EFFECT OF METHANOLIC EXTRACT OF *VINCA ROSEA* LEAVES ON GLYCEMIC CONTROL AND ANTIOXIDANT STATUS IN ALLOXAN INDUCED DIABETES IN RATS

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Summary

In the present study, methanolic extract of *vinca rosea (v.rosea)* leaves was administered orally @ 100 & 200mg/kg body weight to alloxan induced diabetic rats for 15 days. This resulted in significant reduction ($p<0.01$) in blood glucose level in alloxan diabetic rats. The levels of blood glycosylated hemoglobin (HbA1c), serum AST & ALT significantly ($p<0.01$) increased in alloxan induced diabetic rats however, these levels returned to normal in *V.rosea (100&200)* treated rats. There was a significant ($p<0.01$) increase in LPO and reduction in the activities of antioxidant enzymes CAT & SOD in alloxan diabetic rats. The administration of methanolic extracts of *V.rosea* @ 100 & 200mg/kg significantly decreased the LPO in alloxan diabetic rats. CAT level elevated significantly by *V.rosea* (200).However, glibenclamide restored blood glucose, HbA1c, ALT, AST, LPO, CAT and SOD and the restoration was greater than the treated groups. The studies therefore indicated that *V.rosea* possesses antidiabetic activities along with good antioxidant properties and can be used as an adjunct therapy in diabetes.

Keywords: Diabetes, Alloxan, *vinca rosea*

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Introduction

Diabetes mellitus is a syndrome of disordered metabolism, usually due to a combination of hereditary and environmental causes, resulting in abnormally high blood sugar levels (1).Overproduction (excessive hepatic glycogenolysis and gluconeogenesis) and decreased utilization of glucose by the tissue is believed to be the fundamental mechanism underlying hyperglycemia (2). It is considered as one of the five leading causes of death in the world (3). The prevalence of it will be 5.4% by the year 2025, with the global diabetic population reaching to 300 million. Among all the WHO regions, Southeast Asian region are highest affected with maximum global burden of the disease and by the year 2025 there will be nearly 80 million diabetic in the region (4).It has been noticed that certain cases of diabetes mellitus resistant to insulin, sulphonylureas, biguanides, etc responded well when treated with herbal preparations, alone or in combination with insulin or other oral hypoglycemic agents (5). *Vinca rosea* is in the Apocynaceae family, well known for being rich in alkaloids.

The extracts of *Vinca* have demonstrated significant anticancer activity against numerous cell types (6). Its vasodilating and memory-enhancing properties have been shown to alleviate vascular dementia and Alzheimer's disease (7, 8). In this study we have investigated the effect of the methanolic extract of *vinca rosea (v.rosea)* leaves (MVRLEt) on glycemic control and antioxidant status in male wistar rats.

Materials and methods

Animals

Albino Male Wistar rats (150 to 200 g body weight) procured from disease free animal house, Hisar (Haryana, India) were housed in standard environmental conditions (23 ± 1°C, 12-hour light/dark cycles) and fed with standard pellet diet and water ad libitum. The animals were acclimatized in new environment for 2 weeks. All the experiments were performed in accordance with the guidelines of Institutional Animal Ethical Committee.

Plant material

The leaves of *Vinca rosea* were collected from the vicinity of palampur H.P. India. These were washed with distilled water, shade dried, powdered and stored in an air- tight container until for further use. The powder was used for preparation of extracts.

Preperation of methanolic Vinca rosea leaf extract (MVRLEt)

The 100g fine leaf powder of *vinca rosea* was soaked in 800ml of methanol for 24 h with continuous stirring. The mixture was filtered through filter paper. The filterate was vacuum dried in rotary vacuum evaporator. The extract was lyophilized and stored at 4˚c till further use.

Induction of experimental diabetes

Overnight fasted male wistar rats were made diabetic by injecting alloxan monohydrate (SDFCL, Mumbai) @ 120 mg/kg body weight intraperitoneally (in ice cold normal saline). Fasting Blood Glucose (FBG) was measured 72 hours after alloxanization by using glucose oxidase reagent strips with glucometer. All the animals were found diabetic; however rats showing blood glucose level above 200 mg/dl were considered as diabetic and were used in the study.

Antidiabetic activity screening in experimentally induced diabetic rats

A total of 30 rats were used for the present work and they were divided into five groups comprising six rats each. Suspension of *V.rosea* leaf extract & glibenclamide were prepared in 1% (w/v) Tween-80 solution. GroupI and Group II served as normal and diabetic control respectively and received vehicle i.e. 1% Tween-80 solution (1ml/kg,orally).Group III, Group IV received (MVRLEt) @100 and 200mg/kg (p.o) respectively and groupV received standard antidiabetic drug glibenclamide (Daonil, Aventis pharma) @ 5mg/kg (p.o.),once daily for 15 days.

Estimation of Blood Glucose

Fasting blood glucose levels were estimated before the experiment (Day0) and at the end of 10th and 15th day with the help of glucometer (Bayer's) using glucose oxidase reagent strips. For estimation of blood glucose level, a drop of blood was obtained by puncturing the tip of tail vein.

Biochemical determinations

After 15 days of treatment, overnight fasted rats were sacrificed & blood was collected. Glycosylated hemoglobin (HbA1c) was determined in heparanized whole blood (9). The serum was separated and analyzed for enzymes aspartate amino transferase (AST) and alanine amino transferase (ALT) by using siemens kit. A portion of liver tissue was homogenized & the extract was used for the estimation of enzymatic antioxidants; Catalase activity (CAT) [10], reduced glutathione (GSH) [11], superoxide dismutase (SOD) [12] and Lipid peroxidation (LPO) [13].

Statistical analysis

Statistical analysis was carried out using ANOVA followed by Dunnet's test. A 'p' Value < 0.01 was considered to be significant.

Results and Discussion

Oral administration of MVRLEt produces significant ($p<0.01$) and sustained fall in blood glucose level both @ 100 & 200mg/kg on day 10 and 15. However, the fall in blood glucose was greater with MVRLEt @ 200mg/kg but lesser when compared with glibenclamide treated group @ 5mg/kg (p.o) (Table 1). In an earlier study (14) also observed significant reduction in blood glucose in alloxan diabetic rats on oral administration of extract of flowers of *V.rosea* @ 0.5ml/rat/day for 4 weeks.

Tab 1: Effect of daily administration of MVRLEt on blood glucose level in alloxan-induced diabetic rats [mean±SE]. Figure in parenthesis indicates % fall in blood glucose level as compared to Day 0**.**

Group (dose mg/kg,po)	Blood Glucose level (mg/dl)		
	Pretreatment	Post treatment	
	Day0	Day10	Day15
Normal control	75.83^{a}±2.48	76^{a}±2.05	76.3^{a}±2.54
Diabetic control	326.3^{b}±7.14	293.67^{b} ±13.95 (10%)	286.5^{b} ±9.56 (12.2%)
MVRLEt(100)	312.3^{b}±19.76	213.83^{c}±11.50 (31.53%)	202.5^{c} ±8.29 (35.16%)
MVRLEt (200)	302.6^{b} ±17.15	192.17^{c} ±9.79 (36.49%)	174.5^{c} ±8.58 (42.3%)
Glibenclamide (5)	294.3^{b} ±10.35	176.3^{c} ±4.54 (40.09%)	164.5^{c} ±8.14 (44.10%)

[n=6], Means within a column with different superscripts differ at $p<0.01$ using Dunnet's test as a post hoc test

There was a significant ($p<0.01$) increase in HbA1c, serum AST , ALT and LPO levels in alloxan-induced diabetic rats as compared to control rats. The administration of MVRLEt (100&200mg/kg) & glibenclamide caused significant ($p<0.01$) decrease in the values of these

parameters. However, the decrease is much greater in glibenclamide treated rats (Table 2). The increase in the glycosylated levels (HbA1c) is due to interaction of haemoglobin with excess of glucose (15). The rise in glycosylated haemoglobin is a more reliable indicator of the diabetes. A significant ($p<0.01$) reduction in the activities of antioxidant enzymes CAT and SOD was observed in alloxan induced diabetic rats, indicating a free radical damage in diabetic rats. MVRLEt(200mg/kg) significantly ($p<0.01$) elevated CAT. However, glibenclamide significantly ($p<0.01$) restored CAT and SOD (Table 2). Our studies are in agreement with the findings of (Kaleem et al. 2005) who observed decreased concentration of antioxidant enzymes, catalase & glutathione peroxidase in alloxan induced diabetic rats however these levels returned to normal in insulin, *P.nigrum* and *V.rosea* treated rats.

Tab 2: Effect of MVRLEt on biochemical parameters in alloxan diabetic rats (mean±SE)

Group,dose (mg/kg,p.o)	GHbA1c (%)	ALT[1]	AST[2]	LPO [3]	GSH[4]	CAT[5]	SOD[6]
Normal Control	4.94[a]± 0.109	47.5 [a] ± 2.277	73 [a] ± 2.309	6.34 [a] ± 0.0417	0.2 [a] ± 0.017	76.4 [a] ± 3.402	36.22 [a] ± 1.742
Diabetic control	7.64[b]± 0.047	104.3 [b] ± 2.753	139 [b] ± 1.461	10.21 [b] ± 0.672	0.15 [a] ± 0.009	38.87 [b] ± 4.270	10.86 [b] ± 2.111
MVRLEt (100)	7.22[c]± 0.055	84.17 [c] ± 2.94	118.33 [c] ± 3.20	7.86 [c] ± 0.067	0.15 [a] ± 0.015	50.15 [b] ± 1.845	18.78 [b] ± 1.977
MVRLEt (200)	6.78 [c] ± 0.076	75.5 [c] ± 3.03	109.66 [c] ± 3.42	7.33[a]± 0.20	0.19 [a] ± 0.008	58.73 [c] ± 2.245	18.99 [b] ± 5.72
Glibenclamide (5)	5.48 [c] ± 0.057	65.5 [c] ± 1.232	81.0 [a] ± 5.260	6.74 [a] ± 0.257	0.2 [a] ± 0.003	63.65 [a] ± 3.127	27.47 [a] ± 3.821

[n=6], Means within a column with different superscripts differ at $p<0.01$ using Dunnet's test as a post hoc test.

[1, 2](IU/L), [3] nM MDA/g in tissue, [4](nM/g), [5](μmol H2O2 utilized/min/mg of protein), [6](Units/ gm protein)

Conclusion

Oral administration of methanolic extract of *V.rosea* leaves @ 100 & 200 mg/kg to alloxan induced diabetic rats for 15 days, resulted in significant reduction in blood glucose level, HbA1c, serum AST, ALT and LPO levels .Significant increase in the activities of CAT was observed by *V.rosea* leaves @ 200 mg/kg. However, glibenclamide restored blood glucose, HbA1c, ALT, AST, LPO, CAT and SOD and the restoration was greater than the treated groups. Thus, the present study indicated the possible use of *V.rosea* as an adjunct therapy in diabetes.

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