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IN-VIVO ANTIHYPERLIPIDEMIC ACTIVITY AND PRELIMINARY PHYTOCHEMICAL SCREENING OF *BAUHINIA ACUMINATA*

Anusha Govindula *, Musku Sadhana Reddy, Medi Manjula, Musku Soumya Reddy, Nusrath and Perumalla Kalyani

Department of Pharmacology, Vaageswari College of Pharmacy, Beside LMD Police Station, Thimmapur, Karimnagar - 505527, Telangana, India.

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Correspondence to Author:
Anusha Govindula

M. Pharm (PhD),
Department of Pharmacology,
Vaageswari College of Pharmacy,
Beside LMD Police Station,
Thimmapur, Karimnagar - 505527,
Telangana, India.

E-mail: anu.govindula@gmail.com

ABSTRACT: The global burden of CVD is rising due to its increasing prevalence in lower- and middle-income countries where 80% of all global CVD-related deaths occur. Common significant risk factors for dyslipidemia included obesity, diabetes, and dysglycemia. The present study was aimed to investigate the potential antihyperlipidemic activity of ethanolic leaf extract of *Bauhinia acuminata* in atherogenic diet-induced rats. Preliminary phytochemical screening showed the presence of flavonoids, alkaloids, steroids, terpenoids. Acute toxicity study was performed on the rats using OECD Guideline no. 423 and the maximum safe dose was found to be 5000 mg/kg. Animals were grouped into normal control (Group-I), disease control (Group-II), standard group (Group-III) received Standard drug Atorvastatin 10 mg/kg, and test group (Group-IV) received ethanolic extract of the plant with doses 200 mg/kg and 400 mg/kg. The study was conducted for 30 days and hyperlipidemia induced using atherogenic diet. Statistical analysis was performed using Graph Pad Prism and the values are expressed as mean ± SD values. Bodyweight changes were observed every day upon Induction of Hyperlipidemia and it was drastically reduced in the rats with 400 mg/kg dose (190 ± 10) compared with the standard group (180 ± 18.27). There is a significant reduction in the total cholesterol, VLDL, triglycerides, and increase in HDL level along with reduction in random blood Glucose level with ethanolic extract at a dose of 400 mg/kg then 200 mg/kg and the standard atorvastatin. Atherogenic index of the plant extracts 400 mg/kg (1.62) 200 mg/kg (4.69) also compared with normal group (1.37), disease control group (10) and standard group (3.11).

INTRODUCTION: Hyperlipidemia is abnormally elevated levels of any or all lipids or lipoproteins in the blood. It is the most common form of dyslipidemia (which includes any abnormal lipid levels) [1].

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Hyperlipidemia is a family of disorders that are characterized by abnormally high levels of lipids (fats) in the blood. While fats play a vital role in the body's metabolic processes, high blood levels of fats increase the risk of coronary heart disease (CHD).

The recent prevalence of CVD has shown downward trend in many high-income countries, the global burden of CVD is still rising due to its increasing prevalence in lower- and middle-income countries where 80% of all global CVD-related deaths occur.

Common significant risk factors for dyslipidemia included obesity, diabetes, and dysglycemia. The prevalence of dyslipidemia is very high in India, which calls for urgent lifestyle intervention strategies to prevent and manage this important cardiovascular risk factor. Medicinal plants are an important source to treat hyperlipidemia and reduce the risk of certain diseases like atherosclerotic heart disease, heart attack, and heart stroke, but atherosclerosis is the primary cause of death. There are many synthetic antihyperlipidemic drugs in use. It is reported, however, they have several side effects such as constipation, heartburn, mild muscle pain. There is, therefore, a need for more effective, less toxic and cost-effective antihyperlipidemic. Medicinal plants appear to have these desired comparative advantages; hence there is a growing interest in natural antihyperlipidemic from plants.

Moreover, according to the World Health Organization (WHO), medicinal plants used in traditional medicine are one of the definite alternative resources to attain well-being of the world's population. Conservative estimates suggest that about 10% of all flowering plants on earth have at one time, been used by local communities throughout the world but only 1% have gained recognition by modern scientists. There are about 120 plant-based drugs prescribed worldwide and they come from just 95 plant species. Approximately 2, 50,000 species of flowering plants and only 5000 have had their potential assessed. Throughout South-East Asia various parts of numerous Bauhinia species are used in poulticing to reduce swelling and bruises and to ripen ulcerations and boils. Decoctions of various plant parts are taken internally as a febrifugal, antidiarrhoeal and anti dysenteric remedy [2]. Bauhinia species show cytotoxic and antimicrobial activity [4], [5], which may partly support their traditional uses. However, further research is needed to evaluate possible future applications. A very little work was done on this plant, Hence, present study is aimed to investigate antihyperlipidemic activity of *Bauhinia acuminata*.

MATERIALS AND METHODS:

Collection and Authentication of Plant Material: The leaves of *Bauhinia acuminata* were collected in the month of July (2018) from Karimnagar district, Telangana and were authenticated by L. Rasingam, Scientist In-charge, Botanical Survey of India, Deccan Regional Centre, Government of India (BSI/DRC/2018-19/Tech./503).

Preparation of Alcoholic Plant Extract: The leaves of *Bauhinia acuminata* were dried in shade, powdered and extracted with ethanol (55 ºC to 65 ºC) in a Soxhlet apparatus. Before and after extraction the marc was completely dried and weighed. The extract was evaporated under reduced pressure by a rotary vacuum evaporator until all the solvent had been removed to give an extract with a yield of 23.3%.

Experimental Animals: All the experiments were carried out using Wistar male rats (150-180 g). Animals were housed at a temperature 22 ± 1 ºC a 12 h Light 12 dark cycle was followed. All animals were allowed to free access to water and fed normal pellet diet and water *ad libitum*. The composition of atherogenic diet used during the study is given in **Table 1**.

The experimental procedure and protocol used in this study were viewed by the Institutional Animal Ethical Committee (Ref. no: VCP/COLOGY/009/ 11/2018) and were in accordance with the guidelines of the CPCSEA.

TABLE 1: COMPOSITION OF ATHEROGENIC DIET [3]

S. no.	Composition	Normal diet (%)	Atherogenic diet (%)
1	Protein (milk powder)	12	10
2	Carbohydrates (wheat powder)	71	61
3	Sugar	05	05
4	Fat (butter)	05	16
5	Salt	04	04
6	Vitamin	01	02
7	Fibers	02	01
8	Cholesterol	-	01
	Total	100 gm	100 gm

Preliminary Phytochemical Analysis: [9] Preliminary phytochemical analysis was carried out with alcoholic extract adopting standard procedures.

Detection of Alkaloids: About 50 mg of solvent-free extract was treated with little quantity of diluted hydrochloric acid and filtered. The filtrate was tested carefully with various alkaloid reagents.

Mayer's Reagent: solution of potassium mercuric iodide) to a few ml of filtrate, two drops of Mayer's

reagent was added along the sides of the test tube. The formation of a creamy precipitate indicates the presence of alkaloids.

Wagner's Test (Solution of Iodide in Potassium Iodide): To a few ml of filtrate, few drops of Wagner's reagent were added along the sides of the test tube. Formation of reddish-brown precipitate confirms the presence of alkaloids.

Dragendorff's Test (Solution of Potassium Bismuth Iodide): To a few ml filtrates, 1 or 2 drops of dragendorff's reagent were added; formation of a prominent reddish-brown color precipitate indicates the presence of alkaloids.

Hager's Reagent (Saturated Picric Acid Solution): To a few ml of filtrate, 1 or 2 ml of Hager's reagent was added. Formation of a prominent yellow color precipitate indicates the presence of alkaloids.

Detection of Carbohydrates: About 100mg of the extract was dissolved in 5ml of distilled water and filtered. The filtrate was tested for the presence of carbohydrate.

Molish Test (Solution of α-naphthol in Alcohol): To 2 ml of filtrate, 2 drops of alcoholic solution of α-naphthol was added. The mixture was shaken well and 1 ml of concentrated H_2SO_4 was added slowly along the sides of the test tube. The formation of a violet ring at the junction of two liquids indicates the presence of carbohydrates.

Fehling's Test (Solution of Copper Sulphate, Potassium Tartrate and Sodium Hydroxide): Small amount of extract is hydrolyzed with dilute hydrochloric acid and neutralized with alkali a heated with Fehling's solution A and B. Formation of red precipitate indicates the presence of reducing sugars.

Benedict's Test (Solution of Copper Sulphate, Sodium Citrate, and Sodium Carbonate): To 0.5 ml of filtrate 0.5 ml of Benedict's reagent was added. The mixture was heated in boiling water bath for 2 min. Formation of an orange-red precipitate indicates the presence of reducing sugars.

Detection of Glycosides: For detection of glycosides, about 50 mg of extract was hydrolyzed with concentrated hydrochloric acid for 2 h on a water bath, filtered and the hydrolysate was subjected to perform the following tests.

Legal's Test: About 50 mg of the extract was dissolved in pyridine. Sodium nitroprusside solution was added and made alkaline using 10% sodium hydroxide solution. Formation of pink color indicates the presence of glycosides.

Keller-Killiani Test: About 50 mg of the extract was dissolved in 2 ml of glacial acetic acid and two drops of 5% ferric chloride solution and mixed. Then 1 ml of sulphuric acid was added. The reddish-brown color appears at the junction of the two liquid layers and appears bluish-green color indicates the presence of steroidal glycosides.

Detection of Proteins and Amino Acids: About 100mg of the extract was dissolved in 0ml of distilled water and filtered through what man no. filter paper and filtrate were subjected to tests for proteins and amino acids.

Million's Test: To 2 ml of filtrate, 2 ml millions reagent was added and heated to boil. The formation of white precipitate, which turns to red upon heating, indicates the presence of proteins / amino acids.

Biuret Test: To ml of filtrate, ml of 10% sodium hydroxide solution was added and heated to boil to this a drop of copper sulfate solution was added. The formation of purple violet color indicates the presence of proteins.

Ninhydrin Test: To the test solution, a few drops of 5% ninhydrin reagent was added and boiled for few minutes. The formation of violet/blue color indicates the presence of amino acids.

Detection of Phytosterols:

Liebermann-Burchard Test: The extract in chloroform was treated with a few drops of acetic anhydride, few drops of concentrated sulphuric acid were added along the sides of the test tube. Red pink or violet color at the junction of the liquids indicates the presence of steroids / terpenoids and their glycosides

Salkowski Test: The extract in chloroform was treated with a few drops of concentrated sulphuric acid shaken well and allowed to stand. The

formation of yellow-colored layer indicates the presence of tri-terpenes and formation of reddish-brown color layer indicates the presence of steroids.

Detection of Phenolic Compounds and Tannins:
Lead Acetate Test: A small quantity of extract was dissolved in distilled water and to this 3 ml of 10% lead acetate solution was added. The formation of a white precipitate indicates the presence of phenolic compounds.

Gelatin Test: To the test, solution adds 1% gelatin solution containing chloride and heated to boil. The formation of white precipitate indicates the presence of tannins.

Pharmacological Studies:
Induction of Hyperlipidemia: [3] In order to induce hyperlipidemia the method reported by Maruthappan *et al.*, was followed. Male Wistar rats (150-180 g) were given atherogenic diet for 30 days. 16 rats were divided into 4 groups of four animals each.

Acute Toxicity Studies: [10]Acute toxicity study was conducted in Wistar Albino rats as per OECD guideline 423 (Acute Toxic Class Method). It is the principle of the test that, based on a stepwise procedure with the use of a minimum number of animals per step, sufficient information is obtained on the acute toxicity of the test substance to enable its classification. The substance is administered orally to a group of experimental animals at one of the defined doses. The substance is tested using a stepwise procedure, each step using three animals. Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step, *i.e*.

- No further testing is needed,
- Dosing of three additional animals, with the same dose.
- Dosing of three additional animals at the next higher or the next lower dose level.

The test substance is administered in a single dose by gavage using a stomach tube or a suitable intubation canula. In the unusual circumstance that a single dose is not possible, the dose may be given in smaller fractions over a period not exceeding 24 h. Animals are fasted prior to dosing (*e.g.* with the rat, food but not water should be with holdover-night, with the mouse, food but not water should be withheld for 3-4 h). Following the period of fasting, the animals were weighed and the test substance administered. After the substance has been administered, food may be withheld for a further 3-4 h in rats. Three animals are used at each step.

The dose level to be used as the starting dose is selected from one of four fixed levels, 5, 50, 300 and 2000 mg/kg body weight. The available information suggests that mortality is unlikely at the highest starting dose level (2000 mg/kg body weight), a limit test was conducted.

The limit test is primarily used in situations where the experimenter has information indicating that the test material is likely to be nontoxic, *i.e.*, having toxicity only above regulatory limit doses. Information about the toxicity of the test material can be gained from knowledge about similar tested compounds or similar tested mixtures or products, taking into consideration the identity and percentage of components known to be of toxicological significance.

In those situations where there is little or no information about its toxicity, or in which the test material is expected to be toxic, the main test should be performed. Limit test at one dose level of 5000 mg/kg carried out with three animals and observed for tremors, convulsions, salivation, diarrhea, lethargy, sleep body weight changes, *etc*., the alcoholic extracts of *Bauhinia accuminata* was found to be safe at the dose of 5000 mg/kg.

The test extracts were administered orally to overnight night fasted animals at the dose of 200 mg/kg, 400 mg/kg. Animals were observed continuously for a period of 24 h. The dose level of extracts used for the pharmacological study was $1/10^{th}$ and $1/20^{th}$ of the maximum tolerated safe dose found from acute toxicity studies. These were administered one's daily by oral route.

Treatment Protocol: The schedule of dose and diet administration in experimental groups were followed for 30 days, the grouping of animals include;

Group I: Normal diet
Group II: Atherogenic diet
Group III: Atherogenic diet + Atorvastatin
Group IV: Atherogenic diet + alcoholic extract of *Bauhinia acuminata.*

The above-mentioned treatment schedule was followed for the respective groups of animals for 30 days. Blood was withdrawn using the heparinised capillaries from the retro-orbital sinus in animals. The serum was obtained after centrifuging the blood, which was used to estimate the concentration o of biochemical parameters TC, HDL, TG, LDL, and VLDL using relevant lipid profile kits.

RESULTS:

TABLE 2: PRELIMINARY PHYTOCHEMICAL SCREENING

Constituents	Method	Result
Flavonoids:		
Lead acetate test	Sample + lead acetate solution	+ve
Sodium hydroxide test	Sample + sodium hydroxide	+ve
Ferric chloride Test	Sample + ferric chloride	+ve
Alkaloids:		
Hager's test	2-3 ml of sample + hager's reagent	+ve
Wagner's test	2-3 ml of sample + few drops of Wagner's reagent	+ve
Dragendorff's test	2-3 ml of sample + few drops of Dragendorff's reagent	+ve
Carbohydrates:		
Molish test	2-3 ml of sample + few drops of alpha napthol + conc. H_2So_4	+ve
Benedict's test	Mix equal volume of benedict's reagent + sample + heat in boiling water for 5 min	+ve
Steroids:		
Liebermann's-burchard test	2 ml of sample + 2 ml of chloroform + 1-2 ml of acetic anhydride + 2 drops of conc. H_2SO_4	+ve
Salkowski test	2 ml of sample + 2 ml of chloroform + 2 ml of conc. H_2SO_4	+ve
Terpenoids:		
Liebermann's-burchard test	2 ml of sample + 2ml of chloroform + 1-2 ml of acetic anhydride + 2 drops of conc. H_2SO_4	+ve
Salkowski test	2 ml of sample + 2 ml of chloroform + 2 ml of conc. H_2SO_4	+ve

TABLE 3: VARIATIONS IN BODY WEIGHT OF THE RATS

Group	Weight of the rat (1^{st} day)	Weight of the rat (7^{th} day)	Weight of the rat (14^{th} day)	Weight of the rat (30^{th} day)
Normal Control	145 ± 17.32	160 ± 27.08	175 ± 31.09	192 ± 28.72
Hyperlipidemic Control	205 ± 5.77	222.5 ± 18.93	235 ± 17.32	237.5 ± 21.60
Standard Group (Atorvastatin)	205 ± 17.32	202 ± 9.57	192.5 ± 29.86	180 ± 18.27
Ethanolic extract of *B. accuminata* (400 mg/kg)	205 ± 10.01	197.5 ± 12.58	197 ± 5.03	190 ± 10
Ethanolic extract of *B. accuminata* (200 mg/kg)	210 ± 2.80	207 ± 11.12	200 ± 11.54	197 ± 7.55

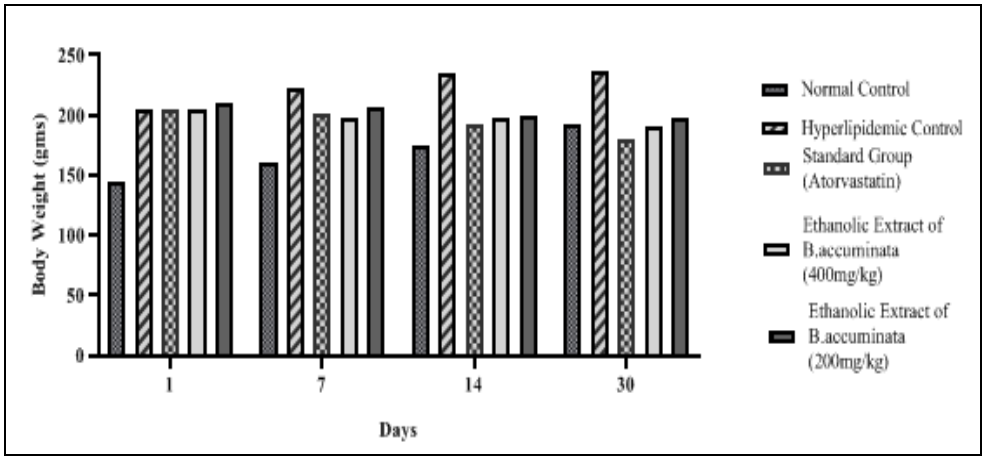

FIG. 1: EFFECT OF ETHANOLIC EXTRACT OF *B. ACCUMINATA* ON BODY WEIGHT CHANGES IN RATS

TABLE 4: COMPARISON OF LIPID PROFILE AND ATHEROGENIC INDEX IN RATS

Lipid profile	Normal control group	Hyperlipidemic control group	Standard group (Atorvastatin)	Ethanolic extract of *B. accuminata* (400 mg/kg)	Ethanolic extract of *B. accuminata* (200 mg/kg)
TC	24.76±14.01	54.32±27.63	48.76±38.52	36.77±1.20	37.62±1.25
TG	104.435±29.93	251.99±12.28	232.9±41.29	145.65±7.14	140.6±7.12
HDL	30.155±5.29	23.38±3.85	42.17±0.45	34.90±5.84	30.77±4.96
LDL	26.28±2.72	176.96±5.58	84.99±33.83	73.76±57.28	114.27±42.30
VLDL	20.885±5.98	53.39±6.69	46.58±8.25	34.13±5.64	38.12±5.29
RBS	72.245±4.77	131.85±9.97	92.52±27.68	88.37±2.50	90.14±2.21
Atherogenic Index	1.57	10	3.11	1.62	4.59

Values are expressed as mean ± SD using ANOVA; P-value < 0.05 considered as significant

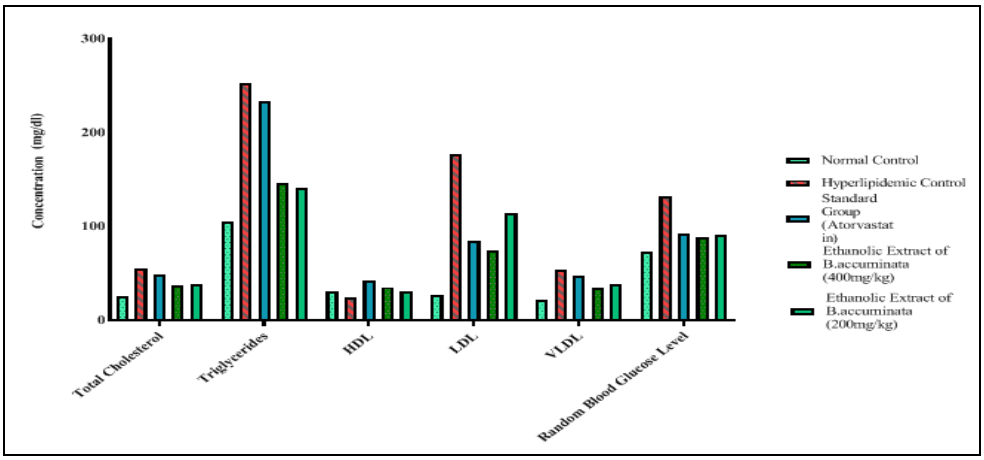

FIG. 2: EFFECT OF ETHANOLIC EXTRACT OF *B. ACCUMINATA* ON ATHEROGENIC DIET INDUCED RATS

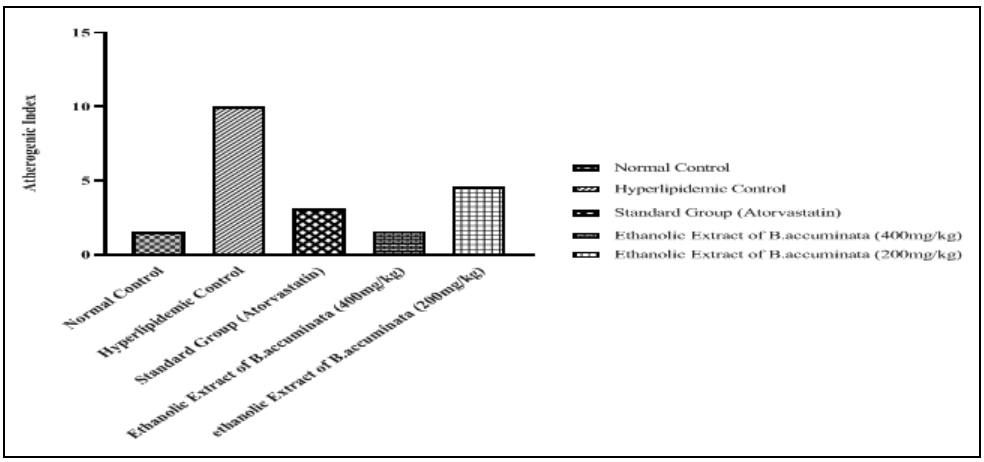

FIG. 3: ATHEROGENIC INDEX

DISCUSSION: Plant-based compounds have a pharmacological activity for the prevention and treatment of complex diseases. Therefore, the researcher provides special attention to the plant-derived metabolites. The antimicrobial, anticancer and antioxidant properties of the medicinal plants depend on the presence of its secondary metabolites *viz.*, phenolic acids, flavonoids, tannins, coumarins, lignins, quinones, stilbenes, and curcuminoids. The secondary metabolites of plants are used as antioxidants, anti-blood-clotting agents, food additives, in the preparation of cosmetics, *etc*.

In the present study, the ethanolic extract of the leaves of *Bauhinia acuminata* has shown significant reduction in the total cholesterol level with a P-value 0.045. There was also a significant reduction in the body weight changes in the male Wistar rats starting from day of dosing till the end of the studying which standard drug Atorvastatin have shown reduction in mean body weight 180 ± 18.27 and the extracts have shown 190 ± 10 (400 mg/kg) 197 ± 7.55 (200 mg/kg) respectively. There exist a little work done on this plant but the species of Bauhinia are proven to be used and screened for antioxidant, anti-inflammatory, cytotoxic, anti-

cancer and antimicrobial activities [7]. The ethanolic extracts of the *Bauhinia acuminata* with 400 mg/kg dose showed significant reduction the TC, TG, LDL, and VLDL levels are compared to the dose of 200 mg/kg and the standard drug atorvastatin. The ethanolic and aqueous extracts of the root of B. variegata (200 and 400 mg/kg body weight) in rats, showed a significant reduction ($P \geq 0.01$) in cholesterol and a significant reduction ($P \geq 0.01$) in triglyceride level. The VLDL level was also significantly ($P \geq 0.05$) reduced, with a significant increase in HDL. The antihyperlipidemic activity of fractions of total methanol extract of leaves of *Bauhinia variegata* was investigated against Triton WR-1339 induced hyperlipidemia in rats. Fractions were administered at a dose of 100 mg/kg orally. Butanol fraction showed a significant reduction ($p<0.05$) in serum cholesterol triglycerides and LDL Level, VLDL and Increase in HDL level in comparison with standard Drug fenofibrate ($p<0.05$) [8].

In the present study ethanolic extract of leaves of *Bauhinia acuminata* also shown a significant reduction in the random blood glucose level with extract at a dose of 400 mg/kg shown 88.37 ± 2.50 and extract at a dose of 200 mg/kg have shown 90.14 ± 2.21 respectively. A study conducted by Manobendro *et al.*, revealed that, in comparison to positive standard, metformin-HCl (100 mg/kg) methanolic extract leaves in lower doses (25 mg/kg and 50 mg/kg) produced promising glucose-lowering property [6]. The methanolic extract of leaves lowered blood glucose to 65.49% and 90.19% at a dose of 25 mg/kg and 50 mg/kg respectively compared to the standard. The blood glucose level of treatment group was compared with the diabetic control group (p-value <0.05). On the other hand, methanolic extract of bark of our experimental plant also showed the significant glucose-lowering property of 70.19% and 76.86% at dose 100 mg/kg and 200 mg/kg respectively compared to the positive control. Comparatively leaves extract was found to be more potent than the bark extract as it produced a remarkable effect at lower doses.

CONCLUSION: The study provides a detailed insight of the antihyperlipidemic activity that may be useful to combat diseases the finding further warrants investigation to decipher the active ingredient underlying the antihyperlipidemic action. With the growing interest of the Western world in complementary and alternative medicines investigations such as these that scientifically examine traditional beliefs and experience are required and are ever-increasingly forthcoming in the literature.

Overall, the use of an effective herbal drug to supplement other drug treatments in controlling Hyperlipidemia and enhancing cardiac functions could be the potential of clinical value if these models are translatable to human clinical studies and outcomes.

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CONFLICTS OF INTEREST: All authors report no conflict of interest.

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