Antihyperlipidemic Activity of *Catharanthus Roseus* L. (Apocyanaceae) Leaf Extract on Triton-induced Hyperlipidemic Rats

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Abstract: *Catharanthus* roseus is a popular ornamental plant used traditionally for a variety of diseases including hyperlipidemia and diabetes. However, pharmacologic studies are needed to support the traditional uses this plant. The study aims to evaluate the ability of *C. roseus* L. leaf extract to lower lipid levels in normoglycemic rats. Thirty (30) Sprague-dawley rats were used, and hyperlipidemia was induced by intraperitoneal injection of Triton WR-1339. The rats were treated with *C. roseus* leaf extracts (50mg/Kg, 150mg/kg, and 250mg/kg BW) or the standard drug, Fenofibrate, for seven days. Results of ANOVA ($P > 0.05$) showed greater reduction of the two concentrations of *C. roseus* extract (150mg/kg, and 250mg/kg) with that of Fenofibrate for total cholesterol (TC), total triglyceride (TG), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) levels. The results demonstrate the potential of *C. roseus* for controlling elevated lipid levels.

Keywords: *Cantharanthus roseus*; antihyperlipidemia; total cholesterol; total triglyceride; high-density lipoprotein; low-density lipoprotein; very low-density lipoprotein

1. INTRODUCTION

1.1 Background

The Department of Health estimated a 2.5% increase in mortality rate per year from cardiovascular diseases (Jambora, 2014). Nelson (2014) stated that hyperlipidemia or elevated blood lipid levels are a known risk factor for the development of cardiovascular diseases such as stroke and ischemic heart disease.

Hyperlipidemia is characterized by elevated serum total cholesterol or total triglyceride levels. A high level of cholesterol in serum, as a result of high dietary intake, is classified as exogenous hyperlipidemia. The role of diet in this process is of primary importance. Hyperlipidemia may also be caused by an inherited defect in lipid metabolism, known as endogenous hyperlipidemia and by disease, known as primary or familial hyperlipidemia (Rubin, 2005). Poorly controlled diabetes is also often associated with elevated lipid levels. All forms of the vascular disease have been noted in Type 2 diabetic patients and hyperlipidemia (Nuovo, 1999). Snipelisky and Ziajka (2012) conducted a quantitative analysis of the relationship of diabetes and hyperlipidemia. A statistically significant relationship was seen between

patients with either glucose intolerance or diabetes and the different lipid levels. In these cases, drug treatment is recommended apart from diet.

Parenteral administration of Triton WR1339 (Tyloxapol) has been shown to increase plasma lipid levels and has been widely used in animal studies to induce acute hyperlipidemia (Al-Qirim et al., 2012; Anandhi et al., 2013; Sikarwar & Patil, 2012; Sivaelango, Kumaran, Revathi, & Jaswant, 2012; Vohra, Gupta, Dureja, & Garg, 2016).

Catharanthus roseus (L.) G. Don. is a short-lived perennial plant with dark green and glossy leaves and is cultivated as an ornamental plant (Evans, 2005). Pharmacological studies have revealed that the plant contains more than 70 different types of alkaloids that are effective in treating various types of cancers. The anticancer drugs vincristine and vinbalstine are obtained from the alkaloids of *C. roseus*. (Evans, 2005; Mustafa and Verpoorte, 2007). Besides anticancer activity, several hypoglycemic studies have been conducted in vivo (Akhtar, Rashid, Wahed, & Ahmed, 2007; Islam et al., 2009; Mostofa et al., 2007; Nammi, Boini, Lodagala, & Behara, 2003; Rasineni, Bellamkonda, Singareddy, Desireddy, 2010). Muralidharan (2014) showed a significant decrease in serum glucose levels and lipid levels (TG, TC, LDL, and VLDL) using aqueous extracts of *C. roseus* leaves in alloxan-induced diabetic rats. Normal and Streptozotocin-induced diabetic rats were used in the study of Islam et al. (2009). All fractions reduced serum TG and TC levels compared to Metformin, an antidiabetic agent.

Although previous studies have indicated the potential of *C. roseus* as an antidiabetic and antihyperlipidemic agent in diabetic rats, there are no studies that focus solely on its use to lower blood lipid levels.

2. METHODOLOGY

2.1 Plant Material

Fresh *C. roseus* leaves were collected from Dasmarinas, Cavite from November to January. The leaves were washed with water to remove extraneous matter and oven dried at 50°C until constant weight. The dried leaves were ground to a coarse powder using a wiley mill (Thomas Scientific, 3383-L30) and were kept in tight, light-resistant containers.

The dried leaves were macerated using 80% ethanol for 48 hours. The mixture was filtered using a Buchner funnel and allowed to evaporate below 50°C using a rotary evaporator.

2.2 Chemicals

Tyloxapol used in the study is of United States Pharmacopeia (USP) grade. Other chemicals used in the study are of analytical grade and were all purchased from Sigma-Aldrich.

2.3 Experimental Animals

Healthy and properly identified male Sprague-dawley rats, each weighing 150 – 200 g, were used. The animals were procured from the Animal House Facility of the Department of Science and Technology (DOST).

2.4 Housing and Feeding Conditions

The rats were maintained under standard laboratory conditions. The temperature in the experimental animal room was set at 22°C (±3°C) with a relative humidity of 50 to 60%. Artificial light was used, with the sequence following 12 hours' light and 12 hours' dark. The animals were individually housed. Conventional rodent dry pellets were used for feeding with an unlimited supply of drinking water.

2.5 Experimental Design

Thirty (30) rats were randomly distributed to six groups with five rats per group (Table 1). The rats in Group I received 0.2 mL/10 g of normal saline solution (NSS) and served as the negative control. Group II, III, IV, V, and VI were all injected with a single dose of 300 mg/kg body weight (BW) of Triton. Group II is the hyperlipidemic (Triton) control, group. Group III, IV, and V are the hyperlipidemic treated group containing 50 mg, 150 mg, and 250 mg/kg body BW of *C. roseus* extract. Group VI received 65 mg/kg BW of Fenofibrate and served as the positive control. The extracts and positive control were given through gastric lavage.

Table 1. Different Experimental Groups and their Corresponding Group Numbers

Group No.	**Treatment**
I	Negative Control (NSS)
II	Hyperlipidemic Control (Triton)
III	Positive Control (Fenofibrate)
IV	50mg/Kg *C. roseus* Extract
V	150mg/Kg C. roseus Extract
VI	250mg/Kg C. roseus Extract

Note. NSS = normal saline solution

2.6 Induction of Hyperlipidemia

The animals were fasted from food and water for 12 hours before testing. Hyperlipidemia was induced by the intraperitoneal administration of Triton WR-1339 (Tyloxapol) in saline suspension at a dose of 300 mg/kg BW.

2.7 Biochemical Analysis

Blood samples were taken from the tail vein, and the sera were subjected to laboratory analysis for lipid profiling (TG, TC, LDL, and VLDL levels). The samples were analyzed by measuring the absorbance by UV spectrophotometer (Shimadzu UV-1200) using wet reagent diagnostic kits. Samples were taken after 24 and 48 hours of induction of hyperlipidemia.

2.4 Statistical Analysis

All data were expressed as Mean ± S.D. (Standard Deviation). Repeated measures analysis of variance (ANOVA) followed by Tukey was used for data analysis using IBM SPSS Statistics version 19. Values with $P < 0.05$ were considered as statistically significant.

3. RESULTS

Table 2 and 3 shows the lipid levels after 24 and 48 hours of treatment. For induction of hyperlipidemia, all treatment groups (II, III, IV, V, & VI) showed a marked increase in TC, TG, LDL and VLDL levels after 24 hours compared to the NSS group (I) with the Triton group (II) showing the greatest elevation in all lipid levels.

Table 2. Cholesterol, and Triglyceride Levels of the Different Experimental Groups after 24 and 48 Hours of Treatment

Group No.	**Cholesterol (mg/dL)**		**Triglycerides (mg/dL)**	
	24 hours	*48 hours*	*24 hours*	*48 hours*
I	56.80±5.78*	53.86±4.81*	34.84±3.23*	33.78±3.50*
II	235.95±16.00*	153.06±14.64*	78.38±8.56*	75.71±9.82*
III	163.70±15.85	63.54±4.33	62.62±15.32	43.07±7.54
IV	144.44±15.35*	78.38±7.42*	53.10±10.29	44.08±8.04
V	175.52±10.14	71.44±9.26	59.80±10.87*	47.51±8.50*
VI	162.60±18.76	59.26±6.16	53.58±10.25*	43.25±6.81*

Note. Values are expressed as Mean ± S.D. in mg/dL, n = 5.
Values with (*) differ significantly at P < 0.05 compared with Fenofibrate (positive control)

Table 3. LDL, and VLDL Levels of the Different Experimental Groups after 24 and 48 Hours of Treatment

Group No.	**LDL (mg/dL)**		**VLDL (mg/dL)**	
	24 hours	*48 hours*	*24 hours*	*48 hours*
I	55.53±9.94*	55.81±7.08*	17.46±1.30	17.47±1.35
II	175.86±10.27*	156.09±9.55*	22.06±1.16	15.97±1.50
III	165.01±10.39	62.32±3.46	26.36±1.94	7.86±1.39
IV	184.22±14.87	61.37±3.30	23.38±1.87	8.43±1.25
V	189.61±11.26*	69.58±10.16*	25.53±2.63	7.62±0.61
VI	202.11±11.20*	69.44±6.69*	25.29±3.52	7.73±0.51

Note. Values are expressed as Mean ± S.D. in mg/dL, n = 5. Values with (*) differ significantly at $P < 0.05$ compared with Fenofibrate (positive control)

A decline in cholesterol levels was observed in all groups after 48 hours of treatment (Figure 1). Multiple comparisons using Tukey revealed a statistically comparable cholesterol-lowering effect of Fenofibrate and the three *C. roseus* extracts (III, IV, V, VI). A statistically significant difference in the triglyceride and LDL-lowering capacity was noted between Fenofibrate (III) and the hyperlipidemic treated groups of 150mg/kg and 250mg/kg extracts (V, VI) (Figure 2 & 3). A marked reduction was seen in Fenofibrate (III) and *C. roseus* extracts (IV, V, VI) in VLDL levels (Figure 4). A significant difference was not observed between the positive control and *C.* roseus extracts in lowering VLDL levels.

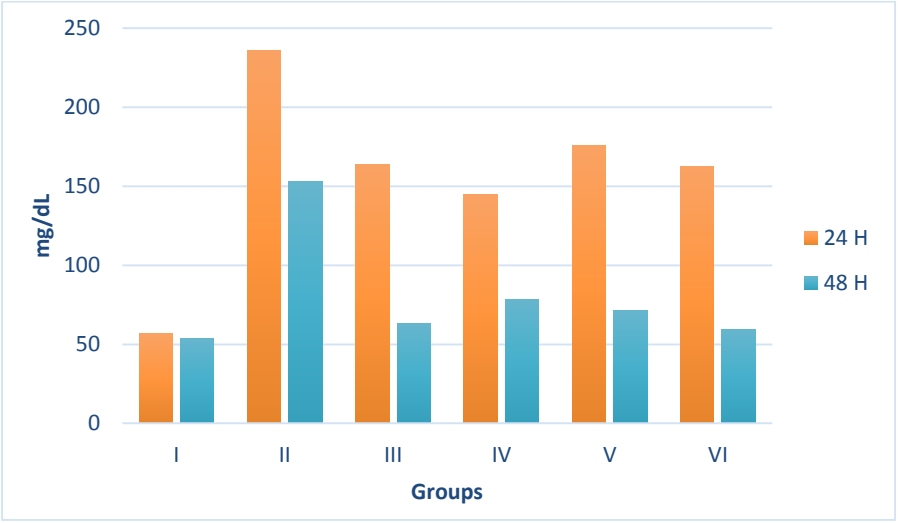

Fig. 1. Mean Total Cholesterol Levels of the Different Treatment Groups Expressed in mg/dL

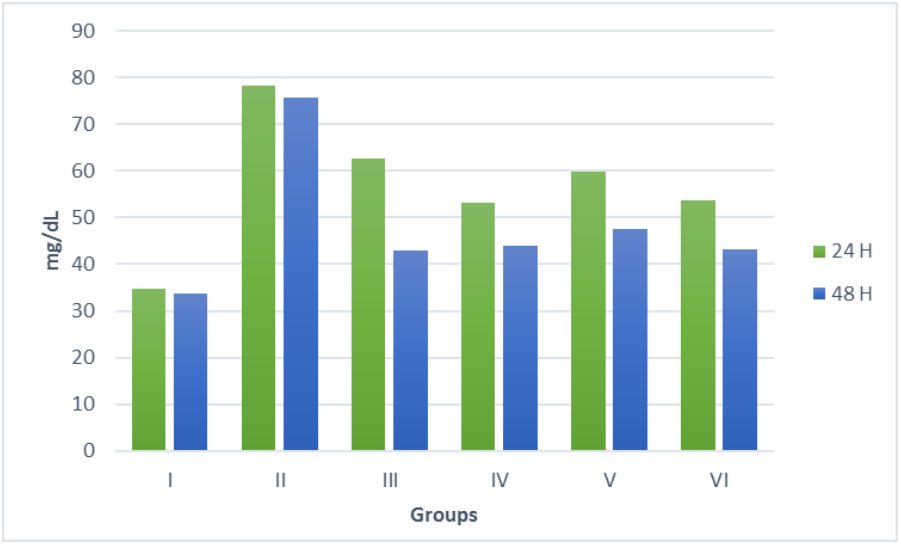

Fig. 2. Mean Total Triglyceride Levels of the Different Treatment Groups Expressed in mg/dL

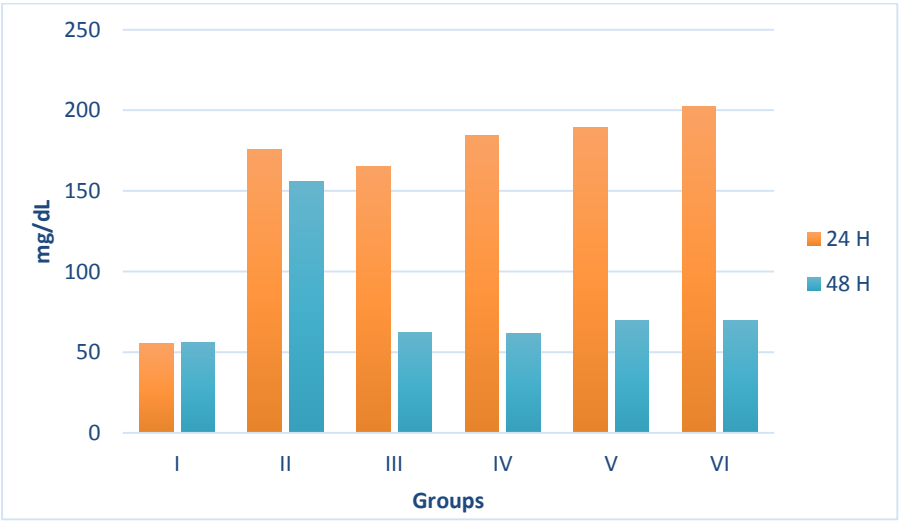

Fig. 3. Mean LDL Levels of the Different Treatment Groups Expressed in mg/dL

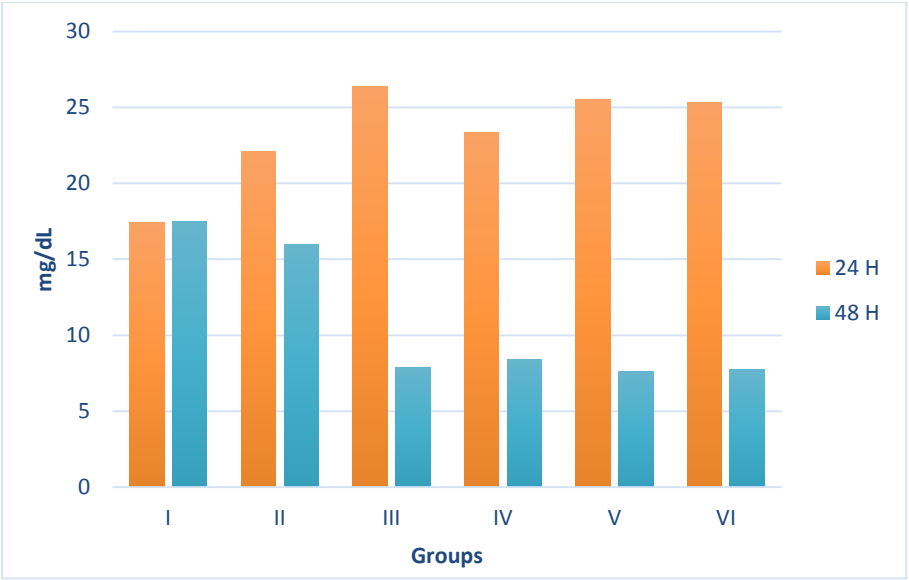

Fig. 4. Mean VLDL Levels of the Different Treatment Groups Expressed in mg/dL

4. DISCUSSION

Diabetes, a chronic metabolic disorder, is often associated with serum lipid level alteration. Deficiency in insulin production causes increased cholesterol concentration resulting to increased free fatty acid release and increased triglyceride synthesis (Giugliano, Ceriello, & Paolisso, 1996; Thirumalaisamy, Prabhakaran, Marimuthu, & Chatterjee, 2013). Homogenous glucose levels of the experimental groups after 24 and 48 hours of treatment using post hoc analysis show that the lipid-lowering effect of the *C. roseus* extracts is not influenced by blood glucose levels.

Triton WR-1339 is a non-ionic detergent that causes elevated levels of lipoproteins in the blood by blocking lipoprotein uptake from peripheral tissues (Anandhi et al., 2013; Vohra et al., 2016). According to Schurr, Schultz, & Parkinson (1972), the induction of hyperlipidemia using this model is rapid, providing peak levels at 20 hours which gradually declines to normal. In the study, Triton was proven to be effective in increasing all lipid levels in the experimental groups after 24 hours as compared to the negative control group. Triton has also been used to successfully induce hyperlipidemia in rats in other studies (Bertges, Mourao, Souza, & Cardoso, 2011). Ngoc, Ngoc, Van, and Phung (2008) used Tyloxapol to determine the hypolidpemic effect of the extracts of *Abelmoschus esculentus*. A significant increase in total cholesterol and triglyceride levels was seen in mice injected with Tyloxapol. The increase noted was more than two folds higher compared to those of the control group. Triton was also utilized to investigate

the potential of a polyherbal preparation as an antihyperlipidemic agent in comparison to Lovastatin. Hyperlipidemia was induced by a single intravenous administration. (Ansarullah, Jadeja, Thounaojam, & Ramachandran, 2009). The results obtained demonstrate the viability of using Triton WR-1339 in antihyperlipidemic studies.

Fenofibrate, a lipid-lowering agent, is indicated as adjunctive therapy to diet to reduce elevated total cholesterol, total triglycerides, LDL and VLDL levels and to increase HDL levels in patients with primary hyperlipidemia or mixed dyslipidemia (Katzung, 2012). Benefits of taking Fenofibrate are also greater for diabetic patients causing significant reductions in total cardiovascular disease events, particularly nonfatal myocardial infarction and coronary revascularization (Scott et al., 2009). From the results obtained, *C. roseus* extracts were comparable in reducing cholesterol and VLDL levels to Fenofibrate. A significant difference, however, was seen in reducing triglyceride and LDL levels using 150mg/kg and 250mg/kg *C. roseus* extracts and Fenofibrate. The greater reduction was observed for the two extracts compared to the positive control. A previous study by Islam, et al. (2008), showed comparable results it lipid-lowering levels. The antidiabetic and hypolipidemic effects of the different ethanolic fractions of *C. roseus* were studied, and a significant reduction in total cholesterol and triglyceride levels was also observed.

The precise mechanism of action and constituents responsible for the reduction of lipid levels are unknown. Reported glucose-lowering effects and antioxidant activities of *C. roseus* may influence lipid levels (Islam et al., 2009; Akhtar et al., 2007; Nammi et al., 2003). Phenols are very widespread and are probably the largest group of secondary plant metabolites. They range from simple structures having a single aromatic ring to highly complex polymeric structures and often exist in glycosidic forms. Phenols may be divided into several classes. Those of pharmaceutical importance are the simple phenolic compounds, tannins, anthraquinones, and flavonoids (Gennaro, 2005). Islam et al. (2009) reported that phytochemical compounds of the plant include flavonoids, known for their antioxidant effects. These compounds can potentially reduce triglyceride and cholesterol levels. Several anthocyanins and flavonols were tested to be present in *C. roseus* by Mustafa and Verpoorte (2007). Among the anthocyanins found on the plant are Petunidin, Malvidin, and Hirsutidin while Kaemferol, Quercetin, and Syringetin-3-robinobioside are the flavonols present in *C. roseus*.

5. CONCLUSION

The study indicates that the ethanolic extracts of *C. roseus* possessed antihyperlipidemic properties. Using repeated measures ANOVA, *C. roseus* extract at doses of 150mg/kg and 250mg/kg showed greater lipid-lowering effect than Fenofibrate. The results of the study may be used to investigate the specific constituents in *C. roseus* extract that are responsible for the

antihyperlipidemic effect. Further pharmacologic studies are also needed to elucidate the lipid-lowering mechanism of *C. roseus*.

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