Hypoglycemic activity and Phyto-chemical screening on *Coccinia* grandis leaf and fruit

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Abstract: Many plant drugs are studied for effecting in the treatment of Diabetes mellitus. In the present study methanolic extract of herb of Coccinia grandis leaf and fruit was evaluated for hypoglycemic effect in Alloxan induced diabetic rats using oral glucose tolerance test. A comparison was made between the actions of methanolic extract Coccinia grandis leaf and fruit and known antidiabetic drug Glibenclamide (5 mg/kg, p.o). The methanolic extract of Coccinia grandis leaf and fruit was administered at doses to normal and diabetic rats. The methanolic extract at 400 mg/kg body weight dose level exhibited significant hypoglycemic activity (P < 0.01 and P < 0.001). Phytochemical screening of methanolic extract of herb of Coccinia grandis leaf and fruit methanolic extract revealed the presence of alkaloids, saponins and steroids, flavonoids etc. It is concluded that methanolic extract of herb of Coccinia grandis leaf and fruit posses anti hyperglycemic activity.

Keywords: Alloxan induced Diabetes, Coccinia grandis, Hypoglycemic activity, Oral glucose tolerance test.

I. Introduction

Medicinal plants have been identified and used throughout human history. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as insects, fungi and herbivorous mammals. At least 12,000 such compounds have been isolated so far; a number estimated to be less than 10% of the total1, 2. From previous studies done on Coccinia grandis and due to the presence of effective phytochemical constituents, methanolic extract of the plant leaves and fruits were selected for evaluating hypoglycemic activity. Various drugs available in the market for hypoglycemic activity and none of the drug are not up to mark for showing its efficacy. From literature survey it was found that Coccinia grandis effective in treatment of anti-bacterial, anti-hepatotoxicity, antiulcer, anti-tussive, anti-oxidant, anthelmintic activity etc3-18. The study period is 4 days. Animals used are male wistar rats. Before performing the hypoglycemic activity of methanolic extract of the plant leaves and fruits extract.

A. Plant Material Collection

II. Materials and Methods

The plant was collected during the month of Jan 2016; Leaves and fruits of *Coccinia grandis* were collected from Tirupati. The plant authentication was done by Department of Botany, Sri Venkateswara University, Tirupathi dist. Chittoor, Andhra Pradesh. Leaves and fruits of *Coccinia grandis* were dried at room temperature for 2-3 days. The dried leaves and fruits of *Coccinia grandis* powdered in a mixture. The extraction was done by using the process of maceration. 150 grams of fine powder was suspended in 200 mL methanol for 72 hours at room temperature. After 72 hours the extract was taken and residue was dried¹⁹.

B. Experimental Animals

Male wistar rats of 150-200 grams weighed were used for present study. The animals were housed in polypropylene cage (6 animals per cage), the standard conditions were maintained (12 hours light and 12 hours dark cycle, 23 ± 5 C and 40-60% humidity). The standard rat diet, water was provided ad libitum. All the animals were collected from the central animal house Joginpally B.R. Pharmacy College, Amdapur X Road, Yenkapally, Moinabad, Hyderabad, Telangana and all experiments were conducted according to the ethical norms approved by CPCSEA, Ethical Committee IAEC reg.no.769/2011/CPCSEA).

C. Preliminary Phytochemical Screening

The methanolic extract of leaves and fruits of *Coccinia grandis* were found large percentage of Alkaloids, glycosides, triterpenoids, flavonoids Phytosterols and tannins²⁰⁻²¹.

D. Induction of Diabetes by Alloxan²²⁻²⁴

Fasting blood glucose was determined after depriving food for 16 h with free access of drinking water. Hyperglycemia was induced by a single i.p. injection of 120 mg/kg of alloxan monohydrate (Avra synthesis. Ltd., Hyderabad, India) prepared in sterile saline. After 3 days of alloxan injection, the hyperglycemic rats (glucose level > 200mg/dl) were separated and divided into different groups comprising of 6 rats each for the hypoglycemic study.

E. Preparation of Test Drug

The test drugs were prepared by 2% tween 80. Both standard and test drugs were given by oral gavage i.e. per oral route at a dose of 0.4 ml/kg body weight. All drugs were prepared freshly before administration.

F. Experimental Procedure

Oral glucose tolerance test on Diabetic rats (OGTT)-Hypoglycemic activity:

The overnight fasted rats of all the groups were loaded with glucose (2gm/kg p.o) 30 minutes after drug administration. Blood samples were collected from the tail vein puncture method prior to drug administration and at 30, 60, 120 minute after glucose loading. Serum glucose levels were measured immediately. The glucose level was estimated using digital glucometer²⁵

Six fasted animals were used in each group.

Rats were divided into following groups.

Group- I - Received Vehicle- Distilled water (Control -ve)-Normal Rats

Group-II – Received 2 gm /kg glucose p.o. (control +ve)-Diabetic Control

Group III - Received standard drug glibenclamide (10mg/kg), p.o.

Group IV - Received methanolic extract of leaves of Coccinia grandis, dose 400mg/kg, p.o.

Group V - Received methanolic extract of fruits of Coccinia grandis, dose 400mg/kg, p.o.

II-IV group rats are diabetic induced by alloxan injection.

III. Statistical Analysis

The obtained results were expressed as Mean \pm SEM. Comparison between control and treatment groups were performed by one way analysis of variance (ANOVA) followed by Dunnets test. The statistical significance criterion was p< 0.05 (95% level). P<0.05 is considered as significant²⁴.

IV. Results

Methanolic extract of Coccinia grandis leaf and fruit (400 mg/kg) significantly decreased blood glucose level in glucose fed rats at 120 minutes when compared with the control group. It also decreased the elevated blood glucose at 60 minutes after the glucose administration. The control group showed significant increase in blood glucose level when compared with the normal group.

Glibenclamide showed its potent anti-diabetic activity at 120 minutes. Also the reduction in elevated blood glucose level at 30 and 60 minutes after the administration of glucose was significant when compared to the control group.

These data suggested that treatment with Methanolic extract of Coccinia grandis leaf and fruit showed better tolerance to exogenously administered glucose.

V. Discussion

OGTT referred to as the glucose tolerance test, measures the body's ability to metabolize glucose, or clear it out of the blood stream. The test reveals how quickly glucose is metabolized from the blood stream for use by cells as an energy source. The methanolic extract of the leaf and fruit part of plant of Coccinia grandis produced hypoglycemia and improved glucose tolerance in diabetic rat's inspite of counter regulatory factors avoiding reduction in blood glucose levels.

Therefore, hypoglycemic activity of MCG could be mediated by stimulation of surviving beta cells to release more insulin and may be through extra-pancreatic mechanisms. The MCG (400mg/kg) dose showed promising results.

Like the plant extract, Glibenclamide also produced a significant reduction in the blood glucose level of diabetic rats. Glibenclamide exert its action mainly by increasing the secretion of insulin. They only work in diabetics with some remaining beta cells. They bind to the ATP-inhibited K+channels in the beta cell membranes and inhibit channel activity, depolarizing the beta cell membrane and increasing Ca2+ influx and hence insulin release 26 .

The comparable effect of the plant extract with Glibenclamide in this study may suggest similar mechanism of action. These findings appear to be in consonance with the earlier suggestion of Jackson and Bressler(1981)

that sulfonylureas such as Glibenclamide have extra-pancreatic hypoglycaemic mechanism of action secondary to their causing insulin secretion and the attendant glucose uptake into and utilization by the tissues.

Therefore, it can be confirmed that, in present investigation significant hypoglycemic potential of Coccinia grandis shrub may be due to flavonoids, alkaloids, tannins, phenols, phytosterols content, which were confirmed by preliminary phytochemical screening.

VI. Conclusion

The extracts showed increase in the glucose tolerance of the rats and decrease in the fasting blood glucose level of diabetic rats, showing the hypoglycaemic activity of the plant which is most pronounced in methanolic extract.

Result from the phytochemical analysis of Coccinia grandis revealed the presence of alkaloids, tannins, saponins, terpenoids, flavonoids, phenolics and glycosides as the possible biologically active principles, which have also been isolated from the other plants and found to possess hypoglycemic and antidiabetic activity. In nutshell the extracts of Coccinia grandis possesses significant hypoglycaemic activity, which is the first claim in this respect.

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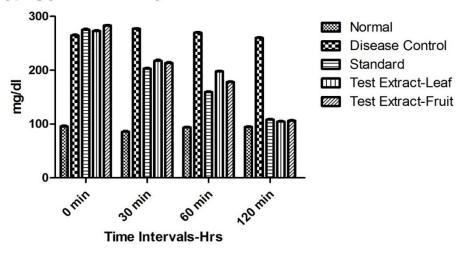
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Phytochemical	70%Methanolic	70%Methanolic
constituents	extract	extract
Alkaloids	+++	+++
Glycosides	++	++
Carbohydrates	-	-
Flavonoids	+++	+++
Saponins	-	-
Tannins	++	++
Steroids	+++	+++
Proteins & Amino acids	-	_
Phytosterols	++	++
Phenols	+++	+++
Triterpenoids	+++	+++

Table No 1: Preliminary phytochemical screening of 70% MCG

Table No 2: Effect of Methanolic extracts of Coccinia grandis on oral glucose tolerance				
test in Diabetic rats				

	Blood glucose level mg/dl			
GROUPS	0 min	30 min	60 min	120 min
	95.69 ± 1.51	85.95 ± 1.37	93.83 ± 0.94	94.51 ± 0.99
Normal				
Control	264.84 ± 1.26^a	277.01 ± 1.19^{a}	269.86 ± 1.01^{a}	260.18 ± 1.12^{a}
(ALX 120mg/kg)				
Standard	275.45 ± 1.52	$203.01 \pm 1.39^{***}$	159.72 ± 1.05***	$108.39 \pm 1.04^{***}$
(GLB 10mg/kg)				
	273.02 ± 1.43	$217.67 \pm 1.60^{***}$	197.94 ± 1.22***	$104.64 \pm 0.89^{***}$
Coccinia grandis leaf (400mg/kg)				
	283.03 ± 1.21	$213.49 \pm 1.34^{\ast\ast\ast}$	178.02 ± 1.18***	106.15 ± 1.03***
Coccinia grandis fruit (400 mg/kg)				



Hypoglycemic Activity-Blood Glucose levels

Graph No 1 Effect of Methanolic extracts of Coccinia grandis on oral glucose tolerance test in Diabetic rats