ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 18, Issue 10, 2025

Online - 2455-3891
Print - 0974-2441

Research Article

PROTECTIVE MODULATORY POTENTIAL OF *AZOLLA PINNATA* EXTRACT ON LIPID METABOLISM, HEPATIC DYSFUNCTIONING, ALONG WITH MITIGATION OF OXIDATIVE STRESS IN TRITON X-100 INDUCED HYPERLIPIDEMIC RATS

MADAN L. KAUSHIK[1], SHAVINDER KUMARI[1]*, MAHENDRA SINGH ASHAWAT[2]

[1]Department of Pharmacology, Aadarsh Vijendra Institute of Pharmaceutical Sciences, Shobhit University, Gangoh, Uttar Pradesh, India.
[2]Department of Pharmaceutics, Laureate Institute of Pharmacy, Bahni, Himachal Pradesh, India.
*Corresponding author: Shavinder Kumari; Email: shavinderkumari.doad@gmail.com

Received: 27 June 2025, Revised and Accepted: 14 August 2025

ABSTRACT

Objective: The objective of the study was to investigate the hypolipidemic and antioxidant effects of ethanolic extract of *Azolla pinnata* (EEAP) in Triton X-100-induced hyperlipidemic rats.

Methods: Hyperlipidemia was induced in male Wistar rats (n=6/group) by intraperitoneal injection of Triton X-100 (100 mg/kg). Animals were divided into five groups: normal control, Triton control, EEAP 125 mg/kg, EEAP 250 mg/kg, and atorvastatin 10 mg/kg. EEAP was prepared by Soxhlet extraction (yield: 17.2%) and screened for phytochemicals. Antioxidant potential was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and nitric oxide scavenging assays. After 7 days of treatment, serum lipid profile, liver and kidney function markers (alanine aminotransferase, aspartate aminotransferase, bilirubin, creatinine, blood urea nitrogen), oxidative stress markers (thiobarbituric acid-reactive substances [TBARS], malondialdehyde [MDA], glutathione [GSH]), and liver histology were analyzed. Data were statistically analyzed using Analysis of Variance followed by Tukey's multiple comparison test.

Results: EEAP showed moderate antioxidant activity (DPPH IC_{50}=379.17 µg/mL, and NO IC_{50}=113.9314 µg/mL) and significantly improved lipid profile by lowering triglyceride (136.18±1.04), total cholesterol (117.18±1.20), low-density lipoprotein (139.12±2.10), and very low-density lipoprotein (48.94±1.20), ***p<0.001 while increasing high-density lipoprotein (33.28±1.10 mg/dL; ***p<0.001). It normalized hepatic markers and reduced TBARS and MDA, with increased GSH. Liver histology showed improved architecture, especially at 250 mg/kg.

Conclusion: *A. pinnata*, a flavonoid and phenolic-rich aquatic fern, remains insufficiently studied for its potential in hyperlipidemia and oxidative stress management. EEAP exerted significant lipid-lowering and antioxidant effects in hyperlipidemic rats, indicating its potential as a natural therapeutic agent for hyperlipidemia and oxidative stress.

Keywords: *Azolla pinnata*, Hyperlipidemia, Triton X-100, Lipid profile, Oxidative stress, Antioxidant activity.

 DOI: http://dx.doi.org/10.22159/ajpcr.2025v18i10.55783. Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

INTRODUCTION

Hyperlipidemia, defined as elevated levels of circulating lipids, particularly low-density lipoprotein cholesterol (LDL-C), is a key risk factor for atherosclerotic cardiovascular diseases (ASCVD) such as coronary artery disease, ischemic stroke, and peripheral artery disease [1,2]. According to the World Health Organization, dyslipidemia significantly contributes to global morbidity and mortality, with ASCVD accounting for more than 17.9 million deaths annually [3]. The burden of hyperlipidemia is increasing worldwide, especially in low and middle-income countries due to rapid urbanization, dietary shifts, sedentary lifestyles, and obesity [4].

Current pharmacological therapies, including statins, fibrates, niacin, and cholesterol absorption inhibitors, are widely prescribed to manage hyperlipidemia. However, their long-term use is often associated with adverse effects such as hepatotoxicity, muscle pain, and increased risk of diabetes, along with issues related to cost and compliance. Consequently, there is an urgent need for safer and more effective therapeutic alternatives, especially those derived from natural sources.

Triton X-100, a non-ionic surfactant, is commonly used in experimental models to induce acute hyperlipidemia by blocking the uptake of lipoproteins from plasma through inhibition of lipoprotein lipase (LPL). A single intraperitoneal injection of Triton X-100 leads to marked increases in serum total cholesterol (TC), triglycerides (TGs), and LDL-C, along with a significant reduction in high-density lipoprotein cholesterol (HDL-C) [5,6]. Moreover, it induces oxidative stress and hepatic dysfunction, thus serving as a useful model to evaluate the lipid-lowering and antioxidant potential of therapeutic agents. Triton X-100 disrupts lipid balance and induces oxidative stress, *A. pinnata* emerges as a promising candidate for investigation, due to its bioactive compounds with potential hypolipidemic and antioxidant effects.

A. pinnata of *Salviniaceae* family, a fast-growing aquatic fern traditionally recognized for its agricultural significance through nitrogen fixation in association with the cyanobacterium *Anabaena azollae.* Beyond its agronomic applications, *A. pinnata* is being explored for its possible medicinal value due to the presence of diverse phytochemicals, including flavonoids, tannins, polyphenols, and saponins. These constituents are known from other plants to exhibit pharmacological activities relevant to lipid metabolism, oxidative stress, and inflammation [7]. Preliminary observations suggest that the bioactive constituents of *A. pinnata* could influence lipid-regulatory pathways. Flavonoids and polyphenols are proposed to attenuate oxidative stress by scavenging reactive oxygen species (ROS), inhibiting lipid peroxidation, and enhancing endogenous antioxidant effect [8]. These actions are particularly relevant in the context of metabolic disorders, where oxidative stress contributes to the progression of cardiovascular disease and hepatic dysfunction. Both are associated complications with that of hypolipidemic.

Although hyperlipidemia and its experimental induction using Triton X-100 are well documented in preclinical studies. The pharmacological evaluation of *A. pinnata* in the context of lipid metabolism remains largely unexplored. Despite evidence supporting its antioxidant and hepatoprotective properties, no comprehensive studies have directly investigated for its potential antihyperlipidemic activity, thereby indicating a significant gap in current research.

The potential lipid-lowering (hypolipidemic) effect of *A. pinnata* is hypothesized to involve modulation of key enzymes such as 3-Hydroxy-3-methylglutaryl coenzyme A reductas (HMG-CoA) reductase and lecithin cholesterol acyltransferase (LCAT), both central to cholesterol biosynthesis and transport. While the precise mechanisms remain to be elucidated, such interactions could feasibly contribute to reductions in serum TC and TG levels. Flavonoids and phenolic compounds present in *A. pinnata* are known to exert hypolipidemic effects by inhibiting HMG-CoA reductase, the rate-limiting enzyme in cholesterol biosynthesis, thereby reducing endogenous cholesterol levels [9]. In addition, these phytoconstituents enhance high-density lipoprotein (HDL) formation through upregulation of LCAT activity and support reverse cholesterol transport, while their antioxidant properties mitigate oxidative stress induced by Triton X-100 [10]. Among the phytoconstituents, flavonoids such as quercetin and rutin have demonstrated antioxidant and anti-inflammatory activities in many herbs, potentially offering protection to vascular endothelium and mitigating the progression of atherosclerosis [11]. Similarly, polyphenols such as anthocyanidins and coumarins may exert protective cardiovascular effects by inhibiting LDL oxidation and improving lipid profiles [12]. Saponins, another key group of compounds, may support cholesterol regulation by inhibiting intestinal absorption and enhancing fecal bile acid excretion [13]. Recent mechanistic studies suggest that natural compounds may modulate the Mevalonate pathway by inhibiting HMG-CoA reductase, reducing cholesterol biosynthesis, and promoting LDL catabolism [14]. Bioactive phytochemicals in *A. pinnata* are hypothesized to suppress LDL oxidation, attenuate ROS generation, and influence nuclear transcription factors such as PPAR-α and RXR, which govern lipid metabolism and inflammatory gene expression [15].

Considering the presence of diverse phytoconstituents, *A. pinnata* represents a promising herb for further investigations as a potential peculiar features therapeutic agent for the management of hyperlipidemia and oxidative stress-associated disorders that still not been scientifically so far reported. Therefore, the present study was designed to investigate the hypolipidemic and antioxidant potential of EEAP in Triton X-100-induced hyperlipidemic rats. The study aimed to evaluate the efficacy of the extract through biochemical lipid profile analysis, antioxidant enzyme assays, histopathological liver assessment, and DPPH-based free radical scavenging activity.

METHODS

Plant material

Fresh *Azolla pinnata* (500–800 g) was collected in August 2023 from a freshwater pond near the College of Horticulture and Forestry, Neri, Hamirpur, Himachal Pradesh, India. The plant material was thoroughly washed to eliminate surface debris and air-dried under shade at ambient temperature for 2 weeks with careful inspection. The dried material was stored in airtight containers until further use. Taxonomic authentication was conducted by Prof. Dr. Shrikant K (SB Arts and KCP Science College, Vijayapur, Karnataka), with an herbarium specimen authentication vide reference identification number- PRCBB-212, dated January 11, 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) was used to induce hyperlipidemia in the rat animal model. Diagnostic kits and reagents were obtained from certified suppliers to ensure accuracy. Diagnostic kits facilitated the biochemical analysis of blood serum samples. In addition, laboratory-grade chemicals and reagents were employed for the phytochemical screening and the quantitative determination of phytoconstituents in the plant extract.

Pharmacognostic evaluation of *A. pinnata*

Coarse leaf powder of *A. pinnata* 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) [16,17]. 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 sulfated ash were evaluated to assess purity and contamination. Ash values were expressed as percentages of initial weight [18].

For extractive values, 5 g of powder was macerated in 100 mL of water, ethanol, or ether, shaken for 6 h, and left to stand for 18 h. 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 h. Extracts were concentrated at 40°C under reduced pressure and stored for further analysis. The percentage yield of ethanolic extract was calculated to support phytochemical profiling [19].

Methodology for preliminary phytochemical screening of *A. pinnata*

The EEAP was qualitatively screened for alkaloids, flavonoids, glycosides, tannins, saponins, terpenoids, and phenolics using standard methods. Quantitative estimation of total polyphenols, flavonoids, and terpenoids was conducted using the Folin-Ciocalteu as standard with suitable wavelength (λ_{max}=760 nm), aluminum chloride (λ_{max}=510 nm), and vanillin-H_2SO_4 (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 analyses were performed in triplicate [18].

Determination of *in vitro* antioxidant activity

1,2-diphenyl-2-picryl-hydroxyl radical (DPPH) assay

The antioxidant activity of the *A pinnata* extracts was assessed 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 (0.2–1.0 mg/mL) and gallic acid (as the standard) were added. The mixture was vortexed and incubated in the dark at room temperature for 30 min. Absorbance was measured at λ_{max}=517 nm [16] using a Shimadzu UV-1900i spectrophotometer.

Nitric oxide (NO) 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 NO 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 min. 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. Controls contained no extract. The assay results provided insights into the antioxidant potential of the plant extracts, contributing to the understanding of their hepatoprotective properties [20,21].

In vivo evaluation of the pharmacological effects of *A pinnata*

An acute oral toxicity study already been conducted in healthy Wistar rats (180–250 g) following Organization for Economic Co-operation and Development (OECD)-423 guidelines [22]. Animals were housed under standard conditions at Laureate Institute of Pharmacy, Kangra, with a 1-week acclimatization.

This study received approval from the Institutional Animal Ethical Committee under proposal number control and supervision of experiments on animal (CCSEA)/LIPH/2023/36 in accordance with the guidelines, the committee for the CCSEA and OECD. Hyperlipidemia was induced in 30 overnight-fasted male Wistar rats by intraperitoneal injection of Triton X-100 at 100 mg/kg. The Triton X-100 solution was freshly prepared in normal saline before administration. The doses

of EEAP were selected based on previous literature demonstrating effective bioactivity of *A. pinnata* [23].

The experimental employed five group of six animals each that was given different treatment for 21 days as follows:

Group 1: The normal control (NC) 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 EEAP (125 mg/kg/p.o.) and Triton X-100 (100 mg/kg, i.p.)

Group 4: Treatment Group 2 was involved in the daily administration of EEAP (250 mg/kg/p.o.) and Triton X-100 (100 mg/kg, i.p.)

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

Inclusion criteria

Healthy male Wistar rats weighing between 180 and 250 g. Animals acclimatized 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 specified weight range were excluded from the study. Additional exclusion criteria included signs of illness, infection, or injury observed during the acclimatization or induction periods. Animals that failed to develop hyperlipidemia following Triton X-100 administration, as well as those with abnormal baseline lipid profiles prior to induction, were also excluded. Furthermore, rats that exhibited a poor response to handling, experienced excessive weight loss or gain, or died during the study period were excluded from the final analysis.

Analyses were performed using ELISA reader. Blood samples were collected by retro-orbital puncture under mild anesthesia. Serum lipid profiles (TC, TGs, HDL, LDL, very low-density lipoprotein [VLDL]) [24,25], and liver biomarkers including bilirubin [26], albumin [27,28], creatinine [29], alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) [30], and blood urea nitrogen (BUN) [31] were measured using standard kits by following the standard procedures prescribed by manufacturer.

At study completion, liver tissues were harvested, homogenized in ice-cold phosphate buffer, and analyzed for malondialdehyde (MDA), glutathione (GSH) levels, and thiobarbituric acid-reactive substances (TBARS) levels. Enzymatic antioxidants were evaluated using ELISA kits to assess oxidative stress [31].

Statistical analysis

The experimental data were statistically analyzed using GraphPad Prism V10.2 for Windows. Both one-way and two-way analysis of variance (ANOVA) 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±Standard Error of the Mean (SEM), with significance levels set at $p<0.05$ to $p<0.0001$.

RESULTS

Extraction of EEAP

The EEAP showed a yield of 17.2%, indicating efficient extraction and the presence of bioactive constituents.

Ethanol was selected over methanol for polyphenol extraction due to its comparable polarity and significantly lower toxicity. Mithraja *et al*. demonstrated that ethanol enabled efficient extraction of phenolic compounds from *A. pinnata*, with substantial yields validated through phytochemical analysis, thereby supporting its use as a safer and effective solvent [32].

Extractive value

Extractive values also revealed an alcohol-soluble extractive value of 12.24%w/w and a water-soluble extractive value of 11.45% w/w, providing insight into the solubility characteristics of the phytoconstituents, which is crucial for understanding the therapeutic potential of herb (Table 1).

Ash values and moisture content

In terms of analytical parameters, the powdered leaves of *A pinnata* showed a total ash content of 21.3%w/w, indicating the mineral composition of the plant. The water-soluble ash was found to be 9.52%w/w, while the acid-insoluble ash and sulfated ash were 4.1% w/w and 11.2% w/w, respectively. The moisture content was recorded at 9.45%, which is essential for assessing the quality of the plant material (Table 2).

Qualitative analysis of phytochemicals

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

Quantitative estimation

The quantitative analysis of the EEAP showed significant levels of bioactive compounds, with total flavonoid content (TFC) measured at 93.23±0.05 mg quercetin, total phenolic content (TPC) at 109.14±0.07 mg gallic acid, and total terpenoid content at 106.21±0.06 mg linalool (Table 4, Figs. 1-3). These values suggest that *A. pinnata* is rich in these compounds, which may play a role in its pharmacological effects.

Table 1: Extraction value for *A. pinnata* Leaves

S. No.	Analytical parameter	*A. pinnata* Percentage (% w/w)
1.	Alcohol soluble extraction value	12.24%w/w
2.	Water-soluble extraction value	11.45%w/w

A. pinnata: Azolla pinnata

Table 2: Analytical parameters for powdered *A. pinnata* leaves

S. No.	Analytical Parameter	*A. pinnata* Percentage (% W/W)
1.	Total ash value	21.3%w/w
2.	Water ash value	9.52%w/w
3.	Acid ash value	4.1%w/w
4.	Sulfated ash	11.2% w/w
5.	Moisture content	9.45%w/w

A. pinnata: Azolla pinnata

Table 3: Preliminary phytochemical screening of EEAP leaves

S. No.	Group of compounds	Observation (EEAP)
1.	Amino Acids	+
2.	Saponins	+
3.	Steroids	−
4.	Alkaloids	−
5.	Glycosides	−
6.	Flavonoids	+
7.	Tannins	+
8.	Terpenoids	+
9.	Phenols	+

(+): Indicates presence, (−): Indicates absence, EEAP: Ethanolic extract of *Azolla pinnata*

Antioxidant assay

The EEAP showed a concentration-dependent increase in DPPH inhibition, with a percentage inhibition of 20.24% at 10 µg/mL and 84.24% at 1000 µg/mL (Fig. 4). The calculated IC_{50} value for *A pinnata* was 379.17 µg/mL, indicating moderate antioxidant activity compared to the reference standard, gallic acid, which showed a much stronger effect with an IC_{50} value of 65.55 µg/mL (Table 5). In the NO scavenging assay, *A pinnata* demonstrated moderate activity, with a percentage inhibition of 33.36% at 100 µg/mL and 81.94% at 1000 µg/mL, compared to the strong activity of rutin, the reference standard, which inhibited 99.59% of NO at 1000 µg/mL (Fig. 5). The calculated IC_{50} value for *A. pinnata* was 113.9314 µg/mL (Table 6).

Table 4: Total phenol, flavonoid, and terpenoid content in *A. pinnata*

S. No.	Ethanolic Extract	Flavonoid (mg QE/100g)	Phenol (mg GAE/100g)	Terpenoid (mg LU/100g)
1.	*A. pinnata*	93.23±0.05 mg	109.14±0.07 mg	106.21±0.06 mg

GAE: Gallic acid, QE: Quercetin, LU: Linalol, *A. pinnata: Azolla pinnata*
EEAP: Ethanolic extract of *Azolla pinnata*. Data is expressed as mean±standard error of the mean (n=3)

Table 5: For IC_{50} value of gallic acid, *A. pinnata*

S. No.	Compound	IC_{50}
1.	Gallic acid	65.5516
2.	*A. pinnata*	379.1694

A. pinnata: Azolla pinnata

Table 6: For IC_{50} value of rutin, *A. pinnata*

S. No.	Compound	IC_{50}
1.	Rutin	35.21905
2.	*A. pinnata*	113.9314

A. pinnata: Azolla pinnata

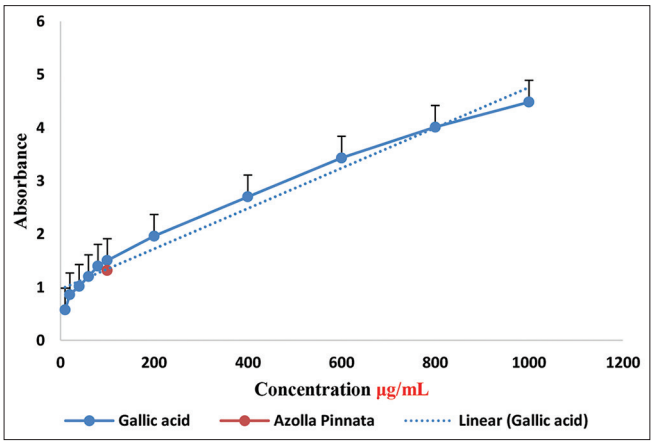

Fig. 1: Total flavonoid content

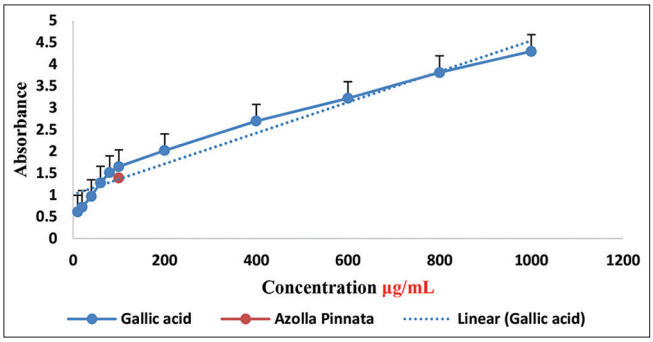

Fig. 2: Total phenol content

In vivo anti-hyperlipidemic activity

Body weight

In vivo anti-hyperlipidemic activity was performed using Male Wistar rats (180–250 g) as per the experimental protocol.

Biochemical analysis

Serum estimations

In vivo pharmacological activities of EEAP were evaluated by assessing its effect on lipid profiles against Triton X-100-induced hyperlipidemic rats. Treatment with the EEAP significantly reduced TG 137.01±1.21 and TC 118.01 ±1.1 levels in a dose-dependent manner (**p<0.005), with the 250 mg/kg dose showing better efficacy (***p<0.001) 136.18±1.04, and 117.18±1.2 as compared to the 125 mg/kg dose (Fig. 7). Furthermore, *A pinnata* significantly improved HDL-C levels, with the higher dose

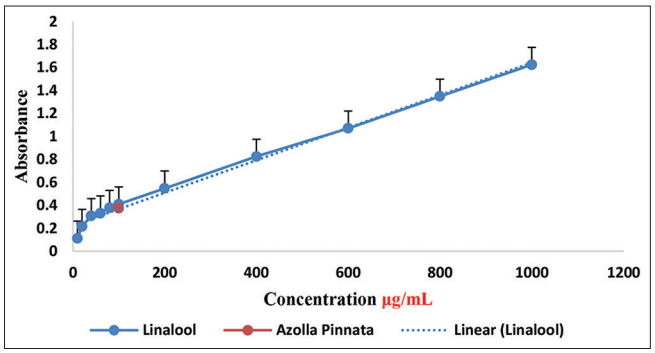

Fig. 3: Total terpenoid content

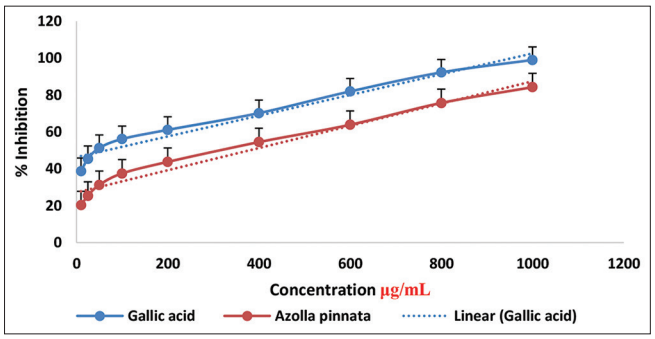

Fig. 4: Curve of 1,2-diphenyl-2-picryl-hydroxyl radical for gallic acid, *Azolla pinnata*

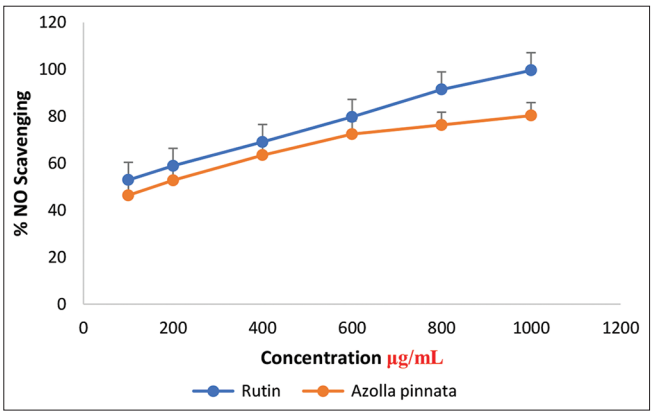

Fig. 5: Curve of NO for rutin, *Azolla pinnata* leaves

exhibiting a greater effect (***p<0.001) 33.281±1.101. Treatment with EEAP significantly reduced creatinine levels 1.199±0.104. Atorvastatin, used as a reference drug, demonstrated the most significant effects (****p<0.0001) on all parameters as compared to @disease group (Fig. 7).

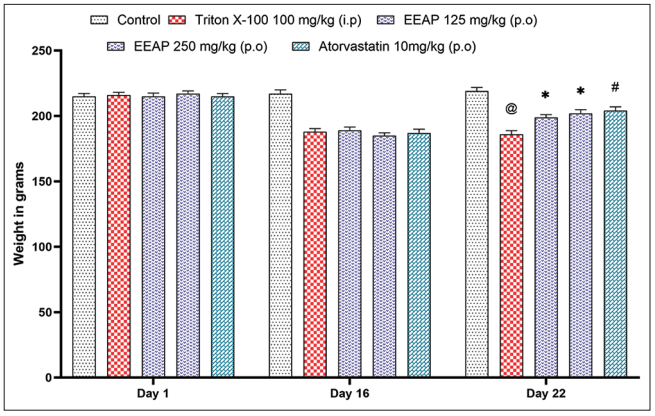

Fig. 6: Effect of ethanolic extract of Azolla pinnata (EEAP) on body weight in Triton X-100 X-100-induced rats. Data were analyzed using two-way analysis of variance followed by Tukey's multiple comparison test. Values are expressed as mean±standard error of the mean (n=6). On day 22, EEAP at 125 mg/kg and 250 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) versus @disease group.**

The EEAP also exhibited hepatoprotective effects, as evidenced by the significant reduction in ALT 83.42±2.09 and AST 123.42 ±2.09 levels (**p<0.005) in rats. The higher dose (250 mg/kg) showed a more pronounced reduction in these liver enzymes 81.84±2.09 and 121.84±2.39, respectively, (***p<0.001) compared to the lower dose, although atorvastatin displayed the most substantial hepatoprotective effect. In addition, treatment with EEAP resulted in significant reductions in serum albumin level, with the 250 mg/kg dose showing superior efficacy 22.94±0.21 (***p<0.001). Atorvastatin again produced the most pronounced effect 19.25±0.21 (****p<0.0001) as compared to @disease group. Potential of *A pinnata* was evaluated by measuring BUN and albumin levels in rats. Treatment with EEAP significantly reduced BUN levels 33.16±1.00 (**p<0.005), indicating the plant's potential to protect. The 250 mg/kg dose 32.01±1.0 (***p<0.001) was more effective than the 125 mg/kg dose, and while atorvastatin demonstrated the most significant reduction, *A. pinnata* 27.15±1.0 (****p<0.0001) exhibited promising protective effects as compared to @disease group (Figs. 8 and 9).

In rats, administration of Triton X-100 led to a significant increase in serum bilirubin levels. Treatment with EEAP resulted in a statistically significant reduction in bilirubin levels 0.581±0.03 (**p<0.005), with the 250 mg/kg dose showing better efficacy 0.554±0.03 (***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) as compared to @disease group. On day 22, EEAP at 125 mg/kg and 250 mg/kg showed a statistically significant increase in body weight (*p < 0.05 and **p < 0.005, respectively). At the same time, Atorvastatin exhibited a highly significant effect (***p < 0.001) compared to the disease group (Fig. 6).

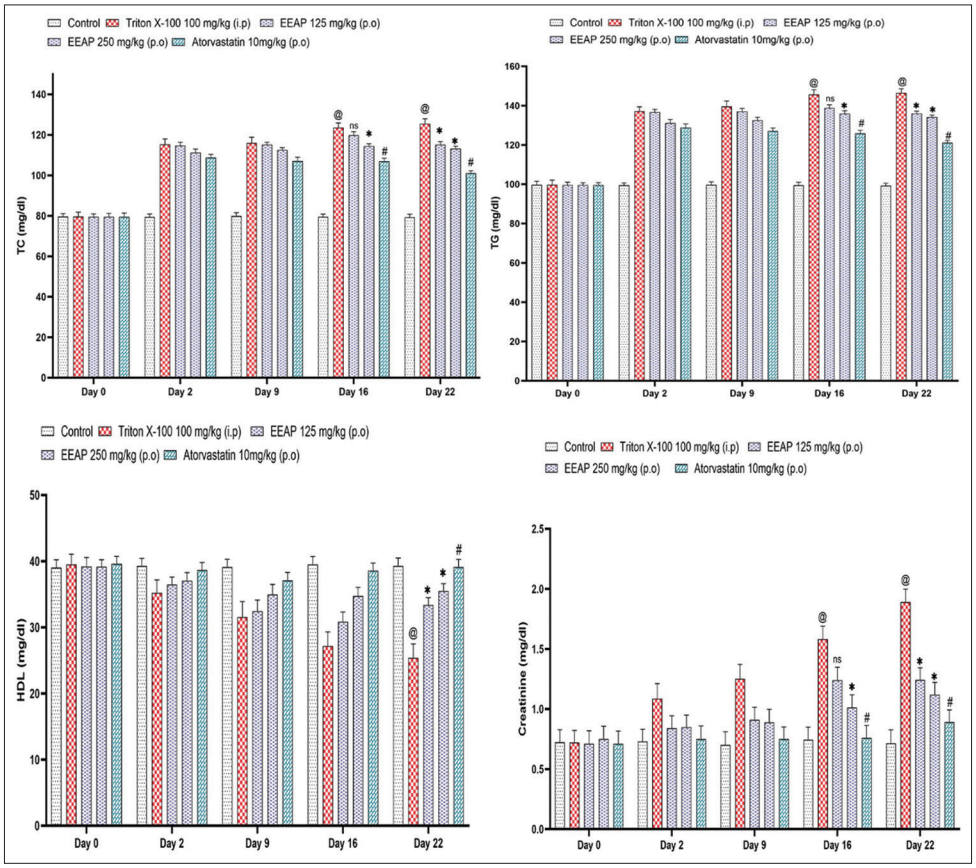

Fig. 7: Effect of ethanolic extract of *Azolla pinnata* (EEAP) on triglyceride, total cholesterol, high-density lipoprotein, and Creatinine Levels in Triton X-100 X-100-induced rats. Data were analyzed using two-way analysis of variance followed by Tukey's multiple comparison test. Values are expressed as mean±standard error of the mean (n=6). On day 22, EEAP at 125 mg/kg and 250 mg/kg showed statistically significant reductions in aforementioned levels (p<0.005 and *** p<0.001, respectively), while Atorvastatin exhibited a very highly significant effect (****p<0.0001) versus @ disease group**

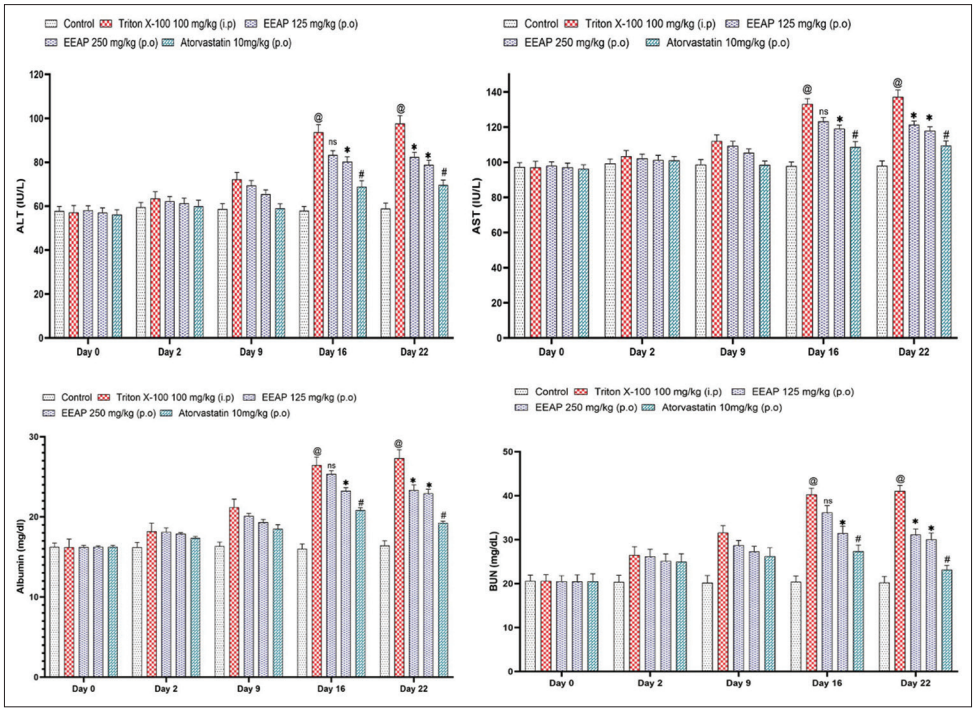

Fig. 8: The impact of ethanolic extract of Azolla pinnata (EEAP) on alanine aminotransferase, aspartate aminotransferase, albumin, BUN concentrations was evaluated in rats subjected to Triton X-100 administration. Statistical analysis was performed using two-way analysis of variance, followed by Tukey's post hoc test to account for multiple comparisons. Data are presented as the mean±standard error of the mean, with a sample size of six animals per group (n=6). EEAP at 125 mg/kg and 250 mg/kg showed statistically significant reductions in earlier earlier-mentioned levels (p<0.005 and ***p<0.001, respectively), while Atorvastatin exhibited a very highly significant effect (****p<0.0001) versus @disease group**

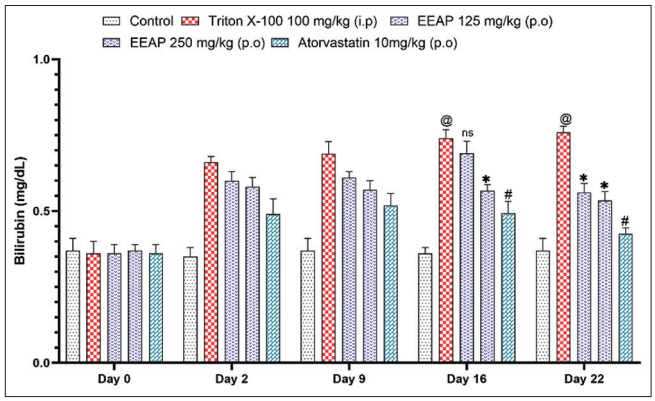

Fig. 9: In rats challenged with Triton X-100, the impact of ethanolic extract of Azolla pinnata (EEAP) on bilirubin levels was evaluated. Results are presented as mean±standard error of the mean (n=6) and analyzed through two-way analysis of variance, with Tukey's post hoc test applied for multiple comparisons. EEAP at 125 mg/kg and 250 mg/kg showed statistically significant reductions in aforementioned levels (p<0.01 and*** p<0.001, respectively), while Atorvastatin exhibited a very highly significant effect (****p<0.0001) versus @disease group**

Finally, oxidative stress markers, including TBARS, MDA, and GSH levels, were assessed to detect the antioxidant potential of *A. pinnata.* The extract significantly reduced TBARS 53.84±2.74 and MDA levels 1.3±0.2 (***p<0.001), indicative of its ability to reduce lipid peroxidation and oxidative damage. Moreover, EEAP significantly elevated hepatic GSH levels 24.83±1.52 (**p<0.005, providing further evidence of its antioxidant capacity. The higher dose of EEAP (250 mg/kg) 26.67±1.35 (***p<0.001) demonstrated greater efficacy in restoring GSH levels compared to the lower dose (125 mg/kg), although atorvastatin showed the most substantial effect in all oxidative stress markers (****p<0.0001) as compared to @disease group. Fig. 10 illustrates the detailed results.

Histopathology of harvested liver for the investigation of EEAP 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, clear hepatic cords, central vein, and normal sinusoids, without pathological alterations (Fig. 11a). In contrast, the Negative Control group (hyperlipidemia-induced) showed pronounced hepatic damage characterized by macrovesicular steatosis (St), inflammatory cell infiltration, hepatocellular necrosis, pyknotic nuclei (PN), ballooning degeneration, and disrupted hepatic cords (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 *A. paniculata* (EEAP) at 125 mg/kg showed partial hepatoprotection, although lipid vacuoles, degenerative hepatocytes, and mild Kupffer cell (KC) proliferation persisted (Fig. 11d). Notably, EEAP at 250 mg/kg resulted in substantial hepatic restoration, exhibiting nearly normal liver histoarchitecture, absence of St or inflammation, binucleated hepatocytes (BH), normal Disse space, and organized hepatic cords, suggesting a dose-dependent therapeutic effect (Fig. 11e).

DISCUSSION

The current study was designed to evaluate the hypolipidemic and antioxidant efficacy of EEAP in Triton X-100-induced hyperlipidemic rats. Triton X-100 is a non-ionic surfactant widely used to induce acute hyperlipidemia by inhibiting LPL activity and blocking lipid uptake in tissues, resulting in elevated levels of serum cholesterol and TGs [33]. Following Triton X-100 administration, significant elevation in serum TC, TGs, LDL-C, and very low-density lipoprotein cholesterol (VLDL-C)

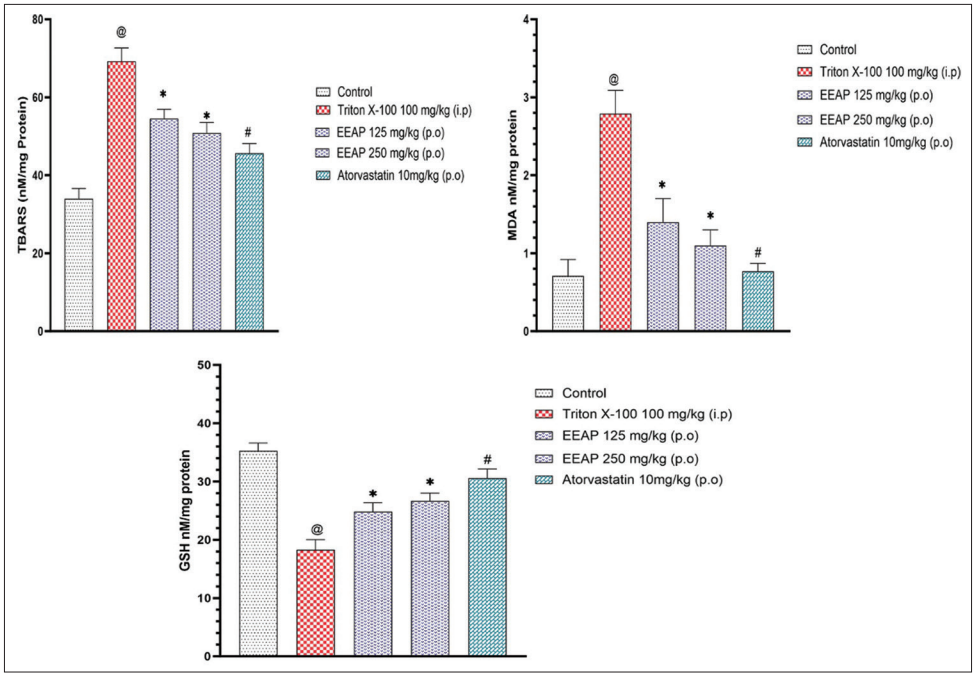

Fig. 10: The effects of ethanolic extract of *Azolla pinnata* (EEAP) on thiobarbituric acid-reactive substances, malondialdehyde, and glutathione levels were evaluated in rats subjected to Triton X-100. Data are presented as mean±standard error of the mean (n=6) and were analyzed using one-way analysis of variance followed by Tukey's *post hoc* test for multiple comparisons. EEAP at 125 mg/kg and 250 mg/kg showed statistically significant reductions in aforementioned levels (p<0.005 and *** p<0.001, respectively), while Atorvastatin exhibited a very highly significant effect (****p<0.0001) versus @disease group**

levels was observed, accompanied by a notable decrease in HDL-C, reflecting the typical dyslipidemic profile [3]. Treatment with EEAP at both tested doses effectively restored lipid parameters toward normal ranges. Notably, the higher dose (125 mg/kg) exhibited a more pronounced lipid-lowering effect, comparable to that of the standard drug atorvastatin, suggesting a dose-dependent hypolipidemic potential.

Preliminary phytochemical evaluation of the EEAP demonstrated the presence of flavonoids, tannins, phenolics, amino acids, saponins, and terpenoids, while steroids, alkaloids, and glycosides were absent. This distinct phytochemical profile suggests that the observed biological effects are primarily attributable to the identified constituents. Among these, amino acids, tannins, and phenolic compounds are extensively documented for their potent antioxidant properties, which may underlie the extract's observed *in vitro* antioxidant activity. These constituents are also associated with additional pharmacological actions, such as anti-inflammatory and hepatoprotective effects, as previously reported in the literature [34].

Triton X-100, administered intraperitoneally at a dose of 100 mg/kg, induces acute hyperlipidemia by inhibiting LPL, thereby impairing the hydrolysis and clearance of TG-rich VLDL particles. This biochemical disturbance leads to a rapid accumulation of circulating TGs and cholesterol. Furthermore, Triton X-100 suppresses LDL catabolism and enhances hepatic lipid synthesis, culminating in excessive hepatic cholesterol and VLDL production. This dyslipidemic state is frequently accompanied by oxidative stress, characterized by increased lipid peroxidation markers such as MDA and TBARS, along with reduced levels of endogenous antioxidants, including GSH, superoxide dismutase (SOD), and catalase (CAT). Hepatocellular damage is evident through elevated serum levels of hepatic enzymes (AST, ALT, ALP) alongside bilirubin and stress markers such as creatinine and BUN. Atorvastatin, a well-established antihyperlipidemic agent, counters these alterations primarily via inhibition of HMG-CoA reductase, thereby reducing hepatic cholesterol biosynthesis and circulating LDL/VLDL levels. In addition, it contributes to partial restoration of antioxidant activity by lowering MDA and TBARS and enhancing the activity of enzymatic antioxidants, resulting in improved liver function indices. *A. pinnata*, rich in polyphenolic and flavonoid content, exhibits potent antioxidant potential, as evidenced by low IC_{50} values in DPPH assays and high levels of total phenolics and flavonoids. Although the extract's direct efficacy in Triton X-100-induced hyperlipidemia models has not been widely explored, its hepatoprotective effects in oxidative damage models, such as lead-induced toxicity, are well-documented. These include enhancements in GSH, SOD, and CAT activities, reductions in MDA, and normalization of liver enzyme levels. These findings suggest that the extract's therapeutic effects may be mediated via ROS scavenging and lipid peroxidation inhibition mechanisms relevant to the pathophysiology of Triton-induced hyperlipidemia. Collectively, both atorvastatin and *A. pinnata* mitigate Triton X-100-induced dyslipidemia through distinct yet complementary mechanisms. Atorvastatin predominantly acts through inhibition of cholesterol biosynthesis and promotion of lipoprotein clearance, while *A. pinnata* confers protection by restoring antioxidant balance and preserving hepatic structural and functional integrity.

Extraction using ethanol yielded 17.2%, indicating a moderate-to-high efficiency for extracting ethanol-soluble constituents from *A. pinnata*. This yield is comparable to previous findings by Mithraja *et al.* [32]. In contrast, conventional aqueous and chloroform extractions typically resulted in yields below 6%. These comparisons reinforce ethanol as an optimal solvent for extracting bioactive metabolites from this herb species.

Quantitative analysis revealed a TPC of 109.14 mg GAE/g and TFC of 93.23 mg QE/g. These values surpass those reported for *Azolla microphylla* (TPC: 90.2 mg GAE/g, TFC: 58.5 mg QE/g) by Nawaz *et al.* and also exceed levels found in *Azolla rubra* [35]. These findings highlight *A. pinnata* as a superior source of phenolic antioxidants, particularly when extracted with ethanol [36]. In addition, the quantified terpenoid content (106.21 mg linalool equivalent/100 g) provides novel quantitative data, as prior studies largely reported terpenoids only qualitatively. This complements the profiling by Abdel Elrasoul *et al.*, [37] detected flavonoids such as quercetin, kaempferol, and vitexin using Ultra-Performance Liquid Chromatography–Mass

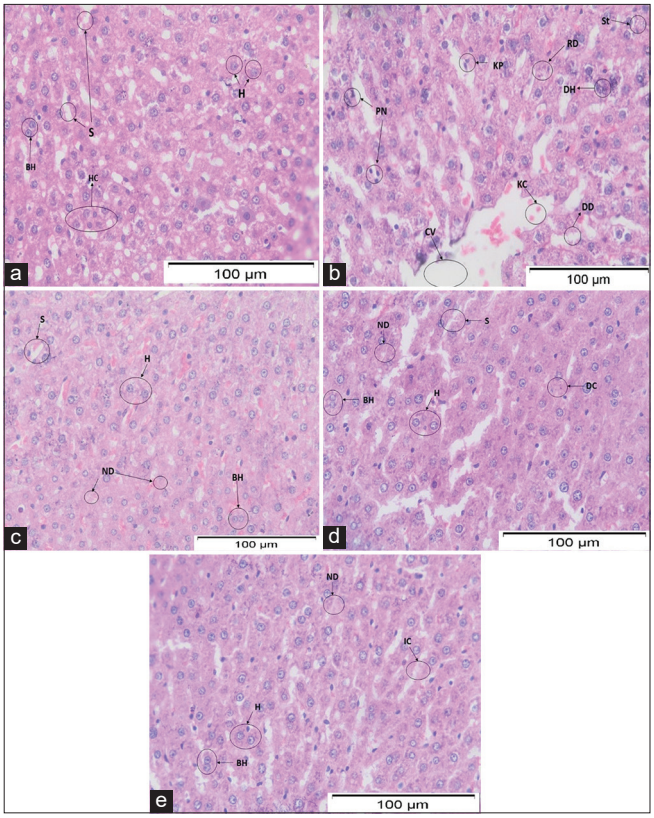

Fig. 11: Histology of liver: (a) Group I Normal control; (b) Group II Disease control (Negative control); (c) Group III- Standard (Positive control) treated with atorvastatin (10mg/kg P.O); (d) Group IV treatment Group I, treated with ethanolic extract of *A. paniculata* (EEAP) (125 mg/kg, p.o.); (e) Group IV treatment Group 2, treated with EEAP (250 mg/kg, p.o.). 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.

Spectrometry (UPLC-MS), compounds known for potent antioxidant and anti-inflammatory effects. Moreover, stress-induced upregulation of flavonoid biosynthesis, as noted by Yehia *et al.*, supports the adaptive accumulation of these bioactives under oxidative stress.

The ethanolic extract demonstrated moderate antioxidant activity, with a DPPH IC_{50} of 379.17 µg/mL and 81.94% NO scavenging at 1000 µg/mL. These are less potent than standard antioxidants like gallic acid and rutin but still indicative of bioactivity. Comparatively Nawaz *et al.*, [38] reported a significantly lower DPPH IC_{50} of 7.32 µg/mL for methanolic extracts of *A. rubra*, nearing that of ascorbic acid (IC_{50} = 1.39 µg/mL), Thiripurasundari and Padmini [39] found high phenolic (87.3 µg GAE/mg) and flavonoid (56.3 µg RU/mg) contents linked to potent DPPH and FRAP activities. Such discrepancies likely reflect differences in extraction solvents, plant source, or seasonal variation. Importantly, *in vivo* study by Ahmed *et al.* demonstrated that *A. pinnata* enhances endogenous antioxidant enzymes, including SOD, CAT, and GSH peroxidase in lead-stressed rats. Furthermore, this investigation evaluated the effects of EEAP on TG levels in Triton X-100-induced hyperlipidemic rats a model in which Triton X-100 inhibits LPL and impairs clearance of TG-rich lipoproteins, causing acute hyperlipidemia. EEAP administered at 125 mg/kg and 250 mg/kg produced statistically significant reductions in TG (***p<0.001 for both), while atorvastatin showed an even stronger effect (****p<0.0001) against @disease group, according to Two-Way ANOVA with Tukey's *post hoc* (mean±SEM; n = 6). These lipid-lowering effects are consistent with other plant extracts rich in phenolics and flavonoids, such as *Cassia tora*, *Ruta chalepensis*, and date fruit extracts, which also mitigated Triton-induced hyperlipidemia by restoring LPL activity and enhancing antioxidant defenses. Collectively, these findings endorse *A. pinnata* as a multifaceted herb with antioxidant and lipid-lowering ability. The reduction in TGs and cholesterol observed with the ethanolic extract is consistent with the findings [34], demonstrated that *A. pinnata* could mitigate lead-induced hepatotoxicity in rats, partly through its antioxidant and anti-inflammatory properties.

The EEAP significantly lowered serum TGs, TC, LDL-C, and VLDL-C levels while increasing HDL-C in Triton X-100-induced hyperlipidemic rats. These lipid-regulating effects were statistically validated using two-way ANOVA followed by Tukey's multiple comparison test, with EEAP at 125 mg/kg and 250 mg/kg showing significant reductions in TG levels (**p<0.001 for both), and atorvastatin showing a very highly significant effect (****p<0.0001) as compared to @disease group, values expressed as mean±SEM (n = 6). *A. pinnata* ethanolic extract, analyzed via UPLC-MS [40], contains flavonoids such as quercetin, kaempferol, and rutin, which activate LCAT to enhance reverse cholesterol transport and raise HDL levels. These compounds also exhibit antioxidant and anti-inflammatory effects, reducing lipid peroxidation and mitigating Triton X-100-induced dyslipidemia. The observed antihyperlipidemic effects align with and expand upon earlier *in vivo* studies, particularly Nawaz *et al.* [35], reported that *A. pinnata* ameliorated lead-induced hepatotoxicity [34] and lipid disturbances in rats by modulating oxidative stress, inflammation, and apoptosis. Lead acetate significantly elevated serum ALT and AST activities, as well as urea and creatinine levels (*p<0.05 vs. control). Apple Polyphenols Extract (APE) supplementation before, during, or after lead acetate exposure significantly reduced these elevations (*p<0.05 vs. lead-only group). APE alone showed no significant effect on liver biomarkers (p > 0.05 vs. control). Although their model focused on lead toxicity, similar molecular mechanisms, especially activation of endogenous antioxidant enzymes and suppression of lipid peroxidation are likely responsible for the lipid-normalizing actions seen in Triton-induced models. The hyperlipidemic state in Triton X-100–treated animals arises primarily from the inhibition of LPL, leading to delayed catabolism of TG-rich lipoproteins and increased hepatic lipid biosynthesis [6]. These effects simulate acute hyperlipidemia, providing a suitable model for evaluating lipid-lowering therapies. Furthermore, studies involving rutin, a key flavonoid also present in *A. pinnata*, have shown similar antihyperlipidemic activities via the upregulation of LPL and LCAT, leading to improved lipid clearance and HDL elevation. In other study, Rutin significantly reduced plasma and tissue lipid levels in streptozotocin-induced diabetic rats (*p<0.05), increasing HDL-C and decreasing LDL-C and VLDL-C. It also reduced HMG-CoA reductase activity in the liver, kidney, and heart, while increasing plasma LPL and LCAT activities (*p<0.05). Glycoprotein levels (hexose, hexosamine, fucose, sialic acid) were significantly lowered in plasma, liver, and kidney. No significant changes were observed in normal rats treated with rutin [41,42]. Thus, our findings add to the growing body of evidence suggesting that the phenolic and flavonoid constituents of *A. pinnata* confer notable therapeutic effects in managing hyperlipidemia and protecting against cardiovascular risk.

The EEAP demonstrated significant hepatoprotective effects in Triton X-100-induced hyperlipidemic rats, evidenced by a dose-dependent reduction in serum ALT and AST, with the 250 mg/kg dose producing a more marked restoration of liver enzyme levels. EEAP also significantly lowered TG levels (***p<0.001 for both 125 and 250 mg/kg), TC, LDL-C, and VLDL-C while increasing HDL-C, compared to the Triton-treated control. These results were statistically validated using Two-Way ANOVA followed by Tukey's multiple comparison test (mean±SEM, n = 6), and atorvastatin displayed a very highly significant reduction (****p<0.0001) in TG levels versus @disease group. Triton X-100 acutely induces hyperlipidemia through inhibition of LPL activity and impaired catabolism of TG-rich lipoproteins, leading to elevated circulating TG and cholesterol an established model for screening lipid-lowering agents. Our findings are congruent with Elrasoul *et al.*, [34], who reported that EEAP ameliorated lead-induced hepatotoxicity by reducing oxidative

stress, inflammation, and hepatocellular damage, thus normalizing ALT, AST, and altered lipid profiles Similarly, Elrasoul *et al*. showed that *A. pinnata* ethanolic extract protected against ranitidine-induced hepatic injury by enhancing antioxidant defenses and reducing hepatic enzyme elevations. Ranitidine significantly increased serum ALT, AST, urea, and creatinine levels in rats ($p<0.05$). Co-treatment with APE significantly reduced these markers compared to the ranitidine-only group ($p<0.05$), while APE alone had no significant effect on liver and kidney function compared to controls. The hepatoprotective and lipid-modulating effects are attributed to the plant's phenolic and flavonoid constituents including quercetin, rutin, tamarixetin, and astragalin with documented antioxidative, membrane-stabilizing, and LPL-activating properties.

The EEAP not only demonstrated hepatoprotective effects but also showed compelling lipid-lowering activity in Triton X-100-induced hyperlipidemic rats. Triton X-100 is a non-ionic surfactant commonly used to produce acute hyperlipidemia by inhibiting LPL, reducing clearance of TG rich lipoproteins, and promoting hepatic cholesterol synthesis and lipid absorption, thus mimicking human lipid disorders. In our study, EEAP administered at 125 mg/kg and 250 mg/kg resulted in significant reductions in serum TGs (***$p<0.001$), while atorvastatin exhibited a very highly significant effect (****$p<0.0001$) against $^{@}$disease group, as determined by two-way ANOVA followed by Tukey's multiple comparison test (mean±SEM, n = 6). These findings corroborate prior research on plant-derived polyphenols, *C. tora* extract significantly reduced TGs, cholesterol, and phospholipids [43] in Triton-treated rats by reactivating LPL activity [44], and *Boerhavia diffusa* extract similarly mitigated hyperlipidemia [45] through antioxidant-mediated pathways [46]. The lipid-lowering efficacy of EEAP may derive from flavonoids such as rutin and quercetin, which promote hepatic uptake of lipids and enhance LPL and LCAT activity. Overall, these results reinforce EEAP's therapeutic potential in managing hyperlipidemia which becomes a major cause behind reducing cardiovascular risk.

The antioxidant and anti-inflammatory activities of *A pinnata* are attributable to its rich content of phenolic compounds, flavonoids, and tannins, which modulate oxidative stress and inflammation. In our study, the EEAP significantly reduced TBARS and MDA levels markers of lipid peroxidation in liver homogenates from Triton X-100-treated rats, and restored hepatic GSH levels, affirming its efficacy in mitigating oxidative damage and enhancing endogenous defense systems. Concurrently, EEAP displayed notable lipid-lowering effects: in Triton X-100-induced hyperlipidemic rats (a model where the surfactant inhibits LPL and blocks peripheral clearance of TG-rich lipoproteins), administration of EEAP at 125 and 250 mg/kg led to statistically significant reductions in TG levels (***$p<0.001$ for both doses), analyzed by two-way ANOVA followed by Tukey's test (mean±SEM, n = 6), while atorvastatin exhibited an even more pronounced effect (****$p<0.0001$) as compared to $^{@}$disease group. These findings align with reports by Abd Elrasoul *et al.*, [37] who observed that *A. pinnata* reduced hepatic MDA and enhanced GSH and antioxidant enzymes in lead-exposed rats, demonstrating anti-oxidative and liver-protective effects [47]. Lead acetate significantly increased hepatic MDA levels (*$p<0.05$), while cotreatment with APE significantly reduced MDA compared to lead-only rats (*$p<0.05$). Lead also decreased hepatic GSH content and SOD and CAT activities (*$p<0.05$ vs. control), which were significantly restored by APE supplementation in the same groups (*$p<0.05$ vs. lead-only). APE alone had no significant impact on oxidative stress markers compared to the control. They also echo studies on other plant extracts such as *C. tora* and *R. chalepensis* which similarly lowered TG levels in Triton-induced hyperlipidemia by improving antioxidant status and lipoprotein metabolism [44]. Taken together, these results reinforce that EEAP exerts a multifaceted therapeutic effect: combating lipid peroxidation, restoring GSH defenses, and significantly lowering TGs in a dose-dependent manner, thus supporting its promise for managing oxidative stress and hyperlipidemia.

In addition to its established hepatoprotective properties, Treatment with EEAP at doses of 125 mg/kg and 250 mg/kg resulted in statistically significant reductions in serum creatinine and BUN compared to untreated controls (***$p<0.001$ for both doses). In contrast, Atorvastatin, used as a standard reference drug, produced a very highly significant effect on these parameters (****$p<0.0001$) versus $^{@}$disease group [48] reported that *A pinnata* mitigated oxidative stress and improved liver and kidney function in *Nile tilapia* exposed to environmental toxins. Treated fish showed significantly lower serum creatinine and BUN levels ($p<0.05$) compared to toxin-exposed controls [40]. Elrasoul *et al*. demonstrated that *Azolla filiculoides* extract significantly reduced markers in rats, including serum creatinine and BUN, with a strong level of significance ($p<0.001$).

The results of this investigates demonstrate that *A. pinnata* possesses a wide range of pharmacological activities, including antioxidant, lipid-lowering, and hepatoprotective effects. The plant's bioactive compounds, such as phenolic compounds, flavonoids, and tannins, likely contribute to these therapeutic properties. This effect has also been reported in prior investigations, supporting the consistency of the observed outcome [49,50]. While the EEAP exhibited moderate antioxidant activity, its promising effects on lipid metabolism and liver function highlight its potential as a natural remedy for managing metabolic disorders and liver-related diseases. Further research is needed to isolate and characterize the specific bioactive compounds responsible for these effects, and clinical trials are necessary to confirm the efficacy and safety of *A. pinnata* in humans.

The histopathological assessment of liver tissues provided critical insights into hepatic alterations induced by Triton X-100 and the protective effects conferred by EEAP treatment. In the NC group, liver sections exhibited preserved hepatic architecture characterized by polygonal hepatocytes arranged in intact hepatic cords, with no observable signs of St, inflammation, or necrosis, serving as a morphological baseline. Conversely, the negative control group subjected to Triton X-100-induced hyperlipidemia showed pronounced centrilobular macrovesicular St, indicative of excessive lipid accumulation. Additional histopathological features included mild lobular inflammation, focal hepatocellular necrosis, PN, and ballooning degeneration, which are hallmark indicators of hepatic injury and steatohepatitis. The Atorvastatin-treated group (Positive Control, 10 mg/kg) demonstrated partial hepatoprotective effects. Liver sections from this group revealed reduced St and inflammation compared to the negative control. Moreover, rare instances of necrotic cells were observed, alongside features such as BH, mild cytoplasmic vacuolation, and relatively normalized KC morphology. However, minor portal tract alterations were still evident, suggesting incomplete reversal of Triton-induced hepatic injury. Treatment with the lower dose of EEAP (125 mg/kg) resulted in modest histological improvement. Although some recovery was evident, residual lipid vacuoles, scattered apoptotic nuclei, mild lobular inflammation, and disorganized hepatic cords persisted, indicating a limited regenerative effect at this concentration. Remarkably, liver histology in rats treated with EEAP at 250 mg/kg exhibited near-complete restoration of normal hepatic architecture. The absence of St, necrosis, or inflammatory infiltration, along with reappearance of well-aligned hepatic cords and normal hepatocyte morphology, highlighted a robust dose-dependent hepatoprotective response.

These findings corroborate earlier investigations. For instance, *Moringa oleifera* extract administered at 400 mg/kg achieved near-normal liver histology and lipid profiles comparable to atorvastatin-treated groups [51,52]. Such studies support the premise that higher doses of phytotherapeutics confer superior hepatoprotection, a pattern consistent with the effects observed in our EEAP-treated groups. Furthermore, polyphenol-rich formulations such as Totum 070 have demonstrated similar dose-dependent improvements in hepatic St and inflammatory resolution in hyperlipidemic models [53].

In summary, our results indicate that EEAP at 250 mg/kg exhibits hepatoprotective effects that are not only dose-dependent but also

comparable, if not superior, to the standard pharmacological agent atorvastatin. These findings align with a growing body of literature advocating the use of polyphenol-rich herbal interventions in the management of hyperlipidemia-induced hepatic dysfunction [54].

CONCLUSION

A. pinnata exhibits strong therapeutic potential owing to its diverse phytochemical profile and multifaceted pharmacological properties. Extract shows a significant lipid-lowering effect along with antioxidant and hepatoprotective potential, largely attributed to its bioactive constituents such as amino acids, tannins, and phenolic compounds. These findings support its traditional use in managing oxidative stress-related disorders and highlight its role in modulating lipid metabolism and protecting against liver and kidney damage. Notably, the higher dose (250 mg/kg) produced superior efficacy, particularly in restoring biochemical parameters and hepatic histology. Histopathological analysis confirmed that *A. pinnata* effectively mitigates hyperlipidemia-induced hepatic injury in a dose-dependent manner, with high-dose treatment achieving near-complete tissue restoration. Overall, these results underscore the potential of *A. pinnata* as a natural therapeutic agent for metabolic and oxidative stress-related diseases and warrant further clinical investigation for its integration into modern medical applications.

ACKNOWLEDGMENT

I sincerely express my gratitude to all the contributors of this article for their invaluable guidance, continuous support, and constructive feedback throughout the preparation of this manuscript. I also extend my heartfelt thanks to the institute for providing the essential research facilities and academic resources that greatly facilitated the successful completion of this work.

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.

AUTHOR'S FUNDING

Not applicable.

REFERENCES

1. Abbasi S, Khan A, Choudhry MW. New insights into the treatment of hyperlipidemia: Pharmacological updates and emerging treatments. Cureus. 2024;16(6):e63078. doi: 10.7759/cureus.63078, PMID 38919858
2. Karr S. Epidemiology and management of hyperlipidemia. Am J Manag Care. 2017;23(9 Suppl):S139-48. PMID 28978219
3. World Health Organization. Noncommunicable Diseases: Risk Factors. The Global Health Observatory; 2023. 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. doi: 10.1038/s41569-021-00541-4, PMID 33833450
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. doi: 10.5152/tjg.2019.18076, PMID 31144662
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. doi: 10.4103/pr.pr_37_17, PMID 29333040
7. Mouradov A, Spangenberg G. Flavonoids: A metabolic network mediating plants adaptation to their real estate. Front Plant Sci. 2014;5:620. doi: 10.3389/fpls.2014.00620, PMID 25426130
8. Debette S, Courbon D, Leone N, Gariépy J, Tzourio C, Dartigues JF, *et al*. Tea consumption is inversely associated with carotid plaques in women. Arterioscler Thromb Vasc Biol. 2008;28(2):353-9. doi: 10.1161/ATVBAHA.107.151928, PMID 18063810
9. Zeka K, Ruparelia K, Arroo RR, Budriesi R, Micucci M. Flavonoids and their metabolites: Prevention in cardiovascular diseases and diabetes. Diseases. 2017 Sep 5;5(3):19. doi: 10.3390/diseases5030019, PMID 32962323
10. Islam B, Sharma C, Adem A, Aburawi E, Ojha S. Insight into the mechanism of polyphenols on the activity of HMGR by molecular docking. Drug Des Dev Ther. 2015 Aug 28;9:4943-51. doi: 10.2147/DDDT.S86705, PMID 26357462
11. Dower JI, Geleijnse JM, Gijsbers L, Schalkwijk C, Kromhout D, Hollman PC. Supplementation of the pure flavonoids epicatechin and quercetin affects some biomarkers of endothelial dysfunction and inflammation in (pre)hypertensive adults: A randomized double-blind, placebo-controlled, crossover trial. J Nutr. 2015;145(7):1459-63. doi: 10.3945/jn.115.211888, PMID 25972527
12. Aviram M, Dornfeld L, Rosenblat M, Volkova N, Kaplan M, Coleman R, *et al*. Pomegranate juice consumption reduces oxidative stress, atherogenic modifications to LDL, and platelet aggregation: Studies in humans and in atherosclerotic apolipoprotein E-deficient mice. Am J Clin Nutr. 2000;71(5):1062-76. doi: 10.1093/ajcn/71.5.1062, PMID 10799367
13. Selvi KV, Aruna S, Rajeshkumar S. Analysis of bioactive metabolites from *Azolla pinnata* against dental caries. Res J Pharm Technol. 2017;10(6):1891-6. doi: 10.5958/0974-360X.2017.00332.8
14. Mahdavi A, Bagherniya M, Fakheran O, Reiner Ž, Xu S, Sahebkar A. Medicinal plants and bioactive natural compounds as inhibitors of HMG-CoA reductase: A literature review. Biofactors. 2020 Nov;46(6):906-26. doi: 10.1002/biof.1684, PMID 33053603
15. Sharma S, Sharma D, Dhobi M, Wang D, Tewari D. An insight to treat cardiovascular diseases through phytochemicals targeting PPAR-α. Mol Cell Biochem. 2024 Mar;479(3):707-32. doi: 10.1007/s11010-023-04755-7, PMID 37171724
16. Khare V, Kushwaha P, Verma S, Gupta A, Srivastava S, Rawat AK. Pharmacognostic evaluation and antioxidant activity of *Urtica dioica* L. China Med. 2012;3(3):128-35. doi: 10.4236/cm.2012.33021
17. 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.
18. 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 May 7;18(5):96-104. doi: 10.22159/ajpcr.2025v1815.54311
19. Mukherjee PK. Quality Control and Evaluation of Herbal Drugs: Evaluating Natural Products and Traditional Medicine. Elsevier; 2019 May 30. Available from: https://shop.elsevier.com/books/quality/control/and/evaluation/of/herbal/drugs/mukherjee/978/0/12/813374/3
20. 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. doi: 10.1089/jmf.2004.7.343, PMID 15383230
21. 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). Int J Pharm Sci Res. 2012 Feb 1;3(2):615. doi: 10.13040/IJPSR.0975-8232.3(2).615-19
22. 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. doi: 10.1155/2024/3769933, PMID 38633362
23. Jerine PS, Sanjay V, Muruganandham L, Sabina EP. Identifying the potential activity of *Azolla pinnata* through *in vitro* assay and SEM. Songklanakarin J Sci Technol. 2021;43(5):1305-11. doi: 10.14456/sjst-psu.2021.170
24. 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. doi: 10.4103/0972-124X.107471, PMID 23633769
25. 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(2):54-61. doi: 10.1007/BF02867401, PMID 23105534

26. Borges LP, Brandão 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. doi: 10.1016/j.cbi.2007.09.005, PMID 17950719
27. 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. doi: 10.5897/AJB2020.17107
28. 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):1165-76. doi: 10.46602/jcsn.v47i5.819
29. 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. doi: 10.3967/0895-3988.2012.06.009, PMID 23228837
30. 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.
31. 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. doi: 10.70945/bjfn.2024.v01i01.06
32. Mithraja MJ, Marimuthu JM, Mahesh M, Paul ZM, Jeeva S. Phytochemical studies on *Azolla pinnata* R. Br., *Marsilea minuta* L. and *Salvinia molesta* Mitch. Asian Pac J Trop Biomed. 2011;1(1):S26-9. doi: 10.1016/S2221-1691(11)60116-0
33. 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 Dec 1;32:100429. doi: 10.1016/j.bcdf.2024.100429
34. Elrasoul MA, Nour Eldeen GN, Arafa MH. *Azolla pinnata* ethanolic extract alleviates lead-induced hepatotoxicity via antioxidant, anti-inflammatory, and anti-apoptotic mechanisms in rats. Environ Sci Pollut Res. 2020;27(12):13356-66. doi: 10.1007/s11356-020-07955-6
35. Nawaz AS, Junaid S, Dileep N, Rakesh KN, Kekuda TR. Antioxidant activity of *Azolla pinnata* and *Azolla rubra* - a comparative study. Scholars Acad J Biosci. 2014;2(10):719-23. doi: 10.36347/sabj.2014.v02i10.013
36. Livingston Raja NR, Ravindran Nair A, Senthilpandian S, Ravi V. Hypolipidemic action of rutin on triton WR-1339 induced hyperlipidemia in rats. J Pre Clin Clin Res. 2021;15(2):51-5. doi: 10.26444/jpccr/136231
37. Abd Elrasoul AS, Mousa AA, Orabi SH, Mohamed MA, Gad-Allah SM, Almeer R, *et al*. Ameliorative effect of *Azolla pinnata* ethanolic extract on ranitidine-induced hepatotoxicity in rats. J Curr Vet Res. 2020;2(2):21-9. doi: 10.21608/jcvr.2020.121532
38. Nawaz N, Rahman MU, Ahmad N, Mehmood A. Comparative study of *in vitro* antioxidant and cytotoxic activities of *Azolla pinnata* and *Azolla rubra*. Asian Pac J Trop Biomed. 2014;4(2):115-21. doi: 10.1016/S2221-1691(14)60219-3
39. Thiripurasundari CJ, Padmini E. Evaluation of phytochemicals and antioxidant potential of *Azolla pinnata* R. Br Res J Pharm Technol. 2018;11(8):3370-4. doi: 10.5958/0974-360X.2018.00620.2
40. Elrasoul AS, Mousa AA, Orabi SH, Mohamed MA, Gad-Allah SM, Almeer R, *et al*. Antioxidant, anti-inflammatory, and anti-apoptotic effects of *Azolla pinnata* ethanolic extract against lead-induced hepatotoxicity in rats. Antioxidants (Basel). 2020;9(10):1014. doi: 10.3390/antiox9101014, PMID 33086604
41. Santhakumar AB, Battino M, Alvarez-Suarez JM. Dietary polyphenols: Structures, bioavailability and protective effects against atherosclerosis. Food Chem Toxicol. 2018;113:49-65. doi: 10.1016/j.fct.2018.01.022, PMID 29360556
42. Stanely Mainzen Prince P, Kannan NK. Protective effect of rutin on lipids, lipoproteins, lipid metabolizing enzymes and glycoproteins in streptozotocin-induced diabetic rats. J Pharm Pharmacol. 2006;58(10):1373-83. doi: 10.1211/jpp.58.10.0011, PMID 17034661
43. Obilineni I, Latha JD, Srikala K, Asha G, Amulya M, Rajani V. Evaluation of antihyperlipidemic activity of leaves of *Cassia tora*. Res J Pharm Technol. 2022;15(2):741-4. doi: 10.52711/0974-360X.2022.00123
44. Awasthi VK, Mahdi F, Chander R, Khanna AK, Saxena JK, Singh R, *et al*. Hypolipidemic activity of *Cassia tora* seeds in hyperlipidemic rats. Indian J Clin Biochem. 2015;30(1):78-83. doi: 10.1007/s12291-013-0412-2, PMID 25646045
45. Bhuvana K, Sridharan D, Gnanapragasam A. Antihyperlipidemic activity of *Boerhavia diffusa* L. In Triton WR 1339 induced hyperlipidemia in rats. Asian J Pharm Clin Res. 2018;11(9):272-5. doi: 10.22159/ajpcr.2018.v11i9.26714
46. Ccs V, Devi SG. Antihyperlipidemic property of *Boerhavia diffusa* leaf extract in streptozotocin-induced diabetic rats. Asian J Pharm Clin Res. 2018;11(4):173-6. doi: 10.22159/ajpcr.2018.v11i4.23226
47. Althaher AR, Alwahsh M, Hasan A, Al-Majali D, Awadallah MW, Al-Qirim T. Anti-hyperlipidemic effect of *Ruta chalepensis* ethanolic extract in Triton WR-1339-induced hyperlipidemia in rats. Appl Sci. 2024;14(19):9017. doi: 10.3390/app14199017
48. Yehia YA, Greish SS, Kamel MS, Abd EL-Rahman AS, Ahmed YM, Gaber MM. Potential role of *A. Pinnata* in mitigating abamectin-induced hepatorenal toxicity and oxidative stress in Nile tilapia, (*Oreochromis niloticus*). J Appl Plant Protect. 2024;13(1):29-38. doi: 10.21608/japp.2024.329067.1015
49. Roy A, Khan A, Ahmad I, Alghamdi S, Rajab BS, Babalghith AO, *et al*. Flavonoids a bioactive compound from medicinal plants and its therapeutic applications. BioMed Res Int. 2022;2022(1):5445291. doi: 10.1155/2022/5445291, PMID 35707379
50. Sun W, Shahrajabian MH. Therapeutic potential of phenolic compounds in medicinal plants-natural health products for human health. Molecules. 2023 Feb 15;28(4):1845. doi: 10.3390/molecules28041845, PMID 36838831
51. Ahmed QA, Mahmood GJ. Comparison of the effect of *Moringa* extract and the drug atorvastatin in alleviating histological and physiological disorders in the aorta and liver caused by hyperlipidemia in male rats. Obstet Gynaecol Forum. 2024;34(3s):2296-307
52. Aborhyem S, Ismail H, Agamy N, Tayel D. Effect of *Moringa oleifera* on lipid profile in rats. J High Inst Public Health. 2016;46(1):8-14. doi: 10.21608/jhiph.2016.20201
53. Langhi C, Vallier M, Bron A, Otero YF, Maura M, Le Joubioux F, *et al*. A polyphenol-rich plant extract prevents hypercholesterolemia and modulates gut microbiota in western diet-fed mice. Front Cardiovasc Med. 2024;11:1342388. doi: 10.3389/fcvm.2024.1342388, PMID 38317864
54. Abdou HM, Yousef MI, Newairy AA, Triton WR. DNA fragmentation, neurotransmitters inhibition, oxidative damage, histopathological and morphometric changes: The protective role of soybean oil. J Basic Appl Zool. 2018;79:51. doi: 10.1186/s41936-018-0065-z