



Effect of *Bougainvillea spectabilis* Leaves on Serum Lipids in Albino Rats Fed with High Fat Diet

H. Saikia*, A. Lama

Department of Pharmacology, Melmaruvathur Adhiparasakthi Institute of Medical Science & Research, Melmaruvathur-603319 India

ABSTRACT

Dyslipidemia is major problem among population those have sedentary life style as well as in diabetics. *Bougainvillea spectabilis* is most commonly found in India as an ornamental plant and has got anti-diabetic action due to presence of one insulin mimicking agent D-pinitol (3-O-methyl-chiroinositol). The present investigation was undertaken to evaluate the effect of alcoholic extract of leaves of *Bougainvillea spectabilis* (AEBSL) on serum lipid profile in albino rats fed with high fat diet (HFD) and to compare it with a standard hypolipidaemic drug simvastatin. Thirty healthy albino rats of both sexes (100-200 g) were randomized into 5 groups of 6 animals each. The groups were treated as follows: Group A: normal diet (ND); Group B: HFD (vanaspati ghee + coconut oil mixture in ratio of 3:2 at 10 ml/kg/day); Group C: HFD+ AEBSL (100 mg/kg/day); Group D: HFD + AEBSL (200 mg/kg/day); Group E: HFD + simvastatin (1.8 mg/kg/day). Lipid profile was estimated after 8 weeks of treatment. AEBSL showed a significant ($p<0.01$) reduction in total cholesterol (TC), Triglyceride (TG), Low density lipoprotein (LDL), Very low density lipoprotein (VLDL) levels and significant ($p<0.01$) increase in high density lipoproteins (HDL) in hypercholesteromic rats (Group C and Group D). AEBSL 200 mg/kg/day found to be more effective than AEBSL 100 mg/kg/day. There is also significant improvement in atherogenic index ($p<0.01$) and increases the percentage of protection AEBSL treated animals. Alcoholic extract of leaves of *Bougainvillea spectabilis* leaves have excellent lipid lowering potentiality.

Keywords: *Bougainvillea spectabilis*, High fat diet, Anti-hyperlipidemic activity, Hyperlipidemia, Atherogenic index.

INTRODUCTION

Atherosclerosis remains the major cause of death and premature disability in developed societies. Current predictions estimate that by the year 2020, cardiovascular diseases notably atherosclerosis, will become the leading global cause of total disease burden. Coronary atherosclerosis causes myocardial infarction and angina pectoris; strokes and transient cerebral ischemia are due to cerebral atherosclerosis. Intermittent Claudication and gangrene are dreaded sequelae of atherosclerosis occurring peripherally. Abnormalities in plasma lipoproteins and derangements in lipid metabolism rank among the most firmly established and the best understood risk factors for atherosclerosis.^[1] During the past few decades, traditional systems of medicines have become a topic of global importance. Current estimates suggest that, in many developing countries, a large proportion of the population relies heavily on traditional practitioners and medicinal plants to meet primary health

care needs. Although modern medicine may be available in these countries, herbal medicines (Phytomedicines) have often maintained popularity for historical and cultural reasons.^[2]

The genus *Bougainvillea* is a native of South America and derived its name from Louis Antione de Bougainville (1729–1811), an admiral in the French Navy who encountered the plant in Brazil in 1768 and first introduced it to the rest of the world.^[3] The genus *Bougainvillea* in the Nyctaginaceae (4 O' clock) family of plants has 18 species, with three that are horticulturally important *B. spectabilis*, *B. glabra* and *B. peruviana*.^[4] The *Bougainvillea spectabilis* leaves (BSL) are reported to have medicinal properties viz. anti-diabetic^[5-7], antiviral^[8], antibacterial^[9], anti-inflammatory^[10], larvicidal^[11] and anti-fertility potential.^[12] D-pinitol (3-o-methyl-chiroinositol) an active constituent has been isolated from leaves of BS.^[7, 13] In spite of the isolation of the hypoglycaemic principle of BSL, the crude extract of the leaves is still being consumed in Northern part of Nigeria (particularly among the Nupe people of Niger State) as a remedy for diabetes.^[14] Information also exists about the *B. glabra* having antiulcer, anti-diarrhoeal, and anti-microbial properties^[15] and also has the anti-diabetic and

*Corresponding author: Dr. Hiteswar Saikia, Department of Pharmacology, Melmaruvathur Adhiparasakthi Institute of Medical Science & Research, Melmaruvathur- 603319 India; Tel.: +91-9791661498; E-mail: hitaisaikia@gmail.com

hypolipidemic activities. [16] Geethan PKMA *et al.*, have demonstrated the antihyperlipidemic effect of D-pinitol in streptozotocin induced type II diabetic Wistar rats. [17] The abnormal lipoprotein profile associated with insulin resistance, known as diabetic dyslipidemia, accounts for part of the elevated cardiovascular risk in patient with type 2 diabetes mellitus. [1] Though different types of hypolipidaemic agents are available to treat hyperlipidemia, there is an increased demand by people to use natural products. Moreover, this plant extract has already shown its anti-diabetic potentiality and diabetes is invariably associated with Dyslipidemia. So, this plant material will be a valuable point of interest in near future. Keeping in view the above idea, the present study has been undertaken to evaluate the effect of AEBSL on serum lipids in albino rats fed with high fat diet and to compare it with a standard hypolipidaemic drug simvastatin.

MATERIALS AND METHODS

Plant material: Fresh, mature, healthy and good quality *Bougainvillea spectabilis* leaves were procured from the Assam Medical College & Hospital campus, during the months of May - June 2006 and botanically identified and authenticated. Leaves were washed thoroughly and shade dried, then powdered by electrical grinder. 500 g of powdered leaves were soaked in sufficient quantity of 70% ethanol and percolated. [5] A greenish, extract was obtained. The extract was collected in glass Petri dishes, kept in a vacuum dessicator. The yield at the end of extraction was 90 grams, 18% of the dry powder. A homogenous aqueous suspension of the extract was made before being administered to the experimental animals.

Acute toxicity study: AEBSL were tested for acute oral toxicity as per our previous study. [5] LD₅₀ was calculated and two doses of 100 mg/kg and 200 mg/kg were chosen for the present study.

Method of Preparation of Simvastatin Suspension: The stock solution was prepared by dissolving 20 mg of simvastatin in 70 ml of normal saline and used as a standard drug in a dose of 1.8 mg/kg body weight for the respective group. The daily dose of simvastatin for rats was calculated by extrapolation from the human dose (20 mg/day) as described by Ghosh. [18]

Method of Preparation of High Fat Diet: Edible coconut oil and vanaspati ghee were procured from the market and a mixture of the two was prepared in a ratio of 2: 3 respectively v/v as per method of Shyamala *et al.* [19] This high fat diet at a dose of 10 ml/kg body weight was fed to the animals per orally daily in addition to normal diet for the entire experimental period to produce hyperlipidemia.

Experimental Design: The experiment was carried out for a period of 8 weeks. 30 numbers of healthy albino rats (Wistar strain) of both sex and weighing approximately 150–200 gm were collected from the Central Animal House of Assam Medical College, Dibrugarh. The animals were allowed to acclimatize to the laboratory environment for one week. They were provided with a standard diet consisting of Bengal gram, wheat, maize and carrot. Water was given *ad libitum* during the entire period of the experiment. Animals were weighed, recorded, numbered and randomly divided into five groups of 6 animals each. All the animals were taken care of under ethical consideration and the experimental protocol was duly approved by institutional ethic committee.

Group–A: (Normal Control Group): Normal saline 10 ml/kg body weight/day.

Group–B: (Hyperlipidaemic Control Group): High fat diet (10 ml/kg body weight/day).

Group–C: (Hyperlipidaemic Test Group): High fat diet + AEBSL (100mg/kg/day).

Group–D: (Hyperlipidaemic Test Group): High fat diet + AEBSL (200mg/kg/day).

Group–E: (Standard Drug Group): High fat diet + Simvastatin (1.8 mg/ kg/day)

All the animals used for the experiment were kept under observation for daily food intake. The drugs were administered to the animals in the doses given above daily, by means of an intragastric feeding tube. At the end of experiment, all the animals were taken group wise and blood collected from each of them for assessing the various parameters of lipid profile.

Method of collection of Blood and Biochemical estimation: Blood was collected from the orbital sinus with the help of a capillary tube by pressing the thumb behind the angle of the jaw resulting in the engorgement of the retro-orbital plexus. [18]

After separation of serum from blood, the various biochemical parameters were estimated in the Department of Pathology, Assam Medical College by using standard kits of Randox, Mumbai. The parameters of lipid profile which were observed were Total Serum Cholesterol (TC), Triglycerides (TG), Low Density Lipoprotein Cholesterol (LDL), Very Low Density Lipoprotein Cholesterol (VLDL), High Density Lipoprotein Cholesterol (HDL) and Atherogenic Index (AI). Serum LDL and VLDL were estimated by calculation based on Friedwald *et al.* [20] Atherogenic Index was calculated by using the formula of Schulpis *et al.* [21] Percentage of protection was calculated based on Dhandapani *et al.* [22]

$$\text{LDL mg/dl} = \text{Total cholesterol} - \text{HDL} - \text{TG}/5$$

$$\text{VLDL mg/dl} = \frac{\text{Serum triglycerides}}{5}$$

$$\text{Atherogenic Index (AI)} = \frac{\text{Total Cholesterol} - \text{HDL}}{\text{HDL}}$$

$$\text{AI of experimental control- AI of treated group}$$

$$\text{Protection (\%)} = \frac{\text{AI of control}}{\text{AI of experimental control- AI of treated group}} \times 100$$

Statistical Analysis- Data obtained were subjected to computerized (Graph Pad Prism 5 Demo version) One-way Analysis of Variance (ANOVA) followed by *Dunnet's multiple comparison test*. The chosen level of significance was $p < 0.01$.

RESULTS

Results of serum lipids estimation were reported as Mean $\pm$ SEM (standard error of mean) of 6 animals at a time from each group. The statistical significance between groups was analyzed using one-way ANOVA, followed by *Dunnet's multiple comparison tests*. The significance was expressed by 'p' values, as mentioned in the table and 'p' value of < 0.01 was considered as significant. Table 1 has shown the different serum lipid parameters at the end of the 8th week of the experiment and Table 2 has shown the atherogenic index and increased in percentage of protection in each treated group.

Table 1: Effects of AEBSL on Serum Lipids at the end of 8th Week

Group & Drugs	Total Cholesterol	Triglycerides	HDL	LDL	VLDL	Atherogenic Index
Group A (Normal control)	89.67±3.77	65.33±1.52	24±1.93	52.60±3.66	13.06±0.30	2.84±0.33
Group B (Hyperlipidemic control)	267.17±6.73 a	217±6.63 a	16.33±1.17 a	207.37±6.47 a	43.40±1.33 a	15.84±1.39 a
Group C (HFD+ AEBS 100 mg/kg)	90.67±1.52 b	80.67±1.45 b	26.83±1.38 b	47.57±2.58 b	16.13±0.29 b	2.43±0.20 b
Group D (HFD+ AEBS 200 mg/kg)	81.83±1.22 b	59.67±0.99 b	30.00±1.39 b	39.90±1.79 b	11.93±0.20 b	1.76±0.12 b
Group E (HFD+ Simvastatin)	74.83±3.76	55.17±1.72	36.50±2.79	27.30±5.58	11.03±0.34	1.14±0.26
F	434.4	449.5	16.64	289.2	449.5	88.91
Df	4,25	4,25	4,25	4,25	4,25	4,25
p	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

Values are expressed as MEAN ± SEM; (n=6).

Test Result of Serum Lipids in mg/dl and Atherogenic index in ratio.

One Way ANOVA followed by Dunnett's multiple comparison tests.

a: $p < 0.001$ when compared with Normal Control Group

b: $p < 0.001$ when compared with Hyperlipidaemic Control Group

Table 2: Atherogenic index and percentage of protection in various groups

Groups	Atherogenic Index	Protection %
Group- A	2.84	-----
Group- B	15.84	-----
Group- C	2.43	85.48%
Group- D	1.76	88.89%
Group- E	1.14	92.80%

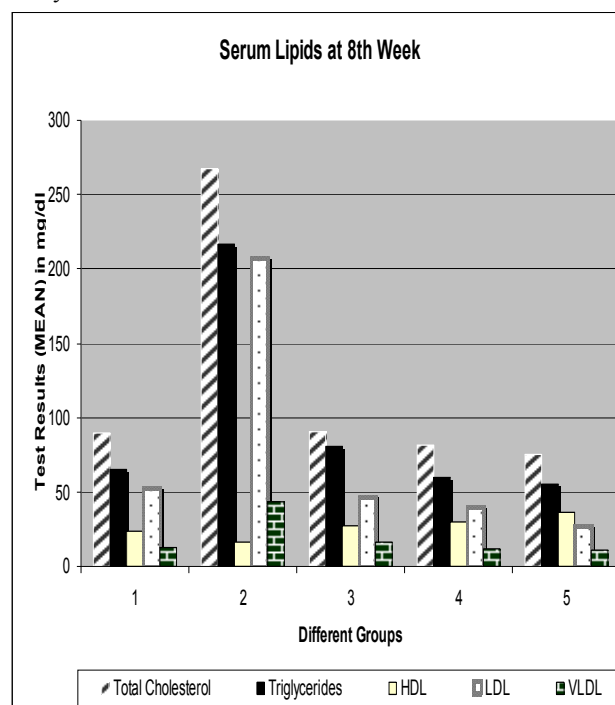
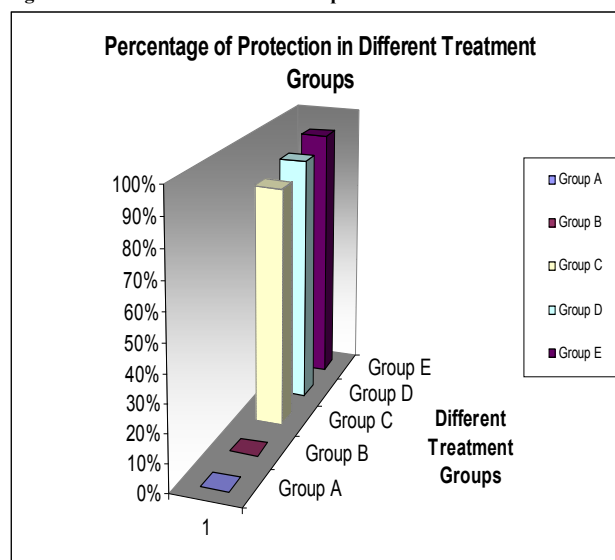
The results reveal that there was a significant increase in all the lipid parameters ($p < 0.01$) except HDL following administration of high fat diet when compared to normal group. It was also seen that concomitant administration of the AEBSL at a dose of 100 mg/kg and 200 mg/kg body weight along with HFD fed animals, showed a significant decrease in all the lipid parameters ($p < 0.01$) with a significant rise in HDL ($p < 0.01$) level as compared to hyperlipidemic control animals.

Standard drug simvastatin, at a dose of 1.8 mg/kg administered along with high fat diet, showed a significant decrease ($p < 0.01$) in all the lipid parameters while there was a significant ($p < 0.01$) increase in HDL level. Both the doses of AEBS (100 mg/kg and 200 mg/kg body weight) treated animals showed decrease in the atherogenic index and increased in percentage of protection. The Hypolipidaemic activity of the test drug AEBSL was found to be less efficacious than that of the standard drug simvastatin in comparison to the control. Fig. 1 & Fig. 2 are the graphical representation of lipid profiles and percentage of protection in different groups respectively.

DISCUSSION

Elevated levels of all lipoproteins except the HDL are associated with increased risk of atherosclerosis. High level of triglycerides and LDL are associated with coronary artery disease [23], which is seen in Group B - HFD fed rats. AEBSL treated rats significantly reduced ($P < 0.01$) TC and TG concentration at both the doses administered when compared with experimental controls animals. Thus, the reduction in serum TC concentration effected by the extract AEBSL is beneficial and may reduce the risk of cardiovascular disease. Those agents that have the ability to lower cholesterol concentration in the blood have been reported to reduce vascular resistance by improving endothelial function. The endothelium is intimately involved in the pathogenesis of atherosclerosis. Oxidative modification of LDL by the endothelium is thought to be an important step in the initiation of atherosclerosis. Oxidized LDL impairs endothelium-mediated relaxation in isolated arterial segments. Hypercholesterolemia and atherosclerosis impair endothelium-mediated vasodilator responses in animal

models and humans. This loss of endothelium-mediated vasodilatation is thought to be involved in the pathogenesis of myocardial ischemia. [24]

**Fig. 1: Effects of AEBSL on Serum Lipids at the end of 8th Week****Fig. 2: Percentage of protection of AEBSL and Standard drug**

The LDL which is popularly known as the “bad cholesterol” is highly atherogenic^[25], because they are primary carriers of plasma cholesterol and builds up slowly in the walls of arteries feeding the heart and brain. As a result of this, it forms plaque that clots the arteries hereby causing atherosclerosis and increasing the risk of high blood pressure which may eventually lead to stroke.^[26] Our results clearly showed that AEBSL treated rats significantly reduced ($P < 0.01$) LDL concentration at both the doses administered when compared with hyperlipidemic controls animals.

The HDL is considered to have anti-atherogenic properties, since there is negative correlation between HDL and risk of cardiovascular disease. It is referred to as the ‘good’ cholesterol because, HDL is involved in transport of cholesterol from peripheral tissues to liver and thereby reducing the amount stored in the tissue and the possibility of developing atherosclerotic plaques.^[26] HDL was increased in our study significantly ($P < 0.01$) in Group C, Group D and Group E when compared with hyperlipidemic controls animals and decreased significantly ($P < 0.01$) in Group B when compared with normal controls animals. This indicates that AEBSL may help to increase transport of peripheral tissue cholesterol to liver and thereby decrease blood cholesterol when concomitantly fed with HFD. Many experts believe that hypertriglyceridaemia, which is frequently a result of elevated VLDL level, is indeed a marker for an increased risk for coronary artery disease, especially in diabetic patient and female subjects, although this risk may not be as great as that for an elevated LDL or low HDL.^[25]

The atherogenic index is the ratio, can be calculated as TC/HDL, Non HDL- Cholesterol/ HDL, LDL/HDL, TG/HDL. Non HDL- Cholesterol is calculated as (TC-HDL).^[27] A total cholesterol /HDL ratio of ≤ 3 connotes a low risk, a ratio of around 4.5 an average risk and ratio of ≥ 8 a high risk of developing coronary artery disease.^[25] Non-HDL cholesterol index may be particularly useful in predicting cardiovascular disease risk in patients with diabetes.^[28] In our study, atherogenic index is decreased significantly ($p < 0.01$) in Group C, Group D and Group E when compared with hyperlipidemic group animals.

A 1% decrease in HDL is associated with a 3-4% increase in the risk of heart disease.^[22] In the present study an increase in plasma HDL with a concomitant increase in percentage of protection in atherogenesis was observed (Table 2 & Fig. 2). By the end of the experimental period, 100 mg/kg, 200mg/kg and the standard drug simvastatin increased the percentage of protection by 85.48 %, 88.89% and 92.80% respectively.

Major complications of Diabetes include aberrant lipid metabolism and vascular wall function. Since alterations in serum lipid profiles are known in diabetes which is likely to increase the risk of coronary heart disease, a reduction in serum lipids, particularly TC, LDL and TG levels should be considered as beneficial in long-term prognosis of diabetic patients. AEBS extract will thus have a potential therapeutic value in combating multifactorial atherosclerotic disorders, which are parts of the major complications of Diabetes. Reducing the risk of atherosclerosis will thus lead to the development of effective and better management of hyperlipidemia.^[16] D-pinitol (3-O-methyl-chiroinositol), an active principle of the traditional antidiabetic plant BS, is claimed to exert insulin-like effects. D-pinitol might exert an insulin-like effect on glucose transport that is independent of

insulin. By analogy with D-chiroinositol, there is a structural similarity of D-pinitol with inositol phosphates involved in the signaling of insulin via PI3K and protein kinase B pathway. D-pinitol exerts an acute and chronic insulin-like antihyperglycaemic effect in diabetic mice. The mechanism of action of D-pinitol does not augment the effect of insulin but might involve an interaction with part of a cellular signalling pathway that links insulin with glucose transport.^[13] This may be the possible mechanism for decreased lipid levels by AEBS (insulin mimicking action), because a principal action of insulin in adipose tissue is to inhibit the activity of the hormone-sensitive lipase, reducing the release of fatty acids as well as glycerol.^[29]

Despite it has beneficial effects on lowering blood glucose as well as lowering serum lipid profile, it may possess some potential of adverse effects in liver and kidney function. Malmo *et al*^[14], has provided some valuable information on the safety/toxicity of the consumption of the absolute Ethanolic extract of BSL. They found that the extract significantly reduced ($p < 0.05$) serum calcium and potassium; and significantly increased ($p < 0.05$) phosphate concentration in a dose dependent manner while it had no significant effect on the sodium concentration. The extract significantly increased ($p < 0.05$) serum urea and creatinine concentrations in a dose dependent manner while it significantly reduced ($p < 0.05$) serum albumin concentration. ALT (alanine aminotransferases) activity in serum and kidney were significantly reduced ($p < 0.05$) at the dose of 100 mg/kg, when compared with controls. Liver AST (aspartate) and serum AST activity was not affected, whereas kidney AST activity was significantly reduced ($p < 0.05$) at both doses when compared with controls. The extract (200 mg/kg) also significantly reduced ($p < 0.05$) Ca^{2+} - Mg^{2+} ATPase activity in the kidney and significantly increased ($p < 0.05$) kidney Na^{+} - K^{+} ATPase activity. In the liver, the extract significantly reduced ($p < 0.05$) Na^{+} - K^{+} ATPase activity at both doses administered while it significantly increased ($p > 0.05$) Ca^{2+} - Mg^{2+} ATPase activity at the dose of 100 mg/kg when compared with controls. They suggest that the repeated administration of BSL extract may compromise the integrity of the kidney and liver and thereby adversely affect their normal functions.

Adebayo *et al*^[30], has investigated the effect of Ethanolic extract of BSL on some haematological and serum lipid parameters in rats during a 7 day administration of the doses of 50, 100 and 200mg/kg body weight orally. They concluded that the extract has some beneficial effects in reduction in serum cholesterol but adversely affect some haematological indices, specially related to red blood cells and white blood cells.

Mishra *et al*^[12] has given some clue of adverse pharmacological effect on mechanism of action of BSL extract on male and female reproductive organs. They evaluated the effect of BSL aqueous extract on fertility of male and female Swiss Albino mice at the dose of 800mg/kg body weight for 30 days. They found that there is significant degeneration of the both gonads (male> female) and declining in the titer of testosterone in males and estrogen in females. They also reported that, extension of reproductive cycle of female mice by 1-2 days with prolonged metaestrus and decrease in serum estrogen level where as in male total count of sperm decreased along with decrease in titer of testosterone. Histological report of males shows that,

reduction in diameter of seminiferous tubules, thickness of germinal epithelium and absence of sperm in the lumen of tubules.

Considering the entire results from this study it was observed that the AEBSL significantly inhibited high fat diet induced hyperlipidemia in albino rats. On comparison, the hypolipidaemic activity of simvastatin was found to be more efficacious than that of AEBSL. *Bougainvillea spectabilis* is the most easily available throughout the world, can be utilized for providing dietary management in the prevention of atherosclerosis in hyperlipidaemic patients as well as Dyslipidemia associated with diabetes. Though it has some adverse effect in various organ systems it deserves further evaluation for its dose in higher animals, from the stand point of its hypolipidaemic as well as hypoglycemic effect in therapy. It holds the hope of development of more purified hypolipidaemic products those have also anti-diabetic effects.

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