

Study of Anti-diabetic Activity of *Vinca Rosea* Extracts In Insulin Induced Mice

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ABSTRACT: The present research study was carried out to evaluate the evaluation of anti-diabetic activity of *Vinca rosea* ethanolic extraction of whole plant extracts in insulin induced diabetic mice for 14 days. The ethanolic whole plant extract at high dose (500 mg/kg) exhibited significant anti-hyperglycemic activity than whole plant extract with at low dose (300 mg/kg) in diabetic rats. The plant ethanolic extracts also expressed improvement in parameters such as, body weight and lipid profile as well as regeneration of β -cells of pancreas in diabetic rats. Histopathological studies reveal the healing of pancreas, by *Vinca rosea* extracts, as a possible mechanism of their anti-diabetic activity.

KEY WORDS- Antidiabetic Activity, *Vinca Rosea*, Insulin Induced Mice, Diabetes mellitus, Marine drug.

List of Abbreviation:	
<i>V. rosa</i>	<i>Vinca Rosea</i>
Conc.	Concentration
Mg	Miligram
MI	Mililitre
Hr	Hour
p.o	Per oral
Fig	Figure
Wt.	Weight
FBG	Fasting blood glucose
TC	Total cholesterol
Aqu	Aqueous

INTRODUCTION

Glucose, a primary source of the energy in living cells and have a serious role in biology. Diabetes can result in raised up blood glucose levels which pose a severe hazard in human health. This is an overgrowing universal public disease and is characterized by lacking of insulin formation or distribution properly in the body, causing the death about 1.6 million people per year globally [1- 3]. Diabetes mellitus (figure 1), commonly known as diabetes, is a metabolic disease that causes high blood sugar. The hormone insulin moves sugar from the blood into your cells to be stored or used for energy. With diabetes, your body either doesn't make enough insulin or can't effectively use the insulin it does make. Untreated high blood sugar from diabetes can damage your nerves, eyes, kidneys, and other organs [2-3].

Vinca rosea (*Catharanthus roseus* L.), a medicinal plants which have been investigated for decades. It is cultivated mostly for its alkaloids, which have anticancer activities. Alkaloids and tannins are two classes of phytochemicals present in *V. rosea* that produces more than 100 monoterpenoids indole alkaloids (TIA) in several organs [4]. The stems and leaves are the main sources of dimeric alkaloids, vinblastine and vinacristine which are indispensable cancer drugs, however roots have antihypertensive, serpentine and ajmalicine property. Traditionally the leaves are used in several regions of the world including India, West Indies as well as Nigeria to control diabetes. The leaves have been well-known to contain about 150 useful alkaloids among other phytochemicals. Significant antihyperglycemic and anti-hypertensive leaf extracts (dichloromethane-methanol or hydroalcoholic) have been reported in laboratory animals [5].

There are a few different types of diabetes-

Type 1 diabetes is an autoimmune disease. The immune system attacks and destroys cells in the pancreas, where insulin is made. It's unclear what causes this attack. About 10 percent of people with diabetes have this type.

Type 2 diabetes occurs when your body becomes resistant to insulin, and sugar builds up in your blood.

Prediabetes occurs when your blood sugar is higher than normal, but it's not high enough for a diagnosis of type 2 diabetes.

Gestational diabetes is high blood sugar during pregnancy. Insulin-blocking hormones produced by the placenta cause this type of diabetes.

A rare condition called diabetes insipidus is not related to diabetes mellitus, although it has a similar name. It's a different condition in which your kidneys remove too much fluid from your body.

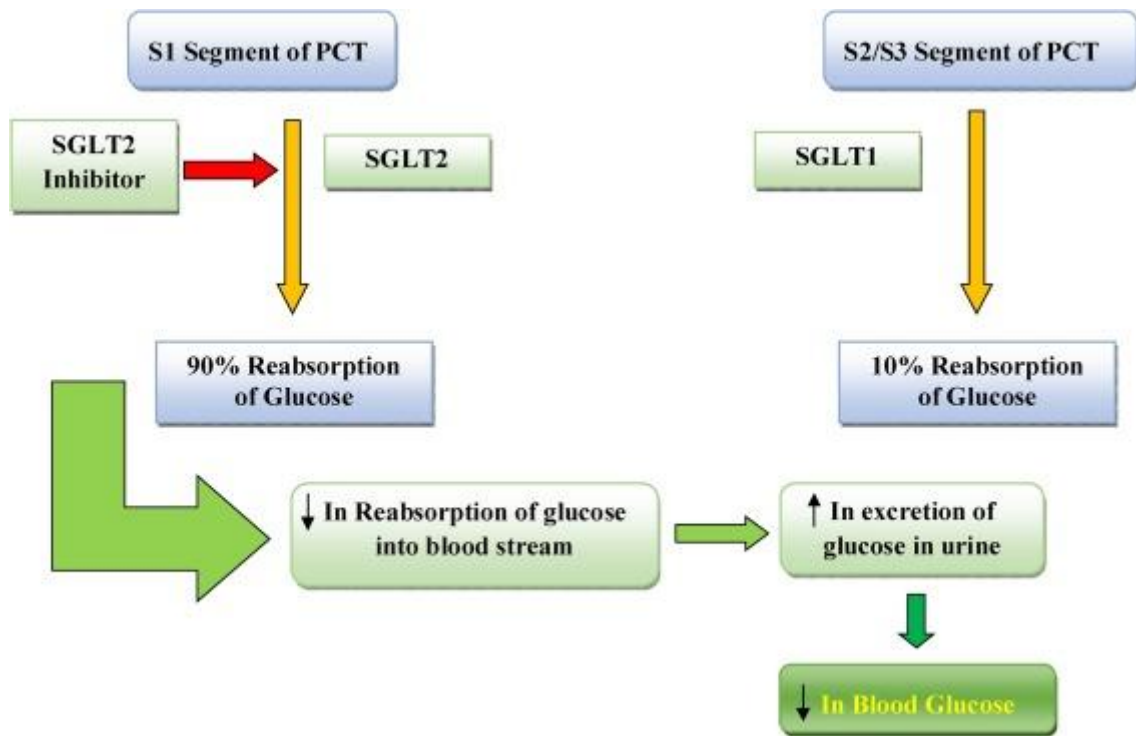


Fig 1: Mechanism of diabetes mellitus

The role of anti-diabetic drugs in treatment

Antidiabetic drugs are not designed to cure diabetes, but they help diabetes patients to keep their condition under control and lower the risk of diabetes complications.

People with diabetes may need to take antidiabetic drugs for their whole lives in order to control their blood glucose levels and avoid hypoglycemia and hyperglycemia.

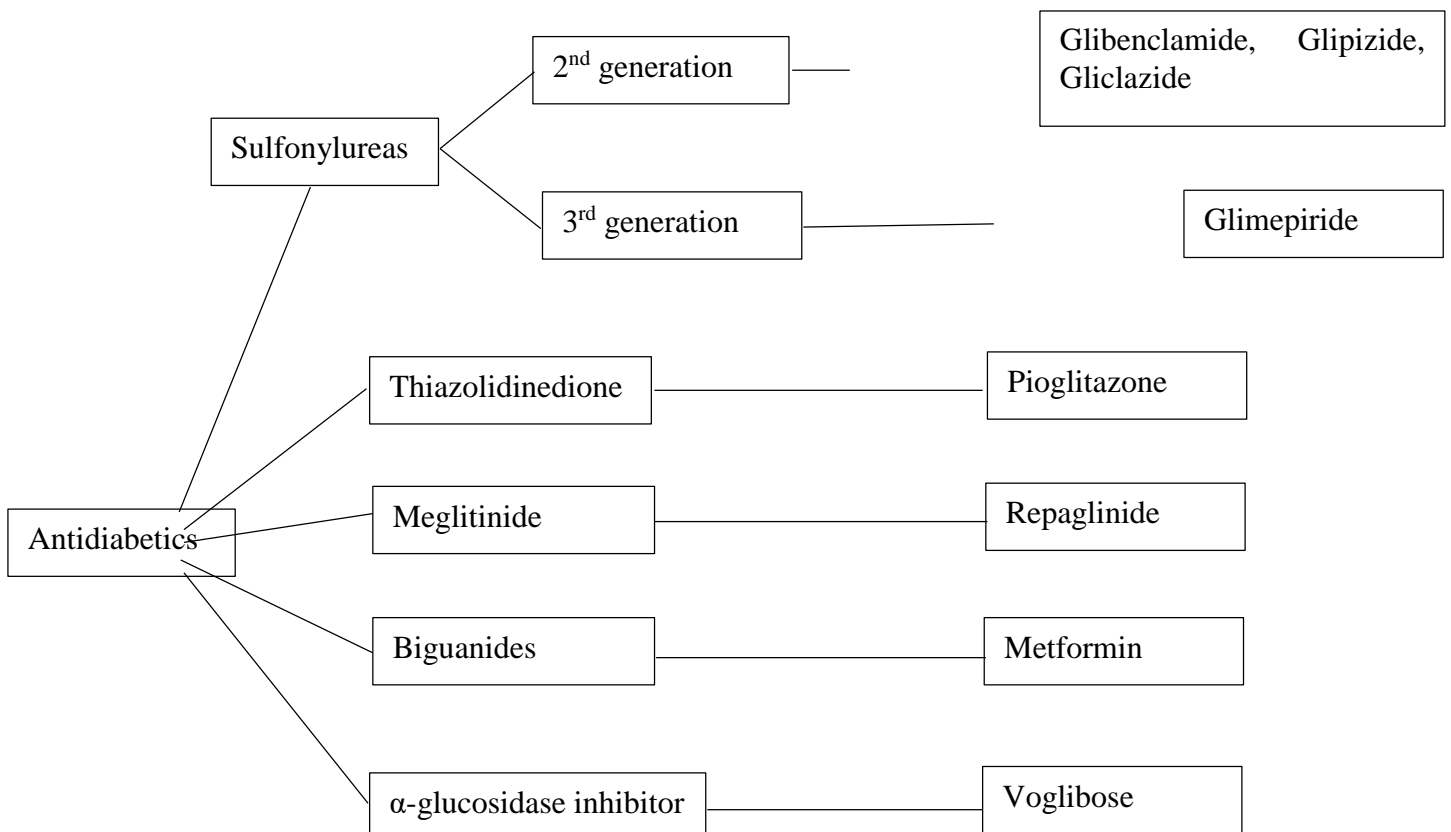


Fig 2: Classification of anti-diabetic drugs

MATERIALS AND METHOD

Plant Material

The basic plant material of *V. rosea* Linn whole plant used for the investigation was obtained from Popular Garden Center Nursery, Kolkata, West Bengal, India. The plant can be identified, authenticated by department of Botanical Survey of India, Kolkata.

Plant Extract

The whole plants were collected and kept for shadow dry. The shade-dried whole plants were subjected to pulverization to get coarse powder. The coarsely powder whole plant (500mg) of *V. 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).

Experimental Animal

Healthy adult Swiss albino mice weighing about 18-20 g between 2 and 3 months of age were used for the study. Animals were housed individually in polypropylene cages, maintained under standard conditions [12 h light and 12 h dark cycle; (25±3) °C]. The animals were fed with standard pellet diet and water *ad libitum*.

Oral Glucose Tolerance Test

Mice were divided into six groups. 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 mice 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.

Acute Oral Toxicity Studies

V. rosea at the dose range of 100 mg–2000 mg/kg were administered orally to different group of mice comprised of a mice in each group. Mortality was observed after 72 hours. Acute toxicity was determined according to the method of Litchfield and Wilcoxon [6].

Experimental Design (Table 1)

Groups of mice, 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.

GROUPS	SPECIMEN
Group 1	Normal control (Saline)
Group 2	Alloxan treated control (150mg/kg. ip)
Group 3	Alloxan (150mg/kg.ip) + <i>V. rosea</i> (300mg/kg,p.o)
Group 4	Alloxan(150mg/kg.ip) + <i>V. rosea</i> (500mg/kg,p.o)
Group 5	Alloxan(150mg/kg) + Standard Drug Glibenclamide (5mg/kg,p.o)

Induction of Diabetes in Experimental Animals

Mice were made diabetic by a single intraperitoneal injection of alloxan monohydrate (150 mg/kg) [7]. 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.

Collection of Blood Sample and Blood Glucose Determination

Blood samples were drawn from tail tip of mice 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 mice and fasting blood sugar was estimated. Serum was separated and analyzed for serum cholesterol, serum triglycerides by enzymatic DHBS colorimetric method, serum HDL, serum LDL, serum creatinine, serum urea and serum alkaline phosphatase hydrolyzed phenol amino antipyrine method 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 5µ thickness were cut and stained by haematoxylin and eosin (H & E) for histological examination.

Statistical Analysis

All the values of body weight, fasting blood sugar, and biochemical estimations were expressed as mean± 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.

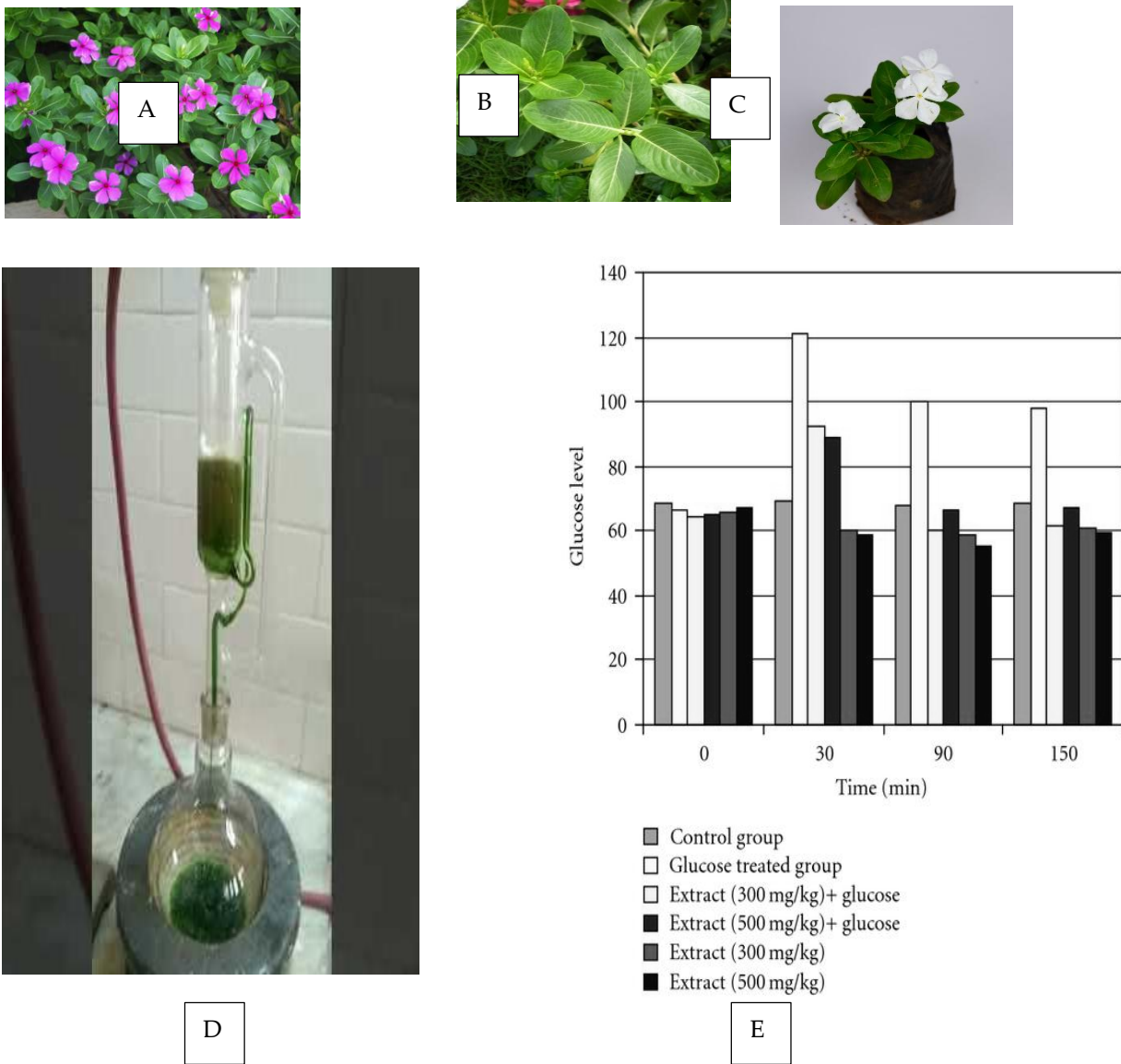


Fig 3: A- Plant material, B- Plant leaves, C- Collected whole plant, D- Plant extraction, E- Statistical analysis

RESULT

Glucose tolerance

The effects of extracts of *V. rosea* (500 mg/kg and 300 mg/kg) on glucose tolerance test are shown in Figure 3. The supplementation of *V. 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 Experimental Results. The acute oral toxicity study of *V. rosea* showed no mortality upto 2000 mg/kg.

Experimental Result

The anti-hyperglycemic effect of the extracts on the fasting blood sugar levels of diabetic rats is shown in Figure 3. 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 *V. 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 (Figure 3). 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 (Figure 3). Alloxan treatment will increase the serum enzymes levels such as cholesterol, LDL, creatinine, urea and alkaline phosphatase and decrease the HDL level, but glibenclamide (5 mg/kg) and whole plant extracts of *V. rosea* reversed the above alloxan induce changes. 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 β -cells with hyperplasia were shown by methanolic extracts.

DISCUSSION

V. roseus has a wide range of applications in the traditional system of medicine especially in cancer and diabetes. During phytochemical investigation, total of 344 compounds including monoterpene indole alkaloids (MIAs) (110), bisindole alkaloids (35), flavonoids (34), phenolic acids (9) and volatile constituents (156) have been reported in the various extracts and fractions of different plant parts of *V. roseus*. The extracts and isolated compounds of *V. roseus* have to exhibit many pharmacological activities such as anticancer/cytotoxic, antidiabetic, antimicrobial, antioxidant, larvicidal and pupicidal. The comparative toxicity of extracts and bioactive compounds investigated in dose dependent manner. The investigation of toxicity showed that the both extracts and isolated compounds are safe to a certain limit beyond that they cause adverse effects. Our study indicates that methanolic extracts of *V. rosea* have good antidiabetic activity. Alcoholic extracts of *V. rosea* exhibited significant anti-hyperglycemic 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 β cells in diabetes have been studied in several animal models. The total β cell mass reflects the balance between the renewal and loss of these cells. It was also suggested that regeneration of islet β cells following destruction by alloxan may be the primary cause of the recovery of alloxan-injected guinea pigs from the effects of the drug [8]. *V. rosea* whole plant alcoholic extracts has been shown to act by β cell regeneration. Similar effects in streptozotocin-treated diabetic animals were reported by pancreas tonic [9], ephedrine [10], and *Gymnema sylvestre* leaf extracts [11]. In our studies, the damage of pancreas in alloxan-treated diabetic control rats (Figure 3 Group II) and regeneration of β cells by glibenclamide (Figure 3 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 (5 mg/kg). This could be due to the possibility that some β -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 *V. rosea* extracts, as a possible mechanism of their antidiabetic activity.

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