Antihyperglycemic activity of *Catharanthus roseus* leaf powder in streptozotocin-induced diabetic rats

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ABSTRACT

Catharanthus roseus Linn (Apocynaceae), is a traditional medicinal plant used to control diabetes, in various regions of the world. In this study we evaluated the possible antidiabetic and hypolipidemic effect of *C. roseus* (*Catharanthus roseus*) leaf powder in diabetic rats. Diabetes was induced by intraperitoneal injection of streptozotocin (STZ, 55 mg/kg body wt) to male Wistar rats. The animals were divided into four groups: Control, control-treated, diabetic, and diabetic-treated group. Diabetic-treated and control-treated rats were treated with *C. roseus* leaf powder suspension in 2 ml distilled water, orally (100 mg/kg body weight/day/60 days). In diabetic rats (D-group) the plasma glucose was increased and the plasma insulin was decreased gradually. In the diabetic-treated group lowering of plasma glucose and an increase in plasma insulin were observed after 15 days and by the end of the experimental period the plasma glucose had almost reached the normal level, but insulin had not. The significant enhancement in plasma total cholesterol, triglycerides, LDL and VLDL-cholesterol, and the atherogenic index of diabetic rats were normalized in diabetic-treated rats. Decreased hepatic and muscle glycogen content and alterations in the activities of enzymes of glucose metabolism (glycogen phosphorylase, hexokinase, phosphofructokinase, pyruvate kinase, and glucose-6-phosphate dehydrogenase), as observed in the diabetic control rats, were prevented with *C. roseus* administration. Our results demonstrated that *C. roseus* with its antidiabetic and hypolipidemic properties could be a potential herbal medicine in treating diabetes.

Key words: Anti *Catharanthus roseus*, plasma insulin, plasma lipids, STZ-induced diabetes

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia and disturbance of carbohydrate, protein, and fat metabolism along with long-term complications affecting the retina, kidney, and nervous system.[1] Different types of oral hypoglycemic agents such as biguanides and sulfonylurea are available along with insulin for the treatment of diabetes. Unfortunately, apart from having a number of side effects, none of the oral synthetic hypoglycemic agents have been successful in maintaining euglycemia or controlling long-term microvascular and macrovascular complications. Although insulin therapy is also used for management of diabetes mellitus, there are several drawbacks, such as, insulin resistance, anorexia nervosa, brain atrophy, and fatty liver after chronic treatment.[2] There is a growing interest in herbal remedies, because of their effectiveness, minimal side effects in clinical experience, and relatively low cost. Herbal drugs or their extracts are prescribed widely; even their biologically active compounds are unknown.[3] In many developing countries, traditional medicine, in particular the herbal medicine, is sometimes the only affordable source of healthcare.[4] Even the WHO (World Health Organization) approves the use of plant drugs for different diseases, including diabetes mellitus.[5] Therefore, studies with plant extracts are useful, to know their efficacy and mechanism of action and safety.

Catharanthus roseus L (Apocynaceae) is a subshrub also known as Medagaskar periwinkle, *Vinca rosea* or *Lanchnera rosea* worldwide. The plant *Catharanthus roseus* (*C. roseus*) has gained acceptance from the pharmaceutical industries, as it is widely used as an infusion in different parts of world, to treat diabetes.[6,7] The fresh juice from the flowers of *C. roseus,* made into a tea, has been used by Ayurvedic

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physicians in India for external use to treat skin problems, dermatitis, eczema, and acne. The ethanol extract of the *C. roseus* flower has been reported to have wound healing activity.[8] The sulfates of *C. roseus* alkaloids, vincristine and vinblastine, are widely used as chemotherapeutic agents against Leukemia and Hodgkin's disease worldwide.[9] Hot water decoction of the leaves and / or the whole plant is used for the treatment of diabetes in several countries.[10] Significant antihyperglycemic activities of the leaf alcoholic extract,[11,12] aqueous extract,[13] and the dichloromethane-methanol extract of leaves and twinges[14] have been reported in laboratory animals. Fresh leaf juice of *C. roseus* has been reported to reduce blood glucose in normal and alloxan diabetic rabbits.[15] Although, earlier reports indicate blood glucose lowering activity in alcoholic extracts of leaves, studies regarding *C. roseus* leaf powder efficacy in the management of hyperglycemia have not been undertaken. In the present study we evaluated the antidiabetic and hypolipidemic activity of *C. roseus* leaf powder suspension in STZ-induced diabetic rats.

MATERIALS AND METHODS

Chemicals

Streptozotocin, lactate dehydrogenase, and glucose-6-phosphate dehydrogenase were obtained from the Sigma Chemical Co., St. Louis, MO, USA. All other chemicals were of analytical grade and procured from Sisco Research Laboratories (P) Ltd., Mumbai, India.

Plant material

Catharanthus roseus (white variety) was taxonomically authenticated by the Department of Botany, Sri Krishnadevaraya University, Anantapur, and the voucher specimen was kept in the herbarium (No. 2235) of our University. Fresh leaves of *C. roseus* (white variety) were collected during September, from the University campus, and the leaves were shade dried and then grinded to fine powder.

Induction of diabetes to experimental animals

Two-to-three-month-old male albino Wistar rats of body weight 150 – 180 g were procured from Sri Venkateswara Enterprises (Bangalore, India), acclimatized for seven days to our animal house, and maintained at standard conditions of temperature and relative humidity, with a 12-hour light / dark cycle. Water and commercial rat feed were provided *ad libitum*. The current study was carried out with prior permission from our Institutional Animal Ethical Committee (Regd. no. 470/01/a/CPCSEA, dt. 24 August, 2001). Diabetes was induced in overnight fasted rats by a single intraperitoneal injection of freshly prepared STZ (55 mg/kg body weight, in ice-cold 0.1 M citrate buffer, pH 4.5, in a volume of 0.1 ml per rat). Seventy-two hours after STZ administration, the plasma glucose level of each rat was determined, for confirmation of diabetes. Rats with plasma glucose level above 250 mg/dl were considered as diabetics and used subsequently.

Experimental design and biochemical analysis

In the present experiment, a total of 32 rats (16 diabetic surviving rats; 16 normal rats) were used. The rats were divided into four groups of eight each: control (C); control rats treated with *C. roseus* (C + CR); diabetic (D), and diabetic animals treated with *C. roseus* (D + CR). Diabetic-treated group and C + CR-group received *C. roseus* leaf powder suspension (100 mg/kg body weight) in 2 ml distilled water daily, for 60 days, through oral intubation, whereas, 2 ml of distilled water was administered to D + CR and C + CR rats. Based on the preliminary experiments on the dose-dependent antihyperglycemic effect of leaf powder, a dose less than 100 mg/kg body weight was not found to be effective in rats. Body weight was monitored at 15-day intervals. During the experimental period, blood was collected from 12-hour fasted rats by means of a capillary tube through the orbital sinus, at 15-day intervals. Plasma glucose was estimated by the glucose oxidase-peroxidase (GOD-POD) method, by using the Span diagnostic kit (Span diagnostic Ltd., Surath, India). Triglycerides, total cholesterol, and HDL-cholesterol were measured by enzymatic colorimetric end point methods using the Span diagnostic reagent kit. LDL and VLDL-cholesterol were obtained by calculations using the formula provided in the cholesterol diagnostic kit booklet. Plasma insulin was estimated by using the radioimmunoassay kit (RIA K-1) from the Bhabha Atomic Research Center (Mumbai, India).

Animal sacrifice

Animals from each experimental group were starved for 16 hours and sacrificed by cervical dislocation. The liver, muscle, and kidneys were removed, washed thoroughly with ice-cold saline and used for analysis.

Glycogen content

Glycogen released from a protein-free supernatant of trichloroacetic acid (TCA)-homogenized tissues were precipitated with 95% ethanol. The precipitated glycogen was then hydrolyzed under acidic condition and the liberated glucose was estimated by the Anthrone method as adapted by Carrol *et al.*[16]

Assay of carbohydrate metabolism enzymes

Liver and muscle homogenates were prepared in 0.1M Tris-HCl buffer, pH 7.4, and used for assay of hexokinase (HK),[17] phosphofructokinase PFK,[18] and pyruvate kinase (PK),[18] and glucose-6-phosphate dehydrogenase (G6PDH)[19] and glycogen phospharylase,[20] were assayed.

Statistical analysis

The results were expressed as means ± S.E. Data were analyzed for significant differences using Duncan's Multiple Range (DMR) test. ($P < 0.05$).

RESULTS

Body weight

Characteristic symptoms of diabetes such as loss of body weight, polyphagia, polydipsia, and polyuria observed in the D-group, were rectified in the D + CR-group. At the end of the 60-day experimental period, the D-group showed 21.9% reduction in body weight, whereas, the C, C + CR, and D + CR-groups showed an increase in body weight of 51.4, 53, and 20.2%, respectively [Figure 1]. However, by the end of the experimental period the D + CR-group showed a significant increase (52.8%) in body weight when compared to the D-group.

Plasma glucose and insulin

Data on the plasma glucose content of the four experimental groups are presented in Figure 2. Group-C and C + CR-rats remained persistently euglycemic throughout the experimental period. In the D-group, the plasma glucose level gradually increased during the experimental period from 390.87 ± 1.18 to 420.25 ± 2.8 mg/dl. A significant antihyperglycemic effect was evident in the D + CR-group from 15 days onwards and the decrease in plasma glucose was 77.7% by 60 days of treatment.

The fasting plasma insulin levels of the four groups of animals during the experimental period are presented in Figure 3. The Control-treated group showed statistically lower insulin levels at 45 and 60 days when compared with the C-group. By the end of the experimental period, D-group plasma insulin was decreased from 11.2 ± 0.18 to 9.33 ± 0.2 μU/ml. Group-D + CR showed significantly higher concentration of insulin at 15 days (12.39 ± 0.5), 30 days (18.70 ± 0.6), 45 days (18.62 ± 0.6), and 60 days (19.87 ± 0.8) with 13.7, 82, 87.7, and 109.1% increase, respectively, when compared with D-group. At the end of experimental period the insulin level of the D + CR-

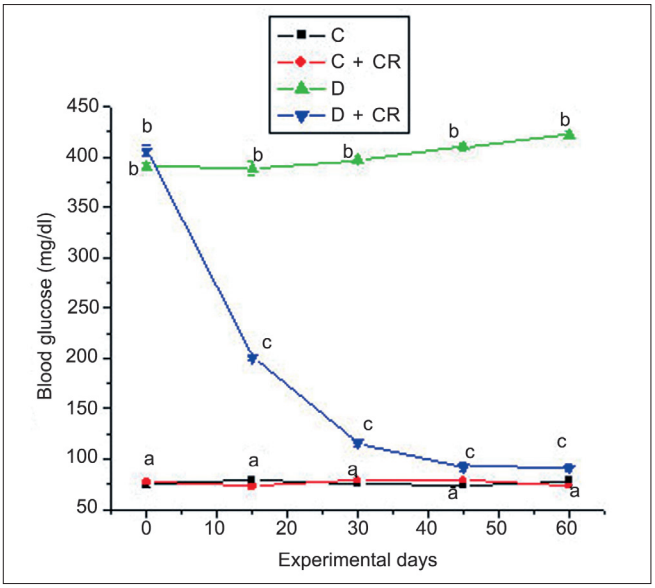

Figure 2: Mean plasma glucose of C, C + CR, D, and D + CR groups during the experimental period. Values are ± S.E., (n = 8 animals). Values not sharing common letters differ significantly at $P < 0.05$ (D.M.R test) among four experimental groups during the corresponding period.

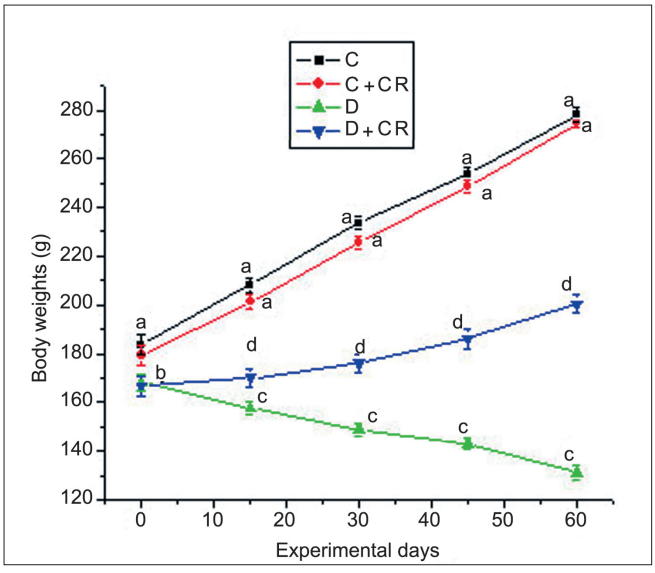

Figure 1: Mean body weight of C, C + CR, D, and D + CR groups during the experimental period. Values are ± S.E., (n = 8 animals). Values not sharing common letters differ significantly at $P < 0.05$ (D.M.R test) among four experimental groups during the corresponding period.

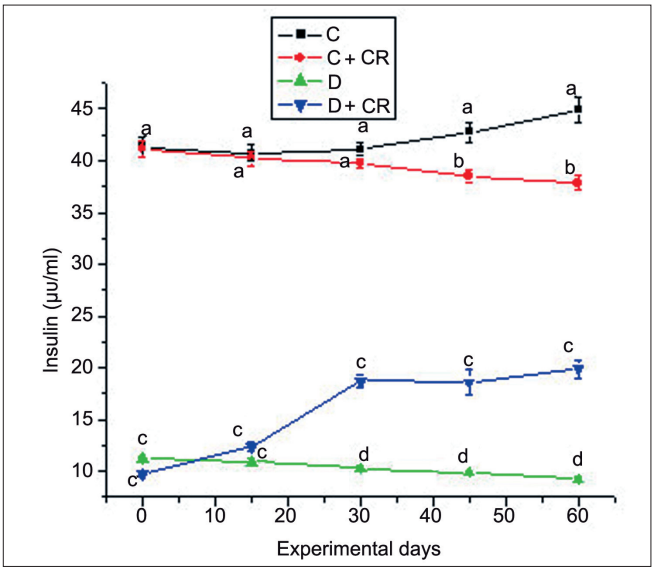

Figure 3: Mean plasma insulin of C, C + CR, D, and D + CR groups during the experimental period. Values are ± S.E., (n = 8 animals). Values not sharing common letters differ significantly at $P < 0.05$ (D.M.R test) among four experimental groups during the corresponding period.

group was significantly higher than the D-group, but still significantly lower than the C-group.

Plasma lipid profile

The plasma lipid profiles of the four groups of animals during the experimental period are represented in Table 1. A significant increase in the plasma total cholesterol (13.2%), triglycerides (17.2%), LDL-cholesterol (37.4%), and VLDL-cholesterol (17.2%), and a significant decrease in HDL-cholesterol (11.31%) in the D-group compared to the C-group, resulted in a significant increase in the atherogenic index (27.9%). A significant decrease in plasma total cholesterol (14.6%), triglycerides (9.8%), LDL-cholesterol (61.8%), VLDL-cholesterol (9.84%), and a significant increase in the HDL-cholesterol concentration (28.3%) in the C + CR-group compared to the C-group resulted in a significant decrease in atherogenic index (33.5%). Group D + CR showed a significant decrease in plasma total cholesterol (8.4%), triglycerides (13.3%), LDL-cholesterol (28.7%), VLDL-cholesterol (13.3%), and the atherogenic index (28.7%), and a significant increase in HDL-cholesterol concentration (24.5%) when compared with the D-group [Table 1].

Glycogen

Group-D showed significantly decreased glycogen content in the liver (60.9%) and muscle (70.3%), when compared to the C-group. In the D + CR-group *C. roseus* treatment resulted in a significant enhancement in the liver (94.8%) and muscle (104%) glycogen content when compared to the D-group [Table 2].

Carbohydrate metabolism enzymes

Table 2 shows activities of carbohydrate metabolic enzymes in the liver and muscle. The STZ diabetic rats (D-group) showed a significantly enhanced hepatic glycogen phosphorylase activity (119.4%) compared to the C-group. The D + CR rats showed decreased activity (48.3%) of glycogen phosphorylase compared to the D-group rats. The STZ-induced diabetic rats showed significantly decreased activities of glycolytic enzymes, both in the liver and muscles, compared to the control rats. The percent decrease in HK, PFK, and PK activities in the liver and muscle of the D-group are 43.2, 37.3, and 32.2%, and 40.7, 58.1, and 37.6%, respectively. *C. roseus* treated diabetic rats, that is, the D + CR-group showed no deviation in the activities of HK, PFK, and PK, both in the liver and muscle compared to the C-group. Thus *C. roseus* treatment in the D + CR-group prevented diabetic-induced alterations in the glycolytic enzyme activities. Hepatic G6PDH activity decreased significantly (54.3%) in the D-group. *C. roseus* treatment resulted in a significant enhancement in the activity of G6PDH in the liver of the C + CR and D + CR-groups (7.0 and 85.5%) compared to the C and D-groups, respectively.

Table 1: Effect of *C. roseus* leaf powder treatment on plasma lipid profile

Parameters	Control	Control + CR	Diabetic	Diabetic + CR
Total cholesterol (mg/dl)	72.86[a] ± 1.07	62.21[b] ± 1.50	82.51[c] ± 2.10	75.60[a] ± 1.10
Tryglycerides (mg/dl)	76.28[a] ± 0.93	68.78[b] ± 0.97	89.37[c] ± 1.98	77.46[a] ±0.94
LDL (mg/dl)	28.17[a] ± 1.03	10.76[b] ± 1.35	38.71[c] ± 1.86	27.61[a] ±0.77
VLDL (mg/dl)	15.25[a] ± 0.19	13.75[b] ± 0.19	17.87[c] ± 0.40	15.49[a] ± 0.18
HDL (mg/dl)	29.43[a] ± 0.45	37.76[b] ± 0.31	26.11[c] ± 0.51	32.50[d] ± 0.27
Atherogenic Index	2.47[a] ± 0.10	1.64[b] ± 0.08	3.16[c] ± 0.20	2.33[a] ± 0.07

Values are ± S.E., (n = 8 animals). Values not sharing common letter differs significantly at $P < 0.05$ (D.M.R test)

DISCUSSION

As expected, STZ-induced D-group rats showed characteristic signs of diabetes such as, polyphagia, polydipsia, and polyuria, failure to gain in body weight, hyperglycemia, hypoinsulinemia, and hyperlipidemia. Inspite of polyphagia, a decrease in the body weight of diabetic rats was possible due to excessive catabolism of fats and protein.[21] In the D + CR-group, ingestion of *C. roseus* leaf powder effectively prevented these diabetic symptoms, indicating the antidiabetic nature of this plant. No visible side effects of *C. roseus* leaf powder were observed in the C + CR-group, representing the non-toxic nature of *C. roseus*. The plasma glucose levels observed in the C + CR and D + CR-groups during the experimental period clearly indicate that *C. roseus* leaf powder does not promote hypoglycemic activity, but exerts an antihyperglycemic effect. Chronic treatment of diabetic rats for a 60-day period with *C. roseus* leaf powder lowered the plasma glucose level to near normal levels. The gradual decrease in the plasma insulin levels of the C + CR-rats during the experimental period resulted in significantly lower values at 45 and 60 days compared to C-rats in the corresponding period. This result with the maintained normoglycemia [Figure 2] in C + CR-rats indicates that *C. roseus* promotes glucose uptake by promoting insulin sensitivity. In the STZ-induced diabetic model, insulin is markedly depleted, but not absent.[22] The hypoinsulinemia observed in STZ-induced diabetic rats is gradually intensified during the experimental period. A significant increase in the plasma insulin levels of the D + CR-group compared to the D-group may be due to the regeneration of the STZ-destructed β-cells, which is probably due to the fact that the pancreas contains stable (quiescent) cells that have the capacity to regenerate.[23,24]

Table 2: Effect of *C. roseus* leaf powder treatment on glycogen and some carbohydrate metabolic enzymes

Parameter	Tissue	Control	Control + CR	Diabetic	Diabetic+ CR
Glycogen	Liver	29.24 ± 0.35[a]	28.89 ± 10.70[a]	11.42 ± 0.36[b]	22.25 ± 0.59[c]
	Muscle	2.36 ± 0.08[a]	2.48 ± 0.07[a]	0.70 ± 0.02[b]	1.43 ± 0.11[c]
Glycogen phosphorylase	Liver	0.131 ± 0.01[a]	0.137 ± 0.01[a]	0.288 ± 0.03[b]	0.149 ± 0.01[c]
Hexokinase	Liver	3.93 ± 0.08[a]	4.052 ± 0.03[a]	2.23 ± 0.04[b]	3.80 ± 0.12[a]
	Muscle	4.39 ± 0.07[a]	4.52 ± 0.08[a]	2.60 ± 0.05[b]	4.53 ± 0.07[a]
Phosphofructokinase	Liver	3.35 ± 0.05[a]	3.59 ± 0.05[b]	2.10 ± 0.05[c]	3.27 ± 0.04[a]
	Muscle	4.17 ± 0.07[a]	4.30 ± 0.07[a]	1.75 ± 0.02[b]	4.15 ± 0.07[a]
Pyruvate kinase	Liver	2.57 ± 0.04[a]	2.76 ± 0.06[b]	1.74 ± 0.02[c]	2.69 ± 0.06[a]
	Muscle	2.87 ± 0.03[a]	2.92 ± 0.05[a]	1.79 ± 0.04[b]	2.78 ± 0.06[a]
G6PDH	Liver	2.43 ± 0.07[a]	2.60 ± 0.04[b]	1.11 ± 0.01[c]	2.06 ± 0.05[d]

Values are ± S.E., (n = 8 animals). Values not sharing common letters differ significantly at $P < 0.05$ (D.M.R test); Glycogen - mg glucose/g tissue; Glycogen phospharylase - µmol of Pi formed /min/ mg protein; Hexokinase - µmol of G6P formed/min/mg protein; Phosphofructokinase - µmol of F16Bis phosphate formed/min/mg protein; Pyruvate kinase - µmol of NADH oxidized/min/ mg protein; G6PDH - µmol of NADP reduced/min/mg protein

Therefore, the surviving cells can proliferate to replace the lost cells. Phytochemicals such as flavonoids and alkaloids present in the *C. roseus* leaf powder[25] may have protected the intact functional β-cells from further deterioration through oxidative stress. Hence, the β-cells remain active and continue to produce insulin. It is also claimed that antioxidants such as flavonoids are possibly beneficial in preventing STZ-induced diabetes by stopping oxidative damage of the pancreas, and increasing insulin secretion by the regeneration of pancreatic β-cells.[26,27]

In this study, we have noticed elevated levels of plasma lipids such as total cholesterol, LDL and VLDL-cholesterol, and triglycerides and decreased level of HDL-cholesterol in the D-group, which are risk factors for coronary heart disease.[28] Insulin increases uptake of fatty acids into the adipose tissue and increases triglyceride synthesis.[29] Moreover, insulin inhibits lipolysis. Lipolysis is not inhibited in the D-group due to the presence of insulin deficiency, leading to hyperlipidemia. It is interesting that treatment with *C. roseus* leaf powder suspension for 60 days brings down the elevated levels of total cholesterol, LDL and VLDL-cholesterol, and triglycerides, and also increases plasma HDL-cholesterol to normal levels in D + CR-rats, indicating the beneficial effect of *C. roseus* in reducing the risk of cardiovascular diseases. Increased levels of HDL-cholesterol, an antiatherogenic lipoprotein, after *C. roseus* administration may be due to an increase in the activity of lecithin cholesterol acyl transferase, which may contribute to the regulation of blood lipids.[30]

The liver plays an important role in buffering postprandial hyperglycemia and is involved in the synthesis of glycogen. Diabetes mellitus is known to impair the normal capacity of the liver to synthesize glycogen.[31] Assessment of glycogen levels serve as a marker for studying the antidiabetic activity of *C. roseus*. In the D-group, the glycogen content in the liver and muscle was reduced compared to control rats. This is in line with previous studies on STZ-diabetic rats.[32] Two months of treatment with *C. roseus* partially prevented the depletion of glycogen in the liver and muscle of STZ-diabetic rats. This could be due to the increased circulatory insulin concentrations observed in the D + CR-group compared to the D-group. Increased activity of hepatic glycogen phosphorylase observed in STZ-diabetic rats might be one contributing factor for the decreased hepatic glycogen content. The elevated hepatic glycogen content and decreased plasma glucose levels of *C. roseus*-treated, STZ-diabetic rats compared to STZ-diabetic control rats could be explained by the diminished activity of the glycogenolytic enzyme, namely, glycogen phosphorylase.

Diabetes mellitus is characterized by partial or total deficiency of insulin, resulting in derangement of carbohydrate metabolism and a decrease in the enzymatic activity of HK and PFK, resulting in the depletion of liver and muscle glycogen.[33] As insulin administration normalizes these alterations in the enzymatic activities, these enzymes represent a method to assess the peripheral utilization of glucose. Glycolytic enzymes (HK, PFK, PK) activity of the D-group was significantly decreased in the liver and muscle, and this was similar to the previous findings.[34,35] An increase in the activity of glycolytic enzymes in *C. roseus*-treated diabetic rats implied that the cellular entry of glucose was facilitated by *C. roseus*, which in turn stimulated the activity of these enzymes. This glucose influx is due to an increase in the circulation of insulin and insulin sensitivity. Glucose-6-phosphate dehydrogenase is the key regulating enzyme of the pentose phosphate pathway and controls the flow of carbon through this pathway. Specifically, the enzyme catalyzes the first reaction in the pathway leading to the production of pentose phosphates and reduces power in the form of Nicotinamide Adenosine Dinucleotide

Phosphate (NADPH) for reductive biosynthesis and maintenance of the redox state of the cell. Alterations in G6PDH activity can significantly alter oxidative stress-induced cell death.[36] *C. roseus* administration increases the G6PDH activity in D + CR- rats. Insulin is reported to stimulate oxidation of glucose by increasing the activation of G6PDH in a dose-dependent manner.[37] Thus, the increased circulatory insulin level observed in *C. roseus*-treated STZ-diabetic rats may cause increased activity of G6PDH in these rats.

Based on our observations of carbohydrate metabolism, the antihyperglycemic effect of this plant appears to be at least in part, due to extra pancreatic activity, including increased glucose utilization by the liver and muscle (glycolysis), enhanced glucose oxidation through shunt pathway, via activation of G6PDH, and decreased glucose production by depression of glycogenolytic enzyme.

CONCLUSIONS

Thus our findings show that oral administration of *C. roseus* leaf powder produces an antihyperglycemic effect, lowers both total cholesterol and triglyceride levels, and at the same time increases HDL-cholesterol in STZ-induced diabetic rats. The antihyperglycemic action of the leaf powder of *C. roseus* is associated with increased plasma insulin concentration and insulin sensitivity. This investigation shows the potential of *C. roseus,* for use as a natural oral agent, with both antihyperglycemic and hypolipidemic effects. Further comprehensive biochemical and pharmacological investigations are needed to elucidate the exact mechanism of the antihyperglycemic effect of *C. roseus*.

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