Research Article

GC-MS Analysis of Phytochemicals and Hypoglycemic Effect of *Catharanthus roseus* in Alloxan-Induced Diabetic Rats

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ABSTRACT

To find out the phytochemical, GCMS, hypoglycemic and hypolipidemic activities of ethanolic leaf extract of *Catharanthus roseus* is the main objective of the present study. The acclimatized animals were injected with Alloxan for induce diabetic condition. Animals were treated with ethanol extract of *Catharanthus roseus* and standard drug glibenclamide. The biochemical parameters such as High density lipoprotein (HDL), Low density lipoprotein (LDL), very low density lipoprotein (VLDL), Total Cholesterol (TC), Triglyceride (TG) along with blood glucose level are evaluated. By using GC-MS Studies 11 compounds were identified in the ethanolic extract of *Catharanthus roseus* leaves. Decreased blood glucose level of the test animals shows that the extract exhibit significant antidiabetic activity and the levels of LDL, VLDL, TG and TC were reduced after the administration. The HDL serum cholesterol levels were significantly increased in ethanol leaf extract of *Catharanthus roseus* administered rats when compared to the diabetic control group. This finding tends to reveal that the hypoglycemic and hypolipidemic effects of *Catharanthus roseus* are similar to the effect of standard drug Glibenclamide. This plant can get in consideration for the searching new drug to treat hyperglycemia from plant source.

Keywords: *Catharanthus roseus,* Phytochemicals, GC-MS, Antidiabetic

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia, abnormal lipid and protein metabolism along with specific long-term complications affecting the retina, kidney and nervous system. Hyperglycemia is an important factor in the development and progression of the complication of diabetes mellitus[1]. Alloxan, a beta-cytotoxin is widely used to induce experimental diabetes in animals. The cytotoxic action of this diabetogenic agents is mediated by reactive oxygen species. Alloxan and the product of its reduction, dialuric acid, establish a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide. Thereafter highly reactive hydroxyl radicals are formed by the fenton reaction. The action of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of β cells of pancreas[2]. Glibenclamide (GLIB), one of the antidiabetic sulphonylureas, produce hypoglycemia by increasing the secretion of insulin from the pancreas**.** It acts by binding sulphonylurea receptor (SUR) of pancreatic β-cells to cause the inhibition of ATP-sensitive K^+ channels[3] and it opens Ca^{2+} channels, increasing intracellular Ca^{2+}. Thus it accelerates the insulin secretion[4]. Moreover, the researchers conducted over last several decades have shown that plant based therapies have a potential to control and treat diabetes[5] and its complications[6]. Over 150 plant extracts and some of their active principles, including coumarins, flavonoids, terpenoids, and most of other secondary plant metabolites, including arginine and glutamic acid and flavonoids are known for the treatment of diabetes[7].

Several flavonoids, glycosides, terpenoids, etc., have been shown to possess anti diabetic properties. Fresh leaf juice of *Catharanthus roseus* has been reported to reduce blood glucose in normal and alloxan diabetic rabbits[8]. Leaves and twigs of *Catharanthus roseus* have been reported to have hypoglycemic activity in streptozotocin induced diabetic rats[9]. In this study the effect of the ethanolic extract of *Catharanthus roseus* in biochemical parameters such as High density lipoprotein (HDL), Low density lipoprotein (LDL), very low density lipoprotein (VLDL), Total Cholesterol, Triglyceride along with blood glucose level are evaluated.

MATERIALS AND METHODS

Plant Material and Preparation of Extract

Fresh leaves of *Catharanthus roseus* were collected from Thanjavur area and were dried under shade for several days. The dried leaves were then grinded to a coarse powder. The coarse powder (100 gm) of *Catharanthus roseus* leaves were extracted with 500 ml of ethanol 95% by continuous hot percolation respectively using soxhlet apparatus until the extraction were completed. After the completion of the extraction, the extracts were filtered and the solvents were removed by distillation under reduced pressure. Greenish black coloured residue was obtained.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The column used for GC programme was elite-1 (100% Dimethyl poly siloxane), 30 x 0.25 mm x 1 µmdf. The equipment was GC Clarus 500 Perkin Elmer. The carrier gas used in GC-MS programme was helium 1 ml/minute (split:10:1). The detector used was mass detector (turbo mass gold-perkin elmer), the software was turbomass 5.4.2. 2 µl of the sample was injected into the GC-MS. The initial oven temperature was 110˚C and it was kept hold for 2 mins, and it was raised upto 200˚C at the rate of 10˚C per min without holding. Further it was raised upto 280˚C at the rate of 5˚C per minute and kept hold for 9 minutes. The injector temperature was 250˚C. The total GC running time was 36 minutes. The library used in the MS was NIST Version-Year 2005. The inlet line temperature of MS program was 200˚C and the source temperature was 200˚C. The electron energy used was 70 ev, the mass scan (m/z) was 45-450. The total MS running time was 36 minutes.

Phytochemical Screening Analysis

Qualitative phytochemical studies analysis was done in the ethanolic and aqueous extract of leaves of *Catharanthus roseus* using the procedure of Chandra Mohan[10].

Chemicals

Alloxan was purchased from Sigma Aldrich Chemicals, Pvt., Ltd., Bangalore. All other chemicals and reagents used were of analytical grade.

Hypoglycemic Drug

Glibenclamide was purchased from Thanjavur Medical College and Hospital [TMCH], Thanjavur.

Animals

We used mature Albino rats. After the adaptation period was over, each group of rats was weighed and marked, and then treated by the specified dose of materials. For inducing diabetes in rats, we used alloxan monohydrate 150 mg/kg (i.p.) solved in saline (9 % sodium chloride solution). Alloxan injection causes apoptosis and necrosis in Langerhans β cells of pancreas that has been used for diabetes induction in different kinds of animals and is similar to human type 1 diabetes.

In this study, animals were assigned to 4 groups having the following characteristics:

Group I

Normal control (saline).

Group II

Alloxan treated control (150 mg/kg.ip).

Group III

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

Group IV

Alloxan (150 mg/kg.ip) + *Catharanthus roseus plant* extract (300 mg/kg, p.o), Plant extract 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 IV are diabetic control rats. Group III and Group IV (which previously received alloxan) are given a fixed standard drug glibenclamide (5 mg/kg) and a fixed dose of plants extract (300 mg/kg, p.o) for 14 consecutive days. And after 14 days, the animals were anesthetized and the blood was collected in tubes, then, levels of serum glucose, lipoproteins (HDL, LDL, and VLDL), triglyceride, and total cholesterol were evaluated by use of enzymatic kits. Experiments were carried out in accordance with Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) Guidelines, and the study was approved by the Institutional Animal Ethical Committee (IAEC/CPCSEA/03/002/11).

Histopathology of the Pancreas of Normal and Induced Diabetic Rats

On the last day of the study, the animals were sacrificed and quickly dissected, and small slices of pancreas samples were fixed in 10 % formalin. Thin sections of the tissue, 5-7µm, were cut and stained with haematoxylin-eosin. The tissue sections were subjected to rehydration by exposing them to the decreasing concentrations of alcohol (10 % - 30 %) and then stained with haematoxylin. The sections were dehydrated by using increasing concentrations of alcohol and then stained with eosin. They were then treated with diphenyxylene (DPX) and examined under microscope.

RESULTS AND DISCUSSION

Table 1 represents the phytocomponents identified in the ethanolic extract of *Catharanthus reseus* leaves using GC-MS Study. 11 compounds were identified in the ethanolic extract of *Catharanthus roseus* leaves.

Fig 1 shows the peak values of phytocomponents identified in the ethanolic extract of *Catharanthus roseus* leaves using GC-MS study.

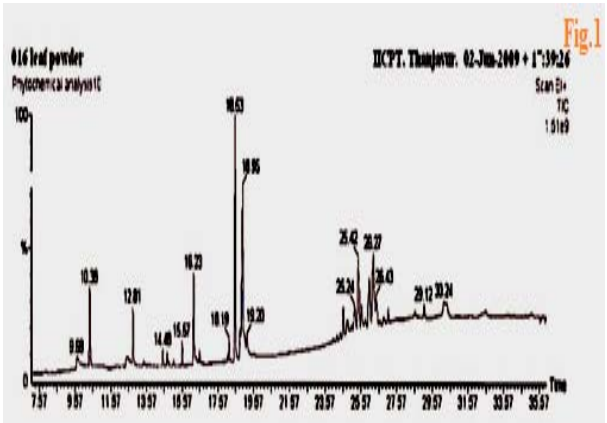

Figure 1: GC-MS chromatogram of ethanol extract of *Catharanthus roseus*

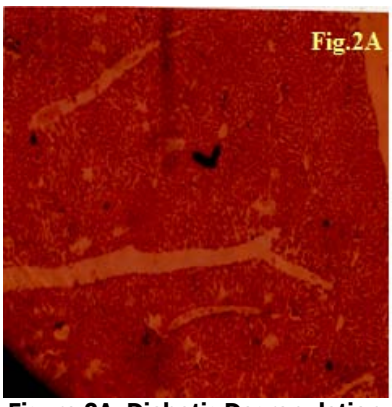

Figure 2A: Diabetic Degranulation

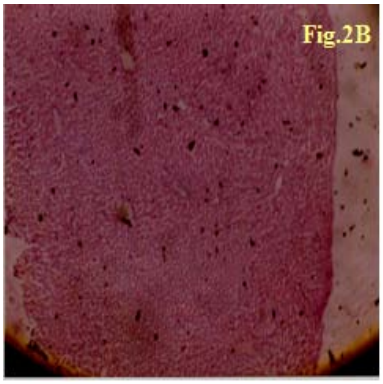

Figure 2B: Plant, less granulation

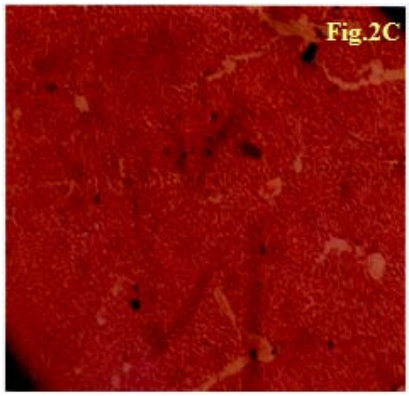

Figure 2C: Glibenclamide, Granulation

Table1: Phytocomponents identified in the ethanolic extract of *Catharanthus roseus* leaves using GC-MS study.

S. No	RT	Name of the compound	Molecular formula	MW	Peak Area%
1	10.38	Dodecanoic acid, methyl ester	$C_{13}H_{26}O_2$	214	9.78
2	12.81	Methyl tetradecanoate	$C_{15}H_{30}O_2$	242	5.49
3	14.48	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	1.71
4	14.73	1,2-Benzenedicarboxylic acid, bis(2-methyl propyl) ester	$C_{16}H_{22}O_4$	278	1.44
5	15.57	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	2.16
6	16.23	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	8.97
7	16.55	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	1.03
8	18.19	9-Octadecenoic acid (Z)-methyl ester	$C_{19}H_{36}O_2$	296	1.80
9	18.53	Phytol	$C_{20}H_{40}O$	296	24.14
10	18.95	9,12,15-Octadecatrienoic acid, (ZZZ)-	$C_{18}H_{32}O_2$	278	27.61
11	26.27	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	15.86

Table 2: Qualitative analysis of phytochemical constituents of aqueous and ethanolic extract of *Catharanthus roseus* leaves.

S. No	Phytochemicals	Aqueous Extract	Ethanol Extract
1	Alkaloids	+	+
2	Flavanoids	+	+
3	Saponins	+	+
4	Tannins	_	+
5	Terpenoids	_	+
6	Glycosides	+	+
7	Phenols	_	+
8	Carbohydrates	_	+
9	Proteins and amino acids	_	+
10	Fixed oils and fats	_	+
11	Polysterols	_	+
12	Gum and mucilages	_	_
13	Volatile oils	_	+
14	Steroids	_	+

The preliminary phytochemical screening as shown in Table 2 revealed the presence of alkaloids, flavonoids, saponins, tannins, terpenoids, glycosides, phenols, carbohydrates, proteins and aminoacids, fixed oils and fats, phytosterols, volatile oils, steroids except gums and mucilages in ethanol extract. The aqueous extract showed positive result for only alkaloids, flavonoids, saponins and glycosides. The negative result for aqueous extract may be due to the inability of water to extract the other fat soluble phytoconstituents.

The hypoglycemic activity of *Catharanthus roseus* may be due to the presence of alkaloids such as catharanthine, leurosine, lochnerine, tetrahydroalstonin, vindoline and vindolinine[11].

Alloxan was reported to cause a significant reduction of insulin-producing β-cells of islets of langerhans, thus inducing hyperglycemia[12]. The increased blood glucose level in diabetic mice as compared to normal ones might be due to glycogenolysis and/or gluconeogenesis[13]. A dose of alloxan up to 140-200 mg/kg body weight was found to be non-lethal[14]. In accordance with the earlier reports, in this study, dose of 150 mg/kg body weight[15] alloxan was selected. Under these conditions, insulin was secreted, but not sufficiently to regulate the blood glucose level, thus leading to the significant increase of blood glucose level in alloxan induced diabetic rats. In the present study, as shown in Table 3, this level was decreased by treating the diabetic rats with ethanolic extract of *Catharanthus roseus.* It was due to the mode of action of the active compounds of this plant material was probably mediated through enhance secretion of insulin

from the β-cell of langerhans or through extrahepancreatic mechanism. The hypoglycemic effect could be due to the presence of phytochemicals such as flavonoids in *Phyllanthus fraternus*[16]. The potency of *Catharanthus roseus* was compared with glibenclamide, a standard antidiabetic drug. It shows that *Catharanthus roseus* exhibited little less hypoglycemic activity than glibenclamide. Enzymic activities of glycogen synthase, glucose-6-phosphate-dehydrogenase, succinate dehydrogenase and malate dehydrogenase was improved in diabetic rats after treatment with extract of *Catharanthus roseus* due to increased metabolization of glucose[9].

The elevated TG, TC, LDL and VLDL level and decreased HDL level in alloxan-induced diabetic mice was in agreement with the previous reports, regarding alteration of these parameters under diabetic condition[17]. This was due to the action of alloxan, which causes excessive catabolism of proteins and amino acids that stimulate lipolysis in the adipose tissue[18]. The diabetes-induced hyperlipidemia might also be due to excess mobilization of fat from the adipose tissue because of underutilization of glucose[19].

In the present study, as depicted in Table 3 high concentration of serum total cholesterol level was observed in alloxan induced diabetic rats compared to normal rats. But there was a reduction in the levels of serum total cholesterol levels in diabetic rats treated with ethanolic extract of *Catharanthus roseus*. The reduction could have resulted from the antioxidant effect of the ethanol extract of *Catharanthus roseus*, whose phytochemical components include flavonoid, which is known for antioxidant effect[20]. The other phytochemicals such as tannin, saponin present in this plant also enhance the hypocholesterolemic activity. The reduction in the level of serum total cholesterol produced by *Catharanthus roseus* treated diabetic rats showed a little less effective when compared with that of glibenclamide treated diabetic rats. It was already reported that the total cholesterol level was significantly decreased in treatment with ethanolic extracts of *Butea monosperma*[21] and *Bergenia ligulata*[22] in alloxan induced diabetic rats.

Table 3: Effect of *Catharanthus roseus* on serum glucose and serum total cholesterol levels in alloxan induced diabetic rats

Groups	Glucose(mg/dl)	Cholesterol(mg/dl)
Group I	85.3 ± 3.1	82.3 ± 7.21
Group II	279.1 ± 9.4	219.3 ± 10.49
Group III	127.3 ± 3.1**	129.0 ± 5.24
Group IV	72.3 ± 8.2**	84.64 ± 6.79

Values were expressed as mean ± SEM, n = 6 by Students 't' test.*P < 0.01 Vs control. **P < 0.001 Vs control

Insulin deficiency results in a marked hypertriglyceridemia mainly due to an ineffective activation of the lipoprotein lipase system[23] and the elevating lipoprotein lipase (LPL) activity would cause an improvement of cahexia and obesity[24].

The elimination of VLDL and chylomicrons also decreases and it causes a marked elevation of triglyceride-rich lipoproteins[25]. In this study, as shown in Table 4 serum triglyceride and VLDL levels were significantly increased in alloxan induced diabetic rats.

Treating with ethanolic extract of *Catharanthus roseus* markedly decreased the levels of serum triglyceride and VLDL in alloxan induced diabetic rats. This result suggests that the ethanolic extract of *Catharanthus roseus* prevent the development of hypertriglyceridaemia in diabetic rats.

The reduction in the levels of serum triglyceride and VLDL could have resulted from the antioxidant effect of the different fractions of ethanol extract of *Catharanthus roseus*, whose phytochemical components include flavonoid, which is known for antioxidant effect[20].

Catharanthus roseus treated diabetic rats showed lesser reduction when compared with glibenclamide treated diabetic rats.

It was reported earlier that both the triglyceride and VLDL levels was significantly reduced in treating the diabetic rats with ethanol extract of *Butea monosperma*[21] and ethanol extract of *Bergenia ligulata*[22] decrease the triglyceride levels in diabetic rats.

Table 4: Effect of *Catharanthus roseus* on serum TGL, serum VLDL, serum HDL and serum LDL levels in alloxan induced diabetic rats

Groups	TGL(mg/dl)	VLDL(mg/dl)	HDL(mg/dl)	LDL(mg/dl)
Group I	75.15 ± 5.03	15.03 ± 1.006	38.6 ± 1.83	41.9 ± 4.37
Group II	116.48 ± 8.5	32.14 ± 1.7	22.93 ± 6.52	171.07 ± 2.27
Group III	87.52* ± 1.45	21.30 ± 2.32	27.24* ± 1.27	87.42** ± 4.14
Group IV	84.58* ± 6.7	16.92 ± 1.34	33.82* ± 2.79	33.9** ± 2.66

Values were expressed as mean ± SEM, n = 6 by Students 't' test.*P < 0.01 Vs control. **P < 0.001 Vs control

HDL cholesterol plays a key role in the protection against oxidative damage of membranes and lipid metabolism by transporting cholesterol from peripheral tissues to the liver through a process known as reverse cholesterol transport. In the present study, (Table 4) the alloxan induced diabetic rats showed a decreased level of serum HDL.

Administration of ethanol extract of *Catharanthus roseus* had influenced the serum HDL cholesterol level, which was nearer to HDL levels of diabetic rats treated with glibenclamide. High level of serum HDL cholesterol is well correlated with increased activity of lipoprotein lipase[26]. Thus the increase in serum HDL cholesterol during the extract treatment might suggest that the extract had influenced the lipoprotein lipase activity.

Reports available that flavonoid increases the HDL cholesterol level. It may augment the activity of lecithin acyl transferase (LCAT), which regulates blood lipids. LCAT plays a key role in the incorporation of free cholesterol into HDL (this may increase HDL) and transferring it back to VLDL and LDL which are taken back later in liver cells. Several studies have showed that increase in HDL cholesterol is associated with decrease in coronary artery disease (CAD)[27]. Thus flavonoids in *Catharanthus roseus* significantly increase the HDL cholesterol level. Increased level of HDL cholesterol was also seen in *Butea monosperma*[21] and *Bergenia ligulata*[22] treated diabetic rats.

Beyond the importance of even modest elevations in LDL cholesterol in people with diabetes, it also appears that LDL cholesterol interacts with risk factors of the metabolic syndrome to magnify the risk of CVD[28]. As a further consequence, through the action of cholesteryl ester transfer protein, a significant amount of the triglyceride content of VLDL is exchanged for cholesterol in LDL particles, leading to the formation of triglyceride-enriched and (Cholesterol-depleted) LDL. These LDL particles are now primed to become smaller and denser through the actions of hepatic lipase-mediated triglyceride hydrolysis[28]. Thus, adverse changes in LDL particles occur as triglyceride levels increase[29].

In this study, as depicted in Table 4, increased concentration of LDL was observed in alloxan induced diabetic rats. Treatment of diabetic rats with ethanol extract of *Catharanthus roseus* resulted in significant decrease in serum LDL level. It was higher than normal and glibenclamide treated diabetic rats. The reduced level of LDL may be possibly due to the presence of flavonoid in ethanol extract of *Catharanthus roseus*. It was stated that flavonoids remove LDL-C from blood by increasing the LDL receptor densities in liver and by binding to apolipoprotein B[30].

Changes of Histopathology of the Pancreas

Ethanolic extract of *Catharanthus roseus* showed slight regeneration of β cells (Fig.2B) when compared with the diabetic control (Fig. 2A). The standard group showed a mild protection from Alloxan induced changes in the pancreatic β cells (Fig. 2C).

CONCLUSION

In conclusion, the present study demonstrated that ethanolic extract of *Catharanthus roseus* leaves exhibit antidiabetic activity by lowering the serum glucose, cholesterol, triglyceride, VLDL and LDL levels and increasing the serum HDL cholesterol levels. The hypoglycemic and hypolipidemic activity of *Catharanthus roseus* was due to the presence of phytochemical components such as alkaloids and flavonoids. Hence, it might help in preventing diabetic complications and serve as a good adjuvant in the present armamentarium of antidiabetic drugs.

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