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PHARMACOLOGICAL EFFECT OF ALANGIUM SALVIFOLIUM ON HIGH FAT DIET INDUCED HYPERLIPIDEMIA IN RATS

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ABSTRACT

Hyperlipidemia, characterized by elevated levels of cholesterol and triglycerides coupled with decreased HDL cholesterol, is a significant lipid metabolism disorder associated with increased risk of cardiovascular diseases. In this preclinical study, we evaluated the anti-hyperlipidemic effects of Alangium salvifolium, a plant traditionally used in folk medicine, using a rat model with high-fat diet (HFD)-induced hyperlipidemia. The study involved administering oral extracts of Alangium salvifolium at doses of 200 mg/kg and 400 mg/kg. Results indicated a significant reduction in serum lipid levels ($P < 0.05$) in treated rats compared to the control group. While the HFD initially caused a marked decrease in HDL cholesterol, treatment with Alangium salvifolium led to a notable increase in HDL levels. The reduction in serum cholesterol and triglycerides, along with the increase in HDL cholesterol, supports the traditional use of Alangium salvifolium as a therapeutic agent for managing hyperlipidemia. This study not only underscores the plant's efficacy in improving lipid profiles but also highlights its potential as a natural alternative in the treatment of hyperlipidemia. Further research is warranted to explore the mechanisms underlying these effects and to evaluate the long-term benefits and safety of Alangium salvifolium in lipid metabolism disorders.

KEYWORDS: Hyperlipidemia, Alangium salvifolium, Cholesterol, Triglycerides, HDL cholesterol.

INTRODUCTION

Hyperlipidemia, characterized by elevated levels of lipids in the blood, is a major risk factor for cardiovascular diseases, which are among the leading causes of mortality worldwide. This condition can result from various factors, including genetic predisposition, sedentary lifestyle, and dietary habits, particularly the consumption of high-fat diets.[1] The traditional management of hyperlipidemia involves lifestyle modifications, such as diet and exercise, and pharmacotherapy. However, these treatments often come with side effects and limitations, prompting the exploration of natural products and herbal medicines as alternative therapeutic options.[2]

Alangium salvifolium is a medicinal plant belonging to the Alangiaceae family, widely used in traditional medicine for its diverse pharmacological properties. The plant is renowned for its anti-inflammatory, antioxidant, and antidiabetic activities, which suggest its potential in managing hyperlipidemia. The various bioactive compounds in Alangium salvifolium, such as alkaloids, flavonoids, and saponins, contribute to its therapeutic effects.[3,4]

Previous studies have indicated that extracts of Alangium salvifolium possess lipid-lowering effects, making it a promising candidate for further investigation in the context of hyperlipidemia induced by high-fat diets. For instance, research has demonstrated that Alangium salvifolium extract can significantly reduce serum cholesterol, triglycerides, and low-density lipoprotein (LDL) levels while increasing high-density lipoprotein (HDL) levels in hyperlipidemic rats. These effects are attributed to the plant's ability to enhance lipid metabolism and reduce oxidative stress, which is often elevated in hyperlipidemic conditions.[5,6]

The high-fat diet model in rats is commonly used to study hyperlipidemia as it closely mimics the human condition. This model involves feeding rats a diet rich in fats, leading to increased lipid levels in the blood and liver, thereby inducing hyperlipidemia. By examining the lipid profile and assessing the histopathological changes in the liver, researchers can evaluate the efficacy of

potential therapeutic agents like Alangium salvifolium.[7,8]

This literature survey highlights the significant potential of Alangium salvifolium in managing hyperlipidemia. By understanding the plant's lipid-lowering mechanisms, we can contribute to developing alternative therapeutic strategies for hyperlipidemia using natural products.

MATERIAL AND METHODOLOGY

Plant collection

The medicinal plant *Alangium salvifolium* (500 gm) was collected locally from Bhopal, M.P. After cleaning, plant parts were dried under shade at room temperature for 3 days and then in oven at 45°C till complete dryness. Dried plant parts were stored in air tight glass containers in dry and cool place to avoid contamination and deterioration.

Authentication of selected traditional plant - The leaves of medicinal plant *Alangium salvifolium* was authenticated by a plant taxonomist in order to confirm its identity and purity.

Extraction of Plant

The air-dried medicinal leaves of plant of *Alangium salvifolium* (500 gm) was coarsely powdered and extracted by continuous hot extraction method by using Soxhlet apparatus with methanol at 60°C for 8 hours, individually. The extract was filtered through Whatman filter paper no.1, and the filtrate was dried using rotary evaporator under reduced pressure (335 mbar) at 40°C and stored in refrigerator (2-4°C) for further activity. The obtained crude extract was stored in a refrigerator at 4°C until time of use. The percentage yield of the extract was calculated using the formula below:

% yield = weight of the extract/weight of plant material × 100

Phytochemical investigation

In order to identify various phyto-constituents namely alkaloids, terpenoids, glycosides, steroids, triterpenoids, flavonoids, carbohydrates, saponins and tannins in different extracts, phytochemical screening was performed using standard procedures.

Quantitative Phytochemical Estimation

Total Phenolic Content Estimation

To determine the total phenolic content, prepare standard solutions of Gallic acid in methanol with concentrations ranging from 20 to 100 µg/ml, and a test sample in methanol or a solvent of similar polarity at 100 µg/ml. Add 0.5 ml of each Gallic acid standard or test sample to 2 ml of Folin-Ciocalteu Reagent (diluted 1:10 with deionized water), followed by 4 ml of sodium carbonate solution. Incubate the mixtures at room temperature for 30 minutes with intermittent shaking. Measure the absorbance at 765 nm using methanol as the blank. Prepare a standard curve from the absorbance readings of the Gallic acid concentrations and determine the line of regression. Use the regression equation to calculate the total phenolic content of the test sample, expressing the results as mg/g or µg/mg Gallic acid equivalents.

Total Flavonoid Content Estimation

To assess total flavonoid content, prepare Rutin solutions in methanol with concentrations ranging from 20 to 100 µg/ml, and prepare a test sample in methanol or a similar solvent at 100 µg/ml. Mix 0.5 ml of each Rutin solution or test sample with 2 ml of distilled water, followed by 0.15 ml of 5% NaNO2 solution. After 6 minutes, add 0.15 ml of 10% AlCl3 solution and let it stand for 6 minutes. Then add 2 ml of 4% NaOH solution and adjust the final volume to 5 ml with water. Mix thoroughly and let the solution stand for 15 minutes. Measure the absorbance at 510 nm using a water blank. Construct a standard curve for Rutin concentrations and determine the regression line. Use the regression equation to calculate the total flavonoid content of the test sample, expressing the results as mg/g or µg/mg Rutin equivalents.

Evaluation of *in-vitro* Antioxidant Activity

1,1- diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Activity

The DPPH (1,1-diphenyl-2-picrylhydrazyl) assay measures antioxidant activity by assessing the reduction of the DPPH free radical, which exhibits a purple color at 517 nm. Antioxidants reduce DPPH to the DPPH-H form, leading to a color change to yellow, indicating the extent of free radical scavenging. The assay involves mixing 1 ml of 0.1 mM DPPH solution (prepared in methanol) with 1 ml of different extract concentrations, followed by vortexing and incubation in the dark at room temperature for 30 minutes. Ascorbic acid serves as the reference standard and methanol as the control. The reduction in DPPH absorbance at 517 nm, measured using a UV spectrophotometer, reflects antioxidant capacity. The antioxidant activity is expressed as the 50% inhibitory concentration (IC50), with lower IC50 values indicating higher antioxidant activity. Percentage inhibition of DPPH radicals is calculated to determine the scavenging effect

% inhibition = [(absorbance of control- absorbance of sample)/absorbance of control] × 100%

Acute Oral Toxicity and Dose Selection

In-vivo acute toxicity study (OECD 423 guidelines)

Acute oral toxicity study: As per OECD 423 guideline for the selection of dose

The acute toxic class method involves a stepwise procedure to assess the toxicity of a substance using 3 animals of a single sex per step. The testing starts with a defined dose, and depending on the observed mortality or morbidity, additional steps are taken. Each step consists of dosing 3 animals, with further steps determined based on whether toxicity is observed. If no toxicity is noted, the process may involve dosing three more animals at the same dose or at a higher/lower dose level. The initial

dose is chosen from four fixed levels: 5, 50, 300, or 2000 mg/kg body weight.

Induction of hyperlipidemia

The high fat diet (HFD) was prepared with a mixture of 2% (w/w) cholesterol and 0.5% (w/w) cholic acid in standard animal chow and administered for 4 weeks except for the normal control group which was fed with standard chow only. At the end of 4th week, total cholesterol level in serum was estimated and the animal with greater than 250 mg/dl level was selected and considered as hyperlipidemic rats.

Experimental design

The animals were grouped into 4 groups each containing 6 rats.

Group I: Normal control fed with standard diet.

Group II: Hyperlipidemic rats

Group III: Hyperlipidemic rats treated with Atorvastatin (30 mg/kg) orally for 2 weeks.

Group IV: Hyperlipidemic rats treated with Ethanolic extract of 200 mg/kg and 400 mg/kg orally for 2 weeks.

Blood samples after 24 hours of last dose was collected from retro-orbital plexus and allowed to coagulate at room temperature which was then centrifuged at 3000 rpm for 10 minutes. The serum was separated and used for the biochemical estimations TC, HDL, LDL, VLDL using the semi-auto analyser and relevant lipid profile kits.

RESULT AND DISCUSSION

The *Alangium salvifolium* used was collected in the month of January 2022 and were dried in shade at room temperature. The dried leaves of *Alangium salvifolium* was crushed and used for the preparation of the extract.

Table 1: Collection of *Alangium salvifolium*.

S. No.	Plant Name	Plant Part Used
1.	*Alangium salvifolium*	Leaves

Plant Extraction

$$\% \text{ yield} = \frac{\text{Weight of extract}}{\text{Weight of plant material used}} \times 100$$

The plant material was extracted by continuous hot percolation using Soxhlet apparatus and the percentage yield calculated by the following formula was found to be 8.41 % (by methanol).

Qualitative Phytochemical Analysis of Plant Extract

The results of qualitative phytochemical analysis of the crude extract of leaves of *Alangium salvifolium* was shown.

Table 2: Phytochemical testing of extract.

S. No.	Experiment	Results	
		Pet. Ether Extract	Methanolic extract
	Test for Carbohydrates		
1	Molisch's Test	-ve	+ve
2	Fehling's Test	-ve	+ve
3	Benedict's Test	-ve	+ve
	Test for Protein & Amino acids		
4	Biuret's Test	-ve	-ve
5	Ninhydrin Test	-ve	-ve
	Test for Glycosides		
6	BorntragerTest	-ve	-ve
7	Killer killaniTest	-ve	-ve
	Test for Alkaloids		
8	Mayer's Test	-ve	+ve
9	Hager's Test	-ve	+ve
10	Wagner's Test	-ve	+ve
	Test for Saponins		
11	Froth Test	+ve	-ve
	Test for Flavonoids		
12	Lead acetate	-ve	-ve
13	Alkaline reagent test	-ve	-ve
	Test for Triterpenoids and Steroids		
14	Libermann-Burchard Test	+ve	+ve
15	Salkowski Test	+ve	+ve
	Test for Tannin and Phenolic Compounds		
16	Ferric Chloride Test	-ve	-ve

17	Gelatin Test	-ve	-ve
18	Lead Acetate Test	-ve	-ve

+ve: Present
-ve: Absent

Quantitative Phytochemical Analysis of Plant Extract
Preliminary phytochemical testing of crude extracts confirmed the presence of Phenolics and flavonoids in plant material. To estimate their amount total phenolic (TPC) and total flavonoid content (TFC) assays were performed.

Total Phenolic Content (TPC) Estimation
Polyphenols, a class of phytochemicals, are widely distributed and act as antioxidants, protecting cells from free radical damage and potentially inhibiting cancer growth. The total phenolic content in methanolic extracts is determined using the Folin-Ciocalteu method with gallic acid as the standard, and results are expressed as mg gallic acid equivalent (GAE) per gram of extract. For A. salvifolium extracts, methanolic extract showed the highest phenolic content at 131.60 mg GAE/g, while ethyl acetate extract had the lowest at 95.30 mg GAE/g. Polar extracts, such as those from alcohol and water, typically have higher total phenolic and flavonoid contents.

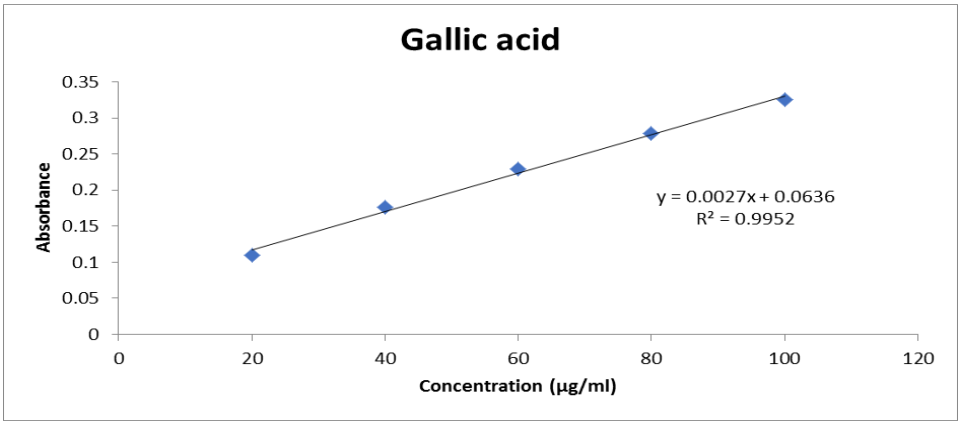


Figure 1: Standard Calibration curve of Gallic acid for Total Phenolic Content.

Table 3: Total Phenolic Content of extracts of A. salvifolium.

Extracts	Total Phenolic content (mg/gm equivalent of Gallic acid)
Methanol	131.60 mg/gm

'x' represents quantity/concentration of phenols which is obtained from the equation: y=0.002×+0.063, where y is absorbance of samples and R^2=0.995.

The total phenolic content (TPC) of Alangium salvifolium leaves was measured and expressed as mg gallic acid equivalents per 100 mg dry weight of the sample. The methanolic extract showed a phenolic content of 131.60 mg/g, while the petroleum ether extract contained no detectable phenolic compounds.

6.3.2 Total Flavonoid Content (TFC) Estimation
Flavonoids are a large group of phenolic compounds with a C15 skeleton, featuring a chroman ring and an aromatic ring, typically found as glycosides rather than aglycones. They are characterized by their C6-C3-C6 carbon core and numerous phenolic OH groups, which enhance their antioxidant activity (Castellano et al., 2013). Flavonoids, a major subclass of polyphenols, are distinguished by their two aromatic rings connected by three carbon atoms forming an oxygenated heterocycle (Pietta, 2000). Known as Vitamin P, flavonoids contribute to plant pigmentation and exhibit significant anti-inflammatory, anti-allergic, and anti-cancer properties.

In the study of Alangium salvifolium leaves, total flavonoid content was determined using a standard curve with rutin (20-100 µg/ml) as a reference. The total flavonoid content was calculated based on the regression equation (y = 0.001x + 0.092, R^2 = 0.979) and measured by UV spectrophotometry at 510 nm with water as the blank.

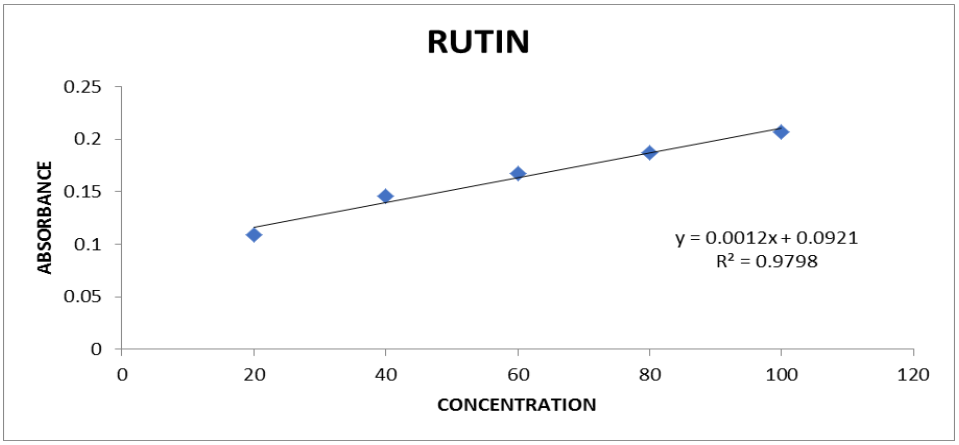


Figure 2: Standard Calibration curve of Rutin for Total Flavonoid Content determination.

Table 4: Total Flavonoid Content of extracts of *A. salvifolium*.

Extracts	Total Flavonoid content (mg/gm equivalent of rutin
Methanol	264.200 mg/gm

'x' represents quantity/concentration of flavonoids which is obtained from the equation: y=0.001×+0.092, Where y=absorbance of samples and R^2=0.979.

The total flavonoids content of the extracts was expressed as percentage of Rutin equivalent per 100 mg dry weight of sample. The total flavonoids estimation of methanolic extract of *Alangium salvifolium* leaves showed the content values of 264.200 mg/gm respectively.

***In vitro* Antioxidant Assays**

DPPH 1, 1- diphenyl-2-picryl hydrazyl Assay

In the evaluation of Alangium salvifolium leaf extracts, all extracts exhibited significant inhibitory activity against the DPPH radical. The IC50 values for the extracts and ascorbic acid (AA) were as follows: Methanol extract (MET) 49.23 µg/mL, Ethyl acetate (EA) 85.78 µg/mL, Petroleum ether (PE) 94.78 µg/mL, and Chloroform (CF) 197.22 µg/mL. The methanol extract demonstrated the highest free radical scavenging ability, while the chloroform extract showed the lowest. The results indicate that polar extracts have stronger antioxidant effects compared to non-polar ones, with the methanol extract's IC50 value being close to that of ascorbic acid, a well-known antioxidant.

Table 5: DPPH radical scavenging activity of Std. Ascorbic acid.

Ascorbic acid (std.)			
S. No.	**Concentration**	**Absorbance of Sample**	**% Inhibition**
1.	20 µg/ml	0.231	59.7561
2.	40 µg/ml	0.298	48.08362
3.	60µg/ml	0.251	56.27178
4.	80µg/ml	0.183	68.11847
5.	100µg/ml	0.158	72.47387
	IC_{50}	11.89	

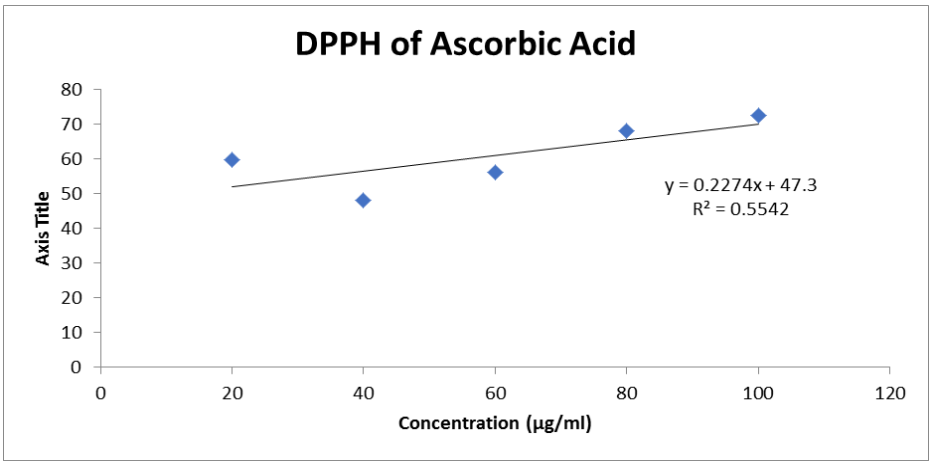


Figure 3: DPPH of Ascorbic acid concentration and Inhibition graph.

Table 6: DPPH radical scavenging activity of Petroleum ether extract of *A. salvifolium*.

Petroleum ether extracts of *A. salvifolium*			
S. No.	**Concentration**	**Absorbance of Sample**	**% Inhibition**
1.	20 µg/ml	0.438	23.69338
2.	40 µg/ml	0.402	29.96516
3.	60µg/ml	0.371	35.36585
4.	80µg/ml	0.314	45.29617
5.	100µg/ml	0.273	52.43902
IC_{50}		94.78	

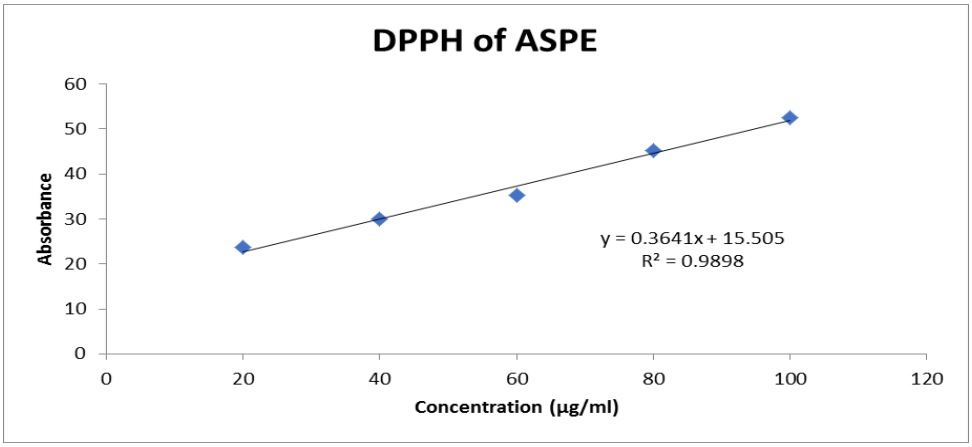


Figure 4: DPPH of *A. salvifolium* Petroleum Ether Concentration and inhibition graph.

Table 7: DPPH radical scavenging activity of Methanolic extract of *A. salvifolium*.

MeOH extract of *A. salvifolium*			
S. No.	**Concentration**	**Absorbance of Sample**	**% Inhibition**
1.	20 µg/ml	0.324	43.55401
2.	40 µg/ml	0.301	47.56098
3.	60µg/ml	0.276	51.91638
4.	80µg/ml	0.242	57.83972
5.	100µg/ml	0.219	61.84669
IC_{50}		49.23	

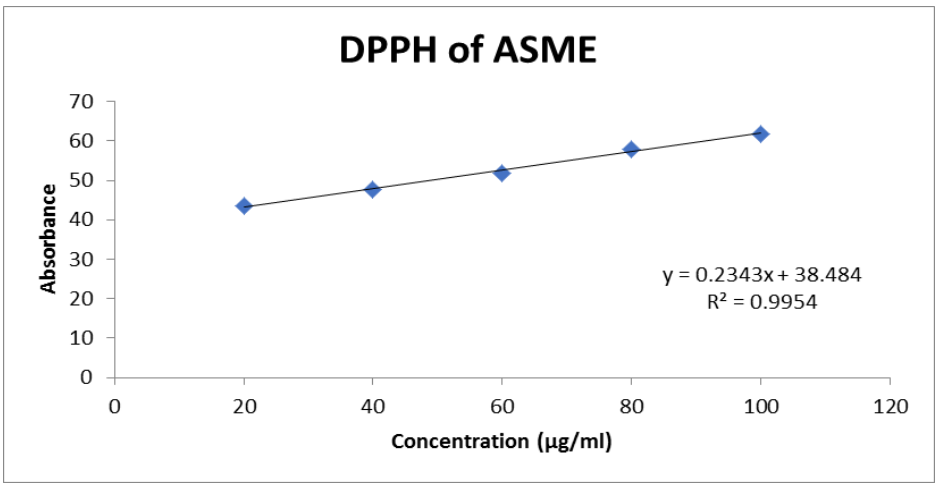


Figure 5: DPPH of *A. salvifolium* MeOH Concentration and Inhibition graph.

Table 8: IC_{50} values of plant extracts of *A. salvifolium*.

***A. salvifolium* extract**	**IC_{50}**
PE	94.78
MET	49.23
AA (Std)	11.89

IC_{50} values of extract, PE=Pet Ether, EA= Ethyl acetate, MET=Methanol, AA=Ascorbic acid.

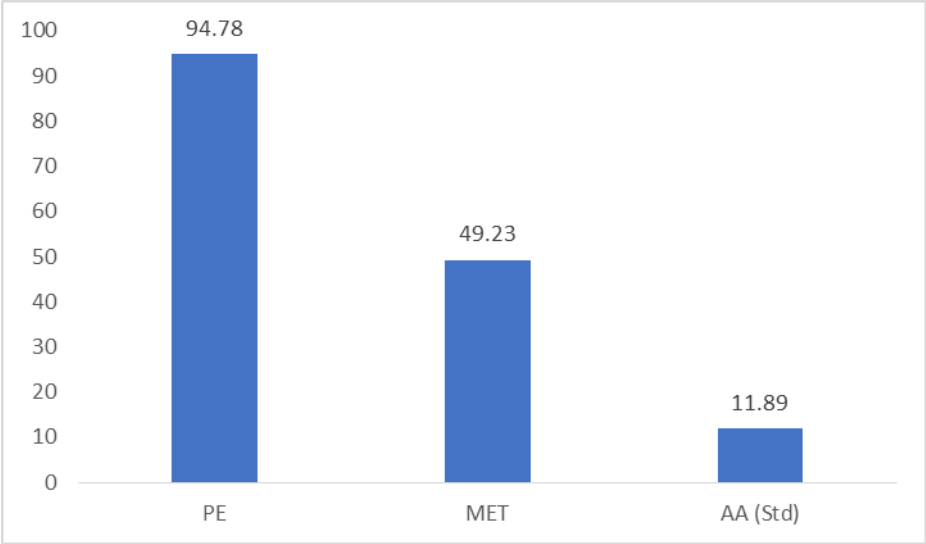


Figure 6: IC_{50} values of different plant extracts of *A. salvifolium*.

Acute Oral Toxicity and Dose Selection

Toxicity testing is a crucial step in drug development to assess the safety of new compounds. According to OECD 423 guidelines, an acute oral toxicity study was conducted using young, healthy adult female Sprague Dawley rats weighing 170-200 grams. The rats were divided into four groups of three animals each and administered one of four dosage levels: 5 mg/kg, 50 mg/kg, 300 mg/kg, and 2000 mg/kg. The animals were monitored for mortality during the first 4 hours post-dosage and for 14 days following administration to evaluate the compound's toxicity.

Table 9: Acute Oral Toxicity of *Alangium salvifolium* extract (in Albino wistar rat).

S. No.	Groups	Observations/ Mortality
1.	5 mg/kg Bodyweight	0/3
2.	50 mg/kg Bodyweight	0/3
3.	300 mg/kg Bodyweight	0/3
4.	2000 mg/kg Bodyweight	0/3

***In vivo*: Anti- diabetic activity (High fat Model)**

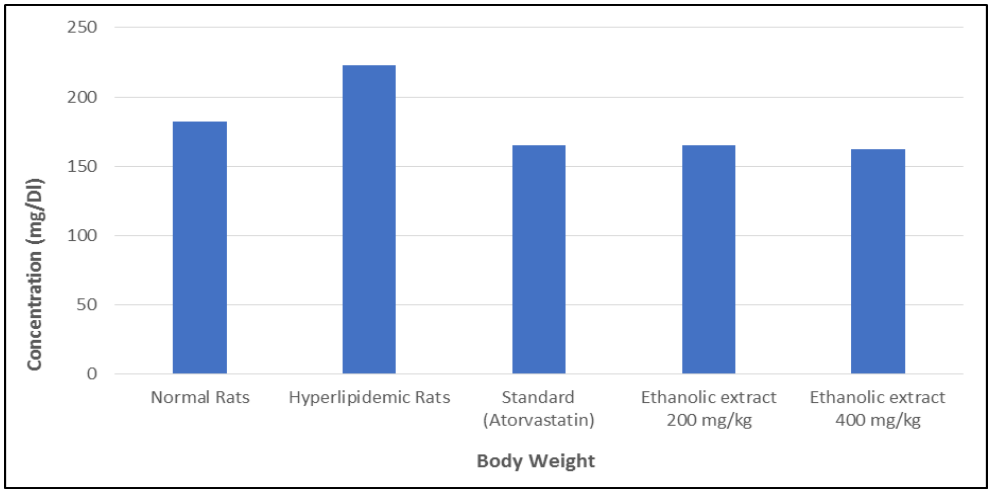
Hyperlipidemia, a group of metabolic disorders characterized by the elevated levels of lipids, is a major modifiable risk factor for atherosclerosis and cardiovascular disease. These lipids include cholesterol, cholesterol esters, phospholipids, and triglycerides.

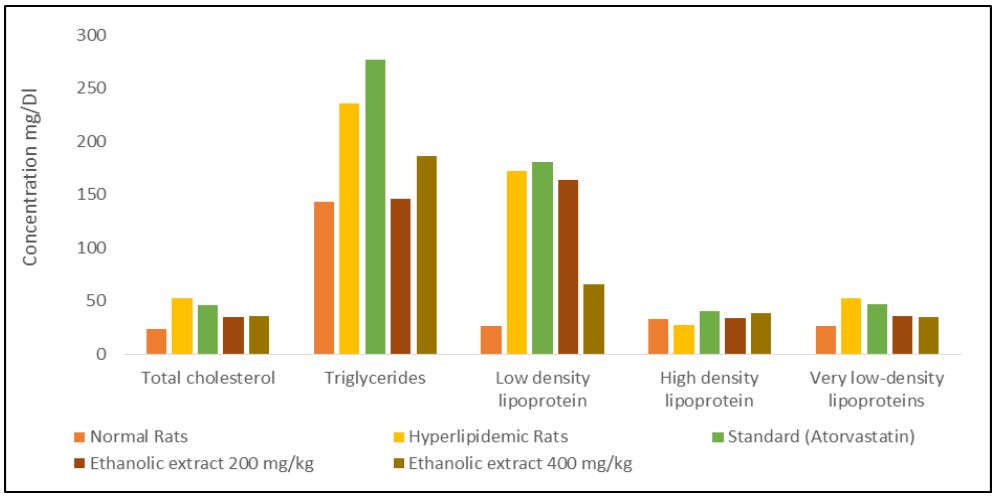
Body weight of rats was significantly increased when they were fed with high fat diet (HFD) for 4 weeks when compared with normal control. By inducing ASE, the body weight reduced significantly ($P < 0.05$), when compared to normal and hyperlipidemic control and the results were expressed.

Table 10: Effect of *Alangium salvifolium* on body weight and lipid profile.

Groups	Body Weight	Total cholesterol	Triglycerides	Low density lipoprotein	High density lipoprotein	Very low-density lipoproteins
Normal Rats	182.5 ± 23.6	23.6 ± 67	142.9 ± 9.1	26.7 ± 38	33.1 ± 4.06	26.5 ± 2.07
Hyperlipidemic Rats	222.5 ± 18.9	52.2 ± 3	235.9 ± 1.6	172.05 ± 3.1	27.1 ± 3.9	52.2 ± 2.0
Standard (Atorvastatin)	165 ± 19.1	45.7 ± 2.2	276.6 ± 10.7	180.7 ± 4.1	40.8 ± 1.7	46.7 ± 2.6
Ethanolic extract 200 mg/kg	165 ± 5.7	35.2 ± 4.8	146.3 ± 3.8	163.5 ± 0.8	33.7 ± 2.4	35.6 ± 2.7
Ethanolic extract 400 mg/kg	162 ± 19.1	35.4 ± 3.5	186.1 ± 4.7	65.5 ± 1.4	38.8 ± 15	35.3 ± 2.7

Values are expressed as Mean ± SD using ANOVA.





CONCLUSION

Hyperlipidemia, characterized by elevated levels of cholesterol and triglycerides, along with reduced HDL cholesterol, is a lipid metabolism disorder. In a preclinical study assessing anti-hyperlipidemic effects, Alangium salvifolium was tested on rats with high fat diet-induced hyperlipidemia. Oral administration of Alangium salvifolium extracts at doses of 200 mg/kg and 400 mg/kg led to a significant reduction in serum lipid levels ($P < 0.05$). The high fat diet initially decreased HDL levels, but treatment with Alangium salvifolium resulted in a notable increase in HDL cholesterol. This study supports the traditional use of Alangium salvifolium, demonstrating its effectiveness in improving lipid profiles and highlighting its potential as an anti-hyperlipidemic agent at the tested doses.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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