ANTI-DIABETIC ACTIVITY OF AQUEOUS EXTRACT OF *TALINUM CUNEIFOLIUM* LINN. IN RATS

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Summary

The antidiabetic effect of aqueous extract of *Talinum cuneifolium* (AETC) was evaluated in normal, glucose fed and alloxan- induced diabetic rats. Oral administration of extract (200 mg/kg and 400 mg/kg body wt) for 7 days resulted in a significant reduction in blood glucose level. The effect was compared with 0.5 mg/kg (i.p) glibenclamide.

Key words: *Talinum cuneifolium,* Aq. Extract of TC, alloxan, glibenclamide, antidiabetic

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Introduction

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 deficiency result in increased concentration 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 the people with diabetes multiply world wide, the disease taken an ever increasing production of national and international health care budgets. It is projected to one 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 be 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 plants medicines are used throughout the world for a range of diabetic presentation [1]. The synthetic hypoglycemic agents used in clinical practices have serious side effects like hematological effects, coma, disturbances of liver and kidney. In addition they are suitable for use during pregnancy [2]. Compared with synthetic drugs, drugs derived from plants are frequently considered to be less toxic with fewer side effects [3].

Talinum cuneifolium Linn. (Protulaceae) commonly known as Ceylone Bachalli. In Indian System of Medicine, the various parts of plants includes leaves and roots are used as treatment of diabetic, mouth ulcer, and aphrodisiac, cough, gastritis, pulmonary tuberculosis, diarrhea and stomachic [4,5].

In the light of the above information the present investigation was undertaken to evaluate the glucose lowering effects of aqueous extract of leaves of *Talinum cuneifolium* in alloxan induced hyperglycemic rats to establish pharmacological evidence in support of the folklore claim.

Materials and Methods

Plant material

Fresh leaves were collected from S.V.U campus, Tirumala gardens of Chittor District of Andhra Pradesh of India and authentified by Asst.Prof.Dr.K.Madava Chetty of the Department of Botany, S.V.University, Tirupati, Andhra Pradesh, India. Voucher specimen [No.TCA1/PRRMCP 06- 10] was deposited at Department of Pharmacognosy for further reference.

Extraction

The leaves were shade dried and powder in a grinder mixture to obtain a coarse powder and then passed through 40 mesh sieves. The powdered leaves (430 g) were defatted with hexane and later extracted with water (cold maceration). The extract was evaporated and dried under reduced pressure. Percentage yield was found to be 20.5%w/w.

Phytochemical screening

A preliminary phytochemical screening of Aq. Extract of TC was carried out as described by Khandelwal K.L. [6].

Animals

Wistar albino rats (200-250g) of both sexes were procured from Sri Venkateshwara Enterprises, Bangalore. Before and during the experiment rats were fed with standard diet (Gold Mohr, Lipton India Ltd). After randomization into various groups and before initiation of experiment, the rats were acclimatized for a period of 7days 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*. Ethical clearance for animal study was obtained from the institutional animal ethics committee. (IAEC/PRRMCP/2006/07)

Toxicity study

An acute toxicity study relating to the determination of LD_{50} value was performed using different doses of the extract according to the method described by Ghosh et.al [7]. From the toxicity study, it was observed that the extract is non-toxic upto dose of 5.0 g/kg body weight and was used in different doses for further studies.

Experimental Design [8-10]

Effects of Aq. Extract of TC on blood glucose levels in normoglycemic rats

In this study the entire groups of animals were fasted over night and administered with respective drugs as per the mentioned dosage schedule. Animals were divided into three groups of six rats in each group. Group-1, 2 and 3 received 1% SCMC (2 ml/kg), 200 mg/kg and 400 mg/kg orally of Aq. Extract of TC respectively. Blood glucose levels were determined at 0 (before drug challenge) 60, 120 min, after drug administration.

Effect of Aq. Extract of TC on blood glucose level on glucose fed hyperglycemic rats (Oral Glucose Tolerance test)

In this study the entire groups of animals were fasted over night and administered with respective drugs as per the mentioned dosage schedule. Animals were divided into four groups of six rats in each group. Group-1, 2, 3 and 4 received glucose 2 g/kg only, glibenclamide 0.5 mg/kg, i.p., 200 mg/kg and 400 mg/kg and glucose 2 g/kg orally half an hour before administration of standard and test extract respectively. Blood glucose levels were determined at 0 (before glucose challenge) 30, 60, 90, 120thmins after glucose administration.

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Effect of Aq. Extract of TC on blood glucose level in alloxan induced diabetic rats

Different groups of rats were used to study the effects of Aq. Extract of TC. The rats were divided into five groups each consisting of six rats. Group-1: Normal control animals received 1% SCMC 2 ml/kg body wt. per orally. Group-2: Alloxan (150 mg/kg body wt.) induced diabetic animals received 1% SCMC 2ml/kg body wt. per orally. Group-3: Alloxan (150 mg/kg body wt.) induced diabetic animals received Glibenclamide 0.5 mg/kg, body wt. intraperitonialy. Group-4: Alloxan (150 mg/kg body wt.) induced diabetic animals received Aq. Extract of TC 200 mg/kg, body wt. per orally**.** Group-5: Alloxan (150 mg/kg body wt.) induced diabetic animals received Aq. Extract of TC 400 mg/kg, body wt. per orally. Significant hyperglycemia was achieved within 48 hours after Alloxan (150 mg/kg b.w. i.p) injection. Alloxan induced diabetic rats with more than 200 mg/dl of blood glucose were considered to be diabetic and used for the study.

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In acute study all the surviving diabetic animals and normal animals were fasted over night. Blood samples were collected from the fasted animals prior to the treatment with above schedule and after administration at each day up to 7days. For glucose determination, blood was obtained by snipping tail with sharp razor [11]. Then the blood glucose levels were determined by using Haemo-Glukotest (20-800R) glucose strips supplied by M/s Boehringer Mannheim India Ltd. These methods, which permit the measurement of blood glucose levels with minimum injury to rat, was previously validated by comparison with glucose oxidase method [12-14].

Statistical Analysis

All values were expressed as mean ± SEM .The data were statistically analyzed by ANOVA followed by Dunnets‘t’ test [15].

Results and Discussion

Phytochemical screening

The preliminary Phytochemical studies of Aq. Extract of TC revealed that presence of alkaloids, tannins, flavonoids, proteins and carbohydrates.

Toxicity study

From the toxicity study it was observed that Aq. Extract of TC was non-toxic and caused no death up to 5 g/kg orally. The results presented in Table-1.

Table-1: Toxicity Study of Aq. Extract of TC

Treatment	Dose(mg/kg body wt)	No. of animals	No. of survival	No. of death	Percentage of morality	LD_{50} valve
Control	1% NaCMC	10	10	0	0	-
EETC	100	10	10	0	0	-
	200	10	10	0	0	-
	400	10	10	0	0	-
	800	10	10	0	0	-
	1600	10	10	0	0	-
	3200	10	10	0	0	-
	5000	10	10	0	0	> 5.0g/kg body wt.

Effect of Aq. Extract of TC on blood glucose in normoglycemic rats

At dose 200 mg/kg and 400 mg/kg of Aq. Extract of TC on fasting blood sugars level were assessed in normal rats at various time interval is shown in table-2.The mean blood glucose level decrease from 76.00 mg/dl to 76.40 mg/dl at dose of 200 mg/kg body weight of Aq. Extract of TC and 77.00 mg/dl to 75.80 mg/dl at dose of 400 mg/kg bodyweight in rats treated with Aq. Extract of TC.

Effect of Aq. Extract of TC on blood glucose level in glucose fed hyperglycemic rats

At dose 200 mg/kg and 400 mg/kg of Aq. Extract of TC blood sugar level were assessed in glucose fed rat at various intervals as shown in table-3. The blood glucose levels decreased from 77.65 mg/dl to 77.83 mg/dl at 200 mg/kg bodyweight and 81.00 mg/dl to 79.53 mg/dl at 400 mg/kg body weight.

Table -2: Effect of Aq. Extract of TC on Blood glucose in normoglycemic rats

GROUPS	Blood glucose levels (mg/dl)		
	Initial	60min	120 min
Group I (n=6)	79.33 ± 1.145	80.00 ± 1.204	78.66 ± 1.364
Group II (n=6)	76.00 ± 0.866	75.90 ± 1.77	76.40 ± 1.82
Group III (n=6)	77.00 ± 0.966	66.83 ± 0.175	75.80 ±1.501

The values are expressed as mean ± SEM. n = number of animals in each group. Statistical significant test for comparison was done by ANOVA, followed by Dunnet's –'t' test. The 60th and 120th min values are compared with initial value.

Table – 3: Effect of Aq. Extract of TC on Blood glucose in glucose fed hyperglycemic normal rats

Groups	Blood glucose levels (mg/dl)				
	Initial	30 min	60min	90 min	120 min
I	82.16±01.30	116.83±0.70	119.50±0.74	104.80±1.75	85.66±1.47
II	77.66±01.20	118.83±1.01*	106.33±1.22*	82.00±1.06*	75.66±1.38*
III	77.65± 1.20	116.53±0.80*	103.00±1.06*	88.53±1.45*	77.83±0.21*
IV	81.00±01.25	120.16±1.38*	111.26±1.08*	94.66±1.30*	79.53±1.25*

The values are expressed as mean ± SEM. n = 6 number of animals in each group. Statistical significant test for comparison was done by ANOVA, followed by Dunnet's –'t' test. The blood glucose values of group II, III and IV are compared with control animal's values. P< 0.05 were taken as* Significant.

Effect of Aq. Extract of TC on blood glucose level in alloxan induced diabetic rats

The antihyperglycemic effect of the extracts on the blood sugar level on diabetic rats is shown in Table-4. The blood glucose level of diabetic animal significantly ($p<0.05$) reduced from 210.15 mg/dl to 105.18 mg/dl at 200 mg/kg body wt. of Aq. Extract of TC and 209.01 mg/dl to 99.73 mg/dl at 400 mg/kg body wt. of Aq. Extract of TC. These results were comparable with 0.5mg/kg of glibenclamide.

Table -4 Effect of EETC on Blood Glucose level in Alloxan induced Diabetic Rats

Groups	Blood glucose levels (mg/dl)						
	1st Day	2nd Day	3rd Day	4th Day	5th Day	6th Day	7th Day
I	81.00 ± 0.59	81.33 ± 0.44	80.91 ± 0.43	80.66 ± 0.54	81.00 ± 0.36	81.00 ± 0.53	81.33 ± 0.49
II	204.83 ± 1.25	212.66 ± 1.45	219.83 ± 1.35	228.16 ± 1.40	237.66 ± 1.80	246.66 ± 2.124	255.83 ± 2.54
III	207.00 ± 1.63	184.00 ± 1.77*	163.83 ± 1.66*	143.16 ± 2.18*	121.83 ± 2.85*	101.33 ± 3.01*	85.33 ± 1.35*
IV	210.15 ± 0.95	199.15 ± 1.20*	180.43 ± 0.95*	160.01 ± 0.95*	137.83 ± 1.75*	117.83 ± 1.30*	105.18 ± 1.60*
V	209.01 ± 1.31	187.50 ± 1.64*	171.86 ± 2.75*	156.42 ± 2.52*	135.00 ± 1.86*	110.83 ± 2.30*	99.73 ± 1.53*

The values are expressed as mean ± SEM. N = 6 number of animals in each group. Statistical significant test for comparison was done by ANOVA, followed by Dunnet's't' test. P< 0.05 were taken as* Significant.

In the recent times many traditionally used medicinally important plants were tested for their anti-diabetic potential by various investigators in experimental animals. These properties were attributed to different formulations, extracts and active principles. Working on the same line, we have undertaken a study on *Talinum Cuneifolium* for its anti-diabetic property.

The Aq. Extract of TC at a dose of 200 mg/kg body wt per orally did not significantly suppress blood glucose levels in over night fasted normoglycemic animals. The same effect was observed at a higher dose level of 400 mg/kg body wt per orally of the Aq. Extract of TC in over night fasted normoglycemic animals after 1st, 2nd and 3rd hour of oral administration, when compared with control group of animals.

The Aq. Extract of *Talinum Cuneifolium* showed significant improvement in glucose tolerance in glucose fed hyperglycemic normal rats. Such an effect may be accounted for, in part, by a decrease in the rate of intestinal glucose absorption, achieved by an extra pancreatic action including the stimulation of peripheral glucose utilization or enhancing glycolytic and glycogenic process with concomitant decrease in glycogenolysis and glyconeogenesis. However, the effect was less significant when compared to standard drug glibenclamide.

Alloxan is the most commonly employed agent for the induction of experimental diabetic animal models of human insulin-dependent diabetes mellitus. There is increasing evidence that alloxan causes diabetes by rapid depletion of β cells, by DNA alkylation and accumulation of cytotoxic free radicals that is suggested to result from initial islet inflammation, followed by infiltration of activated macrophages and lymphocyte in the inflammatory focus. It leads to a reduction in insulin release there by a drastic reduction in plasma insulin concentration leading to stable hyperglycemic states. In this study significant hyperglycemia was achieved within 48 hours after Alloxan (150 mg/kg b.w. i.p) injection. Alloxan induced diabetic rats with more than 200 mg/dl of blood glucose were considered to be diabetic and used for the study.

The studies on antidiabetic activity in alloxanised rats, significant reduction of blood glucose was observed from the 2nd day of the study. The comparable effect of the extract with glibenclamide may suggest similar mode of action since alloxan permanently destroys the pancreatic β cells and the extract lowered blood sugar level in alloxanised rats, indicating that the extent possesses extra pancreatic effects. From the Phytochemical analysis it was found that the major chemical constituents of the extract were flavonoids, and tannins. Over 150 plant extract and some of this active principle including flavonoids are known to be used for the treatment of diabetes [16-19]. On the basis of the above evidences it is possible that the presence of flavonoids and tannins are responsible for the observed antidiabetic activity [20-21].

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