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Anti diabetic activity of methanol/methylene chloride extract of *Terminalia superba* leaves on streptozotocin induced diabetes in rats

Padmashree[1*], Padmavathi P Prabhu[2], Sushanth Pandey[2]

***[1]Department of Pharmacology, Srinivas College of Pharmacy, Valachil, Farangipete Post, Mangalore-574143, Karnataka,India.**

[2]Department of Pharma Chemistry, Srinivas College of Pharmacy, Mangalore,India.

***Corres.author: padmasrikaranth@gmail.com**
Tel No: +91 9480160292

Abstract: Aim of the present work is to evaluate the anti diabetic effect of methanol/methylene chloride extract of *Terminalia superba* leaves in rats. *Terminalia superba* (Combretaceae) is one of the plants used by traditional healers as a remedy for diabetes mellitus. It is widely distributed in the dense humid forests. Effect of various doses (200, 400 mg/kg p.o) extract was studied on streptozotocin induced both diabetic and non diabetic rats. After 2 weeks of the administration of plant extract the normalisation of fasting blood glucose levels, reduction in polyphagia and polydipsia and weight gain by diabetic-treated rats has been observed. The reduction in the glucose level in induced diabetic rats proved that *Terminalia superba* having the wide antidiabetic activity.
Key words: Anti diabetic; Streptozotocin; *Terminalia superba*.

INTRODUCTION:

Diabetes mellitus is a metabolic disorder characterized by fasting hyperglycemia and alterations in carbohydrate, fat and protein metabolism, associated with absolute or relative deficiencies in insulin secretion and/or insulin action.[1] Experimentally, streptozotocin (STZ) or alloxan are used to induce diabetes in rodents. STZ is effective in triggering islet cell death by acute oxidative stress. STZ-induced diabetic rats are one of the animal models of insulin-dependent diabetes mellitus characterized by high fasting blood glucose levels and drastic reduction in plasma insulin concentration.[2] Although different types of oral hypoglycemic agents are available along with insulin for the management of diabetes mellitus, there is a growing interest in herbal remedies due to the side effects associated with these therapeutic agents.[3] Thus plants have played a major role in the discovery of new therapeutic agents.

Terminalia superba (Combretaceae) is one of the plants used by traditional healers as a remedy for diabetes mellitus. *Terminalia superba* is a big tree with deciduous leaves, attaining 60 m of height and leaves are 10 cm long and 5 cm broad. It is widely distributed in the dense humid forests, semi-deciduous forests and also in easily flooded and secondary forests.[4] *Terminalia arjuna, Terminalia belerica, Terminalia chebula, Terminalia catappa and Terminalia pallida* are other species of the same family (Combretaceae) known for their antidiabetic properties.[1]

The present study was undertaken to investigate the anti-hyperglycemia effect of the methanol/methylene chloride extract of *Terminalia superba* leaf on the diabetes induced by a multiple dose of STZ in diabetic rats.

MATERIALS AND METHODS:

Animals:

The experimental animals were male albino Wistar rats (150–250 g body weight) procured from Indian Institute of Sciences were used for this study. They are maintained under standard conditions (temperature 22 ± 20^{0}C, relative humidity 60±5% and 12 h light/dark cycle). They had free access to standard pellet diet and water *ad libitum*. The Institutional Animal Ethics Committee approved the experimental protocol. All the animals received humane care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the "National Academy of Sciences" and published by the "National Institute of Health".

Preparation of plant extracts:

The leaves of *Terminalia superba* used for the investigation were collected from Mangalore. The plant was identified and confirmed by Dr. Noeline J. Pinto, Head of the Department of Botany, St. Agnes College, Mangalore. A voucher specimen (V. No. SCP 356) has been deposited in the Pharmacognosy department of Srinivas College of Pharmacy, Valachil, Mangalore. All fresh plant materials of leaves were sun-dried and ground into powder. The dried powdered materials macerated in 1:1 (v/v, 500 mL) mixtures of methanol/methylene chloride for 7 days with occasional stirring at room temperature. The extracts were filtered and concentrated using a rotor evaporator and dried in an oven at 48^{0}C. The yields of 55 g of *Terminalia superba* crude extracts were 28.3%.

Streptozotocin-induced diabetes

Streptozocin was obtained from Himedia Laboratories, Mumbai. All other chemicals used for this study were of analytical grade. Streptozotocin (55 mg/kg) was dissolved in 0.1M citrate buffer (pH 4.5). Six rats per group were administered by subcutaneous injection. After 48 h, fasting blood glucose levels as well as glycosuria were assessed to confirm the diabetic state. Only rats with a fasting blood glucose level of at least 250 mg/dL and positive urine glucose were considered diabetic and were used in the experiment.

Experimental Design

Male Wistar albino rats weighing 150–200 g (90 to 110 days old) were used. The animals were randomly divided into five groups of six animals each.

Group 1: Normal control (non-diabetic, untreated) rats.
Group 2: Diabetic control (diabetic, untreated) rats.
Group 3: Diabetic test rats administered three units of insulin by subcutaneous injections.
Group 4: Diabetic test rats given *Terminalia superba* extracts at the dose of 200 mg/kg.
Group 5: Diabetic test rats given *Terminalia superba* extracts at the dose of 400 mg/kg.

Treatment of experimental animals with plant extracts was initiated 2 days post streptozotocin injection and was carried out once daily, by orally, for 14 days. Food and water were made freely available.

Measurement of body weight gain, food, water intake and blood glucose

Body weight gain, food and water intakes were monitored daily during the 14 days experimental period. Blood samples for glucose determination were obtained from the tail tip of 12 h fasted rats on day 0 (before streptozotocin administration), days 2 (48 h post streptozotocin injection), 5, 8, 11 and 14th day of the experiments. Blood glucose level was determined using a glucometer (Accu-Check, Roche). Urine glucose was also assessed in fresh urine using glucose indicator sticks (Boerhinger Mannheim, Germany) before and 48 h after streptozotocin administration, for the confirmation of the diabetic state of animals.

Statistical Analysis:

Mean values were obtained by one-way analysis of variance (ANOVA) followed by Dunnet's't' test, using the computer software, Graphpad Prism 5. The significance of difference between and within various groups was determined. The results are expressed as mean±S.E.M. Values of $p < 0.05$ were taken to imply as statistically significant.

RESULTS:

The effects of the *Terminalia superba* extract on the body weight of diabetic rats are shown in **Table 1**. During the 2 weeks of observation of the extract-treated diabetic rats at doses of 400 mg/kg, there were very significant ($p < 0.01$) weight gains relative to day 2. The diabetic rats treated with three units of insulin also showed a very significant ($p < 0.01$) weight increase in the body compared to untreated diabetic rats.

Table 2 shows the effects of the extracts on food and fluid intakes by diabetic rats. When compared to the untreated diabetic rats, untreated diabetic rats had severe polyphagia and polydipsia at the end of the second week of the experiment with respective increase in food and fluid intakes. However, in the presence of *Terminalia superba* extract (200mg/kg and 400 mg/kg), food intake was reduced when compared with diabetic control rats but its not statistically significant ($p>0.05$). Insulin treated diabetic rats also shown reduction in food intake ($p>0.05$). Fluid intakes showed decrease in *Terminalia superba*-treated diabetic rats at doses of both 200 mg/kg and 400 mg/kg when compared with diabetic control rats. Diabetic rats treated with three units of insulin also showed a non significantly lower water intake ($p>0.05$).

Effect of the leaf extracts on fasting blood glucose levels of streptozotocin-diabetic rats are shown in

Table 3. Following a 48 h post streptozotocin injection, all diabetic rats exhibited hyperglycemia, which ranged between 330 and 400 mg/dL while normal control rats showed a normal blood sugar level of 110 mg/dL. After 2 weeks of treatment with the extracts, the glycemic level of 200 mg/kg *Terminalia superba* extract treated diabetic rats dropped significantly from 369.8±4.090 on day 2 to 120.3 ±4.442 mg/dL ($p < 0.01$) on day 14 and from 362.3±5.977 to 116.8±4.854mg/dL ($p < 0.01$) for 400 mg/kg dose respectively. Insulin treated diabetic rats shown very significant decrease in blood sugar level compared to diabetic rats ($p < 0.01$).

Table 1: Effects of *Terminalia superba* extracts on the body weight of STZ-induced diabetic rats.

Group (n=6)	Body weight (g)	
	2days after STZ injection	**14 Days after administration of plant extracts**
Normal control rats	201.0 ± 3.821*	217.5±3.227**
Diabetic control rats	190.2 ± 3.541	162.5±3.227
Diabetic control treated with three units of insulin	197.6 ± 0.9274*	188.8±4.270**
Diabetic test rats (*Terminalia superba* 200mg/kg)	195.6 ± 1.631*	177.0±4.708*
Diabetic test rats (*Terminalia superba* 400mg/kg)	196.0±1.703*	180.5±5.204**

Results are expressed as mean ± SEM, n=6

Table2: Food and fluid intakes of rats treated with *Terminalia superba* extracts for 2 weeks

Group (n=6)	Food intake (g/rat/week)		Fluid intake (mL/rat/week)	
	Week 1	**Week 2**	**Week 1**	**Week 2**
Normal control rats	59.00 ± 1.683	61.00±1.291	22.50±3.227*	21.25±1.493
Diabetic control rats	67.50±2.630	69.50±2.533	42.50±3.227	48.50±2.255
Diabetic control treated with three units of insulin	54.75±5.498*	46.75±2.394	35.00±2.160	37.50±3.227
Diabetic test rats (*Terminalia superba* 200mg/kg)	59.00±1.683	54.50±1.041	32.25±2.175	42.25±3.326
Diabetic test rats (*Terminalia superba* 400mg/kg)	58.00±1.472	50.25±1.250	38.00±2.483	39.50±1.708

Results are expressed as mean ± SEM, n=6

Table 3: Blood glucose level (mg/dL) of rats 2 days post STZ administered and after 14 days of treatment with plant extracts.

Group (n=6)	Glycemia (mg/dL)		
	Before STZ	2 Days after STZ	After 14 days of treatment
Normal control rats	105.0 ±6.455	110.0±4.564**	105.5±2.723**
Diabetic control rats	108.8±4.270	387.8±7.375	373.5±6.886
Diabetic control treated with three units of insulin	97.50±8.539	331.0±5.672**	117.5±2.784**
Diabetic test rats (*Terminalia superba* 200mg/kg)	111.3±4.270	369.8±4.090	120.3 ±4.442**
Diabetic test rats (*Terminalia superba* 400mg/kg)	103.8±8.985	362.3±5.977*	116.8±4.854**

DISCUSSION:

Our results suggest that the methanol/methylene chloride extracts of the leaf of *Terminalia superba* have dose-dependent anti-diabetic activities on streptozotocin-induced diabetes. The metabolic disturbances were corrected after the plant extracts were administered for 2 weeks, as shown by the normalisation of fasting blood glucose levels, reduction in polyphagia and polydipsia and weight gain by diabetic-treated rats but reduction in polyphagia and polydipsia are not statistically significant. The mechanisms by which streptozotocin brings about its diabetic state include selective destruction of pancreatic insulin secreting beta cells, which make cells less active[4,5] and lead to poor glucose utilization by tissues.[6] *Terminalia superba* significantly reduced the high fasting glucose levels in streptozotocin-induced diabetic rats. This suggests that the extracts may possess an insulin like effect on peripheral tissues by either promoting glucose uptake and metabolism, by inhibiting hepatic gluconeogenesis.[7] or absorption of glucose into the muscles and adipose tissues,[8] by the stimulation of a regeneration process and revitalisation of the remaining beta cells[9,10,11].

Pharmacological, biochemical, histological and chemical studies are needed to elucidate the exact mechanism of action of Bauhinia forficata leaf decoction and to isolate any active compounds. Such investigations should also be carried out regarding type 2 diabetes.

CONCLUSION:

Terminalia superba leaf extract possess antidiabetic properties.

REFERENCES:

1. Kameswara Rao B, Renuka Sudarshan P, Rajasekhar MD, Nagaraju N, Appa Rao Ch. Antidiabetic activity of Terminalia pallida fruit in alloxan-induced diabetic rats. J Ethnopharmacol, 2003; 85:169-172.
2. Burcelin R, Eddouks M, Maury J, Kande J, Assan R, Girard J. Excessive glucose production, rather than insulin resistance, accounts for hyperglycemia in recent-onset streptozotocin-diabetic rats. Diabetologia, 1995; 38:283-290
3. Kamesawara BR, Giri R, Kesavulu MM., Apparao CH. Effect of oral administration of bark extracts of *Pterocarpus santalinus* L. on blood glucose level in experimental animals. J of Ethnopharmacology.2000; 74: 69–74.
4. Jacot E, Assal JPH. Regulation de la glycemie. Dans: Pharmacologie des concepts Fondamentaux aux Applications Therapeutiques. Schorderet, in: Frison-Roche et Slatkine (Ed.), 1989; 481–494.
5. Junod A, Lambert AE, Stauffacher W, Renold AE. Diabetogenic action of streptozotocin of dose to metabolic response. J of Clini Inves. 1969; 48: 2129–2139.
6. Marles RJ, Farnsworth N.R. Antidiabetic plants and their active constituents. Phytomedicine. 1995; 22:123–189.

7. Ali L, Azad Khan AK, Mamun MIR, Mosihuzzaman M, Nahar N, Nur-E-Alan M., Rokeya B. Studies on the hypoglycaemic effects of fruits pulp, seed and whole plant of *Momordica charantia* on normal and diabetic model rats. Planta Medica. 1993; 59: 408–412.
8. Kamanyi A, Djamen D, Nkeh B. Hypoglycaemic properties of the aqueous roots extract of *Morinda lucida* (Rubiacea) study in the mouse. Phytotherapy Research. 1994; 8: 369–371.
9. Rokeya B, Nahar N, Ali L, Hassan Z, Nure-E-Alam M, Chowdhury SN, et al. Effects of five medicinal plants on blood glucose levels in non-diabetic and diabetic model rats. Diabetes Research. 1999; 34:219–228.
10. Shanmugasundaram ERB, Gopinath KL, Shanmugasundaram KR, Rajendran VM. Possible regeneration of the islets of Langerhans in streptozotocin-diabetes rats given *Gymnema sylvestre* leaf extracts. J of Ethnopharmacol. 1990; 30: 265–279.
11. Kamtchouing P, Kahpui SM, Djomeni DP, T´edong L, Asongalem EA, Dimo T, 2005. Anti-diabetic activity of methanol/methylene chloride stem bark extracts of *Terminalia superba* and *Canarium schweinfurthii* on streptozotocin-induced diabetic rats. Journal of Ethnopharmacology 104, 306–309.

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