1.45	123.54 ± 1.62	108.68 ± 2.55	96.54 ± 2.10
Gr IV glibenclamide 0.5 mg/ kg	85.34 ± 0.68	126.35 ± 1.50	102.75 ± 2.30	94.65 ± 2.15	81.56 ± 1.20

Table 4 Oral glucose tolerance test on rats ACTI Extract

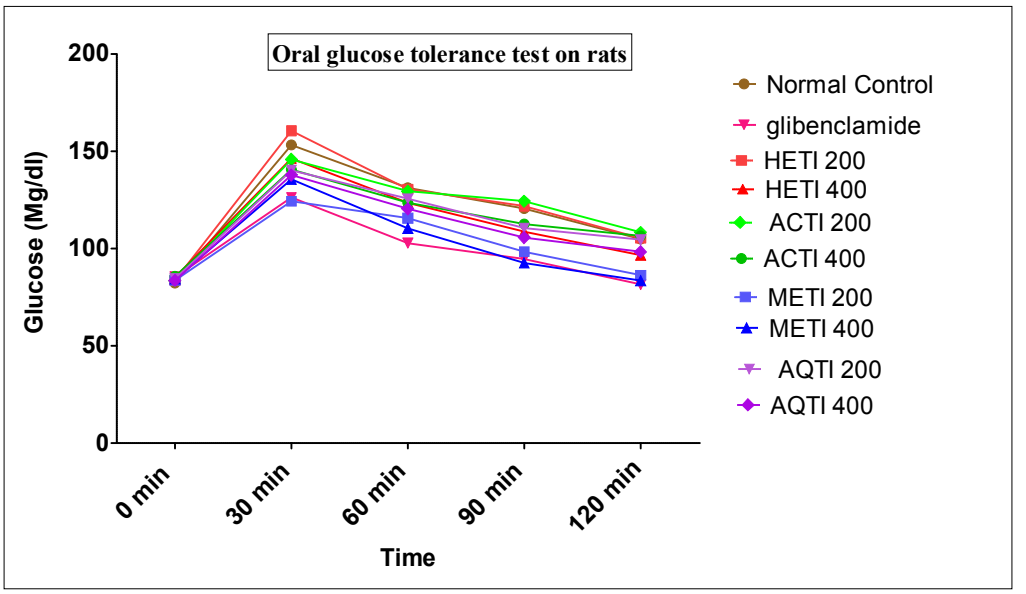
Group	0 min	30 min	60 min	90 min	120 min
Gr I Normal Control	82.26 ± 1.25	153.24 ± 1.50	131.25 ± 2.55	120.54 ± 1.55	104.85 ± 1.60
Gr II ACTI 200	83.56 ± 1.56	145.82 ± 1.24	129.54 ± 1.85	124.35 ± 1.50	108.35 ± 1.24
Gr III ACTI 400	85.69 ± 1.47	140.65 ± 1.45	123.56 ± 1.90	112.58 ± 1.32	106.5 ± 1.28
Gr IV glibenclamide 0.5 mg/ kg	85.34 ± 0.68	126.35 ± 1.50	102.75 ± 2.30	94.65 ± 2.15	81.56 ± 1.20

Table 5 Oral glucose tolerance test on rats METI Extract

Group	0 min	30 min	60 min	90 min	120 min
Gr I Normal Control	82.26 ± 1.25	153.24 ± 1.50	131.25 ± 2.55	120.54 ± 1.55	104.85 ± 1.60
Gr II METI 200	83.65 ± 1.45	124.38 ± 2.50	115.65 ± 1.89	98.32 ± 1.40	86.35 ± 1.20
Gr III METI 400	84.32 ± 1.26	135.62 ± 1.30	110.35 ± 1.65	92.61 ± 1.50	83.54 ± 1.30
Gr IV glibenclamide 0.5 mg/ kg	85.34 ± 0.68	126.35 ± 1.50	102.75 ± 2.30	94.65 ± 2.15	81.56 ± 1.20

Table 6 Oral glucose tolerance test on rats AQTI Extract

Group	0 min	30 min	60 min	90 min	120 min
Gr I Normal Control	82.26 ± 1.25	153.24 ± 1.50	131.25 ± 2.55	120.54 ± 1.55	104.85 ± 1.60
Gr II AQTI 200	84.52 ± 1.40	140.25 ± 1.23	125.64 ± 1.56	110.54 ± 1.23	104.52 ± 1.28
Gr III AQTI 400	83.54 ± 1.36	137.85 ± 1.42	120.47 ± 1.34	105.64 ± 1.65	98.36 ± 1.36
Gr IV glibenclamide 0.5 mg/ kg	85.34 ± 0.68	126.35 ± 1.50	102.75 ± 2.30	94.65 ± 2.15	81.56 ± 1.20



Graph 3 Oral glucose tolerance test on rats

Table 7 Effect of HETI on Blood glucose level in Acute Model

Group	0 h	1 h	2 h	4 h	6 h	8 h
Gr I Normal Control	85.3 ± 1.3	84.3 ± 1.0	86.6 ± 1.2	87.4 ± 1.2	86.5 ± 1.5	86.3 ± 1.4
Gr II Diabetic Control	290.6 ± 1.2	284.5 ± 2.3***	280.2 ± 1.5***	285.6 ± 1.5***	285.4 ± 1.6***	290.7 ± 1.2***
Gr III HETI 200	286.5 ± 1.2	270.5 ± 1.8^{ns}	258.3 ± 2.3^{ns}	242.7 ± 1.5^{ns}	226.4 ± 1.6^{ns}	206.4 ± 1.5^{ns}
Gr IV HETI 400	285.2 ± 1.4	262.2 ± 1.6*	248.6 ± 2.4*	226.8 ± 1.4*	212.5 ± 1.5*	184.6 ± 1.4*
Gr V glibenclamide 0.5 mg/ kg	290.6 ± 1.2	256.2 ± 1.6**	235.4 ± 1.3**	216.8 ± 1.5**	180.5 ± 1.4**	164.7 ± 1.2**
Values Expressed as Mean ±SEM (n=6) animals *P<0.05 **P<0.01 ***P<0.001 compared to control (one way ANOVA followed by dunnett's multiple comparison test) (P value for diabetic control Vs normal control, P value for remaining groups III, IV & V Vs diabetic control), ns= not significant						

Table 8 Effect of ACTI on Blood glucose level in Acute Model

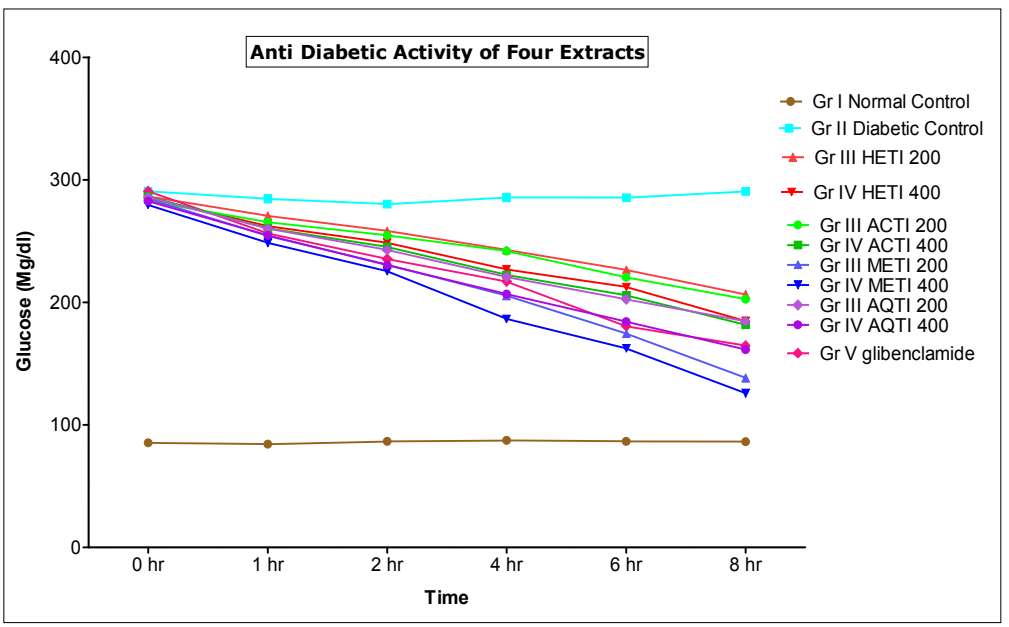
Group	0 h	1 h	2 h	4 h	6 h	8 h
Gr I Normal Control	85.3 ± 1.3	84.3 ± 1.0	86.6 ± 1.2	87.4 ± 1.2	86.5 ± 1.5	86.3 ± 1.4
Gr II Diabetic Control	290.6 ± 1.2	284.5 ± 2.3***	280.2 ± 1.5***	285.6 ± 1.5***	285.4 ± 1.6***	290.7 ± 1.2***
Gr III ACTI 200	282.6 ± 2.0	265.4 ± 2.3^{ns}	254.6 ± 1.5^{ns}	241.8 ± 1.6^{ns}	220.4 ± 1.2^{ns}	202.5 ± 1.4^{ns}
Gr IV ACTI 400	286.4 ± 2.1	260.4 ± 1.2*	245.2 ± 1.6*	222.4 ± 1.6*	205.7 ± 1.2*	181.6 ± 1.4*
Gr V glibenclamide 0.5 mg/ kg	290.6 ± 1.2	256.2 ± 1.6**	235.4 ± 1.3**	216.8 ± 1.5**	180.5 ± 1.4**	164.7 ± 1.2**
Values Expressed as Mean ±SEM (n=6) animals *P<0.05 **P<0.01 ***P<0.001 compared to control (one way ANOVA followed by dunnett's multiple comparison test) (P value for diabetic control Vs normal control, P value for remaining groups III, IV & V Vs diabetic control), ns= not significant						

Table 9 Effect of METI on Blood glucose level in Acute Model

Group	0 h	1 h	2 h	4 h	6 h	8 h
Gr I Normal Control	85.3 ± 1.3	84.3 ± 1.0	86.6 ± 1.2	87.4 ± 1.2	86.5 ± 1.5	86.3 ± 1.4
Gr II Diabetic Control	290.6 ± 1.2	284.5 ± 2.3***	280.2 ± 1.5***	285.6 ± 1.5***	285.4 ± 1.6***	290.7 ± 1.2***
Gr III METI 200	284.5 ± 1.2	254.2 ± 1.2*	230.7 ± 1.4*	205.4 ± 1.4*	174.5 ± 1.2*	138.4 ± 1.4*
Gr IV METI 400	279.5 ± 1.4	248.6 ± 1.2**	225.4 ± 1.5**	186.5 ± 1.3**	162.3 ± 1.2**	125.8 ± 1.4**
Gr V glibenclamide 0.5 mg/ kg	290.6 ± 1.2	256.2 ± 1.6*	235.4 ± 1.3*	216.8 ± 1.5*	180.5 ± 1.4*	164.7 ± 1.2*
Values Expressed as Mean ±SEM (n=6) animals *P<0.05 **P<0.01 ***P<0.001 compared to control (one way ANOVA followed by dunnett's multiple comparison test) (P value for diabetic control Vs normal control, P value for remaining groups III, IV & V Vs diabetic control), ns= not significant						

Table 10 Effect of AQTI on Blood glucose level in Acute Model

Group	0 h	1 h	2 h	4 h	6 h	8 h
Gr I Normal Control	85.3 ± 1.3	84.3 ± 1.0	86.6 ± 1.2	87.4 ± 1.2	86.5 ± 1.5	86.3 ± 1.4
Gr II Diabetic Control	290.6 ± 1.2	284.5 ± 2.3***	280.2 ± 1.5***	285.6 ± 1.5***	285.4 ± 1.6***	290.7 ± 1.2***
Gr III AQTI 200	285.6 ± 1.3	260.2 ± 2.1*	242.8 ± 1.4*	220.6 ± 1.2*	202.4 ± 1.2*	184.5 ± 1.5*
Gr IV AQTI 400	282.5 ± 1.5	254.6 ± 1.5**	230.4 ± 1.2**	206.7 ± 1.7**	184.2 ± 1.3**	161.4 ± 1.6**
Gr V glibenclamide 0.5 mg/ kg	290.6 ± 1.2	256.2 ± 1.6*	235.4 ± 1.3*	216.8 ± 1.5*	180.5 ± 1.4*	164.7 ± 1.2*
Values Expressed as Mean ±SEM (n=6) animals *P<0.05 **P<0.01 ***P<0.001 compared to control (one way ANOVA followed by dunnett's multiple comparison test) (P value for diabetic control Vs normal control, P value for remaining groups III, IV & V Vs diabetic control), ns= not significant						



Graph 4: anti-diabetic activity of four extracts

CONCLUSION

T. indicum has the ability to inhibit amylase enzyme with IC_{50} value of 91.3 µg/ml. IC_{50} value of METI was 46.72µg/ml whereas remaining three extracts have low percentage of inhibition. METI has as efficiency to inhibit enzyme and also inhibit glucose uptake.

Acute Anti diabetic activity of four extracts was evaluated in STZ-Nicotinamide induced rat model. METI extract has significantly decreased (P<0.01) the glucose levels in drug induced rats along with standard drug.

The results of our study suggest that *Trichodesma indicum* plant extracts has rich anti-diabetic property in both *in vitro* and *in vivo* analysis. METI and AQTI showed significant activity in diseased rats in lowering of blood glucose levels.

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REFERENCES

1. Charak. Charaksamhita, edited by Sastry CS. published by Chowkambha, Sanskrit series office, Varanasi, 1969.
2. Franswart NR, Akerele O, Bingel AS, Soejarto D and Guo ZG. Medicinal plants theory. Bulliten of W.H.O. 1985; 63: 965-981.
3. Franswart NR and Moris. Higher plants and sleeping gaint of drug development. Am.J.Pharma. Edu. 1976; 149: 46-52.
4. Batugal P. Inventory and documentation of medicinal plants in 14 Asia Pacific countries. Medicinal plants Research in Asia. International Plant Genetic Resources Institute (IPGRI) Volume 1, 2004.
5. Ayensu ES. World Medicinal Plant Resources. Conservation for Productive Agriculture. [V.L. Chopra and T.N. Khoshoo, (eds.)]. ICAR, New Delhi, India.1996, 11-42.
6. Salleh MK, Natesh S, Osman A, and Kadir AA. Medicinal and aromatic plants: Strategies and technologies for conservation. Proceedings of the Symposium Stateof the- Art Strategies and Technologies for Conservation of Medicinal and Aromatic Plants. Ministry of Science, Technology and Environment, Malaysia and Forest Research Institute, Malaysia, 1997.
7. Kumar S, Hassan SA, Dwivedi S, Kukreja AK, Sharma A, Singh AK, Sharma S, and Tewari R. Proceedings of the National Seminar on the Frontiers of Research and Development in Medicinal Plants, Central Institute of Medicinal and Aromatic Plants (CIMAP). Lucknow, India. Journal Medicinal and Aromatic Plant Sciences. Vol. 22/4A and Vol. 23/1A, 2000.
8. Narendra K, Sowjanya KM, Swathi J and Krishna Satya A. Phytochemical Evaluation & Antimicrobial Efficiency of Threatened Medicinal Plant of Andhra Pradesh *Pterospermum xylocarpum* (Thada Tree). Int Res J Pharm. 2013; 4 *(2):* 155-160.
9. Leon S, Alon HM, Paul FS and Larry NS. Comprehensive pharmacy review Lippincott & wilkins, 2004; 5*:*1033-1055.
10. Perianayagam JB, Sharma SK and Pillai KK. Anti-inflammatory activity of *Trichodesma indicum* root extract in experimental animals. J of Ethnopharmacology, 2006; Vol 104 *(3):* 410-414.
11. Srikanth K, Murugesan T, Anil Kumar Ch, Suba, V, Das, AK, Sinha S, Arunachalam G and Manikandan L. Effect of *Trichodesma indicum* extract on cough reflex induced by sulphur dioxide in mice. Phytomedicine, 2002; Vol. 9 *(1):* 75-77.
12. Perianayagam JB, Sharma SK and Pillai KK. Evaluation of antidiarrheal potential of *Trichodesma indicum* root extract in rats. Methods Find Exp Clin Pharmacol. 2005; 27 *(8):* 533-537.
13. Manani Lata M, Kakrani Purvi and Saluja Ajay K. Evaluation of Diuretic Activity of *Trichodesma indicum* R.Br. In Rats. Int J Pharm Bio Sci. 2014; 5 *(2):*129 – 133.
14. Khan T, Ahmad M, Khan R, Khan H, and Choudhary MI. Phytotoxic and insecticidal activities of medicinal plants of Pakistan, *Trichodesma indicum*, *Aconitum laeve* and *Sauromatum guttatum*. Journal of the Chemical Society of Pakistan. 2008; Vol. 30 (2): 251–255.
15. Anusha K, Shwetha Balakrishnan, Sindhu S and Sekar Babu Hariram. Screening the Metal Chelating Efficacy of *Trichodesma indicum*. Res J Pharma Bio Che Sci. 2014; 5*(2)*; 1259-1262.
16. Alarmal Mangai S and Subban Ravi. Comparative Corrosion Inhibition Effect of Imidazole Compounds and of *Trichodesma indicum* (Linn) R. Br. on C38 Steel in 1 M HCl Medium. Jrnl of Chemistry, Vol. 2013. Doi No.10.1155/2013/527286.
17. Alarmal Mangai S and Ravi Subban. *In vitro* Cyto toxicity of *Trichodesma indium* Linn.R.Br. Extracts Against Three Human Cancer Cell Lines. Asian J Pharm Clin Res. 2014; Vol 7*(3):* 103-105.
18. Shekib LA, Iraq EI, Abo Baker SM. Studies on amylase inhibitors in some Egyptian legume seeds. Plant Foods Human Nutr, 1988; 38: 325–332.
19. Gupta daksha, chandrashekher KS and Girish pal. *In vitro* antidiabetic activity of pentacyclic tritrprnoida and fatty acid ester from *Bauhinia purpurea*. Int J Pharmcol Pharmceut Tech, 2013; 2(1):25-28.
20. Bacteriol J, Goncalves T, Maria C, and Loureiro-Dias. Aspects of glucose uptake in Saccaharomyces cerevesiae. Journal of Bacteriology, 1994; 176(5):1511-1513.
21. Dinesh Kumar, Mitra A and Manjunatha M. *In vitro* and *in vivo* studies of antidiabetic Indian medicinal plants: A review. J Herb Medi Toxi, 2009; 3(2): 9-14.
22. Shirwaikar A, Rajendran K. Effect of aqueous bark extract of *Garuga pinnata* Roxb. in streptozotocin–nicotinamide induced type-II diabetes mellitus. Journal of Ethnopharmacology, 2006; 107: 285-290.
23. Shirwaikar A, Rajendran K, Dinesh C, Ramgopal B. Antidiabetic activity of aqueous leaf extracts of *Annona squamosa* in streptozotocin–nicotinamide type 2 diabetic rats. J Ethnopharmacol, 2004; 91*:* 71–75.
24. Matsui T, Tanaka T, Tamura S, Toshima A, Tamaya K and Miyata Y. α – glucosidase inhibitory profile of catechins and theaflavins. J Agric Food Chem, 2007; 55*:*99-105.
25. Hara Y, Honda M. The inhibition of α-amylase by tea polyphenols. Agric Biol Chem, 1990; 54*:*1939-45.
26. Latner A. Carbohydrate metabolism, abnormalities of post absorptive blood sugar level. Clinical Biochemistry. Saunders, Philadelphia, Pa, USA, 1958; pp-48.
27. Anderson T, Schein PS, McMenamin MG and Cooney DA. Streptozotocin diabetes correlation with extent of depression of pancreatic islet nicotinamide adenine dinucleotide. Journal of Clinical Investigation, 1974; 54: 672 – 677.

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