Antihyperglycemic and antihyperlipidaemic activities of root extracts of *Calotropis procera* (Ait.) R.Br on streptozotocin induced diabetic rats.

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Abstract

The root extracts of *Calotropis procera* were 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) Pet. ether, methanol and aqueous extracts of roots of *C. procera* at dose of 250 mg/kg, b. wt were administered as a single dose, per day to diabetes rats for the period of 15 days, respectively. After extracts treatment, the blood glucose levels were decreased 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 respectively. The effect of *C. procera* on blood glucose level was measured in the diabetic rats. Serum lipid profile like total cholesterol (TC), triglycerides (TG), phospholipids (PL), low density lipoprotein (LDL), very low density lipoprotein (VLDL) and high density lipoprotein (HDL) 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.

Keywords: *Calotropis procera,* Glibenclamide, Hyperglycemia, Streptozotocin.

1. 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 (Bennet, 1998). 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 (Aiman, 1970). 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 (Dinesh Puri, 1997; Ratnakar, 1996).

C. procera (Aselepiadaceae) is a small plant and found in tropical and subtropical regions of India. The wood is used as cheap fuel and latex is used in tanning industry, and pharmacologically 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 (Qudrat-I-Khuda, 1969) triterpenes from root bark of *C. procera* (Ansari, 1999).

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.

2. Materials and Methods

2.1. Materials and Methods

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.

2.2. Preparation of the extract

The powdered *C. procera* root material (1000g) was extracted separately using pet. Ether, methanol by Soxhlet and aqueous extract by cold maceration. The extracts were dried under reduced pressure.

2.3. 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 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.

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2.4. 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 their toxicity in mice by administrating, a single oral pet. ether, 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.

2.5. Preliminary phytochemical screening

The extracts were subjected to preliminary screening for various active phytochemical constituents (Kokate, 1994).

2.6. Drugs and chemicals

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.

2.7. 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.

3. Experimental design: Long-term experiment

3.1. 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 (Pulok K Mukarjee, 2002). 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.

3.2. 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) (Augusti, 1996)

- 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 first 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 (Harold varley, 1983).

3.3. 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 Richterich, 1981). LDL cholesterol was estimated indirectly by Friedwald's method (Friedwald, 1972). Triglycerides (TG) were determined using Hantzsch condensation method (MacDonald, 1970).

3.4. 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.

4. Results

4.1. Preliminary phytochemical test

Phytochemical studies indicated that extracts of roots of *C. procera* contains alkaloids, flavanoids, glycosides, saponins and terpenes.

4.2. 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 up-to 2500 g/kg b. wt. acute toxicity studies revealed the non-toxic nature of the pet. ether, methanol and aqueous extracts of the roots of *C. procera*.

4.3. 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 level significantly increased from 93.89±1.47 to 238.2±2.22 mg/dl. Pet. Ether, methanol and aqueous extracts (250 mg/kg) given up-to 15 days. After extracts treatment, the blood glucose levels were decreased 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 respectively, Whereas in glibenclamide treated rats, blood glucose levels were decreased from 237.0±1.00 to 136.8±3.11mg/dL.

4.4. 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 indicated significantly (p<0.001) decreased total cholesterol, triglycerides, phospholipids and LDL and VLDL cholesterol and significantly (p<0.001) increased HDL.

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

Treatment mg/kg	Blood glucose level (mg/dl)			
	Day 1	Day 5	Day 10	Day 15
Normal control	123.89±1.47	124.2±2.26	123.4±2.46	123.2±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	136.8±3.11
Pet. Ether extract (R) 250	235.6±2.30	219.8±2.74	215.2±1.92	203.2±1.59
Methanol extract (R) 250	234.8±1.48	208.8±1.58	197.6±1.58	144.2±2.22
Aqueous extract (R) 250	235.2±1.58	230.2±3.11	214.2±2.91	163.2±1.30

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

Treatment mg/kg	Changes in mg/dL level					
	Serum total cholesterol	Triglycerides	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 (R) 250	142.8± 0.2	130.0±0.22	19.6±4.8	45.3±3.2	26.8±4.6	188.0±2.2
Methanol extract (R) 250	138.8± 4.6	122.8±2.2	20.5± 2.8	44.8±4.2	18.6 ± 8.2	180.4±0.2
Aqueous extract (R) 250	144.0± 8.6	132.2±0.2	18.4±4.8	47.8±6.2	22.8±2.2	192.0±4.8

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.

5. Discussion

Diabetic mellitus is a metabolic disease associated with impaired glucose metabolism which in effect alters intermediary metabolism of lipids and proteins adversely. Most of the complications of the diabetic state are initiated by the generation of free radicals: for instance LDH oxidative modification, leading to atherosclerosis

(Felmeden 2003; Bhakdi, 2004) occurs only in the presence of free radicals.

The presence study was conducted to study the antihyperglycemic and antihyperlipidaemic activities of extracts of *C. procera* in rats as well as to provide on introductory approach for the evaluation of its traditional preparation in order to scientifically validate the therapeutic preparation of this plant in the control of diabetes.

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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