ANTI-DIABETIC ACTIVITY OF ALCOHOLIC EXTRACT OF TALINUM CUNEIFOLIUM IN RATS

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Summary

The alcoholic extract of *Talinum cuneifolium* (Protulaceae) was studied for antidiabetic activity. The study was performed using Alloxan induced diabetic model at dose of 200mg/kg, 400mg/kg body weight of rat of AETC for seven days and the effects were compared with reference standards, Glibenclamide 0.5mg/kg. The plant extract at 400mg/kg significantly lowered the blood sugar level of hyperglycemic rats. From the toxicity study it was observed that EETC is non-toxic of showed the presence of steroid, flavonoids, tannins alkaloid, proteins and carbohydrates.

Keywords: Talinum cuneifolium, Antidiabetic, Alloxan, AETC

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Introduction

Diabetes mellitus (DM) is a metabolic disorder affecting carbohydrate, fat and protein metabolism. The world wide survey reported that the DM is affecting 10% of the population [1].The treatment of DM is based on oral hypoglycemic agents and insulin. However, DM is also treated with Indian traditional medicine using antidiabetic medicinal plants [2-7] 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 not suitable for use during pregnancy [8]. Compared with synthetic drugs, drugs derived from plants are frequently considered to be less toxic with fewer side effects [9]. Therefore, the search for more effective and safer antidiabetic agent as becomes an area of active research.

Talinum cuneifolium Lin. (protulaceae) commonly known as ceylone bachalli. The leaves and roots are medicinally important parts. The powdered leaves are used in treatment of diabetic, mouth ulcer, and aphrodisiac, roots are used for cough, gastritis and pulmonary tuberculosis [10-11].

In this study, we have evaluated the glucose lowering effects of ethanolic extract of leaves of Talinum cuneifolium in Alloxan hyperglycemic rats to establish pharmacological evidence in support of the folklore claim.

Material 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, Tirupathi. A.P. A voucher specimen [No.TCA1/PRRMCP 06- 10] was deposited at Department of Pharmacognosy for further reference.

Extraction

The leaves, shade dried powder in a grinder mixture to obtain a coarse powder and then passed through 40 mesh sieve. The powdered leaves (430g) were defatted with hexane and later extracted (soxhlet) using alcohol. The extract evaporated to dryness, gave a residue 17.5%w/w.

Phytochemical screening:

The chemical constituents of the extract were identified by qualitative test. [12].(Table-1).

Animals:

Albino wistar rats of either sex weighing (200-250g) were employed for study. They were housed in standard environmental conditions and fed with standard rodent diet with water ad libitum. Ethical clearance for animal study was obtained from the institutional animal ethics committee.

Toxicity study:

An acute toxicity study to determine LD_{50} valve was performed using different doses of the extract according to the method described by Ghoshet.al [13]. It was observed that extract is non-toxic up to dose 3.2kg/body wt. (Table-2).

Effects of EETC On Blood Glucose Levels In Normoglycemic Rats:

Animal were divided into three groups of six rats in each group
Group-1: Animals received 1% SCMC 2ml/kg body wt. per orally.
Group-2: Animals received EETC 200mg/kg body wt. per orally.
Group-3: Animals received EETC 400mg/kg body wt. per orally.

In this study the entire groups of animals were fasted over night and administered with respective drugs as per the above mentioned dosage schedule. Blood glucose levels were determined at 0 (before drug challenge) 60, 120min, after drug administration.

Effect of EETC On Blood Glucose Level On Glucose Fed Hyperglycemic Rats (Oral Glucose Tolerance Test):

The animals were divided into four groups of six rats in each group
Group-1: Animals received glucose at a dose 2g/kg body wt. per orally.
Group-2: Animals received glibenclamide 0.5mg/kg body wt. and glucose Solution at a dose 2g/kg body wt. per orally.
Group-3: Animals received EETC 200mg/kg body wt. and glucose Solution at a dose 2g/kg body wt. per orally.
Group-4: Animals received EETC 400mg/kg body wt. and glucose Solution at a dose 2g/kg body wt. per orally.

In this study, the entire group of animals were fasted and treated with above dosage schedule orally. The EETC 200mg/kg, 400mg/kg and 0.5mg/kg glibenclamide were administered half an hour before administration of glucose solution. Blood glucose levels were determined at o (before glucose challenge) 30, 60, 90, 120thmins after glucose administration.

Effect of EETC on Blood Glucose Level in Alloxan Induced Diabetic Rats.

Different routes of rats were used to study the effects of EETC. The rats were divided into five groups each consisting of six rats.

Group-1: Normal control animals received 1% SCMC 2ml/kg body wt. per Orally.

Group-2: Alloxan (150mg/kg body wt.) induced diabetic animals received 1% SCMC 2ml/kg body wt. per orally.

Group-3: Alloxan (150mg/kg body wt.) induced diabetic animals received Glibenclamide 0.5mg/kg, body wt. per orally.

Group-4: Alloxan (150mg/kg body wt.) induced diabetic animals received EETC 200mg/kg, body wt. per orally**.**

Group-5: Alloxan (150mg/kg body wt.) induced diabetic animals received EETC 400mg/kg, body wt. per orally. .

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 snipping tail with sharp razor [14-15]. 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 [16-18].

Statistical Analysis:

All values were expressed as mean ± SEM .The data were statistically analyzed by student t-test [19].

Results

Phytochemical screening:

The results of the preliminary phytochemical screening of Ethanolic extract of Talinum Cuneifolium as shown in Table-1

Table-1
Phytochemical Screening

Sl.no	Tests	EETC
1	Alkaloids	+
2	Tannins	+
3	Flavonoids	+
4	steroids	+
5	Proteins	+
6	Carbohydrates	+

'-' absence; '+' presence.

Toxicity Study:

From the toxicity study it was observed that EETC is non-toxic and caused no death upto 3.2g/kg orally. It is safe and used in different doses for further studies. The results presented in Table-2.

Table-2
Toxicity Study

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	>3.2g/kg body wt

Effect of EETC on Blood Glucose in Normoglycemic Rats:

At dose 200mg/kg and 400mg/kg of EETC on fasting blood sugars level were assessed in normal rats at various time interval.The results were shown in Table-3 and figure-1.The mean blood glucose level decrease from 75.50 to 75.90 at dose of 200mg/kg body weight of EETC and 77.00mg% to 73.00 at dose of 400mg/kg bodyweight in rats treated with EETC.

Effect of EETC on Blood Glucose Level in Glucose Fed Hyperglycemic Rats:

At dose 200mg/kg and 400mg/kg of EETC blood sugar level were assessed in glucose fed rat at various intervals as shown in Table-4 and figure-2. The blood glucose levels decreased from 78.50 to 76.83 at 200mg/kg bodyweight and 80.16 to 78.83 at 400mg/kg body weight.

Effect of EETC on Blood Glucose Level in Alloxan Induced Diabetic Rats:

The antihyperglycemic effect of the extracts on the blood sugar level on diabetic rats as shown in Table-5 and figure-3. The blood glucose level of diabetic animal significantly ($p<0.05$) reduced from 211.6 to 103.16 at 200mg/kg body wt. of EETC and 207mg/dl to 94.83 mg/dl at 400mg/kg body wt. of EETC.these results are comparable with 0.5mg/kg of. Glibenclamide.

Table -3
Effect of EETC 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)	75.50 ± 0.844	73.00 ± 2.352	75.90 ± 1.777
Group III (n=6)	77.00 ± 0.966	62.83 ± 0.158	73.80 ±1.201

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.

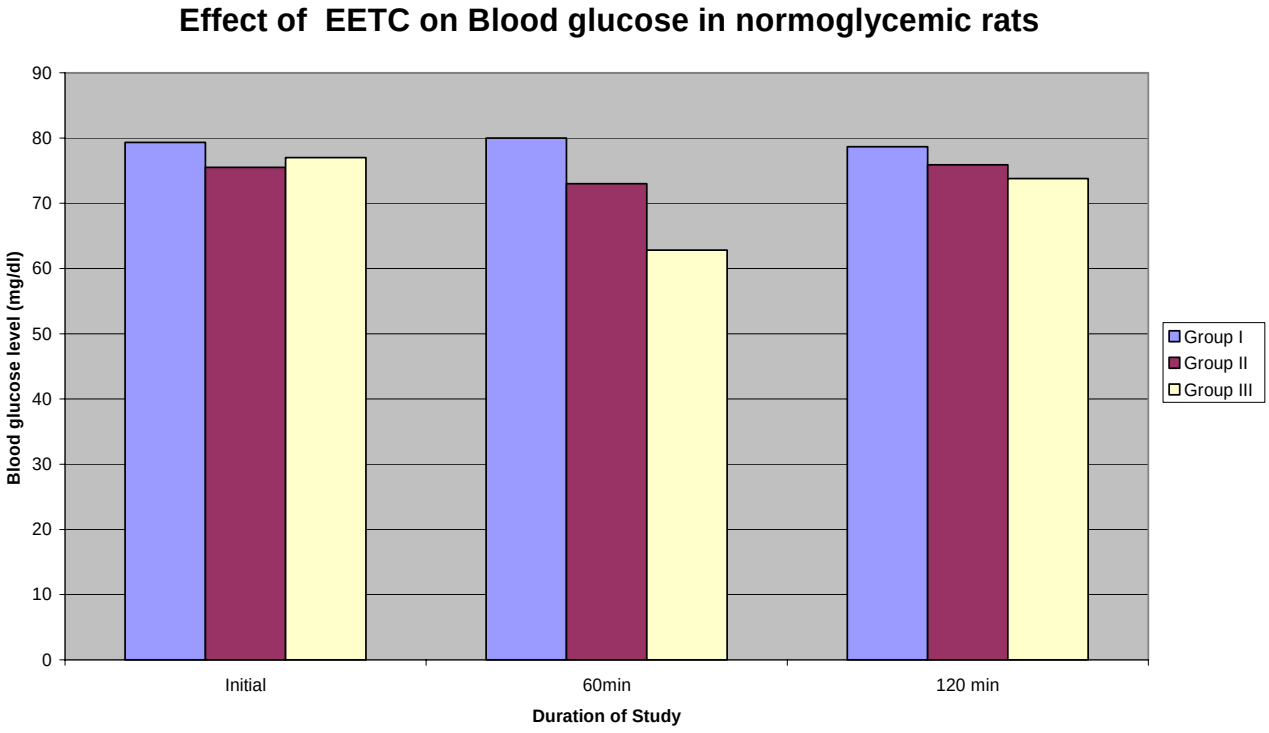

Figure-1

Table - 4
Effect of EETC 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	78.50±00.99	127.00±1.43*	103.00±1.06*	87.83±1.53*	76.83±0.91*
IV	80.16±01.35	133.16±1.40*	110.66±1.28*	92.66±1.20*	78.83±1.30*

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 blood glucose values of group II, III and IV are compared with control animal's values.
*** - Significant.**

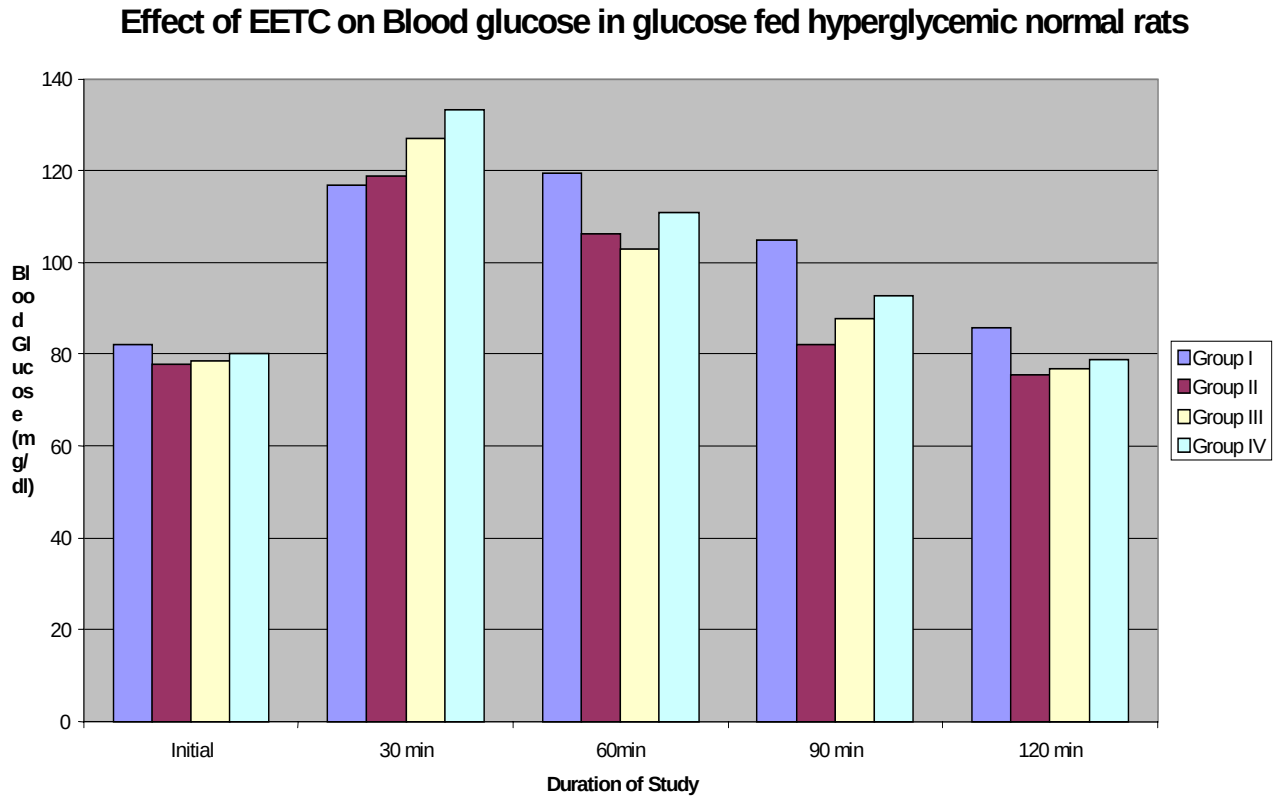

Figure-2

Table -5

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	211.16 ± 1.08	195.16 ± 1.40*	175.33 ± 0.84*	158.00 ± 1.15*	137.83 ± 1.85*	118.83 ± 1.40*	103.16 ± 1.70*
V	207.00 ± 1.41	185.50 ± 1.54*	169.50 ± 2.51*	152.30 ± 2.83*	130.80 ± 2.22*	112.3 ± 2.4726*	94.83 ± 1.73*

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.

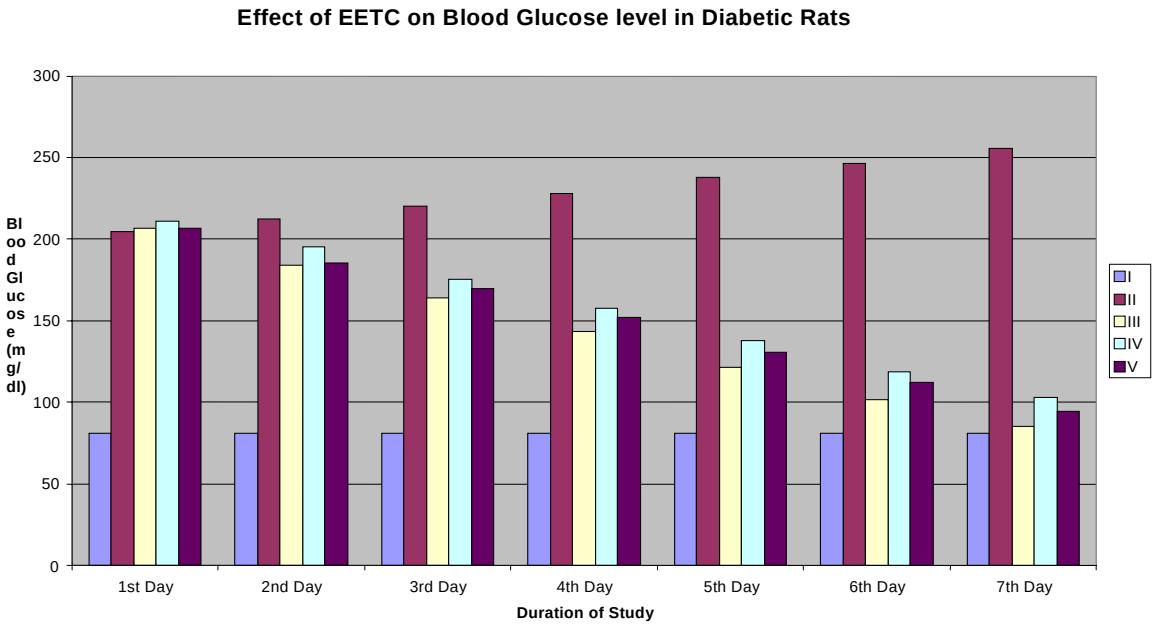

Figure-3

Discussion

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.

Indian system of Medicine (ISM) refers the uses of *Talinum Cuneifolium* as a

- Leaves and roots are medicinally important parts.
- Powdered leaf is used in treatment of diabetes, mouth ulcers, and aphrodisiac.
- Roots posses tonic properties are used for cough, gastritis and pulmonary tuberculosis.
- They are also used to treat dehydrating diarrhea.
- The fresh leaves are used as stomachic.

Preliminary Phytochemical analysis of the Ethanol (EETC) extract of the leaves of *Talinum Cuneifolium* showed that the plant has a rich possession of phytochemicals like alkaloids, flavonoids, steroids, glycosides, phenols, Tannins etc.

Acute oral toxicity studies revealed the non-toxic nature of the ethanol (EETC) extract *Talinum Cuneifolium***.** No lethality was observed or any profound toxic reactions found at a dose of 3200mg/kg body wt. p.o. This indirectly pronounces the safety profile on the plant extracts.

The ethanol (EETC) extract at a dose of 200mg/kg body wt p.o. did not significantly suppress blood glucose levels in over night fasted normoglycemic animals. The same effect was observed at a higher dose level of 400mg/kg body wt p.o. of the ethanol (EETC) extract in over night fasted normoglycemic animals after 1^{st} ,2^{nd} and 3^{rd} hour of oral administration, when compared with control group of animals.

The ethanol (EETC) extract *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 (150mg/kg b.w. i.p) injection. Alloxan induced diabetic rats with more than 200mg/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, steroids and tannins. Over 150 plant extract and some of this active principle including flavonoids are known to be used for the treatments of diabetes [20-23] on the basis of the above evidences it is possible that the presence of flavonoids and tannins are responsible for the observed antidiabetic activity [24-25].

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