Journal of Carcinogenesis
Covering Genesis to Therapeutics

Journal of Carcinogenesis, ISSN: 1477-3163
2025; Vol 24: Issue 4s, https://carcinogenesis.com/

Modulatory Effect of *Hydrilla Verticillata* Extract on Lipid Profile, Hepatic Dysfunctioning and Mitigation of Oxidative Stress in Triton X-100 Induced Hyperlipidemic Rats

Madan L. Kaushik[1], Shavinder Kumari[2*], Mahendra Singh Ashawat[3]

[1] Professor, Department of Pharmacology, Aadarsh Vijendra Institute of Pharmaceutical Sciences (AVIPS), Shobhit University, Gangoh, Saharanpur U.P., India, 247341.

Email ID: kaushikmadan2011@gmail.com, ORCID:0000-0001-7859-3959

[*2]Research Scholar, Department of Pharmacology, Aadarsh Vijendra Institute of Pharmaceutical Sciences (AVIPS), Shobhit University, Gangoh, Saharanpur U.P., India, 247341.

ORCID: 0000-0002-1167-6642.

[3]Professor cum principal, Department of Pharmaceutics, Laureate Institute of Pharmacy, Kathog, Distt. Kangra, H.P., 177101.

Email ID: msaresearchg@gmail.com, ORCID: 0000-0001-7188-426X

***Corresponding Author:**

Shavinder Kumari

Email ID: shavinderkumari.doad@gmail.com

ABSTRACT

Background: *Hydrilla verticillata* is an aquatic plant with documented ethnomedicinal relevance. This study investigated the phytochemical composition and assessed the antioxidant, antihyperlipidemic, and hepatoprotective activities of the ethanolic extract of *Hydrilla verticillata* (EEHV) using *in vitro* and *in vivo* experimental models.

Methods: Hyperlipidemia was induced in Wistar rats via Triton X-100. Animals received EEHV at 200 and 400 mg/kg, compared with atorvastatin (10 mg/kg). Phytochemical screening, extractive yield determination, and standardized assays assessed total phenolic (TPC), flavonoid (TFC), and terpenoid content. Antioxidant capacity evaluated via DPPH and nitric oxide radical scavenging assay. Biochemical markers, oxidative stress parameters (TBARS, MDA, GSH), and liver histology were analyzed. Data were analyzed using ANOVA followed by Tukey's post hoc test.

Results: EEHV showed 18.2% yield and contained phenolics, flavonoids, tannins, terpenoids, alkaloids, and saponins. TPC, TFC, and terpenoids were quantified as 166.28 ± 0.03 mg GAE/100g, 120.07 ± 0.07 mg QE/100g, and 146.92 mg linalool equivalents/100g, respectively. EEHV exhibited dose-dependent antioxidant effects with IC_{50} values of 245.99 µg/mL (DPPH) and 233.66 µg/mL (NO). *In vivo*, EEHV significantly improved lipid profiles, reduced ALT, AST, bilirubin, creatinine, and BUN levels, and restored antioxidant status, especially at 400 mg/kg (***$p < 0.001$). Histopathology confirmed hepatic tissue recovery comparable to the standard.

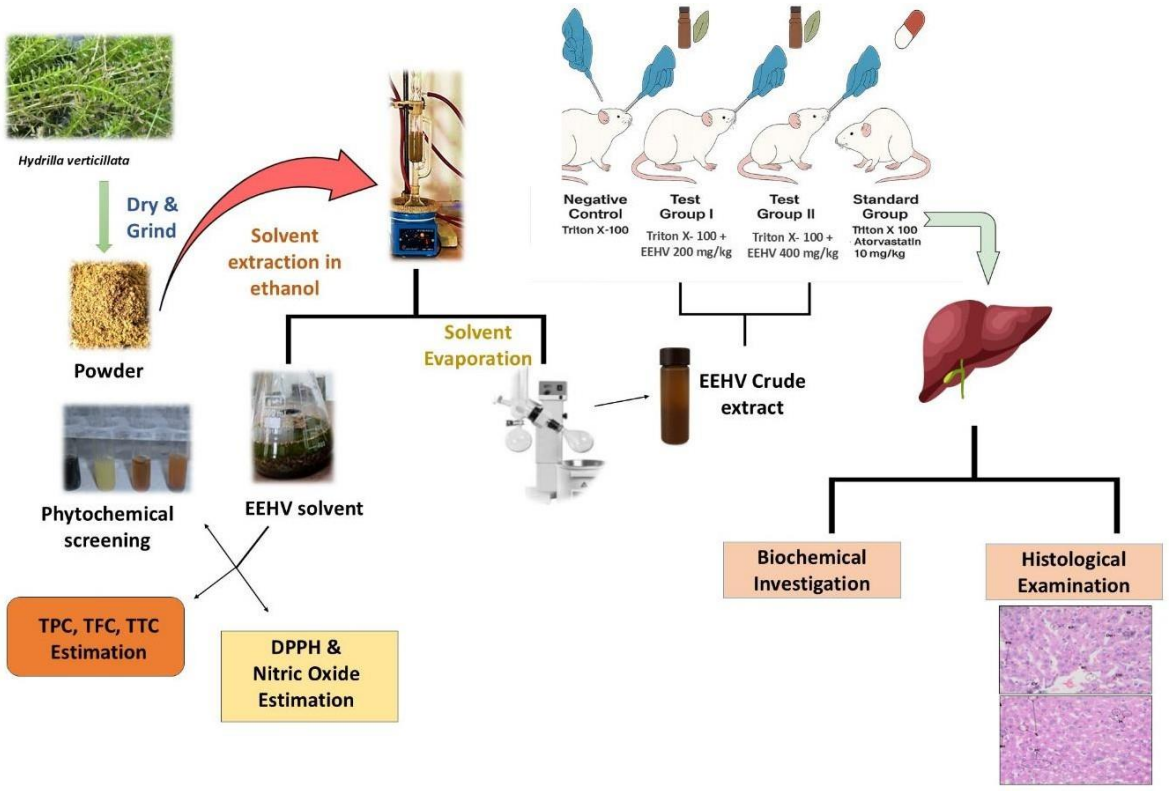
Conclusion: EEHV demonstrates significant antioxidant, antihyperlipidemic, and hepatoprotective activities, particularly at 400 mg/kg, indicating potential as a natural therapeutic agent for oxidative and metabolic disorders. This study offers a comprehensive evaluation of EEHV's pharmacological promise.

Keywords: *H. verticillata, hyperlipidemia, antioxidant activity, Triton X-100, hepatoprotective*

How to Cite: Madan L. Kaushik, Shavinder Kumari, Mahendra Singh Ashawat, (2025) Modulatory Effect of Hydrilla Verticillata Extract on Lipid Profile, Hepatic Dysfunctioning and Mitigation of Oxidative Stress in Triton X-100 Induced Hyperlipidemic Rats, *Journal of Carcinogenesis, Vol.24, No.4s*, 250-266

1. INTRODUCTION

Hyperlipidemia, characterized by a marked decrease in high-density lipoprotein cholesterol (HDL-C) and increased blood lipid levels, especially low-density lipoprotein cholesterol (LDL-C) which is a prominent modifiable risk factor implicated in the pathogenesis of atherosclerotic cardiovascular diseases (ASCVD), such as coronary artery disease, ischemic stroke, and peripheral arterial disorders [1,2]. The World Health Organization (WHO) recognizes dyslipidemia as a major contributor to global health burdens, with ASCVD responsible for over 17.9 million deaths annually [3]. Notably, the global incidence of hyperlipidemia is on the rise, especially in low- and middle-income countries, driven by factors such as urbanization, shifts toward calorie-dense diets, reduced physical activity, and the growing prevalence of obesity [4].



To date, only a limited number of pharmacological agents such as statins, fibrates, and cholesterol absorption inhibitors are standard therapies for lipid regulation; however, their long-term use is often restricted by adverse effects including myopathy, hepatotoxicity, and poor patient compliance. Furthermore, a notable subset of patients does not attain target lipid levels despite these interventions. These limitations have catalyzed growing research interest in exploring alternative lipid-lowering approaches, particularly phytotherapeutics derived from medicinal plants, which are generally associated with fewer side effects and better tolerability.

A commonly employed method for experimentally inducing hyperlipidemia is the administration of Triton X-100, a non-ionic surfactant. A single intraperitoneal dose of Triton X-100 at 100 mg/kg body weight effectively elevates serum lipid levels by interfering with normal lipid metabolism, resulting in marked increases in total cholesterol, triglycerides, and LDL-C, alongside a reduction in HDL-C [5, 6]. This model is further characterized by elevated hepatic enzyme levels and oxidative stress markers, thereby offering a robust system for assessing the lipid-modulating and antioxidant potential of bioactive compounds.

Hydrilla verticillata is a fast-growing submerged aquatic plant, predominantly recognized for its invasive nature and significant ecological impact on freshwater ecosystems. However, beyond its reputation as a weed, this species has recently attracted considerable scientific interest due to its diverse pharmacological potential. Traditionally utilized in herbal medicine, the entire plant has been applied for therapeutic purposes, a practice substantiated by phytochemical studies identifying a diverse range of bioactive compounds, including saponins, flavonoids, alkaloids, glycosides, steroids, tannins, phenolics, and terpenoids [7]. These phytoconstituents are well-recognized by many researchers for their diverse therapeutic effects, including antioxidant, anti-inflammatory, hepatoprotective, and lipid-lowering activities [7–9].

The flavonoids and phenolic constituents present in *H. verticillata* exhibit potent free radical scavenging and lipid

peroxidation-inhibiting activities, thereby mitigating oxidative stress a key contributor to the onset and progression of hyperlipidemia and its associated disorders. Saponins are postulated to lower serum cholesterol by impeding intestinal cholesterol absorption and enhancing bile acid excretion [10]. Additionally, terpenoids and alkaloids may exert regulatory effects on lipid metabolism through modulation of enzymatic pathways involved in cholesterol synthesis and transport, such as HMG-CoA reductase and lecithin cholesterol acyltransferase (LCAT). Flavonoids and phenolics in *H. verticillata* exhibit hypolipidemic activity by inhibiting HMG-CoA reductase, the key enzyme in cholesterol synthesis, leading to reduced endogenous cholesterol levels [11]. The lipid-lowering effects of *H. verticillata* are therefore thought to result from its capacity to influence these metabolic enzymes, reduce systemic lipid load, and enhance hepatic antioxidant effect. Moreover, its bioactive components may offer vascular protection by limiting oxidative impairment and suppressing inflammatory mediators implicated in atherosclerosis and cardiovascular pathologies [12].

H. verticillata presents a compelling case for further investigation as a plant based therapeutic agent for the management of hyperlipidemia and related oxidative stress disorders. However, comprehensive studies including pharmacodynamic, toxicological, and clinical evaluations are necessary to fully validate its efficacy and safety before it can be considered for widespread therapeutic and nutraceutical uses.

2. MATERIAL AND METHODS

Plant material

Fresh *H. verticillata* (500–800 g) was collected in August 2023, from a freshwater pond near the College of Horticulture and Forestry, Neri, Hamirpur, Himachal Pradesh. The samples were thoroughly washed to remove surface impurities, then air-dried under ambient, shaded conditions for two weeks with periodic inspection. Dried material was stored in airtight containers. Taxonomic authentication was conducted by Prof. Dr. Shrikant K (SB Arts and KCP Science College, Vijayapur, Karnataka), with the herbarium specimen authentication number- PRCBB-208 dated 11/1/2023.

Chemicals and Glassware

Atorvastatin, a marketed formulation of Pfizer, was utilized as the reference standard drug for this study. Triton X-100 (Sigma Aldrich) and Atorvastatin (Dr. Reddy's Laboratories) were used. Diagnostic kits and reagents were obtained from certified suppliers to ensure accuracy. Standard diagnostic kits facilitated the biochemical analysis of blood serum samples. In addition, laboratory-grade chemicals and reagents were employed for the phytochemical screening and quantitative determination of phytoconstituents within plant extract.

Pharmacognostic evaluation of *H. verticillata*

Coarse leaf powder of *H. verticillata* was assessed for key physicochemical parameters, including moisture content, ash values, and extractive yields. All procedures followed standard protocols using analytical-grade reagents sulfuric acid (Himedia, Mumbai), ethanol, chloroform, and petroleum ether (60–80 °C) (S.D. Fine Chemicals, India) [13, 14]. Moisture content was determined by drying 1.5 g of powdered sample at 105 °C until constant weight, followed by cooling in a desiccator. Total ash, water-soluble ash, acid-insoluble ash, and sulphated ash were evaluated to assess purity and contamination. Ash values were expressed as percentages of initial weight [15].

For extractive values, 5 g of powder was macerated in 100 mL ethanol, shaken for 6 hours, and then left to stand for 18 hours. Post-filtration, 25 mL of each extract was evaporated at 100 °C to determine solvent-soluble constituents. Soxhlet extraction of 100 g of powder was also conducted using solvents of increasing polarity for 18–20 hours. Extracts were concentrated at 40 °C under reduced pressure and stored at 4-5 ^{0}C for further analysis. The percentage yield of ethanolic extract was calculated to support phytochemical profiling [16].

Methodology for Qualitative Phytochemical Screening of *H. verticillata*

The ethanolic extract of *H. verticillata* was qualitatively screened for alkaloids, flavonoids, glycosides, tannins, saponins, terpenoids, and phenolics using standard assay methods. Quantitative estimation of total polyphenols, flavonoids, and terpenoids was conducted using the Folin–Ciocalteu at λ_{max} = 760 nm, aluminium chloride at λ_{max} = 510 nm, and vanillin H_2SO_4 at λ_{max} = 608 nm methods, respectively. Results were expressed as mg GAE, QE, and LU per 100 g of extract, based on standard calibration curves. All the analysis were performed in triplicate [15].

Determination of *In-vitro* Antioxidant Activity

DPPH (1,2-Diphenyl-2-Picryl-hydroxyl radical) Assay

The antioxidant potential of the *H. verticillata* extracts was determined using the DPPH assay as per methodology described by Wintola and Afolayan (2015), with slight modifications. A 0.135 mM DPPH solution in methanol was prepared. To 1 mL of this solution, 1 mL of plant extract at varying concentrations (0.2 to 1.0 mg/mL) and by using standard gallic acid were added. The mixture was vortexed and incubated in darkness at room temperature for 30 minutes. Absorbance was measured at λ_{max} = 517 nm using a Shimadzu UV-1900i spectrophotometer [13].

Nitric Oxide Scavenging Activity

Crude ethanolic extracts (10 mg/mL) were diluted to 100–1000 µg/mL with distilled water. Griess reagent was freshly prepared by mixing equal parts of 1% sulphanilamide and 0.1% naphthyl ethylenediamine dihydrochloride in 2.5% phosphoric acid. For the nitric oxide scavenging assay, 0.5 mL of 10 mM sodium nitroprusside in phosphate-buffered saline was mixed with 1 mL of each extract dilution and incubated at 25 °C for 180 minutes. After incubation, an equal volume of Griess reagent was added. Absorbance was measured at λ_{max} = 546 nm using a Shimadzu UV-1900i spectrophotometer, with rutin as the standard and control without extract. The assay results provided insights into the antioxidant potential of the plant extracts, contributing to the understanding of their hepato-protective properties [17], [18].

In Vivo* Evaluation of the Pharmacological Effects of *H. verticillata

An acute oral toxicity study was already been conducted in healthy Wistar rats (180–250 g) following OECD-423 guidelines [19]. Animals were housed under standard conditions at anima house of Laureate Institute of Pharmacy, Kangra, with a one-week acclimatization.

The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) under approval number CCSEA/LIPH/2023/36, in compliance with the guidelines of the Committee for the Control and Supervision of Experiments on Animals (CCSEA) and the Organisation for Economic Co-operation and Development (OECD). The Triton X-100 solution was freshly prepared in normal saline prior to administration. Hyperlipidemia was induced after overnight-fasted in 30 male Wistar rats by intraperitoneal injection of Triton X-100 at dose of 100 mg/kg. EEHV doses were chosen based on prior reports of *Hydrilla verticillata* bioactivity [20].

This experiment employed five group of six animals each that were given different treatment for 21 days as follows:

Group 1: The normal control group received standard pellet food with free access to water.

Group 2: The negative control group received Triton X-100 (100 mg/kg, i.p.)

Group 3: Treatment group-1 was involved in the daily administration of EEHV (200 mg/kg/p.o.) & Triton X-100 (100 mg/kg, i.p.)

Group 4: Treatment group-2 was involved in the daily administration of EEHV (400 mg/kg/p.o.) & Triton X-100 (100 mg/kg, i.p.)

Group 5: The positive control group was administered atorvastatin (10 mg/kg/p.o.) & Triton X-100 (100 mg/kg, i.p.)

Inclusion Criteria

Healthy male Wistar rats weighing between 180–250 grams. Animals acclimatised to laboratory conditions for a minimum of 7 days before experimentation. Rats induced with hyperlipidemia using a Triton x-1000 for a specified period (e.g., 21 das), showing elevated lipid profile parameters (TC, TG, LDL-C. etc). Only animals with successful induction of hyperlipidemia (confirmed biochemically) were included for treatment and evaluation.

Exclusion Criteria

Female rats or animals outside the defined weight range. Rats exhibiting signs of illness, infection, or injury during the acclimatization or induction period. Animals failing to develop hyperlipidemia after Triton X-100 administration. Rats with abnormal baseline lipid levels before induction. Animals that showed poor response to handling, excessive weight loss/gain, or mortality during the study period.

Analyses were performed using a Shimadzu UV-Visible Spectrophotometer, Mispa Excel autoanalyzer, and Remi ELISA reader. Blood samples were collected weekly by retro-orbital puncture under mild anaesthesia. Serum lipid profiles (total cholesterol, triglycerides, HDL, LDL, VLDL) [21, 22]. and liver biomarkers including bilirubin, [23], albumin [24, 25], creatinine [26], ALT, AST, ALP [27], and BUN [28] were measured using standard kits by following the standard procedures prescribed by the manufacturer.

After completion of study protocol liver harvested and liver homogenized in ice-cold phosphate buffer, and analyzed for malondialdehyde (MDA) levels via TBARS assay. Enzymatic antioxidants were quantified using ELISA kits to assess oxidative stress. [28].

Statistical Analysis

The results were statistically analyzed using GraphPad Prism V10.2 for Windows. Both one-way and two-way ANOVA (Analysis of Variance) methods were employed to assess the mean differences observed among the various experimental groups. Post-hoc analysis was conducted using Tukey's multiple comparison test. The results are expressed as Mean ± SEM, with significance levels set at $p < 0.05$ to $p < 0.0001$.

3. RESULTS

Extraction of EEHV

The EEHV showed a yield of 18.2 %, indicating efficient extraction and the presence of bioactive constituents.

Extractive Value

Extractive values also revealed an alcohol-soluble extractive value of 13.57 % w/w and a water-soluble extractive value of 12.01 % w/w, providing insight into the solubility characteristics of the phytoconstituents, which is crucial for understanding the plant's therapeutic potential (Table 1).

Table 1: Extraction value for *H. verticillata* Leaves

S. No.	Analytical Parameters	*H. verticillata* Percentage (%W/W)
1.	Alcohol soluble Extraction Value	13.57 % w/w
2.	Water soluble Extraction Value	12.01 % w/w

Ash Values & Moisture content

In terms of analytical parameters, the powdered leaves of *H. verticillata* showed a total ash content of 19.24 % w/w, indicating the mineral composition of the plant. The water-soluble ash was found to be 8.21 % w/w, while the acid-insoluble ash and sulphated ash were 3.74 % w/w and 10.7 % w/w, respectively. The moisture content was recorded at 8.69 %, which is essential for assessing the quality of the plant material (Table 2).

Table 2: Analytical Parameters for Powdered *H. verticillata* Leaves

S. No.	Analytical Parameters	*H. verticillata* leaves Percentage (W/W)
1.	Total ash value	19.24 % w/w
2.	Water ash value	8.21 % w/w
3.	Acid ash value	3.74 % w/w
4.	Sulfated ash	10.7 % w/w
5.	Moisture content	8.69 % w/w

Qualitative analysis of phytochemicals

Preliminary phytochemical screening confirmed the presence of, tannins, Saponins, alkaloids, terpenoids, flavonoids and phenols, suggesting potential antioxidant and anti-hyperlipidemic activity. glycosides, steroids and amino acids were absent, reflecting a selective phytochemical profile (Table 3).

Table 3: Preliminary phytochemical Screening of Ethanolic Extract of *H. Verticillata* Leaves

S. No.	Chemical constituent	Ethanolic extract of *H. Verticillata* leaves
1.	Amino Acids	-
2.	Saponins	+
3.	Steroids	-
4.	Alkaloids	+
5.	Glycosides	-
6.	Flavonoids	+
7.	Tannins	+
8.	Terpenoids	+
9.	Phenols	+

(+): Indicates presence, (-): indicates absence

Quantitative estimation

The quantitative analysis of the ethanolic extract of *H. Verticillata* showed significant levels of bioactive compounds, with total flavonoid content measured 120.07 ± 0.07 mg quercetin, total phenolic content 166.28 ± 0.03 mg gallic acid, and total terpenoid content 146.92 ± 0.08 mg linalool (Table 4, Fig 1-3). These values suggest that *H. verticillata* is rich in these compounds, which could have significant pharmacological applications.

Table 4: Total Phenol, Flavonoid, & Terpenoid Content in *H. verticillata*

S. No.	Ethanolic Extract of *H. verticillata*	Flavonoid (mg QE/100g)	Phenol (mg GAE/100g)	Terpenoid (mg LU/100g)
1.	EEHV	120.07 ± 0.07 mg	166.28 ± 0.03 mg	146.92 ± 0.08 mg

[GAE: Gallic acid, QE: Quercetin, LU: Linalol, EEHV: ethanolic extract of *H. verticillata*. Data is represented as mean ± SEM (n=3)]

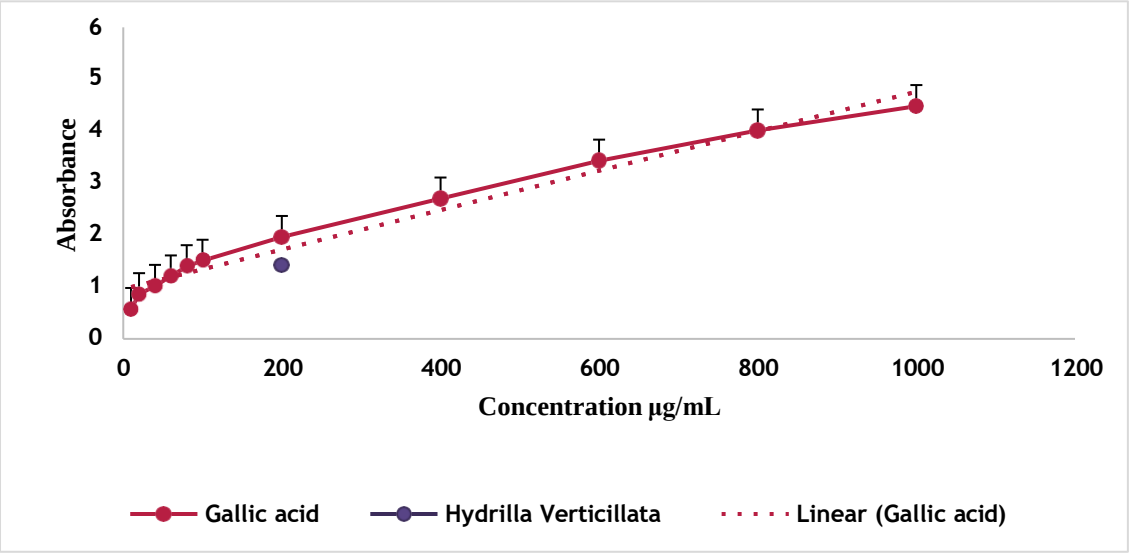


Figure 1: Total Flavonoid Content

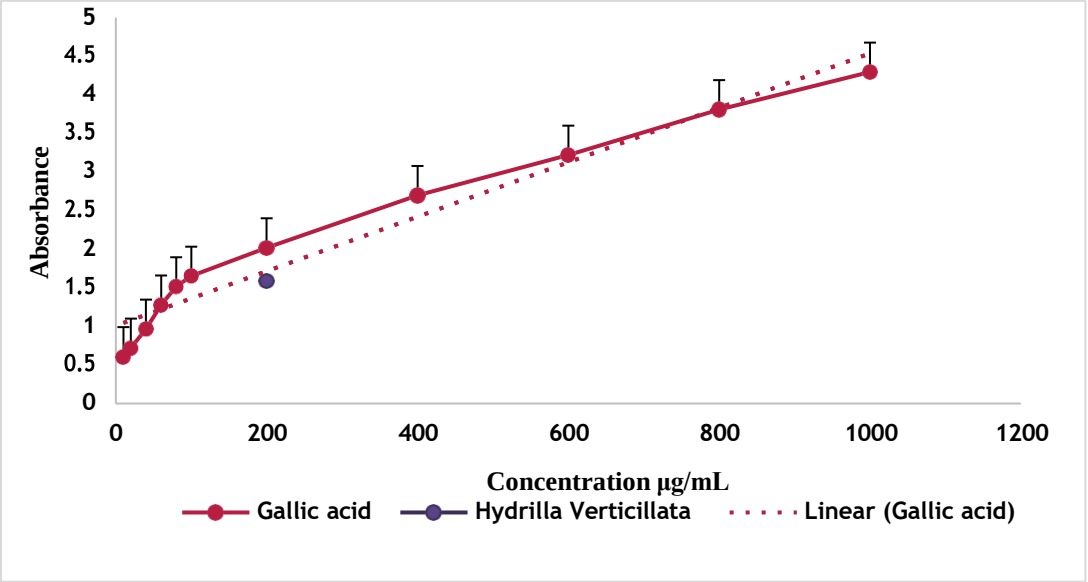


Figure 2: Total Phenol Content

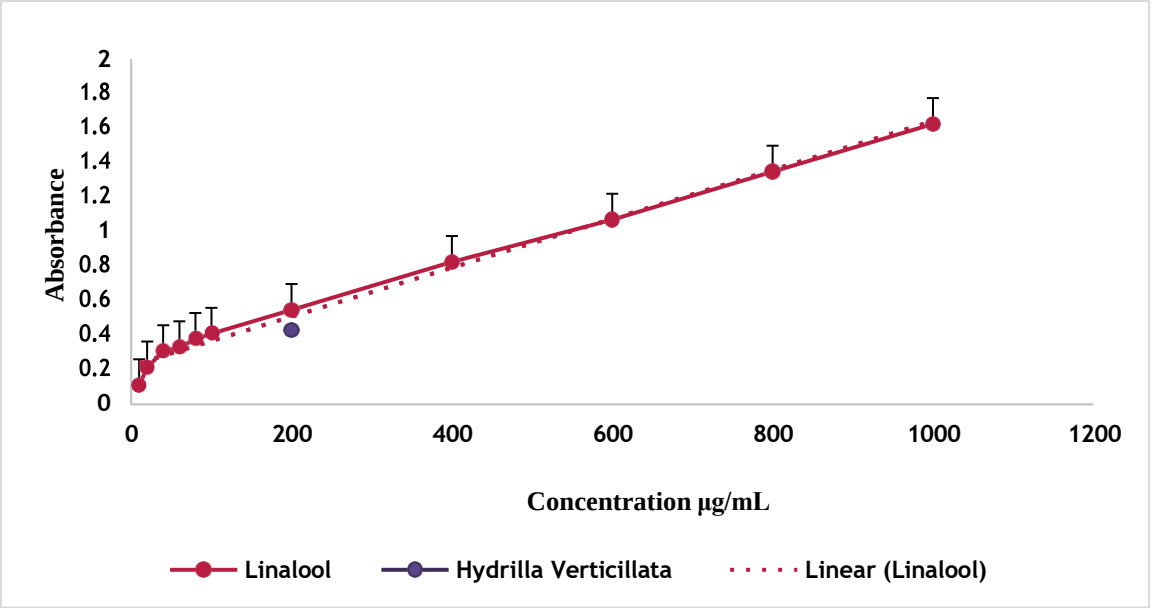


Figure 3: Total Terpenoid Content

Antioxidant Assay

The EEHV showed a concentration-dependent increase in DPPH inhibition, with a percentage inhibition of 29.65% at 10 µg/ml to 90.96% at 1000 µg/ml (Fig 4). The calculated IC_{50} value for *H. verticillata* was 245.99 µg/ml, indicating moderate antioxidant activity compared to the reference standard, gallic acid, which showed a much stronger effect (IC_{50} =65.55 µg/ml) (Table 5). In the nitric oxide scavenging assay, *H. verticillata* demonstrated moderate activity, with a percentage inhibition of 41.29 % at 100 µg/ml and 91.16 % at 1000 µg/ml, compared to the strong activity of rutin, the reference standard, which inhibited 99.59% of nitric oxide at 1000 µg/ml (Fig 5). The calculated IC_{50} value for *H. verticillata* was 233.663 µg/ml found less effective as compared to that of the reference, rutin with an IC_{50} value of 35.22 µg/mL (Table 6).

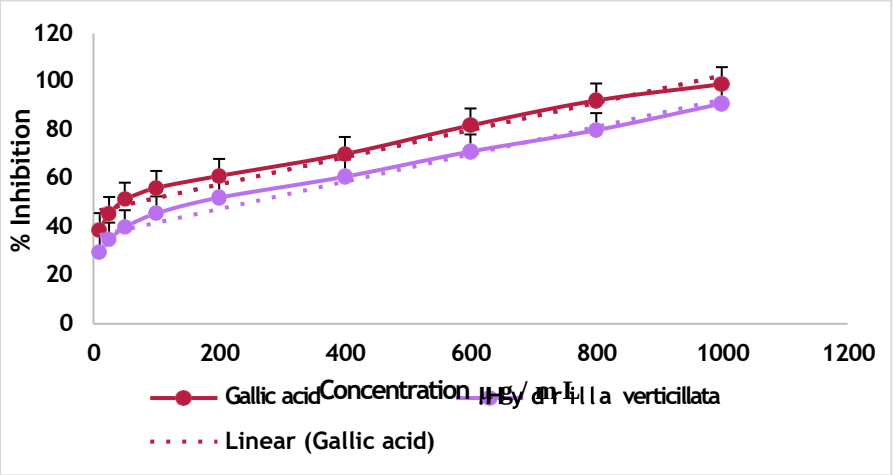


Figure 4: Curve of DPPH for Gallic acid, *H. verticillate*

Table 5: For IC_{50} value of Gallic acid, *H. verticillata*

S. No.	Compound	IC_{50}
1.	Gallic acid	65.5516
2.	*H. verticillata*	245.9894

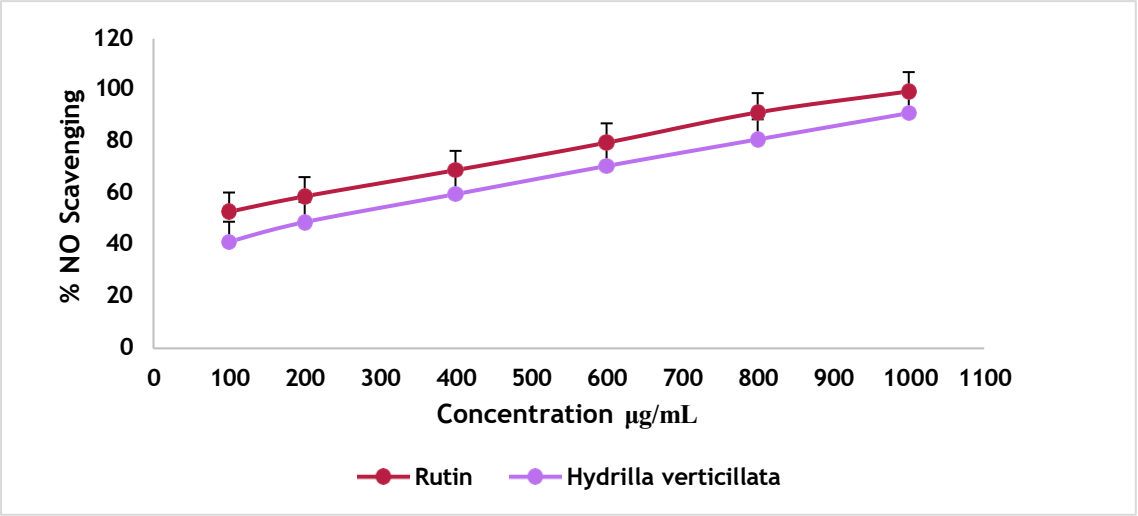


Figure 5: Curve of NO for Rutin, H. verticillata leaves

Table 6: For IC_{50} value of Rutin, H. verticillata

S. No.	Compound	IC_{50}
1.	**Rutin**	35.21905
2.	***H. verticillata***	233.663

***In-vivo* anti-hyperlipidemic activity**

Body Weight

The effect of EEHV on body weight BW was evaluated in Triton X-100-induced hyperlipidemic rats. Body weights were measured on Days 1, 16, and 22 to monitor changes over time. Administration of Triton X-100 (100 mg/kg, i.p.) induced a significant decrease in body weight by Day 22 (196.00 ± 2.80 g) compared to the normal control group (229.00 ± 2.75 g), indicating successful induction of the hyperlipidemic condition. Treatment with EEHV at doses of 200 mg/kg and 400 mg/kg (p.o.) produced a dose-dependent improvement in body weight, restoring it to 208.80 ± 2.20 g and 212.00 ± 2.78 g, respectively, by Day 22. These improvements were statistically significant when compared to the Triton-induced group, with *p < 0.05 and **p < 0.005, respectively. The higher dose of EEHV (400 mg/kg) demonstrated superior efficacy, nearly restoring body weight to control levels. Atorvastatin (10 mg/kg, p.o.), used as the reference standard, exhibited the most significant effect, increasing body weight to 214.13 ± 2.82 g by Day 22 (***p < 0.001), reflecting a highly significant protective effect against Triton X-100-induced ($^{@}$disease group). body weight loss. All data are presented as mean ± SEM (n = 6 per group) and were evaluated using Two-Way ANOVA followed by Tukey's multiple comparison test.

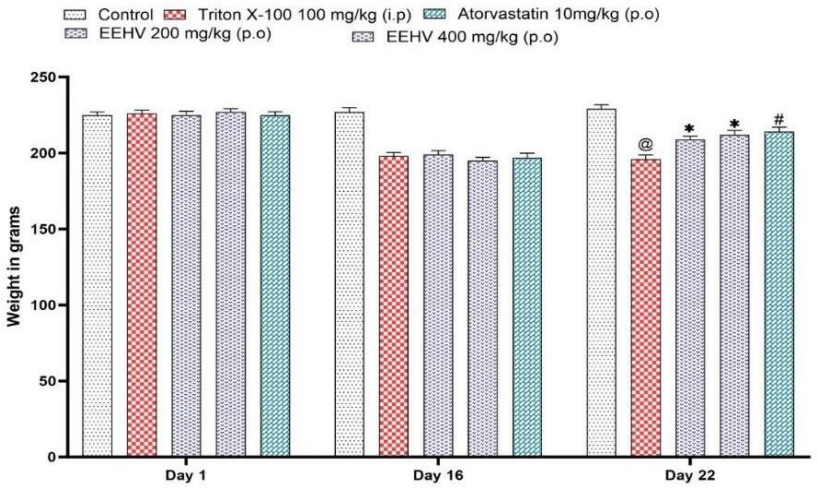


Figure 6: Effect of EEHV on Body Weight in Triton X-100 Induced Rats. Data were analyzed using Two-Way ANOVA followed by Tukey's multiple comparison test. Values are expressed as mean ± SEM (n=6). On day 22, EEHV at 200 mg/kg and 400 mg/kg showed statistically significant increase in body weight (*p < 0.05 and **p < 0.005, respectively), while Atorvastatin exhibited a very highly significant effect (***p < 0.001) vs @disease group.

Biochemical analysis:

Serum estimations

In vivo pharmacological activities of EEHV were evaluated by assessing its effect on lipid profiles against Triton X-100-induced hyperlipidemic rats. Treatment with the EEHV significantly reduced triglyceride (TG) 137.165 ± 1.46 and total cholesterol (TC) 115.865 ± 1.60 levels in a dose-dependent manner (**p < 0.005), with the 400 mg/kg dose showing better efficacy (***p < 0.001) 129.890 ± 1.58, and 113.790 ± 1.80 as compared to the 200 mg/kg dose (Fig 7). Furthermore, *H. verticillata* significantly improved high-density lipoprotein cholesterol (HDL-C) levels, with the higher dose exhibiting a greater effect (***p < 0.001) 34.581 ± 1.471. Treatment with EEHV significantly reduced creatinine levels 0.971 ± 0.102. Atorvastatin, used as a reference drug, demonstrated the most significant effects (****p < 0.0001) on all parameters as compared to @disease group. (Fig 7).

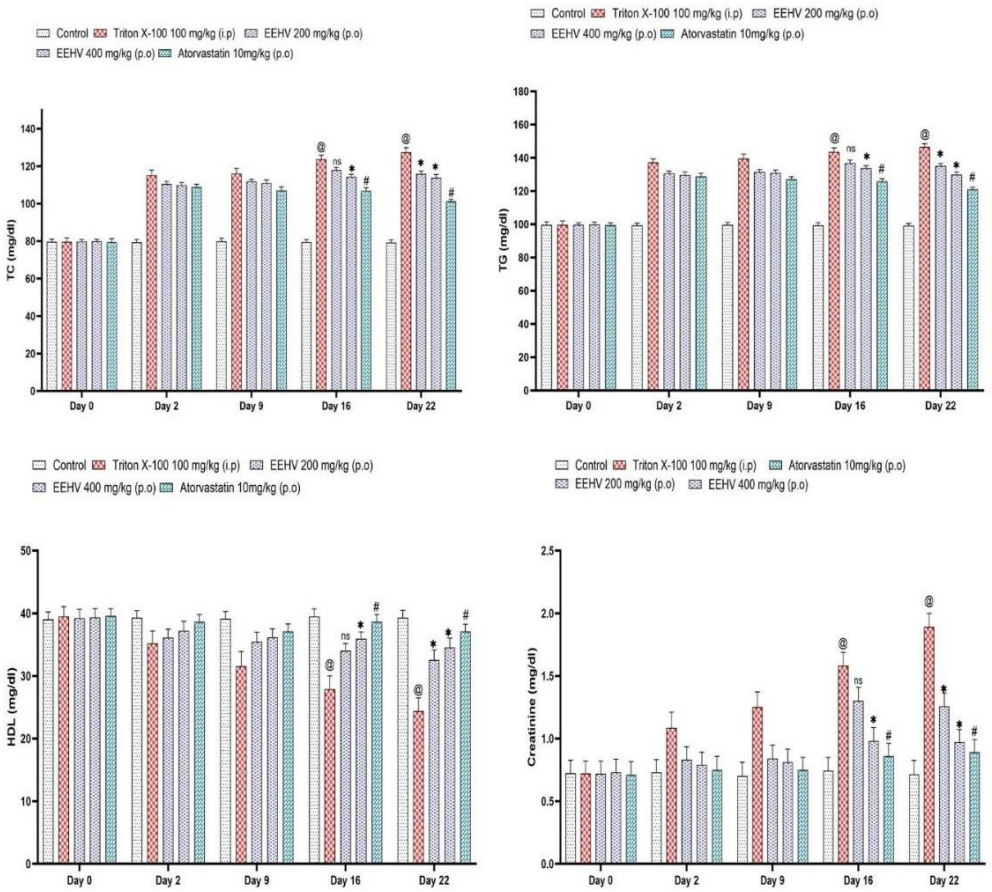


Figure 7: Effect of EEHV on TG, TC, HDL, and Creatinine Levels in Triton X-100 Induced Rats. Data were analyzed using Two-Way ANOVA followed by Tukey's multiple comparison test. Values are expressed as mean ± SEM (n=6). On day 22, EEHV at 200 mg/kg and 400 mg/kg showed statistically significant reductions in levels of aforementioned biomarkers **p < 0.005 and ***p < 0.001, respectively), while Atorvastatin exhibited a very highly significant effect (****p < 0.0001) vs @disease group.

The *EEHV* also exhibited hepatoprotective effects, as evidenced by the significant reduction in alanine aminotransferase (ALT) 81.79 ± 2.17 and aspartate aminotransferase (AST) 120.49 ± 2.37 levels (**p < 0.005) in rats. The higher dose (400 mg/kg) showed a more pronounced reduction in these liver enzymes 71.25 ± 2.74 and 112.15 ± 2.94 respectively, (***p < 0.001) compared to the lower dose, although atorvastatin displayed the most substantial hepatoprotective effect. Additionally, treatment with EEHV resulted in significant reductions in serum albumin level, with the 400 mg/kg dose showing superior efficacy 20.45 ± 0.45 (***p < 0.001). Standard drug atorvastatin produced the most pronounced effect 19.25 ± 0.21 (****p < 0.0001) against @disease group. Potential of *H. verticillata* was evaluated by measuring blood urea nitrogen (BUN), albumin levels in rats. Treatment with EEHV significantly reduced BUN levels 31.21 ± 1.20

(**p < 0.005), indicating the plant's potential to protect. The 400 mg/kg dose 29.11 ± 1.20 (***p < 0.001) was more effective than the 200 mg/kg dose, and while atorvastatin demonstrated the most significant reduction, *H. verticillata* 27.15 ± 1.0 ($^{\#}$p < 0.0001) exhibited promising protective effects against $^{@}$disease group (Fig- 8).

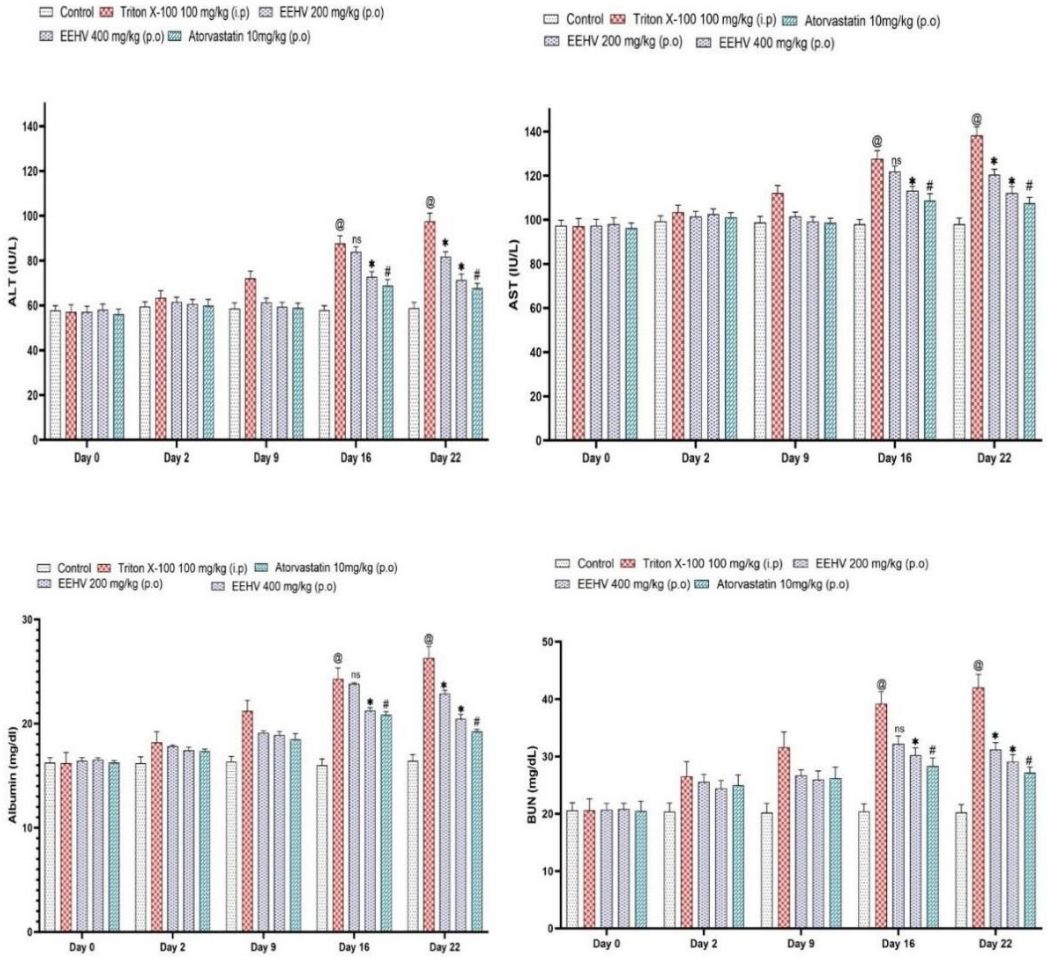


Figure 8: The impact of EEHV on ALT, AST, Albumin, and BUN concentrations was evaluated in rats subjected to Triton X-100 administration. Statistical analysis was performed using Two-Way ANOVA, followed by Tukey's post-hoc test to account for multiple comparisons. Data are presented as the mean ± standard error of the mean (SEM), with a sample size of six animals per group (n=6). EEHV at 200 mg/kg and 400 mg/kg showed statistically significant reductions in earlier mentioned levels (**p < 0.005 and ***p < 0.001, respectively), while Atorvastatin exhibited a very highly significant effect (****p < 0.0001) vs $^{@}$disease group.

In rats, administration of Triton X-100 led to a significant increase in serum bilirubin levels. Treatment with EEHV resulted in a statistically significant reduction in bilirubin levels 0.551 ± 0.03 (**P<0.005), with the 400 mg/kg dose showing better efficacy 0.521 ± 0.02 (***p < 0.001) compared to the lower dose. Atorvastatin exhibited the most substantial effect, inducing a highly significant decrease in bilirubin levels 0.425 ± 0.02 (****p < 0.0001) when compared with $^{@}$disease group (Fig- 9).

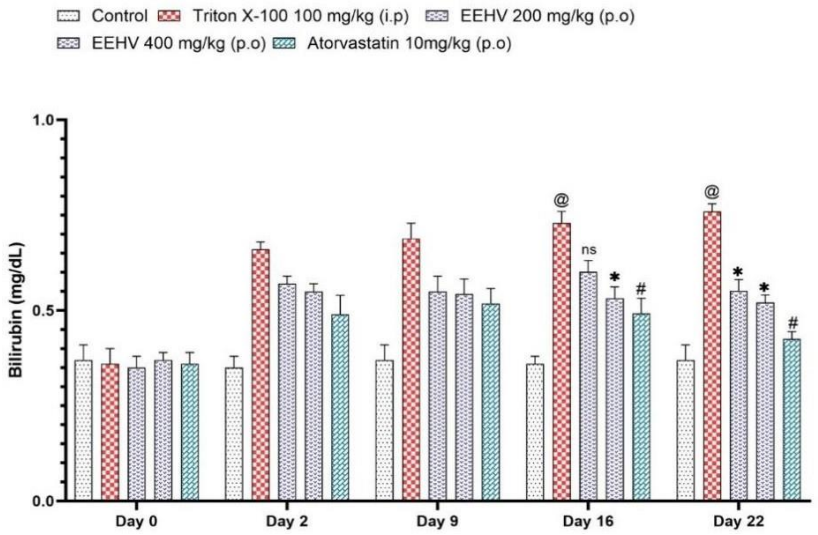


Figure 9: In rats challenged with Triton X-100, the impact of EEHV on bilirubin levels was evaluated. Results are presented as mean ± SEM (n=6) and analyzed via Two-Way ANOVA, with Tukey's post-hoc test applied for multiple comparisons. EEHV at 200 mg/kg and 400 mg/kg showed statistically significant reductions in level of bilirubin (**p < 0.005 and ***p < 0.001, respectively), while Atorvastatin exhibited a very highly significant effect (****p < 0.0001) vs @disease group.

Oxidative stress markers, including thiobarbituric acid-reactive substances (TBARS), GSH, and MDA levels, were assessed to detect the antioxidant potential of *H. verticillata.* The extract significantly reduced TBARS 51.250 ± 2.64 and MDA levels 1.1 ± 0.2 (***p < 0.001), indicative of its ability to reduce lipid peroxidation and oxidative damage. Moreover, EEHV significantly elevated hepatic GSH levels 27.54 ± 1.33 (**P<0.005, providing further evidence of its antioxidant capacity. The higher dose of EEHV (400 mg/kg) 28.47 ± 1.20 (***p < 0.001) demonstrated greater efficacy in restoring GSH levels compared to the lower dose (200 mg/kg), although atorvastatin showed the most substantial effect in all oxidative stress markers. Fig 10 illustrates the detailed results at the significant level of (****p < 0.0001) as compared to @disease group.

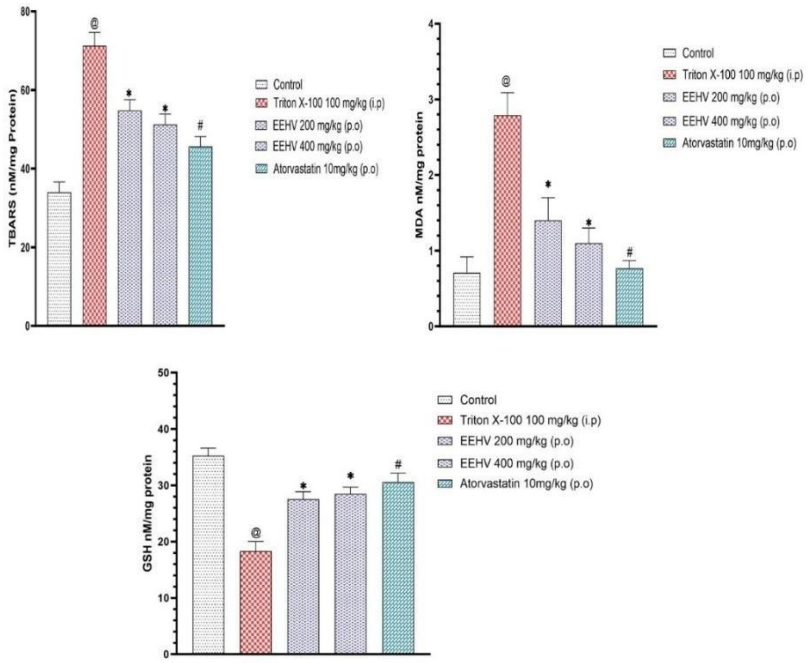


Figure 10: The effects of EEHV on TBARS, GSH, MDA levels were evaluated in rats subjected to Triton X-100. Data are represented as mean ± SEM (n=6) and were obtained using one-way ANOVA followed by Tukey's post-hoc test for multiple comparisons. EEHV at 200 mg/kg and 400 mg/kg showed statistically significant alteration in the level of aforementioned biomarkers (**p < 0.005 and ***p < 0.001, respectively), while Atorvastatin exhibited highly significant effect (****p < 0.0001) vs @disease group.

Histopathology of harvested liver for the investigation of EEHV effects against Triton X-100 induced hyperlipidemia

The histological evaluation of liver tissues across all groups revealed significant differences in response to hyperlipidemia and treatment. The Control group exhibited normal hepatic architecture, with intact hepatocytes (H), clear hepatic cords (HC), central vein (CV), and normal sinusoids (S), without pathological alterations (Fig. 11a). In contrast, the Negative Control group (hyperlipidemia-induced) showed pronounced hepatic damage characterized by macrovesicular steatosis (St), inflammatory cell (IC) infiltration, hepatocellular necrosis, pyknotic nuclei (PN), ballooning degeneration, and disrupted hepatic cords (DC) (Fig. 11b). The Positive Control group (atorvastatin-treated) displayed moderate morphological recovery, with decreased lipid accumulation and minimal inflammatory response (Fig. 11c). The group treated with ethanolic extract of EEHV at 200 mg/kg showed partial hepatoprotection, although lipid vacuoles, degenerative hepatocytes (DH), and Bile Duct (BD), Lobular Vein (LV) slight inflammation persisted (Fig. 11d). Notably, EEHV at 400 mg/kg resulted in substantial hepatic restoration, exhibiting nearly normal Lobular Vein (LV) no sign of inflammation, absence of, Degenerative Hepatocytes (DH), Destructed Hepatic Cords (DC), and organized Hepatocytes (H) and Sinusoids (S), suggesting a dose-dependent therapeutic effect (Fig. 11e).

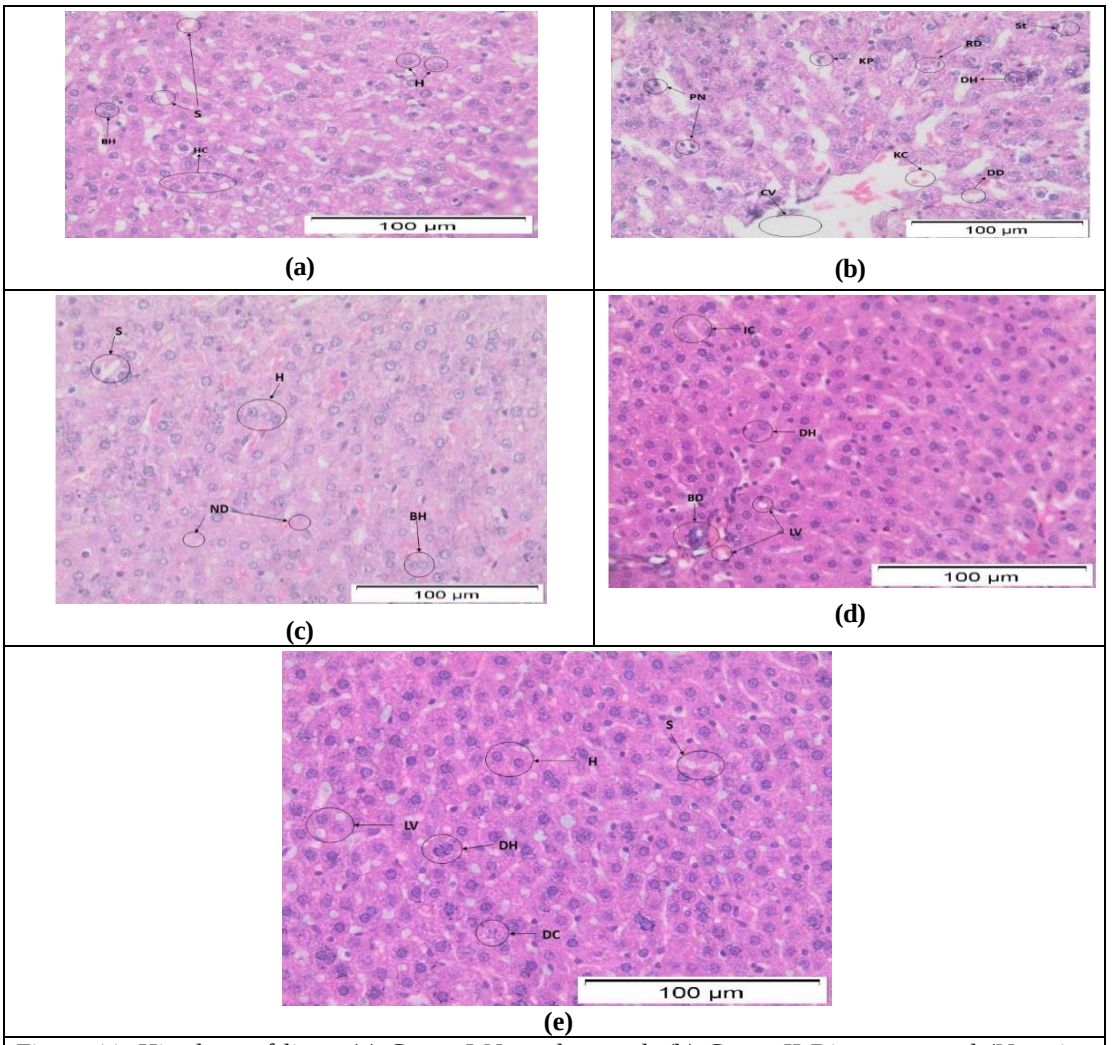


Figure 11: Histology of liver: (a) Group I Normal control; (b) Group II Disease control (Negative control); (c) Group III- Standard (Positive control) treated with atorvastatin (10mg/kg P.O); (d) Group IV Treatment Group I, treated with EEHV (200 mg/kg, p.o.); (e) Group IV Treatment Group 2, treated with EEHV (400 mg/kg, p.o.)

Abbreviations: S= Sinusoids; H= Hepatocytes; BH= Binucleated hepatocytes; HC= Hepatic cords; PN= Pyknotic nucleus; CV= Central Vein; KP= Kupffer cell proliferation; St= Steatosis; DH= Degenerative Hepatocytes; KC= Kupffer cells; DD= Dilated Disse Space; ND= Normal Disse Space; DC= Destructed Hepatic Cords; IC= Inflammatory Cells; LV= Lobular Vein; BD= Bile Duct.

4. DISCUSSION

The present investigation elucidates the pharmacological potential of *H. verticillata* through a comprehensive assessment of its phytochemical profile and bioactivity in established experimental models. Triton X-100 was employed as a well-established agent to induce acute hyperlipidemia due to its ability to interfere with lipid metabolism by inhibiting lipoprotein lipase activity, thereby leading to elevated circulating lipid levels [29]. Preliminary phytochemical screening of the ethanolic extract (EEHV) revealed the presence of tannins, saponins, alkaloids, terpenoids, flavonoids, and phenolic compounds, whereas glycosides, steroids, and amino acids were absent. The presence of these identified secondary plant metabolites scientifically supports the versatile pharmacological properties of the plant extract. The detection of tannins and phenolics both widely recognized for their antioxidant, anti-inflammatory, and hepatoprotective activities offers a biochemical rationale for the extract's antioxidant efficacy, consistent with previous reports on the therapeutic relevance of these phytoconstituents [30]. After preliminary qualitative identification of phytoconstituents with in the EEHV. Study was further proceeded for quantitative estimation of bioactives to better corelate with dose dependent pharmacological effects.

Ethanolic extraction yielded 18.2%, indicating favorable efficiency for isolating ethanol-soluble constituents from *H. verticillata*. This yield surpasses previously reported values obtained via ultrasonic-assisted extraction (UAE), which yielded 10.3% under comparable conditions [26], thereby affirming ethanol's broad-spectrum solubilizing capacity for polar and semi-polar phytochemicals. Quantitative phytochemical profiling of EEHV revealed a high content of total phenolics (166.28 ± 0.03 mg GAE/ 100g), flavonoids (120.07 ± 0.07 mg QE/ 100g), and terpenoids (146.92 ± 0.08 mg linalool equivalents/100 g). These values significantly exceed those reported in earlier qualitative and semi-quantitative analyses, marking a substantial advancement in the phytochemical characterization of this species. For instance, Pandi Prabha and Rajkumar (2015) identified phytol a key terpenoid comprising 70% of the ethanolic fraction via GC-MS but did not report standardized quantitative equivalents. Similarly, studies such as Boossayarat Petpheng (2024) have consistently noted the presence of flavonoids and phenols, yet lacked quantitative precision. Thus, the present study provides one of the first comprehensive and standardized quantifications of EEHV's phytochemical composition, facilitating meaningful cross-comparisons across species and extraction methodologies [7, 31].

The antioxidant potential of EEHV was validated through in vitro assays, wherein the extract exhibited concentration-dependent radical scavenging activity. In the DPPH assay, EEHV demonstrated 29.65% inhibition at 10 µg/mL, reaching 90.96% at 1000 µg/mL, with an IC_{50} of 245.99 µg/mL, indicating moderate potency relative to gallic acid (IC_{50} = 65.55 µg/mL). In the nitric oxide scavenging assay, EEHV showed 91.16% inhibition at the highest concentration, with an IC_{50} of 233.66 µg/mL, while rutin demonstrated near-complete inhibition (99.6%). These values confirm notable antioxidant potential, though somewhat less potent than standard references. Behera and Satapathy (2023) reported lower IC_{50} values for methanolic and n-hexane extracts (16.84 µg/mL and 36.26 µg/mL, respectively), highlighting the influence of solvent polarity and phytochemical specificity on antioxidant responses [32]. Moreover, an Indonesian study reported a higher IC_{50} (608.45 µg/mL) for EEHV, further underscoring the improved potency observed in our extract [33].

Following the demonstration of *in vitro* antioxidant efficacy, the extract was subsequently investigated for its potential lipid-lowering activity in an *in vivo* hyperlipidemic screening model. Comparative analysis of EEHV's *in-vivo* lipid-modulating effects revealed promising, dose-dependent improvements in a Triton X-100–induced hyperlipidemic rat model. EEHV significantly reduced TG at 200 mg/kg =137 mg/dL and 400 mg/kg =129 mg/dL, and TC at 200 mg/kg =116 mg/dL and 400 mg/kg =114 mg/dL, with the higher dose exhibiting more pronounced effects with significant values of $^{**}p < 0.005$ to $^{***}p < 0.001$), and markedly increased HDL-C (34.6 mg/dL, $^{***}p < 0.001$). Furthermore, serum creatinine levels were reduced (0.97 mg/dL), whereas atorvastatin, the reference drug, produced even greater reductions across all parameters ($^{****}p < 0.0001$) vs $^{@}$disease group [34]. In a stress-induced vascular dysfunction model mimicking oxidative and lipid metabolic disturbances *H. verticillata* improved vascular outcomes, indirectly supporting its role in lipid regulation and endothelial health [35]. Additionally, EEHV demonstrated significant anti-adipogenic effects in vitro, attributed to its high phytol content, indicating potential systemic metabolic benefits [12]. Collectively, these results establish EEHV as a promising natural lipid-lowering agent, supported by consistent reductions in TG and TC, HDL-C elevation, and improved hepatic function.

Triton X-100 (100 mg/kg, i.p.) significantly reduced body weight by Day 22 (196.00 ± 2.80 g) compared to the normal control (229.00 ± 2.75 g), indicating successful hyperlipidemia induction. Treatment with EEHV at 200 mg/kg and 400 mg/kg (p.o.) significantly improved body weight to 208.80 ± 2.20 g (*$p < 0.05$) and 212.00 ± 2.78 g (**$p < 0.005$), respectively, in a dose-dependent manner. The higher dose showed greater efficacy, nearly restoring weight to control levels. Atorvastatin (10 mg/kg, p.o.) was most effective, increasing weight to 214.13 ± 2.82 g (***$p < 0.001$) as compared to $^{@}$disease group. These results are consistent with earlier studies where plant extracts, such as *Syzygium cumini* and *Argyreia nervosa*, demonstrated similar improvements in body weight and lipid profiles in Triton-induced models. The observed effects suggest that EEHV may exert protective metabolic effects comparable to standard antihyperlipidemic agents [36- 38].

Considering the liver's pivotal role in lipid metabolism, the hepatoprotective potential of EEHV was subsequently examined. This protective role was further substantiated by EEHV's ability to restore hepatic enzyme markers in Triton X-100–intoxicated rats. Both ALT and AST were significantly reduced at 200 mg/kg and 400 mg/kg doses (***$p < 0.001$), with atorvastatin again demonstrating maximal efficacy (****$p < 0.0001$) against $^{@}$disease group. These findings are consistent with earlier studies involving *H. verticillata* in aquatic species, where dietary administration mitigated heavy metal-induced hepatotoxicity and normalized hepatic architecture [39]. Similarly, *Hygrophila auriculata* extracts were shown to restore liver enzymes and improve histopathological outcomes in CCl_4-induced liver injury models, with alkaloid fractions providing efficacy comparable to silymarin [40]. In the current study, EEHV also significantly decreased serum bilirubin levels (0.551 ± 0.03 at 200 mg/kg and 0.521 ± 0.02 at 400 mg/kg; **$p < 0.005$ and ***$p < 0.001$), and increased albumin levels (20.45 ± 0.45, ***$p < 0.001$), further confirming hepatocellular recovery. The observed reductions in blood urea nitrogen (BUN: 31.21 ± 1.20 and 29.11 ± 1.20 at respective doses) further corroborating the extract's multiorgan protective profile.

In vivo antioxidant efficacy of EEHV was also evident through significant modulation of oxidative stress markers. TBARS and MDA levels were markedly reduced (51.25 ± 2.64 and 1.1 ± 0.2, ***$p < 0.001$), while GSH levels increased (27.54 ± 1.33 and 28.47 ± 1.20 at 200 and 400 mg/kg, respectively; **$p < 0.005$, ***$p < 0.001$). These outcomes were statistically validated via one-way ANOVA followed by Tukey's post hoc test ($n = 6$), with atorvastatin exerting the most potent antioxidant effects (****$p < 0.0001$) when compared with $^{@}$disease group. [41]. These findings align with established roles of TBARS and GSH as markers of lipid peroxidation and antioxidant defence [42, 43]. Comparable antioxidant responses were reported in *H. auriculata*, with enhanced GPx, GST, SOD, and CAT activities in diabetic and toxin-induced models [44].

Histopathological evaluation supported these biochemical findings. The normal control group exhibited well-preserved hepatic architecture with polygonal hepatocytes, organized hepatic cords, central nuclei, and intact sinusoids. In contrast, the hyperlipidemic group (negative control) showed extensive hepatic degeneration macrovesicular steatosis, inflammation, necrosis, pyknotic nuclei, and architectural disruption typical of steatohepatitis (Raj *et al*., 2010). Atorvastatin treatment (positive control) led to partial histological restoration, with decreased lipid accumulation, reduced inflammatory foci, binucleation of hepatocytes, and improved hepatic cord alignment, albeit with minor residual changes [40]. Treatment with EEHV at 200 mg/kg yielded modest improvements, including reduced steatosis and necrosis, though lipid vacuoles and mild lobular inflammation persisted. However, at 400 mg/kg, EEHV induced substantial morphological recovery, evidenced by restored hepatic cords, absence of necrosis, reappearance of sinusoidal architecture, and normalized hepatocytes indicative of a robust dose-dependent protective response. These results mirror prior observations with *H. auriculata*, where 200 mg/kg significantly attenuated hepatic damage and restored near-normal histology [39]. Similar hepatoprotective trends have been noted in botanicals such as *Moringa oleifera* and *Totum 070*, both demonstrating dose-dependent reversal of hepatic steatosis and necroinflammation [45, 46]. Together, these results establish EEHV as a potent candidate for integrated management of oxidative stress, hyperlipidemia, and hepatic dysfunction. Further investigations are warranted to elucidate its mechanisms and potential clinical applicability.

5. CONCLUSION

H. verticillata demonstrates substantial therapeutic potential owing to its rich phytochemical composition and broad-spectrum pharmacological activities. The ethanolic extract exhibits marked lipid-lowering effects, along with notable antioxidant and hepatoprotective properties, largely attributable to its bioactive constituents such as flavonoids, phenolic acids, saponins, alkaloids, and terpenoids. These findings align with its ethnomedicinal usage in managing oxidative stress-associated disorders and underscore its role in modulating lipid metabolism and conferring protection against hepatic and renal impairment. Notably, administration of the higher dose (400 mg/kg) produced superior efficacy, particularly in normalizing biochemical parameters and restoring hepatic architecture. Histopathological evaluations confirmed that *H. verticillata* effectively attenuates hyperlipidemia-induced hepatic injury in a dose-dependent manner, with the high-dose treatment achieving near-complete histological recovery. Collectively, these results highlight the promise of *H. verticillata* as a natural therapeutic agent for the management of metabolic and oxidative stress-related conditions and support its further evaluation in preclinical and clinical settings for integration into modern pharmacotherapy.

Author's contributions

Dr. Madan L. Kaushik conceptualized and supervised the study, provided guidance on the experimental design and data analysis, and critically reviewed the manuscript. Shavinder Kumari carried out the research, performed the experiments, analyzed the data, interpreted the findings, and drafted the manuscript. All authors contributed to the critical revision of the manuscript for intellectual content and approved the final version for submission.

Conflicts of interest

Not applicable.

Funding Statement

N/A

Ethics Committee- Institutional Animal Ethics Committee

Date of approval- 28/12/23

REFERENCES

[1] Abbasi S, Khan A, Choudhry MW. New insights into the treatment of hyperlipidemia: pharmacological updates and emerging treatments. Cureus. 2024;16(6). https://doi.org/10.7759/cureus.63078

[2] Wan, H., Wu, H., Wei, Y., Wang, S., & Ji, Y. (2025). Novel lipid profiles and atherosclerotic cardiovascular disease risk: insights from a latent profile analysis. Lipids in Health and Disease, 24(1), 71. https://doi.org/10.1186/s12944-025-02471-3

[3] World Health Organization. Noncommunicable diseases: Risk factors. The Global Health Observatory. 2023 [cited 2025 Jul 16]. Available from: https://www.who.int/data/gho/data/themes/topics/topic-details/GHO/ncd-risk-factors

[4] Pirillo A, Casula M, Olmastroni E, Norata GD, Catapano AL. Global epidemiology of dyslipidaemias. Nat Rev Cardiol. 2021;18(10):689–700 https://doi.org/10.1038/s41569-021-00541-4.

[5] Parwin A, Najmi AK, Ismail MV, Kaundal M, Akhtar M. Protective effects of alendronate in Triton X-100-induced hyperlipidemia in rats. Turk J Gastroenterol. 2019;30(6):557–64. https://doi.org/10.5152/tjg.2019.18076

[6] Saleem U, Riaz S, Ahmad B, Saleem M. Pharmacological screening of Trachyspermum ammi for antihyperlipidemic activity in Triton X-100 induced hyperlipidemia rat model. Pharmacogn Res. 2017;9(Suppl 1):S34–40. https://doi.org/10.4103/pr.pr_37_17

[7] Pandi Prabha S, Rajkumar J. Phytochemical screening and bioactive potential of Hydrilla verticillata. J Chem Pharm Res. 2015;7(3):1809–15. https://www.researchgate.net/publication/306180562_Phytochemical_screening_and_bioactive_potential_of_Hydrilla_verticillata

[8] Behera B, Meher RK, Mir SA, Nayak B, Satapathy KB. Phytochemical profiling, in vitro analysis for anti-inflammatory, immunomodulatory activities, structural elucidation and in silico evaluation of potential selective COX-2 and TNF-α inhibitor from Hydrilla verticillata (Lf) Royle. J Biomol Struct Dyn. 2025;43(2):859–73. https://doi.org/10.1080/07391102.2023.2283871

[9] Rajkumar J. Hepatotoxic effect of lead and hepatoprotective effect of Hydrilla verticillata on hepatic transcriptional and physiological response in edible fish Labeo rohita. Drug Chem Toxicol. 2022;45(3). https://doi.org/10.1080/01480545.2020.1815762

[10] Pal DK, Nimse SB. Screening of the antioxidant activity of Hydrilla verticillata plant. Asian J Chem. 2006;18(4):3004. https://asianpubs.org/index.php/ajchem/article/view/20722

[11] Kensa VM, Neelamegam R. GC-MS determination of bioactive constituents of Hydrilla verticillata (Lf) Royle collected from unpolluted and polluted water sources. Asian J Biol. 2016;1(1):1-6. 10.9734/AJOB/2016/30954

[12] Prabha SP, Sadhana S, Karthik C, Caroline DG. In vitro evaluation of Hydrilla verticillata for anti-adipogenesis activity on 3T3 L1 cell lines. Pharmacogn Mag. 2020;16(05). https://doi.org/10.4103/pm.pm_234_20

[13] Khare V, Kushwaha P, Verma S, Gupta A, Srivastava S, Rawat AKS. Pharmacognostic evaluation and antioxidant activity of Urtica dioica L. Chin Med. 2012;3(3):128–35. https://doi.org/10.4236/cm.2012.33021

[14] Gami B, Parabia MH. Pharmacognostic evaluation of bark and seeds of Mimusops elengi L. Int J Pharm Pharm Sci. 2010;2(Suppl 4):110–3. https://innovareacademics.in/journal/ijpps/Vol2Suppl4/813.pdf

[15] Kaushik ML, Goutam N, Ashawat MS. Anti-Nephrolithiasis activity of ethanol extract of Ipomoea carnea

Jacq. against ethylene glycol-induced urolithiasis in rats. Asian J Pharm Clin Res. 2025;18(5):96–104. Available from: https://journals.innovareacademics.in/index.php/ajpcr/article/view/54311

[16] Mukherjee PK. Quality control and evaluation of herbal drugs: Evaluating natural products and traditional medicine. Elsevier; 2019 May 30. https://shop.elsevier.com/books/quality-control-and-evaluation-of-herbal-drugs/mukherjee/978-0-12-813374-3.

[17] Jagetia GC, Baliga MS. The evaluation of nitric oxide scavenging activity of certain Indian medicinal plants in vitro: a preliminary study. J Med Food. 2004;7(3):343–8. https://doi.org/10.1089/jmf.2004.7.343.

[18] Jayachandra K, Maheswaran A, Murali M. In-vitro evaluation of nitric oxide scavenging activity of methanolic and aqueous extract of syzygium cumini Linn. Bark (Myrtaceae). International journal of pharmaceutical sciences and research. 2012 Feb 1;3(2):615. http://dx.doi.org/10.13040/IJPSR.0975-8232.3(2).615-19\

[19] Ballesteros-Ramírez R, Lasso P, Urueña C, Saturno J, Fiorentino S. Assessment of acute and chronic toxicity in Wistar rats (Rattus norvegicus) and New Zealand rabbits (Oryctolagus cuniculus) of an enriched polyphenol extract obtained from Caesalpinia spinosa. J Toxicol. 2024;2024:3769933. https://doi.org/10.1155/2024/3769933

[20] Kensa VM, Neelamegam R. Evaluation of wound healing activity of Hydrilla verticillata (LF) Royle collected from unpolluted and polluted water sources. Int J Pharm Pharm Sci. 2014;6(3):417-23. https://www.cabidigitallibrary.org/doi/full/10.5555/20153018162

[21] Penumarthy S, Penmetsa GS, Mannem S. Assessment of serum levels of triglycerides, total cholesterol, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol in periodontitis patients. J Indian Soc Periodontol. 2013;17(1):30–5. https://doi.org/10.4103/0972-124X.107471

[22] Sahu S, Chawla R, Uppal B. Comparison of two methods of estimation of low-density lipoprotein cholesterol, the direct versus Friedewald estimation. Indian J Clin Biochem. 2005;20:54–61. https://doi.org/10.1007/BF02867401

[23] Borges LP, Brandao R, Godoi B, Nogueira CW, Zeni G. Oral administration of diphenyl diselenide protects against cadmium-induced liver damage in rats. Chem Biol Interact. 2008;171(1):15–25. https://doi.org/10.1016/j.cbi.2007.09.005

[24] Chukwudoruo CS, Osuji-Kalu-Ibe NC, Igwe KO, Iheme CI, Ujowundu FN, Mba BA. Serum total protein concentration and liver enzymes activities in albino rats model administered with ethanolic leaf extract of Ficus capensis. Afr J Biotechnol. 2021;20(4):164–8. https://doi.org/10.5897/AJB2020.17107

[25] Alum EU, Umoru GU, Uti DE, Aja PM, Ugwu OP, Orji OU, et al. Hepato-protective effect of ethanol leaf extract of Datura stramonium in alloxan-induced diabetic albino rats. J Chem Soc Niger. 2022;47(5). https://doi.org/10.46602/jcsn.v47i5.819

[26] Chargui I, Grissa I, Bensassi F, Hrira MY, Haouem S, Haouas Z, et al. Oxidative stress, biochemical and histopathological alterations in the liver and kidney of female rats exposed to low doses of deltamethrin (DM): a molecular assessment. Biomed Environ Sci. 2012;25(6):672–83. https://doi.org/10.3967/0895-3988.2012.06.009

[27] Olatosin TM, Akinduko DS, Uche CZ. Evaluation of the hepatoprotective efficacy of Moringa oleifera seed oil on CCl_4-induced liver damage in Wistar albino rats. Int J Eng Sci. 2013;2(11):13–8. https://www.semanticscholar.org/paper/Evaluation-of-the-Hepatoprotective-Efficacy-of-Seed-Olatosin/ad2f5451b3e5039617c347dde61a4347efd356f6

[28] Friday OA, Eke MO. Inclusive diet of Balanites aegyptiaca Del defatted protein meal and protein concentrate on organ weight and diabetic conditioned liver of Wister albino rat. Bangladesh J Food Nutr. 2024;1(1):13–25. https://doi.org/10.70945/bjfn.2024.v01i01.06

[29] Inamdar S, Ahmad M, Tazneem B, Hashmi S, Kulkarni B, Aldhabi BM, et al. Molecular docking analysis of Ficus religiosa L. fruit extracts with HMG-COA reductase inhibitors, anti-hyperlipidemic effects of its in Albino wistar rats, triggered by Triton-X 100 and High fat diet. Bioact Carbohydr Diet Fibre. 2024;32:100429. https://doi.org/10.1016/j.bcdf.2024.100429

[30] Wang C, Zhang SH, Wang PF, Li W, Lu J. Effects of ammonium on the antioxidative response in Hydrilla verticillata (Lf) Royle plants. Ecotoxicol Environ Saf. 2010;73(2):189–95. https://doi.org/10.1016/j.ecoenv.2009.08.012

[31] Petpheng B, Mudtaleb B, Piboon W, Paichid N, Sangkharak K. The extraction of phytol from Hydrilla verticillata using ultrasonic-assisted extraction (UAE), the analysis of antibacterial activity, and the utilization of residual extract for polyhydroxyalkanoate (PHA) production. Biomass Convers Biorefin. 2024;14(18):22007–17. https://doi.org/10.1007/s13399-023-04331-5

[32] Behera B, Satapathy KB. Studies on in vitro evaluation of antibacterial and antioxidant activity of Hydrilla verticillata (Lf) Royle. Plant Sci Today. 2023;10(4):66–71. https://doi.org/10.14719/pst.2312

[33] Joselin M. Karakterisasi simplisia dan skrining fitokimia serta uji aktivitas antioksidan ekstrak air dan ekstrak etanol hidrilla (Hydrilla verticillata (Lf) Royle). 2014. https://repositori.usu.ac.id/handle/123456789/77207

[34] Farheen M, Unnisa SQ, Fatima Z, Jabeen N. Invivo & Invitro hypolipidemic effect of herbal extracts on Triton X-100 induced hyperlipidemia. [unpublished]. https://doi.org/10.9790/3008-1506020116.

[35] Bhavsar V, Vaghasiya J, Trivedi V, Vashi A, Maheshwari T, Thaker P. Investigation of Hydrilla verticillata (Linn. f.) Royle in stress-induced vascular dysfunction in rats. Nutrire. 2023;48(2):42. https://doi.org/10.1186/s41110-023-00228-5

[36] Khanna AK, Rizvi F, Chander R. Lipid lowering activity of Phyllanthus niruri in hyperlipemic rats. Journal of ethnopharmacology. 2002 Sep 1;82(1):19-22. https://doi.org/10.1016/S0378-8741(02)00136-8

[37] Singh S, Singh L, Sagar BP, Das M. Evaluation of Antihyperlipidemic Activity of Ethanolic Extract of Syzygium cumini in Triton X-100 Induced Hyperlipidemic Rats. International Journal of Pharmacy & Pharmaceutical Research. 2018;12(3):40-54. https://ijppr.humanjournals.com/wp-content/uploads/2018/07/4.Shailendra-Singh-Lalit-Singh-B.P.S.Sagar-Manas-Das.pdf

[38] Goud VS, Babu SR, Srinivas N. ANTIHYPERLIPIDEMIC ACTIVITY OF ARGYREIA NERVOSA ROOT EXTRACT IN TRITON X-100 INDUCED HYPERLIPIDEMIC RATS. https://doi.org/10.20959/wjpr20164-5770.

[39] Prabha SP, Karthik C, Priya GS. Evaluation of protective effect of aquatic weed Hydrilla verticillata against lead-induced acute liver toxicity in fish. Int J Pharm Sci Res. 2021;12(1):183–7. https://doi.org/10.13040/IJPSR.0975-8232.12(1).183-87

[40] Raj VP, Chandrasekhar RH, Vijayan P, Dhanaraj SA, Rao MC, Rao VJ, et al. In vitro and in vivo hepatoprotective effects of the total alkaloid fraction of Hygrophila auriculata leaves. Indian J Pharmacol. 2010;42(2):99–104. https://doi.org/10.4103/0253-7613.64500

[41] Rastogi S, Bachhar V, Joshi V, Kumar S, Panjwani D, Singh L, Haldhar R. Therapeutic potential of Glycyrrhiza glabra L. extract and atorvastatin in HFD-induced MAFLD: in vitro and in vivo exploration. 3 Biotech. 2025 Jun;15(6):155. https://doi.org/10.1007/s13205-025-04322-5

[42] Malpotra M, Garg M, Singh N, Sadhu SD, Chopra R, Sharma B. An overview of bioactive components and phytopharmaceutical potentials of Hygrophila auriculata—a herbaceous medicinal plant. Phytomed Plus. 2025:100737. https://doi.org/10.1016/j.phyplu.2025.100737

[43] Tsikas D. Assessment of lipid peroxidation by measuring malondialdehyde (MDA) and relatives in biological samples: Analytical and biological challenges. Analytical biochemistry. 2017 May 1;524:13-30. https://doi.org/10.1016/j.ab.2016.10.021

[44] Vijayakumar M, Govindarajan R, Rao GMM, Rao CV, Shirwaikar A, Mehrotra S, et al. Action of Hygrophila auriculata against streptozotocin-induced oxidative stress. J Ethnopharmacol. 2006;104(3):356–61. https://doi.org/10.1016/j.jep.2005.09.030

[45] Anwar F, Latif S, Ashraf M, Gilani AH. Moringa oleifera: a food plant with multiple medicinal uses. Phytother Res. 2007;21(1):17–25.https://doi.org/10.1002/ptr.2023

[46] Mizelman E, Chilibeck PD, Hanifi A, Kaviani M, Brenna E, Zello GA. A low-glycemic index, high-fiber, pulse-based diet improves lipid profile, but does not affect performance in soccer players. Nutrients. 2020;12(5):1324. https://doi.org/10.3390/nu12051324