K Jayaseelan
Department of Zoology and Biotechnology, A.V.V.M. Sri Pushpam College (Autonomous), Poondi, Thanjavur, Tamilnadu, India

J Kalidoss
Department of Zoology and Biotechnology, A.V.V.M. Sri Pushpam College (Autonomous), Poondi, Thanjavur, Tamilnadu, India

Evaluation of antihyperglycemic and antihyperlipidaemic activity of ethanolic extract of *Catharanthus roseus* (Linn.) in streptozotocin-induced diabetic rats

K Jayaseelan and J Kalidoss

Abstract
In India, native remedies have been used in diabetes treatment. Plants have always been an ideal source of drugs and many of the plants either directly or indirectly are used to formulate the present available drugs. The present investigation was to find out the antihyperglycemic and antihyperlipidaemic effects of ethanolic leaves extracts of *Catharanthus roseus* in normal and streptozotocin (STZ) induced diabetic rats. Diabetes was induced in adult wistar rats by injecting streptozotocin (STZ, 50 mg/kg) intraperitoneally. The ethanolic extract of *C. roseus* was orally administered at 100mg/kg/day while glibenclamide was administered at 50mg/kg/day. All animals were treated for 28 days before being sacrificed. The blood glucose levels and serum lipid profile like total cholesterol (TC), triglycerides (TG), phospholipids (PL), low density lipoprotein (LDL), high density lipoprotein (HDL), very low density lipoprotein (VLDL) were determined in all the rats. The ethanolic extract of *C. roseus* exhibited significant reduction in blood glucose levels, TC, LDL, VLDL and an increase in HDL levels in diabetic rats when compared to the standard drugs. The above results indicate that the plant leaves are capable of ameliorating hyperglycemia in STZ induced diabetic rats. Hence this plant has a potential source for the isolation of new orally active agent for diabetes mellitus. The present investigation established pharmacological evidence to support the traditional claim of this plant being used as an antidiabetic.

Keywords: *Catharanthus roseus*, diabetes mellitus, streptozotocin, blood glucose level, plasma lipid

Introduction

Plants are wealthy source of secondary metabolites with interesting biological activities (Dineshkumar *et al.,* 2015) [1]. Plants have been used as medicine for thousands of years and also a hallmark in the search of new medicine (Vimalavady A, *et al.,* 2012) [2]. Many plant species have been used in traditional medicine to treat many health problems. Plant derived compounds plays a major role in primary health care as therapeutic remedies in many developing countries. Medical plants play an essential role in the management of diabetes mellitus where resources are inadequate especially in developing countries. Diabetes mellitus is a group of metabolic diseases with higher concentrations of glucose in the blood because of improper insulin production from pancreas or inactivity of cells to the insulin. It is a stern health problem being the third greatest death cause all over the world, and if it is not treated properly, it causes many complications affecting various organs in the body (El-Hilaly J, *et al.,* 2007) [3]. The chronic hyperglycemia of diabetes is associated with long term damage, dysfunction, and failure of various organs (Lyra R, *et al.,* 2006) [4]. Hyperglycemia results due to interruption in the group of metabolism of carbohydrates, proteins and lipids, resulting from defects in insulin secretion, action or both (Nyholm B, *et al.,* 2000) [5]. Currently there are over 150 million diabetics worldwide and this number is likely to increase due to increase in sedentary (inactive) lifestyle, energy rich diet consumption and obesity (Yajnik CS, *et al.,* 2001) [6]. In modern medicine, there is still no satisfactory effective therapy available to cure diabetes (Piedrola G, *et al.,* 2001) [7].

Catharanthus roseus L (Apocynaceae) is a sub shrub also known as Madagascar periwinkle, *Vinca rosea* (or) *Lochnera rosea* worldwide. It is native to the Caribbean Basin and has historically been used to treat a wide assortment of diseases.

Correspondence
K Jayaseelan
Department of Zoology and Biotechnology, A.V.V.M. Sri Pushpam College (Autonomous), Poondi, Thanjavur, Tamilnadu, India

The plant *Catharanthus roseus* (*C. roseus*) is used by the pharmaceutical industries to treat diabetes and it is widely used as an infusion in different parts of world (Heijden RV, *et al.,* 2004) [8].The fresh juice from the flowers of *C. roseus* is used to prepare tea and widely accepted by Ayurvedic physicians in India for external use to treat dermatitis, skin problems, eczema and acne. The ethanol extract of *C. roseus* flower has been reported to have wound healing activity (Nayak BS, *et al.,* 2006) [9]. Hence the present study was therefore undertaken to investigate the anti-diabetic, antihyperlipidaemic activities and safety potentials of the ethanol leaf extract of *Catharanthus roseus* in experimental diabetic models.

Materials and Methods
Collection, identification, and authentication of selected plant
Fresh, healthy, and young leaves of *Catharanthus roseus* were collected from Saliyamangalam, Thanjavur district, Tamil Nadu, India and authenticated by professionals in the Department of Botany, St. Joseph's College, Tiruchirappalli, India. The voucher specimen number of the plant is GDK 001.

Preparation of plant extracts
The shade dried leaves were taken and then made into fine powder. About 500 g of dry powder was extracted with ethanol (80%) at 70 °C by continuous hot percolation using Soxhlet apparatus. The extraction was continued for 24 hrs, and the ethanolic extract was then filtered and kept at 40 °C for 24 hrs in hot air oven to evaporate the ethanol from it. A dark brown residue was obtained. The residue was kept separately in airtight containers and stored in a deep freezer.

Animal studies
Animals were divided into four groups (6 animals in each)
Group 1: Normal Control
Group 2: Diabetic induced model (Streptozotocin 50mg/kg)
Group 3: Diabetic (STZ) + Ethanolic Extract of *C. roseus* (100mg/kg)
Group 4: Diabetic (STZ) + Standard drug (Glibenclamide, 50 mg/kg)

Animals
Albino rats of 4 weeks old, weighing 150-210 g were used for the present study. The animals are maintained in animal house at standard temperature (24±2 °C) and relative humidity (30-70) with a 12:12 light: dark cycle. The animals were fed with standard pellet diet and water. The animals were handled according to good laboratory practice (GLP). Ethical clearance was obtained from institutional animal ethical committee and conducted according to the Indian National Science Academy guidelines for the use and care of experimental animal.

Induction of diabetes
Diabetes was induced by the intraperitoneal (i.p.) injection of streptozotocin (STZ) at a dose of 50 mg/kg in distilled water (1 mL/kg) to 16 h fasted rats. After 5 days, fasting blood glucose levels were measured and the animals with blood glucose concentration level above 250 mg/dL was considered to be diabetic. To overcome the hypoglycemia which occurred during the first 24 h following the STZ administration, diabetic rats were orally given 5% glucose solution (Sezik, E, *et al.,* 2005) [10].

Examination of Body weight
Body weight of rats from each group was measured on initial stage and 28th day. Weight of the animal was measured using standard digital weight balance to get accuracy.

Biochemical Parameter
Serum glucose was estimated by the oxidase method (Trinder P, *et al.,* 1969) [11]. The total cholesterol was estimated by Allain method (Allain CC, *et al.,* 1974) [12]. Triglyceride was estimated by the Werner method (Werner M, *et al.,* 1981) [13]. HDL cholesterol was separated by adding phosphotungstic acid magnesium chloride to the fresh samples to precipitate other lipoproteins and the HDL cholesterol was estimated by Allain method. The concentration of LDL, VLDL cholesterol was calculated by using the Friedwald formula (Friedwalds WT, *et al.,* 1972) [14]. Heamoglobin was estimated by the method of Dacie and Lewis (Dacie JV *et al.,* 1968) [15].

Estimation of Anti-Oxidants
The estimation of anti-oxidants Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx) and Glutathione (GSH) was measured by the following methods. The activity of superoxide dismutase (SOD) was assayed by the method of Kakkar (Kakkar P, *et al.,* 1984) [16]. Catalase (CAT) was estimated by the method of Sinha (Sinha AK, *et al.,* 1972) [17]. Glutathione peroxidase (GPx) was measured by the method described by Rotruck (Rotruck JT, *et al.,* 1973) [18]. The estimation of non-enzymatic antioxidants, Glutathione (GSH) was measured by the method of Ellman (Ellmann GL, *et al.,* 1959) [19], Ascorbic acid was estimated by the method of Omaye (Omaye ST, *et al.,* 1979) [20] and Vitamin E was estimated by the method of Baker (Baker H, *et al.,* 1980) [21].

Statistical Analysis
All results are presented as Mean ± SEM. Data were analyzed by the student "t" test. Groups for the pair of observations depend upon each other. Results were considered statistically at *P*<0.001.

Table 1: Examination of Body weight

S. No	Groups	Body Weight (gm)	
		Initial (0th Day)	Final (28th Day)
1.	Normal (Control)	205.15 ± 19.12	230.21 ± 19.14
2.	Diabetic (STZ)	182.04± 1.2	149.11 ± 11.4*
3.	Diabetic (STZ) + *C. roseus* ethanolic extract (100 mg/kg b. wt)	198.06 ± 19.43*	226.02 ± 11.41**
4.	Diabetic (STZ) + Glibenclamide (50 mg/kg b.wt)	202 ± 11.25**	225 ±12.35**

Values are expressed as Mean ± SD (n=6)
Values are statistically significant at *P*<0.01 Vs Control
Values are statistically significant at **P*<0.001Vs Control by student T test

Table 2: Estimation of Blood glucose, Plasma insulin and Haemoglobin

S. No	Groups	Blood Glucose (mg/dL)	Plasma Insulin (µU/ml)	Total Hemoglobin (mg/mL)
1.	Normal (Control)	86.44 ± 8.81	16.27 ± 0.13	12.47 ± 1.35
2.	Diabetic (STZ)	285.11 ± 19.33	7.28 ± 1.54	8.97 ± 0.49
3.	Diabetic (STZ) + *C. roseus* ethanolic extract (100 mg/kg b.wt)	90.04 ± 3.12**	15.68 ± 1.05**	13.99 ± 0.45**
4.	Diabetic (STZ) + Glibenclamide (50 mg/kg b.wt)	87.27 ± 6.23**	16.32 ± 2.43**	14.88 ± 3.12**

Values are expressed as Mean ± SD (n=6)
Values are statistically significant at *$P<0.01$ Vs Control
Values are statistically significant at **$P<0.001$ Vs Control by student T test

Table 3: Estimation of Serum Lipid Profile

S. No.	Groups	TGL (mg/dl)	HDL (mg/dl)	VLDL (mg/dl)	LDL (mg/dl)	Total C Cholesterol	Serum Phospho-Lipid (mg/dL)
1.	Normal (Control)	77.15±6.68	39.6±2.71	17.03±1.51	43.9±4.25	98.16±8.76	106.61 ± 5.09
2.	Diabetic (STZ)	133.32±10.05	25.31±1.64	32.14±2.86	96.47±8.12	215.2±7.23	167.18 ± 4.27
3.	Diabetic (STZ) + *C. roseus* ethanolic extract (100 mg/kg b.wt)	89.49±7.45**	38.27±2.56**	21.52±3.4**	68.27±5.28**	115.6±6.90**	119.48 ± 5.36**
4.	Diabetic (STZ) + Glibenclamide (50 mg/kg b.wt)	82.04±6.71**	38.91±5.14**	18.41±1.34**	41.9±2.66**	103.2±4.8**	109.73 ± 5.44**

Values are expressed as Mean ± SD (n=6)
Values are statistically significant at *$P<0.01$ Vs Control
Values are statistically significant at **$P<0.001$ Vs Control by student T test

Table 4: Effect of *C. roseus* ethanolic leaves extract on enzymatic and non enzymatic levels in control and experimental animals

S. No	Groups	SOD (mg%)	CAT (mg%)	GPx (mg%)	GSH (mg%)	Vitamin C	Vitamin E	Plasma TBARS (nmol/ml)
1.	Normal (Control)	3.86 ± 0.16	14.98 ± 0.79	6.08 ± 0.21	26.31 ± 1.35	1.64 ± 0.09	1.96 ± 0.08	3.07 ± 0.15
2.	Diabetic (STZ)	2.08 ± 0.07	7.35 ± 0.27	3.02 ± 0.17	18.67 ± 0.92	0.91 ± 0.05	0.62 ± 0.15	7.14 ± 0.25
3.	Diabetic (STZ) + *C. roseus* ethanolic extract (100 mg/kg b.wt)	3.68 ± 0.12 **	12.96 ± 0.52**	5.89 ± 0.24**	24.12 ± 1.38**	1.17 ± 0.11**	1.41 ± 0.13**	3.14 ± 0.17**
4.	Diabetic (STZ) + Glibenclamide (5 mg/kg b.wt)	3.54 ± 0.15 **	12.91 ± 0.55**	5.71± 0.25**	23.78 ± 1.58**	1.81 ± 0.15**	1.63 ± 0.12**	3.55± 0.11**

Discussion

Traditional herbal system of medicine are used throughout the world and herbs which is the original source for most of the drugs have been used from centuries. Medicinal plants are the major source of therapeutic agents that contains many chemical compounds which are used to cure human diseases. Recent discovery and advancement in medicinal and aromatic plants have lead to the augmentation of health care of mankind (Vijayaraj R, *et al.,* 2016) [22]. Medicinal plants in traditional medicine systems have been used since ancient times for the treatment and management of diabetes mellitus (DM) throughout the world (Jung M, *et al.,* 2006) [23]. Currently, medicinal plants continue to play a vital role in the management of DM, especially in developing countries, where many people do not have access to conventional anti-diabetic therapies(Grover JK, *et al.,* 2002) [24].

STZ is well known for its selective pancreatic islet cell toxicity and has been widely used in inducing diabetes mellitus in animals. STZ is taken up by the β cells via the glucose transporter GLUTZ and causes alkylation of DNA (Delancy CA, *et al.,* 1995) [25] and reduction of ATP and NAD+ content (Heller B, *et al.,* 1994) [26]. STZ induces severe and irreversible hyperglycemia in experimental animals. With STZ there is no incidence of spontaneous

revision and recovery of islets, which results in more than 90% of rats becoming diabetic (Mitra SK, *et al.,* 1995) [27]. It is evident from the present investigation that streptozotocin administration at the dose of 50 mg/kg body weight causes significant diabetogenic response in albino rats.

Glucose is an important fuel for liver and muscle metabolism. In the present study, glucose level is decreased due to the lack of insulin in the diabetic state, which results in the inactivation of glycogen synthetase system. When diabetic rats were treated with ethanolic extract of *C. roseus* leaves, significantly lowered (*p*<0.001) blood glucose level at dose of 100 mg/kg body weight. The ethanolic extract showed a significant improvement in their ability to control the blood glucose level. This effect is comparable to standard drug Glibenclamide.

Hypercholesteremia is primary factor involved in the development of atherosclerosis and coronary heart disease which are the secondary complications of diabetes (Ananthan R, *et al.,* 2003) [28]. In the present study STZ induced diabetic rats show a significant (*P*<0.001) increase of total cholesterol, triglycerides, phospholipids and low density lipoproteins (LDL) and very low density lipoprotein (VLDL) and significant (*p*<0.001) decrease in high density lipoprotein (HDL) cholesterol in serum when compared with normal control. When diabetic rats were treated with

ethanolic leaves extract of *C. roseus* significantly lowered ($p<0.001$) the total cholesterol, triglycerides, phospholipids, LDL and VLDL cholesterol and significantly ($p<0.001$) increased HDL cholesterol.

Antioxidants from plant materials terminate the action and generation of free radicals thereby protecting the body from various diseases (Lai LS, *et al.,* 2001) [29]. The enzymes SOD and CAT are major antioxidant defense systems of the body which protect the cell membrane and other cellular constituents against oxidative damage that occurred due to free radical species (Umamaheswari M, *et al.,* 2008) [30]. The enzymatic antioxidant is the nature protector against lipid peroxidation. SOD, CAT, and GPx enzymes are important scavengers of hydrogen peroxide and superoxide ion. These enzymes prevent generation of hydroxyl radical and protect the cellular constituents from oxidative damage (Scott MD, *et al.,* 1991) [31]. It was also observed that the ethanolic leaves extract of *C. roseus* significantly increased the SOD, CAT, GPx, GSH, Vitamin C and E activity in STZ induced diabetic in rats. This show *C. roseus* can reduce reactive free radicals that might lessen oxidative damage to the tissues and improve the antioxidant enzymes.

Conclusion
Our finding indicates that the ethanolic extract of *C. roseus* leaves are useful for treatment of diabetes associated with hyperlipidemia. Further chemical and pharmacological investigations are required to elucidate the exact mechanism of action of this extract and to isolate the active principles responsible for such effects.

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