

Research Article



The Effect of *Thunbergia Fragens Roxb* in Experimental Diabetes and Their Effects on Key Metabolic Enzymes Involved in Carbohydrate Metabolism

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ABSTRACT

Diabetes mellitus (DM) is a metabolic disease, characterized by hyperglycemia resulting from insulin action Secretion together with impaired metabolism of glucose, lipids and protein. The WHO Expert Committee recommended that the important to investigate the hypoglycemic agents from plant origin, which were used in traditional medicine for the treatment of diabetes mellitus. *Thunbergia fragrans* Roxb belong to the family Acanthaceae. The anti-hyperglycemic effect of hydroalcoholic extract of *Thunbergia fragrans* Roxb was evaluated in diabetic rats using alloxan monohydrate 150mg/kg for pilot study (4 weeks) so as to assess their effect in mild (Plasma sugar > 180mg/dl duration 28days) diabetes mellitus. In the pilot study maximum reduction of 33% and 35% glucose levels was observed in animals receiving 200mg and 400mg/kg per day of hydro alcoholic extract of *Thunbergia fragrans* Roxb (HAETF) after 4 weeks of treatment, respectively. The alteration in hepatic glycogen content and hepatic glucokinase, hexokinase and glucose-6-phosphate levels in diabetic rats were partially restored by hydroalcoholic extract of *Thunbergia fragrans* Roxb.

Keywords: Diabetic mellitus, *Thunbergia fragrans* Roxb, Alloxan monohydrate.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease, characterized by hyperglycemia resulting from insulin action Secretion together with impaired metabolism of glucose, lipids and protein.^{1,2} The total number of people with Diabetes according to the International

Diabetic Federation (IDF), the global prevalence of diabetes is predicted to grow from 366 million by 2003.³ The raise in the incidence of diabetes mellitus is due to changing lifestyle, dietary patterns, and low birth weight has contributed to the increased diabetic ratio around worldwide.⁴ In modern medicine, there is no satisfactory effective therapy to cure diabetes mellitus. The management of diabetes mellitus by insulin therapy has several drawbacks like insulin resistance⁵ and in chronic treatment cause anorexia nervosa, brain atrophy and fatty liver.⁶

The oral hypoglycemic drugs are sulfonyl ureas and biguanides is also associated with side effects.⁷ Herbal medicines are used for primary health care, by about 80% of the world population particularly in the developing countries, because of better cultural acceptability, safety, efficacy, potent, inexpensive, and lesser side effects.⁸ The plant drugs are frequently considered to be less toxic when compared to synthetic drugs.⁹

The WHO Expert Committee recommended that the important to investigate the hypoglycemic agents from plant origin, which were used in traditional medicine for the treatment of diabetes mellitus.¹⁰ The oral hyperglycemic agent have been focused on plant used in

traditional medicine because that may be a better treatment than currently used synthetic drugs.¹¹

Thunbergia fragrans Roxb belong to the family Acanthaceae, is a large woody climber grown in India, Burma and China. A decoction made of leaves of this plant used in stomach complaint and fungal infection.¹² Entire plant contains "Trigonelline" a protein,¹³ flowers contains apogenin 7-o glucuronide, and daucosterol¹⁴ were reported. The anti-hyperglycemic activity of this plant has also not been assessed against hyperglycemia. Therefore, the primary objectives of this study were to assess the anti-hyperglycemic efficacy of this plant against hyperglycemia (in utilizing alloxan as diabetogenic agent at a dose of 150mg/kg).

MATERIALS AND METHODS

Plant Material

Fresh leaves of *Thunbergia fragrans* Roxb(TF) was collected from Alagar hills, Madurai, Tamil Nadu. The plants were authenticated by Dr. D. Stephen, Lecturer, Dept. of Botany, The American College, Madurai, Tamil Nadu.

Preparation of Hydro Alcoholic Extract of TF leaves

The leaves of TF were dried in the shade. Then the shaded dried plants were powdered to get coarse powder. And about 500gms of the dried coarse powder of TF were soaked in the extractor and macerated for 30hrs with petroleum ether, followed by Chloroform. Then it is extracted with ethanol: water (70:30) by continuous hot percolation technique using Soxhlet apparatus for 72hrs. Crude extract were distilled under vacuum condition.



alcoholic extract of *Thunbergia fragrans* at different doses of 200 mg/kg & 400 mg/kg was significantly increased to 222.50 ± 2.65 & 225.45 ± 2.95 respectively as compared to non-treated diabetic animals.

In group III treated animals also showed an increase in body weight significantly as compared to diabetic rats.

Effect of HAETF on Blood Glucose Levels

In all groups prior to alloxan administration the basal level of plasma glucose of the rats were not significantly higher in the rats selected for the study.

In contrast non-diabetic control remained steadily euglycemic throughout the course of study.

In pilot study (mild diabetics) the Table no 1 also shows the effect of treatment of various doses of Hydro-alcoholic extract *Thunbergia fragrans* (HAETF) at a dose of 200 mg/kg & 400mg/kg respectively on plasma glucose levels. Blood glucose level was increased significantly to 172.46 ± 1.82 & 225.22 ± 3.32 at 14th & 28th day of treatment respectively, in the diabetic animals as compared to normal animals.

In the HAETF treated groups (both doses), significant anti-hyperglycemic ($p < 0.001$) effect was evident from the 2nd week onwards, the decrease in blood sugar was maximum on completion of the 4th week in the group receiving 200 mg/kg & 400mg/kg of *Thunbergia fragrans* respectively, where as in group III treated animals receiving glipizide at a dose of 5mg/kg also restored the blood sugar level near to normal range.

Effect of HAETF on Glycogen Content

Glycogen content of liver tissue was estimated on the 28th day in non-diabetic control, diabetic control, treated group and positive control group as shown in Table no 2.

In diabetic control liver glycogen content decreased significantly by 79.59% as compared to non-diabetic control.

Treatment with glipizide, HAETF at two doses (200mg/kg & 400mg/kg) led to 73.75%, 62.64% and 67.23% increase in liver glycogen content in comparison to diabetic control.

Table 1: Effect of 4 week treatment with various doses of hydro-alcoholic extract of *Thunbergia fragrans* (HAETF) on glucose levels (mg %) and body weight in alloxan diabetic rats.

Groups	Body Weight (gm)		Blood Glucose Levels (mg)		
	Initial Body weight	Final Body weight	0 th Day	14 th Day	28 th Day
Group I	212.27 ± 2.80	225.00 ± 4.28	81.00 ± 2.56	80.00 ± 1.71	72.00 ± 1.71
Group II	197.50 ± 2.14	157.50 ± 2.14 ^{*a}	157.72 ± 2.61	172.46 ± 1.82 ^{*a}	225.22 ± 3.32 ^{*a}
Group III	215.00 ± 2.56	217.14 ± 3.26 ^{*b}	181.72 ± 2.61	155.00 ± 2.56 ^{*b}	138.67 ± 2.58 ^{*b}
Group IV	215.00 ± 4.28	222.50 ± 2.65 ^{*b}	192.08 ± 1.50	162.50 ± 2.14 ^{*b}	151.91 ± 1.93 ^{*b}
Group V	219.30 ± 6.54	225.45 ± 2.95 ^{*b}	191.91 ± 1.93	164.00 ± 1.71 ^{*b}	146.85 ± 1.50 ^{*b}

Values are expressed as Mean ± SEM.

*a values were significantly different from normal control (G 1) at ($P < 0.001$)

*b values were significantly different from diabetic control (G 2) at ($P < 0.001$)

Effect of HAETF on Hepatic Enzymes

To establish diabetic, plasma glucose was determined 72hrs after alloxan administration. Only those rats with over 180 mg% were included in the study.

On the 28th day, hepatic enzymes Hexokinase & Glucokinase and substrate Glucose-6-phosphate were estimated in saline controls (group I), diabetic control (group II), and treatment controls (groups III, IV, V). The result has been compiled in Table no 2. As compared to non-diabetic control values, mean level of enzymes Hexokinase & Glucokinase and substrate Glucose-6-phosphate values decreased in diabetic control. The respective percentage decrease was 54.75%, 80.84% and 65.84% in diabetic control. Treatment with HAETF at two doses (200mg/kg & 400mg/kg) for 28 days led to rise in percentage of these parameter by 21.25%, 60.49%, 43.26%, and 31.03%, 67.50%, 48.51% respectively ($P < 0.001$) as compared to diabetic control. Also treatment with glipizide 5mg/kg for 28 days led to rise in percentage of these parameters by 33.33%, 73.01% and 57.62% respectively ($P < 0.001$) as compared to diabetic control.

Effect of HAETF on Hematological Parameter

Table no 3 values shows the hematological parameters of group I to group V treated animals. At the end of 28 days of the study period, no statistically significant differences were seen in the mean WBC and RBC counts, HB & Platelet values as compared to the non-diabetic animals.

Histopathological Study

In histopathological study, the Figure-1-A showed normal acini and normal cellular population in the islets of langerhans in pancreas of non-diabetic rats (group-I). Figure-1-B showed extensive damage and reduced number of islets of langerhans in pancreas of diabetic rats (group-II). Figure-1-C showed restoration of normal cellular population size of islets with hyperplasia by glipizide (group-III). Figure-1 D and E showed partial restoration of normal cellular population and enlarged size of β -cells with hyperplasia in HAETF treated groups (group IV & group V).



Table 2: Effect of administration of various doses of hydro-alcoholic extract of *Thunbergia fragrans* (HAETF) on glycogen content (mg/gm tissue) of liver tissue of rats and enzymes involved in carbohydrate metabolism.

Groups	Liver tissue glycogen content (mg/g tissue)	Enzymes involved in carbohydrate metabolism		
		Hexokinase (µg/mg)	Glucose-6-phosphate (µg/mg)	Glucokinase (µg/mg)
Group I	44.85 ± 1.30	0.221 ± 0.003	0.407 ± 0.004	27.51 ± 0.75
Group II	9.15 ± 0.28 ^{*a}	0.100 ± 0.003 ^{*a}	0.139 ± 0.002 ^{*a}	5.27 ± 0.10 ^{*a}
Group III	34.86 ± 1.37 ^{*b}	0.150 ± 0.005 ^{*b}	0.328 ± 0.004 ^{*b}	19.53 ± 0.90 ^{*b}
Group IV	25.30 ± 1.31 ^{*b}	0.127 ± 0.002 ^{*b}	0.245 ± 0.004 ^{*b}	13.34 ± 0.58 ^{*b}
Group V	27.93 ± 1.45 ^{*b}	0.145 ± 0.002 ^{*b}	0.270 ± 0.008 ^{*b}	16.25 ± 1.10 ^{*b}

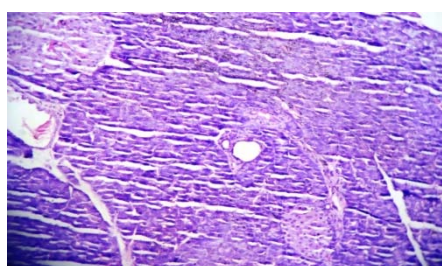
Values are expressed as Mean ± SEM; ^{*a} values were significantly different from normal control (G 1) at (P<0.001)

^{*b} values were significantly different from diabetic control (G 2) at (P<0.001)

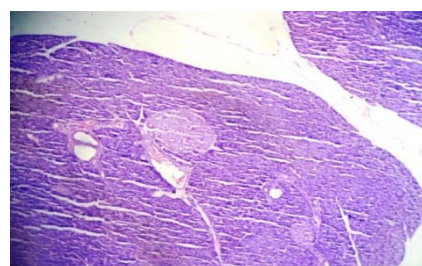
Table 3: Effect of administration of various doses of hydro-alcoholic extract of *Thunbergia fragrans* (HAETF) on hematological parameters.

Groups	WBC × 10 ³ /µL	RBC × 10 ⁶ /µL	HB % gm/Dl	Platelet × 10 ³ /mL
Group I	8.58 ± 0.62	6.50 ± 0.30	12.55 ± 0.62	310.40 ± 40.10
Group II	8.30 ± 0.70	6.95 ± 0.36	12.15 ± 0.46	300.6 ± 28.10
Group III	7.55 ± 0.52	6.78 ± 0.32	12.30 ± 0.50	287.40 ± 26.40
Group IV	7.60 ± 0.50	7.05 ± 0.25	12.12 ± 0.55	302.70 ± 26.40
Group V	7.95 ± 0.65	7.10 ± 0.38	11.70 ± 0.30	290.20 ± 25.30

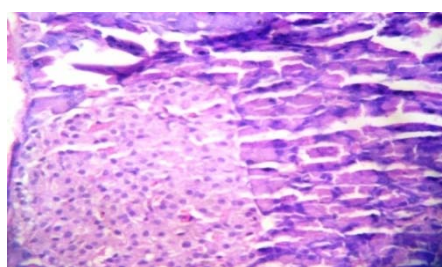
^{*}Values were not significantly different from normal and diabetic control.



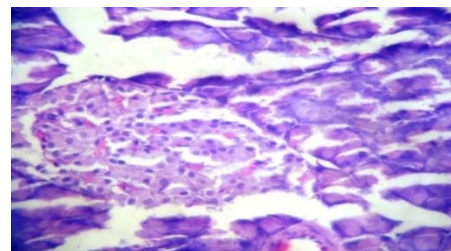
A-Group I: Normal Control (Saline)
It shows normal numbers and volume of the islets cells



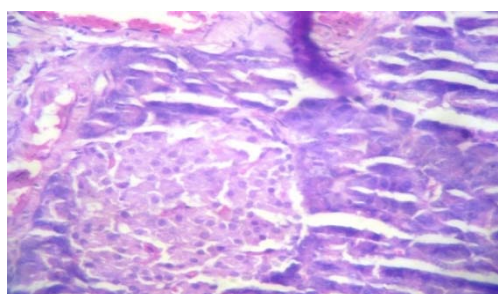
B-Group II: Toxic Control [The numbers of islets cells were severely decreased, islets cells were severely swelled.]



C-Group III: Positive Control (Alloxan monohydrate + Glipizide)
The numbers of islets cells were decreased, islets cells were mildly swelled



D-Group IV: Treatment group (Alloxan monohydrate + T.fragrans 200mg/kg) [The numbers of islets cells were decreased, islets cells were also swelled]



The numbers of islets cells were decreased, islets cells also moderately swelled.

E-Group V: Treatment group (Alloxan monohydrate + T.fragrans 400mg/kg)

Figure 1: Histopathology Study of Pancreas of Rats

DISCUSSION

Currently available drug regimens for the management of diabetes mellitus have certain drawbacks²² and therefore, there is a need for safer and more effective anti-diabetic drugs. This study was therefore undertaken to assess anti-hyperglycemic property of *Thunbergia fragrans* which have been reported in Thai to be useful in diabetes mellitus.

In the current study diabetes mellitus was induced by alloxan monohydrate at a dose of 150 mg/kg i.p. Alloxan causes enormous reduction in insulin release through the destruction of β cells of the islets of Langerhans. The mechanism of alloxan action was completely described elsewhere.^{23,24} In our study we have observed a significant increase in plasma glucose level in alloxan induced diabetic rats, whereas treatment with glipizide (5mg/kg), hydro-alcoholic extract of *Thunbergia fragrans* at two different doses (200mg/kg & 400mg/kg) showed significant anti-hyperglycemic activity in mild degree of hyperglycemia.

In mild diabetes, the maximum percent reduction in glucose level was seen in groups receiving 400mg/kg per day of HAETF.

This could be due to potentiation of insulin effect of plasma by increasing their pancreatic secretion of insulin from existing β -cells of islets of Langerhans or its release from bound insulin.

The significant and consistent anti-diabetic effect of HAETF in alloxan-induced diabetic rats is also due to enhanced glucose utilization by peripheral tissues. The body weights were decreased in alloxan-induced diabetic rats.

Administration of HAETF at two doses increased body weight in alloxan-induced diabetic rats. The ability of HAETF to protect massive body weight loss seems to be due to its ability to reduce hyperglycemia.

As reported earlier, in the current study the liver glycogen content was reduced significantly in diabetic control as compared to non-diabetic control.²⁵

Treatment with HAETF at two doses prevented this alteration in glycogen content of liver tissue. But could not normalize the content of glycogen of the non-diabetic control.

This prevention of depletion of glycogen in liver is possibly due to either stimulation of insulin release from β -cells or due to the insulinomimetic activity of some components of the plants resulting in direct peripheral glucose uptake.²⁶

Decreased enzymatic activity of hexokinase, glucokinase and substrate glucose-6-phosphate has been reported in diabetic animals resulting in depletion of liver and muscle glycogen.²⁷

The present study also had similar results. Treatment with HAETF significantly increased the hexokinase & glucokinase activity and glucose-6-phosphate level in the liver, indicating an overall increase in glucose influx thus *Thunbergia fragrans* seems to have an overall effect in increase in glucose utilization.

Studies also assess this plant extract showed no adverse effect on hematological parameters including WBC and RBC counts, HB, Platelets.

Thus this plant extract can be presumed to be free from toxicological effects.

Histopathological studies revealed that HAETF and Glipizide significantly improved the histological architecture of the islets of Langerhans.

The groups treated with HAETF (200mg/kg & 400mg/kg) and glipizide (5mg/kg) showed greater persistence of islets of Langerhans & lesser degree of necrotic changes as compared to the untreated alloxan-induced diabetic rats.

CONCLUSION

In our study we have observed a significant increase in plasma glucose level in alloxan induced diabetic rats, whereas treatment with glipizide (5mg/kg), hydro-alcoholic extract of *Thunbergia fragrans* at two different doses (200mg/kg & 400mg/kg) showed significant anti-hyperglycemic activity in mild degree of hyperglycemia.

In mild diabetes, the maximum percent reduction in glucose level was seen in groups receiving 400mg/kg per day of HAETF.

Further investigation is necessary to determine the exact phytoconstituents responsible for the anti-diabetic effect.

REFERENCES

1. American diabetes Association. Diagnosis and classification of diabetes care. 33, 2010, S62-S69.
2. Lin Y, Sun Z. Current views on type 2 diabetes. Journal of Endocrinol, 204, 2010, 1-11.
3. Whiting DR, Guariguata L, Weil C, Shaw J. IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. Diabetes Res Clin Pract, 94, 2011, 311-321.
4. IDF. Diabetes Atlas 4th Edition, International diabetes federation. President of international diabetes federation calls for concerted action to stop diabetes epidemic. Montreal Canada. 2009.
5. Piedrola G, Nov E, Escobar F and Garcia-Robles R. White blood cell count and insulin resistant in patients with coronary artery disease. Ann.Endocrinol (Paris), 62,2001, 7-10.
6. Tobias Y.J.A, Pinto A and Nerziroglu F. Anorexia nervosa, diabetes mellitus, brain atrophy and fatty liver. Int.J.Eat.Disord, 30, 2001, 350-353.
7. Rang, H.P, Dale M.M. The Endocrine System Pharmacology, 2nd edn. Longman, Harlow, 1991, 504-508.



8. Pullaiah T and Chandrasekhar Naidu K. Anti-diabetic plants in India and herbal based anti-diabetic research, Regency Publication, New Delhi, 2000, 3-9.
9. Pari L and Uma Maheshwari J. Antihyperglycemic activity of *Musa sapientum* flowers: Effect on lipid peroxidation in alloxan diabetic rats. *J Ethnopharmacol*, 14, 2000, 1-3.
10. Alarcon-Aguilera FJ, Roman-Ramos R, and Perez-Gutierrez S. Study of anti-hyperglycemic effect of plants used as anti diabetics. *J Ethnopharmacol*, 61, 1998, 101-110.
11. Hu X, Sato J, Oshida Y, Yu M, Bajotto G and Sato Y. Effect of Goshajinki-gan (Chinese herbal medicine: Niu-che-sen-qj-wan) on insulin resistance in streptozotocin induced diabetic rats. *Diabetes Res. Clin. Pract*, 59, 2003, 103-111.
12. Yoganasimhan S.N. *Medicinal Plants of India*, 2, 2000, 546.
13. Fischer H and Nielsen B J, *Phytochemistry*, 278, 1988, 2581.
14. Nair A.G.R and Subramanian S S. *Curr. Sci. (India)*, 43, 1974, 472.
15. Al-Shamaony L, Al-Khazraji S.M, Twaiji H.A. Hypoglycemic effect of *Artemisia herba alba*. II. Effect of a valuable extract on some blood parameters in diabetic animals. *Journal of Ethno Pharmacology*, 43, 1994, 167–171.
16. Trinder P. Determination of blood glucose using an oxidase peroxidase system with a non-carcinogenic chromogen. *Journal of Clinical Pathology*, 22, 1969, 158–161.
17. Crane R.K, Sols A. Animal tissue hexokinases. In: Colowick, S. P. Kaplan N.O. (Eds) *Methods in Enzymology*. Academic Press, New York, 1, 1955, 277–282.
18. Marjorie A.S. Phosphatase of liver-1 glucose-6-phosphate. *Journal of Biological Chemistry*. 184, 1950, 647-659.
19. Fiske C.H, Subbaraw Y. The colorimetric determination of phosphorus. *Journal of Biological Chemistry*, 66, 1925, 375–400.
20. Castano J.G, Nieto A, Feli J.E. Inactivation of phosphofructokinase by glucagon in rat hepatocytes. *Journal of Biological Chemistry*. 254, 1925, 5576-5579.
21. Morales M.A, Jabbagy A.J, Terenzi H.P. Mutations affecting accumulation of glycogen. *Neurospora News Letter*. 20, 1973, 24–25.
22. Knatterud G, Klimt CR, Levin ME, Jacobsen ME, Goldner MG. Effect of hypoglycemic agents on vascular complication in patients with adult onset diabetes: section VIII. Mortality and selected non fatal events with insulin treatment. *Journal of the American Medical Association*. 240(1), 1978, 37–42.
23. Lazarow A. Alloxan diabetes and mechanisms of beta cell damage by chemical agents. *Expt diab*. 23, 1964, 49-69.
24. Colca JR, Kotagel N, Brooks CL, Lacy PE, Landt M and McDaniel ML. Alloxan inhibition of a Ca^{2+} and calmodulin-dependent protein kinase in pancreatic islets. *J.Biol.chem*. 25, 1983, 7260-7263.
25. Welihinda J and Karunanayake EH. Extra pancreatic effects of *Eugenia jambolana* in rats. *J Ethno Pharmacol*. 17(3), 1986, 247–255.
26. Lolitkar M M, and Rao M.R.R. Pharmacology of a hypoglycemic principle isolated from the fruits of *Eugenia jambolana* Linn. *Indian Journal of Pharmacy*. 28(5), 1966, 129–133.
27. Hikino H, Kobayashi M, Suzuki Y and Konno C. Mechanism of hypoglycemic activity of aconitan A, a glycan from *Aconitum carmichaeli* roots. *Journal of Ethnopharmacology*. 25(3), 1989, 295–304.

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