



Evaluation of Antidiabetic activity of crude extracts of bark of *Terminalia paniculata* Roth in Streptozotocin induced Diabetes in Wister Rats

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Abstract:

The objective of the present study was to evaluate antidiabetic activity of petroleum ether (PETP) and ethanolic (ETP) extracts of bark of *Terminalia paniculata* Roth (Family: Combretaceae) in normal and Streptozotocin induced diabetic rats. The extracts of *Terminalia paniculata* was investigated for antidiabetic activity in Streptozotocin induced diabetic rats by oral administration of extract 100 mg/kg body weight for single-dose one day and multiple-dose seven days. The effect was compared with oral dose of 0.5 mg/kg body weight Gilbenclamide. The determination of serum glucose level was estimated by the enzymatic glucose oxidase method. The results showed that Petroleum ether and Ethyl acetate extracts of bark of *Terminalia paniculata* significantly lowered the serum glucose levels. The efficacy of Petroleum ether extract was better than Ethyl acetate extract. Phytochemical study showed the presence of alkaloids, carbohydrate, flavanoids, phenolics, steroids and alkaloids. Therefore, this study shows that *Terminalia paniculata* has antidiabetic action and the extracts should be further be subjected to bioactivity graded drug discovery to isolate a lead compound responsible for this activity.

Keywords: *Terminalia paniculata*, Combretaceae, Streptozotocin, Diabetes mellitus, Wister rats, Gilbenclamide.

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INTRODUCTION

Diabetes mellitus is a chronic metabolic disease, as old as mankind, characterized by hyperglycemia associated with impairment in insulin secretion/action with altered carbohydrate, protein and lipid metabolism and increased risk of vascular complication (1). The function of insulin is to maintain normal blood glucose levels either by suppression of glucose output from liver or by the stimulation of glucose uptake and its metabolism. Insufficient release of insulin or loss of insulin action at target tissues causes abnormal glucose and lipid metabolism, which results in elevated glucose levels in blood, the hall mark of diabetes. Type-1 diabetes results from autoimmune destruction of pancreatic beta cells resulting insulin deficiency. In conventional therapy Type-1 diabetes is

managed with exogenous insulin and Type-2 with oral hypoglycemic agents such as sulfonyl ureas, metformin acarbose etc.

Before the introduction of insulin in 1922, the treatment of Diabetes mellitus relied heavily on dietary measure which included the use of traditional plant therapies. Many traditional plant treatments exist for diabetes (2, 3, 4). However, few have received scientific or medicinal scrutiny and the WHO has recommended the traditional plant therapy warranted further evaluation (5). Insulin therapy affords effective glycemic control, yet its drawbacks such as ineffectiveness on oral administration, short shelf life, need for constant refrigeration and hypoglycemia on excess dosage limits its use (6).

Diabetes mellitus has recently been identified by Indian Council of Medical Research (ICMR) as one

of the refractory disease for which satisfactory treatment is not available in modern allopathic system of medicine and suitable herbal preparations are to be investigated. Therefore efforts continue to find insulin substitutes from synthetic or plants sources. Researchers in India have documented the use of over 150 plants in various families with hypoglycemic activity (7).

Terminalia paniculata Roth (Family: Combretaceae) commonly known as Kindal or Kinjal is a tropical wild tree with large distribution in Western Ghats of India. Traditionally, flower juice and bark of *Terminalia paniculata* have been used as a remedy for cholera for the treatment of inflamed parotid glands and in menstrual disorders. It is used as a cardiogenic and diuretic. Timber is useful for shipbuilding and as a substitute for teak. Fruits are used for tanning and dyeing. However, till date, there has been no investigation supporting the pharmacological properties of this plant. Due to paucity of scientific information regarding the effect of *Terminalia paniculata* on blood glucose. This study was therefore aimed to investigate the hypoglycemic activity of petroleum ether, ethyl acetate and ethanolic extract of *Terminalia paniculata* bark (PETP, EATP and ETP) in Streptozotocin (STZ) induced diabetic male Wister rats.

MATERIALS AND METHODS

Collection of Plant Material

The Bark of *Terminalia Paniculata* Roth was collected from the Devarakadu (preserved forest) of Hosagunda Village of Sagar Taluk, Shivamogga District under the supervision of localities and forest officials. The plant material was identified and authenticated by Resident Botanist, Prof. R.

Manjunatha of Botany Department, DVS College of Arts and Science, Shivamogga.

Extraction

Freshly collected Bark of *Terminalia Paniculata* Roth were dried in shade and pulverized to get a coarse powder. A weighed quantity of powder (830 g.) was used for extraction.

Dried powder of bark was exhaustively extracted successively using Petroleum ether (60-80°C) (PETP), Ethyl acetate (EATP) and Ethanol (95%) (ETP) respectively in Soxhlet apparatus. All the extracts were concentrated by rotary flash evaporator, under reduced pressure and controlled temperature, followed by freeze drying and stored at 40 °C for further use.

All the crude fractions PETP, EATP and ETP were subjected to qualitative phytochemical investigation using standard tests to identify the types of phyto constituents (8,9).

Animals

Young adult male wister rats 7-8 weeks old, weighing 150-200g were procured from inbred disease free animal house, National Institute of Pharmacy, Shivamogga, Karnataka, India. The animals were housed in polypropylene cages in standard husbandry conditions, 12 hr light and 12 hr dark cycle at 25 ± 2 °C. Before and during the experiments, the rats were fed with standard laboratory pellet diet and water *ad libitum*. Wister rats were acclimatized to the laboratory condition for at least 15 days prior to the experiment and were maintained in a well ventilated animal house. The experimental protocol was approved by the Institutional animal Ethical Committee (IAEC) and the care of the laboratory animals was taken as per the CPCSEA regulation.

Drugs and Chemicals

Streptozotocin (STZ) commonly known as (N-Methylnitroso carboxyl)-D glucose procured from

HI-Media Mumbai. Standard drug Glidenclamide (GLB) obtained from Aventis Pharma, Ltd. Goa. Standard kits for Glucose were obtained from Erba Mannheim, Manufactured by Transasia Biomedical Ltd Baddi, India.

Preparation of drugs

Weighed quantity of Pet ether (PETP), ethyl acetate (EATP) and ethanol (EtTP) of *Terminalia Paniculata Roth* were suspended in water using 0.5% tragacanth and administered orally to experimental rats. Suspension of extract was prepared freshly. The extracts were administered at a constant volume of ~10 ml/kg for each rat.

Evaluation of anti-diabetic effect of PETP, EATP and EtTP in standardized STZ-induced diabetic rats

Induction of Diabetes mellitus

All the rats were fasted overnight before the administration of Streptozotocin. Diabetic condition was induced in male wister rats by single intravenous injection of Streptozotocin dissolved in 0.1 M Sodium citrate buffer pH 4.5 at the dose of 50 mg/kg.p.o. After injection they had free access to food and water. The development of diabetes was conformed after 48 hours of Streptozotocin injection. The rats having fasting blood glucose level 200 mg/dl were considered as diabetic and used for experimentation (10). Diabetic rats were randomized in to different groups based on their serum glucose level.

Experimental design for Single-dose one-day study

The experimental rats were divided into six groups of six rats each and treated as follows.

Group 1: Normal control (NC) received 0.5% tragacanth (10 ml/kg, p.o.)

Group 2: Diabetic control (DC) received 0.5% tragacanth (10 ml/kg, p.o.)

Group 3: DC rats treated with PETP (100 mg/kg, p.o.)

Group 4: DC rats treated with EATP (100 mg/kg, p.o.)

Group 5: DC rats treated with EtTP (100 mg/kg, p.o.)

Group 6: DC rats treated with Gilbenclamide (10 mg/kg, p.o.)

Blood samples were collected at 0, 1, 3, 5, and 7 h after extracts/GLB administration (single-dose one-day study). Serum glucose was estimated by the enzymatic glucose oxidase method. Percent reduction in Glycemia was calculated with respect to the initial (0 hr).

Experimental design for Multiple-dose seven-day study

The animals treated with respective doses of PETP, EATP, EtTP were further treated for seven consecutive days (Multiple-dose seven-day study). The following groups of animals were further treated with single daily doses for another 7 days in order to evaluate the chronic effect of extracts/GLB treatment on hyperglycemia.

Group 1: Normal control (NC) received 0.5% tragacanth (10 ml/kg, p.o./day)

Group 2: Diabetic control (DC) received 0.5% tragacanth (10 ml/kg, p.o./day)

Group 3: DC rats treated with PETP (100 mg/kg, p.o./day)

Group 4: DC rats treated with EATP (100 mg/kg, p.o./day)

Group 5: DC rats treated with EtTP (100 mg/kg, p.o./day)

Group 6: DC rats treated with Gilbenclamide (0.5 mg/kg, p.o./day)

Blood samples were collected at 0 hr, 1st, 3rd, 5th, and 7th day after extracts/GLB administration (multiple-dose seven-day study). Serum glucose was estimated by the enzymatic glucose oxidase method. Percent reduction in Glycemia was calculated with respect to the initial (0 hr).

Statistical analysis

The data were expressed as Mean \pm S.E.M for six rats in each group. Statistical comparisons were performed by one-way ANOVA followed by Dunnet's multiple comparison tests. $P < 0.05$ were considered significant (11).

RESULTS

The preliminary Phytochemical studies indicated the presence of steroids, alkaloids, flavanoids, carbohydrates, phenolics, saponins and glycosides in the PETP, EATP and EtTP extracts of bark of *Terminalia paniculata*.

Evaluation of anti-diabetic effect of PETP, EATP and EtTP in standardized Streptozotocin-induced diabetic rats

Single-dose one-day study

A single dose of PETP, EATP and EtTP (100 mg/kg) treatment exhibited reduction in SG levels at different time intervals compared to basal levels (0 hr). However, administration of GLB showed significant ($P < 0.05$; $P < 0.001$) reduction in SG levels with maximum reduction at 7 hr post GLB treatment compared to their basal levels, whereas, PETP treated animals showed dose dependent percentage reduction in SG levels compared to their basal levels (Table 1 and Figure. 1).

Multiple-dose seven-day study

Repeated administration of PETP, EATP, EtTP (100 mg/kg) for 7 days, showed significantly ($P < 0.05$; $P < 0.01$) reduced levels of SG compared to respective basal values (0 day). On 7th day, tested doses of PETP and EATP showed significantly ($P < 0.001$) greater percentage reduction in glycemia compared to GLB treated adiabatic control (Table 2 and Figure 2). The change in

body weight of rats was determined after 3rd, 5th and 7th day post induction (Table 3).

DISCUSSION

In the present study, the hypoglycemic activity of Petroleum ether (PETP), Ethyl acetate (EATP) and ethanol (EtTP) extracts of stem bark of *Terminalia paniculata* Roth was evaluated in Streptozotocin induced diabetic rats. The present data suggested that PETP and EATP significantly reduced hyperglycemia which is comparable to that of standard drug Gilbenclamide used in treatment of type II diabetes mellitus in both single dose one day and multiple dose seven day diabetic studies. The standard drug Gilbenclamide stimulates insulin secretion from beta cells of islets of langerhans. The efficacy of the PETP was found to be better than EATP. This could be mediated by improving the glycemic control mechanism and increasing insulin secretion from remnant beta cells in diabetic rats. The phytochemical examination of petroleum ether (PETP) and Ethyl acetate (EATP) extract of stem bark of *Terminalia paniculata* revealed the presence of alkaloids, carbohydrate, flavanoids, tannins and phenolics. There have been reports about flavanoids, phenolics and steroidal glycosides acting as bioactive antidiabetic principles (12, 13). Then the observed antidiabetic activity of title plant may be attributed to the presence of these bioactive principles and their synergistic properties. Therefore we conclude that the petroleum ether (PETP) and Ethyl acetate (EATP) of *Terminalia paniculata* has endowed with antidiabetic in standardized Streptozotocin induced diabetic rats, justifying its use in traditional system of medicine. These extracts also showed improvement in the parameter like body weight.

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necessary laboratory and other requisite facilities to carry out this work.

ETHICAL CLEARANCE

The research work was approved by Institutional Animal Ethical Committee (NCP/IAEC/CLEAR/25/02/2009-10, dated 09/03/2010).

Table 1: Effect of bark extracts of Terminalia paniculata (PETP, EATP and EtTP) on Serum glucose levels in Streptozotocin induced male diabetic rats (Single-dose one-day study)

Group	Serum glucose (mg/dl)/ Time						
	0hrs	1hrs	3 rd hrs	5 th hrs	7 th hrs	On 7 th Day	Change in body weight (g)
Normal	96.17±5.46	101.17±4.22	97.83±5.15	94.33±4.06	92.67±2.86	94.50±3.47	36.17±2.01
Diabetic Control	343.83±5.64	348.50±10.85	359.50±13.79	356.33±16.76	371.00±15.36	399.17±12.5	-17.83±0.79
Glibenciamide	333.33±6.04	319.33±6.19*	304.83±8.60*	264.83±5.62**	226.83±8.61**	102.17±4.85**	8.67±0.88
PETP	337.33±7.94	317.50±19.9	299.83±28.28*	274.83±19.11**	238.33±12.79**	126.33±8.35**	4.17±0.48
EATP	335.83±7.56	333.17±27.59	312.67±23.34	287.50±23.91*	254.17±17.62**	147.50±14.13**	7.33±0.76
EtTP	341.67±7.75	348.17±19.62	300.17±18.15	280.83±20.50*	265.33±14.51**	166.50±11.58**	5.83±1.51

Values are mean ± S.E.; n=6 in each group.

* P<0.05 and ** P<0.01 when compared to Diabetic control.

Table 2: Effect of bark extracts of Terminalia paniculata (PETP, EATP and EtTP) on Serum glucose levels in Streptozotocin induced male diabetic rats (Multiple-dose seven-day study)

Group	Serum glucose level (mg/dl) /Day				
	0hrs	1 st day	3 rd day	5 th day	7 th day
Normal	96.17 ± 5.46	92.67 ± 2.86	89.17 ± 2.96	89.17 ± 2.96	94.50 ± 3.47
Diabetic Control	343.83 ± 5.64	371.00 ± 15.36	375.50 ± 16.10	379.50 ± 19.59	399.17 ± 12.5
Gilbenclamide	333.33 ± 6.04	226.83 ± 8.61**	199.00 ± 4.49**	158.67 ± 14.34**	102.17 ± 4.85**
PETP	337.33 ± 7.94	238.33 ± 12.79**	217.83 ± 7.48**	170.33 ± 3.47**	126.33 ± 8.35**
EATP	335.83 ± 7.56	254.17 ± 17.62**	233.50 ± 11.00**	198.17 ± 7.54**	147.50 ± 14.13**
EtTP	341.67 ± 7.75	265.33 ± 14.51**	254.50 ± 11.74**	220.00 ± 2.42**	166.50 ± 11.58**

Values are mean ± S.E.; n=6 in each group* P<0.05 and ** P<0.01 when compared to Diabetic control.

Table 3: Effects of bark extracts of Terminalia paniculata (PETP, EATP and EtTP) on body weight in Streptozotocin induced Diabetic rats

Group	Change in Bodyweight (g.) post induction days		
	3 rd day	5 th day	7 th day
Normal	9.83±1.64	15.00±3.04	36.50±4.70
Diabetic Control	7.17±1.14	-13.50±1.52	24.00±5.19
Gilbenclamide	5.33±1.45	10.00±2.85	18.00±5.43
PETP	4.17±1.60	6.50±1.54	12.67±2.70
EATP	2.17±0.65	3.50±0.76	7.50±1.23
EtTP	2.17±1.17	3.83±1.38	5.83±1.51

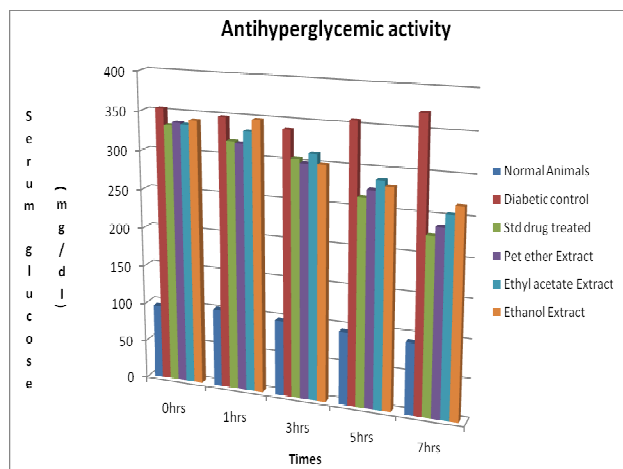


Fig. 1 Effect of extracts in one day study

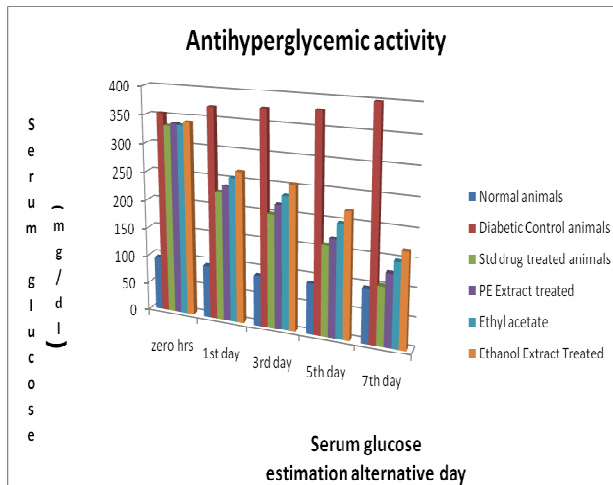


Fig. 2 Effect of extracts in multiple day study

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