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Research Article

Antidiabetic Activity of Methanol Extract of *Canthium parviflorum* Lam. Leaves

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ABSTRACT

The present investigation was carried out to study the antidiabetic activity of the methanol extract from the leaves of *Canthium parviflorum* Lam. (Family- Rubiaceae) in adult Wistar rats at 100, 200 and 400 mg/kg p.o. respectively using normoglycemic, glucose loaded and alloxan induced hyperglycaemic models. Metformin (250 mg/kg, p.o.) was used as reference standard for the activity comparison. Results of the study revealed significant antidiabetic activity of the methanol extract in a dose dependent manner. The antidiabetic activity showed by the extract may be due to by promoting the insulin release from the undestroyed β-cells or its action may be similar to insulin. Our study supports the folklore claims to its utilization as an antidiabetic medicinal plant and and suggests that leaves possess promising therapeutic activity for the maintenance of diabetes mellitus.

Introduction

Canthium parviflorum Lam. (Family- Rubiaceae) is a thorny sub-scandent shrub with spreading branches and wildly distributed in forests and dry localities. The leaves and fruits are edible and used as herbal medicine to treat diabetes among major tribal groups in South India [1-3]. Traditionally, the leaves and roots are reported to possess astringent, diuretic, febrifuge and anthelmintic properties [4]. Leaf paste is applied to the affected part to treat scabies and ring worm infections [5]. The crude ethanol extract of leaves reported anticancer and antimicrobial activity [6-7].Significant antioxidant and diuretic activity of the leaf extracts has been reported by previous authors [8-9]. The present investigation is carried out to assess the antidiabetic activity of the methanol extract of leaves on alloxan induced diabetic rats.

Materials and methods

Plant material

Fresh leaves of *C. Parviflorum* were collected from Lonavala, Pune (Maharashtra, India) and authenticated by the Botanist Dr. Jayanthi, Botanical Survey of India, Pune. A voucher specimen (No.CACRUP3 dated 31/10/2012) is preserved in the herbarium for future reference. The collected leaves were cleaned under water, shade dried, powdered and stored in cool place until further use.

Drugs and chemicals

Metformin was procured from Franco India Pvt. Ltd. Mumbai, India. Alloxan monohydrate was obtained from Loba Chem, Mumbai, India. All the other chemicals used were of analytical grade.

Experimental animals

The experimental protocol was approved by the institutional animal ethics committee of K.B.H.S.S Trust's Institute of Pharmacy, Malegaon (Maharashtra, India) which was registered with CPCSEA (Committee for the purpose of control and supervision of experiments on animals), Govt. of India (Registration no.IAEC2012/05/2015). Adult Wistar albino rats of either sex (150-200 g) were used for the study. The animals were carefully monitored by keeping in standard environmental condition (12:12 hours) light and dark cycle at 25 ± 2°C with 60-70% relative humidity. Prior to setting up the experiment the animals were carefully marked on different parts of the body, which were later used for their identification during the course of the study.

Preparation of the extract

The air-dried powdered leaves was defatted with petroleum ether for 24 h followed by extracting with methanol using a

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soxhlet apparatus for 48 h. After extraction, the liquid extract was concentrated under vacuum to yield dry extract. The dried residue (extract) was preserved in a desiccator until further use [10].

Screening for Antidiabetic activity

The selected animals were put in to polypropylene cages for randomization and were divided into various groups each containing six animals and numbered. Metformin (250 mg/kg, p.o.) was used as reference drug for activity comparison. The test samples were separately suspended in 0.5% w/v sodium carboxy methyl cellulose and used for the study.

Using normoglycaemic rats

The study was performed on overnight fasted normal rats, which were equally divided into five groups of six rats in each. The baseline blood glucose concentration of the overnight fasted rats were measured before administration of the test samples and taken as zero time (0 h). The control animals received only vehicle (2 ml/kg) through oral route as Group I, while the reference control group (Group II) rats received metformin (250 mg/kg, p.o.). Group III to V animals received either 100, 200 or 300 mg/kg of the methanol extract in a similar manner.After the single dose of treatment, blood was collected through the tail vein of each rat and the glucose level was measured with a pre-standardized blood glucose monitor (Accu-Check Active, Roche Diagnostics, Germany). The blood glucose levels were measured after 1, 2, 4 and 8 h of administration of test samples [11]. The results are presented in Table 1.

Oral Glucose Tolerance Test (OGTT)

Overnight fasted normal rats were divided into five groups of six rats in each. Group l and Group II served as a solvent control and positive control and received either vehicle (2 ml/kg) or metformin (250 mg/kg) through oral route. Other groups of animals (Group III to V) received either 100, 200 or 300 mg/kg of the methanol extract in a similar manner. Thirty minutes after administration of the test samples, the rats of all groups were given glucose (3 g/kg, p.o).The blood samples were collected from the tail vein just prior to glucose administration and at 30, 60, 150 and 180 min after the glucose loading and the levels of blood glucose were measured [12-13].The results are depicted in Table 2.

Using alloxan induced diabetic rats

Experimental diabetes was induced in overnight fasted rats by single intraperitoneal injection (120 mg/kg) of alloxan monohydrate in normal saline. After 1 h, the animals were provided with standard laboratory diet and water *ad libitum*. The blood glucose level was checked before alloxanisation and at every 24 h after alloxanisation as per the method cited earlier. Animals showing fasting blood glucose more than 200 mg/dl were considered diabetic and divided into different groups each comprising of six animals.This condition was noticed at the end of 48 h after alloxanisation.

The selected animals were divided into five groups. GroupI served as solvent control and received only vehicle (2 ml/kg) through oral route. GroupII served as positive control and received metformin (250 mg/kg, p.o.). Other groups (Group III to V) received the extracts at 100, 200 or 300 mg/kg in a similar manner. The level of blood glucose of each animal was measured at 0, 1, 2, 4 and 8 h respectively after administration of single dose of test samples.The results are given in Table 3.

Statistical analysis

All values are expressed as mean ± SEM. Statistical analysis was performed by One-way Analysis of Variance (ANOVA) followed by Dunnet's t-test. A 'p' value less than 0.05 was considered significant.

Results and discussion

Results of the normoglycaemic study of *C. parviflorum*(Table 1) illustrated that the methanol extract reduced the blood glucose concentration in a dose dependent manner. Significant activity was noticed at 8 h for 200 mg/kg dose and 4 h for 300 mg/kg dose level respectively. The percentage reduction of the blood glucose was 32.12 for 300 mg/kg dose level compared to metformin that registered a reduction of 39.75% of the glucose concentration in the blood at the end of 8 h. The study revealed comparable activity of the methanol extract with respect to the standard drug metformin. In Oral glucose tolerance test (Table 2), experimental induction of hyperglycemia by oral administration of glucose (3 g/kg, p.o) resulted an increase in blood glucose concentration after 30 min of administration. The extract treated groups reveled decrease in the glucose concentration with time, in a dose dependent manner. However, significant activity was noticed with 200 and 300 mg/kg dose levels. Comparable activity with the reference drug metformin was noticed at 300 mg/kg dose level.

Single dose of administration of alloxan showed increasing of blood sugar more than two folds than the normal blood sugar level within 48 h[14]. In this study (Table 3), the methanol extract showed reduction in the blood glucose concentration in a dose dependent manner. Significant antidiabetic activity was observed at 200 and 300 mg/kg dose levels. The percentage reduction of blood glucose concentration at the end of 8 h for the extract was found to be 13.22, 29.89 and 53.69 at 100, 200 and 400 mg/kg respectively. Metformin on the other hand registered 61.06% decrease when compared with control group.

Alloxan is known to cause direct and selective cytoxicity to the pancreatic β-cells by causing cell membrane disruption after its intracellular accumulation [15] resulting in a decrease in endogenous insulin secretion and release, which leads to decreased glucose utilization by the tissues [16-17].In our present study, we have observed that test extracts of *C. parviflorum* leaf could reverse the hyperglycaemic condition in diabetic rats and brought about hypoglycaemic action because blood glucose once lowered by the extracts did not increase again throughout experiment as compared to

untreated alloxanized control, where the blood glucose level was always remaining above the initials. The possible mechanism of action of the methanol extract may be due to by promoting the insulin release from the undestroyed β-cells or its action may be similar to insulin [18-19].

The results of the study were quite interesting since the antidiabetic activity of the leaf extract was comparable with metformin.The study further provided a scientific support to the folklore use of the*C. Parviflorum* and suggest that the leaves are endowed with promising therapeutic activity for the maintenance of diabetes mellitus.

Conclusion

Table 1: Effect of *C parviflorum* extract on the blood glucose level in normal rats

Group	Treatment	Dose	Blood glucose concentration (mg/dl)				
			0 h	1 h	2 h	4 h	8 h
I	Control	2 ml/kg	78.73±2.13	77.36±2.12	78.4±2.36	76.93±2.13	77.60±2.02
II	Metformin	250 mg/kg	80.5±3.63	70.5±2.23*(12.42%)	65.16±2.9**(19.05%)	61.5±2.11**(23.60%)	48.5±2.34**(39.7%)
III	Extract	100 mg/kg	79.83±2.68	78.16±2.42(2.09%)	76.33±2.74(4.38%)	73.5±2.52(7.93%)	69.33±2.6(13.15%)
IV	Extract	200 mg/kg	79.5±3.12	76.16±3(4.20%)	74.5±3.3(6.29%)	69.13±3.2(13.04%)	61.36±2.46*(22.81%)
V	Extract	300 mg/kg	81.83±2	76.33±2.04(6.72%)	72.66±2.1(11.20%)	65.16±2.21*(20.37%)	55.54±2.29**(32.12%)

Results expressed as Mean ± SEM from six observations (n = 6). *p<0.05, **p<0.01when compared with control group. Figures in parentheses denote percentage reduction

Table 2: Effect of *C. parviflorum* extract on Oral Glucose Tolerance Test

Group	Treatment	Dose	Blood glucose concentration (mg / dl)				
			0 min	30 min	60 min	120 min	180 min
I	Control	2 ml/kg	79.66±2.29	117.83±2.3	126.83±8.18	132.66±8.1	129.83±8.27
II	Metformin	250 mg/kg	80.5±2.11	118.66±2.18	100.5±5.05*(15.3%)	88.5±5.74**(25.41%)	72.5±5.74**(38.9%)
III	Extract	100 mg/kg	81±2.08	123.83±2.18	119.83±4.22(3.23%)	115.83±4.44(6.46%)	111.66±5.6(9.83%)
IV	Extract	200 mg/kg	79±2.12	121±2.11	113.56±4.79(6.14%)	106.10±5.36(12.31%)	96.78±6.19*(20.01%)
V	Extract	300 mg/kg	80.5±2.07	120.33±2.29	105.73±5.04(12.13%)	95.17±5.82*(20.90%)	80.53±6.58**(33.07%)

Results expressed as Mean ± SEM from six observations (n = 6). *p<0.05, **p<0.01 when compared with control group. Figures in parentheses denote percentage reduction

Table 3: Effect of *C. parviflorum* extract in alloxan induced diabetic rats

Group	Treatment	Dose	Blood glucose concentration (mg/dl)				
			0 h	1 h	2 h	4 h	8 h
I	Control	2 ml/kg	227.5±7.48	236.33±7.43	238.16±7.36	243.33±6	246±5.72
II	Metformin	250 mg/kg	228.16±10.56	191±10.84*(16.28%)	151.16±10.89**(33.74%)	109±11.13**(52.22%)	88.83±10.87**(61.06%)
III	Extract	100 mg/kg	229.50±10.32	220.16±9.92(4.06%)	210±9.52(8.49%)	205.8±9.31(10.32%)	199.16±13.9(13.22%)
IV	Extract	200 mg/kg	224.43±10.85	210.83±11.15(6.05%)	196.10±10.52(12.62%)	181.83±16.02(18.98%)	157.33±15.92*(29.89%)
V	Extract	300 mg/kg	223.62±12.37	204±11.88(8.77%)	194.13±11.82(13.18%)	137.33±16.77*(38.58%)	103.54±12.17**(53.69%)

Results expressed as Mean ± SEM from six observations (n = 6). *p<0.05, **p<0.01 when compared with control group. Figures in parentheses denote percentage reduction

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