Antidiabetic and Hypolipidemic Effects of Different Fractions of *Catharanthus Roseus* (Linn.) on Normal and Streptozotocin-induced Diabetic Rats

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Abstract

The antidiabetic and hypolipidemic effects of petroleum-ether, ethyl acetate and chloroform fractions from ethanolic extract of the leaves of *Catharanthus roseus* (C. roseus) were investigated in normal and streptozotocin-induced diabetic rats (SIDRs). Single doses (150 mg/kg, i.p.) of *C. roseus* extracts in the fasting blood glucose (FBG) levels were determined in normal and SIDRs on 0, 1, 2, 3, 6, 10, 16, and 24th hours and serum triglyceride (TG) and serum total cholesterol (TC) levels were determined after 24th hour. In normoglycemic rats and in SIDRs, petroleum-ether and ethyl acetate fraction of *C. roseus* reduced blood glucose level significantly. In case of hypolipidemic effects, all fractions reduced serum total cholesterol but the ethyl acetate fraction of *C. roseus* was the most effective. All fractions of *C. roseus* reduced serum triglyceride level but the ethyl acetate fraction reduced triglyceride level at the highest. The antidiabetic and hypolipidemic activities were compared to metformin HCl (150 mg/kg). Of all the three fractions, ethyl acetate fractions were the best in activity. Ethyl acetate fraction of *C. roseus* was found to contain flavonoids and alkaloids. The mechanism underlying the antidiabetic activity is probably increased glycogenesis, decreased gluconeogenesis or decreased absorption of glucose from intestine.

Keywords: *Catharanthus roseus*; Antidiabetic; Hypolipidemic; Streptozotocin-induced diabetic rat.

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1. 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 [1]. Hyperglycemia is an important factor in the development and progression of the complications of diabetes mellitus [2]. The pathogenesis of diabetes mellitus is managed by insulin and oral administration of hypoglycemic drugs such as sulfonylureas and biguanides [3]. Unfortunately, apart from having a number of side effects, none of the oral synthetic hypoglycemic agents has been successful in
maintaining euglycaemia and controlling long-term microvascular and macrovascular complications [3-5]. The toxicity of oral antidiabetic agents differs widely in clinical manifestations, severity, and treatment [6]. The use of herbal medicines for the treatment of diabetes mellitus has gained importance throughout the world. The World Health Organization also recommended and encouraged this practice especially in countries where access to the conventional treatment of diabetes is not adequate [7]. There is an increased demand to use natural products with antidiabetic activity due to the side effects associated with the use of insulin and oral hypoglycemic agents [8, 9]. The available literature showed that there are more than 400 plant species having hypoglycemic activity [10-12]. Though some of these plants have great reputation in the indigenous system of medicine for their antidiabetic activities, many remain to be scientifically established. Hypercholesterolemia and hypertriglyceridemia are common complications of diabetes mellitus in addition to hyperglycemia [13-15]. The frequency of hyperlipidemia in diabetes is indeed very high, depending on the type of diabetes and its degree of control [16].

Periwinkle Catharanthus roseus Linn. (Nayantara) is an herbaceous ever-blossoming sub-shrub, grown as an ornamental plant in gardens all over Bangladesh. It has been reported that the juice of fresh leaves of Catharanthus reduces blood glucose in normal and alloxan-induced diabetic rabbits [17]. B. Antia and J. Okonon have demonstrated significant reduction in the levels of total cholesterol, triglycerides, LDL (low-density lipoprotein) and VLDL (very low-density lipoprotein) cholesterol using the fresh leaf juice of catharanthus in normal rats [18]. Leaf extract of Catharanthus is also shown to have blood glucose decreasing property [19]. S. Satyanrayana and colleagues have shown the leaf juice of Catharanthus and the seed powder of fenugreek have their hypoglycemic action individually and in combination on rabbits [20].

Some research have been performed on ethanolic crude extract of the plant yet the glucose lowering effects of petroleum ether, chloroform and ethyl acetate fractions isolated from the ethanolic extract have not been done. This fractionation effects will help us to determine which fraction is more potent. This finding will indicate which compounds are actually responsible for antidiabetic properties. In this study the effects of petroleum ether, ethyl acetate and chloroform extracts isolated from ethanolic extracts of C. roseus on fasting blood glucose (FBG) and lipid biochemical parameters such as serum total cholesterol (TC) and serum triglyceride (TG) were investigated on normal and SIDRs where metformin-HCl is used as standard drug. Thus the hypoglycemic, antihyperglycemic and hypolipidemic effects of the plant has been investigated.

2. Materials and Methods

2.1. Plant material

Fresh leaves of C. roseus were collected from the medicinal plant garden of our University and were dried under shadow for several days. The dried leaves were then ground to a coarse powder. The authenticity of the C. roseus was identified by Mr. A.H.M. Mahbubur Rahman, Department of Botany, Rajshahi University. Voucher
2.2. Reagents

Metformin HCl was the generous gift sample from Square Pharmaceuticals Ltd., Pabna, Bangladesh. Both the streptozotocin-HCl and DMSO were purchased from Loba Chemie, Bombay, India. DMSO (dimethyl sulfoxide) was used to dissolve metformin and the extracts of \textit{C. roseus}, since these substances are insoluble in water and other available inert solvents [21].

2.3. Preparation of ethanol extracts

Dried leaves of \textit{C. roseus} were soaked for 5-7 days in 2 liter of 95\% ethanol with occasional shaking and stirring. Then, they were passed through cotton and then filtered through filter paper. The remaining parts were filtered again under the same procedure. Then the solvent i.e., ethanol was allowed to evaporate using rotary evaporator at temperature 40-45\(^\circ\)C. Thus the highly concentrated ethanolic extract was obtained.

2.4. Fractionation of ethanol extract

Crude rectified spirit extract was diluted by addition of 150 ml distilled water to obtain aqueous solution. The aqueous solution is then treated with 50 ml petroleum ether for three times. The upper fraction was collected in each time of fractionation by using separating funnel. The aqueous fraction is then treated with chloroform 50 ml for three times. The lower fraction was collected for getting chloroform extract. The remaining aqueous fraction was again treated with 50 ml ethyl acetate for three times. The upper fraction was collected for getting ethyl acetate fraction.

The fractions of the different solvents were then evaporated by rotary evaporator. The remaining portions of the different fractions were then dried by using mild sunlight. The dried extracts were then preserved in the freeze for the experimental use [22].

2.5. Phytochemical screening tests

The following phytochemical screening methods [23] were used for tests:

\textit{Test for saponins}: Boiled 300 mg of extract with 5 ml water for two minutes. Mixtures was cooled and mixed vigorously and left it for three minutes. The formation of frothing indicates the presence of saponins.

\textit{Test for tannins}: To an aliquot of the extract added sodium chloride to make to 2\% strength. Filtered and mixed with 1\% gelatin solution. Precipitation indicates the presence of tannins.
Test for triterpenes: 300 mg of extract mixed with 5 ml chloroform and warmed for 30 minutes. The chloroform solution is then treated with a small volume of concentrated sulfuric acid and mixed properly. The appearance of red color indicates the presence of triterpenes.

Test for alkaloids: 300 mg of extract was digested with 2 M HCl. Acidic filtrate was mixed with amyl alcohol at room temperature, and examined the alcoholic layer for the pink colour, which indicates the presence of alkaloids.

Test for flavonoids: The presence of flavonoids was determined using 1% aluminium chloride solution in methanol, concentrated HCl, magnesium turnins and potassium hydroxide solution.

2.6. Animal experiments

A total number of 50 long-Evans female rats weighing about 150-180 mg age 2 months were purchased from animal house of International Centre for Diarrhoal Disease Research, Bangladesh (ICDDR, B). Prior to the commencement of the experiment, all the rats were acclimatized to the new environmental condition for a period of one week. During the experimental period, the rats were kept in a well-ventilated animal house at room temperature of 25°C and were supplied with standard pellets supplied from ICDDR, B and fresh drinking water ad libitum. They were kept in cages and maintained in well-ventilated room under conditions of natural light and dark cycle. Animals were fasted for 16 h prior to drug administration allowing access only to water and were deprived of both food and water during the experiment.

2.7. Induction of diabetes

The rats were randomly divided into 10 groups, each containing 5 rats. After fasting 16 h, rats of group (VI-X) were rendered diabetic by injecting intraperitoneally a freshly prepared solution of streptozotocin (45 mg/kg) in 0.1 mol/L citrate buffer, pH 4.5, in volume 1 ml/kg [24], after a base line glucose estimation was done. After 48 hours blood glucose content was measured by using BioLand G-423 Glucose Test Meter (BioLand, Germany) using Blood sample from the tail vein of the rats. When the condition of diabetes was established animals with blood glucose levels above 11.1 mmol/L were selected for the study [25].

2.8. Effect on diabetic rats

Group I served as a nondiabetic control while group VI for diabetic control group. Group II served as nondiabetic metformin control while group VII served as diabetic metformin controlled group. Group II and VII were treated with metformin HCl (150 mg/kg, i.p.) for 24 hours experiment. Group III, IV, V and group VIII, IX and X were treated with the pet-ether, ethyl acetate and chloroform fractions of ethanolic extract of *C. roseus* at 150
mg/kg for 24 hours experiment. The reference drug and the extracts were administered intraperitonially to the rats.

2.9. Collection of blood and serum and determination of blood glucose, serum total cholesterol and serum triglycerides

Blood samples were collected from tail vein of each rat of a group before and also at 0, 1, 2, 3, 6, 10, 16, and 24th hours of one day experiment. The samples were analyzed for blood glucose content by using BioLand G-423 glucose test meter (BioLand Germany). Then the rats were sacrificed and about 1-2 ml of blood was collected directly from the heart by syringes, centrifuged at 4000 rpm for 10 minutes and the serum was obtained for the determination of TC and TG. Serum TC and TG concentrations were analyzed by measuring absorbance by UV spectrophotometer (Shimidzu UV-1200, Tokyo, Japan), using wet reagent diagnostic kits (Boehringer Mannheim, GmbH) according to manufacturer’s protocol.

2.10. Statistical analysis

Data were expressed as mean ± standard error of mean (SEM). Statistical comparisons were performed by one-way ANOVA followed by Dunnett's Multiple Comparison Test (DMCT) and the values were considered statistically significant when $p<0.01$. Statistical calculations and the graphs were prepared using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, CA, USA, www.graphpad.com).

3. Results and Discussions

The effects of different fractions of the ethanolic extracts of *C. roseus* on the FBG, serum TC and serum TG levels were investigated in the control and streptozotocin-induced diabetic rats using metformin HCl as standard antidiabetic agent.

3.1. Effect of different fractions of *C. roseus* on fasting blood glucose level in normoglycemic rats

The mean blood glucose concentration of controlled and fractions of *C. roseus*–treated animals (after intraperitoneal administration of a single dose) on 0, 1, 2, 3, 6, 10, 16, and 24th hours are shown in Fig. 1. Hypoglycemia was observed in animals treated with *C. roseus* extracts. The significant reduction ($p<0.01$) of 48.34% for petroleum ether-CR was observed at 10th hour of the experiment. The chloroform extract of *C. roseus* has no significant effect on the blood glucose level. Chloroform-CR fraction showed some toxic effect because some rats were died. This may be due to the presence of some alkaloids with anticancer properties of the plant. The ethyl acetate fraction of *C. roseus* reduced FBG by 40.68% was observed at 24th hour for ethyl acetate. This may be due to the
presence of hypoglycemic alkaloids (catharanthin, leurosine, lochnerine, tetrahydroalstonin, vindoline and vindolinine) \( p < 0.01 \) [26]. A significant reduction \( p < 0.01 \) in blood glucose of 52.55% was observed for metformin-HCl at 6\(^{th}\) hour after treatment in comparison to control. All treatment is done with a single dose of (150 mg/kg body weight).

3.2. Effect of different fractions of \textit{C. roseus} on fasting blood glucose level in streptozotocin-induced diabetic rats

Fig. 1. Effect of different fractions of \textit{C. roseus} (150mg/kg) on Fasting Blood Glucose level (mmol/L) in normal rats. * and \( \Delta \) (***) indicate significant changes in FBG level compared to normal rats after treatment \( p < 0.05, \ p < 0.01 \) respectively. The result is expressed as mean±SEM.

Fig. 2. Effect of different fractions of \textit{C. roseus} on the FBG level on diabetic rats compared to normal rats. \( \Psi \) (***) indicates significant change in blood glucose level compared with normal control group \( p < 0.001 \). *, \( \Delta \) (**) and \( \delta \) (***) indicate significant changes in FBG level in SIDRs after treatment \( p < 0.05, \ p < 0.01 \) and \( p < 0.001 \), respectively. The result is expressed as mean ± SEM.
The mean blood glucose concentration of controlled and fractions of *C. roseus*–treated animals (after intraperitoneal administration of a single dose) on 0, 1, 2, 3, 6, 10, 16, and 24th hours are shown in Fig. 2. Hypoglycemia was observed in animals treated with *C. roseus* extracts. The significant reduction (*p*<0.001) of 51.67% for petroleum ether-CR occurs at 10th hour of the experiment. Ethyl acetate-CR fraction showed maximum reduction (*p*<0.001) of 49.22%, observed at 24th hour. Metformin caused maximum reduction (*p*<0.001) of blood glucose level of 49.62% on 10th hour of experiment after treatment in comparison to controlled diabetic rats. All treatment is done with a single dose of (150 mg/kg body weight).

### 3.3. Effects of different fractions of *Catharanthus roseus* on total cholesterol and triglyceride levels in normal rats

The mean serum total cholesterol and triglyceride levels of control and extracts of *C. roseus*–treated animals (after intraperitoneal administration of a single dose) on the 24th hour are shown in Fig. 3 and Fig. 4. Hypolipidemia was observed in animals treated with *C. roseus* extracts.

![Fig. 3. Effect of different fractions of *C. roseus* on the total cholesterol (mmol/L) in normal rats. *, Δ (**) and δ (***) indicate significant change in serum total cholesterol compared with normal control group (*p*< 0.05). The result is expressed as mean± SEM.](image1)

![Fig. 4. Effect of different fractions of *C. roseus* on the triglyceride (mmol/L) in normal rats. Δ (**) indicates significant change in serum triglyceride compared with normal control group (*p*< 0.01). The result is expressed as mean ± SEM.](image2)
During the effect of different fractions of *C. roseus* (Fig. 3) on the cholesterol level of normal rats CHCl₃-CR, ethyl acetate-CR and pet ether-CR fractions decreased cholesterol level to 87.46% (not significant), 68.03% and 69.99% respectively. So the ethyl acetate-CR fraction showed maximum reduction of 31.97%, which is similar to metformin (31.01%) also.

During the effects of different fractions of *C. roseus* (Fig. 4) on serum triglyceride level of normal rats the chloroform-CR, ethyl acetate-CR and petroleum ether-CR showed serum triglyceride level of 73.11%, 55.64% and 60.48% respectively. Maximum reduction of serum triglyceride level of 44.36% was observed for ethyl acetate-CR.

### 3.4. Effects of *C. roseus* on total cholesterol and triglyceride levels in streptozotocin-induced diabetic rats

During the effects of different fractions of *C. roseus* (Fig. 5) on SIDRs CHCl₃-CR, ethyl acetate-CR and pet ether-CR showed total cholesterol level of 68.81%, 64.33% and 68.28% respectively where metformin reduces 64.61%. Maximum reduction of 35.67% was observed for ethyl acetate-CR, which is also similar to metformin action.

![Fig. 5. Effect of different fractions of *C. roseus* on the serum total cholesterol (mmol/L) on diabetic rats compared to normal rats.](image1)

![Fig. 6. Effect of different fractions of *C. roseus* on the serum triglyceride (mmol/L) on diabetic rats compared to normal rats.](image2)
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During the effects of different fractions of *C. roseus* (Fig. 6) on serum triglyceride level of normal rats the CHCl_3-CR, ethyl acetate-CR and petroleum ether-CR showed serum triglyceride level of 73.11%, 55.64% and 60.48%, respectively where metformin reduces to 80.64%. Maximum reduction of serum triglyceride level of 44.36% was observed for ethyl acetate-CR.

However, the serum total cholesterol and triglyceride lowering efficiency of ethyl acetate fraction of *C. roseus* was found higher than other fractions. All the plant extracts were found to have the antihyperlipidemic activity in SIDRs.

3.5. Phytochemical screening test result

The phytochemical screening test result is shown in the Table 1.

| Chemical constituents of different fractions of *C. roseus* | Saponin | Tanins | Triterpines | Alkaloids | Flavonoids |
|------------------------------------------------------------|--------|--------|-------------|-----------|------------|
| Petroleum ether-CR                                          | -      | +      | -           | +         | +          |
| Chloroform-CR                                               | -      | -      | -           | +         | +          |
| Ethyl acetate-CR                                            | -      | -      | -           | +         | +          |

(-) – Not detected; (+) - Detected

In the mid-1960s streptozotocin was found to be selectively toxic to the β-cells of the pancreatic islets, the cells that normally regulate blood glucose levels by producing the hormone insulin. This suggested the drug’s use as an animal model of type I diabetes [27]. It is well established that biguanides like metformin produce hypoglycemia by increasing the secretion of insulin from the pancreas [28] and these compounds are active in mild Streptozotocin-induced diabetes whereas they are inactive in intense streptozotocin diabetes (nearly all β-cells have been destroyed). However, since our results showed that metformin reduced the blood glucose levels in hyperglycemic rats, the state of diabetes is not severe. Streptozotocin-treated animals receiving the extracts of *C. roseus* showed rapid normalization of blood glucose levels in comparison to the control and this could be due to the possibility that some β-cells are still surviving to exert their insulin releasing effect by different fractions of *C. roseus*. No histological studies were carried out to prove this and it is not possible to explain the detailed mechanism of antidiabetic action of the fractions of *C. roseus*. The active principles in the *C. roseus*, which may be responsible for the hypoglycemic and hypolipidemic actions, are unknown. This may be due to the presence of hypoglycemic alkaloids (catharanthin, leurosine, lochnerine, tetrahydroaflstomin, vindoline and vindolinine) [26]. This suggests that the the plant extracts studied in this protocol might have bound to insulin receptors to act as insulin secretagogue, like biguanides. Other probable mechanisms by which the extracts of *C. roseus* lowered blood glucose levels in diabetic rats might be by increasing glycogenesis, inhibiting gluconeogenesis in the liver, or inhibiting the absorption of glucose from the intestine or these might have improved insulin resistance. Further experiments are needed.
to determine the actual mechanism of action of the active constituents of the relative plant fractions. In the present study, there was a significant reduction in the levels of total cholesterol and triglycerides. This reduction could have resulted from the antioxidant effect of the different fractions of ethanol extract of *C. roseus*, whose phytochemical components include flavonoid, which is known for antioxidant effect. Further investigations are warranted to identify the hypolipidemic mechanism of the active principles in *C. roseus*.

4. Conclusion

Plant medicines (phytotherapies) have a long history as treatment for diabetes. With a disturbing rise in the prevalence of this metabolic disease and associated healthcare costs, interest in alternative or complementary therapies has grown. Our study have shown that the ethyl acetate fraction of ethanolic extract of *C. roseus* is most effective in glucose lowering effect in normal and streptozotocin-induced hyperglycemic rats. In the present study, there was a significant reduction in the levels of total cholesterol and triglycerides. This reduction could have resulted from the antioxidant effect of the extracts of *C. roseus*, whose phytochemical components include flavonoid, which is known for antioxidant effect. This also may be due to the presence of hypoglycemic alkaloids (catharanthin, leurosine, lochnerine, tetrahydralstonin, vindoline and vindolinine) [26]. Our preliminary phytochemical analysis has indicated that flavonoids and alkaloids have been reported to exert potent hypoglycemic and hypolipidemic effects. It needs to be determined as to which components of *C. roseus* are responsible for the hypoglycemic and hypolipidemic activity exhibited by the ethyl acetate fraction. However this study will pave the way for plant based specific treatment of diabetes avoiding the complications of artificial drug substances.

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