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Phytochemical and anti hyperlipidemic activity of leaves extract of lagerstromia microcarpa

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Abstract

The present study investigated the phytochemicals and in-vivo anti hyperlipidemic activity of hydroalcoholic extract of leaves of *lagerstroemia microcarpa* wight. According to phytochemical study, the plant contains a variety of secondary metabolites with pharmacologically significant qualities, including flavonoids, phenol, saponins, proteins, carbohydrates, and alkaloids. Hyperlipidaemia induced by the diet was significantly reduced in rodents by the hydroalcoholic extract. In a dose-dependent manner, all treatment groups led to a substantial reduction in "serum levels of total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C), and high density lipoprotein cholesterol (HDL-C)", which was comparable to the standard drug Atorvastatin. In addition, "the hydroalcoholic extract of *Lagerstroemia microcarpa* leaves extract (300mg/kg)" significantly reduced serum LDL-C levels.

Keywords; Antihyperlipidemia activity, *Lagerstromia microcarpa*, triglycerides, total cholesterol.

INTRODUCTION

A disorder known as hyperlipidemia occurs when the blood has an excess of fatty compounds known as lipids, primarily cholesterol and triglycerides. It is also referred to as hyperlipoproteinemia due to the fact that these excess lipids are transported through the bloodstream in conjunction with proteins. This is the only way that these fats may stay dissolved in the bloodstream, which causes hypertension, heart attacks, strokes, atherosclerosis, and other conditions.

Humans have been using natural products— "plants, animals, microbes, and marine organisms"—in medicines to cure and relieve illnesses since the beginning of time. As far back as 60,000 years ago, fossil evidence suggests that humans were already making use of plants for medicinal purposes. Early humans must have faced a significant challenge when utilising natural products as remedies.[1]

A moderate to big deciduous tree, *L. microcarpa* Wall (Lythraceae) may grow up to "30 meters in height and 2.4 to 3.0 meters in diameter", with a neat, cylindrical bole that is 12 to 15 meters long. From Bombay to Kerala, *L. microcarpa* is a plant that has been used to cure colds, coughs, chronic bronchitis, diabetes mellitus, and asthma. In the plant, the primary components include "steroid, terpenoids, phenols, flavonoids, alkaloids, ellagic acid, and tannins". [2] [3].

Many active ingredients in the plant are used in pharmaceutical products, such as ellagitannins found in leaves and fruits, alanine, isoleucine, α -aminobutyric acid, and menthionine found in "leaf extract, lageracetel, amyl alcohol, ellagic acid, β -sitosterol, and a tannin compound called lagertannin; 3,3', 4-tri-O-methylellagic acid, and 3-O-methylellagic acid." Additionally, it is in charge of displaying a number of significant properties, including anti-inflammatory, anti-hyperlipidemia, antioxidant, and metabolic syndrome properties [4] [5]. *Lagerstroemia microcarpa* wight (Leaves) is examined for its in-vivo antihyperlipidemic properties in rodents.

MATERIAL AND METHOD

Collection of plant

In March 2022, *Lagerstroemia microcarpa* leaves were gathered from the Bhopal neighbourhood. In the Department of Botany at Career College, Bhopal, Dr. Jaswinder Mehta conducted the authentication of the leaves. Its reference number is /Career/Herb/2022/092. In the shade, the leaves were dry.

EXTRACTION BY MACERATION:

Soxhlet's device ground up 75 gram of shade-dried plant material into a coarse powder and used petroleum ether to extract it. Until the material had been defatted, the extraction process was continued. Using Soxhlet's equipment, the defatted mark of *Lagerstroemia microcarpa* was extracted using a "hydroalcoholic solvent (Ethanol water; 80:20) for 48 hours". The microcarpa was then filtered and dried at 40°C using a vacuum evaporator [6]

$$\text{Percent Yeild} = \frac{\text{Actual yeild}}{\text{Theoretical yeild}} \times 100\%$$

Phytochemical Screening

Many of the current medicinal drugs are indirectly derived from medicinal plants, which are traditional pharmaceutical commodities. The two primary bioactive components of phytochemical materials include alkaloids, terpenoids, phenols, flavonoids, and other secondary bioactive components. The primary bioactive components include sugar, vitamins, amino acids, and chlorophyll. The standard protocols for extract were followed to conduct phytochemical analyses.[7]

Estimation of total flavonoids content

The aluminium chloride technique was used to determine the total flavonoid content [44]. In 10 millilitres of methanol, 10 milligrammes of quercetin were dissolved, and several aliquots were made with concentrations ranging from 5 to 25 microgrammes per millilitre. After use, the dried extracts were filtered after dissolving in 10 millilitres of methanol. With a concentration of one milligramme per millilitre, the flavonoid content was determined using three millilitres of this solution. Using "1 ml of 2% AlCl₃ methanolic solution" added to three millilitres of extract or standard, the absorbance was measured at 420 nm after letting the mixture remain at room temperature for fifteen minutes. [8]

Total phenol content estimation

Using a modified Folin-Ciocalteu method, we determined the extract's total phenol content [45]. Gallic acid dissolved

in methanol at concentrations ranging from 10 milligrammes to 50 microgrammes per millilitre was used to create different aliquots. Filtered 10 millilitres of methanol containing 10 milligrammes of dried extract. A two millilitre (1 mg/ml) volume of this extract was used to evaluate the phenol content. One millilitre of Folin-Ciocalteu reagent, diluted 1:10 v/v with distilled water, and one millilitre of sodium carbonate, with a concentration of 7.5 g/l, were mixed with two millilitres of extract and each standard. After letting the mixture sit for 10 minutes and being vortexed for 15 seconds, colour development may begin. The absorption at 765 nm was measured using a spectrophotometer. [9]

In vivo antihyperlipidemia activity of Lagerstroemia macrocarpa

Animals

In the present investigation, "Animal's Albino rats (SD strain)" of either sex, weighed 150–200g, were employed. In addition to receiving a typical food, tap water, and labium, they were also exposed to a 12-hour light and 12-hour dark cycle. Before the investigations were conducted, the animals were accustomed to the laboratory environment. An "Institutional Animal Ethics Committee" authorized the experimental procedure. The animals were cared for in accordance with the regulations set out by the Ministry of Environment and Forests, Government of India's "Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA)". The Animal Ethics Committee of the institution authorized the experiment protocol.

Drug and extract

1% w/v gum acacia was used to suspend the extract in distilled water. One percent carboxymethyl cellulose (CMC) was used to suspend the reference medication atorvastatin in distilled water. As vehicles, the control group was given "1% CMC solution and 1% w/v gum acacia in distilled water".

Acute oral toxicity study

In accordance with the Organisation for Economic Co-operation and Development's (OECD 423) recommendation, "adult albino rats (SD strain) weighing 150–200g of either sex" were utilised for the acute toxicity investigation after fasting for several hours. Both male and female mice were fasted for the whole night in four groups. At 200, 600, and 2000 mg/kg, the mice in the other groups were given "hydroalcoholic extract of *Lagerstroemia microcarpa* in 0.5% CMC", whereas the animals in the first control group were given a 0.5% CMC suspension in

distilled water. For the initial four hours, the animals were closely monitored for any signs of toxicity, including higher motor activity, salivation, convulsions, stupor, and mortality. For the next 24 hours, observations were performed at regular intervals. No deaths were observed throughout the research period, and the animals were examined further for a total of 14 days.

Diet induced hyperlipidemia in rats

Caused hyperlipidaemia by diet. Compared to the normal control group, "the serum levels of TC, TG, LDL-C, VLDL-C, and HDL-C" exhibited a substantial increase in the hyperlipidemia control group. Serum levels of HDL-C, VLDL-C, TC, and TG significantly decreased in all therapy groups. Besides this, the extract from the leaves of *Lagerstroemia microcarpa* considerably reduced the blood LDL-C levels. [10]

Table 1 Diet-induced hyperlipidemia model

S. No.	Groups	Treatments	No. of Animals
1.	"Normal	Vehicles (1 mL of 1% gum acacia and 1% CMC)	6
2.	Hyperlipidemic control	High cholesterol diet	6
3.	Treated with Standard (Atorvastatin)	High cholesterol diet + Atorvastatin (50mg/kg, p.o.)	6
4.	Treated with HELM 200mg/kg	High cholesterol diet + HELM (200mg/kg, p.o.)	6
5.	Treated with HELM 300mg/kg	High cholesterol diet + HELM (300mg/kg, p.o.)	6

Lipid profile

On the eighth day in the instance of diet-induced hyperlipidemia, the blood lipid profile was assessed. "Employing commercially available kits (Erba; Transasia Bio-Medicals Ltd., Daman, India), the levels of high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), and total cholesterol (TC) were calculated. VLDL-C, or very low-density lipoprotein cholesterol, was computed as TG/5. Levels of LDL-cholesterol (LDL-C) were determined."

RESULT AND DISCUSSION

Table 2 % Yield of hydroalcoholic extract of *Lagerstroemia microcarpa*

S. No.	Extracts	% Yield (w/w)
1.	Pet. ether	1.69%
2.	Hydroalcoholic	8.61%

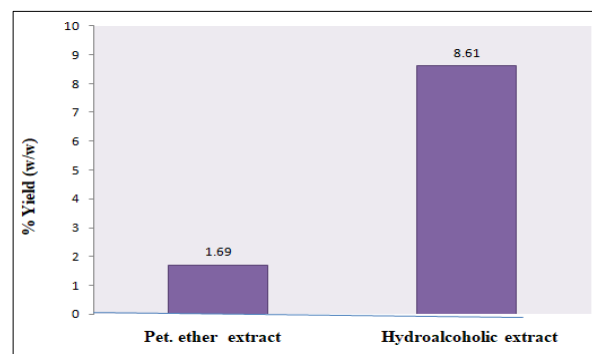


Figure 1 Lagerstroemia microcarpa hydroalcoholic extract yield was 8.61%, whereas Pet. ether yield was 1.69%.

Table 3 Phytochemical screening of extract of *Lagerstroemia microcarpa*

S. No.	Constituents	Hydroalcoholic extract
1.	Alkaloids	-ve
	Mayer's Test	
	Wagner's Test	
	Dragendroff's Test	
	Hager's Test	
2.	Glycosides	-ve
	Legal's Test	
3.	Flavonoids Lead acetate Alkaline test	-ve
4.	Phenol Ferric chloride test	+ve

5.	Proteins Xanthoproteic test	+ve
6.	Carbohydrates	-ve
	Molisch's Test	
	Benedict's Test	-ve
	Fehling's Test	+ve
7.	Saponins Froth Test	+ve
8.	Diterpenes Copper acetate test	-ve
9.	Tannins Gelatin Test	+ve

Total flavonoids content estimation (TFC)

" $Y = 0.030X - 0.008$, $R^2 = 0.998$, where X is the quercetin equivalent (QE) and Y is the absorbance", was the formula based on the calibration curve that was used to determine the total flavonoid concentration as quercetin equivalent (mg/100mg).

Table 4 Preparation of Calibration curve of Quercetin

S. No.	Concentration	Mean absorbance
1	5	0.191
2	10	0.348
3	15	0.514
4	20	0.652
5	25	0.812

Average of three determinations

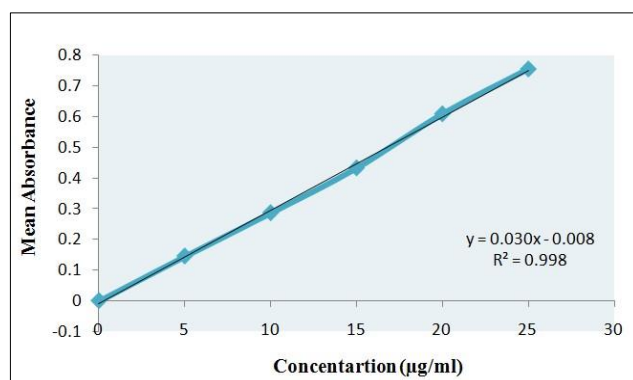


Figure 2 of calibration curve of Quercetin

Estimation of total phenolic content

The standard compound used is gallic acid, and the total phenols were expressed as mg/100 mg gallic acid equivalent via the standard curve equation: $y = 0.018x + 0.016$, $R^2 = 0.998$, where x is "the total phenolic content in the hydroalcoholic extract of Lagerstroemia microcarpa" and y

is the absorbance at 765 nm. Gallic acid equivalents (mg/100 mg of extract) were used to express the findings.

Table 5 Preparation of calibration curve of Gallic acid

S. No.	Concentration	Mean absorbance
1	10	0.214
2	20	0.405
3	30	0.576
4	40	0.762
5	50	0.944

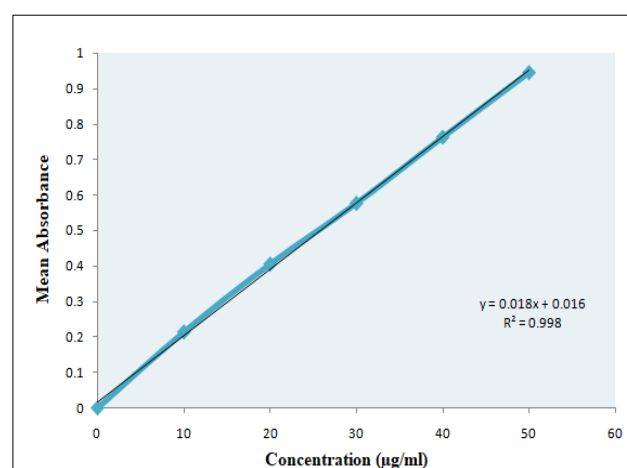


Figure 3 of calibration curve of Gallic acid

Table 6 Estimation of total flavonoids and phenolic content of extract of Lagerstroemia microcarpa

S. No.	Extract	Total flavonoids content (mg/ 100 mg of dried extract)	Total phenolic content (mg/ 100 mg of dried extract)
1.	Hydroalcoholic	0.856	0.641

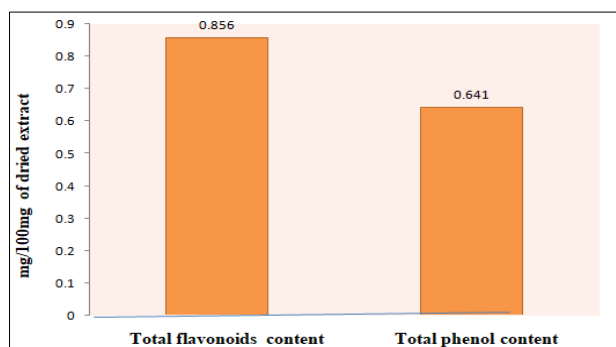


Figure 4 of total flavonoids and phenolic content

Table 7 Effect of Lagerstroemia microcarpa leaves extract on serum lipid profile of diet-induced hyperlipidemia in rats

Group (n = 6)	TC (mg/dL)	TG (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	VLDL-C (mg/dL)
“Normal	131.64±3.12	100.46±2.14	63.12±2.47	51.43±2.54	21.09±0.48
Hyperlipidemia control	324.23±3.45*	301.80±9.57*	101.56±5.66*	162.91±6.32	61.76±1.58*
Atorvastatin	160.00±5.51**	161.99±0.24**	65.87±1.71†	61.53±5.44**	33.60±0.27**
Treated with HELM 200mg/kg	241.26±2.14**	107.42±3.21**	65.50±4.14†	154.08±2.11	22.68±0.62**
Treated with HELM 300mg/kg”	203.56±6.54**	130.51±4.11**	61.99±3.33†	116.46±6.11**	27.10±1.04**

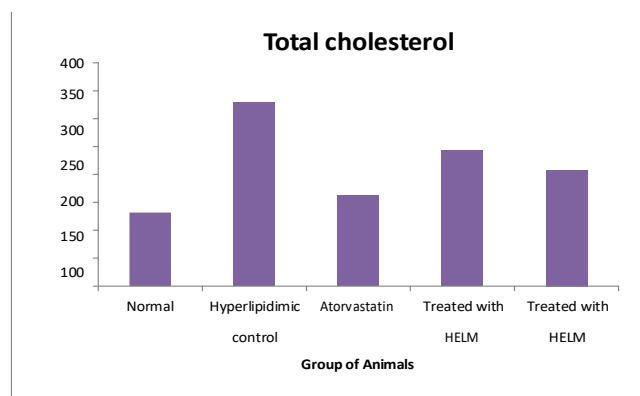


Figure 5 Effect of HELM on Total Cholesterol

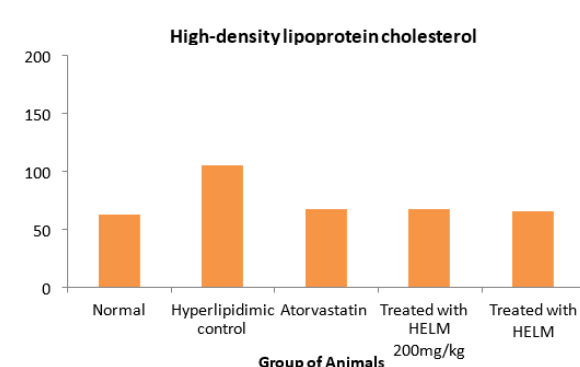


Figure 7 Effect of HELM on HDL-C

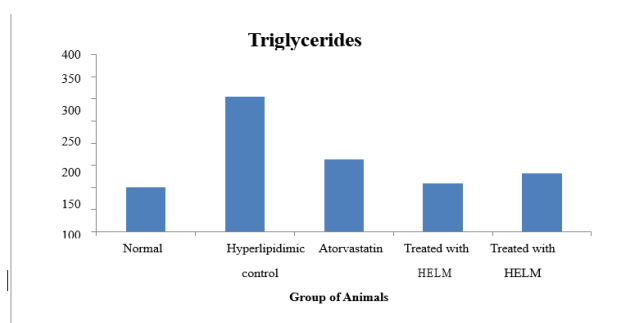


Figure 6 Effect of HELM on Triglyceride

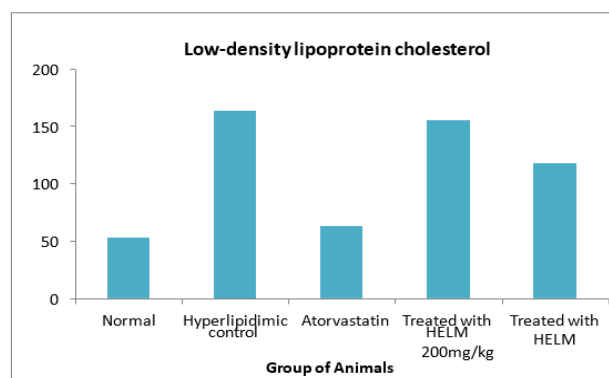


Figure 8 Effect of HELM on LDL-C

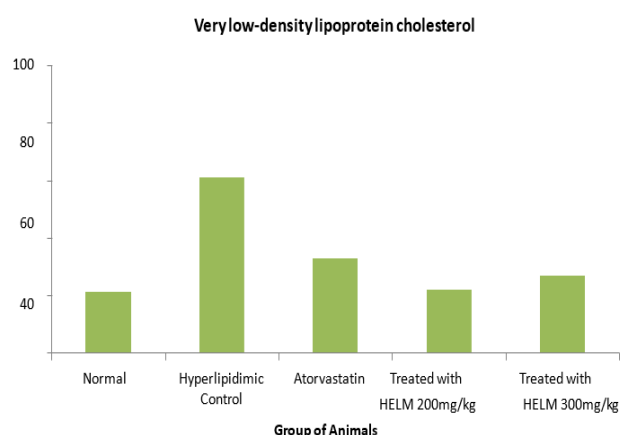


Figure 9 Effect of HELM on VDL-C

CONCLUSION

The result of our study being reported for the first time provide clear evidence that hydroalcoholic extract from *Lagerstromia microcarpa* leaves posses antihyperlipidemia activity and could useful for the development of new antihyperlipidemia drugs. However further pharmacological and toxicological study will be necessary.

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