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Antidiabetic activity of aqueous root extract of *Merremia tridentata* (L.) Hall. f. in streptozotocin-induced-diabetic rats

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ABSTRACT

Objective: To investigate the antidiabetic effect of aqueous extract of *Merremia tridentata (M. tridentata)* root (MTRAE) in normal, glucose-loaded hyperglycemic and streptozotocin (STZ)-induced diabetic rats. **Methods:** Oral administration of MTRAE at the doses of 50, 100 and 150 mg/kg was studied in normal, glucose-loaded and STZ-diabetic rats. The three doses caused significant reduction in blood glucose levels in all the models. **Results:** The effect was more pronounced in 100 and 150 mg/kg than 50 mg/kg. MTRAE also showed significant increase in serum insulin, body weight and glycogen content in liver and skeletal muscle of STZ-induced diabetic rats while there was significant reduction in the levels of serum triglyceride and total cholesterol. MTRAE also showed significant antilipidperoxidative effect in the pancreas of STZ-induced diabetic rats. The antidiabetic effect of *M. tridentata* was compared with glibenclamide, a well known hypoglycemic drug. **Conclusions:** The results indicate that aqueous extract of *M. tridentata* root possesses significant antidiabetic activity.

1. Introduction

Diabetes mellitus (DM) consists of a group of syndromes characterized by hyperglycemia; altered metabolism of lipids, carbohydrates, and proteins; and an increased risk of complications[1]. Apart from currently available therapeutic options for diabetes like oral hypoglycemic agents and insulin, which have limitations of their own, many herbal medicines have been recommended for the treatment of diabetes[2]. A variety of ingredients present in medicinal plants are thought to act on a variety of targets by various modes and mechanisms. They have the potential to impart therapeutic effect in complicated disorders like diabetes and its complications[3]. Hence the present study was carried out to evaluate the antidiabetic activity of *Merremia tridentata* (L.) Hall. f. (Family: Convolvulaceae) (*M. tridentata*) .

M. tridentata commonly known as "Mudiarkunthal" or "Savulikodi" or "Thrippan Pullu" in Tamil and "Prasarini" in Sanskrit is reported to possess a number of medicinal values[4]. It is a thick climbing herb with woody rootstock spreading on the walls and on the grounds. It grows in all plain districts of Tamilnadu, Western peninsula and Bengal[5]. The plant is considered bitter, astringent, tonic and it's used in the treatment of rheumatism, piles, swellings and urinary disorders[4]. The previous studies conducted on *M. tridentata* showed strong wound healing, anti-inflammatory and anti-arthritic activities[6,7]. It is also used as a supplementary feed to the grass *Panicum maximum* for young West African Dwarf Sheep[8]. The aerial parts of the *M. tridentata* contain flavonoids, diosmetin, luteolin, and their 7-O- β -D-glucosides[9]. Ergosine alkaloids have been isolated from the seeds of *M. tridentata*[10]. Pyrolidine alkaloids like hygrins and nicotine have been isolated from the root and the aerial parts of *M. tridentata*[10]. The acetone extract of root possess high phenolic contents and rich potential of antioxidant activity[11]. Moreover K. Arunachalam *et al.*[12] also revealed the HPTLC analysis of acetone extract of root has six phenolic and two flavonoid compounds existing. Bio-flavonoids are well-known for their multi-directional biological activities including anti-diabetic efficacy[13]. Numerous studies have been carried out to explore their potential role in the treatment of diabetes[14]. Considering the medicinal potential of this plant, *M. tridentata* root was chosen for the study.

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2. Materials and methods

2.1. Chemicals

Streptozotocin was purchased from SISCO Research Laboratories Ltd., Mumbai. Glibenclamide was obtained as a gift sample from Sanofi Aventis India Ltd. All other chemicals and reagents used were of analytical grade.

2. 2. Plant material and extraction

M. tridentata was collected from Coimbatore, Tamil Nadu State, India during November 2008, authenticated and deposited in the Department of Botany Herbarium, Bharathiar University with voucher number BUBH - 2895. The freshly collected plant roots were washed thoroughly in tap water, shade dried at room temperature (25 ℃) powdered, and it was macerated with distilled water for 48 h at room temperature with shaker. It was then filtered through Whatman filter paper. The filtrate was air dried and stored in refrigerator for further use as *M. tridentata* root aqueous extract (MTRAE). During experiment the crude extract was diluted with distilled water just before administration to animals.

2.3. Experimental animals

Healthy adult male albino Wistar rats (150-200 g), in-house bred at the Animal House of Kovai Medical Centre Hospital College of Pharmacy, Coimbatore, Tamil Nadu, India were used for the study. Rats were housed in polypropylene cages lined with husk in standard environmental conditions [temperature (25±2) ℃, relative humidity (55±10)% and 12:12 light:dark cycle]. The rats were fed on a standard pellet diet (Amrut rat and mice feed, Sangli, India) ad libitum and had free access to water. The experimental protocol was subjected to the scrutiny of the Institutional Animal Ethics Committee and was cleared by the same before beginning the experiment (No. KMCRET/M.Sc./4/2010-11).

2.4. Experimental design

Antidiabetic activity of MTRAE was assessed in normal, glucoseloaded hyperglycemic and streptozotocin-induced diabetic rats. In all studies, the animals were fasted overnight for 16 h with free access to water throughout the duration of the experiment.

2.4.1. Evaluation of extract on normal healthy rats

This study was carried out according to the method of Kar *et al*.[15] At the end of the fasting period, taken as zero time (0 h), blood was withdrawn from the retro-orbital plexus of the eye under mild ether anesthesia. Serum was separated by centrifugation and glucose was estimated. The animals were then randomly divided into four groups of six animals each. Group I served as control and received distilled water. Groups II, III and IV received MTRAE orally at the dose of 50, 100, 150 mg/kg. Blood glucose levels were determined 1, 2, 3 and 4 h following treatment.

2.4.2. Evaluation of extract in oral glucose tolerance test[16]

Healthy rats were divided into four groups of six animals each: Group I served as control received only vehicle (distilled water) and Groups II, III and IV received MTRAE orally at the dose level of 50, 100 and 150 mg/kg, respectively. All the animals were given glucose (2 g/kg) 60 min after dosing. Blood samples were collected from the retro-orbital plexus of the eye just prior to (0 h) and at 30, 60, 90 and 120 min after the glucose loading, and blood glucose levels were estimated.

2.4.3. Evaluation of extract in streptozotocin-induced diabetic rats[17]

Experimental diabetes was induced by single intraperitoneal injection of 55 mg/kg of streptozotocin (STZ), freshly dissolved in cold citrate buffer, pH 4.5. Control animals received only citrate buffer. After 5 days of STZ injection, animals with fasting blood glucose above 250 mg/dL were considered as diabetic and included in the study. The animals were randomly assigned into six groups of six animals each and received the following treatments: Group I: Normal control + distilled water, Group II: Diabetic + distilled water, Group III: Diabetic + MTRAE (50 mg/kg), Group IV: Diabetic + MTRAE (100 mg/kg), Group V: Diabetic + MTRAE (150 mg/kg) and Group VI: Diabetic + glibenclamide (10 mg/kg). The freshly prepared solutions were orally administered daily for 21 days. Body weights and blood glucose analysis was done weekly on overnight fasted animals. At the end of the experimental period, the animals were fasted an overnight and blood was collected for various biochemical estimations. The animals were sacrificed by cervical decapitation. Organs like liver, pancreas and skeletal muscle were dissected out, immediately rinsed in ice cold saline and stored for further biochemical estimations.

2.4.4. Biochemical analysis

Serum glucose analysis was done by GOD-POD method using Glucose Estimation Kit (Erba Diagnostics, India). Other serum estimations were done spectrophotometrically using standard kits available which included serum insulin (RIA kit provided by BRIT, BARC, India), serum triglycerides (GPO-Trinder method, Erba Diagnostics), and serum total cholesterol (CHOP-PAP method, Erba Diagnostics). Glycogen was estimated in liver and skeletal muscle by the method of Good *et al*[18]. *In vivo* lipid peroxidation, expressed as TBARS (thiobarbituric acid reactive substances) was estimated in the pancreatic tissue homogenate according to the method of Ohkawa *et al*.[19]

2.4.5. Acute oral toxicity study

Acute oral toxicity of MTRAE was performed on Swiss female albino mice, according to OECD Guideline 423. Two groups of three mice each were used for the study. Group I served as control and received distilled water. Group II received single oral dose of MTRAE (2 000 mg/kg). The animals were observed for gross behavioral, neurological, autonomic and toxic effects at short intervals of time for 24 h and then daily for 14 days. Food consumption was monitored daily and body weights were recorded weekly. On 14th day, animals were sacrificed and all the organs were removed for gross pathological examination.

2.5. Statistical analysis

All values are expressed as mean±S.E.M. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. The results were considered statistically significant if $P < 0.05$.

3. Results

3.1. Effect of MTRAE on normoglycemic rats

Results of the effect of graded doses of MTRAE on blood glucose level of normal healthy rats are presented in Figure 1. MTRAE produced peak hypoglycemia at 3 h. Dose dependent blood glucose reduction was observed in animals treated with 50, 100 and 150 mg/kg (13.44%, 24.72% and 26.2%, respectively). Blood glucose levels were restored in all treatment groups by 4h.

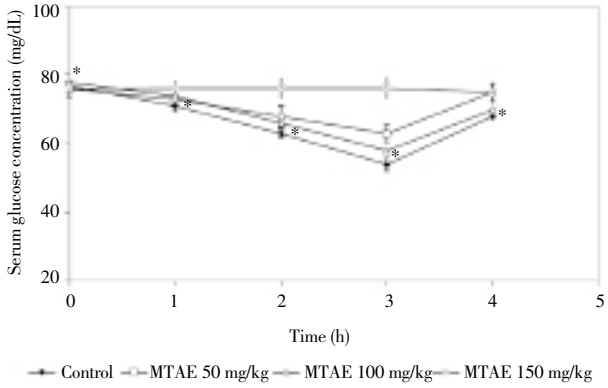

Figure 1. Effect of MTRAE on blood glucose levels in normoglycemic rats.
Each value is expressed as mean of six observations. *$P < 0.05$ when compared with values of 0h of the same group.

3.2. Effect of MTRAE on oral glucose tolerance in normal rats

MTRAE, when administered 60 min. prior to glucose loading produced significant reduction ($P<0.05$) in the rise in blood glucose levels at 60 min. after glucose administration. MTRAE at doses of 50, 100 and 150 mg/kg produced 13.04%, 20.15% and 14.36% reduction in blood glucose respectively when compared to vehicle treated group at 60 min (Figure 2).

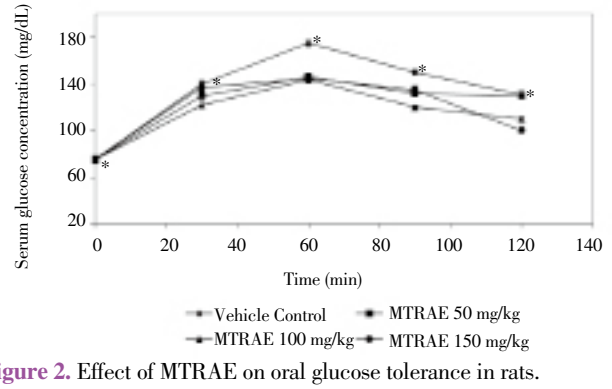

Figure 2. Effect of MTRAE on oral glucose tolerance in rats.

3.3. Effect of MTRAE on fasting blood glucose and body weight in STZ-induced diabetic rats

The effect of repeated oral administration of MTRAE on blood glucose levels in STZ-diabetic rats is presented in Figure 3. MTRAE, administered at three different doses of 50, 100, 150 mg/kg to STZ-treated diabetic rats caused significant ($P<0.001$) reduction of blood glucose levels which was related to dose and duration of treatment. Maximum reduction was observed on day 21 (28.56%, 49.65% and 48.58%, respectively). MTRAE 50 mg/kg exhibited maximum glucose lowering effect in diabetic rats compared to the other two doses. Glibenclamide exhibited 61.66% reduction in blood glucose levels at the end of the study when compared to diabetic control. STZ produced significant loss in body weight as compared to normal animals during the study. Diabetic control continued to lose weight till the end of the study while MTRAE at all the three doses (50, 100 and 150 mg/kg) showed significant improvement ($P<0.05$) in body weight compared to diabetic control (Figure 4).

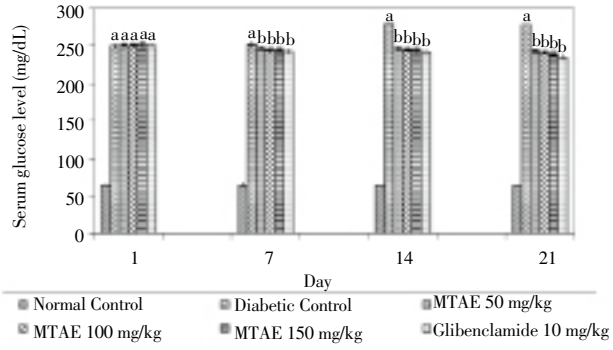

Figure 3. Effect of MTRAE on blood glucose levels of STZ-induced diabetic rats.
Each value is expressed as mean±S.E.M. ($n = 6$). [a]$P< 0.001$ when compared to corresponding values of the normal control. [b]$P<0.001$ when compared to corresponding values of the diabetic control.

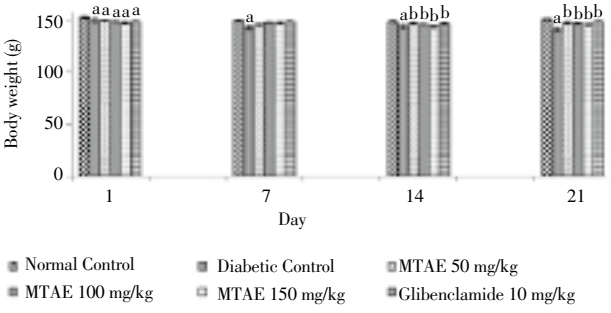

Figure 4. Effect of MTRAE on body weight of STZ-induced diabetic rats.

3.4. Effect of MTRAE on serum insulin in STZ-induced diabetic rats

STZ caused a significant decrease in serum insulin. Administration of MTRAE at all the three doses (50, 100 and 150 mg/kg) caused significant ($P < 0.01$) increase in insulin levels at the end of the study. Of the three doses, 50 mg/kg showed maximum increase which was comparable to glibenclamide (Table 1).

3.5. Effect of MTRAE on serum lipids in STZ-induced diabetic rats

MTRAE showed a dose related significant ($P< 0.01$) reduction in triglycerides (13.91%, 22.62% and 25.11% for 50, 100 and 150 mg/kg, respectively) compared to pretreatment levels (Table 1). MTRAE at the doses of 100 and 150 mg/kg was more effective than 50 mg/kg in reducing the cholesterol levels.

3.6. Effect of MTRAE on glycogen content in STZ-induced diabetic rats

Glycogen content in liver and skeletal muscle decreased significantly ($P<0.001$) in diabetic control compared to normal control (Table 1). Administration of MTRAE at the doses of 100 and 150 mg/kg for 21 days resulted in significant ($P<0.001$) increase in the glycogen levels in both the liver and skeletal muscle. However, with none of the dose levels, the values were restored to normal.

3.7. Effect of MTRAE on pancreatic lipid peroxidation in STZ-induced diabetic rats

There was a significant elevation in the level of TBARS in the diabetic control when compared with the corresponding normal control. However, the oral administration of MTRAE and glibenclamide tended to bring these values back to normal (Table 1).

3.8. Acute oral toxicity study

In acute toxicity study, MTRAE treated animals did not show any change in their behavioral pattern. There was no significant difference in the body weights and food consumption when compared to the vehicle treated group. Also, no gross pathological changes were seen. Thus, it was concluded that MTRAE was safe at 2 000 mg/kg.

4. Discussion

This study was undertaken to evaluate the hypoglycemic activity of MTRAE in normal, glucose-loaded hyperglycemic and streptozotocin-induced diabetic rats. In normoglycemic rats, MTRAE showed dose dependent hypoglycemic effect at 3 h. From OGTT it could be concluded that dose of 100 mg/kg showed the maximum improvement in glucose tolerance. STZ significantly induced hyperglycemia accompanied by hypoinsulinemia. Oral administration of MTRAE for 21 days caused a significant decrease in blood glucose levels. The possible mechanism by which MTRAE mediated its antidiabetic effect could be by potentiation of pancreatic secretion of insulin from existing β-cells of islets, as was evident by the significant increase in the level of insulin in the extract treated animals. The hypoglycemic activity of MTRAE was compared with glibenclamide, a standard hypoglycemic drug. From the results of the present study, it may be suggested that the mechanism of action of MTRAE may be similar to glibenclamide action. Diabetes mellitus impairs the normal capacity of the liver to synthesize glycogen. Synthase phosphatase activates glycogen synthase resulting in glycogenesis and this activation appears to be defective in diabetes[20]. Skeletal muscle is also a major site of insulin-stimulated glucose uptake[21]. Decrease in both muscle and hepatic glycogen was observed in this study. Treatment with MTRAE (100 and 150 mg/kg) for 21 days significantly increased muscle and liver glycogen indicating that the defective glycogen storage of the diabetic state was partially corrected by the extract. Hypercholesteremia and hypertriglyceridemia are primary factors involved in the development of atherosclerosis and coronary heart disease which are the secondary complications of diabetes[22]. MTRAE significantly reduced serum triglycerides and total cholesterol in STZ-diabetic rats. Thus, it is reasonable to conclude that MTRAE could modulate blood lipid abnormalities. In diabetes, tissue damage is considered to be mediated by free radicals by attacking membranes through peroxidation of unsaturated fatty acids[23-29]. Lipid peroxidation eventually leads to extensive membrane damage and dysfunction[30]. Decreased lipid peroxidation and improved antioxidant status may be one of the mechanisms by which drug treatment could contribute to the prevention of diabetic complications[31]. In our study, MTRAE significantly attenuated the increased lipid peroxidation which could be due to the antioxidant effect of flavonoids, detected in the preliminary phytochemical screening of the extract. Thus, the significant antidiabetic effect of MTRAE could be due to the presence of various phytoconstituents detected in the phytochemical screening which alone or in synergism can impart therapeutic effect.

In conclusion, it can be stated, that the aqueous root

Table 1
Effect of MTRAE on serum insulin, serum lipids, glycogen content in liver and skeletal muscle and lipid peroxidation in pancreas of STZ treated diabetic rats.

Experimental group	Serum insulin (U/mL)#		Triglyceride (mg/dL)#		Total cholesterol (mg/dL)#		Glycogen (mg/g of wet tissue)#		TBARS (μM/g of wet tissue)#
	Day 1	Day 21	Day 1	Day 21	Day 1	Day 21	Liver	Skeletal muscle	
Normal control	19.21±0.50	18.56±0.45	80.24±2.37	82.79±5.06	55.00±1.28	66.00±3.21	41.46±1.39	7.45±0.22	0.38±0.01
Diabetic control	8.57±0.26	6.01±0.34	162.10±6.62	218.11±7.69	95.00±2.38	139.47±5.93	14.31±1.03[d]	3.53±0.16[d]	0.64±0.02[d]
Diabetic+ MTAE(50 mg/kg)	8.34±0.42	10.81±0.38[a,c]	162.65±7.04	148.62±4.86[a,c]	97.99±2.34	88.11±2.75	20.85±1.00	4.09±0.13	0.54±0.01
Diabetic+ MTAE(100 mg/kg)	8.10±0.41	14.76±0.43[a,c]	162.10±6.15	133.245±7.59[a,c]	99.65±2.81	59.65±2.79[b,c]	28.73±1.14[c]	4.81±0.10[c]	0.46±0.01[c]
Diabetic+ MTAE(150 mg/kg)	8.14±0.45	14.85±0.55[a,c]	157.56±8.32	125.47±4.81[a,c]	100.43±1.53	60.50±3.79[b,c]	30.74±1.25[c]	4.69±0.11[c]	0.47±0.01[c]
Diabetic+ glibenclamide (10 mg/kg)	8.14±0.40	18.32±0.30[a,c]	158.01±7.45	102.41±3.51[a,c]	98.19±2.13	55.91±2.81[b,c]	32.58±0.96[c]	5.07±0.10[c]	0.41 ±0.05[c]

Each value is mean±S.E.M. ($n = 6$), [a] $P < 0.01$ when compared to the day 1 values of the same group, [b] $P < 0.001$ when compared to the day 1 values of the same group, [c] $P < 0.001$ when compared to the corresponding values of the diabetic control, [d] $P < 0.001$ when compared with the corresponding values of the normal control.

extract of *M. tridentata* has beneficial effects, in reducing the elevated blood glucose level and lipid profile of STZ-induced-diabetic rats, but has no effect on normal rats. Thus, justifying the claim made by Ayurvedic classics. However, longer duration studies on chronic models are necessary to elucidate the exact mechanism of action so as to develop it as a potent antidiabetic drug.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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