UPI Journal of Pharmaceutical Medical, and Health Sciences
Content Available at www.uniquepubinternational.com ISSN: 2581-4532

Open Access **Research Article**

EVALUATION OF HYPOLIPIDEMIC ACTIVITY OF HIBISCUS IN HIGH-FAT DIET-INDUCED HYPERLIPIDEMIA IN WISTAR RATS

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DOI: https://doi.org/10.37022/jpmhs.v8i3.145

Article History
Received: 04-07-2025
Revised: 26-07-2025
Accepted: 20-09-2025

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Keywords: Hibiscus, Rosuvastatin, Hyperlipidemia,Hypolipidemic activity,Cholesterol.

Abstract

The global rise in obesity is largely linked to increased consumption of dietary fats, contributing to a higher prevalence of hyperlipidemia. This study aimed to evaluate the hypolipidemic activity of Hibiscus using an in vivo animal model. As current treatments like statins, though effective, are associated with potential side effects, there is growing interest in safer, plant-based alternatives. Hibiscus, a plant commonly found in India, is traditionally known for its lipid-lowering properties. The objective was to assess and compare the efficacy of an aqueous Hibiscus extract with Rosuvastatin in treating high-fat diet-induced hyperlipidemia in male Wistar rats. Hyperlipidemia was induced over 30 days, followed by a 30-day treatment period. Rats received Hibiscus extract orally at 2.5 g/kg and 5 g/kg, while the standard group received Rosuvastatin (10 mg/kg). Body weight, serum lipid profiles, and atherogenic indices were measured on days 0, 15, and 30. Data were analyzed using paired and unpaired Student's t-tests. The Rosuvastatin group showed significant reductions in total cholesterol (TC), triglycerides (TG), LDL-C, and VLDL-C, along with increased HDL-C levels ($p < 0.001$). Hibiscus also produced significant but comparatively lower improvements in lipid parameters. Despite its lower efficacy, Hibiscus demonstrated a favorable safety profile and notable hypolipidemic effects, supporting its potential as a natural alternative in hyperlipidemia management. Further research is warranted to explore its mechanisms and long-term benefits.

Introduction

Chronic diseases, particularly non-communicable diseases (NCDs), have become a major global health concern, especially in developing countries like India. NCDs, including cardiovascular diseases (CVDs), diabetes, cancer, and chronic respiratory diseases, now account for over 50% of deaths in India. Lifestyle changes, poor diet, inactivity, and substance abuse are leading risk factors [1-3]. Among these, hyperlipidemia-a metabolic disorder characterized by elevated levels of blood lipids, particularly cholesterol and triglycerides (TG)-is a key contributor to CVDs. It often remains asymptomatic but can lead to atherosclerosis and severe complications if untreated. LDL and VLDL cholesterol increase cardiovascular risk, while HDL is considered protective [4]. Current pharmaceutical treatments for hyperlipidemia are costly and may cause side effects, prompting interest in plant-based alternatives. Natural compounds found in medicinal plants offer promising, affordable options for lipid management and NCD prevention [5-6].

Aim and Objectives

Goal

To identify traditional Hibiscus-based remedies for hyperlipidemia using preclinical exploratory models and investigate their bioactivity-guided fractionation to determine the components responsible for the hypolipidemic effect.

Objectives:

1. To identify traditional plant-based medicines, particularly Hibiscus, used in the treatment of hyperlipidemia.
2. To develop and use a series of solvents for extracting active compounds from selected plant materials.

3. To conduct preliminary phytochemical screening of various extracts.
4. To evaluate the hypolipidemic potential of these extracts using a standard in vivo screening model.
5. To determine the hypolipidemic activity of the most effective extract through activity-guided fractionation.
6. To assess the biological activity of the active fractions in a hypolipidemic screening model.
7. To identify and characterize the bioactive constituents responsible for the observed hypolipidemic effect.
8. To propose a scientific explanation for the mechanism by which these phytoconstituents exert their lipid-lowering activity.

Plan of Work

The following components constitute the plan of work:
Step 1: Plant Selection
Step 2: Collection and Preparation of Hibiscus Material for Extraction
Step 3: Extraction of Plant Material Using Solvent Systems
Step 4: Phytochemical Screening of the Extracts
Step 5: Induction of Hyperlipidemia in Animal Models
Step 6: Evaluation of Hypolipidemic Activity of Extracts
Step 7: Bioactivity-Guided Fractionation of Active Extract
Step 8: Isolation and Identification of Active Phytoconstituents
Step 9: Mechanism Elucidation and Validation

Plant Profile

Table 01: Plant Profile [7-8]

Category	Details
Botanical Name	Hibiscus
Kingdom	Plantae
Order	Malvales
Family	Malvaceae
Genus	Hibiscus
Species	H. cannabinus

French Variations of Chanvre

1. Kénaf
2. Ketmie à feuilles de chanvre (Belgium)
3. Chanvre de roselle
4. Chanvre de Gambo
5. Chanvre de Guinée
6. Chanvre du Deccan
7. Chanvre de Bombay
8. Jute from Java
9. Jute from Siam

German Variations

1. Ambari
2. Dekkanhanf
3. Gambohanf
4. Hanfeibisch
5. Javajute
6. Kenaf
7. Rosellahanf
8. Roselle
9. Siamjute

Portuguese Variations:

1. Quenafe
2. Cânhamo rosella
3. Juta-de-java
4. American Spanish Variations:
5. Yute de Java
6. Yute de Siam
7. Yute de Gambo
8. Yute de Rosella

Brazilian Portuguese

1. Phonafe
2. Cânhamo-brasileiro
3. Papoula-de-são-francisco

African Language:

1. Chanvre-related names may vary by region.

Indian Languages

2. Manipur, Himachal Pradesh, Bihari, India: Shougri Kudrum
3. West Bengal, India: Pundi Palle
4. Marathi: Ambaadi
5. Tamil (India): Pulichakeerai, Palungu
6. Tulu (India): Gongura

Figure 01: Leaves of Hibiscus

Hibiscus (Malvaceae Family)

Hibiscus, commonly called "rose mallow," includes several species, notably Hibiscus rosa-sinensis, known for its large, colorful flowers. It is native to warm, subtropical, and tropical regions. Popular varieties include tropical hibiscus, rose of Sharon, and tough hibiscus. It is widely used in decoration and its tea, made from both hot and cold infusions, is globally enjoyed due to its vivid color, sour taste, and high vitamin C content [9].

Botanical Characteristics

Hibiscus rosa-sinensis is a member of the Magnoliopsida class, with leaves, stems, and roots. It is a eudicot, with plants exhibiting features like weblike veins and two cotyledons. The flowers have

five oval petals and a central column with yellow anthers, developing into a cup-shaped calyx [10].

Plant Biology

The plant contains several phytochemicals, including phlobatannins, flavonoids, saponins, and terpenoids. The flower colors, such as red, yellow, and white, contain unique compounds like cyanidin-3,5-diglucoside and quercetin. The plant's pharmacological effects are linked to these compounds and may have medicinal properties, such as anti-inflammatory, antimicrobial, and antioxidant effects.

The stems and leaves also contain malvalic acids, fatty acids, and β-sitosterol. The root bark contains cyclopropenoids, and various compounds in the plant may contribute to its potential therapeutic benefits, such as reducing cholesterol and preventing cancer. The plant's antioxidant properties are attributed to anthocyanins, especially in the flowers, which are also used as natural dyes for textiles.

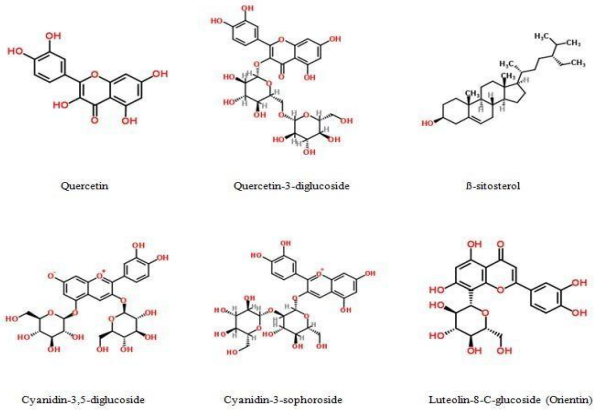

Figure 02: Some of the structures of the several bioactive compounds derived from H. rosa-sinensisare shown in Figure 1.ß-sitosterol, quercetin, quercetin-3-diglucoside, cyanidin-3,5-diglucoside, cyanidin-3-sophoroside, and luteolin-8-C-glucoside are the components that make up this compound [11].

Traditional Uses of Hibiscus [12-13]

Religious & Cultural Symbolism:

1. In India, the hibiscus is sacred to Hindu deities Kali and Ganesha, often depicted together in Bengali art.
2. In Hawaii and Tahiti, women wear hibiscus flowers behind their ears to signify relationship status.
3. The yellow hibiscus is the state flower of Hawaii.

Folklore & Childhood Play:

1. In the Philippines (called gumamela), children crush flowers and leaves to make bubbles using papaya stems.
2. In Waray (Eastern Samar), it's known as Tarukanga.

Literary Significance

1. Inspired the title of "Purple Hibiscus" by Nigerian author Chimamanda Ngozi Adichie.

Textile Use

1. Hibiscus bark can be soaked to produce strong bast fibers.

Pharmacological Effects of Hibiscus cannabinus

Phytochemical Benefits

- Exhibits antibacterial, antifungal, and antioxidant activities using different solvents.

Cytotoxic Properties:

- Lignans from H. cannabinus have shown cytotoxic effects, potentially useful in cancer treatment.

Wound Healing

- Helps stop bleeding and supports tissue repair.

Anti-Inflammatory Action

- Leaf extracts (methanol and water-based) reduce inflammation.

Antioxidant Defense

- Capable of neutralizing free radicals, suggesting strong antioxidant properties.

Methodology

Study Location

- Conducted at Central Animal House, Sura Laboratory, and Department of Pharmacology in Hyderabad, Telangana, India.

Objective:

- To evaluate the **hypolipidemic effects** of **Hibiscus liquid extract** in rats with diet-induced hyperlipidemia.

Plant Extraction

- *Hibiscus* leaves were dried in shade for 7 days, powdered, and processed to produce an aqueous extract.

Animal Model

- **Subjects:** 30 healthy male Wistar rats (180–200g, 18 weeks old).
- **Housing:** 2 per cage, controlled environment (22 ± 2°C, 12-hour light/dark cycle).
- **Ethics:** Approved by the Institutional Animal Ethics Committee (AJIMS), following CPCSEA guidelines.
- **Acclimatization:** 1 week before the experiment.

Experimental Design (60 Days Total)

Group	Treatment
Group I	Control – normal saline (5 ml/kg)
Group II	Hyperlipidemic Control – high-fat diet + saline
Group III	Rosuvastatin (10 mg/kg) – standard drug
Group IV	Hibiscus extract (2.5 g/kg)
Group V	Hibiscus extract (5.0 g/kg)

- All treatments were given orally once daily via gavage.
- Groups II–V were fed a **high-cholesterol diet (HCD)** for 60 days.

High-Cholesterol Diet Composition

- 300g rat food + 300g coconut oil + 5g cholesterol + 5g deoxycholic acid mixed and formed into cakes.

Data Collection

- **Body Weight:** Measured on days 0, 15, and 30 of the treatment phase.
- **Blood Samples:** Collected via retro-orbital puncture under ether anesthesia (after 12-hr fasting).
- **Serum Analysis:** Performed using enzymatic kits and auto-analyzer (Lablife Robochem, RFCL Ltd).

Biochemical Parameters Analyzed

1. **Lipid Profile:** TC, TG, HDL-C, LDL-C, VLDL-C (using Friedewald formula:
   TG/5 = VLDL,
   LDL = TC – (HDL + VLDL)).
2. **Atherogenic Index (AI):**
   AI = TC / HDL
   % Protection = [(AI control – AI treated)/AI control] × 100
3. **Lipid Change Rate:**
   % Change = [(Lipid level difference) / Day 0 value] × 100

Statistical Analysis

- Results expressed as **Mean ± SD (n=6)**.
- **T-tests (paired and unpaired)** conducted using **SPSS**.
- Significance set at **p < 0.05**.

Results and Discussion

Following 30 days of treatment, significant changes were observed in the blood lipid profiles of rats administered rosuvastatin (10 mg/kg, p.o.) and hibiscus aqueous extract (2.5 g/kg and 5 g/kg, p.o.). In the hyperlipidemic control group, levels of total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL-C), and very low-density lipoprotein (VLDL-C) were significantly elevated ($p < 0.001$), while high-density lipoprotein (HDL-C) levels were significantly reduced ($p < 0.05$) when compared to the normal control group. By day 30, both rosuvastatin and hibiscus-treated groups showed a significant reduction in TC, TG, LDL-C, and VLDL-C levels, along with a marked increase in HDL-C levels ($p < 0.001$). Intragroup comparisons between day 15 and day 30 revealed continued improvements in lipid profiles, particularly in the rosuvastatin group, with significant decreases in TC, TG, LDL-C, and VLDL-C, and a concurrent increase in HDL-C ($p < 0.001$). Rate-of-change analysis on day 30 indicated that both hibiscus-treated groups and the rosuvastatin group effectively lowered harmful lipid parameters, with Test Group B (Hibiscus 5 g/kg) demonstrating a greater increase in HDL-C compared to the rosuvastatin group. Furthermore, the atherogenic index (AI) significantly increased in the hyperlipidemic group ($p < 0.001$), while rosuvastatin and hibiscus treatments significantly lowered AI, suggesting their potential protective effect against atherosclerosis.

Table 01: A comparison of serum lipid levels and the atherogenic index (AI) across different groups on Days 0, 15, and 30.

Groups	Days	C (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)	VLDL-C (mg/dl)	LDL-C (mg/dl)	AI
N	0	103.45±25.10	82.14±2.25	41.45±1.52	17.52±2.16	46.24±12.10	2.42±1.63
	15	103.26±10.24	80.46±1.39	40.36±1.51	16.14±2.36	45.14±10.14	2.12±0.14
	30	101.14±1.25	78.14±2.68	39.15±2.41	14.25±1.78	43.59±06.24	2.24±0.39
H	0	291.81±12.14	211.51±11.90	41.02±1.01	41.60±2.13	204.42±10.10	6.71±0.41
	15	291.04±18.95	211.97±11.35	36.77±4.38	42.39±2.27	206.69±21.02	6.89±0.99
	30	280.23±15.15	207.81±9.52	38.01±4.43	41.56±1.90	199.16±18.36	6.83±0.72
R	0	290.08±03.15	213.51±11.12	40.01±1.97	41.21±2.06	201.88±12.88	6.99±0.83
	15	190.13±12.26	155.22±12.22	51.69±3.33	30.60±3.20	95.62±15.00	3.03±0.39
	30	165.03±16.95	115.01±16.68	52.85±5.10	22.45±2.22	72.70±15.13	2.26±0.25
T_A	0	292.14±16.41	204.15±12.52	43.14±7.17	40.83±2.50	211.70±19.65	7.02±1.24
	15	270.29±13.28	186.82±13.87	48.49±4.82	37.36±2.77	186.44±14.66	5.65±0.53
	30	254.35±12.13	169.70±15.34	53.30±2.60	33.94±3.07	169.26±14.40	4.82±0.30
T_B	0	292.95±14.06	200.41±10.21	34.12±4.31	40.48±2.66	218.19±14.19	7.69±0.96
	15	241.13±19.20	165.05±19.10	50.19±2.47	34.01±3.23	158.59±19.02	4.68±0.49
	30	220.15±19.58	144.46±21.21	48.07±3.08	29.30±2.26	131.31±19.54	3.60±0.44

Six perceptions are used to illustrate the information, together with their Cruel and Standard Deviation (SD). The values are significantly different from both the normal control (N) and the hyperlipidemic control (H), with a p-value of 0.001.

The results of the Student's unpaired t-test confirm this.The results of the Student's matched t- test are as follows:Pairs (a1 and a2), (a1 and a3):There is no significant difference ($p>0.05$), no critical difference ($p>0.05$), values that are measurably crucial ($p<0.001$), values that are factuallycritical ($p<0.001$), values that are factuallycritical (d1 and d2), values that are factually critical (d1 and d3), values that are factually critical (e1 and e2), values that are factually critical (e1 and e3).

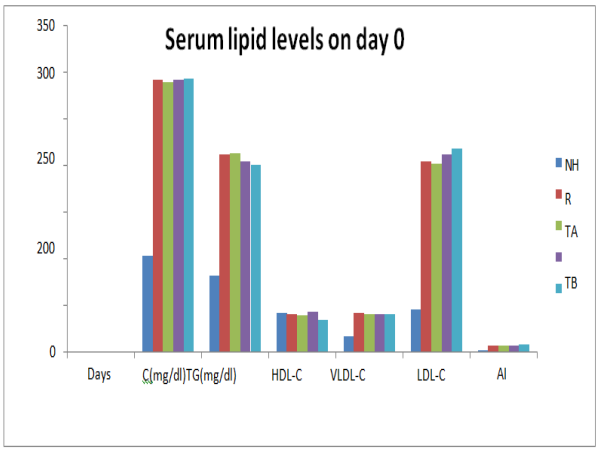

Figure 01: Serum lipid levels on day 0.

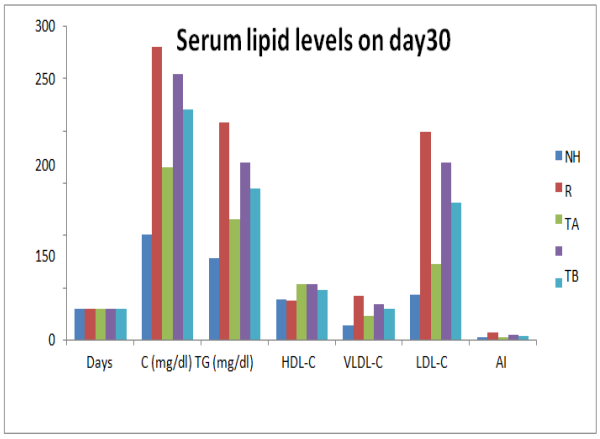

Figure 02: Serum lipid levels on day 30.

Table 02: On Days 15 and 30 of the study, the following table presents the mean percentage change (%) in blood lipid levels from Day 0 values across the various groups.

Groups	Day	Mean percentage change (%)				
		C	TG	HDL-C	VLDL-C	LDL-C
N	15	0.35	5.10	1.89	5.12	5.26
	30	4.12	6.98	0.10	9.61	2.02
H	15	0.63	7.05	9.61	0.52	0.43
	30	4.41	3.12	6.95	3.13	4.26
R	15	34.03	28.12	23.16	31.15	50.36
	30	43.21	47.16	35.12	47.26	68.12
T_A	15	8.12	9.41	14.14	9.05	10.41
	30	14.24	17.20	27.62	17.14	20.14
T_B	15	18.16	17.10	36.12	17.14	29.12
	30	27.12	30.45	52.12	25.14	36.19

Data are expressed as the mean of 6 observations.

Weight Change Analysis: On Day 30, the hyperlipidemic group showed a significant increase in body weight ($p < 0.01$), whereas the Rosuvastatin group and Test Group B exhibited a significant decrease in weight ($p < 0.001$). However, no significant change in weight was observed in Test Group A ($p > 0.05$) (refer to Table 03).

Table 03: Atherogenic Index (AI) in Different Groups on Day 30

Groups	Atherogenic Index	Protection (%)
N	4.36	-
H	9.20	-
R	5.12	61.12
T_A	6.14	37.50
T_B	5.14	53.19

Data presented as the average of six observations (mean).

Table 4: Weight Gain in Different Groups on Day 30 Compared to Day 0.

Groups	Weight Gain (gm)
N	7.12 ± 1.62
H	18.10 ± 2.20 *
R	9.1 ± 3.40 #
T_A	12.1 ± 1.26 @
T_B	10.02 ± 1.51 $

Data are presented as the mean ± standard deviation (SD) of six observations. The results of the Student's unpaired t-test indicate a significant difference compared to the normal control group (N) with $p < 0.01$, marked by an asterisk (*). A significant difference compared to the hyperlipidemic control group (H) is indicated with a hash (#), also at $p < 0.01$. No significant difference compared to the hyperlipidemic control group is marked with an at symbol (@), where $p > 0.05$. Additionally, a dollar sign ($) indicates a significant difference compared to the hyperlipidemic group at $p < 0.05$.

Discussion

The role of diet in causing hyperlipidemia and atherosclerosis is well established, with high-fat, high-cholesterol diets increasing LDL-C and decreasing HDL-C, promoting an atherogenic lipid profile [14]. Cholesterol and sodium cholate disrupt cholesterol excretion, contributing to hypercholesterolemia in animal models. Both Rosuvastatin and Hibiscus showed significant cholesterol-lowering and HDL-C enhancing effects, though Hibiscus had a slower impact on lipid changes compared to Rosuvastatin. Rosuvastatin lowers cholesterol by inhibiting HMG-CoA reductase, while Hibiscus's mechanism is less clear but may involve increasing HDL-C and promoting reverse cholesterol transport.

Phytochemicals in Hibiscus, including flavonoids, saponins, tannins, and plant sterols, likely contribute to its lipid-lowering and antioxidant effects by enhancing cholesterol excretion and lipoprotein metabolism. Both treatments improved protective HDL-C and reduced harmful LDL-C and VLDL-C levels, with Hibiscus showing a

more pronounced HDL-C increase, which is particularly beneficial for populations with low HDL-C.

Hibiscus also significantly reduced triglycerides and the atherogenic index without adverse effects, indicating its potential as a safe, effective hypolipidemic agent [15]. While statins remain the clinical standard due to their potent effects and pleiotropic benefits, Hibiscus holds promise for treating hyperlipidemia and cardiovascular risk and warrants further clinical investigation. Traditionally, it has also been valued as a cardioprotective agent.

Conclusion

The results of this study indicate that Rosuvastatin and Hibiscus extract may have synergistically contributed to reductions in weight and cholesterol levels, particularly with respect to improvements in blood lipid profiles and the rate of weight gain. Recent research highlights Hibiscus's anti-atherothrombotic and cardioprotective properties, which are largely attributed to its significant hypolipidemic effects. These therapeutic benefits position Hibiscus as a promising candidate for the management of cardiovascular disorders, especially within the Indian population. However, to substantiate these findings, further detailed investigations are warranted. Additionally, comprehensive case-control studies are essential to establish the therapeutic efficacy and safety of Hibiscus extract in humans.

Acknowledgement

The authors are thankful to the management of for their support in carrying out this work.

Conflicts of interest

The authors declare no conflicts of interest.

Finding

Nil

Ethical Approval

Not Applicable

Inform Consent

Not Applicable

Author Contribution

Both Authors contributed equally

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