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Evaluation of Antihperglycemic Activity of Extracts of *Calotropis procera* (Ait.) R.Br on Streptozotocin Induced Diabetic Rats.

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Abstract: The extracts of *Calotropis procera* were as investigated for its anti-hyperglycemic, effect in Male Wister Albino rats. Diabetes was induced by administration of single dose of streptozotocin (STZ, 50 mg/kg, I.P). The pet ether, methanol and aqueous extracts of leaves of *C. procera* at dose of 250 mg/kg, per oral were administered as single dose per day to diabetes-induced rats for a period of 15 days. The effect of *C. procera* on blood glucose level was measured in the diabetic rats. Serum lipid profile (Total cholesterol, triglycerides, phospholipids, low density, very low density and high density lipoprotein) also were measured in the diabetic rats. The activities were also compared to that effect produced by a standard anti-diabetic agent, glibenclamide 500µg/kg. The present investigation established pharmacological evidence to support the folklore claim that it is an anti-diabetic agent.

Key words: *Calotropis procera* · Glibenclamide · Hyperglycemia · Streptozotocin

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder affecting approximately 10% of the global population. Besides hyperglycemia, several other factors including dislipidemia or hyperlipidemia are involved in the development of micro and macro vascular complications of diabetes which are the major causes of morbidity and death [1]. Plants have played a major role in the introduction of new therapeutic agents. A medicinal plant, *Galega officinalis*, led to the discovery and synthesis of metformin [2]. Despite considerable progress in the treatment of diabetes by oral hypoglycemic agents, search for newer drugs continues because the existing synthetic drugs have several limitations. In recent times there has been a renewed interest in the plant remedies [3, 4].

C. procera (Aselepiadaceae) is a small plant and found in tropical and sub tropical regions of India. The wood is used as cheap fuel and latex is used in tanning industry and pharmacologically the latex is used as a wound healing agent by traditional healers and as an abortificient in folk medicines. The plants were considered sacred and the leaves were used in Vedic times in sun worship. Polysaccharides, four cardenolids were isolated from leaves [5] triterpenes from root bark of *C. procera* [6].

The plant selected for this present work is locally available in the Salem district and has been used for long a time in local folklore medicine for the treatment of diabetics.

MATERIALS AND METHODS

Plant Material: The plant materials were collected in the forest area of Ghaziabad (Uttar Pradesh). The plant was authenticated and identified at raw material herbarium and museum, National Institute of Science Communication and Information Resources, Delhi. The plant material was shade dried at room temperature for 10 d, coarsely powdered with the help of a hand-grinding mill and the powder was passed through sieve No.60 and used for extraction.

Preparation of the Extract: The powdered *C. procera* leaf material (1000g) was extracted separately using pet. ether, methanol by Soxhlet and aqueous extract by cold maceration. The extracts were dried under reduced pressure.

Animals: Male albino rats, 9-12 weeks old with an average weight of 150-175 g were used for the study. They were housed in polypropylene cages and fed with a standard chow diet and water *ad libitum.* The animals

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were exposed to an alternating 12 h and light cycle. Before each experiment, the animals were fasted for at least 18 h. The experimental protocols were approved by Institutional Animal Ethical Committee.

Toxicity Evaluation in Mice: Male albino mice, 6-8 weeks old with an average weight of 25-30 g were used for the study. The methanol and aqueous extracts were tested for its toxicity in mice by administration, a single oral methanol and aqueous extracts of *C. procera* in different dose to different groups of mice (6 mice were used for each group). The control group received Tween. Mortality and general behavior of the animals were observed periodically for 48 h. The animals were observed continuously for the initial 4 h followed by 6 h, 24 h and 48 h after drug administration. The parameters observed were grooming, hyperactivity, sedation, respiratory rate and convulsion.

Preliminary Phytochemical Screening: The extracts were subjected to preliminary screening for various active phytochemical constituents [7].

Drugs and Chemicals Used: Alloxan monohydrate was purchased from S.D Fine chemicals Ltd., Boisar. Glibenclamide was procured from Aventis Pharma, Mumbai, India. All other chemicals were obtained from local sources and were of analytical grade.

Preparation of Extract, Reference and STZ: The extract was administered orally to rats, as a suspension in 1% Carboxy Methyl Cellulose (CMC). Glibenclamide (500 µg/kg) was suspended with 1% w/v CMC and administered orally (Standard drug). STZ was dissolved in 0.9% ice-cold saline immediately before use.

Experimental Design: Long Term Experiment

Streptozotocin-induced Diabetic Rats: Streptozotocin (STZ) was dissolved in 0.9% ice-cold saline immediately before use. Diabetes was induced in rats by intra peritoneal (i.p) injection of streptozotocin at a dose of 50 mg/kg, dissolved in saline [8]. Forty eight hours after streptozotocin administration, blood samples were drawn from tail and glucose levels determined to confirm diabetes. The diabetic rats exhibiting blood glucose levels higher than 200 mg/dl were selected for the studies.

Experimental Procedure: In experiment, a total of 30 rats were used (36 diabetic surviving rats, 6 control rats) for the execution of the experiment. The rats were divided as follows into six groups.

Group I	Control rats (Vehicle treated)
Group II	Diabetic control (Received 0.5 ml of 5% Tween 80)
Group III	Diabetic rats given Glibenclamide 500 µg/kg (Received 0.5 ml of 5% Tween 80) [9]
Group IV, V and VI	Diabetic rats given pet. ether, methanol and aqueous extracts of *C. procera* 250 mg/kg b. wt,

Blood samples were collected from the tail for glucose estimation just before drug administration on the 1st day and 1 h after drug administration on days 4, 7, 10 and 15. Blood samples were collected and centrifuged to separate serum for estimation of lipid profile and other biochemical parameters [10].

Anti-hyperlipidaemic Activity: Total cholesterol, HDL- C, LDL-C, VLDL-C and triglycerides were analyzed from serum. Total cholesterol was estimated according to Liebermann Burchard Reaction Method [11]. LDL cholesterol was estimated indirectly by Friedwald's method [12]. Triglycerides (TG) were determined using Hantzsch condensation method [13].

Statistical Evaluation: All the data are presented as mean ±SEM, n= 6. The differences between groups were evaluated by one-way analysis of variance (ANOVA) followed by the Dunnette multiple comparisons test. $P<0.01$ was considered to be significant.

RESULTS

Preliminary Phytochemical Test: Phytochemical studies indicated that aqueous extract of leaves of *C. procera* contains alkaloidas, flavanoids, glycosides, saponins and terpenes.

Acute Toxicity Studies: In performing preliminary test for pharmacological activity in rats, aqueous extract did not produce any significant changes in the behavioral or neurological responses upto 2500 g/kg b. wt. acute toxicity studies revealed the non-toxic nature of the pet. ether, ethanol and aqueous extracts of the leaves of *C. procera.*

Antihyperglycemic Activity: The effects of extracts on blood glucose levels in diabetic rats are reported in Table 1. Blood glucose levels of the STZ treated rats were significantly higher than those in normal rats. In STZ (50 mg/kg) induced rats, the blood glucose

Table 1: Effect of extracts of *C. procer*a on blood glucose level on streptozotocin-induced diabetes in rats

	Blood glucose level (mg/dl)			
Treatment mg/kg	Day 1	Day 5	Day 10	Day 15
Normal control	93.89±1.47	94.2±2.26	94.4±2.46	93.2±0.22
Diabetic control	238.2±2.22	242.6±2.24	240.8±4.24	248.6±4.48
Glibenclamide 500 µg/kg	237.0±1.00	204.8±2.58	192.6±2.07	146.8±3.11
Pet.ether extract (L)	238.6±2.30	218.2±1.92	198.8±1.82	154.0±1.58
Methanol extract (L)	236.0±1.58	205.2±2.77	199.4±2.00	142.6±2.07
Aqueous extract (L)	238.6±2-64	217.4±2.07	198.6±2.30	164.4±2.70

Values are mean ±SEM, n= 6 (One way ANOVA Followed by Dunnette multiple Comparisons test).

Super script *, **, denotes statistically significance of P<0.05, P<0.01, P<0.001, when compared with respective diabetic control

Table 2: Antihyperglycemic effects of extracts of leaves of *C. procera* on STZ induce diabetic rats

	Changes in mg/dL level					
Treatment mg/kg	Serum total cholesterol	Triglyceride	Serum HDL	Serum LDL	Serum VLDL	Serum Phospholipids
Normal control	81.5±7.9	89.3±7.5	23.8±2.1	35.3±3.3	13.3±1.7	146.3±7.7
Diabetic control	195.3±11.6*	170.3±5.1	13.3±3.1*	71.7±8.3*	30.3±1.1*	255.8±10.6*
Glibenclamide 500 µg/kg	133.7±9.2**	114.7±2.8**	19.2±1.0**	40.2±4.5**	17.8±2.0**	178.8±7.6**
Pet.ether extract (L)	154.7±10.4*	127.0±4.6**	22.2±0.9**	47.2±6.7*	20.7±2.1**	193.0±10.4**
Methanol extract (L)	138.2±9.5**	120.5±3.2**	18.9±0.9**	42.2±5.3**	18.0±2.0**	180.2±8.4**
Aqueous extract (L)	152.8±8.2**	130.6±6.2**	19.2±1.10**	46.7±6.3**	22.6± 2.2*	192.6±4.6**

Values are mean ±SEM, n= 6 (One way ANOVA Followed by Dunnette multiple Comparisons test).

Super script *, **, denotes statistically significance of P<0.05, P<0.01, P<0.001, when compared with respective diabetic control

level significantly increased from 93.89±1.47 to 238.2±2.22 mg/dl. Extracts (250 mg/kg) given upto 15th day after STZ treatment, showed decreased blood glucose levels significantly from 238.6±2.30 to 198.8±1.8 and 236.0±1.58 to 154.0±1.58, 236.0±1.58 to 142.6±2.07 and 238.6±2-64 to 164.4±2.70 mg/dL, whereas glibenclamide treated rats, blood glucose levels were decreased from 237.0±1.00 to 136.8±3.11mg/dL, respectively.

Antihyperlipidaemic Activity: The lipid profiles in control and experimental rats are depicted in Table 2 in STZ induced diabetic rats, there was a significant (P<0.001) increase of total cholesterol, triglycerides, phospholipids and low density lipoproteins (LDL) and very low density lipoprotein (VLDL) cholesterol and significant (p<0.001) decreases in high density lipoprotein (HDL) cholesterol in serum compared with normal control. The extracts treated rats were significantly (p<0.001) decreased the total cholesterol, triglycerides, phospholipids and LDL and VLDL cholesterol and significantly (p<0.001) increased HDL.

DISCUSSION

The results showed that the intraperitoneal administration of STZ (50 mg/kg) effectively induced diabetes in normal animals.

It has been established that diabetes mellitus altered the normal metabolism of lipids in diabetic rats. It is seen that cholesterol and triglycerides are elevated in the diabetic condition; such an elevation represented the risk factor for coronary heart disease. There was a significant reduction in the cholesterol and triglycerides level of diabetic rats after *C. procera* treatment for 15 days.

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