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The juice of fresh leaves of *Catharanthus roseus* Linn. reduces blood glucose in normal and alloxan diabetic rabbits

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Abstract

Background: The leaf juice or water decoction of *Catharanthus roseus* L. (Apocyanaceae) is used as a folk medicine for the treatment of diabetes all over the world. In the present investigation, the leaf juice of *C. roseus* has been evaluated for its hypoglycemic activity in normal and alloxan-induced diabetic rabbits.

Methods: The blood glucose lowering activity of the leaf juice was studied in normal and alloxan-induced (100 mg/kg, i.v.) diabetic rabbits, after oral administration at doses of 0.5, 0.75 and 1.0 ml/kg body weight. Blood samples were collected from the marginal ear vein before and also at 4, 6, 8, 10, 12, 16, 18, 20 & 24 h after drug administration and blood glucose was analyzed by Nelson-Somogyi's method using a visible spectrophotometer. The data was compared statistically by using Student's *t*-test.

Results: The leaf juice of *C. roseus* produced dose-dependent reduction in blood glucose of both normal and diabetic rabbits and comparable with that of the standard drug, glibenclamide. The results indicate a prolonged action in reduction of blood glucose by *C. roseus* and the mode of action of the active compound(s) of *C. roseus* is probably mediated through enhance secretion of insulin from the β-cells of Langerhans or through extrapancreatic mechanism.

Conclusions: The present study clearly indicated a significant antidiabetic activity with the leaf juice of *Catharanthus roseus* and supports the traditional usage of the fresh leaves by Ayurvedic physicians for the control of diabetes.

Background

Diabetes mellitus is a major endocrine disorder affecting nearly 10% of the population all over the World [1]. Inspite of the introduction of hypoglycemic agents, diabetes and the related complications continue to be a major medical problem. Since time immemorial, patients with non-insulin dependent diabetes mellitus have been treated orally by folklore with a variety of plant extracts. In the indigenous Indian system of medicine (Ayurveda), a mention was made on good number of plants for the cure of diabetes or 'madhumeha' and some of them have been experimentally evaluated and the active principles were isolated [2–8]. However, search for new antidiabetic drugs continues.

Catharanthus roseus (L.) G. Don (Apocyanaceae) is known with various names (Madagascar periwinkle; *Vinca rosea*; *Lochnera rosea*) in India and all over the world. Water decoction of the leaves and/or the whole plant is used as household remedy for diabetes in several countries [9]. Traditionally, in India seven flowers/leaves are used at a time whereas in the Cook Islands 18 leaves boiled in a kettle of water and in West Indies roots of plants infused in whiskey are used to control diabetes [10–12]. Earlier reports indicate significant blood glucose lowering activity with hydroalcoholic or dichloromethane-methanol extracts of leaves of *C. roseus* in laboratory animals [13–19]. The use of fresh leaf juice of *C. roseus* has been in practice by Ayurvedic physicians in India with beneficial action. Hence, in the present study the leaf juice of *C. roseus* has been evaluated for hypoglycemic activity in normal and alloxan diabetic rabbits.

Methods

Plant material

Fresh leaves of *C. roseus* (white variety) were collected from our University campus and were crushed in a stainless steel mortar and squeezed by means of a fine cloth to separate the juice. The authenticity of the plant was done by botanist Dr. M. Venkaiah, Department of Botany, Andhra University and the voucher specimen was kept in the herbarium (No. 0614) of our University.

Chemicals Used

Glibenclamide was provided as a generous gift sample by Hoechst Pharmaceuticals, Mumbai, while alloxan (Sigma Chemical Company, USA) was a generous gift sample from Dr. Reddy's Laboratories, Hyderabad. All other reagents used were of analytical grade.

Animal Experiments

Adult albino rabbits (B.N. Ghosh & Co., Kolkata) of either sex weighing 1.5 – 2 kg were used in the study. They were divided into 10 groups of five each and were provided with a standard diet and water *ad libitum*. All the rabbits were kept in cages with wide square mesh at the bottom to avoid coprophagy and maintained in a well-ventilated animal house with 12 h light and dark cycle. They were fasted for 18 h prior to the experiment, allowing access to water only, and were deprived of both food and water during the 24 h monitoring period of the experiment after the treatment either with the drug or distilled water (control) to minimize the changes in plasma volume. The same procedure has been followed for each treatment. The experimental protocol has been approved by the Institutional Animal Ethics Committee and by the animal regulatory body of the government (Regd. No. 516/01/A/CPCSEA)

Groups I to IV consist of normal animals, as well as the normal control, group V. Groups I, II & III were given the leaf juice of *C. roseus* orally through intragastric intubation at doses of 0.5, 0.75 and 1.0 ml/kg body weight respectively. The gastric tube was washed with distilled water after drug administration, such that each animal received a total volume of 3 ml. Group IV was given glibenclamide at a dose of 40 μg/kg orally in a total volume of 3 ml for each animal. Group V, the normal control received 3 ml of distilled water.

Groups VI to X were rendered diabetic by injecting alloxan (100 mg/kg, i.v) into the marginal ear vein after a base line blood glucose (95.3, 99.7, 105.1, 91.0 and 101.3 mg/dl respectively) estimation was done. After two weeks when the condition of diabetes was stabilized, rabbits with blood glucose above 300 mg/dl were selected for the study.

Animals in groups VI, VII & VIII received the leaf juice of *C. roseus* orally at doses of 0.5, 0.75 and 1.0 ml/kg respectively. Group IX received glibenclamide orally at a dose of 40 μg/kg. Group X served as diabetic control.

Collection of blood and analytical procedure

Blood samples (approx. 0.3 ml) were collected by puncturing the marginal ear vein of each rabbit of a group before and also at 4, 6, 8, 10, 12, 16, 18, 20 & 24 h after oral administration of the drug. The samples were collected into glass vials containing a small quantity of a mixture of potassium oxalate and sodium fluoride as anticoagulant. They were stored at 4 °C in a refrigerator before the analysis of glucose by Nelson-Somogyi's method [20–22] using a visible spectrophotometer.

Data and Statistical Analysis

Data was expressed as mean ± standard error of means. Statistical analysis was made by using Student's unpaired *t*-test.

Results

The leaf juice of *C. roseus* produced a dose-dependent hypoglycemia in normal rabbits. It produced maximum reduction in blood glucose of 16.7% (6 h, $p < 0.05$), 28.6% (18 h, $p < 0.05$), and 31.9% (20 h, $p < 0.01$) with doses of 0.5, 0.75 and 1.0 ml/kg body weight respectively (Table 1). Glibenclamide (40 μg/kg) produced a significant ($p < 0.01$) reduction in blood glucose compared to control (31.9%, 8 h).

Dose-dependent reduction in blood glucose was also observed in alloxan-induced diabetic rabbits treated with *C. roseus*. The percent reduction in blood glucose tended to be higher in the diabetic condition compared to the normal state. A significant reduction ($p < 0.001$) in blood

Table 1: Percentage blood glucose reduction produced by *C. roseus* after oral administration in normal rabbits.

Group (n = 5)	Dose	Initial Blood glucose (mg/dl)	Percent blood glucose reduction								
			4	6	8	10	12	16	18	20	24 (h)
Control	---	105.6	-3.1 ± 5.3	-2.4 ± 6.4	-0.4 ± 6.2	-3.6 ± 5.0	-6.9 ± 6.3	-4.5 ± 4.2	-0.4 ± 9.9	5.9 ± 1.5	-1.5 ± 4.6
C. roseus	0.50 ml/kg	99.2	11.5 ± 0.7*	16.7 ± 1.2*	15.3 ± 3.1	12.6 ± 2.4*	4.9 ± 8.0	4.6 ± 6.6	8.1 ± 2.1	8.9 ± 1.9	1.0 ± 4.4
C. roseus	0.75 ml/kg	101.4	13.9 ± 5.1*	17.1 ± 1.9*	19.5 ± 3.4*	19.7 ± 5.7*	22.8 ± 3.1**	27.0 ± 0.7***	28.6 ± 0.4*	14.7 ± 6.2	4.3 ± 7.1
C. roseus	1.0 ml/kg	108.5	14.1 ± 3.3*	17.5 ± 3.2*	19.2 ± 2.6*	20.8 ± 6.1*	24.4 ± 4.4**	29.6 ± 4.2***	30.7 ± 5.2*	31.9 ± 5.3**	30.0 ± 1.4***
Glibenclamide	40 μg/kg	104.2	25.5 ± 4.1**	29.7 ± 4.9**	31.9 ± 4.8**	29.8 ± 5.2**	28.3 ± 4.7**	23.1 ± 3.6**	21.9 ± 3.6	19.8 ± 5.4	17.3 ± 4.7

Values are mean percent blood glucose reduction (± S.E.M.) of five animals. Significant difference from control at corresponding intervals: *p < 0.05, **p < 0.01, ***p < 0.001

Table 2: Percentage blood glucose reduction produced by *C. roseus* after oral administration in alloxan-induced diabetic rabbits.

Group (n = 5)	Dose	Initial Blood glucose (mg/dl)	Percent blood glucose reduction								
			4	6	8	10	12	16	18	20	24 (h)
Control	---	324.7	1.2 ± 0.3	0.7 ± 0.3	0.1 ± 0.3	-0.5 ± 0.4	-0.5 ± 0.2	0.2 ± 0.5	-0.6 ± 1.9	-0.7 ± 0.1	-0.2 ± 0.3
C. roseus	0.50 ml/kg	316.0	10.5 ± 1.9**	14.9 ± 0.8***	19.6 ± 0.5***	16.85 ± 0.8***	13.4 ± 4.9**	8.9 ± 1.9**	7.7 ± 2.4*	6.3 ± 1.3***	4.4 ± 1.6*
C. roseus	0.75 ml/kg	304.9	14.6 ± 4.5*	16.7 ± 2.5***	22.2 ± 3.8***	24.5 ± 7.4**	27.8 ± 6.5**	27.4 ± 3.5***	31.4 ± 5.6***	25.8 ± 5.1***	19.4 ± 6.4*
C. roseus	1.0 ml/kg	319.2	16.8 ± 5.8*	20.9 ± 5.5**	24.1 ± 2.9***	26.6 ± 4.9***	27.5 ± 9.2*	30.4 ± 6.9*	32.7 ± 6.0***	36.5 ± 4.8***	31.8 ± 8.5**
Glibenclamide	40 μg/kg	323.9	22.6 ± 3.6***	28.5 ± 5.5***	34.9 ± 4.6***	28.7 ± 6.5**	24.8 ± 5.7**	24.6 ± 4.1***	21.3 ± 5.6**	17.4 ± 9.8	14.5± 5.9*

Values are mean percent blood glucose reduction (± S.E.M.) of five animals. Significant difference from control at corresponding intervals: *p < 0.05, **p < 0.01, ***p < 0.001

glucose of 19.6% (8 h), 31.4% (18 h) and 36.5% (20 h) was observed with *C. roseus* at doses of 0.5, 0.75 and 1.0 ml/kg body weight respectively (Table 2). Glibenclamide (40 μg/kg) produced a significant reduction ($p < 0.001$) in blood glucose compared to diabetic control at the 8 h (34.9%).

Discussion

Diabetes mellitus is possibly the world's largest growing metabolic disease, and as the knowledge on the heterogeneity of this disorder is advanced, the need for more appropriate therapy increases [23]. Traditional plant medicines are used throughout the world for a range of diabetic complications. The study of such medicines might offer a natural key to unlock a diabetologist's pharmacy for the future.

Leaves and flowers of *C. roseus* are used traditionally by diabetic patients in India and are taken as water decoction. Due to this reason the leaf juice of the plant was evaluated and the data also confirmed the traditional indications. Earlier investigations [13–19] on the antidiabetic activity of the organic extracts of *C. roseus* by various authors also substantiate the results of our studies in rabbits. Moreover, the fact that the juice has a more prolonged effect (at 1.0 ml/kg) than the glibenclamide dose in the period 18–24 h after treatment indicates a prolonged duration of antidiabetic action and could be due to multiple sites of action possessed by the active principles of *C. roseus*.

Alloxan, a beta-cytotoxin causes a massive destruction of β-cells of the islets of Langerhans resulting in reduced synthesis and release of insulin [24–26]. It is well established that sulphonylureas produce hypoglycemia by increasing the secretion of insulin from pancreas and these compounds are active in mild alloxan-induced diabetes whereas they are inactive in intense alloxan diabetes (nearly all β-cells have been destroyed) [27,28]. Since our results showed that glibenclamide reduced blood glucose levels in hyperglycemic animals, the state of diabetes is not severe. Alloxan-treated animals receiving the leaf juice of *C. roseus* showed rapid normalization of blood glucose levels in comparison to control and this could be due to the possibility that some β-cells are still surviving to act upon by *C. roseus* to exert its insulin releasing effect. More-

over, like sulphonylureas oral administration of *C. roseus* produced hypoglycemia in normal animals. This suggests that the mode of action of the active ingredients of *C. roseus* is probably mediated by an enhanced secretion of insulin, like sulphonylureas. However, the possibility that enhanced tissue glucose utilization by *C. roseus* cannot be ruled out. Further work is obviously required to fractionate, purify and identify the active aqueous principle(s) present in the leaves of *Catharanthus roseus*.

Conclusions

Our study clearly indicated a significant antidiabetic activity with the leaf juice of *Catharanthus roseus* and supports the traditional usage of fresh leaves by the Ayurvedic physicians for the control of diabetes. Hence it might help in preventing diabetic complications and serve as a good adjuvant in the present armamentarium of antidiabetic drugs.

References

1. Burke JP, Williams K, Narayan KMV, Leibson C, Haffner SM and Stern MP: **A population perspective on diabetes prevention: whom should we target for preventing weight gain?** *Diabetes Care* 2003, **26:**1999-2004.
2. Chopra RN, Nayar SL and Chopra IC: **Glossary of Indian Medicinal Plants,.** *New Delhi, CSIR* 1956.
3. Al-Awadi FM and Gumaa KA: **Studies on the activity of individual plants of an antidiabetic plant mixture.** *Acta Diabetologica Latina* 1987, **24:**37-41.
4. Ivorra MD, Paya M and Villar A: **A review of natural products and plants as potential antidiabetic drugs.** *J Ethnopharmacol* 1989, **27:**243-75.
5. Alarcon-Aguilara FJ, Roman-Ramos R, Perez-Gutierrez S, Aguilar-Contreras A, Contreras-Webar CC and Flores-Saenz JL: **Study of the anti-hyperglycemic effect of plants used as antidiabetics.** *J Ethnopharmacol* 1998, **61:**101-10.
6. Chattopadhyay RR: **A comparative evaluation of some blood glucose lowering agents of plant origin.** *J Ethnopharmacol* 1999, **67:**367-72.
7. Ajit K, Choudhary BK and Bandhopadhyay NG: **Preliminary studies on the inorganic constituents of some indigenous hypoglycemic herbs on oral glucose tolerance test.** *J Ethnopharmacol* 1999, **64:**179-84.
8. Grover JK, Yadav S and Vats V: **Medicinal plants of India with antidiabetic potential.** *J Ethnopharmacol* 2002, **81:**81-100.
9. Don G: ***Catharanthus roseus*.** In: *Medicinal Plants of the World* Edited by: *Ross IA. Totowa, New Jersey, Human Press*; 1999:109-18.
10. Cowley RC and Bennett FC: ***Vinca rosea*.** *Australation J Pharm* 1928, **9:**61.
11. Kirtikar KR and Basu BD: ***Vinca rosea.*** In: *Indian Medicinal Plants (II ed.) Allahabad, India, Lalit Mohan Basu Publications* 1933, **Vol III:**1559-60.
12. Sastry BN: **The wealth of India.** *New Delhi, CSIR, Publication and Information Directorate* 1953:205.
13. Pillay PP, Nair CPM and Santi Kumari TN: ***Lochnera rosea* as a potential source of hypotensive and other remedies.** *Bull Research Inst Univ Kerala, Ser. A6* 1959, **1:**51-4.
14. Gordon SH, Marvin G and Marry RA: **Alkaloids of *Vinca rosea* : A preliminary report on hypoglycemic activity.** *Lloydia* 1964, **27:**361-63.
15. Stolle K and Greoger D: ***Catharanthus roseus* – a new medicinal plant.** *Pharm Zentralh Deut* 1967, **106:**285-306.
16. Ghosh RK and Gupta I: **Effect of *Vinca rosea* and *Ficus racemososus* on hyperglycemia in rats.** *Ind J Animal Health* 1980, **19:**145-48.
17. Swanston-Flatt SK, Day C, Flatt PR, Gould BJ and Bailey CJ: **Glycaemic effects of traditional European plant treatments for diabetes. Studies in normal and streptozotocin diabetic mice.** *Diabetes Res* 1989, **10:**69-73.
18. Chattopadhyay RR, Sarkar SK, Ganguli S, Banerjee RN and Basu TK: **Hypoglycemic and antihyperglycemic effect of leaves of Vinca rosea Linn.** *Ind J Physiol Pharmacol* 1991, **35:**145-51.
19. Singh SN, Vats P, Suri S, Shyam R, Kumria MML, Ranganathan S and Sridharan K: **Effect of an antidiabetic extract of Catharanthus roseus on enzymic activities in streptozotocin induced diabetic rats.** *J Ethnopharmacol* 2001, **76:**269-77.
20. Nelson N: **A photometric adaptation of the Somogyi's method for the determination of glucose.** *J Biol Chem* 1944, **153:**375-80.
21. Somogyi N: **A new reagent for the determination of sugars.** *J Biol Chem* 1945, **160:**61-75.
22. Hawk PB and Bernard LO: **Practical Physiological Chemistry.** *New York, McGraw Hill Co* XIII1954:573-75.
23. Baily CJ and Flatt PR: **Antidiabetic drugs, new developments.** *Ind Biotech* 1986, **6:**139-42.
24. Lazarow A: **Alloxan diabetes and mechanism of β-cell damage by chemical agents.** In: *Experimental Diabetes* Edited by: *Lazarow A. Oxford, Blackwell Scientific Publication*; 1964:49-69.
25. Rerup CC: **Drugs producing diabetes through damage of insulin secreting cells.** *Pharmacol Rev* 1970, **22:**485-520.
26. Colca JR, Kotagel N, Brooks CL, Lacy PE, Landt M and McDanield ML: **Alloxan inhibition of Ca^{2+} and calmodulin-dependent protein kinase in pancreatic islets.** *J Biol Chem* 1983, **225:**7260-63.
27. Yallow RS, Black H, Villazan M and Berson SA: **Comparison of plasma insulin levels following administration of tolbutamide and glucose.** *Diabetes* 1960, **9:**356-62.
28. Grodsky GM, Epstein GH, Fanska R and Karam JH: **Pancreatic action of sulphonylureas.** *Fed Proc* 1971, **36:**2719-28.

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