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Antihyperlipidemic effect of *Bougainvillea glabra* leaves in triton wr-1339 induced hyperlipidemic rats

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ABSTRACT

Hyperlipidemia is the highest risk factor of coronary heart disease. Herbal treatment for hyperlipidemia has no side effects and is relatively cheap and locally available, *Bougainvillea glabra* was selected and the present study on anti-hyperlipidemic activity of extract of *Bougainvillea glabra* leaves against triton induced hyperlipidemia in rats. Ethanolic extract, aqueous extract, chloroform fraction of ethanolic extract and ethylacetate fraction of ethanolic extract administered at different doses to the triton induced hyperlipidemic rats. *Bougainvillea glabra* has shown a significant decrease in the levels of serum cholesterol, triglyceride, LDL and significant increase in the level of serum HDL.

Keywords: *Bougainvillea glabra*, Hyperlipidemia, TC, TG, LDL, HDL.

INTRODUCTION

Hyperlipidemia is the highest risk factor of coronary heart disease [1]. Lipid metabolism maintains a sophisticated balance between synthesis and degradation. When the hyperlipidemia misbalanced which may causes hypertriglyceridemia and hypercholesterolemia [2]. Hyperlipidemias properties to increased serum cholesterol, low density and decrease high density lipoprotein are the risk factor for coronary heart diseases. Hyperlipidemia accompanying lipid profile disorders are reflected to cause the atherosclerotic disease [3].

The main aim of study with hyperlipidaemia is to reduce the risk of developing ischemic heart disease or the occurrence of further atherosclerotic disease [4]. Presently obtainable hypolipidemic drugs have been related with number of side effects compare with allopathic drugs, herbal treatment for hyperlipidemia has shown no side effects [5]. Medicinal plants are effective in reducing the lipid levels in the system.

The genus *Bougainvillea* in the Nyctaginaceae family of plants has 18 species, with three that are horticulturally important *Bougainvillea spectabilis*, *B. glabra* and *B. peruviana*. *Bougainvillea glabra* have been traditional used disorders like diarrhoea, reduce stomach acidity, obesity, cough and sore throat, decoction of dried flowers for blood vessels and leucorrhoea and decoction of the stem in hepatitis [6-9].

However an attempt has been made to investigate the effects of *Bougainvillea glabra* leaves on triton wr-1339 induced hyperlipidemic rats.

MATERIALS AND METHODS

Collection and authentication of plant

The leaves of *Bougainvillea glabra* were collected from local area of Bhopal and identified in the Botany Department, Dr. Hari Singh Gour Vishwavidyalaya, Sagar (M.P.). The voucher specimen (Herb. No. Bot/Her/592) has been deposited in the same department for future reference.

Preparation of extract

The all plant materials were washed with tap water, shade dried and coarsely powdered by grinder. 500 g of powdered plant material was packed in Soxhlet apparatus and successively extracted with petroleum ether (60-80°C) and 95% ethanol. The marc left after the ethanol extraction was macerated with distilled water for 24 h. To ensure complete extraction, few drops were collected from the thimble, which found to have no residue on evaporation. Before extraction with next solvent the marc was air dried to remove the adhering solvent. The solvents were distilled off under reduced pressure below 45°C to afford extracts.

For fractionation, ethanolic extract (30 g) was suspended in water and then fractionated successively with chloroform and ethyl acetate to yield their respective fractions.

Preliminary phytochemical screening of *Bougainvillea glabra*

The preliminary phytochemical investigation was carried out with leaves of *Bougainvillea glabra* for qualitative identification of phytochemical constituents. Phytochemical tests were carried out by standard methods [10-11].

Animals

Albino Wistar rats of either sex weighing between 150 and 180 g were used for the present study. The animals were maintained under standard environmental conditions and were fed with standard pellet diet and water *ad libitum*. The guidelines of CPCSEA, India, were strictly followed during the maintenance and experiment.

Chemicals

Biochemical kits for the estimation of total triglycerides (GPO-POD), total cholesterol (CHOD-POD), high density lipoprotein (PEG) were purchased from Crest Biosystems Kits (India) and Triton WR-1339 was purchased from Sigma Chemical Co., St Louis, MO, USA.

Acute toxicity study

Acute toxicity study was carried out for the extracts of TD following Organization of economic co-operation and development (OECD) guidelines (OECD guideline, 2001) [12]. The extract was dissolved in distilled water in a dose of 2 g/kg body weight and orally administered to overnight-fasted, healthy rats (n= 6). The animals were observed continuously for 24 h for mortality.

Triton induced hyperlipidemia

In the acute experiment the rats were divided into control, triton, and triton plus ethanolic extract, aqueous extract and chloroform fraction and ethyl acetate fraction of ethanolic extract of *Bougainvillea glabra* leaves treated groups containing six animals in each. Triton was administered (400mg/kg) by intraperitoneal injection. Various groups of animal for each extract and fraction of *Bougainvillea glabra* are as follows in triton induced hyperlipidemic model:

Group I	-	Control (administered vehicle only)
Group II	-	Triton treated
Group III	-	Triton treated + Ethanolic extract-EE (100 mg/kg body wt.)
Group IV	-	Triton treated + Ethanolic extract (200 mg/kg body wt.)
Group V	-	Triton treated + Ethanolic extract (300 mg/kg body wt.)
Group VI	-	Triton treated + Aqueous extract-AE (100 mg/kg body wt.)
Group VII	-	Triton treated + Aqueous extract (200 mg/kg body wt.)
Group VIII	-	Triton treated + Aqueous extract (300 mg/kg body wt.)
Group IX	-	Triton treated + Chloroform fraction of ethanolic extract- CF (50 mg/kg body wt.)
Group X	-	Triton treated + Chloroform fraction of ethanolic extract (100 mg/kg body wt.)
Group XI	-	Triton treated + Chloroform fraction of ethanolic extract (150 mg/kg body wt.)
Group XII	-	Triton treated + Ethylacetate fraction of ethanolic extract- EAF (50 mg/kg body wt.)
Group XIII	-	Triton treated + Ethylacetate fraction of ethanolic extract (100 mg/kg body wt.)
Group XIV	-	Triton treated + Ethylacetate fraction of ethanolic extract (150 mg/kg body wt.)

Estimation of lipid profile

Lipid profiles of all the rats were determined at end of experiment. The blood samples were collected from each rat by retro-orbital venepuncture of the overnight fasted rats into micro centrifuge tubes containing heparin (10 µl, 1000 IU/ml). Biochemical parameters were estimated using commercially available diagnostic kits.

Statistical analysis

The results are expressed as mean \pm S.E.M. Data were analyzed using one-way analysis of variance (ANOVA) test. $P < 0.05$ was considered statistically significant in all the cases.

RESULTS

Preliminary phytochemical screening of *Bougainvillea glabra*

Phytochemical screening of *Bougainvillea glabra* revealed the presence of carbohydrates, steroids/triterpenoids, saponins, phenolics, flavonoids and alkaloids.

Effect of *Bougainvillea glabra* leaves on serum lipid levels in triton induced hyperlipidemic rats

Triton treatment resulted in total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL) and reduction in high density-lipoprotein levels (HDL) as compared to the Triton treated rats as noted at end of the study (Table 1). When Triton treated with EE, AE, CF and EAF at the end of study showed most significant ($P < 0.01$) reduction in TC levels, TG levels and LDL levels. Conversely, HDL cholesterol levels were significantly ($P < 0.01$) increased in the EE, AE, CF and EAF treated groups at the end of treatment as compared to Triton treated group (Table 1).

Table 1: Effect of *Bougainvillea glabra* leaves on serum lipid levels in triton induced hyperlipidemic rats

	Total cholesterol (mg/dl serum)	Triglyceride (mg/dl serum)	HDL-cholesterol (mg/dl serum)	LDL-cholesterol (mg/dl serum)
Control rats	98.04 \pm 3.29	56.70 \pm 2.35	46.12 \pm 2.13	44.10 \pm 2.40
Triton treated rats	321.0 \pm 7.48**	382.0 \pm 8.18**	90.14 \pm 7.21*	157.10 \pm 8.46**
EE (100mg/kg)	245.15 \pm 8.26** (\downarrow 24.95%)	233.26 \pm 4.19** (\downarrow 39.0%)	92.38 \pm 3.92 ^{NS} (\uparrow 2.07%)	122.67 \pm 6.04** (\downarrow 22.13%)
EE (200mg/kg)	228.45 \pm 7.76** (\downarrow 29.11%)	221.36 \pm 5.64** (\downarrow 42.77%)	94.24 \pm 4.38* (\uparrow 5.04%)	98.78 \pm 5.19** (\downarrow 37.42%)
EE (300mg/kg)	203.7 \pm 4.62** (\downarrow 37.0%)	203.3 \pm 7.02** (\downarrow 47.12%)	97.32 \pm 4.16** (\uparrow 8.59%)	79.12 \pm 4.16** (\downarrow 50.60%)
AE (100mg/kg)	293.56 \pm 5.32** (\downarrow 9.69%)	343.24 \pm 3.10 ^{NS} (\downarrow 10.74%)	90.72 \pm 3.26 ^{NS} (\uparrow 0.63%)	142.19 \pm 11.82** (\downarrow 9.17%)
AE (200mg/kg)	273.17 \pm 3.26** (\downarrow 15.95%)	322.22 \pm 3.14 ^{NS} (\downarrow 16.0%)	90.95 \pm 2.52 ^{NS} (\uparrow 1.07%)	121.37 \pm 8.64** (\downarrow 23.13%)
AE (300mg/kg)	261.43 \pm 5.66** (\downarrow 19.15%)	300.42 \pm 5.56** (\downarrow 21.33%)	93.74 \pm 3.68** (\uparrow 4.16%)	106.18 \pm 2.98** (\downarrow 32.93%)
CF (50mg/kg)	267.22 \pm 6.71** (\downarrow 17.42%)	302.70 \pm 7.13** (\downarrow 21.57%)	92.24 \pm 4.11 ^{NS} (\uparrow 2.09%)	129.21 \pm 12.16** (\downarrow 18.41%)
CF (100mg/kg)	250.22 \pm 5.11** (\downarrow 22.5%)	265.20 \pm 5.14** (\downarrow 31.71%)	93.66 \pm 4.14 ^{NS} (\uparrow 4.02%)	108.70 \pm 7.04** (\downarrow 31.46%)
CF (150mg/kg)	239.3 \pm 4.54** (\downarrow 25.66%)	246.24 \pm 5.24** (\downarrow 36.65%)	94.3 \pm 3.14** (\uparrow 5.09%)	88.31 \pm 3.18** (\downarrow 44.61%)
EAF (50mg/kg)	237.74 \pm 9.25** (\downarrow 26.96%)	216.81 \pm 5.81** (\downarrow 43.62%)	92.31 \pm 4.21* (\uparrow 2.16%)	111.48 \pm 7.12** (\downarrow 29.17%)
EAF (100mg/kg)	219.46 \pm 6.72** (\downarrow 32.69%)	189.28 \pm 5.16** (\downarrow 51.74%)	95.12 \pm 4.26 ^{NS} (\uparrow 6.13%)	90.17 \pm 4.52** (\downarrow 43.17%)
EAF (150mg/kg)	190.64 \pm 6.12** (\downarrow 41.30%)	166.48 \pm 5.85** (\downarrow 57.27%)	99.25 \pm 4.26* (\uparrow 9.86%)	70.64 \pm 4.76** (\downarrow 55.51%)

Percentage reversal- \uparrow : Increase, \downarrow : Decrease Values are mean \pm SEM for 6 animals in each group, ** $P < 0.01$ (Considered very significant), * $P < 0.05$ (Considered significant), $P > 0.05$ (Considered non significant), triton group compared with control and triton group with drug treated group.

DISCUSSION

Triton model [13] has been successfully used for evaluation of lipid lowering activity of drugs [14-16]. Triton WR-1339 acts as a surfactant to block the uptake of lipoproteins from the circulation by extra hepatic tissues, resulting in an increase in the level of circulatory lipoproteins [13]. Present investigations shows that ethanolic extract, chloroform /ethyl acetate fraction of ethanolic extract of *Bougainvillea glabra* (leaves) caused a significant decrease in the total cholesterol, triglyceride and LDL-cholesterol level followed by an increase in HDL-cholesterol level in triton induced hyperlipemic rats. The maximum activity was found in 150 mg/kg body weight dose of ethyl acetate fraction of ethanolic extract of *Bougainvillea glabra*. It has been shown that flavonoids and phenolics present in ethanolic extract and ethylacetate fraction prevent cholesterol absorption, interfere with its enterohepatic circulation and increase its fecal excretion [17]. Flavonoids have been shown to form complex with plasma lipids [18]. An increased faecal elimination of bile acids followed by increased formation of bile acids from endogenous cholesterol might also contribute to the lowering of cholesterol [19].

CONCLUSION

The findings of the present work indicate the usefulness of the extracts of *Bougainvillea glabra* in the treatment of hyperlipidemia. However, further studies are needed to evaluate the safety profile of the plant beneficial anti hyperlipidemia agent.

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