



## ANTIDIABETIC ACTIVITY OF VINCA ROSEA EXTRACTS IN ALLOXAN-INDUCED DIABETIC RATS

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### ABSTRACT

The present study was carried out to evaluate the antidiabetic activity of *Vinca rosea* methanolic whole plant extracts in Alloxan induced diabetic rats for 14 days. The methanolic whole plant extract at high dose (500 mg/kg) exhibited significant anti-hyperglycemic activity than whole plant extract at low dose (300 mg/kg) in diabetic rats. The methanolic extracts also showed improvement in parameters like body weight and lipid profile as well as regeneration of  $\beta$ -cells of pancreas in diabetic rats. Histopathological studies reinforce the healing of pancreas, by methanolic *Vinca rosea* extracts, as a possible mechanism of their antidiabetic activity.

**KEYWORDS:** *Vinca rosea*, Histopathological, Alloxan.

### 1. INTRODUCTION

Diabetes mellitus is one of the common metabolic disorders with micro-and macrovascular complications that results in significant morbidity and mortality. It is considered as one of the five leading causes of death in the world.<sup>[1, 2]</sup> In modern medicine no satisfactory effective therapy is still available to cure diabetes mellitus.<sup>[3]</sup> There is increasing demand by patients to use natural products with antidiabetic activity due to side effects associated with the use of insulin and oral hypoglycemic agents.<sup>[4,6]</sup> There are numerous traditional medicinal plants reported to have hypoglycemic properties such as *Allium sativum* (Garlic), *Azadirachta indica* (Neem), *Vinca rosea* (Nayantara), *Trigonella foenum* (Fenugreek), *Momordica charantia* (Bitter ground), *Ocimum santum* (Tulsi). Many of these are less effective in lowering glucose levels in severe diabetes.

*Vinca rosea* (*C.roseus*) Linn. (Apocynaceae) is an herbaceous subshrub also known as Madagascar periwinkle, *Vinca rosea*, or *Lchnera rosea* worldwide. It is cultivated mainly for its alkaloids, which are having anticancer activities.<sup>[7]</sup> The two classes of active compounds in *Vinca* are alkaloids and tannins. *Catharanthus roseus* produces more than 100 monoterpenoids indole alkaloids (TIA) in different organs.<sup>[8]</sup> The leaves and stems are the sources of dimeric alkaloids, vinacristine and vinblastine that are indispensable cancer drugs, while roots have

antihypertensive, ajmalicine and serpentine.<sup>[9]</sup> The leaves are used traditionally in various regions of the world including India, West Indies as well as Nigeria to control diabetes.<sup>[10]</sup> The leaves have been known to contain 150 useful alkaloids among other pharmacologically active compounds. Significant antihyperglycemic and hypotensive activity of the leaf extracts (hydroalcoholic or dichloromethane-methanol) have been reported in laboratory animals.<sup>[11]</sup> Fresh leaf juice of *C.roseus* has been reported to reduce blood glucose in normal and alloxan diabetic rabbits.<sup>[12]</sup> Leaves and twigs of *Catharanthus roseus* have been reported to have hypoglycaemic activity in streptozotocin induced diabetic rats.<sup>[13]</sup> In this study the prolonged effect (up to 14 day) of the methanolic extracts of whole plant of *Vinca rosea* in fasting blood glucose (FBG) and biochemical parameters such as serum total cholesterol (TC), LDL, HDL, creatinine, urea, and alkaline phosphatase were studied in Alloxan induced diabetic rats. Hence on the above fact no study has been carried out on methanolic extracts of whole plant of *Vinca rosea* in streptozotocin induced diabetic rats. Thus the present study is an attempt to test the antidiabetic activity of whole plant of the *Vinca rosea*.

### 2. MATERIALS AND METHODS

**2.1. Plant Material:** The basic plant material of *Vinca rosea* Linn whole plant used for the investigation was obtained from c camp, Near market, Kurnool Dist,

Andhra Pradesh, India. The plant can be identified authenticated by department of Botany research office (Botanist) Acharya nagarjuna university, Guntur.

**2.2. Alcoholic Extraction:** The whole plants were collected and shade dried. The shade-dried whole plants were subjected to pulverization to get coarse powder. The coarsely powder whole plant (1 kg) of *Vinca rosea* Linn was used for extraction with methanol in Soxhlet apparatus. The extract was evaporated to dryness under vacuum and dried in vacuum desiccator (15.5% w/w).

**2.3. Animals:** Wistar albino rats (8–10 weeks) of both sexes were obtained from the animal house of Kurnool Medical College, Kurnool. Before and during the experiment, rats were fed with standard diet (Gold Mober, Lipton India Ltd). After randomization into various groups and before initiation of experiment, the rats were acclimatized for a period of 7 days under standard environmental conditions of temperature, relative humidity and dark/light cycle. Animals described as fasting were deprived of food and water for 16 hours ad libitum.

**2.4. Oral Glucose Tolerance Test:** Rats were divided into six groups containing six animals in each group. All animals fasted before treatment. Group I was kept as vehicle control which received 5% Tween 80 p.o., group II received glucose only, group III received methanolic extract 300 mg/kg, group IV received methanolic extract 500 mg/kg and group V and VI received only extracts (300 mg/kg and 500 mg/kg) only in a vehicle, respectively. The rats of group III and IV were loaded with glucose (3 g/kg, p.o.) 30 minutes after drug administration. Blood samples were collected from puncturing the retro orbital sinus just prior to drug administration, and 30, 90, 150 minutes after loading glucose. Serum glucose level was measured immediately by using glucose estimation kit (Span Diagnostic Pvt. Ltd. Surat, India).

**2.5. Acute Oral Toxicity Studies:** *Vinca rosea* at the dose range of 100 mg–2000 mg/kg were administered orally to different group of rats comprised of ten rats in each group. Mortality was observed after 72 hours. Acute toxicity was determined according to the method of Litchfield and Wilcoxon.<sup>[14]</sup>

**2.6. Experimental Design:** Five groups of rats, six in each received the following treatment schedule.

Group I: Normal control (saline).

Group II: Alloxan treated control (150 mg/kg.ip).

Group III: Alloxan (150 mg/kg.ip) + *Vinca rosea*. Whole plants extract (300 mg/kg, p.o),

Group IV: Alloxan (150 mg/kg.ip) + *Vinca rosea*. Whole plants extract (500mg/kg, p.o),

Group V: Alloxan (150 mg/kg.ip) + Standard drug, Glibenclamide (5 mg/kg, p.o).

Whole plant extracts and standard drug glibenclamide (5 mg/kg) and saline were administered with the help of feeding cannula. Group I serve as normal control, which received saline for 14 days. Group II to Group V are diabetic control rats. Group III to Group V (which previously received alloxan) are given a fixed dose whole plants extract (300 mg/kg, p.o), (500 mg/kg, p.o) and standard drug glibenclamide (5 mg/kg) for 14 consecutive days.

**2.7. Induction of Diabetes in Experimental Animals:** Rats were made diabetic by a single intraperitoneal injection of alloxan monohydrate (150 mg/kg).<sup>[15]</sup> Alloxan was first weighed individually for each animal according to the body weight and then solubilized with 0.2 ml saline (154 mM NaCl) just prior to injection. Two days after alloxan injection, rats with plasma glucose levels of >140 mg/dl were included in the study. Treatment with plant extracts was started 48 h after alloxan injection.

**2.8. Collection of Blood Sample and Blood Glucose Determination:** Blood samples were drawn from tail tip of rat at weekly intervals till the end of study (i.e., 2 weeks). Fasting blood glucose estimation and body weight measurement were done on day 1, 7 and 14 of the study. Blood glucose estimation can be done by one touch electronic glucometer using glucose test strips.

On day 14, blood was collected from retro-orbital plexus under mild ether anesthesia from overnight fasted rats and fasting blood sugar was estimated.<sup>[16]</sup> Serum was separated and analyzed for serum cholesterol<sup>[17]</sup>, serum triglycerides by enzymatic DHBS colorimetric method<sup>[18]</sup>, serum HDL<sup>[19]</sup>, serum LDL<sup>[20]</sup>, serum creatinine<sup>[21]</sup>, serum urea<sup>[22]</sup> and serum alkaline phosphatase hydrolyzed phenol amino antipyrine method<sup>[23]</sup> was estimated. The whole pancreas from each animal was removed after sacrificing the animal and was collected in 10% formaline solution and immediately processed by the paraffin technique. Sections of 5 $\mu$  thickness were cut and stained by haematoxylin and eosin (H & E) for histological examination.

**2.9. Statistical Analysis:** All the values of body weight, fasting blood sugar and biochemical estimations were expressed as mean $\pm$ standard error of mean (S.E.M.) and analyzed for ANOVA and post hoc Dunnett's *t*-test. Differences between groups were considered significant at  $P < .01$  levels.

### 3. RESULTS

**3.1. Glucose Tolerance:** The effects of extracts of *Vinca rosea* (500 mg/kg and 300 mg/kg) on glucose tolerance test are shown in Figure 2. The supplementation of *Vinca rosea* improved the glucose tolerance in the fasted normal rats. After that serum glucose level was lowered significantly ( $P < .05$ ) at 90 minutes and varied significantly ( $P < .01$ ) lowered at 150 minutes. Extract

also showed significant hypoglycemic effect after 90 minutes of treatment.

**3.2. Experimental Results:** The acute oral toxicity study of *Vinca rosea* showed no mortality upto 2000 mg/kg. Administration of alloxan (150 mg/kg, i.p.) lead to 1.5-fold elevation of fasting blood glucose levels, which was maintained over a period of 2 weeks. Two weeks of daily treatment of various extract of *Vinca rosea* lead to a dosedependent fall in blood sugar levels by 25%–50%. Effect was maximum till 14 days of treatment. Vehicle control animals were found to be slightly increased in their body weight but diabetic rats showed significant reduction in body weight during 14 days. Alloxan caused body weight reduction, which is reversed by whole plant extract at high dose (500 mg/kg) is more effectively than whole plant extract at low dose (300 mg/kg) after 14

days of treatment. Alloxan treatment will increase the serum enzymes levels such as cholesterol, LDL, creatinine, urea and alkaline phosphatase and decrease the HDL level, but glibenclamide (5mg/kg) and whole plant extracts of *Vinca rosea* reversed the above alloxan induce changes (Table 1). Histopathological studies (Figure 1) showed normal acini and normal cellular population in the islets of Langerhans in pancreas of control rats (Group I). Extensive damage to the islets of Langerhans and reduced dimensions of islets (Group II), restoration of normal cellular population size of islets with hyperplasia by glibenclamide (Group V) were also shown. The partial restoration of normal cellular population and enlarged size of  $\beta$ -cells with hyperplasia were shown by methanolic extracts (Group III & Group IV).

**Table 1: Effect of various groups of *Vinca rosea* on serum profile in alloxan (150 mg/kg, i.p.) induced diabetic albino rats after 14 days of treatment.**

Groups	Cholesterol(mg/dl)	H.D.L(mg/dl)	L.D.L(mg/dl)	Creatinine(mg/dl)	Urea(mg/dl)	Alkaline Phosphatase (mg/dl)
Normal control	145.36 $\pm$ 3.2	36.83 $\pm$ 2.5	91.32 $\pm$ 1.2	0.54 $\pm$ 0.3	31.83 $\pm$ 2.2	120 $\pm$ 3.2
Diabetic control	271.16 $\pm$ 10.5	30.00 $\pm$ 1.9	189 $\pm$ 12.4	2.4 $\pm$ 0.1	62.6 $\pm$ 1.8	276.00 $\pm$ 3.6
Alloxan +Whole plant extract (300 mg/kg,p.o)	184.32 $\pm$ 2.5*	34.22 $\pm$ 4.3*	120.27 $\pm$ 1.4*	0.98 $\pm$ 0.3*	43.32 $\pm$ 3.8*	146.35 $\pm$ 4.9*
Alloxan +Whole plant extract (500 mg/kg,p.o)	158.46 $\pm$ 5.6*	36.63 $\pm$ 2.1*	93.65 $\pm$ 3.6*	0.60 $\pm$ 0.2*	32.33 $\pm$ 2.0*	135.55 $\pm$ 4.9*
Alloxan+glibenclamide (5 mg/kg)	145.42 $\pm$ 5.3*	36.73 $\pm$ 1.5*	92.35 $\pm$ 3.1*	0.58 $\pm$ 0.1*	31.24 $\pm$ 4.0*	130.75 $\pm$ 2.9*

Values are given as mean  $\pm$  SEM for groups of six animals each \* $P < .01$  (Dunnet  $t$ -test). Diabetic control was compared with the vehicle control and extract treated groups were compared with the diabetic control.

#### 4. DISCUSSION

In light of the results, our study indicates that methanolic extracts of *Vinca rosea* have good antidiabetic activity. Alcoholic extracts of *Vinca rosea* exhibited significant antihyperglycemic activities in alloxan-induced hyperglycemic rats without significant change in body weight; they can also improve the condition of Diabetic mellitus as indicated by parameters like body weight & lipid profile along with serum creatinine, serum urea and serum alkaline phosphatase. The renewal of  $\beta$  cells in diabetes have been studied in several animal models. The total  $\beta$  cell mass reflects the balance between the renewal and loss of these cells. It was also suggested that regeneration of islet  $\beta$  cells following destruction by alloxan may be the primary cause of the recovery of alloxan-injected guinea pigs from the effects of the drug.<sup>[24]</sup> *Vinca rosea* whole plant alcoholic extracts has been shown to act by  $\beta$  cell regeneration. Similar effects in streptozotocin-treated diabetic animals were reported by pancreas tonic<sup>[25]</sup>, ephedrine<sup>[26]</sup> and *Gymnema sylvestre* leaf extracts.<sup>[27]</sup>

In our studies, the damage of pancreas in alloxan-treated diabetic control rats (Group II) and regeneration of  $\beta$  cells by glibenclamide (Group V) was observed. It is found that methanolic whole plant extract at high dose

(500 mg/kg) is more effective than whole plant extract at low dose (300 mg/kg) after 14 days of treatment. Hence the above discussion reveals that methanolic whole plant extract at high dose (500 mg/kg) is more effective and shows similar curative effect as standard that is, glibenclamide (5mg/kg). This could be due to the possibility that some  $\beta$ -cells are still surviving to act upon by *Vinca rosea* extract to exert its insulin releasing effect. Histopathological studies reinforce the healing of pancreas, by *Vinca rosea* extracts, as a possible mechanism of their antidiabetic activity.

#### 5. CONCLUSIONS

The whole plant extracts did not show a consistent effect on normal blood sugar levels but it effectively reversed the alloxan-induced changes in the blood sugar level and the beta-cell population in the pancreas. It also showed a protective effect when it was given prior to alloxan administration. The action of whole plant extracts on the pancreatic beta-cells and absence of acute toxicity may offer a new hope to the diabetics in future. From the above discussion it conclude that alcoholic whole plant extracts of *Vinca rosea* at high dose (500 mg/kg) exhibited significant antihyperglycemic activity than whole plant extract at low dose (300 mg/kg) in alloxan-induced diabetic rats. These extracts also showed

improvement in parameters like body weight and lipid profile as well as regeneration of  $\beta$  cells of pancreas and so might be of value in diabetes treatment. Further investigation is necessary to determine the exact phytoconstituents (s) responsible for antidiabetic effect.

## REFERENCES

- V. Vats, S. P. Yadav and J. K. Grover, "Ethanol extract of *Ocimum sanctum* leaves partially attenuates streptozotocin-induced alterations in glycogen content and carbohydrate metabolism in rats," *Journal of Ethnopharmacology*, 2004; 90(1): 155–160.
- G. P. S. Kumar, P. Arulselvan, D. S. Kumar, and S. P. Subramanian, "Anti-diabetic activity of fruits of *Terminalia chebula* on streptozotocin induced diabetic rats," *Journal of Health Science*, 2006; 52(3): 283–291.
- G. Sumana and S. A. Suryawashi, "Effect of *Vinca rosea* extracts in treatment of alloxan diabetes in male albino rats," *Indian Journal of Experimental Biology*, 2001; 39: 748–758.
- R. R. Holman and R. C. Turner, "Oral agents and insulin in the treatment of NIDDM," in *Text Book of Diabetes*, J. Pickup and G. Williams, Eds., pp. 467–469, Blackwell, Oxford, UK, 1991.
- B. Kameswara Rao, M. M. Kesavulu and Ch. Apparao, "Antihyperglycemic activity of *Momordica cymbalaria* in alloxan diabetic rats," *Journal of Ethnopharmacology*, 2001; 78(1): 67–71.
- B. Kameswara Rao, R. Giri, M. M. Kesavulu and Ch. Apparao, "Herbal medicine," in *The Management by Indigenous Resources*, J. S. Bajaj, Ed., pp. 375–377, Diabetes Mellitus in Developing Countries. Interprint, New Delhi, India, 1997.
- C. A. Jaleel, R. Gopi, G. M. A. Lakshmanan, and R. Panneerselvam, "Triadimefon induced changes in the antioxidant metabolism and ajmalicine production in *Catharanthus roseus* (L.) G. Don," *Plant Science*, 2006; 171(2): 271–276.
- M. A. Jordan, D. Thrower and L. Wilson, "Mechanism of inhibition of cell proliferation by *Vinca* alkaloids," *Cancer Research*, 1991; 51(8): 2212–2222.
- R. N. Kulkarni, K. Baskaran, R. S. Chandrashekar and S. Kumar, "Inheritance of morphological traits of periwinkle mutants with modified contents and yields of leaf and root alkaloids," *Plant Breeding*, 1999; 118(1): 71–74.
- R. C. Cowley and F. C. Bennett, "*Vinca rosea*," *Australian Journal of Pharmacy*, 1928; 9: 61.
- P. P. Pillay, C. P. M. Nair and T. N. Santi Kumari, "*Lochnera rosea* as a potential source of hypotensive and other remedies," *Bulletin of Research Institute of the University of Kerala*, 1959; 1: 51–54.
- S. Nammi, K. M. Boini, S. D. Lodagala, and R. B. S. Behara, "The juice of fresh leaves of *Catharanthus roseus* Linn. Reduces blood glucose in normal and alloxan diabetic rabbits," *BMC Complementary and Alternative Medicine*, 2003; 3: article 4.
- S. N. Singh, P. Vats, S. Suri et al., "Effect of an antidiabetic extract of *Catharanthus roseus* on enzymic activities in streptozotocin induced diabetic rats," *Journal of Ethnopharmacology*, 2001; 76(3): 269–277.
- J. T. Litchfield and F. A. Wilcoxon, "Simplified method of evaluating dose effect experiments," *Journal of Pharmacology and Experimental Therapeutics*, 1949; 96: 99–133.
- R. V. Aruna, B. Ramesh and V. N. R. Kartha, "Effect of betacarotene on protein glycosylation in alloxan induced diabetic rats," *Indian Journal of Experimental Biology*, 1999; 37(4): 399–401.
- B. P. Giordano, W. Thrash, L. Hollenbaugh et al., "Performance of seven blood glucose testing systems at high altitude," *The Diabetes Educator*, 1989; 15(5): 444–448.
- P. Roeschlau, E. Bernt, and W. Gruber, "Enzymatic determination of total cholesterol in serum," *Zeitschrift für klinische Chemie und Klinische Biochemie*, 1974; 12(5): 226.
- P. H. Mueller, R. M. Schmuelling, and H. M. Liebich, "A fully enzymatic triglyceride determination," *Journal of Clinical Chemistry and Clinical Biochemistry*, 1977; 15(9): 457–464.
- C. C. Allain, L. S. Poon and C. S. G. Chan, "Enzymatic determination of total serum cholesterol," *Clinical Chemistry*, 1974; 20(4): 470–475.