

# Anti-Diabetic Activity of *Terminalia catappa* Linn. Leaf Extracts in Alloxan-Induced Diabetic Rats

SYED MANSOOR AHMED, VRUSHABENDRA SWAMY BM, P GOPKUMAR R DHANAPAL and VM CHANDRASHEKARA

Department of Pharmacology, M.M.U College of Pharmacy (S.M.A.); Department of Pharmacology (V.S.B.M.), Department of Pharmaceutics (R.D.), Rural college of Pharmacy; Department of Pharmaceutical Chemistry, S.A.C College of Pharmacy (P.G.); Department of Pharmacology, H.S.K College of Pharmacy, Bagalkot (V.M.C.), Karnataka, India.

Received April 4, 2005; Revised May 6, 2005; Accepted June 6, 2005

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

## ABSTRACT

In view of suggested anti diabetic potential, effect of aqueous and cold extracts of *Terminalia catappa* Linn (Combretaceae) leaves, on fasting blood sugar levels and serum biochemical analysis in alloxan-induced diabetic rats was investigated. All the extracts of *Terminalia catappa* produced a significant anti diabetic activity at dose levels of 1/5<sup>th</sup> of their lethal doses. Concurrent histological studies of the pancreas of these animals showed regeneration by aqueous and cold extracts which were earlier necrosed by alloxan.

**Keywords:** Anti-diabetic activity, *Terminalia catappa*, Alloxan, Aqueous extract, Cold extract

Diabetes mellitus (DM) is a chronic disease caused by inherited and/or acquired deficiency in production of insulin by the pancreas, or by ineffectiveness of insulin produced, such a deficiency results in increased concentration of glucose in the blood, which in turn damages many of the body's systems in particular the blood vessels and nerves. As the number of the people with diabetes multiplies world wide, the disease has taken an ever-increasing share of national and international health care budgets. It is projected to become of the world's main disablers and killers with in the next 25 years. Regions with greatest potential are Asia and Africa, where DM rates could rise to two-to-three- folds campened with 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 presentation.

*Terminalia catappa* Linn (Combretaceae) is found in the warmer parts of India. It is also known as Indian Almond, Malabar Almond, and Tropical Almond [1]. The various extracts of leaves and bark of the plant have been reported to be anticancer, antioxidant [2], anti-HIV reverse transcriptase [3], hepatoprotective [4], anti-inflammatory [5], anti-hepatitis [6], and aphrodisiac [7]. The phytochemicals of this plant include tannins (punicalagin, punicalin, terflavins A and B, tergalagin, tercatatin, chebulagic acid, geranin, granatin B, corilagin) [8], flavanoids (isovitexin, vitexin, isoorientin, rutin)

[9] and triterpinoids (ursolic acid, 2 $\alpha$ , 3 $\beta$ , 23-trihydroxyurs-12-en-28 oic acid) [10].

Tannins have been reported to posses anti-diabetic activity [11].

In the light of the above information the present investigation was undertaken to evaluate the anti-diabetic potential of *Terminalia catappa* Linn. leaves extracts on fasting blood sugar and serum biochemical analysis.

## MATERIAL AND METHODS

### Plant Material

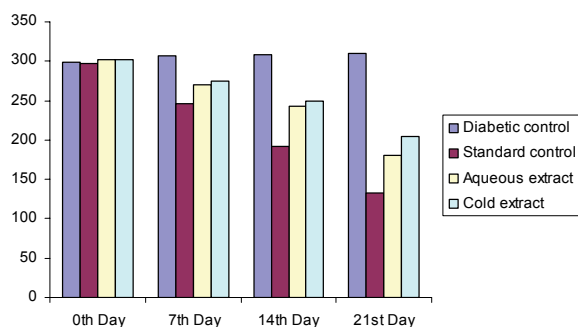
Fresh tender leaves of *Terminalia catappa* were collected from Mysore botanical garden and were authenticated by botanist of M.M.U college of Pharmacy, Ramanagar. A voucher specimen (RTCL) is kept in our laboratory for future reference.

### Preparation of Extracts

The leaves were shade dried at room temperature. The dried leaves were subjected to size reduction to coarse powder by using dry grinder and passed through sieve no 40.

**Aqueous extraction.** The powder of *Terminalia catappa* leaves were packed in a Soxhlet apparatus and extracted with distilled water for 18 hours. The obtained extract (7.5%) was dried at 45°C in hot air oven till solid/semisolid mass was obtained.

**Cold extraction.** Approximately about 100 g of the powdered leaves is taken in a 2000 ml conical flask with



**Fig 1.** Comparative effect of aqueous and cold extract of leaves of *Terminalia catappa* on blood glucose level in alloxan (150 mg/kg) induced diabetes in rats.

500 ml of distilled water and 10 ml of chloroform added as a preservative (maceration process). It was extracted up to one week with daily 2 hours stirring with a mechanical stirrer. After 7 days the extract was filtered through muslin cloth and marc was discarded and its filtrate (8.2%) dried in a hot air oven at 45°C till solid/semisolid mass, was produced.

Both the extracts were stored in air tight container in refrigerator below 10°C. The suspensions of aqueous and cold extracts were prepared by using normal saline as solvent for administration to experimental animals.

#### Animals Used

Wistar albino rats (150-200 g) and Wistar albino mice (20-25 g) of both sexes were procured from Indian Institute of Sciences, Bangalore, India. Before and during the experiment rats were fed with standard diet (Gold Mohr, Lipton India Ltd). After randomization to 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 hours *ad libitum*.

#### Sample Collection

Blood samples were collected by the 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).

#### Experimental Design

All the animals were randomly divided in the five groups with six in each group. Group I, II and III were administered saline, diabetic, and standard drug (glibenclamide, 10 mg/kg per day p.o) control, respectively. Preliminary oral LD<sub>50</sub> doses of aqueous and cold extract of *Terminalia catappa* in mice were found to be 215, 230 mg/kg respectively. Group IV and V were treated with leaves extracts in one-fifth of LD<sub>50</sub> doses of aqueous extract (43g/kg per day p.o) and cold extract (46 mg/kg per day p.o), respectively.

#### 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) [12]. Alloxan was first weighed individually for each animals according to the weight and solubilized with 0.2ml 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 hours after alloxan injection. Blood sample were drawn at weekly intervals till end of study (i.e. 3 weeks). Fasting blood glucose estimation and body weight measurement were done on day of 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 was estimated [13]. Serum was separated and analyzed for serum cholesterol [14], serum triglycerides by enzymatic DHBS colorimetric method [15], serum HDL [16], serum LDL [17], serum creatinine [18], serum urea [19] and serum alkaline phosphatase by hydrolyzed phenol amino antipyrine method [20].

The whole pancreas from each animal was removed after killing the animals, was placed in 10% formaline solution, and immediately processed by the paraffin technique. Sections of 5µm 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-E).

#### Statistical Analysis

All the values of body weight, fasting blood sugar, and biochemical estimations were expressed as mean ± standard error of mean (SEM) and analyzed using Student 't' test.

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

Group No	Treatment	Dose (mg/kg P.O)	Average body weight (g) ± SEM			
			Day 1	Day 7	Day 14	Day 21
I	Vehicle control	0.2 ml <sup>a</sup>	200.1 ± 1.9	201.83 ± 1.02	203.00 ± 1.05	205.83 ± 1.52
II	Diabetic control	0.2 ml <sup>b</sup>	206.2 ± 2.1	176.00 ± 5.2	162.33 ± 2.51	148.83 ± 1.62
III	Glibenclamide	10	206.8 ± 2.2	198.00 ± 1.31**	195.21 ± 2.33***	192.00 ± 3.96***
IV	Aqueous extract	43	207.3 ± 2.07	196.16 ± 1.70*	190.21 ± 1.72**	181.22 ± 4.3**
V	Cold extract	46	206.9 ± 1.83	197.02 ± 2.2*	189.72 ± 3.1**	180.47 ± 5.2**

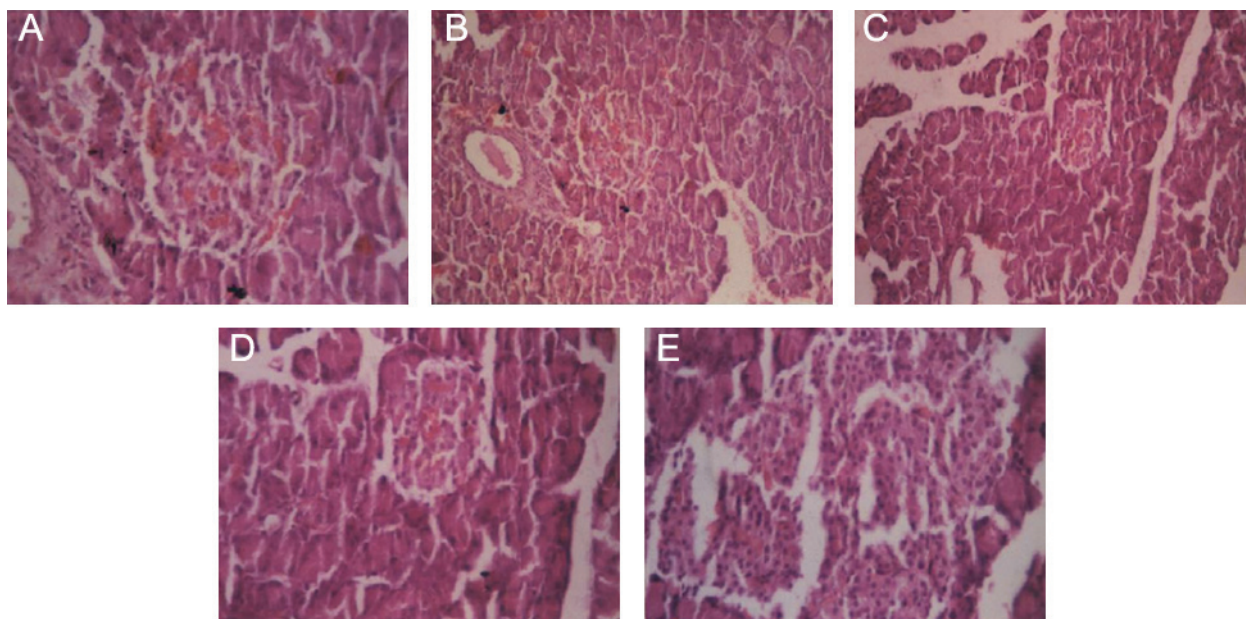
Values are given in average body weight (g) ±SEM for groups of six animals each.

<sup>a</sup> Normal saline.

<sup>b</sup> Normal saline + alloxan.

Significance vs. control group.

\*  $p < 0.05$ . \*\*  $p < 0.01$ . \*\*\*  $p < 0.001$ .



**Fig 2.** Photomicrographs of rat pancreas stained by haematoxylin and eosin of (A) untreated and (B) alloxan induced diabetic rats and effects of (C) glibenclamide, (D) aqueous extract, (E) cold extract of *Terminalia catappa*. Microscope magnification: 400 $\times$ .

## RESULTS

The anti diabetic effects of the extracts on the fasting blood sugar levels of diabetic one shown in Fig 1. Administration of alloxan (150 mg/kg, i.p) led to 1.5-fold elevation of fasting blood glucose levels, which was maintained for period of 3 weeks. Three weeks of daily treatment of extracts led to a dose-dependent fall in blood sugar levels by 25-62%. Effect seems to reach maximum after 15 days of treatment and remained constant in third week.

Vehicle control animals were found to be stable in their body weight while diabetic rats showed significant reduction in body weight during 21 days (Table 1). Alloxan caused weight reduction, which was reversed by aqueous and cold extracts of *Terminalia catappa* after 7 days of treatment.

Serum cholesterol, serum triglycerides, serum LDL, serum creatinine, serum urea, and serum alkaline phosphatase levels were decreased significantly by glibenclamide ( $p < 0.001$ ), aqueous extract ( $p < 0.001$ ) and cold extract ( $p < 0.01$ ) of *Terminalia catappa*, after 21 days of treatment compared with diabetic control. HDL levels were increased by glibenclamide ( $p < 0.001$ ),

aqueous extract ( $p < 0.001$ ) and cold extract ( $p < 0.01$ ) compared with diabetic control (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  $\beta$ -cells with hyperplasia was shown by aqueous and cold extracts (Fig 2D-E).

## DISCUSSION AND CONCLUSION

In the light of the results, our study indicates that *Terminalia catappa* leaves extracts have anti-diabetic activity. Aqueous and cold extracts of *Terminalia catappa* exhibited significant anti- hyperglycemic activities in alloxan-induced hyperglycemic rats without significant change in body weight. They also improved conditions of DM as indicated by parameters like bodyweight, and lipid profiles along with serum

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

Group No.	Treatment	Dose (mg/kg P.O)	Serum cholesterol	Serum triglycerides	Serum HDL cholesterol	Serum LDL cholesterol	Serum creatinine	Serum urea	Serum alkaline phosphatase
I	Vehicle control	0.2 ml <sup>a</sup>	151.00 $\pm$ 6.2	86.83 $\pm$ 5.5	37.00 $\pm$ 1.5	93.23 $\pm$ 5.5	0.52 $\pm$ 0.1	23.66 $\pm$ 1.4	116.16 $\pm$ 2.6
II	Diabetic control	0.2 ml <sup>b</sup>	269.33 $\pm$ 15.5	200.83 $\pm$ 11.1	30.00 $\pm$ 1.4	199.16 $\pm$ 14.2	1.35 $\pm$ 0.1	61.00 $\pm$ 1.9	314.50 $\pm$ 5.9
III	Glibenclamide	10	146.83 $\pm$ 6.1***	108.00 $\pm$ 6.1***	51.50 $\pm$ 1.9***	73.73 $\pm$ 6.7***	0.58 $\pm$ 0.1***	30.00 $\pm$ 2.2***	130.16 $\pm$ 4.7***
IV	Aqueous extract	43	154.83 $\pm$ 3.8***	115 $\pm$ 6.1***	40.83 $\pm$ 1.2***	94.56 $\pm$ 2.7**	0.63 $\pm$ 0.1***	31.83 $\pm$ 1.0***	133.66 $\pm$ 5.9***
V	Cold extract	46	162.8 $\pm$ 4.1**	122 $\pm$ 6.1**	44.22 $\pm$ 1.6**	101.02 $\pm$ 3.5**	0.72 $\pm$ 0.1**	35.22 $\pm$ 1.7**	140.22 $\pm$ 4.8**

<sup>a</sup> Normal saline

<sup>b</sup> Normal saline + alloxan.

Significance vs. control group.

\* $p < 0.05$ . \*\* $p < 0.01$ . \*\*\* $p < 0.001$ .



creatinine, serum urea, and serum alkaline phosphatase. The number of functionally intact  $\beta$ -cells in the islet organ is of decisive importance the development course and outcome of DM. The renewal of  $\beta$ -cells in diabetes has been studied in several animal models. The total  $\beta$ -cell mass reflects the balance between the renewal and loss of these cells. It was also suggested that regeneration of islet  $\beta$ -cells following destruction by alloxan may be the primary cause of the recovery of alloxan – injected guinea pigs from the effects of the drug [21]. In alloxan-induced diabetes, (-)-epicatechin [22] and *Vinca rosea* extract [23] have also shown to act by  $\beta$ -cell regeneration. Similar effect in streptozotocin treated diabetic animals were reported by pancreas tonic [24], ephedrine [25], and *Gymnema Sylvestre* leaf extracts [26].

In our studies, damage to pancreas in alloxan-treated diabetic control (Fig 2B), and regeneration of  $\beta$ -cells by glibenclamide (Fig 2C) was observed. A comparable regeneration was also shown by aqueous and cold extracts of *Terminalia catappa* (Fig 2D and Fig 2E). This effect may be due to  $\beta$ -carotene, which was reported to be constituents of *Terminalia catappa* [27]. The beneficial role of  $\beta$ -carotene in reducing diabetic complications like glycosylation in alloxan-induced diabetic rats [12] had been reported previously. Photomicrographical data in our studies confirm healing of pancreas by *Terminalia catappa* leaves extracts, as a plausible mechanism of their anti diabetic activity.

Aqueous and cold extract of *Terminalia catappa* leaves exhibited significant anti hyperglycemic activities in alloxan-induced diabetic rats. These extracts showed improvement in parameters like body weight and lipid profile as well as regeneration of  $\beta$ -cells of pancreas and so might be of value in diabetes treatment.

## REFERENCES

- Nadkarni KM. *Indian Materia Medica*. 4<sup>th</sup> Edition, 1976. Vol 1:1205 Popular Prakashn Bombay.
- Masuda T, Yonemori Y, Oyama Y, Takeda T, Tanaka T. Evaluation of the antioxidant activity of environmental plants: activity of the leaf extracts from seashore plants. *J Agric Food Chem* 1999;**47**:1749-54.
- Tan GT, Pezzulo JM, Kinghom AD, Hughes SH. Evaluation of natural products as inhibitors of human immunodeficiency virus type 1 (HIV-1) reverse transcriptase. *J Nat Products* 1991;**54**:143-54.
- Lin CC, Hsu JM, Ujiie T. Evaluation of the antioxidant and hepatoprotective activity of *Terminalia catappa*. *Amer J Chin Med* 1997;**27**:153-63.
- Pawar SP, Pal SC, Kasture VS. Anti inflammatory activity of *Terminalia catappa* Linn. Pharmacy focus in the new millennium abstracts. 1997. p. 110.
- Chen JH, Li TY, Lin TC. Folk medicine *Terminalia catappa* and its major tannins component, punicalagin, are effective against bleomycin-induced genotoxicity in Chinese hamster ovary cells. *Cancer lett* 2000;**152**:115-22.
- Ratnasooriya WD, Dharmansiri. Effects of *Terminalia catappa* seeds on sexual behaviour and fertility of male rats. *Asian J Androl* 2000;**2**:213-9.
- Jeng-Leun M, Pei-Tzu K, Charng-Charng C. *Food Research International* 2003;**36**:97-104.
- Yun-Lian L, Yueh-Hsiung K, Ming-Shi S, Chein-Chin C, Jun-Chih O. *J Chinese Chem Soc* 2000;**47**:253-6.
- Fan YM, Xu LZ, Gao J, Wang Y, Tang SH, Zhao SN, Zhang ZX. *Fitoterapia* 2004;**75**:253-60.
- Teotia S, Singh M. Hypoglycemic effect of *Prunus amygdalus* seeds in albino rabbits. *Indian J Exp Biol*. 1997;**35**:295-296.
- Aruna RV, Ramesh B, Kartha VN. Effect of beta carotene on protein glysylation in alloxan induced diabetic rats. *Indian J Exp Biol* 1999;**37**:399-401.
- Giordano BP, Thrash W, Hollenbaugh L, Dube WP, Hodges C, Swain A, et al. Performance of serum blood glucose testing systems at high altitude. *Diabetes Educ* 1989;**15**:444-8.
- Roeschlau P, Bernt P, Gruber W. Enzymatic determination of total cholesterol in serum. *Z Klini Chem Klini Bioch* 1974;**12**:226.
- Muller PH, Schmulling RM, Liebich HM, Eggstgein M. A fully enzymatic triglyceride determination. *J Clin Chem Clini Biochem* 1977;**15**:457-64.
- Allain CC, Poon LS, Chan CS, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. *Clin Chemistry* 1974;**20**:470-5.
- Friedewald WT, Levy RI, Fedrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chemistry* 1972;**18**:499-502.
- Bowers LD. Kinetic serum creatinine assays I. The role of various factors in determining specificity. *Clin Chemistry* 1980;**26**:551-4.
- Wilson BW. Automatic estimation of urea using urease and alkaline phenol. *Clin Chemistry* 1966;**12**:360-8.
- Sasaki M. A new ultra micro method for the determination of serum alkaline phosphates. Use of Berthelot's reaction for the estimation of phenol released by enzymatic activity. *Igaku To Seibutsugaku* 1966;**70**:208-14.
- Gorray KC, Baskin D, Brodsky J, Fujimoto WY. Responses of pancreatic  $\beta$  cells to alloxan and streptozotocin in the guinea pig. *Pancreas* 1986;**1**:130-8.
- Chakravarthy BK, Gupta S, Gode KD. Functional beta cell regeneration in the islets of pancreas in alloxan induced diabetic rats by (-)-epicatechin. *Life Sci*. 1982;**31**:2693-7.
- Ghosh S, Suryawanshi SA. Effect of *Vinca rosea* extracts in treatment of alloxan diabetes in male albino rats. *Indian J Exp Biol* 2001;**30**:748-59.
- Rao RM, Salem FA, Gleason-Jordan I. Anti diabetic effects of dietary supplement 'Pancreas Tonic'. *J Natl Med Assoc* 1998;**90**:614-8.
- Xiu LM, Miura AB, Yamamoto K, Kobayashi T, Song QH, Cyong JC. Pancreatic islet regeneration by ephedrine in mice with streptozotocin-induced diabetes. *Amer J Chin Med* 2001;**29**:493-500.
- Shanugasundaram ER, Gopinath KL, Shanmugasundaram R, Rajendra VM. Possible regeneration of the islets of Langerhans in streptozotocin-diabetic rats given *Gymnema sylvestre* leaf extracts. *J Ethnopharmacol* 1990;**30**:265-79.
- Duke JA. Handbook of phytochemical constituents of GRAS herb and other economic plants. *CRC press*, Boca Raton, FL, 1992.

**Address correspondence to:** Dr. Syed Mansoor Ahmed, Department of Pharmacology, M.M.U College of Pharmacy, Karnataka, India. E-mail: [mansoor276783@yahoo.com](mailto:mansoor276783@yahoo.com)