

Effects of *Turnera ulmifolia* (Linn.) Leaves on Blood Glucose Level in Normal and Alloxan-Induced Diabetic Rats

D. PRABU, M. NAPPINAI, K. PONNUDURAI, A. THIRUGNANASAMBANTHAN, S. SRINIVASAN and M. RAMVIKAS

For author affiliations, see end of text.

Received July 23, 2008; Revised February 21, 2009; Accepted May 17, 2009

This paper is available online at <http://ijpt.iuims.ac.ir>

ABSTRACT

The methanolic extract of leaves of *Turnera ulmifolia* L was evaluated for its effect on blood glucose in alloxan-induced diabetic and euglycemic rats. The extract were administered at three doses of 100, 200 and 400 mg/kg, p.o. Glibenclamide (10 mg/kg, p.o.) was used as standard for control group. In euglycemic rat, extract were administered at three doses of 100, 200 and 400 mg/kg, p.o. Extract at 400 mg/kg and glibenclamide significantly reduced blood glucose level in normal and alloxan-induced diabetic rats and raised the liver glycogen content significantly. This indicates that the leaves extract of *Turnera ulmifolia* L posses anti-hyperglycemic activity.

Keywords: *Turnera ulmifolia*, Euglycemic, Antihyperglycemic, Alloxan-induced diabetes, Rats

Diabetes mellitus is abnormal carbohydrate metabolism and mainly characterized by hyperglycemia. According to world health organization (WHO), the prevalence of diabetes is likely to increase by 35% [1]. Currently, there are over 150 million diabetics patients worldwide and this is likely to increase to 300 million or more by the year 2025 [2]. Though different types of oral hypoglycemic agents are available along with insulin for the treatment of diabetes mellitus, there is a growing interest in herbal remedies because of perceived effectiveness and minimal side effects. Cell damage caused by free radicals appears to be a major contributor to aging and to degenerative disease of aging such as cancer, diabetes, cataract, and brain dysfunction [3]. Free radical formation is controlled naturally by various beneficial antioxidant compounds. Many plant derived substances are becoming increasingly known for their antioxidant activity. The plants containing nutrients serve as protectors against a wide variety of environmental stresses [4].

The plant source of the *Turnera ulmifolia* L. (Turneraceae) can grow to a height of about 2 – 3 feet tall. The leaf is more like an ovate shape. It is commonly-found in India, Brazil, Japan, China and many countries. The margin of leaf is dentate. The leaves branch out alternately. It is traditionally used for treatment of chest ailments, biliousness, indigestion and

rheumatism, wound, diabetic disorder and hepatitis [5]. The leaves contain sterol, flavonoids and terpenes and seed contain the unusual fatty acids 9, 10-epoxy-octadec-cis-12-enoic acid [6]. Anti-oxidant capacity, peroxidation suppression and thiobarbituric acid reactive substance inhibition in concentration-dependent assays show that the anti-oxidant activities are strongly correlated with phenolic compound [7]. The leaves extract showed anti-inflammatory activity in rat colitis [8]. Aerial part of the plants showed anti-inflammatory and an anti-ulcerogenic activity [9]. The present study report the preliminary phytochemical screening and anti-hyperglycemic activity of methanolic extract of *Turnera ulmifolia* leaves in euglycemic and alloxan induced diabetic rats.

MATERIALS AND METHODS

Plant materials

Fresh leaves of *Turnera ulmifolia*. L was collected from Kancheepuram District, Tamilnadu, India, during the months of October 2006. The identity of the leaves was confirmed by Plant Anatomy Research Centre, Chennai, Tamilnadu, India. Leaves were dried under shade, pulverised by a mechanical grinder and passed through a 40 mesh and then stored in a well-closed container for further use.

Preparation of the extract

The dried leaves powder (500 g) was extracted with methanol (90% w/v) for 24 h using a Soxhlet extractor. This methanol extract was concentrated to dryness under reduced pressure and controlled temperature (50-60°C) to yield solid masses (18.7%) that were completely free from solvents and subjected to preliminary phytochemical tests [10].

Chemical, drug and instruments

Alloxan (Sigma, USA), Tween 80 (Lab Chemicals, Pasle) and glibenclamide (Euglucan, Nicholas Piramal, Mumbai) were used for the study. Blood glucose levels of rats were determined using Glucometer (mg/dL) and UV-visible spectrophotometer 1601 model (Shimadzu).

Animals

Inbred colony of adult wistar albino rats (150-200g) of either sex were used for the pharmacological activities. They were kept in polypropylene cages at 25±2°C, with relative humidity 45-55% under 12h:12h light and dark cycles. All the animals were acclimatized to the laboratory conditions for a week before use. They were fed with standard animal feed (Hidustan Lever Ltd., Bangalore, India.) and water *ad libitum*. The test extracts and the standard drugs were administered in the form of a suspension in water using 1% Tween 80 as suspending agent. The study has got the clearance from the Institutional Animal Ethical Committee (IAEC) as per the directions of the CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals).

Experimental methods

Wistar albino rats were randomly divided into six groups six rats each. Group I (non diabetic control) and Group II (Diabetic control) were given 2 ml/kg, p.o. of 1 % Tween 80. Group III, IV and V were administered methanolic extract at 100 mg, 200mg and 400 mg/kg, p.o., respectively. Group VI were treated with glibenclamide (10mg/kg, p.o.).

Induction of diabetic

Hyperglycemia was induced by intra-peritoneal administration of alloxan monohydrate (150 mg/kg, i.p.). Blood samples (0.2 ml) were collected from the tail vein of each rat by venous puncture using hypodermic needle, every time for blood glucose determination using Glucometer (mg/ dL).

Evaluation of hypoglycemic effect of euglycemic rats

Wistar albino rats (150-200 gm) either sex were fasted over night for about 18 hours and were divided in to four groups of six rats each. Group I (control) received 2 ml /kg, p.o. of 1% Tween 80, Group II, III and IV were administered 100, 200, 400 mg/ kg body weight of methanolic extract of *Turnera ulmifolia* L. Group V received glibenclamide (10 mg/kg body

weight, p.o). The extract and standard were dissolved in 1% Tween 80 water and normal saline respectively. The blood samples were obtained through the tail vein puncturing with hypodermic needle at the interval of initial, 1st, 3rd, 5th hours [11]. The blood glucose level per group was determined using Glucometer (Roche DiagnostisGmbH, Germany).

Evaluation of anti-diabetic activity on alloxan induced diabetic rats

The animals were kept fasting for 24 hr with water *ad libitum* and injected intraperitoneally at a dose of 150 mg/kg of body weight of alloxan monohydrate freshly-prepared in normal saline solution. After one hour of alloxan administration, animals were given food *ad libitum* and 1 ml of glucose i.p. to combat severe hypoglycerin. After 72 hours of alloxan injection, the animals were tested for evidence of diabetes by estimating their blood glucose level using Glucometer [11, 12]. The blood glucose level more than 150 mg/dL was considered diabetes. The blood samples were obtained through the tail vein puncturing with hypodermic needles. About 0.2 ml of blood was withdrawn at interval of initial, 1st, 2nd, 4th, 8th hours and 7th day of administration of single dose for acute and study respectively. All the animals were scarified by decapitation method after 7 days and liver were utilized for the estimation of glycogen [13].

Statistical analysis

The data were subjected to statistical analysis using SPSS 11.0 for Windows. The values are represented as the mean ± SEM for six rats. Statistical significance was determined by one-way ANOVA, followed by Dunnet's test. Values with $p < 0.05$ were considered statistically significant.

RESULTS

The phytochemical studies of *Turnera ulmifolia* L showed presence of alkaloids, saponnins, tannins, flavanoids, sterol and phenols. Effects of *Turnera ulmifolia* L extract on euglycemic rats at 100 mg was not significant, while it was significant at 200 mg on 3rd hour ($p < 0.05$) and 5th hour ($p < 0.01$). At 400mg of extract and glibenclamide at all hours significantly ($p < 0.01$) reduced the blood glucose when compared to control (Table 1). The diabetic rats showed significant elevation in blood glucose levels at various intervals, where as extract treatment at 400mg and standard drug significantly ($p < 0.01$) reduced the glucose level in blood at 4th, 8th, and 7th day, when compared to the hyperglycemic control group. Treatment of 400 mg at 2nd hour and dose 200mg at all hours significantly ($p < 0.05$) reduced the glucose level in blood. The diabetic rats in the study also showed a marked reduction in hepatic glycogen synthesis. This was regulated to normal range following the administration of 200mg, 400mg and standard group, when compared to hyperglycemic control group (Table 2).

Table 1. Effect of *Turnera ulmifolia* L on blood glucose level in euglycemic rats

Group	Blood Glucose level (mg/ dL) Mean±SEM			
	Fasting	1 h	3h	5h
I	96.72±6.03	94.81±7.21	94.02±4.21	92.42±7.40
III	94.86±7.72	91.08±6.78 ^{NS}	89.78±3.24 ^{NS}	88.63±2.82 ^{NS}
IV	96.78±4.96	86.38±4.22 ^{NS}	75.42±4.88*	73.68±6.72**
V	93.41±6.78	80.42±3.75**	66.82±3.42**	57.23±4.03**
VI	94.73±5.43	78.42±6.08**	63.42±5.06**	55.42±4.56**

Group I-Normal control

Group II-100mg/kg bw,p.o. METU administered

Group III-200mg/kg bw,p.o. METU administered

Group III-400mg/kg bw,p.o. METU administered

Group IV-10mg/kg bw,p.o. glibenclamide administered

n = 6, NS- Not Significant, * $p < 0.05$, ** $p < 0.01$ when compared with control Group.**Table 2.** Effect of *Turnera ulmifolia* L extract on blood glucose level of alloxan-induced diabetic rats

Group	Blood Glucose level (mg/ dL) (Mean±SEM)						Content of Glycogen
	Initial	1 h	2h	4h	8h	7 th day	
I	101.82±6.03	103.31±4.31	102.42±3.47	105.38±4.43	103.4± 6.72	102.84±4.84	754.25 ± 20.84
II	186.48±2.47	190.92±1.02	184.93±3.43	197.42±3.33	208.82±4.31	225.48±3.78	596.53± 26.43
III	188.14±6.03	181.41±2.04 ^{NS}	176.48±3.24 ^{NS}	175.48±2.42 ^{NS}	170.9±0.42 ^{NS}	164.14±4.78 ^{NS}	632.43 ± 31.12 ^{NS}
IV	185.54±1.06	178.48±2.08 ^{NS}	170.42±4.03	154.48±2.78*	146.4±0.68*	120.72±2.12*	730.27 ± 43.73*
V	187.42±1.82	172.19±0.67 ^{NS}	154.02±2.18*	130.18±1.82**	122.47±3.44**	118.5±6.23**	798.51 ± 27.34**
VI	184.78±1.83	173.48±0.78 ^{NS}	149.41±1.03*	124.43±0.83**	112.2±1.48**	101.4±6.83*	830.67 ± 34.52**

Group I-Normal control

Group II-Diabetic control

Group III-Alloxan -induced diabetic rats + 100mg/kg bw,p.o. METU administered

Group IV-Alloxan -induced diabetic rats + 200mg/kg bw,p.o. METU administered

Group V-Alloxan -induced diabetic rats + 400mg/kg bw,p.o. METU administered

Group VI-Alloxan -induced diabetic rats + 10mg/kg bw,p.o. glibenclamide administered

n = 6, NS- Not Significant, * $p < 0.05$, ** $p < 0.01$ when compared with diabetic control group.

DISCUSSION

The present study is a preliminary assessment of the antihyperglycemic and euglycemic effect of methanolic extract of *Turnera ulmifolia* L in alloxan-induced diabetic rat. Methanolic extract showed antihyperglycemic and euglycemic effect at different doses. The extract at 100mg/kg, body weight produced mild effect. Dose of 200 mg/kg body weight produced reasonably good effect in diabetic rats and higher dose (400mg/kg body weight) showed maximum effect. Hyperglycemia is associated with the generation of reactive oxygen species (ROS) causing oxidative damage particularly to heart, kidney, eyes, nerves, liver, pancreases, small and large vessels and gastrointestinal system [11]. The increased levels of plasma glucose in alloxan-induced diabetic rats were lowered by *Turnera ulmifolia* L administration. Flavonoids, sterols, triterpenoids, alkaloids and phenolics are known to be bioactive antidiabetic principles [14, 15]. Flavonoids are known to regenerate the damaged beta cells in the alloxan-induced diabetic rats [16]. Phenolics are found to be effective antihyperglycemic agents [17]. Various phyto-nutrients and anti-oxidant may possess scavenging activity of free radicals.

Alloxan, a beta-cytotoxin induces chemical diabetes in a wide variety of animal species through damage of insulin secreting cell [18]. It is well established that sulphonylureas produce hypoglycemia by increasing the secretion of insulin from the pancreas [19, 20]. these

compounds are active in mild alloxan-induced diabetes whereas they are inactive in intense alloxan diabetes (nearly all beta-cells have been destroyed). No histological studies were carried out to prove this and it is not possible to explain the detailed mechanism of antidiabetic action of *Turnera ulmifolia*. However, since our results showed that glibenclamide reduced the blood glucose levels in hyperglycemic animals, the state of diabetes is not severe. Alloxan-treated animals receiving the leaves extraction of *Turnera ulmifolia* L. showed rapid normalization of blood glucose levels in comparison to the control and this could be due to the possibility that some beta-cells are still surviving to exert their insulin-releasing effect by *Turnera ulmifolia*. Moreover, like sulphonylureas, oral administration of *Turnera ulmifolia* leaves extraction produced hypoglycemia in normal animals. This suggests that the mode of action of the active constituents of *Turnera ulmifolia* is probably mediated by an enhanced secretion of insulin, like sulphonylureas. However, the possibility of enhanced tissue uptake by *Turnera ulmifolia* cannot be ruled out.

Insulin is a stimulator of glycogen synthase system. On the other hand, insulin inhibits glycogenolysis and in lack of insulin, glycogenolysis is not under inhibition of insulin and, therefore, glycogen content of the liver decreases [21]. Glycogen is the primary intracellular storable form of glucose and its levels in various tissues especially skeletal muscle are a direct reflection of insulin activity as insulin promotes intracellular

glycogen deposition by stimulating glycogen synthase and inhibiting glycogen phosphorylase. Since alloxan causes selective destruction of beta-cells of islets of Langerhans resulting in marked decrease in insulin levels, it is rational that glycogen levels in tissues (skeletal muscle and liver) decrease as they depend on insulin for influx of glucose [22, 23]. Moreover, this alteration in muscle and hepatic glycogen content is normalized by insulin treatment [24, 25]. Results showed that hepatic and skeletal glycogen content decreased drastically in diabetic controls which have also been reported earlier [26]. Administration of *Turnera ulmifolia* significantly increases hepatic glycogen levels in diabetic rats. This focuses the one possible way of antidiabetogenic action of this extract by improvement of glycogenesis process in muscle and liver. Further experiments are needed to identify the active components of the leave extraction to determine its mechanism of action. Conclusively, it is evident that methanolic extract of *Turnera ulmifolia* contains antihyperglycemic agents capable of lowering blood glucose level in hyperglycemic and normal rats.

ACKNOWLEDGMENT

The authors are grateful to Department of Pharmacology, C.L.Baid Metha College of Pharmacy, Chennai, India, for the constant support and encouragement throughout this study.

REFERENCES

- King H, Aubert RE, Herman WH. Global burden of diabetes 1995-2025 prevalence, numerical estimates and projections. *Diabetes Care* 1998; 21:1414-31.
- Boyle JP, Honeycutt AA, Narayan KM, Hoerger TJ, Geiss LS, Chen H, Thompson TJ. Projection of diabetes burden through 2050: impact of changing demography and disease prevalence in the US. *Diabetes Care* 2001; 24:1936-40.
- The Wealth of India - A dictionary of Indian raw materials and industrial products. 1959. CSIR, New Delhi, India. Vol-10, 314-5.
- Halliwell B. Free radicals, anti-oxidant and Human Disease: Curiosity, cause or consequence. *Lancet* 1995; 344:721-4.
- Havsteen B. Flavonoids, a class of natural products of High pharmacological potency. *Biochem Pharm* 1983; 32:1141-8.
- Kallappa MH. Fatty acids in seed oil from *Turnera ulmifolia*. *Phytochemistry* 1993; 34:1363-5.
- Nascimento MA, Silva AK, Franca LCB, Quignard ELJ, Lopez JA, Almeida MG. *Turnera ulmifolia* L (Turneraceae): Preliminary study of its antioxidant activity. *J Ethnopharmacol* 2006; 97:1387-91.
- Galvez J, Souza Gracioso JD, Camuesco D, Galvez J, Vilegas W, Souza Brito ARM, Zarzuelo A. Intestinal anti-inflammatory activity of a lyophilized infusion of *Turnera ulmifolia* in TNBS rat colitis. *Fitoterapia* 2006; 77:515-20.
- Antonio MA, Souza Brito ARM. Oral anti-inflammatory and anti-ulcerogenic activities of hydroalcoholic extract and partitioned fractions of *Turnera ulmifolia* (Turneraceae). *J Ethnopharmacol* 1998; 61:215-28.
- Kokate CK, Purohith AP, Gokhale SB. Pharmacognosy, 1990. Nirali Prakashan, Pune 120.
- Tunali, S., Yanardag, R. Effect of Vanadyl sulfate on the status of lipid parameters and on stomach and spleen tissue of streptozotocin-induced diabetic rats. *Pharmacol Res* 2006; 53:271-7. Reshmik R, Fatima A, Sinilal B, and Latha MS. Antidiabetic effect of a herbal drug in alloxan-diabetic rats. *Ind Drugs* 2001; 38:319-22.
- Dash GK, Patro CP, Maiti AK. A study on the anti-hyperglycemic effect of *Premna corymbosa* Rott. Roots. *J Nat Remd* 2005; 5:31-4.
- Seifter S, Dayton S, Movic B, Mutwzer E. The estimation of glycogen with the anthrone reagent. *Arch Biochem* 1985; 25:191-201.
- Oliver-Bever B. Medicinal Plants in Tropical West Africa. 1986. Cambridge University Press, London. 245-67.
- Kameswara Rao B, Kesavulu MM, Giri R, Appa Rao CH. Antidiabetic and hypolipidemic effects of *Momordica cymbalaria* Hook fruit powder in Alloxan diabetic rats. *J Ethnopharmacol* 1999; 67:103-9.
- Chakravarthy BK, Gupta S, Gambir SS, Gode KD. Pancreatic beta cell regeneration. A novel antidiabetic mechanism of *Pterocarpus marsupium* Roxb. *Ind J Pharmacol* 1980; 12:123-7.
- Manickam M, Ramanathan M, Farboodinay Jahromi MA, Chansouria JPN, Ray AB. Antihyperglycemic activity of phenolics from *Pterocarpus marsupium*. *J Nat Prod* 1997; 60:609-60.
- Rerup CC. Drugs producing diabetes through damage of insulin secreting cells. *Pharmacol Rev* 1970; 22:485-520.
- Yellow RS, Black H, Villazan M, Berson SA. Comparison of plasma insulin levels following administration of tolbutamide and glucose. *Diabetes* 1960; 9:356-62.
- Grodsky GM, Epstein GH, Fanska R, Karam JH. Pancreatic action of sulphonylureas. *Fed Proceed* 1971; 36: 2719-28.
- Vats V, Yadav SP, Grover JK. Ethanolic extract of *Ocimum sanctum* leaves partially attenuates streptozotocin-induced alterations in glycogen content and carbohydrate metabolism in rats. *J Ethnopharmacol* 2004; 90 155-60.
- Whittonm PD, Hems DA. Glycogen synthesis in the perfused liver of streptozotocin-diabetic rats. *Biochem J* 1975; 150:153-65.
- Golden S, Wals PA, Okajima F. Glycogen synthesis by hepatocytes from diabetic rats. *Biochem J* 1979; 182:727-34.
- Weber G, Lea MA, Fisher EA. Regulatory pattern of liver carbohydrate metabolizing enzymes; insulin as an inducer of key glycolytic enzymes. *Enz Biol Clin* 1966; 7:11-24.
- Steiner DF, King J. Induced synthesis of hepatic uridine diphosphate glucose glycogen glycosyltransferase after administration of insulin to alloxan diabetic rats. *J Biol.Chem* 1964; 239:1292-8.
- Hikino H, Kobayashi M, Suzuki Y, Konno C. Mechanism of hypoglycemic activity of aconitan A, a glycan from *Aconitum carmichaeli* roots. *J Ethnopharmacol* 1989; 25:295-304.

CURRENT AUTHOR ADDRESSES

- D. Prabu, Department of Pharmacology, C.L.Baid Metha College of Pharmacy, Thoraipakkam, Chennai -600097, Tamilnadu, India. E-mail: prabu.pharma@gmail.com (Corresponding author)
- M. Nappinnai, Department of Pharmaceutics, C.L.Baid Metha College of Pharmacy, Thoraipakkam, Chennai -600097, Tamilnadu, India.
- K. Ponnudurai, Department of Pharmacology, Nandini Nagar Mahavidyalaya College of Pharmacy, Nandini nagar, Nawabganj, Gonda, Uttarpradesh - 271303, India.
- A. Thirugnanasambanthan, Department of Pharmaceutical Chemistry, K.L.E. Society's College of Pharmacy, Rajajinagar, Bangalore-560010, Karnataka, India.
- S. Srinivasan 5, 5Department of Statistics, Madras Christian College, Chennai-600 045, Tamilnadu, India.
- M. Ramvikas, Department of Pharmaceutics, C.L.Baid Metha College of Pharmacy, Thoraipakkam, Chennai -600097, Tamilnadu, India.