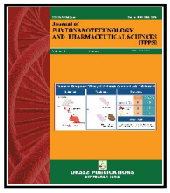
Journal of Phytonanotechnology and Pharmaceutical Sciences 5(4): 62-71, 2025

Online ISSN:2583-0376

http://jpps.ukaazpublications.com

DOI: http://dx.doi.org/10.54085/jpps.2025.5.4.8

Journal of Phytonanotechnology and Pharmaceutical Sciences



Original Article : Open Access

In vivo and *in vitro* evaluation of antiatherosclerotic and antioxidative activity of ethanolic extracts of *Luffa echinata* Roxb. and *Tribulus terrestris* L.

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Article Info

Article history

Received 19 October 2025

Revised 21 November 2025

Accepted 22 November 2025

Published Online 30 December 2025

Keywords

Atherosclerosis

Oxidative stress

Luffa echinata Roxb

Tribulus terrestris L.

Triton X-100

Abstract

Atherosclerosis is one of the most important vascular diseases pertaining to the arteries, that occurs due to the deposition of fats, lipids and various inflammatory materials in the inner lining of the blood vessels which restricts the flow of blood from the heart to different organs by forming atheromatous plaques. The aim of this study is to determine the antiatherosclerotic and antioxidative activity of ethanolic leaf extracts of *Luffa echinata* Roxb. and *Tribulus terrestris* L. To investigate the activity of the extracts, *in vitro* and *in vivo* methods are being utilized. Under the *in vitro* methods, HMG CoA reductase inhibition assay and lipoprotein lipase activation assay are carried out, whereas for *in vivo* methods, HFD induced hyperlipidemia and Triton X-100 induced hyperlipidemia models are being conducted. About 54 adult Wistar rats were selected and divided into 9 groups, where each group was subjected to different treatment as per the predetermined experimental design. Two doses of the extracts, *i.e.*, 200 mg/kg and 400 mg/kg were selected which were given individually as well as in combination. Serum lipid profile (LDL, VLDL, HDL, TG, TC) and oxidative stress markers (CAT, MDA, SOD) were the parameters evaluated along with the histopathological examination of the heart and liver slides post the experiment and it was observed and concluded that the *T. terrestris* possesses preferential lipid lowering activity along with antioxidative effect in comparison to *L. echinata*.

1. Introduction

Atherosclerosis is defined as a progressive cardiovascular condition that occurs due to the accumulation of cholesterol and fats at the walls of the blood vessels which forms plaque that later confines and restricts blood flow. It is considered as a commonest risk factor for development of various CVDs. The initial step involved in the pathogenesis of atherosclerosis is the destruction of the endometrial lining of the vessels where the lipids get piled up and undergoes oxidation leading to migration of macrophages and development of foam cells (Mota *et al*., 2017). Oxidative stress is regarded as a common etiological factor for development of atherosclerosis. It usually occurs when there is an imbalance between the antioxidants and reactive oxygen species (ROS), as a result, the increased levels of free radical's attacks and destroys the important biomolecules of the body like proteins, lipids, carbohydrates, amino acids, *etc.* This increases lipid oxidation in the blood vessels, thus accelerating plaque formation (Batty *et al*., 2022; Thank *et al.,* 2025; Rani *et al.,* 2025).

L. echinata, belonging to the family Cucurbitaceae, is a medicinal plant commonly known as Chikni torai, loofah, sponge gourd, or bitter luffa. It falls under the phylum Tracheophyta and the genus *Luffa,* species *echinata* Roxb., with synonyms including *Momordica echinata* and *Luffa amara*. The leaves of *L. echinata* are traditionally valued for their therapeutic importance and have attracted scientific interest due to their potential antioxidant and cardioprotective properties, justifying their selection for evaluating antiatherosclerotic activity (Mondal *et al.,* 2023) (Figure 1).

T. terrestris, a member of the family Zygophyllaceae, is widely recognized for its medicinal relevance and is commonly known as Gokshura, cowhage, puncture vine, tribulus, or gokru. Classified under the phylum Tracheophyta, genus *Tribulus*, and species *terrestris* Linn., the plant has synonyms such as *Tribulus maximus* and *Tribulus orientalis*. The leaves of *T. terrestris* are traditionally used in herbal medicine and are scientifically explored for their antioxidant and cardioprotective potential, supporting their inclusion in the present study to assess antiatherosclerotic and antioxidative activities (Chhatre *et al.,* 2014) (Figure 2).

These plants widely used in traditional systems of medicine for the management of metabolic and cardiovascular disorders. The present study aims to evaluate the antiatherosclerotic and antioxidative potential of ethanolic leaf extracts of these plants, considering the crucial role of oxidative stress and dyslipidemia in the development of atherosclerosis.

2. Materials and Methods

2.1 Collection and authentication of plants

The dried leaves of both plant species were collected from a local herbarium and subsequently subjected to taxonomic identification and authentication. *Luffa echinata* Roxb. was identified and

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authenticated by Dr. Madhava Chetty, Professor and Head, Department of Botany, Tirupathi University, Hyderabad, and assigned the Voucher Specimen Number 382. Similarly, *Tribulus terrestris* L. was authenticated by the same authority and assigned the Voucher Specimen Number 383. The authenticated specimens were preserved for future reference.

Figure 1: ***Luffa echinata.***

Figure 2: ***Tribulus terrestris.***

2.2 Preparation of plant extracts

The dried powdered leaves of the plants were collected and placed in a separate container into which ethanol was added in a ratio of 1:2 for about 7 days. Later on, the last day, the contents of the container were filtered through a muslin cloth and the ethanol present is distilled off by evaporation to obtain the bioactive-extracts of both the plants (Banu *et al.,* 2024).

2.3 Phytochemical screening

The extracts of *L. echinata* and *T. terrestris* were subjected to preliminary phytochemical screening using standard qualitative tests to evaluate the presence of various chemical constituents, including alkaloids, flavonoids, tannins, glycosides, carbohydrates, proteins, steroids, and saponins (Naaz *et al.,* 2024).

2.4 GC-MS analysis

GC-MS analysis was performed using a SHIMADZU QP-2010 GC-MS system equipped with a Turbo Gold mass detector and Turbo Mass 5.2 software. Separation was achieved on a fused silica capillary column coated with Elite-5 ms (5% diphenyl, 95% dimethylpolysiloxane; 30 m × 0.25 mm i.d. × 0.25 µm film thickness). Helium was used as the carrier gas at a constant flow rate of 1 ml/min, and 2 µl of seed extract was injected into the system. the GC oven temperature was maintained at 110°C with a 2 min hold, while the injector temperature was set at 250°C. The inlet line and ion source temperatures were maintained at 200°C. Mass spectra were recorded under electron ionization at 70 eV, with a scan period of 0.5 s over a mass range of 45-450 Da, and the total MS detection time was 31.2 min. Identification of compounds was carried out by comparing the obtained mass spectra with reference spectra available in the NIST mass spectral database (Banu *et al.,* 2025; Gomathi *et al.,* 2015).

2.5 Experimental animals

Animals were procured only after obtaining prior approval from the Institutional Animal Ethics Committee (IAEC). Male Wistar rats weighing 150-200 g were obtained from Vyas Laboratories, Telangana. The animals were housed under controlled environmental conditions (temperature 20-25°C) with a 12 h light/12 h dark cycle, in accordance with the guidelines of the Committee for the Control and Supervision of Experiments on Animals (CCSEA), Government of India. The experimental work was carried out at Shadan Women's College of Pharmacy, Khairtabad, Hyderabad, as per the approved protocol (IAEC Protocol No.: IAEC-07/SES-2025/41/109).

2.6 Acute toxicity studies

Acute oral toxicity studies were carried out in accordance with OECD guideline 423. Graded doses of both plant extracts were administered orally up to a maximum dose of 2000 mg/kg body weight. The animals were closely observed for a period of 14 days for the appearance of any clinical signs of toxicity or mortality. No mortality or treatment-related adverse effects were observed during the study period. Based on these findings, doses of 200 mg/kg and 400 mg/kg were selected for subsequent pharmacological evaluation (Banu and Das, 2025).

2.7 *In vitro* methods

2.7.1 HMG-CoA reductase inhibition assay

HMG-CoA reductase is a key rate-limiting enzyme involved in hepatic cholesterol biosynthesis. The inhibitory potential of the test substance was evaluated based on its ability to suppress the activity of HMG-CoA reductase. A reaction mixture was prepared containing the isolated HMG-CoA reductase enzyme, substrate, cofactor (NADPH), buffer, and the test extract. The mixture was incubated at 37°C for a specified duration. Enzyme activity was determined by measuring the decrease in NADPH absorbance at 360 nm using a spectrophotometer, which reflects the extent of HMG-CoA reductase inhibition (Liu *et al.,* 2016).

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

2.7.2 Lipoprotein lipase activation assay

Lipoprotein lipase (LPL) is a key enzyme involved in the hydrolysis of triglycerides into free fatty acids. Enhancement of free fatty acid release reflects increased LPL activity and contributes to the evaluation of the anti-atherosclerotic potential of the test extracts. Freshly isolated LPL enzyme obtained from blood or adipose tissue was incubated in a reaction medium containing triglyceride substrate,

isopropanol, and the test compound under appropriate experimental conditions for 20-30 min. The extent of triglyceride hydrolysis was determined by quantifying the liberated free fatty acids using a fluorometric method. The inhibitory concentration (IC_{50}) value of the extract was subsequently calculated (Liu *et al.,* 2016).

2.8 *In vivo* methods

2.8.1 High fat diet induced hyperlipidemia model

This method is based on the induction of hyperlipidemia using a high-fat (atherogenic) diet, followed by evaluation of the antihyperlipidemic potential of the test substance based on its ability to reduce diet-induced dyslipidemia. Adult Wistar rats weighing 150-200 g were acclimatized for 7 days prior to the study. Hyperlipidemia was induced by replacing the normal laboratory diet with an atherogenic diet comprising cheese, butter, coconut, and peanut. The test extract and standard drug were administered orally throughout the experimental period. The study was carried out for 2-4 weeks, during which body weights were recorded before, during, and at the end of the experiment. At the conclusion of the study, blood samples were collected from the retro-orbital venous plexus for biochemical analysis (Vesnina *et al.,* 2023; Feng *et al.,* 2019).

2.8.2 Triton X-100 induced hyperlipidemia

Triton is a non-ionic surfactant known to inhibit lipid clearance, thereby producing a marked elevation in plasma lipid levels. In this method, Wistar rats were fasted overnight with free access to water. On the following day, the animals were divided into different groups and hyperlipidemia was induced by intraperitoneal administration of triton at a dose of 400 mg/kg body weight, except in the normal control group. Simultaneously, the test extracts were administered orally. Blood samples were collected at 24 and 48 h after triton administration and subjected to biochemical estimation of lipid parameters (Vesnina *et al.,* 2023).

Table 1: Experimental design

Groups	Treatment	Dose	No. of rats
Group-I	Normal Saline	1 ml of 0.9% w/v p.o	6
Group-II	Toxic dose (Triton X-100, HFD)	400 mg/kg i.p	6
Group-III	TD + Atorvastatin	10 mg/kg	6
Group-IV	TD + P1 (D1)	200 mg/kg p.o	6
Group-V	TD + P1 (D2)	400 mg/kg p.o	6
Group-VI	TD + P2 (D1)	200 mg/kg p.o	6
Group-VII	TD + P2 (D2)	400 mg/kg p.o	6
Group-VIII	TD + P1(D1) + P2 (D1)	200 mg/kg + 200 mg/kg p.o	6
Group-IX	TD + P1 (D2) + P2 (D2)	400 mg/kg + 400 mg/kg p.o	6

The animals were randomly divided into nine groups, each consisting of six rats, to evaluate the antihyperlipidemic potential of the test extracts against Triton X-100 and high-fat diet-induced hyperlipidemia. Group-I served as the normal control and received normal saline (1 ml of 0.9% w/v, p.o.). Group-II acted as the toxic control and was administered Triton X-100 (400 mg/kg, i.p.) along with a high-fat diet to induce hyperlipidemia. Group-III received Triton X-100 followed by atorvastatin (10 mg/kg) as the standard treatment. Group-IV and Group-V received Triton X-100 followed by plant extract *L. echinata,* P1(at doses of 200 mg/kg and 400 mg/kg (p.o.), respectively. Group-VI and Group-VII received Triton X-100 followed by plant extract *T. terrestris* P2 at doses of 200 mg/kg and 400 mg/kg (p.o.), respectively. Group-VIII received Triton X-100, followed by a low-dose combination of P1 (200 mg/kg) and P2 (200 mg/kg, p.o.), while Group-IX received Triton X-100, followed by a high-dose combination of P1 (400 mg/kg) and P2 (400 mg/kg, p.o.).

3. Results

3.1 Phytochemical evaluation tests

Preliminary phytochemical screening showed that both *L. echinata* and *T. terrestris* were rich in flavonoids and glycosides. *L. echinata* exhibited high levels of terpenoids and proteins and amino acids, with moderate amounts of carbohydrates, quinones, and reducing sugars, while tannins and steroids were absent. In contrast, *T. terrestris* showed a very high presence of saponins and a strong presence of steroids, along with moderate levels of alkaloids and tannins, whereas quinones and reducing sugars were absent. Overall, *T. terrestris* demonstrated a comparatively richer saponin and steroid profile, while *L. echinata* was characterized by higher terpenoid and protein content.

3.2 GC-MS analysis

Plant-1: *L. echinata*

GC-MS analysis of *L. echinata* identified several bioactive compounds with therapeutic relevance. Sulphamide (RT 41.45 min; 0.66%) exhibited antidiabetic, antimicrobial, and antioxidant potential. Hydroxycinnamic acid (31.4 min; 0.12%) is associated with cardioprotective and anti-inflammatory effects, while salicylic acid (27.3 min; 0.09%) showed antidiabetic and antidepressant activity. Eburnamenine (29.1 min; 0.085%) demonstrated anticholinergic and antihyperlipidemic properties, and heptasiloxane (26.1 min; 0.06%) is linked to arteriosclerosis prevention. Notably, cucurbitacin (22.7 min; 20%) was the most abundant constituent, indicating strong antioxidant and antihyperlipidemic potential (Figure 3 and Table 3).

Table 2: Phytochemical evaluation

Chemical constituents	Plant:1 *L. echinata*	Plant:2 *T. terrestris*
Alkaloids	+	++
Glycosides	++	++
Terpenoids	+++	+
Tannins	–	++
Flavonoids	+++	+++
Steroids	–	+++
Carbohydrates	++	+
Saponins	+	++++
Quinines	++	–
Protein and amino acids	+++	+
Reducing sugars	++	–

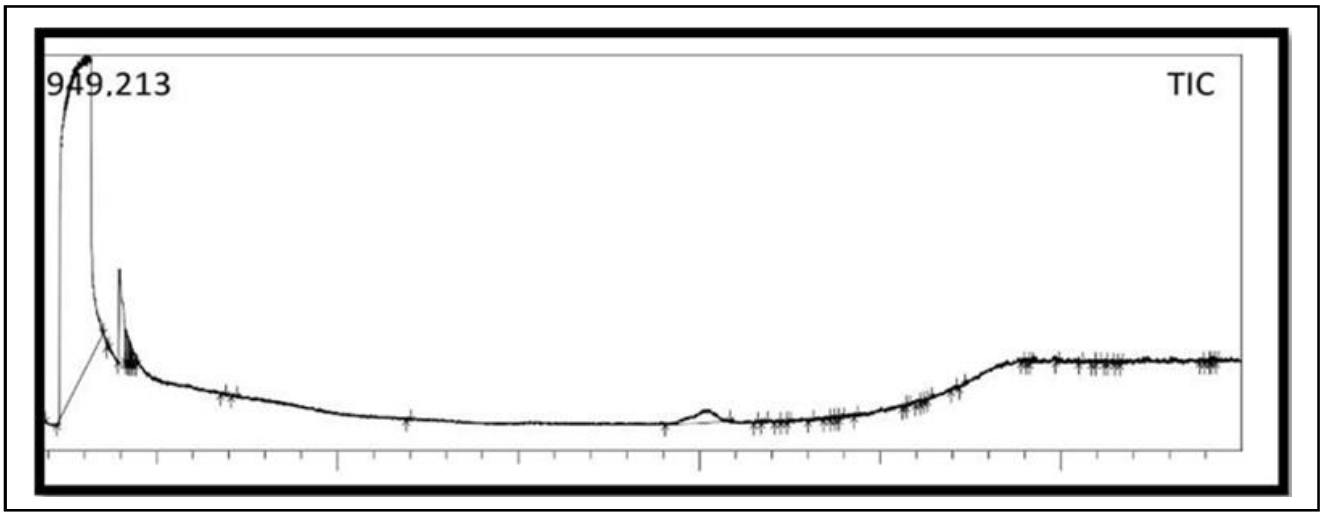

Figure 3: GC-MS chromatogram of *L. echinata*.

Table 3: GCMS and significances of *L. echinata*

Retention time	Chemical constituents	Area%	Significance
41.45	Sulphamide	0.66	Inhibit diabetes, microbial infection and oxidative stress
31.4	Hydroxycinnamic acid	0.12	CVD protection, prevents inflammation
27.3	Salicic acid	0.09	Antidiabetic, antidepressant
29.1	Eburnamenine	0.085	Anticholinergic, antihyperlipidemic
26.1	Heptasiloxane	0.06	Prevention of arteriosclerosis
22.7	Cucurbitacin	20	Antioxidant, antihyperlipidaemia

Plant-2: *T. terrestris*

GC-MS analysis revealed the presence of five major phytochemical constituents with diverse pharmacological relevance. Octasiloxane (retention time 19.56; area 0.45%) exhibited antioxidant and antibacterial activities. Sarasapogein (26.98; 0.14%) is associated with antitumor and antifungal effects and offers protection against coronary artery diseases. 2-Hydro-4-methoxybenzoic acid (28.087; 0.09%) demonstrated antidiabetic, antidepressant, and anti-atherosclerotic properties. Diethyl phthalate (12.232; 0.47%) showed hypolipidemic and anti-atherosclerotic potential. Neotigogenin (25.889; 0.91%), the compound with the highest area percentage, is known for its hypoglycemic and anti-hyperlipidemic activities, indicating its possible role in metabolic disorder management (Figure 4 and Table 4).

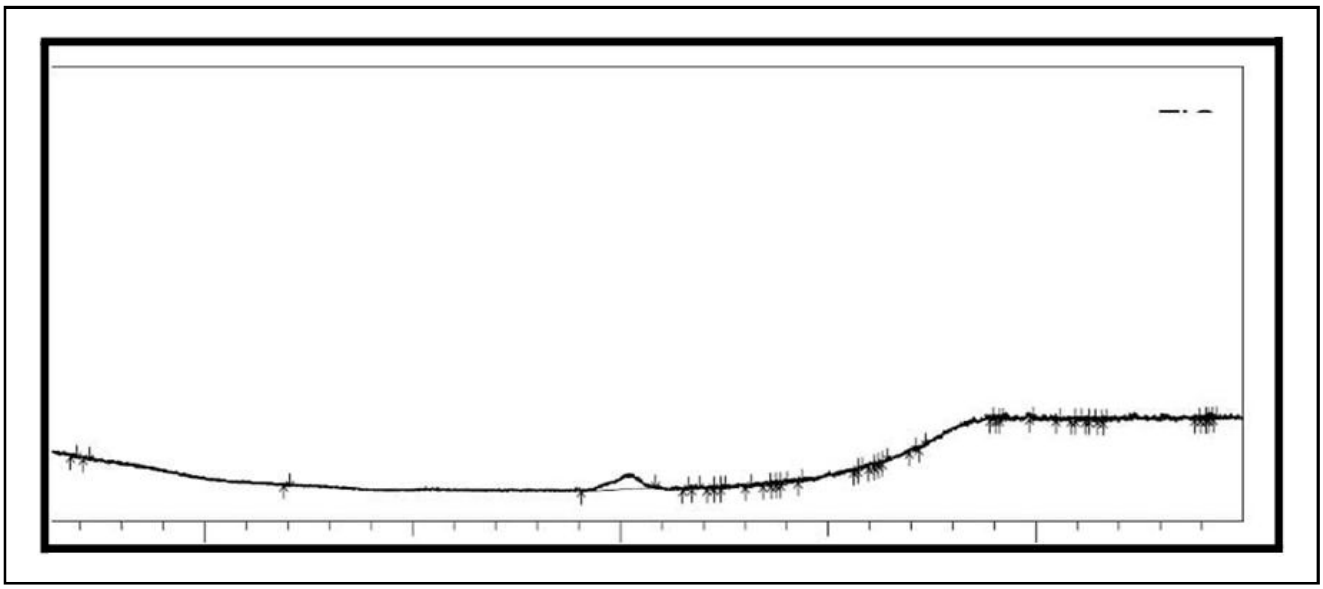

Figure 4: GC-MS chromatogram of *T. terrestris*.

Table 4: GC-MS and significances of *T. terrestris*

Retention time	Chemical constituents	Area%	Applications
19.56	Octasiloxane	0.45	Antioxidants, antibacterial
26.98	Sarasapogein	0.14	Antitumor, antifungal, coronary artery diseases
28.087	2-hydro 4 methoxybenzoic acid	0.09	Antidiabetic, antidepressant, antiatherosclerotic
12.232	Diethyl phthalate	0.47	Hypolipidemic, antiatherosclerotic
25.889	Neotigogenin	0.91	Hypoglycemia, antihyperlipidemia

3.3 *In vitro* studies

3.3.1 HMG-CoA reductase assay

The HMG-CoA reductase assay showed a concentration-dependent inhibitory effect for atorvastatin as well as both plant extracts. Atorvastatin produced the highest inhibition at all tested concentrations. Among the plant samples, Plant-2 exhibited greater inhibitory activity compared to Plant-1. Overall, both plant extracts demonstrated significant HMG-CoA reductase inhibitory potential, with Plant-2 showing comparatively superior antihyperlipidemic activity.

Table 4: HMG-CoA reductase inhibitory activity of atorvastatin and plant extracts

Concentration	Atorvastatin	Plant-1	Plant-2
120	81.58 ± 1.02	62.56 ± 1.25	75.01 ± 0.49
100	75.25 ± 1.25	55.45 ± 1.12	62.45 ± 1.99
80	70.25 ± 1.25	48.00 ± 1.85	52.32 ± 1.12
60	64.01 ± 1.01	38.45 ± 1.90	47.03 ± 0.55
40	58.99 ± 0.79	31.02 ± 1.65	41.56 ± 0.86
20	53.00 ± 1.05	20.01 ± 1.95	33.03 ± 1.05
10	48.25 ± 0.30	10.01 ± 0.52	21.00 ± 1.24

3.3.2 Lipoprotein lipase assay

The lipoprotein lipase assay revealed that both plant extracts significantly enhanced LPL activity compared to atorvastatin. Among the extracts, Plant-1 consistently showed higher LPL activation than Plant-2 across the tested concentrations. Atorvastatin exhibited comparatively lower stimulation of LPL activity, indicating that the plant extracts may have a stronger potential to promote lipid metabolism.

Table 5: Effect of atorvastatin and plant extracts on lipoprotein lipase activity

Concentration	Atorvastatin	Plant-1	Plant-2
25	1.82 ± 0.24	10.00 ± 0.38	8.50 ± 0.35
50	2.12 ± 0.12	8.00 ± 0.12	8.25 ± 0.28
100	1.24 ± 0.21	8.12 ± 0.58	7.90 ± 032
200	2.24 ± 0.18	8.40 ± 0.14	8.92 ± 0.24
400	1.47 ± 0.23	9.20 ± 0.18	7.45 ± 0.25

3.4 *In vivo* studies

3.4.1 Effect of EEP1 and P2 on serum lipid profile of HFD rats

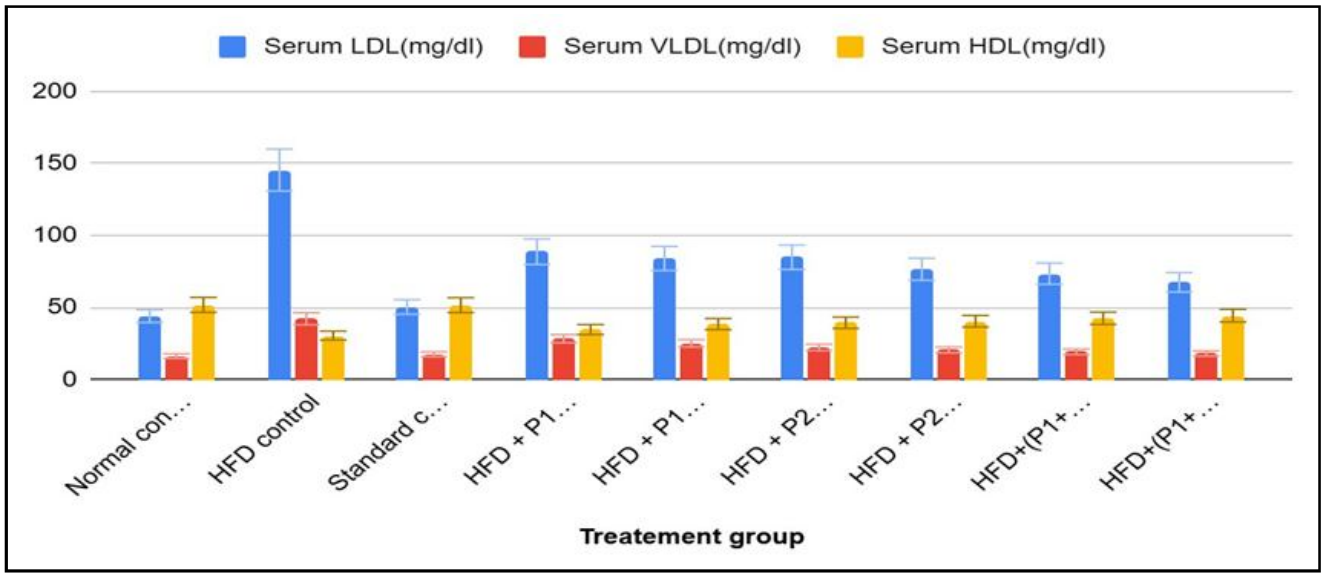

Figure 5: The graph here represents the serum lipid profile (LDL, VLDL and HDL) of HFD induced rats. From the graph, it is observed that the combination dose (400 mg/kg) of both the plants resembles with that of the standard drug action.

3.4.2 Effect of P1and P2 on Serum TG, TC, CH:HD ratio of HFD rats

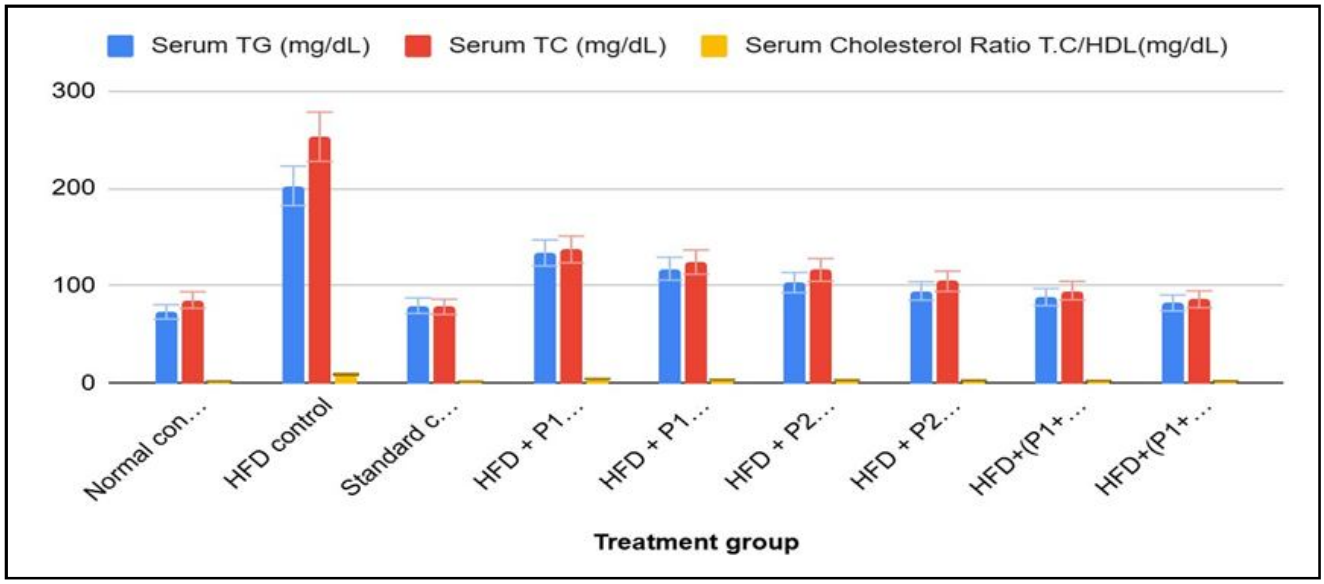

Figure 6: The graph shows the serum TG, TC levels in HFD rats, the combination dose is known to give desirable and yet more similar action with atorvastatin.

3.4.3 Effect of EELE, TT on serum lipid profile of Triton-X100 rats

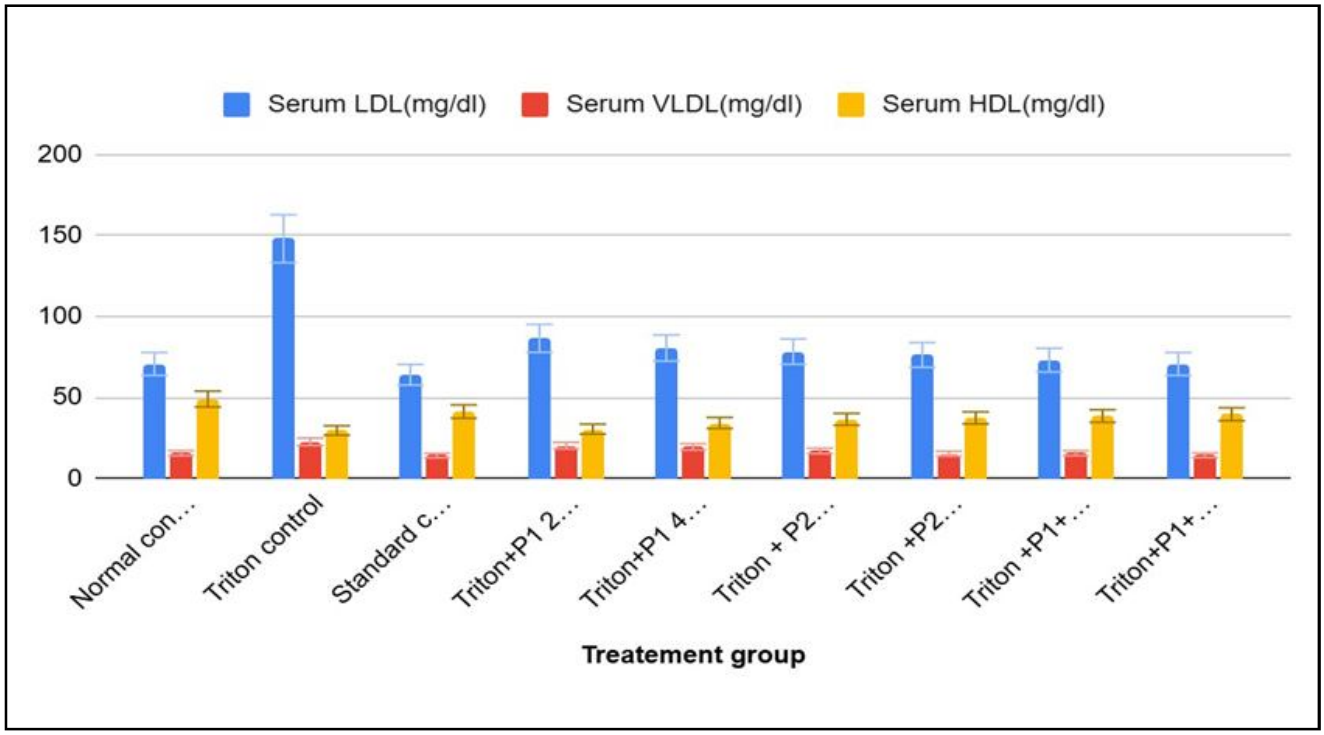

Figure 7: Effect of EELE, TT on serum lipid profile of Triton-X100 rats.

3.4.4 Effect of EEP1 and EEP2 on serum TG, TC, CHl-HDL ratio of Triton-X100 rats

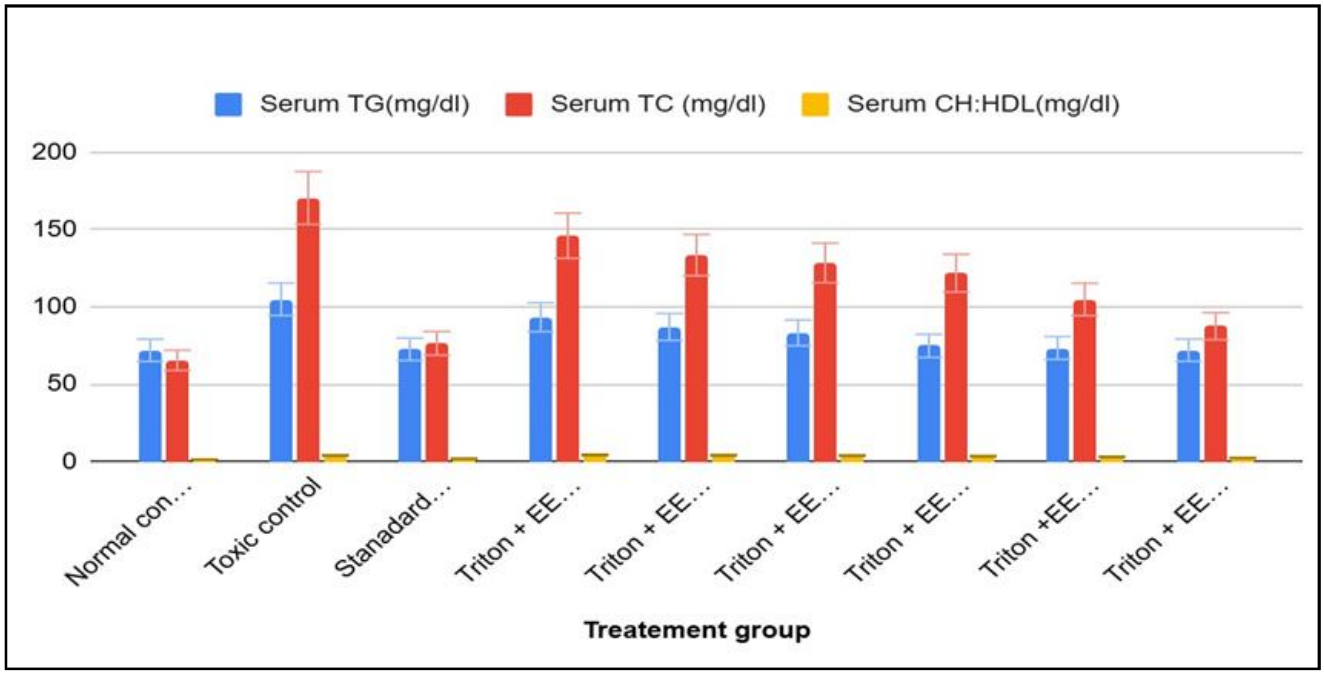

Figure 8: Effect of EEP1 and EEP2 on serum TG, TC, CHl-HDL ratio of Triton-X100 rats.

3.4.5 Effect on oxidative stress marker of Triton -X100 induced hyperlipidemia

The graph indicates the levels of free radicals in triton treated animals. It is clearly visible that the combination doses of plants showed decreased levels of oxidative stress markers, *i.e.,* SOD, CAT, and MDA.

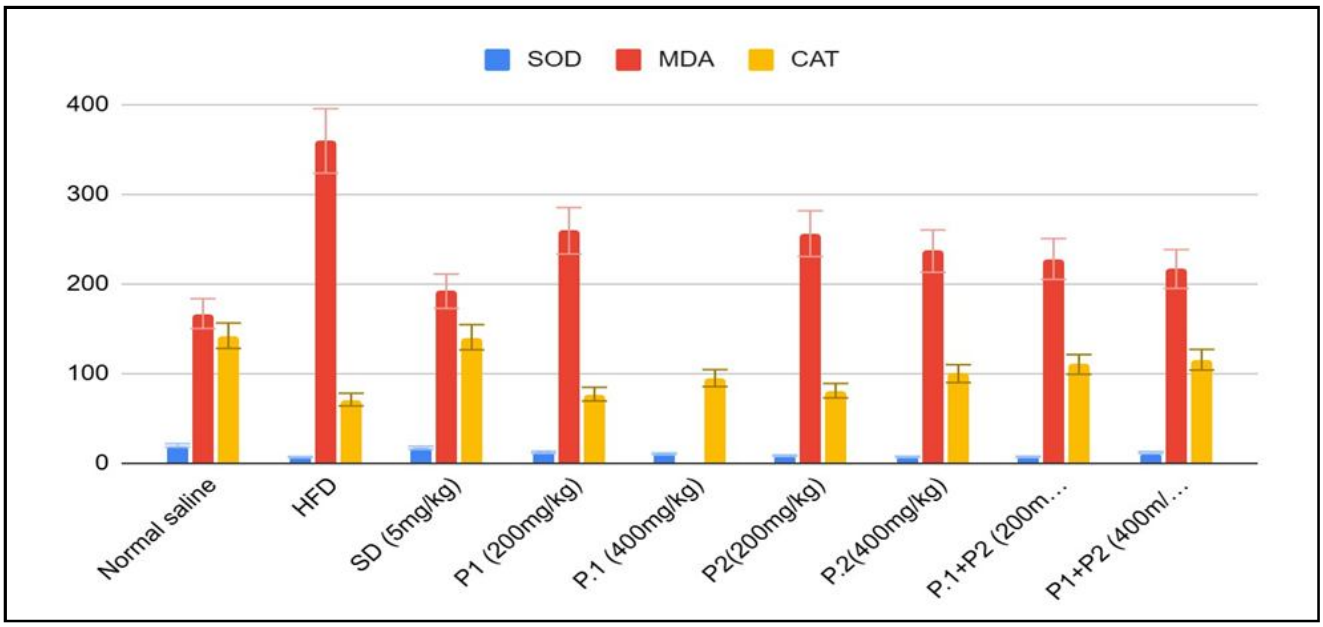

Figure 9: Effect on serum TG of Triton -X 100 induced hyperlipidemia.

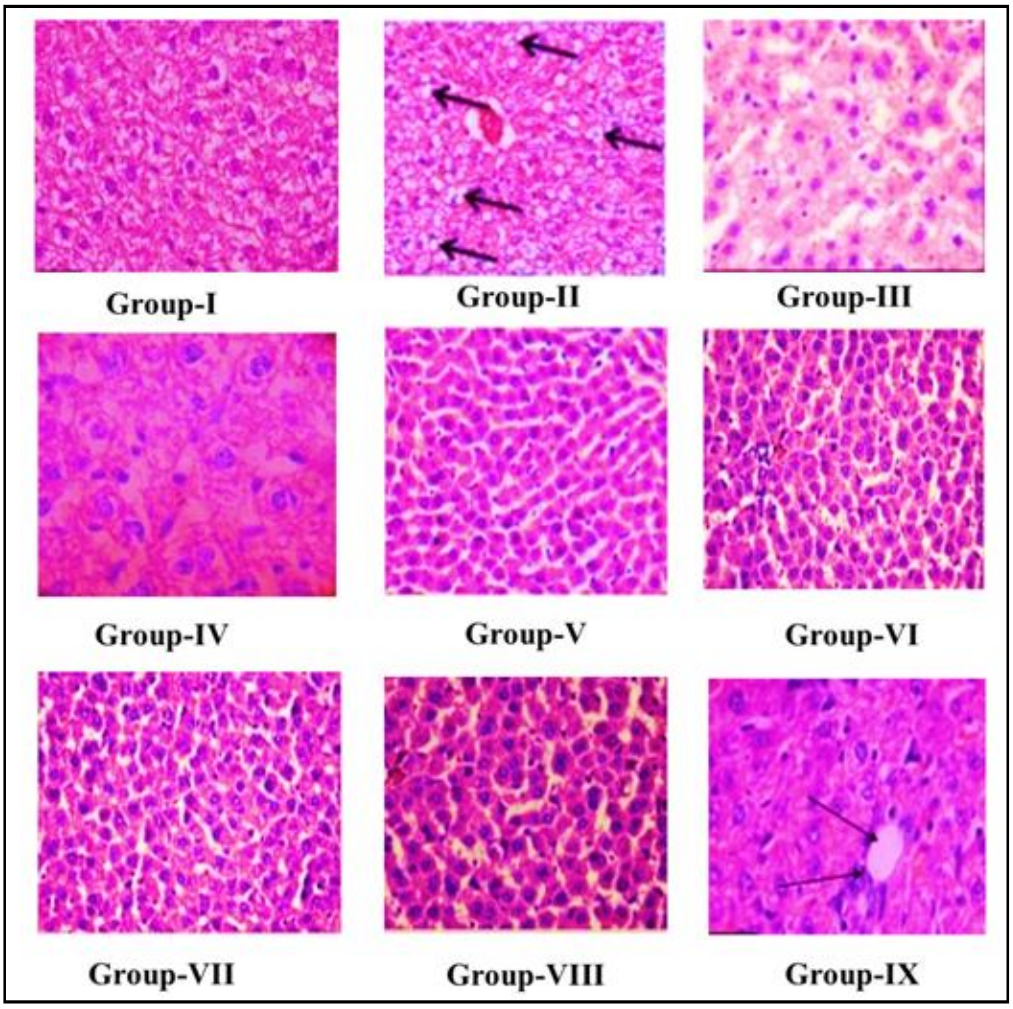

Figure 10: Microscopic liver examination.

3.5 Histopathological results

Microscopic examination of the liver revealed distinct histological changes across the experimental groups. The normal control group (I) displayed typical liver architecture, while the toxic control Group-II, treated with Triton, showed noticeable alterations in hepatic structure. The standard control Group-III, treated with atorvastatin, exhibited liver morphology comparable to the normal group. In the EELE-treated groups, dose 1 Group-IV showed fatty infiltration and visible granular droplets, whereas dose 2 Group-V demonstrated reduced infiltration and lipid droplets, though some dilation was observed. The EETT-treated groups showed congestion in hepatic and portal veins at dose 1 Group-VI and reduced congestion and dilation at dose 2 Group-VII. Combined treatment with EELE and EETT (doses 1 and 2; Group-VIII and IX) indicated liver histology closely resembling the normal group, with higher doses showing more prominent restoration of normal liver structure.

4. Discussion

Post-experimental analysis revealed that animals fed a high-fat diet (HFD) exhibited significant modulation of lipid profiles following treatment with the plant extracts. Notably, there was a marked reduction in low-density lipoprotein (LDL) levels, with percentage reductions of 88, 84, 84, and 76%, surpassing the 50% reduction observed in the standard drug-treated group. Concurrently, high-density lipoprotein (HDL) levels were elevated to 34, 38, 39, and 40%, approaching the 51% increase seen in the standard group. Similar trends were observed for triglycerides (TG) and total cholesterol (TC), which were effectively lowered, indicating a favorable impact on overall lipid metabolism.

Enzyme activity analyses further supported these findings. Plant-2 demonstrated potent modulatory effects, with IC_{50} values of 65.85 for HMG-CoA reductase inhibition (standard: 9.3) and 8.7 for lipoprotein lipase (LPL) activation (standard: 1.82). This indicates that Plant-2 not only inhibited cholesterol synthesis effectively, but also enhanced lipid clearance *via* LPL activation. While individual responses highlighted the efficacy of each extract, cumulative responses from combined treatments showed even greater potency and consistency, suggesting synergistic or additive effects in normalizing lipid metabolism.

Overall, these results highlight the potential of Plant-2 as a promising natural antihyperlipidemic agent, capable of improving lipid profiles and modulating key enzymes involved in cholesterol synthesis and lipid clearance, comparable to or in some aspects exceeding the standard pharmacological intervention.

5. Conclusion

The primary objective of the present study was to evaluate the antiatherosclerotic and antioxidative potential of the selected herbal extracts. These plants were chosen not only for their well-documented traditional use but also due to their minimal or negligible adverse effects. Prior to experimentation, the diverse secondary metabolites present in the herbs were characterized using GC-MS analysis. Based on the findings of this study, Plant-2 demonstrated superior anti-hyperlipidemic activity, whereas Plant-1 exhibited more pronounced antioxidative effects with modest lipid-lowering potential. Overall, a combined or synergistic approach may be particularly advantageous, providing both anti-atherosclerotic and antioxidant benefits.

Availability of data and material

All data are provided within the manuscript.

Authorship contribution statement

Mehnoor Farheen: Contributed to supervision, conceptualization, project administration, validation, and overall guidance of the study; **Sarah Maryam:** Contributed to data curation, investigation, methodology, and drafting of the manuscript; **Shaika Razia:** Contributed to writing the original draft, reviewing and editing the manuscript, and methodology; **Syeda Qadar Unnisa:** Contributed to software handling, data analysis, and visualization of results; **D. Ramakrishna:** Contributed to resources, methodology, and formal analysis; **Meraj Fatima:** Contributed to investigation, data curation, and validation of experimental results.

Consent for publication

All authors gave their full consent for publication and submission to this journal.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

Funding

The author(s) stated that the work presented in this article received no associated funding.

Ethics approval

Approved by the Institutional Animal Ethics Committee (IAEC), Protocol No. IAEC-07/SES-2025/41/109.

Acknowledgements

We extend our heartfelt gratitude to Shadan Women's College of Pharmacy, Khairtabad, Hyderabad, Telangana, India, for their constant encouragement, infrastructure facilities, and access to academic resources that greatly supported the completion of this research work.

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Citation **Mehnoor Farheen, Sarah Maryam, Shaika Razia, Syeda Qadar Unnisa, D. Ramakrishna and Meraj Fatima (2025). *In vivo* and *in vitro* evaluation of antiatherosclerotic and antioxidative activity of ethanolic extracts of *Luffa echinata* Roxb. and *Tribulus terrestris* L. J. Phytonanotech. Pharmaceut. Sci., 5(4):62-71. http://dx.doi.org/10.54085/jpps.2025.5.4.8**