RESEARCH PAPER

Hepatoprotective potential of Phyllanthus niruri through in vivo pharmacological activity, molecular docking studies and its HPTLC characterization

Prashant Kaushik[1], Pradeep Kumar Mohanty[2]

[1]LNCT University Bhopal, Department of Pharmacology, School of Pharmaceutical Sciences, Madhya Pradesh, India, Email: Prashantkaushik03@gmail.com, ORCID: 0009-0008-7968-5386

[2]LNCT University Bhopal, Department of Pharmacology, School of Pharmaceutical Sciences, Madhya Pradesh, India, ORCID: 0009-0004-5753-2427

ABSTRACT

Background: Phyllanthus niruri has been traditionally used for its antidiabetic, antilipidemic, diuretic, antimicrobic, and antineoplastic properties; however, its hepatoprotective potential remains scientifically unexplored.

Aim: Addressing this gap, the current study investigated the hepatoprotective effects of polyphenol enriched extract of Phyllanthus niruri (PNE) in Wistar albino rats subjected to acetaminophen-induced hepatotoxicity.

Methodology: HPTLC fingerprinting commenced to characterize the phytoconstituents, acetaminophen rodent model used to exert the hepatoprotective properties and their molecular docking with proteins linked to explore hepatotoxicity.

Results: The present findings revealed a significant hepatoprotective effect by improving serum biochemical parameters. Total glyceride compared to disease control were significant for the PNE (400mg/kg) 65.22 ± 6.86, Liver enzymes showed a marked significant improvement in PNE treated groups AST (33.49 ± 3.60), ALT (74.08 ± 5.86) and ALP (75.85 ± 6.37) in respect to disease control group. Antioxidant parameters showed a significant improvement in PNE treated groups SOD (7.24 ± 0.24), CAT (14.06 ± 0.76), Gpx (10.57 ± 0.54) and MDA (0.58 ± 0.08) with respect to diseased groups. Antioxidant parameters showed a significant improvement in PNE treated groups SOD (7.24 ± 0.24), CAT (14.06 ± 0.76), Gpx (10.57 ± 0.54) and MDA (0.58 ± 0.08) with respect to diseased groups. Histological analysis showed that PNE effectively mitigated liver tissue damage. HPTLC analysis identified polyphenolic compounds such as quercetin and caffeic acid in PNE. In Silico, findings indicated that quercetin and caffeic acid exhibit strong and stable interactions with SOD and TNF-α. These stable bindings suggest their antioxidant and anti-inflammatory properties.

Conclusion: PNE exhibited hepatoprotective potential by modulating liver enzymes, hyperlipidemia, inflammation, oxidative stress, and restoring liver histological architecture.

Keywords: Acetaminophen, Computer simulation, Chromatography, Phyllanthus niruri, Polyphenol, Liver

How to cite this article: Kaushik P, Mohanty PK. Hepatoprotective Potential of Phyllanthus niruri through in vivo Pharmacological Activity, Molecular Docking Studies and Its HPTLC Characterization. Int J Drug Deliv Technol. 2026;16(67s):922-930. DOI: 10.25258/ijddt.16.67s.78

INTRODUCTION

The liver, an extremely delicate organ in the human body, is crucial to preserving homeostasis. This organ performs vital functions, including metabolism and detoxification. The liver is responsible for the majority of the body's harmful chemical metabolism. These toxicants mostly flow into the body within the gastrointestinal tract and once absorbed, they passed to the liver via the hepatic vein (1).

The most common diseases in the world are liver ailments, which are typically brought on by medicines, chemicals, and alcohol. In wealthy countries, Metabolic Syndrome liver disease is often diagnosed with persistent liver disease. Several different hepatic problems cause the disease. Some of these anomalies are brought on by an abnormal buildup of fat in the liver, which eventually causes inflammation and liver cirrhosis (2). Oxidative stress brought on by the high-fat diet, acetaminophen and

other chemical substances eventually culminates in active oxygen. These free oxygen radicals injure cells by oxidizing their constituent parts (3).

Hepatotoxicity, caused by exposure to drug and chemical products, is emerging as a major metabolic and public health challenge worldwide. It contributes to approximately 5% of all hospital admissions and is implicated in nearly 50.0% of acute liver failure events globally (Roy et al. 2024). Globally, drug-induced liver injury (DILI), one of the most substantial contributors to hepatotoxicity, shows substantial variability in incidence across geographic regions. Global data estimates the annual incidence rate of DILI to be between 13.90 and 24.0 cases per 100,000 inhabitants. These rising figures clearly indicate that hepatotoxicity is not only a growing clinical burden but also a significant financial strain, especially in developing and under developing nation across Asia and its sub-continent, Africa countries, and South America, where access to hepatology care and pharmacovigilance systems remains limited (5).

Plant-based medicine has numerous bioactivities, including anti-inflammatory, anti-autoimmune, antidiabetic, antineoplastic, and antioxidant properties. To manage and treat illnesses, the trend in therapeutic selection has shifted from synthetic to natural remedies, suggesting that people are getting back in touch with nature. In recent decades, herbal medicine has expanded rapidly and become more popular in both developed and developing nations, including India (6). Furthermore, because of its natural origins, ease of accessibility and availability, and lack of adverse effects, herbal therapy has been used for millennia and is well recognized worldwide as a treatment plan for illness prevention. In the present scenario, we have all observed that there is no effective therapy to prevent hepatotoxicity. Contemporarily, several efficacious and less toxic plant-based medicines have been developed to protect against hepatotoxicity (6).

Phyllanthus niruri, commonly known as Bhumi Amla, exhibits significant hepatoprotective potential due to its rich composition of phytochemicals such as lignans (phyllanthin, hypophyllanthin), flavonoids, polyphenols, alkaloids, and tannins. These compounds exert antioxidants, anti-inflammatory, antiviral, and antifibrotic effects, helping to stabilize liver enzymes, protect hepatocytes, and inhibit lipid peroxidation. Studies show its efficacy in managing hepatitis B, non-alcoholic fatty liver disease, and liver fibrosis, while enhancing bile flow and detoxification pathways. Its antioxidant properties neutralize free radicals, preventing hepatic cell damage and improving liver regeneration (7,8). This makes P. niruri a promising natural remedy for liver and metabolic disorders, but evidence-based studies revealed that P. niruri is still lacking in hepatoprotective activity. With due, the present study aims to test the hepatoprotective effect of P. niruri extract using a rodent model and explore its action mechanism through molecular docking.

MATERIALS AND METHODS

Procurement of sample and determination of total polyphenolic content

P. niruri polyphenols-enriched extract was obtained as a gift sample from MotherSoul Private Limited, Indore, Madhya Pradesh, India

DPPH inhibition activity

The ability of PNE to scavenge DPPH (2,2-diphenyl-1-picrylhydrazyl) was tested according to the defined protocol. The different concentrations of samples were mixed with a 0.1 mM DPPH solution and left to incubate at 37°C in the dark for 30 min. Thereafter, the absorbance was measured at 517 nm to determine the scavenging potential. The reference compound was utilized as ascorbic acid, and IC50 values were calculated (10).

$$\text{Percentage inhibition} = (1\text{-}A_{sample}/A_{control}) \times 100$$

3 HPTLC determination of Quercetin and Caffeic acid in Phyllanthus niruri extract (PNE)

The extract was dissolved in HPLC-grade methanol to prepare the solution (50 mg/mL of extract and 0.5 mg/mL of standards), which was then filtered using a Millipore filter (0.2 µm). The extract and standard (quercetin and caffeic acid) were applied to TLC plates using the following parameters: The application rate was 150 nL/s, the band length was 6 mm, the track margin was 4 mm, and the side edge and bottom were 1.5 and 2 cm apart, respectively. A pre-saturated TLC chamber containing mobile phases such as formic acid, ethyl acetate, and toluene (0.5: 4.5: 5 v/v/v) was used for TLC fingerprinting. After TLC development the plate was scanned for the compound analysis for both standard and extract (10).

Experimental animal models

Wistar albino rats weighing 175-200 g were used for the in vivo hepatoprotective properties in accordance with the previous report. All the animals were housed in regular laboratory settings and provided a standard diet throughout the research. The experimental design for the animal investigation is mentioned in Figure 1. Silymarin is used as a reference medication for the purposes of comparison. At the conclusion of the trial, the body weight of each animal was measured. Following animal sacrifice, serum was carefully isolated and kept at -4°C

for additional analysis after blood samples were taken, allowed to clot for 20 minutes, and centrifuged for 10 minutes at 1350 rpm. Additionally, the animals were put to death and slaughtered so that kidney tissue could be taken from each one for histological and antioxidant investigation (11,12).

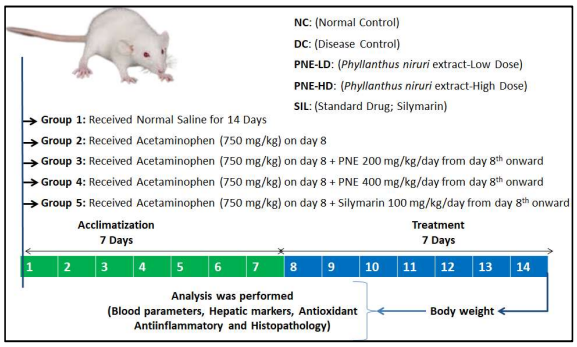

Figure 1: Outline acetaminophen induced in vivo model, standard treatment silymarin and different doses of Phyllanthus niruri extract (PNE)

Biochemical Estimation

At the end of the experiments, all the animals were anaesthetized, and withdrawn blood for biochemical analysis using biochemical reagents and commercial kits.

Total cholesterol (TC), Triglycerides (TG) High density lipoprotein (HDL), Low density lipoprotein (LDL, aspartate transferase (AST), alanine aminotransferase (ALT), aspartate aminotransferase (ALP), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and MDA levels were estimated. Further, assessment of inflammatory biomarkers such as TNF-α and IL-6 was performed as per protocol using an ELISA reader (10,11,12).

Histopathology

The rats were euthanized with CO2 in an appropriate chamber at the end of the experiments. The portion of liver was immersed in formalin for histological evaluation. Also prepared liver homogenates for biochemical analysis (13).

Molecular docking studies

Retrieval of ligands: The quercetin and caffeic acid were downloaded from PubChem. 3D structures of these ligands were docked against selected target proteins (14).

Retrieval and refinement of receptor proteins: The RCSB Protein Data Bank provided the three-dimensional structures of SID and TNF-α 6, and these structures were downloaded in PDB format. Before docking investigations, the Auto dock Vina software was utilized to optimize the protein structures of receptor proteins. To construct and optimize the receptor proteins, ligands and water molecules were eliminated.

Docking protocol: To employ Auto Dock Vina to perform molecular docking analysis, both ligands and proteins' PDB files were transformed to PDBQT. The grid parameters were utilised to calculate using the "Grid" feature of AutoDock. The protein-ligand interactions were further utilised for docked pose analysis of the ligands at the binding sites of the target proteins (14).

Statistical analysis

The data was statistically represented as mean and standard deviation using the Tukey test and One Way ANOVA. Statistical significance was defined as p values below 0.05. The toxic group was contrasted with the control and treatment groups.

RESULTS

Antioxidant potential of Phyllanthus niruri extract (PNE)

The antioxidant potential of Phyllanthus niruri extract (PNE) was evaluated using the DPPH radical scavenging assay, a widely accepted biochemical test. In this method, antioxidant compounds present in the extracts react with the stable purple DPPH solution, reducing it to a yellow-colored product, which indicates free radical neutralization. The results revealed that PNE demonstrated a significant, concentration-dependent inhibitory effect on DPPH radicals within the tested range of 25–500 µg/ml. The IC50 value, a measure of antioxidant strength, was determined as 34.34 ± 5.18 µg/mL for Phyllanthus niruri extract (PNE) compared to 38.36 ± 3.36 ug/mL for the standard reference, confirming the antioxidant efficacy of PNE.

Simultaneous identification of Quercetin and Caffeic acid in Phyllanthus niruri extract (PNE)

In this study, HPTLC was employed to identify quercetin and caffeic acid. To achieve optimal separation of these compounds, different solvent systems with varied compositions were tested. Among them, the mobile phase consisting of formic acid, ethyl acetate, and toluene (0.5: 4.5: 5 v/v/v) provided the most effective resolution compared to previously reported methods. This optimized solvent system enabled clear and distinct separation of polyphenolic quercetin and caffeic acid compounds. The chromatographic profiling of the extract showed well-resolved bands, confirming the effective separation of all bioactive polyphenolics. Furthermore, their characteristic fluorescence bands were distinctly visible under UV light at 254 nm. Quercetin and caffeic acid were identified as the PNE

spots with Rf values of 0.57, and 0.68, respectively (Figure 2).

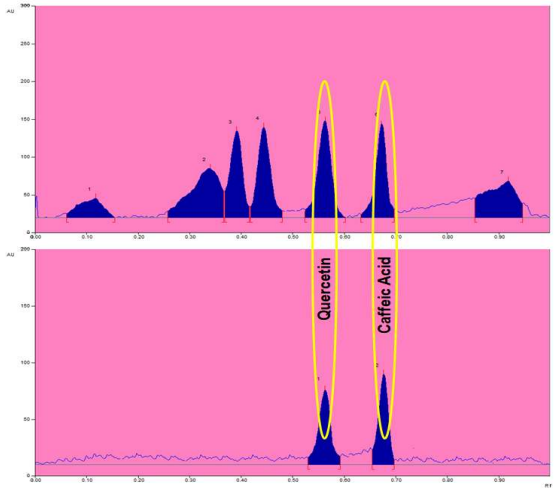

Figure 2. Developed HPTLC chromatograms of Phyllanthus niruri extract (PNE) and standard.

Measurement of body weight

Measurement of bodyweight before and after experimentation demonstrated a marked loss in weight among the acetaminophen-intoxicated groups, strongly indicating drug-induced hepatotoxicity. Animals treated with a high dose of Phyllanthus niruri extract (PNE)or the standard compound showed a significant restoration of bodyweight (Figure 3), underscoring their protective, hepatorestorative potential.

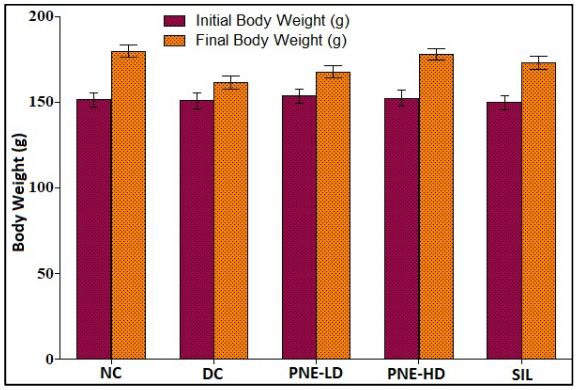

Figure 3: Showing effects of Phyllanthus niruri extract (PNE) versus silymarin on the body weight of experimental rats.

Effects of Phyllanthus niruri extract (PNE) on Serum Lipid Profiles

The levels of TG, TC, LDL-C, and HDL-C in normal and acetaminophen-intoxicated rats are presented in Table 1A. The disease control (DC) group showed a significant reduction in HDL-C along with decreased TG, TC, and LDL-C levels compared to the normal control (NC) group. Oral administration of PNE significantly restored TG, TC, and LDL-C levels, although no notable improvement in HDL-C was observed in the PNE-LD group compared to DC. These findings suggest that PNE exhibits dose-dependent effects on lipid profile regulation, with ameliorative potential like that of silymarin.

Table 1: Effect of Phyllanthus niruri extract (PNE) on Lipid profile, Liver function, Antioxidant enzymes and Anti-inflammatory status in experimental rats

Parameters	Experimental Group				
	Normal Control	Diseased Control	*Phyllanthus niruri* extract (PNE) -Low Dose	*Phyllanthus niruri* extract (PNE) -High Dose	Silymarin (SIL)
(A) Lipid profile					
TC (mg/dl)	67.79 ± 6.75	117.18 ± 9.52$^{###}$	95.41 ± 6.62*	75.25 ± 4.73***	79.53 ± 6.52***
TG (mg/dl)	48.52 ± 6.07	88.69 ± 7.65$^{###}$	73.25 ± 5.46ns	63.46 ± 5.29**	65.22 ± 6.86**
LDL-C (mg/dl)	41.54 ± 3.48	81.72 ± 5.92$^{###}$	60.16 ± 5.01**	47.57 ± 5.49***	44.96 ± 3.43***
HDL-C (mg/dl)	23.69 ± 0.82	15.30 ± 2.13$^{##}$	18.19 ± 0.93ns	21.06 ± 2.59*	20.21 ± 1.19*
(B) Liver function test					
AST (U/L)	27.46 ± 3.44	55.48 ± 5.20$^{###}$	44.47 ± 5.02	37.05 ± 4.33**	33.49 ± 3.60***
ALT (U/L)	49.52 ± 5.70	130.62 ± 10.51$^{###}$	106.37 ± 8.00*	55.37 ± 6.51***	74.08 ± 5.86***
ALP (U/L)	70.11 ± 6.36	150.15 ± 9.01$^{###}$	124.36 ± 8.34*	63.81 ± 8.33***	75.85 ± 6.37***
(C) Kidney function test					
Urea (mg/dl)	60.33 ± 4.52	203.07 ± 9.17$^{###}$	175.15 ± 8.32**	76.45 ± 7.24***	103.86 ± 6.29***
Uric acid (mg/dl)	8.35 ± 0.70	13.31 ± 1.22$^{###}$	11.22 ± 1.26	8.87 ± 0.74**	10.30 ± 0.72*

Creatinine (mg/dl)	0.69 ± 0.10	0.88 ± 0.10	0.83 ± 0.11	0.73 ± 0.09	0.68 ± 0.06
(D) Antioxidant level					
SOD (U/mg protein)	7.98 ± 0.15	3.77 ± 0.18###	5.09 ± 0.55**	7.54 ± 0.32***	7.24 ± 0.24***
CAT (U/mg protein)	17.21 ± 0.41	11.54 ± 0.81###	13.21 ± 0.41*	16.23 ± 0.37***	14.06 ± 0.76**
GPx (U/mg protein)	11.09 ± 0.44	5.31 ± 0.57###	7.72 ± 0.62**	10.04 ± 0.65***	10.57 ± 0.54***
MDA (U/mg protein)	0.61 ± 0.10	0.87 ± 0.08##	0.75 ± 0.08	0.70 ± 0.06*	0.58 ± 0.08**

Data are expressed as mean ± SD (n = 6). Values with superscripts (#) is significantly different between normal control vs hepatotoxic control and superscripts (*) is significantly different between hepatotoxic control vs treatment groups. The value $p < 0.05$ considered significant. The symbol represents the significance level such as #/* ($p < 0.05$); ##/** ($p < 0.01$) and ###/*** ($p < 0.001$).

Keys: Total Cholesterol (TC)), Triglycerides (TG), Low-Density Lipoprotein Cholesterol (LDL-C), High-Density Lipoprotein Cholesterol (HDL-C), Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx), Malondialdehyde (MDA)

Effects of Phyllanthus niruri extract (PNE) on Serum Liver Profiles

Table 1B summarizes the impact of Phyllanthus niruri extract (PNE) supplementation on liver abnormalities. In the disease control (DC) group, serum levels of ALT, AST, and ALP were markedly elevated compared to the normal control (NC) group, indicating liver injury. Administration of PNE for 2 weeks significantly reduced these elevated enzyme levels. Higher dose of PNE shows more pronounced improvements in all the parameters. While the lower dose of PNE successfully restored AST and ALP levels, it was less effective for ALT. The results demonstrate a hepatoprotective effect comparable to that of silymarin.

Estimation of the Antioxidant status of liver tissue in the experimental groups

Oxidative stress (OS) is a hallmark of acetaminophen-induced hepatotoxicity, primarily due to excessive reactive oxygen species generation that impairs hepatic antioxidant defenses. In this study, results of antioxidant enzymes SOD, CAT, and GPx revealed a marked increase in SOD in the acetaminophen-induced toxic group after treatment with PNE. Such a pattern indicates an adaptive, yet overwhelmed, antioxidant response due to ongoing oxidative injury. Notably, treatment with higher doses of test compound and standard control effectively normalized these parameters, demonstrating significant antioxidant and hepatoprotective efficacy. Comparable effects were observed between the highest treatment dose and the standard, as detailed in Table 1 C.

Estimation of inflammatory markers in the experimental groups

The levels of key inflammatory markers TNF-α and IL-6 were significantly elevated in the acetaminophen-intoxicated (toxic) group, indicating pronounced hepatic inflammation and injury. Scientific studies consistently link the increased expression of these cytokines to acute hepatocellular damage and oxidative stress. Treatment with PNE, especially at a high dose, led to a marked decrease in these cytokines, effectively restoring their expression towards normal values, like the effects observed with standard silymarin treatment, as shown in Table 1D. These results highlight the strong anti-inflammatory and hepatoprotective capabilities of Phyllanthus niruri extract (PNE), suggesting that it can mitigate acetaminophen-induced liver injury by modulating inflammatory responses and protecting hepatic tissue.

Histological observations of the liver

All liver function test (LFT) findings were closely correlated with the histopathological alterations observed in the liver photomicrographs (Figure 4). Histological analysis revealed well-defined vesicular nuclei and preserved liver architecture in the control group (NC). In contrast, the acetaminophen-intoxicated group (DC) exhibited marked cellular degeneration, infiltrating lymphocytes, centrilobular cell degeneration, and pronounced hepatic necrosis. Low dose of PNE demonstrated mild degenerative changes and increased intercellular spaces within proliferative hepatocytes, while groups high dose of PNE and silymarin showed restoration of normal hepatic architecture with clear, well-defined nuclei. Notably, treatment with PNE effectively attenuated acetaminophen-induced histopathological damage, supporting its hepatoprotective potential.

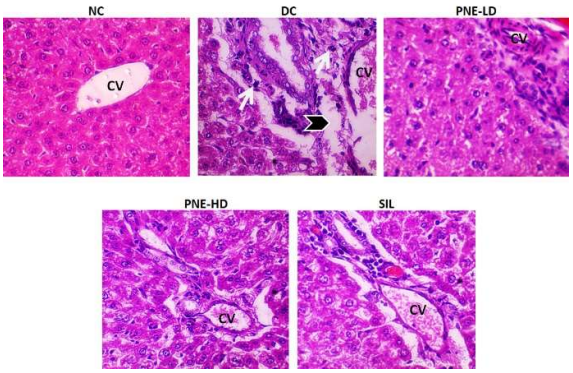

Figure 4: Photomicrograph of the liver stained with HE. The normal control (NC) group shows normal portal triad along with normal hepatocytes with the central vein (CV). Disease control (DC) group shows degenerated cells with fading nuclei (white arrow), irregular hepatic cords (head arrow), with the disarranged central vacuole (CV). After treatment with Phyllanthus niruri extract (PNE)and standard, histological architecture of the hepatic cells ameliorates to normal in experimental rats. Keys: Normal control (NC), Disease control (DC), Phyllanthus niruri extract low dose (PNE LD), Phyllanthus niruri extract high dose (PNE HD), Silymarin (SIL)

Molecular Docking Inhibition of TNF-α and IL-6

In-silico analysis demonstrated that quercetin and caffeic acid possess strong and stable affinities toward the active sites of two pivotal inflammatory mediators, SOD and TNF-α. The key interacting residues involved in the binding of quercetin and caffeic acid to SOD and TNF-α are summarized in Table 2, Figure 5, highlighting their pivotal role in inhibiting inflammation-associated proteins. Collectively, these findings support the hypothesis that these phytochemicals (quercetin and caffeic acid) exert significant antioxidant and anti-inflammatory effects, contributing to protective therapeutic roles.

Table 2. The results of molecular docking of quercetin and caffeic with SOD and TNF-α.

Compounds	Proteins	Bonding energy	H-bonds Interactions
Quercetin	(A) SOD	-8.9kcal/mol	Ile312(A), Asp353(A)
	(B) TNF-α	-8.8kcal/mol	Arg267(A), Arg346(A), Ala310(A), Phe348(A), Gly304(A)
Caffeic acid	(C) SOD	-6.3kcal/mol	Asp353(A), Arg303(A), Gly309(A), Ile312(A), Ala310(A)
	(D) TNF-α	-6.1kcal/mol	Arg346(A), Arg267(A), Asn352(A)

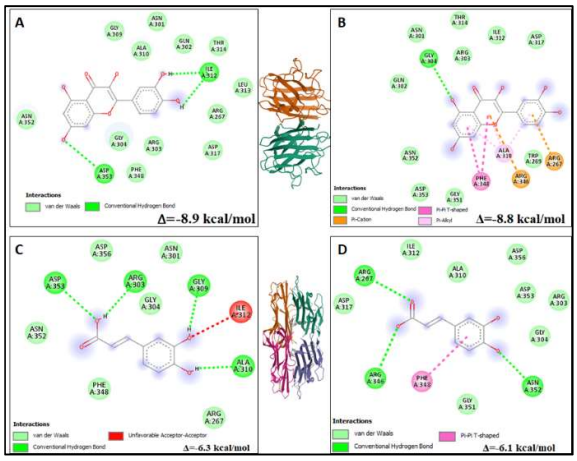

Figure 5. Docking view of the binding interaction of quercetin with SOD (A) and TNF- α (B), as well as caffeic acid SOD (C) and TNF-α (D).

DISCUSSION

Paracetamol (acetaminophen) is a widely utilized over-the-counter antipyretic and analgesic agent. While safe at therapeutic doses, exceeding recommended levels results in hepatotoxicity due to the accumulation of its toxic metabolite, N-acetyl-p-benzoquinone imine (NAPQI), causing DILI. Acetaminophen remains the leading cause of DILI in the United States (15). Patients with pre-existing liver conditions or chronic alcohol consumption are particularly vulnerable, warranting cautious dosing, typically recommended at ≤2,000 mg daily. The increasing prevalence of combination medications, including over-the-counter cold remedies and prescription analgesics containing acetaminophen, complicates toxicity recognition and management. This polypharmacy elevates the risk of inadvertent overdose (16). According to many researchers of the world and present findings, herbal medicine may play a preventive role in mitigating the side effects of paracetamol due to their multi-mechanistic approaches. Herbal medicine prevents pathophysiological processes by vanquishing oxidative stress, inflammation, and hepatic necrosis to combat hepatotoxicity (17). This finding is strongly correlated with other reported studies.

Oxidative stress (OS) and lipid peroxidation play a pivotal role in exacerbating acetaminophen-induced hepatotoxicity. The oxidation of cellular lipids and

proteins is closely linked to disruptions in live enzymes, structural integrity and physiological functions. The results of the present study clearly indicate hepatocellular damage in the acetaminophen-induced animal model, marked by significant elevation in AST, ALT, and ALP levels (18). However, treatment with both low and high doses of PNE effectively restored these levels to normal. Furthermore, previous studies have demonstrated that acetaminophen-induced free radicals overwhelm antioxidant enzymes such as SOD, CAT, and GPx as observed in the present findings (3). These antioxidant enzymes are essential for neutralizing oxidative free radicals and serve as the primary cellular defense mechanism against oxidative stress in living organisms (19). Treatment with PNE significantly restored the activities of SOD, CAT, and GPx. The higher dose of PNE exhibited greater antioxidant efficacy compared to the lower dose and demonstrated effects comparable to the standard treatment. Moreover, PNE significantly inhibits the DPPH. These findings confirm the antioxidant and hepatoprotective potential of PNE in this investigation.

The antioxidant potential of the plant extract is mainly due to the free hydroxyl groups in polyphenols, which scavenge free radicals and inhibit reactive oxygen species formation. Phenols and flavonoids (ArOH) reduce oxidative stress by donating hydrogen atoms to radicals, suggesting that polyphenol and flavonoid-rich foods may help lower the risk of hepatotoxicity. Therefore, biologically and pharmacologically believe that phenolic and flavonoids rich foods may reduce the risk of hepatotoxicity or liver injury (7,19).

Proinflammatory cytokines, including TNF-α and IL-6, play critical roles in mediating cellular inflammation and are closely linked to the pathophysiology of acetaminophen-induced hepatotoxicity (20). Elevated levels of TNF-α in liver tissues are indicative of acetaminophen toxicity in the experimental groups, with similar patterns observed for IL-6. Treatment with PNE significantly normalized the expression of these inflammatory cytokines, aligning with previous studies that have demonstrated the anti-inflammatory effects of PNE in rodent models of acetaminophen-induced liver injury. These findings highlight the potent anti-inflammatory properties of the polyphenol-enriched extract of Phyllanthus niruri. Published studies highlight polyphenolic compounds as potential therapeutic agents for preventing acetaminophen-induced liver toxicity. In line with previous research, this study also confirmed increased TNF-α and IL-6 levels, emphasizing the inflammatory nature of the liver damage observed.

Acetaminophen administration caused significant impairment of liver histological architecture and was associated with a reduction in body weight. Histopathological analysis revealed severe cellular degeneration, infiltration of lymphocytes, centrilobular cell degeneration, and extensive hepatic necrosis. These pathological changes corresponded with biochemical evidence of elevated inflammatory markers, increased oxidative stress, and decreased antioxidant enzyme levels in liver tissues (16,21). Treatment with the polyphenol-enriched extract (PNE) effectively mitigated these adverse effects, restoring the liver's histological structure close to normal. The histopathological outcomes were consistent with the biochemical findings and corroborate previous studies, underscoring the hepatoprotective potential of PNE against acetaminophen-induced liver injury.

Molecular docking simulations were employed to investigate the interactions of quercetin and caffeic acid with the active sites of two key enzymes, SOD and TNF-α. The docking analysis predicted their binding modes and provided insights into their possible hepatoprotective activity (22). Both compounds exhibited an excellent fit within the proteins' active sites, showing favorable binding energy. These observations highlight their pivotal role in inhibiting oxidative stress and inflammation-associated proteins. Collectively, these findings suggest that the bioactive compounds in PNE possess significant antioxidant and anti-inflammatory result to hepatoprotective potential.

Quercetin is a potent antioxidant that protects liver cells from toxins such as ethanol, acetaminophen, and carbon tetrachloride by scavenging free radicals, reducing lipid peroxidation, and boosting glutathione levels, thereby mitigating oxidative stress and inflammation. Studies show that quercetin also suppresses inflammatory cytokines like TNF-α and IL-1β, reducing liver inflammation (23). Similarly, caffeic acid exhibit hepatoprotective effects by activating antioxidant pathways, scavenging free radicals, and enhancing the production of antioxidant enzymes like SOD and CAT, as well as increasing glutathione levels. Caffeic acid also activates the Keap1-Nrf2 signaling pathway, crucial for defense against oxidative stress, and helps prevent increases in liver enzymes (ALT, AST) and lipid peroxidation linked to liver injury (22,24). Quercetin and caffeic acid enriched extract of Phyllanthus niruri appears to offer a protective effect against ethanol-induced liver injury in rats, likely due to the synergistic action of these compounds through multiple biological mechanisms.

CONCLUSIONS

In summary, the current findings strongly support the hepatoprotective effects of a polyphenol-enriched

extract of Phyllanthus niruri against acetaminophen-induced liver injury in rats. Phyllanthus niruri exerts this protective effect through multiple mechanisms, including enhancing antioxidant defense against oxidative stress, reducing proinflammatory cytokine expression, lowering hyperlipidemia, and modulating liver enzymes. These results suggest that Phyllanthus niruri extract holds promise as a therapeutic agent for managing acetaminophen-induced liver damage. However, additional molecular and clinical studies are needed to further confirm and expand upon these results.

Language: English (United States)
Conflict of Interest: Declared no conflict of interest
Funding: No source

Authors contributions: All authors contributed to the research work and manuscript equally

REFERENCES

1. Singh D, Cho WC, Upadhyay G. Drug-induced liver toxicity and prevention by herbal antioxidants: an overview. Frontiers in Physiology. 2016;6:363. doi.org/10.3389/fphys.2015.00363
2. Idalsoaga F, Kulkarni AV, Mousa OY, Arrese M, Arab JP. Non-alcoholic fatty liver disease and alcohol-related liver disease: two intertwined entities. Frontiers in Medicine. 2020;7:448. doi: 10.3389/fmed.2020.00448
3. Du K, Ramachandran A, Jaeschke H. Oxidative stress during acetaminophen hepatotoxicity: sources, pathophysiological role and therapeutic potential. Redox Biology. 2016;10: 148–156. doi: 10.1016/j.redox.2016.10.001
4. Roy S, Shah Z, Chakraborthy GS. A brief overview of drug-induced liver damage. The Egyptian Journal of Internal Medicine. 2024 May 30;36(1):56. doi.org/10.1186/s43162-024-00315-7
5. Tiwari V, Shandily S, Albert J, Mishra V, Dikkatwar M, Singh R, Sah SK, Chand S. Corrigendum to “Insights into medication-induced liver injury: Understanding and management strategies”. Toxicol. Report. 2025;14:102068. doi.org/10.1016/j.toxrep.2025.102068
6. Ibrahim M, Parveen B, Zahiruddin S, Gautam G, Parveen R, Khan MA, et al. Analysis of polyphenols in Aegle marmelos leaf and ameliorative efficacy against diabetic mice through restoration of antioxidant and anti-inflammatory status. Journal of Food Biochemistry. 2022;46(4):e13852. doi.org/10.1111/jfbc.13852
7. Khan S, Ibrahim M. A systematic review on hepatoprotective potential of grape and polyphenolic compounds: Molecular mechanism and future prospective. Natural Resources for Human Health. 2022; 3: 196–213. doi.org/10.53365/nrfhh/156261
8. Pratima H, Shiraguppi A, Joojagar P, Shah K, Cheeraladinni SS, Singh PS, Mendem SK, Chauhan NS. Phytochemical profile and hepatoprotective potentiality of Phyllanthus genus: a review. Journal of Pharmacy and Pharmacology. 2025;77(2):189-205. doi.org/10.1093/jpp/rgae040
9. Liu L, Wang B, Ma Y, Sun K, Wang P, Li M, Dong J, Qin M, Li M, Wei C, Tan Y. A review of Phyllanthus urinaria L. in the treatment of liver disease: viral hepatitis, liver fibrosis/cirrhosis and hepatocellular carcinoma. Frontiers in Pharmacology. 2024;15:1443667. doi.org/10.3389/fphar.2024.1443667
10. Fahim M, Ibrahim M, Zahiruddin S, Parveen R, Khan W, Ahmad S, et al. TLC-bioautography identification and GC-MS analysis of antimicrobial and antioxidant active compounds in Musa× paradisiaca L. fruit pulp essential oil. Phytochemical Analysis. 2019;30(3):332-45. doi.org/10.1002/pca.2816
11. Hasanein P, Sharifi M. Effects of rosmarinic acid on acetaminophen-induced hepatotoxicity in male Wistar rats. Pharmaceutical Biology. 2017;55(1):1809-16. doi.org/10.1080/13880209.2017.1331248
12. Yan M, Huo Y, Yin S, Hu H. Mechanisms of acetaminophen-induced liver injury and its implications for therapeutic interventions. Redox biology. 2018;17:274-83. Redox Biology. doi: 10.1016/j.redox.2018.04.019
13. Rahman A, Ibrahim M, Siddiqui JI, Ansari SA, Ahmad S. Metabolomic profiling and antidiabetic potential of Rumex vesicarius seed extract in high-fat diet and streptozotocin-induced diabeticrat. Brazilian Journal of Pharmaceutical Sciences. 2022;58:e21032. doi.org/10.1590/s2175-97902022e21032
14. Jaramillo-Morales OA, Díaz-Cervantes E, Via LD, Garcia-Argaez AN, Espinosa-Juárez JV, Ovando-Zambrano JC, et al. Hepatoprotective activity, in silico analysis, and molecular docking study of verbascoside from Leucophyllum frutescens in rats with post-necrotic liver damage. Scientia Pharmaceutica. 2023;91(3):40. doi.org/10.3390/scipharm91030040
15. Yoon E, Babar A, Choudhary M, Kutner M, Pyrsopoulos N. Acetaminophen-induced hepatotoxicity: a comprehensive update. Journal of Clinical and Translational Hepatology. 2016;4(2):131. doi: 10.14218/JCTH.2015.00052
16. Rotundo L, Pyrsopoulos N. Liver injury induced by paracetamol and challenges associated with intentional and unintentional use. World Journal of Hepatology. 2020;12(4):125. doi.org/10.4254/wjh.v12.i4.125

17. Chang L, Xu D, Zhu J, Ge G, Kong X, Zhou Y. Herbal therapy for the treatment of acetaminophen-associated liver injury: recent advances and future perspectives. Frontiers in pharmacology. 2020; 11: 313. doi.org/10.3389/fphar.2020.00313
18. Jaeschke H, Ramachandran A. Oxidant stress and lipid peroxidation in acetaminophen hepatotoxicity. Reactive Oxygen Specie. 2018;5(15):145. doi.org/10.20455/ros.2018.835
19. Ismail NA, Okasha SH, Dhawan A, Abdel-Rahman AO, Shaker OG, Sadik NA. Antioxidant enzyme activities in hepatic tissue from children with chronic cholestatic liver disease. Saudi Journal of Gastroenterology. 2010;16(2):90-4. doi.org/10.4103/1319-3767.61234
20. Li Q, Chen F, Wang F. The immunological mechanisms and therapeutic potential in drug-induced liver injury: Lessons learned from acetaminophen hepatotoxicity. Cell & Bioscience. 2022;12(1):187. doi: 10.1186/s13578-022-00921-4
21. Wu CT, Deng JS, Huang WC, Shieh PC, Chung MI, Huang GJ. Salvianolic acid C against acetaminophen-induced acute liver injury by attenuating inflammation, oxidative stress, and apoptosis through inhibition of the Keap1/Nrf2/HO-1 signaling. Oxidative Medicine and Cellular Longevity. 2019;2019(1):9056845. doi.org/10.1155/2019/9056845
22. Ojo OO, Ogunleke T, Ajeoge J, Olorunsogo OO. Experimental and molecular docking studies of quercetin and vitamin E with diabetes-associated mitochondrial-ATPase as anti-apoptotic therapeutic strategies. Journal of Diabetes & Metabolic Disorders. 2022;21(2):1717-29. doi.org/10.1007/s40200-022-01132-x
23. Chen X. Protective effects of quercetin on liver injury induced by ethanol. Pharmacognosy Magazine. 2010; 6(22):135. doi.org/10.4103/0973-1296.62900
24. Li M, Wang XF, Shi JJ, Li YP, Yang N, Zhai S, Dang SS. Caffeic acid phenethyl ester inhibits liver fibrosis in rats. World Journal of Gastroenterolpgy. 2015;21(13):3893. doi.org/10.3748/wjg.v21.i13.3893