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Antidiabetic activity of *Terminalia catappa Linn* fruits

A.N. Nagappa [a,*], P.A. Thakurdesai [a], N. Venkat Rao [b], Jiwan Singh [b]

[a] *Pharmacy Group, Birla Institute of Technology and Sciences, Pilani-333031, Rajasthan, India*
[b] *Pharmacology Department, S.C.S. College of pharmacy, Harapanahalli-583131, Davanagere (Dist), Karnataka, India*

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Abstract

In view of alleged antidiabetic potential, effect of the petroleum ether, methanol, and aqueous extracts of *Terminalia catappa Linn* (combretaceae) fruit, on fasting blood sugar levels and serum biochemical analysis in alloxan-induced diabetic rats were investigated. All the three extracts of *Terminalia catappa* produced a significant antidiabetic activity at dose levels 1/5 of their lethal doses. Concurrent histological studies of the pancreas of these animals showed comparable regeneration by methanolic and aqueous extracts which were earlier, necrosed by alloxan.

Keywords: Antidiabetic activity; *Terminalia catappa Linn* fruit; Alloxan-induced diabetes

1. Introduction

Diabetes mellitus (DM) is a chronic disease caused by inherited and/or acquired deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced. Such a deficiency results in increased concentrations of glucose in the blood, which in turn damage many of the body's systems, in particular the blood vessels and nerves.

As the number of people with diabetes multiply worldwide, the disease takes an ever-increasing proportion of national and international health care budgets. It is projected to become one of the world's main disablers and killers within the next 25 years. Regions with greatest potential are Asia and Africa, where DM rates could rise to two- to three-folds than the present rates. Apart from currently available therapeutic options, many herbal medicines have been recommended for the treatment of diabetes. Traditional plant medicines are used throughout the world for a range of diabetic presentations.

Terminalia catappa Linn (combretaceae) is found throughout the warmer parts of India and called as Indian Almond, Malabar Almond, Tropical Almond. It is a medium sized tree with leaves clustered towards the ends of the branches. The various extracts of leaves and bark of the plant have been reported to be anticancer antioxidant (Masuda et al., 1999), anti-HIV reverse transcriptase (Tan et al., 1991) and hepatoprotective (Lin et al., 1997), antiinflammatory (Lin et al., 1999), hepatitis (Chen et al., 2000), and aphrodisiac (Ratnasooriya and Dharmasiri, 2000). Fruit of *Terminalia catappa* contains cyanidin-3-glucoside, corilagin (Topoisomerase I and II inhibitor (Hecht et al., 1992; Kashiwada et al., 1993), and Xanthin oxidase inhibitor (Hatano et al., 1990), ellagic-acid (anti-HIV (Tan et al., 1991), Xanthin oxidase inhibitor (Hatano et al., 1990)), anti asthmatic compound, gallic-acid (Dorsch and Wagner, 1991), and pentosans. They are also reported to contains phytochemicals which are indicative of it's potential in treatment of DB e.g. brevifolin-carboxylic-acid (Shimizu et al., 1989) and ellagic-acid (Basnet et al., 1993), which are an aldose reductase inhibitors. Euginic acid have also showed anticataract (Shimizu et al., 1989) activity. *Terminalia catappa* is rich in tannins that are reported to be antidiabetic (Teotia and Singh, 1997).

In view of alleged antidiabetic potential of *Terminalia catappa*, we have investigated effect of extracts of its fruit on fasting blood sugar levels and serum biochemical analysis in alloxan-induced diabetic rats.

2. Materials and methods

2.1. Plant material

Fresh, unriped, green fruits of *Terminalia catappa* were collected in the month of October from the herbal garden of

* Corresponding author. Tel.: +91-159-7645074x413.
E-mail address: anantha@bits-pilani.ac.in (A.N. Nagappa).

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S.C.S. college of pharmacy, Harapanahalli and were authenticated by botanist Prof. K. Prabhu, Department of pharmacognosy, S.C.S. college of pharmacy. A voucher specimen of the herbarium has been deposited at the same department, S.C.S. college of pharmacy, Harapanahalli, India.

2.2. Preparation of extracts

The fruits were cut into pieces and shade dried at room temperature. The dried fruits were subjected to size reduction to a coarse powder by using dry grinder and passed through sieve. This powder was packed into soxhlet apparatus and extracted successively with petroleum ether (60–80°), methanol, and distilled water (yield 2, 8, and 8%, respectively). All the extracts were dried at 45 °C in hot air oven till solid to semisolid mass was obtained and were stored in airtight containers in refrigerator below 10 °C. The suspensions of methanol and petroleum ether extracts were prepared by using 0.5% Tween-80 (SD fine chemicals, Mumbai, India) in normal saline and solution of aqueous extract was prepared by using normal saline as solvent for the experiment.

2.3. Animals

Wistar albino rats (150–200 g) or Wistar albino mice (20–25 g) of both sexes were obtained from the experimental animal facility of S.C.S. College of pharmacy, Harapanahalli. Before and during the experiment, rats were fed with standard diet (Gold Moher, Lipton India Ltd). After randomization into various groups and before initiation of experiment, the rats were acclimatized for a period of 7 days under standard environmental conditions of temperature, relative humidity, and dark/light cycle. Animals described as fasting were deprived of food and water for 16 h ad libitum.

2.4. Sample collection

Blood samples were collected by retro-orbital plexus puncture method and blood glucose levels were estimated using an electronic glucometer (Miles Inc, USA) and glucostix (Bayer diagnostic India Ltd., Baroda).

2.5. Experimental design

All the animals were randomly divided into the six groups with six animals in each group. Group A, B, and C were served as saline, diabetic, and standard drug (glibenclamide, 10 mg/kg per day p.o) control, respectively. Preliminary oral LD_{50} doses of petroleum ether, methanol, and aqueous extract of *Terminalia catappa* in mice were found to be 343, 195, and 210 mg/kg, respectively. Groups D, E, and F were treated fruit extracts in one-fifth of LD_{50} doses of the petroleum ether extract (68 mg/kg per day p.o.), methanol extract (40 mg/kg per day p.o), and aqueous extract (42 mg/kg per day p.o), respectively.

2.6. Assessment of extracts on alloxan-induced diabetic animals

Rats were made diabetic by a single intraperitoneal injection of alloxan monohydrate (Loba Chemie, Bombay; 150 mg/kg; Aruna et al., 1999). Alloxan was first weighed individually for each animal according to the weight and then solubilized with 0.2 ml saline (154 mM NaCl) just prior to injection. Two days after alloxan injection, rats with plasma glucose levels of >140 mg/dl were included in the study. Treatment with plant extracts was started 48 h after alloxan injection. Blood samples were drawn at weekly intervals till the end of study (i.e. 3 weeks). Fasting blood glucose estimation and body weight measurement ware done on day 1, 7, and 21 of the study.

On day 21, blood was collected by cardiac puncture under mild ether anesthesia from overnight fasted rats and fasting blood sugar (Giordano et al., 1989) was estimated. Serum was separated and analyzed for serum cholesterol (Roeschlau et al., 1974), serum triglycerides by enzymatic DHBS colorimetric method (Muller et al., 1977), serum HDL (Allain et al., 1974), serum LDL (Friedewald et al., 1972), serum creatinine (Bowers, 1980), serum urea (Wilson, 1966), serum alkaline phosphatase hydrolyzed phenol amino antipyrine method (Sasaki, 1966) were estimated.

The whole pancreas from each animal was removed after sacrificing the animal and was collected in 10% formaline solution, and immediately processed by the paraffin technique. Sections of 5 μ thickness were cut and stained by haematoxylin and eosin (H & E) for histological examination. The photomicrographs of histological studies are presented in Fig. 2(A–F).

2.7. Statistical analysis

All the values of body weight, fasting blood sugar, and biochemical estimations were expressed as mean ± standard error of mean (S.E.M.) and analyzed for ANOVA and post hoc Dunnet's *t*-test. Differences between groups were considered significant at $P < 0.01$ levels.

3. Results

The anti-hyperglycemic effect of the extracts on the fasting blood sugar levels of diabetic rats is shown in Figs. 1 and 2. Administration of alloxan (150 mg/kg, i.p.) led to 1.5-fold elevation of fasting blood glucose levels, which was maintained over a period of 3 weeks. Three weeks of daily treatment of various extract of *Terminalia catappa* led to a dose-dependent fall in blood sugar levels by 25–62%. Effect seems to reach maximum after 15 days of treatment and remains constant in third week.

Vehicle control animals were found to be stable in their body weight but diabetic rats showed significant reduction in body weight during 21 days (Table 1). Alloxan caused

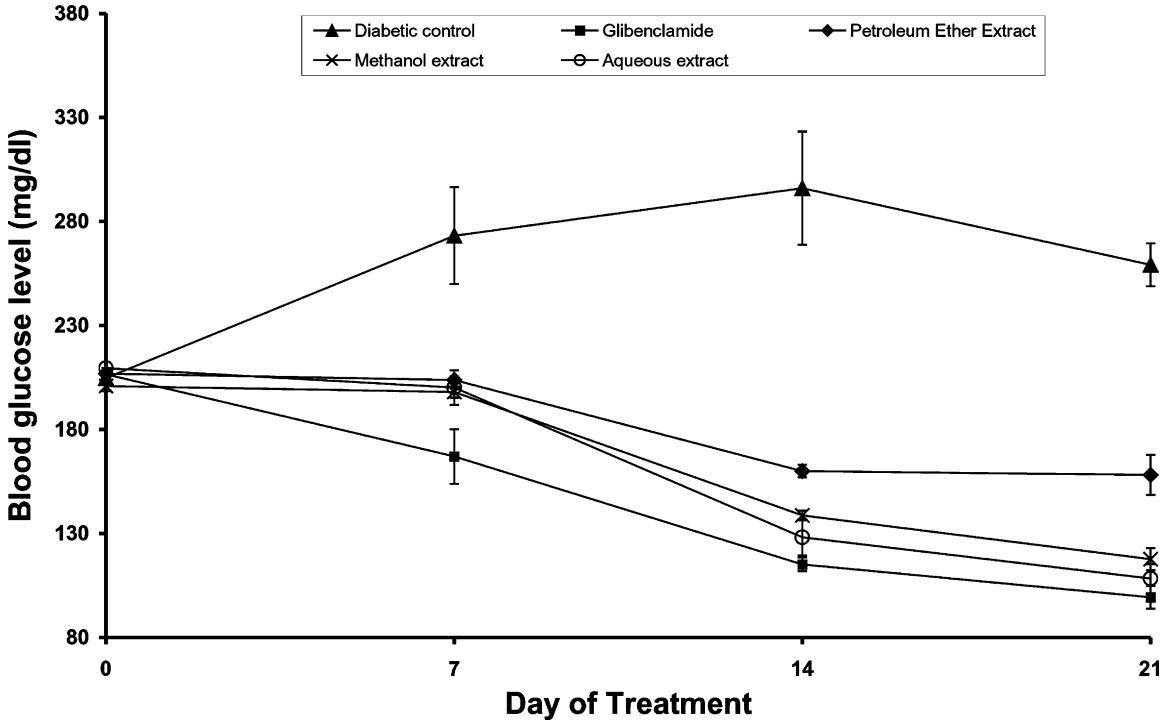

Fig. 1. Comparative effect of different extract of fruits of *Terminalia catappa* on blood glucose level in alloxan (150 mg/kg)-induced diabetis in rats.

body weight reduction, which is reversed by aqueous and methanol extracts of *Terminalia catappa* after 7 days of treatment but petroleum ether extract failed to cause such reversal (Table 1).

Serum cholesterol, serum tryglycerides, serum LDL, serum creatinine, serum urea, and serum alkaline phosphatase levels were decreased significantly by glibenclamide and all the extracts of *Terminalia catappa* due to 21 days of treatment except petroleum ether extract (which had no effect on chlesterol, triglycerides, and LDL levels). HDL levels were increased by glibenclamide, methanol, and aqueous extracts but not by petroleum ether extracts (Table 2).

Photomicrographs (Fig. 2) showed normal acini, and normal cellular population in the islets of langerhans in pancreas of vehicle-treated rats (A). Extensive damage to the islets of langerhans and reduced dimensions of islets (B), restoration of normal cellular population size of islets with hyperplasia by Glibenclamide (C) was also shown. The partial restoration of normal cellular population and enlarged size of β-cells with hyperplasia was shown by methanol and aqueous extract but not by petroleum ether extract (Fig. 2D–F).

4. Discussion

In light of the results, our study indicates that *Terminalia catappa* fruit extracts have good antidiabetic activity. Methanol and aqueous extracts of *Terminalia catappa* exhibited significant anti-hyperglycemic activities in alloxan-induced hyperglycemic rats without significant change in

Table 1
The effect of 3-week treatment with various extracts of *Terminalia catappa* on body weight (g) after alloxan (150 mg/kg i.p.) induced diabetes in rats

Gr. No.	Treatment	Dose (mg/kg p.o)	Average body weight (g) ±SEM			
			Day 1	Day 7	Day 14	Day 21
A	Vehicle control	0.2 ml[a]	200.50 ± 2.84	201.83 ± 1.04	203.00 ± 1.06	205.83 ± 1.49
B	Diabetic control	0.2 ml[b]	205.50 ± 4.88	175.00 ± 8.16*	160.33 ± 2.51*	148.83 ± 1.72*
C	Glibenclamide control	10	206.66 ± 2.23	198.00 ± 1.50	195.16 ± 2.48	192.00 ± 3.96
D	Petroleum ether extract	68	209.66 ± 2.37	177.50 ± 1.89*	163.50 ± 3.35*	160.66 ± 2.04*
E	Methanol extract	40	207.33 ± 2.33	185.50 ± 1.89	180.33 ± 2.41	165.50 ± 1.89*
F	Aqueous extract	42	206.83 ± 2.07	195.16 ± 1.70	190.16 ± 3.62	180.16 ± 2.78*

Values are given in average body weight (g) ±SEM for groups of six animals each.
[a] Vehicle (0.5% Tween 80 solution in normal saline).
[b] Alloxan single dose of 150 mg/kg i.p in normal saline on day 0.
* $P < 0.05$ as compared to vehicle control on corresponding day.

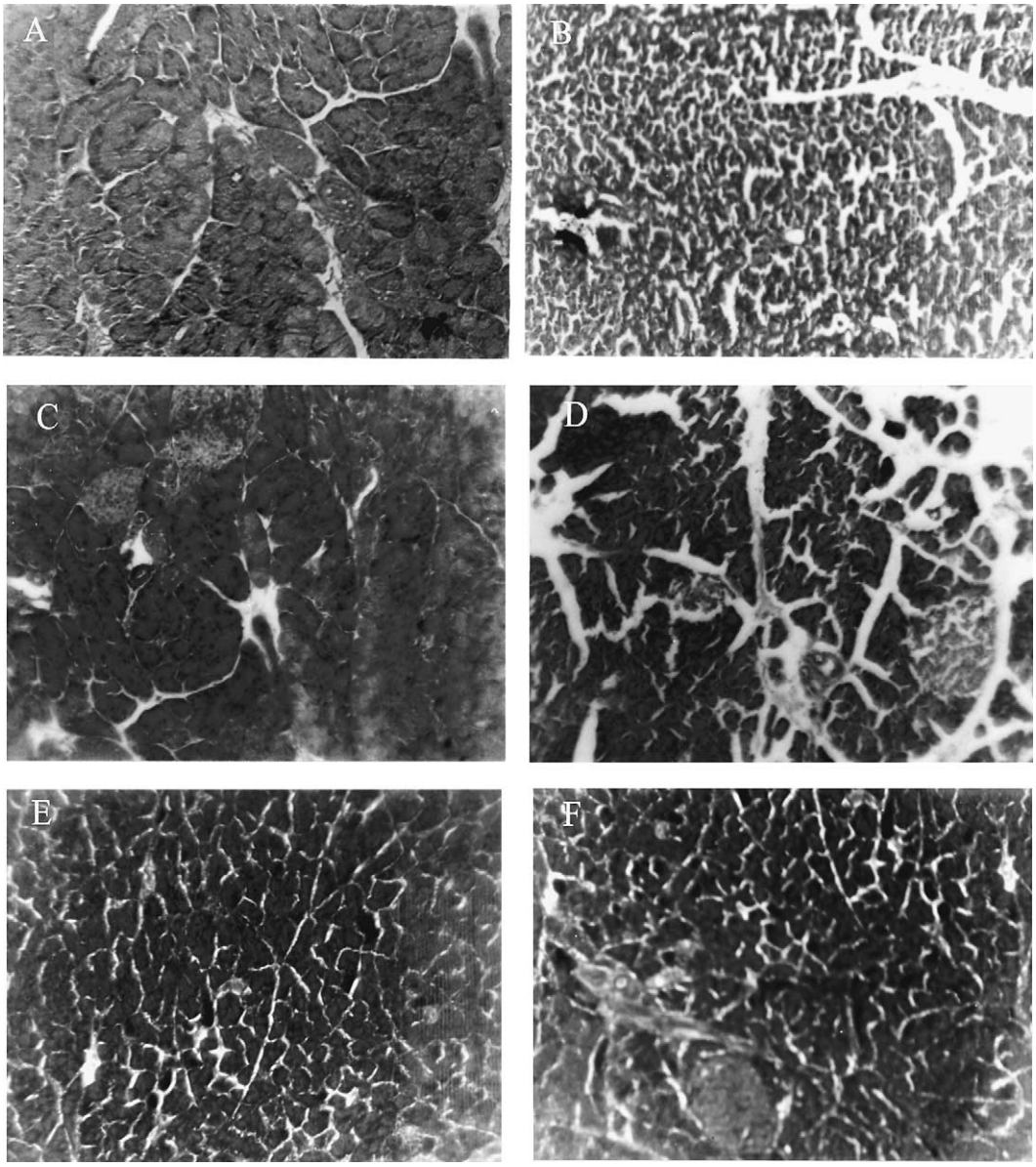

Fig. 2. Photomicrographs rat pancreas stained by haematoxylin and eosin of untreated (A) and alloxan-induced diabetic (B) rats and effects of (C) glibenclamide, (D) petroleum ether extract, (E) methanol extract, and (F) aqueous extract of *Terminalia catappa*. Microscope magnification (400×).

Table 2
Effect of various extracts of *Terminalia catappa* on serum profile in alloxan (150 mg/kg, i.p.)-induced diabetic albino rats after 21 days of treatment

Gr. No.	Treatment	Dose (mg/kg p.o)	Serum cholesterol	Serum triglycerides	Serum H.D.L. cholesterol	Serum L.D.L cholesterol	Serum creatinine	Serum urea	Serum alkaline phosphatase
A	Vehicle control	0.2 ml[a]	151.00 ± 6.2	86.83 ± 5.5	37.00 ± 1.5	93.23 ± 5.1	0.52 ± 0.1	23.66 ± 1.4	116.16 ± 2.6
B	Diabetic control	0.2 ml[b]	269.33 ± 15.5	200.83 ± 11.1	30.00 ± 1.4	199.16 ± 14.2	1.35 ± 0.1	61.00 ± 1.9	314.50 ± 5.9
C	Glibenclamide control	10	146.83 ± 6.1*	108.00 ± 6.1*	51.50 ± 1.9*	73.73 ± 6.7*	0.58 ± 0.0*	30.00 ± 2.2*	130.16 ± 4.7*
D	Petroleum ether extract	68	289.16 ± 15.6	193.33 ± 6.0	35.23 ± 2.1	215.16 ± 14.0	0.70 ± 0.1*	36.83 ± 2.2*	254.50 ± 9.7*
E	Methanol extract	40	174.83 ± 4.9*	131.66 ± 7.9*	45.66 ± 1.5*	102.83 ± 5.9*	0.52 ± 0.1*	33.00 ± 1.7*	163.83 ± 18.2*
F	Aqueous extract	42	158.50 ± 6.5*	115.50 ± 6.1*	40.83 ± 1.9*	94.56 ± 4.8*	0.63 ± 0.1*	31.83 ± 1.0*	133.66 ± 5.9*

Values are given as mean ± SEM for groups of six animals each.

[a] Vehicle (0.5% Tween 80 solution in normal saline).

[b] Alloxan single dose of 150 mg/kg i.p in normal saline on day 0.

* $P < 0.01$ (Dunnet *t*-test), diabetic control was compared with the vehicle control and extract treated groups were compared with the diabetic control.

body weight They can also improve the condition of DB as indicated by parameters like body weight and lipid profile along with serum creatinine, serum urea, and serum alkaline phosphatase.

The number of functionally intact β-cells in the islet organ is of decisive importance for the development course and outcome of DB. The renewal of β-cells in diabetes has been studied in several animal models. The total β-cell mass reflects the balance between the renewal and loss of these cells. It was also suggested that regeneration of islet β-cells following destruction by alloxan may be the primary cause of the recovery of alloxan-injected guinea pigs from the effects of the drug (Gorray et al., 1986). In alloxan-induced diabetes, (−)-Epicatechin (Chakravarthy et al., 1982) and *Vinca rosea* extracts (Ghosh and Suryawanshi, 2001) has also been shown to act by β-cell regeneration. Similar effects in streptozotocin-treated diabetic animals were reported by pancreas tonic (Rao et al., 1998), ephedrine (Xiu et al., 2001), and *Gymnema sylvestre* leaf extracts (Shanmugasundaram et al., 1990).

In our studies, the damage of pancreas in alloxan-treated diabetic control rats (Fig. 2B) and regeneration of β-cells by glibenclamide (Fig. 2C) was observed. The comparable regeneration was also shown by methanolic and aqueous extracts of *Terminalia catappa* fruit (Fig. 2D–F). This effect may be due to the β-carotine, which was reported to be constituent of *Terminalia catappa* fruit (Duke, 1992). Beneficial role of β-carotene in reducing diabetic complications like glycosylation in alloxan-induced diabetic rats (Aruna et al., 1999) had been reported previously. Photomicrographical data in our studies reinforce healing of pancreas, by *Terminalia catappa* fruit extracts, as a plausible mechanism of their antidiabetic activity.

5. Conclusions

Methanolic and aqueous extracts of *Terminalia catappa* fruit exhibited significant antihyperglycemic activities in alloxan-induced diabetic rats. These extracts showed improvement in parameters like body weight and lipid profile as well as regeneration of β-cells of pancreas and so might be of value in diabetes treatment.

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