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EVALUATION OF THE ANTIHYPERLIPIDEMIC POTENTIAL OF JUSTICIA BETONICA (L.) AND VIGNA TRILOBATA (L.) VERDC. IN EXPERIMENTAL RAT MODELS OF HYPERLIPIDEMIA

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ABSTRACT

Background: Hyperlipidemia is a major metabolic disorder that significantly contributes to the development of cardiovascular diseases through abnormal lipid metabolism and oxidative stress. Although synthetic lipid-lowering agents are clinically effective, their long-term use is often associated with adverse effects, creating a need for safer plant-derived alternatives.

Objective: The present study aimed to evaluate the antihyperlipidemic potential of the ethanolic extract of Justicia betonica (L.) and the aqueous extract of Vigna trilobata (L.) Verdc. using acute and chronic experimental models of hyperlipidemia.

Methods: The extracts were prepared by Soxhlet extraction and subjected to preliminary phytochemical screening, chromatographic isolation, HPLC, FT-IR, and LC-HRMS characterization. Acute oral toxicity was evaluated according to OECD Guideline 423. Antihyperlipidemic activity was assessed using Triton WR-1339-induced acute and high cholesterol diet-induced chronic hyperlipidemic rat models. Serum lipid profile parameters, including total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and very low-density lipoprotein cholesterol (VLDL-C), were estimated using commercial diagnostic kits.

Results: Both extracts were found to be safe at the tested dose levels with no mortality or treatment-related toxicity. Phytochemical investigation confirmed the presence of flavonoids, phenolics, tannins, alkaloids, terpenoids, and other bioactive constituents. In both experimental models, treatment with the extracts significantly reduced serum TC, TG, LDL-C, and VLDL-C levels while increasing HDL-C in a dose-dependent manner. The 500 mg/kg dose of Justicia betonica produced the greatest lipid-lowering effect, followed by Vigna trilobata, with results approaching those of the standard drug, simvastatin.

Conclusion: The findings demonstrate that Justicia betonica and Vigna trilobata possess significant antihyperlipidemic activity, which may be attributed to their rich phytochemical composition and antioxidant properties. These results support their potential as promising natural therapeutic agents for the management of hyperlipidemia and related cardiovascular disorders.

KEYWORDS: Justicia betonica; Vigna trilobata; Hyperlipidemia; Triton WR-1339; High cholesterol diet; Antihyperlipidemic activity; Phytochemicals; Experimental rats; Lipid profile; Simvastatin.

INTRODUCTION

Hyperlipidemia is a common metabolic disorder characterized by abnormal elevations in circulating lipids, including total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and very low-density lipoprotein cholesterol (VLDL-C), together with a reduction in high-density lipoprotein cholesterol (HDL-C). Persistent disturbances in lipid metabolism contribute significantly to the development of atherosclerosis and are strongly associated with cardiovascular diseases such as coronary artery disease, myocardial infarction, ischemic stroke, and peripheral vascular disorders. As the global prevalence of obesity, diabetes mellitus, and sedentary lifestyles continues to increase, hyperlipidemia has emerged as a major public health concern requiring effective long-term therapeutic management.[1] Current pharmacological interventions, including statins, fibrates, cholesterol absorption inhibitors, and PCSK9 inhibitors, have substantially improved the management of dyslipidemia. Nevertheless, prolonged therapy is often associated with adverse reactions such as hepatic dysfunction, muscle toxicity, gastrointestinal disturbances, and poor patient compliance. These limitations have encouraged researchers to explore medicinal plants as safer and cost-effective alternatives. Herbal medicines contain diverse phytochemicals that act through multiple biological pathways and have attracted considerable interest because of their therapeutic efficacy, lower incidence of adverse effects, and long history of traditional use.[2]

Natural products rich in flavonoids, phenolic acids, tannins, terpenoids, alkaloids, saponins, and phytosterols have demonstrated beneficial effects on lipid metabolism. These bioactive constituents exhibit antioxidant properties, inhibit lipid peroxidation, improve endogenous antioxidant defense mechanisms, regulate cholesterol biosynthesis, enhance reverse cholesterol transport, and reduce oxidative damage associated with hyperlipidemia. Consequently, medicinal plants possessing these phytochemicals are considered promising candidates for the development of novel antihyperlipidemic agents.[3] Justicia betonica (L.), belonging to the family Acanthaceae, is a medicinal plant traditionally employed in folk medicine for the treatment of inflammatory conditions, microbial infections, skin disorders, and several chronic ailments. Phytochemical investigations have revealed that the plant contains abundant flavonoids, phenolic compounds, tannins, terpenoids, alkaloids, glycosides, and steroids, which are known to possess antioxidant and pharmacological activities. The antioxidant potential of these constituents suggests that the plant may protect against oxidative stress-induced lipid abnormalities and cardiovascular complications. Vigna trilobata (L.) Verdc., a member of the family Fabaceae, is an underutilized medicinal legume with considerable nutritional and therapeutic value. Previous studies have reported the occurrence of flavonoids, isoflavonoids, phenolic compounds, sterols, tannins, and other secondary metabolites exhibiting antioxidant, anti-inflammatory, antimicrobial, and metabolic regulatory activities. Despite these promising phytochemical characteristics, comprehensive pharmacological investigations evaluating its lipid-lowering efficacy remain limited, indicating the need for systematic experimental studies.[4] Experimental animal models are widely employed to investigate the efficacy of candidate antihyperlipidemic agents before clinical evaluation. Among these, the Triton WR-1339-induced hyperlipidemic model provides a rapid method for assessing acute lipid-lowering activity by producing transient hyperlipidemia through inhibition of plasma lipoprotein clearance. Conversely, the high cholesterol diet-induced hyperlipidemic model closely resembles chronic dyslipidemia observed in humans and enables the evaluation of long-term therapeutic effects on lipid metabolism. The combined use of these experimental models provides a comprehensive assessment of the antihyperlipidemic potential of medicinal plant extracts.[5] In our previous phytochemical investigation, the ethanolic extract of Justicia betonica and the aqueous extract of Vigna trilobata were found to contain abundant phenolics, flavonoids, tannins, and other biologically active constituents. Chromatographic and spectroscopic analyses, including TLC, HPLC, FT-IR, and LC-HRMS, confirmed the chemical diversity of both extracts and suggested their potential as natural antioxidant sources. These findings provided a strong scientific basis for investigating their pharmacological activity against experimentally induced hyperlipidemia. Therefore, the present study was designed to evaluate the antihyperlipidemic activity of the ethanolic extract of Justicia betonica (L.) and the aqueous extract of Vigna trilobata (L.) Verdc. using Triton WR-1339-induced acute and high cholesterol diet-induced chronic hyperlipidemic rat models. The lipid-lowering efficacy of both extracts at different dose levels was compared with simvastatin as the reference standard by assessing serum lipid profile parameters, including TC, TG, HDL-C, LDL-C, and VLDL-C. The findings of this study are expected to provide experimental evidence supporting the therapeutic potential of these medicinal plants and contribute to the development of safe, plant-derived interventions for the management of hyperlipidemia and associated cardiovascular disorders.

MATERIALS AND METHODS

MATERIALS:

The ethanolic extract of Justicia betonica (L.) and the aqueous extract of Vigna trilobata (L.) Verdc. prepared by Soxhlet extraction were used for the pharmacological investigation. Triton WR-1339 (Tyloxapol) was procured from Sigma-Aldrich, St. Louis, MO, USA. Simvastatin was received as a gift sample from Sun Pharmaceutical Industries Ltd., India. Cholesterol and Carboxymethyl Cellulose (CMC) were purchased from Loba Chemie Pvt. Ltd., Mumbai, India. Commercial diagnostic kits for the estimation of total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were procured from Transasia Bio-Medicals Ltd., Mumbai, India, and the biochemical estimations were carried out using a semi-automatic biochemical analyzer (Erba Chem-5 Plus V2, Erba Mannheim, Germany). All chemicals, solvents, and reagents used in the study were of analytical reagent (AR) grade.

Collection and Authentication of Plant Material

Fresh leaves of Justicia betonica (L.) and the whole plant of Vigna trilobata (L.) Verdc. were collected from the local region of Chhatrapati Sambhajinagar, Maharashtra, India. The plant materials were authenticated by Dr. Arvind S. Dhabe, Senior Professor and Head, Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Chhatrapati Sambhajinagar, Maharashtra, India. The authenticated specimens were used for the present investigation.

Preparation of Plant Extracts

The collected plant materials were washed, shade-dried, pulverized, and extracted separately using the Soxhlet extraction method. The ethanolic extract of Justicia betonica and the aqueous extract of Vigna trilobata were concentrated under reduced pressure using a rotary vacuum evaporator and stored at 4°C in airtight containers until further pharmacological evaluation.[6]

Preliminary Phytochemical Screening

The prepared extracts were subjected to preliminary qualitative phytochemical screening using standard pharmacognostic methods to identify major classes of secondary metabolites, including alkaloids, flavonoids, phenolics, tannins, glycosides, saponins, steroids, terpenoids, proteins, carbohydrates, and fixed oils.[7-8]

Phytochemical Isolation and Characterization

The ethanolic extract of Justicia betonica (L.) and the aqueous extract of Vigna trilobata (L.) Verdc. were subjected to phytochemical isolation by silica gel column chromatography, and the fractions were monitored by thin-layer chromatography (TLC). The phytochemical profiles were further characterized using High-Performance Liquid Chromatography (HPLC), Fourier Transform Infrared (FT-IR) spectroscopy, and Liquid Chromatography–High Resolution Mass Spectrometry (LC–HRMS) to establish the chromatographic fingerprints and identify the major bioactive constituents present in both extracts.[9]

IN VIVO PHARMACOLOGICAL EVALUATIONS:

Procurement of Experimental Animals

Healthy adult male Wistar albino rats weighing 180–220 g were procured from Deshpande Laboratories Pvt. Ltd., Bhosari, Pune, Maharashtra, India, a CPCSEA-registered animal breeding facility (Registration No. 1410/C/11/CPCSEA). The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India (Approval No.: DL/IAEC/02/2026/CPCSEA). The animals were housed under standard laboratory conditions (temperature 22 ± 2°C, relative humidity 55 ± 10%, and 12 h light/12 h dark cycle) with free access to standard pellet diet and water ad libitum. The animals were acclimatized for 7 days before the commencement of the experiment.[10-11]

Acute toxicity study

The acute oral toxicity study of the ethanolic extract of Justicia betonica (L.) and the aqueous extract of Vigna trilobata (L.) Verdc. was performed according to OECD Guideline 423 (Acute Toxic Class Method). Three healthy female experimental animals were administered the extracts orally in a stepwise manner at predetermined dose levels. Following treatment, the animals were closely observed for clinical signs of toxicity, behavioural changes, body weight, food and water intake, and mortality during the first 24 h and subsequently for 14 days. The absence of mortality and significant toxic effects was used to estimate the safety of the extracts and to select appropriate doses for the subsequent antihyperlipidemic study.[11-13]

Evaluation of Antihyperlipidemic Activity

Triton WR-1339-Induced Hyperlipidemic Model (Acute Study)

The acute antihyperlipidemic activity of the ethanolic extract of Justicia betonica (L.) and the aqueous extract of Vigna trilobata (L.) Verdc. was evaluated using the Triton WR-1339-induced hyperlipidemic rat model. Healthy adult male Wistar albino rats weighing 180–220 g were acclimatized to laboratory conditions for 7 days before the experiment. The animals were randomly divided into nine groups (n = 6). The test extracts and standard drug were administered orally once daily for 6 consecutive days. On Day 7, one hour after the final treatment, hyperlipidemia was induced in all groups except the normal control by a single intraperitoneal injection of Triton WR-1339. Blood samples were collected from the retro-orbital plexus at 6 h and 24 h after Triton administration and centrifuged at 2500 rpm for 10 min to separate serum for the estimation of serum lipid profile, including total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and very low-density lipoprotein cholesterol (VLDL-C).[13-21]

Table 1: Experimental Design for Triton WR-1339-Induced Acute Hyperlipidemic Model

Group	Experimental Treatment	Dose	Route
G1	Normal Control (1% CMC)	10 mL/kg	p.o.
G2	Hyperlipidemic Control (Triton WR-1339 + 1% CMC)	Triton WR-1339 + Vehicle	i.p. + p.o.
G3	Standard (Simvastatin + Triton WR-1339)	10 mg/kg	p.o.
G4	Justicia betonica Extract + Triton WR-1339	125 mg/kg	p.o.
G5	Justicia betonica Extract + Triton WR-1339	250 mg/kg	p.o.
G6	Justicia betonica Extract + Triton WR-1339	500 mg/kg	p.o.
G7	Vigna trilobata Extract + Triton WR-1339	125 mg/kg	p.o.
G8	Vigna trilobata Extract + Triton WR-1339	250 mg/kg	p.o.
G9	Vigna trilobata Extract + Triton WR-1339	500 mg/kg	p.o.

Abbreviations: CMC, Carboxymethyl cellulose; p.o., per oral; i.p., intraperitoneal; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; VLDL-C, very low-density lipoprotein cholesterol.

High Cholesterol Diet-Induced Hyperlipidemic Model (Chronic Study)

The chronic antihyperlipidemic activity of the ethanolic extract of Justicia betonica (L.) and the aqueous extract of Vigna trilobata (L.) Verdc. was evaluated using a high cholesterol diet (HCD)-induced hyperlipidemic rat model. Healthy male Wistar albino rats (180–220 g) were acclimatized for 7 days and randomly divided into nine groups (n = 6). Except for the normal control group (G1), all animals were fed a high cholesterol diet continuously for 30 days to induce hyperlipidemia. The normal control group received a standard pellet diet throughout the study.

The treatment groups received the respective plant extracts or standard drug orally once daily from the 16th to the 30th day of the experimental period. Simvastatin (10 mg/kg, p.o.) was used as the reference standard. At the end of the study,

overnight-fasted animals were anesthetized, and blood samples were collected through the retro-orbital plexus. The blood was centrifuged at 2500 rpm for 10 min to obtain serum for the estimation of biochemical parameters including total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and very low-density lipoprotein cholesterol (VLDL-C). After blood collection, the animals were sacrificed, and liver tissues were excised for histopathological examination.[22-26]

Table 2: Experimental design for high cholesterol diet-induced chronic hyperlipidemic model

Group	Experimental Treatment	Dose	Duration
G1	Normal Control (Standard pellet diet + 1% CMC)	10 mL/kg	30 days
G2	Hyperlipidemic Control (HCD + 1% CMC)	Vehicle	30 days
G3	Standard (HCD + Simvastatin)	10 mg/kg, p.o.	Days 16–30
G4	HCD + Justicia betonica Extract	125 mg/kg, p.o.	Days 16–30
G5	HCD + Justicia betonica Extract	250 mg/kg, p.o.	Days 16–30
G6	HCD + Justicia betonica Extract	500 mg/kg, p.o.	Days 16–30
G7	HCD + Vigna trilobata Extract	125 mg/kg, p.o.	Days 16–30
G8	HCD + Vigna trilobata Extract	250 mg/kg, p.o.	Days 16–30
G9	HCD + Vigna trilobata Extract	500 mg/kg, p.o.	Days 16–30

Abbreviations: **HCD, High Cholesterol Diet; CMC, Carboxymethyl Cellulose; p.o., per oral; TC, Total Cholesterol; TG, Triglycerides; HDL-C, High-Density Lipoprotein Cholesterol; LDL-C, Low-Density Lipoprotein Cholesterol; VLDL-C, Very Low-Density Lipoprotein Cholesterol.**

Biochemical Analysis

At the end of the experimental period, blood samples were collected from the retro-orbital plexus under light anesthesia and centrifuged at 2500 rpm for 10 min to obtain serum. The serum samples were analyzed for lipid profile parameters, including total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and very low-density lipoprotein cholesterol (VLDL-C), using commercially available diagnostic kits and a semi-automatic biochemical analyzer according to the manufacturer's instructions.[27-34]

Blood Collection Schedule

Blood samples were collected at predetermined intervals to evaluate changes in the serum lipid profile during the study. To minimize animal stress and comply with CPCSEA/IAEC guidelines for safe blood collection, each rat was sampled at a maximum of three time points throughout the experimental period. Blood samples were collected on Day 0 (baseline), Day 15, and Day 30, and the obtained serum was used for biochemical estimations.[35-40]

Table 3: Blood Collection Schedule

Sampling Time	Study Day	Parameters Evaluated
Baseline	Day 0	Initial lipid profile (TC, TG, HDL-C, LDL-C, VLDL-C)
Mid-study	Day 15	Serum lipid profile
Final	Day 30	Serum lipid profile and biochemical analysis

Abbreviations: TC, Total Cholesterol; TG, Triglycerides; HDL-C, High-Density Lipoprotein Cholesterol; LDL-C, Low-Density Lipoprotein Cholesterol; VLDL-C, Very Low-Density Lipoprotein Cholesterol.

Statistical Analysis

All experimental data were expressed as mean ± standard error of the mean (SEM) for six animals per group (n = 6). Statistical analysis was performed using GraphPad Prism software (Version 9.0, GraphPad Software Inc., San Diego, CA, USA). Differences among the experimental groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test for post hoc analysis. A value of $p < 0.05$ was considered statistically significant.[41-43]

RESULTS AND DISCUSSIONS:

Preliminary Phytochemical Investigation

The preliminary phytochemical screening of the ethanolic extract of Justicia betonica (L.) and the aqueous extract of Vigna trilobata (L.) Verdc. revealed that the extracts contain alkaloids, flavonoids, phenolic compounds, tannins, glycosides, saponins, steroids, terpenoids, proteins, amino acids, and fixed oils. The ethanolic extract of Justicia betonica was found to contain more phenolics, flavonoids, tannins, and triterpenoids than the aqueous extract of Vigna trilobata. The phytochemical screening results confirmed the richness of the extracts in phytoconstituents and their potential for the antihyperlipidemic study.

Phytochemical Isolation and Characterization

The ethanolic extract of Justicia betonica (L.) and the aqueous extract of Vigna trilobata (L.) Verdc. were analysed using thin-layer chromatography (TLC), Fourier-transform infrared (FT-IR) spectroscopy, high-performance liquid chromatography (HPLC), and liquid chromatography-high-resolution mass spectrometry (LC–HRMS) to identify the phytoconstituents present in the extracts. The TLC analysis showed good separation of spots with different Rf values, while FT-IR spectroscopy confirmed the presence of functional groups characteristic of phenolics, flavonoids, carbonyl,

and aromatic compounds in the extracts. The HPLC fingerprints of the extracts showed distinct peaks at 3.67 and 2.76 min for Justicia betonica and Vigna trilobata, respectively, which indicated the presence of phytoconstituents in the extracts. In addition, the LC–HRMS analysis yielded characteristic molecular ion peaks for the two extracts, indicating the presence of low- and high-molecular-weight compounds. Overall, the phytochemical isolation and characterization results demonstrated that the two extracts were rich in phytoconstituents and hence were suitable for the antihyperlipidemic study.

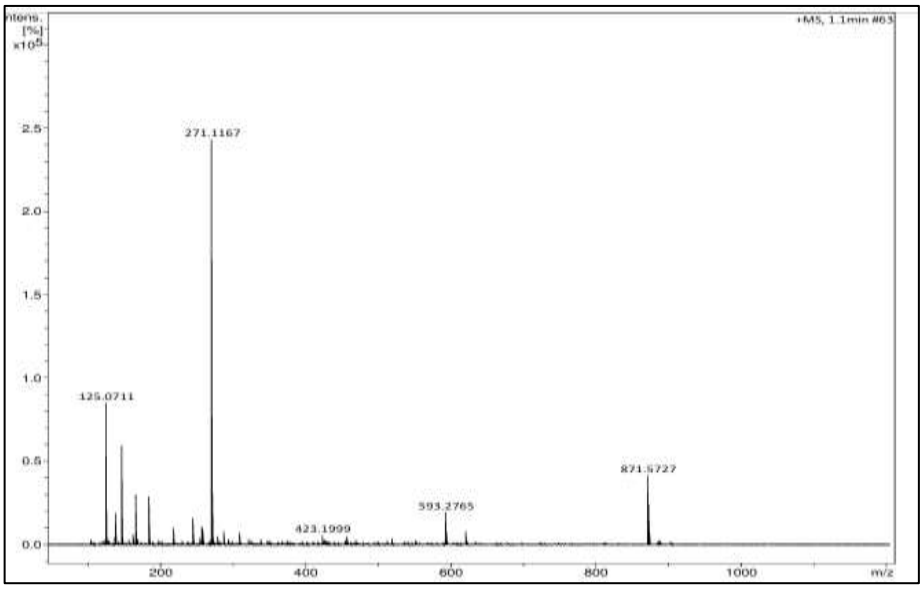

Figure 1: LC–HRMS positive ion mass spectrum of the ethanolic extract of Justicia betonica (L.) showing the characteristic molecular ion peaks.

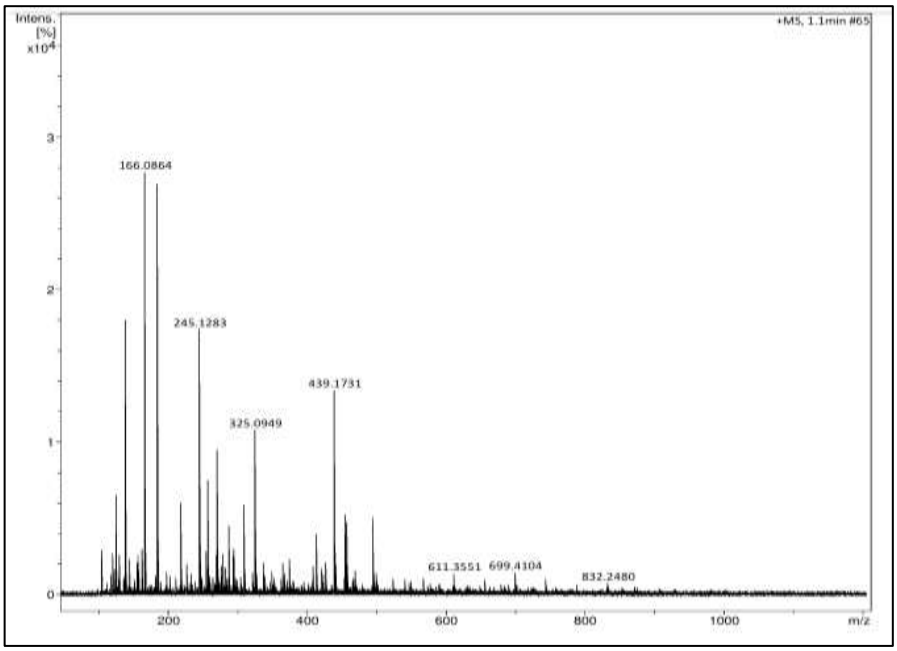

Figure 2: LC–HRMS positive ion mass spectrum of the aqueous extract of Vigna trilobata (L.) Verdc. showing the characteristic molecular ion peaks.

IN VIVO PHARMACOLOGICAL EVALUATIONS:

Acute Toxicity Study

The acute oral toxicity of the ethanolic extract of Justicia betonica (L.) and the aqueous extract of Vigna trilobata (L.) Verdc. was investigated according to OECD Guideline 423 (Acute Toxic Class Method). Three healthy experimental animals were used to establish a stepwise oral dose of the test extract. The animals were observed for clinical signs of toxicity, behavioural changes, body weight, food and water intake, and mortality during the first 24 h and for 14 days after the treatment. The absence of mortality and toxic effects was used to estimate the safety of the extracts and to select doses for the antihyperlipidemic study.

Table 4: Acute oral toxicity study

Parameter	Justicia betonica	Vigna trilobata
Mortality	Nil	Nil
Clinical signs of toxicity	Absent	Absent
Behavioural changes	Absent	Absent
Body weight changes	Normal	Normal
Food and water intake	Normal	Normal
Observation period	14 days	14 days

The acute oral toxicity study according to OECD Guideline 423 showed that the ethanolic extract of Justicia betonica and the aqueous extract of Vigna trilobata did not induce mortality and toxic effects during the 14-day observation period. In addition, no significant changes in the animals' behaviour, body weight, food and water intake, and general health status were observed after the treatment, which indicated the safety of the extracts (Table 4). Therefore, the doses of 125, 250, and 500 mg/kg were selected for the antihyperlipidemic study.

Evaluation of Antihyperlipidemic Activity

Triton WR-1339-Induced Hyperlipidemic Model (Acute Study)

The acute antihyperlipidemic activity of the ethanolic extract of Justicia betonica (L.) and the aqueous extract of Vigna trilobata (L.) Verdc. was evaluated using the Triton WR-1339-induced hyperlipidemic rat model. Healthy adult male Wistar albino rats (180–220 g) were acclimatized for 7 days before the commencement of the experiment. The animals were randomly divided into nine groups (n = 6). The test extracts and standard drug were administered to the animals orally once a day for 6 consecutive days. On the 7th day, hyperlipidemia was induced in all groups except the normal control by a single intraperitoneal injection of Triton WR-1339. Blood samples were collected from the retro-orbital plexus 6 h and 24 h after the induction of hyperlipidemia and centrifuged at 2500 rpm for 10 min to obtain the serum. Table 5 estimated the total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and very low-density lipoprotein cholesterol (VLDL-C) levels in the serum samples after completion of study. However, Figure 3 represented the results of antihyperlipidemic activity of developed extract in experimental animals.

Table 5: Effect of Ethanolic Extract of Justicia betonica and Aqueous Extract of Vigna trilobata on Serum Lipid Profile in Triton WR-1339-Induced Hyperlipidemic Rats (24 h post-induction).

Group	TC (mg/dL)	TG (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	VLDL-C (mg/dL)
G1	94.2 ± 3.8	88.5 ± 4.6	39.4 ± 2.1	37.8 ± 2.9	17.7 ± 0.9
G2	255.6 ± 9.2	228.4 ± 10.1	21.8 ± 1.5	185.3 ± 8.2	45.7 ± 2.0
G3	122.8 ± 5.9	110.3 ± 6.4	36.1 ± 2.0	65.4 ± 4.3	22.1 ± 1.3
G4	208.7 ± 7.5	185.2 ± 8.3	25.9 ± 1.7	148.2 ± 6.8	37.0 ± 1.7
G5	165.4 ± 6.8	142.6 ± 7.1	31.2 ± 1.9	107.8 ± 5.6	28.5 ± 1.4
G6	128.9 ± 5.4	115.7 ± 6.2	35.3 ± 2.2	72.6 ± 4.5	23.1 ± 1.2
G7	215.3 ± 8.1	192.8 ± 8.9	25.1 ± 1.6	153.7 ± 7.1	38.6 ± 1.8
G8	175.6 ± 7.2	150.4 ± 7.8	30.4 ± 1.8	117.2 ± 6.0	30.1 ± 1.6
G9	140.2 ± 6.1	122.5 ± 6.5	33.8 ± 2.0	85.1 ± 4.9	24.5 ± 1.3

Values are Mean ± SEM (n=6). ***p < 0.001 vs G1; *p < 0.05, **p < 0.01 vs G2.

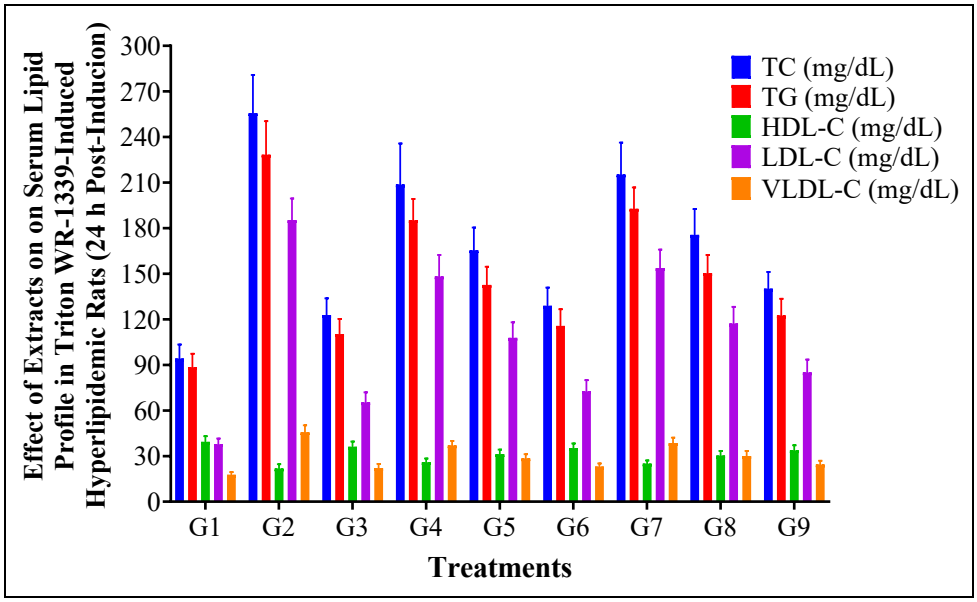

Figure 3: Effect of Ethanolic Extract of Justicia betonica and Aqueous Extract of Vigna trilobata on Serum Lipid Profile in Triton WR-1339-Induced Hyperlipidemic Rats (24 h post-induction).

High Cholesterol Diet-Induced Hyperlipidemic Model (Chronic Study)

The chronic antihyperlipidemic activity of the ethanolic extract of Justicia betonica (L.) and the aqueous extract of Vigna trilobata (L.) Verdc. was evaluated using the high cholesterol diet (HCD)-induced hyperlipidemic rat model. Healthy male Wistar albino rats (180–220 g) were acclimatized for 7 days and randomly divided into nine groups (n = 6). Except for the normal control group (G1), all other animals received a high cholesterol diet for 30 consecutive days to induce hyperlipidemia. The normal control group received the standard pellet diet throughout the experimental period. The test extracts and standard drug were administered to the respective groups orally once a day from day 16 to day 30 of the experiment. Simvastatin (10 mg/kg, p.o.) was used as the reference standard. At the end of the experimental period, the animals were sacrificed under overnight fasting, and blood samples were collected from the retro-orbital plexus. The blood samples were centrifuged at 2500 rpm for 10 min to obtain the serum, which was used to estimate the TC, TG, HDL-C, LDL-C, and VLDL-C levels (Table 6). The results for HCD-induced hyperlipidemic model as represented under Figure 4. Following the sacrifice of the animals, the liver tissues were collected for histopathological examination.

Table 6: Effect of Ethanolic Extract of Justicia betonica and Aqueous Extract of Vigna trilobata on on Serum Lipid Profile in High Cholesterol Diet -Induced Hyperlipidemic Rats (After 30 Days).

Group	TC (mg/dL)	TG (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	VLDL-C (mg/dL)
G1	96.5 ± 4.1	90.2 ± 4.8	40.2 ± 2.3	38.4 ± 3.0	18.0 ± 1.0
G2	268.4 ± 10.3	235.7 ± 11.2	20.5 ± 1.4	195.6 ± 9.1	47.1 ± 2.2
G3	125.7 ± 6.2	115.8 ± 6.8	37.4 ± 2.2	68.2 ± 4.7	23.2 ± 1.4
G4	215.3 ± 8.4	192.6 ± 9.5	26.3 ± 1.8	155.4 ± 7.3	38.5 ± 1.9
G5	172.8 ± 7.1	148.9 ± 7.9	32.1 ± 2.0	112.5 ± 6.1	29.8 ± 1.6
G6	132.6 ± 5.8	118.4 ± 6.5	36.5 ± 2.1	75.3 ± 4.8	23.7 ± 1.3
G7	222.5 ± 8.7	198.7 ± 9.8	25.7 ± 1.7	160.8 ± 7.6	39.7 ± 2.0
G8	180.4 ± 7.5	155.2 ± 8.2	31.5 ± 1.9	121.6 ± 6.4	31.0 ± 1.6
G9	145.9 ± 6.3	126.8 ± 7.0	34.8 ± 2.0	88.7 ± 5.2	25.4 ± 1.4

Values are Mean ± SEM (n=6). ***p < 0.001 vs G1; *p < 0.05, **p < 0.01 vs G2.

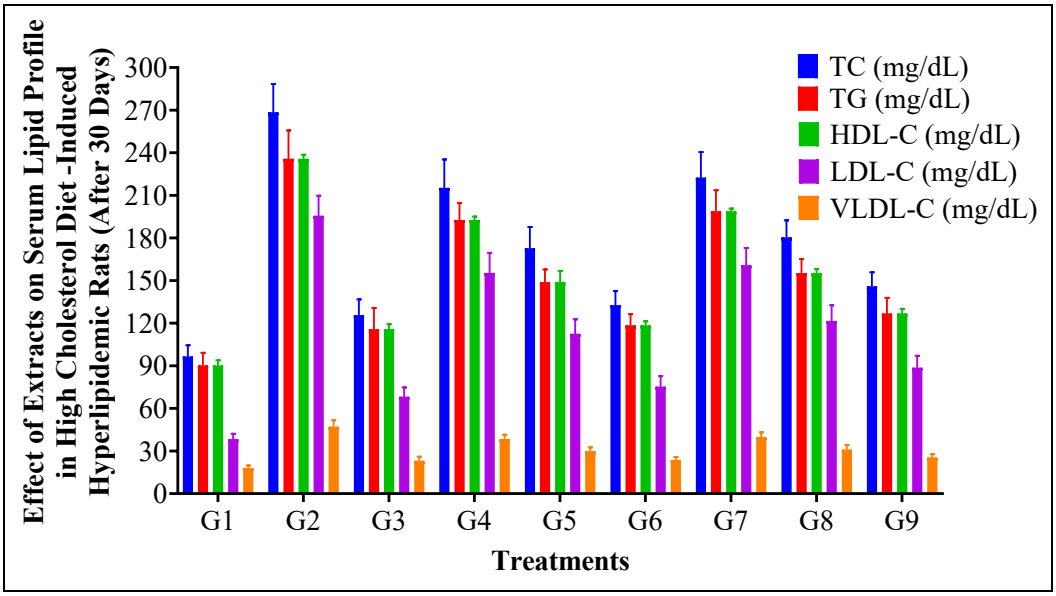

Figure 4: Effect of Ethanolic Extract of Justicia betonica and Aqueous Extract of Vigna trilobata on Serum Lipid Profile in Triton WR-1339-Induced Hyperlipidemic Rats (After 30 Days).

Biochemical Analysis and Blood Collection Schedule

The blood samples were collected from the retro-orbital plexus of the animals under light anesthesia and centrifuged at 2500 rpm for 10 min to obtain the serum. The serum samples were analysed for TC, TG, HDL-C, LDL-C, and VLDL-C levels using commercial diagnostic kits. The blood samples were collected on day 0 (baseline), day 15, and day 30 of the experiment according to the schedule depicted in Table 3 in the materials sections. The results were expressed as mean ± standard error of the mean (SEM) for six animals per group (n = 6). The data were analysed using GraphPad Prism software (Version 9.0). The differences among the experimental groups were evaluated using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. A p-value of less than 0.05 was considered statistically significant. The results of the antihyperlipidemic study showed that both extracts produced a significant dose-dependent reduction in TC, TG, LDL-C, and VLDL-C levels and an increase in HDL-C levels in the acute and chronic hyperlipidemic models. In addition, the histopathological examination of the liver tissues of the treated groups showed reduced lipid deposits compared to the hyperlipidemic control group.

CONCLUSION

The present investigation demonstrated that the ethanolic extract of Justicia betonica (L.) and the aqueous extract of Vigna trilobata (L.) Verdc. possess significant antihyperlipidemic activity in both acute and chronic experimental models of hyperlipidemia. Acute oral toxicity evaluation according to OECD Guideline 423 confirmed that both extracts were safe and well tolerated, with no evidence of mortality or treatment-related toxicity. Phytochemical screening and chromatographic characterization revealed the presence of flavonoids, phenolic compounds, tannins, terpenoids, alkaloids, and other bioactive constituents that may contribute to their pharmacological effects. Administration of both extracts produced a dose-dependent improvement in serum lipid parameters by significantly reducing total cholesterol, triglycerides, LDL-C, and VLDL-C while increasing HDL-C compared with the hyperlipidemic control group. Among the tested doses, the 500 mg/kg extract of Justicia betonica exhibited the highest antihyperlipidemic activity, followed by Vigna trilobata, with efficacy comparable to the standard drug simvastatin. The observed lipid-lowering effects are likely associated with the synergistic action of the phytoconstituents identified during phytochemical characterization. Overall, the study provides experimental evidence supporting the traditional medicinal use of Justicia betonica and Vigna trilobata for the management of hyperlipidemia. These findings indicate that both plants represent promising natural sources of antihyperlipidemic agents and warrant further investigations to isolate the active compounds, elucidate their molecular mechanisms of action, and evaluate their long-term safety and clinical efficacy.

FUNDING

None.

CONCFILCT OF INTREST

The authors declare that there is no conflict of interest.

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