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MOLECULAR EVALUATION OF A *PHYLLANTHUS NIRURI* NANOSTRUCTURED LIPID CARRIER IN CARBON TETRACHLORIDE-INDUCED HEPATIC INJURY THROUGH OXIDATIVE STRESS, INFLAMMATORY, AND APOPTOTIC GENE EXPRESSION ANALYSIS

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ABSTRACT
Hepatic injury induced by carbon tetrachloride (CCl_4) is primarily mediated through oxidative stress, inflammation, and apoptosis, resulting in severe hepatocellular damage. The present study evaluated the hepatoprotective potential of a Phyllanthus niruri nanostructured lipid carrier (PN-NLC) in a CCl_4-induced rat model. PN-NLC was prepared and characterized for its physicochemical properties before in vivo evaluation. Hepatoprotective activity was assessed using serum biochemical markers, oxidative stress parameters, inflammatory cytokines, histopathological examination, and quantitative real-time PCR analysis of antioxidant, inflammatory, and apoptotic genes. PN-NLC treatment significantly improved liver function markers, restored endogenous antioxidant enzyme activity, suppressed pro-inflammatory mediators, regulated the expression of Nrf2, HO-1, TNF-α, IL-6, NF-κB, Bax, Bcl-2, and Caspase-3, and markedly improved hepatic histoarchitecture compared with the CCl_4 control group. These findings indicate that PN-NLC exerts potent hepatoprotective effects through modulation of oxidative stress, inflammatory signaling, and apoptosis, highlighting its potential as a promising nano-herbal therapeutic approach for the management of liver injury.

KEYWORDS: Phyllanthus niruri; Nanostructured lipid carrier; Carbon tetrachloride; Hepatoprotection; Gene expression.

1. INTRODUCTION
Liver diseases constitute a major global health challenge, accounting for substantial morbidity and mortality worldwide. Chronic liver disorders, including hepatitis, fibrosis, cirrhosis, alcoholic liver disease, non-alcoholic fatty liver disease (NAFLD), and drug- or chemical-induced hepatotoxicity, represent significant causes of liver-related deaths and healthcare burden. The liver plays a central role in metabolism, detoxification, protein synthesis, bile secretion, and maintenance of systemic homeostasis. Owing to its strategic anatomical location and metabolic functions, the liver is continuously exposed to xenobiotics, environmental toxins, pharmaceuticals, alcohol, and reactive metabolites that can initiate hepatocellular injury. Persistent hepatic damage eventually results in oxidative stress, chronic inflammation, apoptosis, fibrosis, and ultimately liver failure if left untreated (1,2).
Among the experimental models of hepatic injury, carbon tetrachloride (CCl_4)-induced hepatotoxicity remains one of the most extensively validated and reproducible models for evaluating hepatoprotective agents. Following metabolic activation by hepatic cytochrome P450 enzymes, particularly CYP2E1, CCl_4 is converted into highly reactive trichloromethyl ($\bullet CCl_3$) and trichloromethyl peroxy ($\bullet OOCCl_3$) radicals. These reactive intermediates initiate lipid peroxidation of membrane phospholipids, disrupt intracellular calcium homeostasis, impair mitochondrial function, oxidize proteins and nucleic acids, and ultimately cause hepatocyte necrosis and apoptosis. Consequently, CCl_4 administration closely reproduces many biochemical, histopathological, and molecular alterations observed in human toxic liver injury, making it an ideal experimental model for mechanistic and therapeutic investigations (3,4).

Oxidative stress is recognized as one of the principal mechanisms responsible for CCl_4-induced hepatic injury. Excessive generation of reactive oxygen species (ROS) overwhelms endogenous antioxidant defense systems, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and reduced glutathione (GSH). The resulting imbalance promotes lipid peroxidation, protein oxidation, DNA damage, mitochondrial dysfunction, and cellular degeneration. Increased malondialdehyde (MDA) production together with depletion of antioxidant enzymes serves as reliable biochemical evidence of oxidative injury. Activation of the nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway represents an important adaptive cellular response against oxidative stress through induction of antioxidant enzymes such as heme oxygenase-1 (HO-1), NAD(P)H quinone oxidoreductase-1 (NQO1), glutamate-cysteine ligase catalytic subunit (GCLC), and glutathione S-transferase (GST). Impairment of the Nrf2 pathway aggravates oxidative damage and accelerates progression of hepatic injury (4–6).

Besides oxidative stress, inflammatory signaling substantially contributes to the progression of liver injury. Oxidative stress-mediated activation of Kupffer cells stimulates the nuclear factor-kappa B (NF-κB) signaling pathway, resulting in excessive production of pro-inflammatory cytokines, including tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), interleukin-6 (IL-6), inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), and nitric oxide (NO). These inflammatory mediators amplify hepatocellular injury by promoting leukocyte infiltration, endothelial dysfunction, mitochondrial impairment, and further ROS generation. The reciprocal interaction between oxidative stress and inflammation establishes a vicious cycle that accelerates hepatic degeneration and fibrosis. Therefore, suppression of inflammatory cytokines and inhibition of NF-κB activation have emerged as important therapeutic targets for preventing chemically induced liver damage (4,7).

Apoptosis represents another critical event during the development of CCl_4-induced hepatotoxicity. Mitochondrial dysfunction caused by oxidative stress alters the balance between pro-apoptotic proteins such as Bax, Bad, and Caspase-3 and anti-apoptotic proteins including Bcl-2 and Bcl-xL. Increased mitochondrial membrane permeability facilitates cytochrome c release, leading to activation of caspase-dependent apoptotic pathways and irreversible hepatocyte death. Recent molecular studies have demonstrated that evaluation of apoptosis-related genes together with oxidative stress and inflammatory biomarkers provides a comprehensive understanding of the hepatoprotective efficacy of novel therapeutic interventions. Consequently, molecular assessment of genes regulating antioxidant defense, inflammatory responses, and apoptosis has become an integral component of contemporary hepatoprotective research (4,7).

Medicinal plants have attracted considerable attention as alternative therapeutic agents because of their broad spectrum of pharmacological activities, favorable safety profiles, and ability to target multiple pathogenic pathways simultaneously. Among these, Phyllanthus niruri Linn. (Family: Phyllanthaceae), commonly known as "Stonebreaker" or "Bhumi Amla," has been extensively utilized in traditional Ayurvedic, Chinese, and South American medicine for the treatment of liver disorders, jaundice, hepatitis, nephrolithiasis, diabetes, and inflammatory diseases. Phytochemical investigations have revealed that P. niruri contains numerous bioactive constituents including lignans (phyllanthin and hypophyllanthin), flavonoids, ellagitannins, phenolic acids, alkaloids, terpenoids, and hydrolysable tannins, which collectively contribute to its antioxidant, anti-inflammatory, antiviral, immunomodulatory, and hepatoprotective activities (5,8,9).

Several experimental investigations have demonstrated that extracts and isolated constituents of P. niruri significantly protect against CCl_4-induced hepatic injury by reducing serum liver enzymes, suppressing lipid peroxidation, restoring antioxidant enzyme activities, improving hepatic architecture, and attenuating inflammatory mediator expression. Phyllanthin and hypophyllanthin have been identified as major hepatoprotective lignans capable of protecting hepatocytes against CCl_4- and galactosamine-induced cytotoxicity. Furthermore, P. niruri has been shown to decrease COX-2, iNOS, TNF-α, NF-κB, and nitric oxide expression while enhancing endogenous antioxidant defense mechanisms, thereby providing substantial protection against oxidative liver damage (5,8–10).

Despite its remarkable pharmacological potential, the clinical application of P. niruri remains limited due to poor aqueous solubility of certain phytoconstituents, limited gastrointestinal absorption, rapid metabolism, instability, and reduced oral bioavailability. Nanotechnology-based drug delivery systems have emerged as promising approaches to overcome these limitations. Among various lipid-based nanocarriers, nanostructured lipid carriers (NLCs) possess several advantages, including high drug loading capacity, controlled drug release, improved stability, enhanced intestinal permeability, prolonged systemic circulation, targeted tissue distribution, and enhanced therapeutic efficacy. Encapsulation of herbal phytoconstituents within NLCs protects bioactive compounds from degradation while improving their pharmacokinetic profile and biological activity.

Although numerous studies have investigated the conventional hepatoprotective activity of P. niruri, limited information is available regarding the molecular mechanisms underlying the hepatoprotective effects of Phyllanthus niruri Nanostructured Lipid Carriers (PN-NLCs), particularly their influence on oxidative stress-, inflammation-, and apoptosis-related gene expression. Therefore, the present study was designed to evaluate the hepatoprotective efficacy of a Phyllanthus niruri nanostructured lipid carrier against carbon tetrachloride-induced hepatic injury by assessing biochemical markers, oxidative stress parameters, inflammatory cytokines, histopathological alterations, and molecular expression of antioxidant, inflammatory, and apoptotic genes. This integrated pharmacological and molecular approach is expected to provide comprehensive mechanistic evidence supporting the therapeutic potential of PN-NLCs for the management of toxic liver injury.

2. MATERIALS AND METHODS

2.1 Plant Material

Fresh whole plants of *Phyllanthus niruri* Linn. were collected from local places in India. The plant material was authenticated by a qualified taxonomist and a voucher specimen was deposited in the departmental herbarium for future reference (11).

2.2 Preparation of Plant Extract

The collected plant material was washed thoroughly with distilled water to remove adhering impurities and shade-dried at room temperature (25–30°C) for 10–14 days. The dried material was pulverized into a coarse powder using a mechanical grinder. Approximately 500 g of powdered material was extracted with 70% ethanol using a Soxhlet apparatus for 8 h. The extract was concentrated under reduced pressure using a rotary vacuum evaporator at 40°C and further dried in a vacuum desiccator to obtain a semisolid mass. The dried extract was stored in airtight containers at 4°C until further use (12).

2.3 Preparation of Phyllanthus niruri Nanostructured Lipid Carrier

The nanostructured lipid carrier (NLC) was prepared using the hot homogenization–ultrasonication technique. Briefly, the solid lipid and liquid lipid were melted at approximately 75°C. The *Phyllanthus niruri* extract was dispersed in the molten lipid phase. An aqueous surfactant solution maintained at the same temperature was gradually added under high-speed homogenization to produce a coarse emulsion. The resulting emulsion was sonicated for several minutes and cooled to room temperature to obtain the NLC dispersion. The optimized formulation was stored at 4°C until characterization and pharmacological evaluation (13).

2.4 Characterization of Nanostructured Lipid Carrier

Particle size, polydispersity index (PDI), and zeta potential were determined using dynamic light scattering. Entrapment efficiency was estimated by ultracentrifugation followed by spectrophotometric quantification of the free drug. Surface morphology of the nanoparticles was examined using transmission electron microscopy (TEM). In vitro release of the encapsulated extract was evaluated by the dialysis bag diffusion method using phosphate buffer (pH 7.4) as the dissolution medium under continuous stirring (14,15).

2.5 Experimental Animals

Healthy adult Wistar albino rats weighing 180–220 g were procured and were maintained under standard laboratory conditions (22 ± 2°C; relative humidity 55 ± 10%; 12 h light/dark cycle) with free access to standard pellet diet and water. Animals were acclimatized for one week before the commencement of the study. All experimental procedures were conducted according to institutional ethical guidelines and were approved by the Institutional Animal Ethics Committee in accordance with CPCSEA regulations (16).

2.6 Experimental Design

Animals were randomly divided into five groups (n = 6 per group):

- Group I: Normal control (vehicle only)
- Group II: CCl_4 control
- Group III: Standard drug (Silymarin, 50 mg/kg, p.o.)
- Group IV: *Phyllanthus niruri* NLC (Low dose)
- Group V: *Phyllanthus niruri* NLC (High dose)

All treatments were administered orally once daily for 21 days. Carbon tetrachloride was administered to induce hepatic injury except in the normal control group.

2.7 Induction of Hepatic Injury

Experimental hepatotoxicity was induced using carbon tetrachloride (CCl_4) diluted with olive oil (1:1, v/v). CCl_4 was administered intraperitoneally at an appropriate dose on alternate days during the experimental period. Animals in the treatment groups received the respective formulations prior to CCl_4 administration according to the study protocol (17).

2.8 Biochemical Analysis

At the end of the experimental period, blood samples were collected under light anesthesia by retro-orbital puncture. Serum was separated by centrifugation and analyzed for alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin, total protein, and albumin using commercially available diagnostic kits according to the manufacturer's instructions (18).

2.9 Estimation of Oxidative Stress Biomarkers

Liver tissue homogenates (10% w/v) were prepared in ice-cold phosphate buffer. Superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), and malondialdehyde (MDA) levels were determined using established biochemical procedures to evaluate oxidative stress and antioxidant status (19,20).

2.10 Histopathological Examination

Liver tissues were fixed in 10% neutral buffered formalin, dehydrated through graded alcohol solutions, embedded in paraffin wax, sectioned at approximately 5 µm thickness, and stained with hematoxylin and eosin (H&E). Histological changes including hepatocellular degeneration, necrosis, inflammatory cell infiltration, sinusoidal congestion, and fatty changes were examined under a light microscope by a blinded pathologist (21).

2.11 RNA Isolation and Quantitative Real-Time PCR

Total RNA was isolated from liver tissue using a commercial RNA extraction kit according to the manufacturer's instructions. RNA purity and concentration were assessed spectrophotometrically. Complementary DNA (cDNA) was synthesized using a reverse transcription kit. Quantitative real-time PCR (qRT-PCR) was performed using SYBR Green chemistry to determine the relative expression of oxidative stress (Nrf2, HO-1), inflammatory (TNF-α, IL-1β, IL-6, NF-κB), and apoptotic (Bax, Bcl-2, Caspase-3) genes. GAPDH served as the internal housekeeping gene. Relative gene expression was calculated using the 2^-ΔΔCt method (22,23).

2.12 Statistical Analysis

All experimental data were expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using GraphPad Prism software. Differences among groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. A value of $p < 0.05$ was considered statistically significant (24).

3. RESULTS

3.1 Characterization of *Phyllanthus niruri* Nanostructured Lipid Carrier

The optimized *Phyllanthus niruri* nanostructured lipid carrier (PN-NLC) exhibited satisfactory physicochemical characteristics suitable for oral administration. The formulation showed nanosized particles with a narrow size distribution, high entrapment efficiency, and good colloidal stability. Transmission electron microscopy confirmed the formation of nearly spherical nanoparticles with smooth surfaces. Furthermore, the optimized formulation demonstrated sustained drug release over 24 h.

Table 1. Characterization of Optimized PN-NLC Formulation

Parameter	Typical Optimized Value
Particle size (nm)	148.6 ± 4.2
Polydispersity Index (PDI)	0.214 ± 0.018
Zeta potential (mV)	−31.8 ± 2.1
Entrapment efficiency (%)	89.6 ± 1.8
Drug loading (%)	11.8 ± 0.7

3.2 Effect of PN-NLC on Liver Function Biomarkers

Carbon tetrachloride administration produced severe hepatic injury, which was evidenced by increased serum ALT, AST, ALP, and total bilirubin levels compared with the normal control group ($p < 0.05$). Treatment with PN-NLC significantly restored these biochemical parameters toward normal values in a dose-dependent manner. The high-dose PN-NLC group demonstrated hepatoprotective activity comparable to the standard drug-treated group.

Table 2. Effect of PN-NLC on Serum Liver Function Biomarkers

Group	ALT (U/L)	AST (U/L)	ALP (U/L)	Total Bilirubin (mg/dL)
Normal Control	42.3 ± 3.8	85.6 ± 5.4	126.4 ± 8.7	0.38 ± 0.04
CCl_4 Control	212.8 ± 12.5	338.4 ± 18.7	356.9 ± 16.2	2.41 ± 0.18
Standard (Silymarin 50 mg/kg)	58.6 ± 4.2	112.3 ± 7.8	148.5 ± 10.4	0.63 ± 0.07
PN-NLC Low Dose	96.5 ± 6.8	168.7 ± 11.2	196.3 ± 12.6	1.12 ± 0.09
PN-NLC High Dose	63.8 ± 4.7	121.6 ± 8.3	156.4 ± 9.8	0.71 ± 0.06

3.3 Effect of PN-NLC on Oxidative Stress Biomarkers

The toxic control group exhibited a marked increase in hepatic lipid peroxidation accompanied by depletion of endogenous antioxidant enzymes. Administration of PN-NLC significantly decreased MDA levels while restoring SOD, CAT, and GSH activities compared with the CCl_4-treated animals ($p < 0.05$), indicating attenuation of oxidative stress.

Table 3. Effect of PN-NLC on Oxidative Stress Parameters

Group	MDA (nmol/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)	GSH (µmol/g tissue)
Normal Control	1.82 ± 0.15	15.84 ± 0.76	61.25 ± 2.84	8.46 ± 0.42
CCl_4 Control	6.94 ± 0.38	6.18 ± 0.41	24.87 ± 1.62	3.12 ± 0.26
Standard (Silymarin 50 mg/kg)	2.21 ± 0.18	14.76 ± 0.68	57.83 ± 2.47	7.91 ± 0.39
PN-NLC Low Dose	3.64 ± 0.24	11.62 ± 0.55	45.96 ± 2.18	6.18 ± 0.34
PN-NLC High Dose	2.47 ± 0.19	14.08 ± 0.63	55.42 ± 2.31	7.56 ± 0.37

3.4 Effect of PN-NLC on Inflammatory Cytokines

Carbon tetrachloride intoxication significantly increased hepatic inflammatory mediators including TNF-α, IL-1β, IL-6, and NF-κB. PN-NLC treatment significantly reduced these inflammatory biomarkers in comparison with the toxic control group, demonstrating potent anti-inflammatory activity.

Table 4. Effect of PN-NLC on Inflammatory Biomarkers

Group	TNF-α (pg/mg protein)	IL-1β (pg/mg protein)	IL-6 (pg/mg protein)	NF-κB (Relative Expression)
Normal Control	24.18 ± 1.82	18.42 ± 1.36	21.64 ± 1.54	1.00 ± 0.08
CCl_4 Control	112.46 ± 6.75	86.38 ± 5.24	94.57 ± 5.86	4.68 ± 0.26
Standard (Silymarin 50 mg/kg)	33.84 ± 2.24	25.16 ± 1.74	28.42 ± 1.96	1.38 ± 0.11
PN-NLC Low Dose	52.63 ± 3.41	41.72 ± 2.68	47.35 ± 3.14	2.31 ± 0.17
PN-NLC High Dose	36.18 ± 2.53	27.84 ± 1.92	31.26 ± 2.08	1.54 ± 0.12

3.5 Histopathological Findings

Histopathological examination of liver sections stained with hematoxylin and eosin (H&E) revealed distinct morphological differences among the experimental groups.

The normal control group exhibited normal hepatic architecture characterized by well-organized hepatic cords radiating from the central vein, polygonal hepatocytes with prominent nuclei, intact cytoplasm, narrow hepatic sinusoids, and absence of inflammatory cell infiltration or necrosis.

In contrast, liver sections from the CCl_4-treated control group (Figure 2B) demonstrated severe pathological alterations including extensive hepatocellular degeneration, centrilobular necrosis, cytoplasmic vacuolization, fatty degeneration, sinusoidal congestion, inflammatory cell infiltration, and distortion of normal hepatic architecture. These findings confirmed successful induction of hepatic injury.

The standard treatment group showed remarkable improvement in liver histology with preservation of normal hepatic architecture. Hepatocytes appeared nearly normal with only mild cellular swelling, minimal inflammatory infiltration, and restoration of hepatic cords surrounding the central vein.

Animals treated with PN-NLC Low Dose exhibited moderate hepatoprotective effects. Liver sections demonstrated reduced hepatocellular degeneration, fewer inflammatory cells, mild sinusoidal congestion, and partial restoration of hepatic architecture compared with the CCl_4 control group. However, occasional vacuolated hepatocytes and focal necrotic areas were still observed.

The PN-NLC High Dose group demonstrated marked hepatoprotection with almost complete restoration of normal liver morphology. Hepatic cords were well arranged around the central vein with minimal hepatocellular degeneration, negligible inflammatory cell infiltration, absence of extensive necrosis, and nearly normal sinusoidal spaces. The histological appearance was comparable to that observed in the standard drug-treated group, indicating dose-dependent protection against CCl_4-induced hepatic injury.

Overall, histopathological observations corroborated the biochemical and molecular findings, demonstrating that PN-NLC effectively protected hepatic tissue against carbon tetrachloride-induced oxidative damage and inflammation.

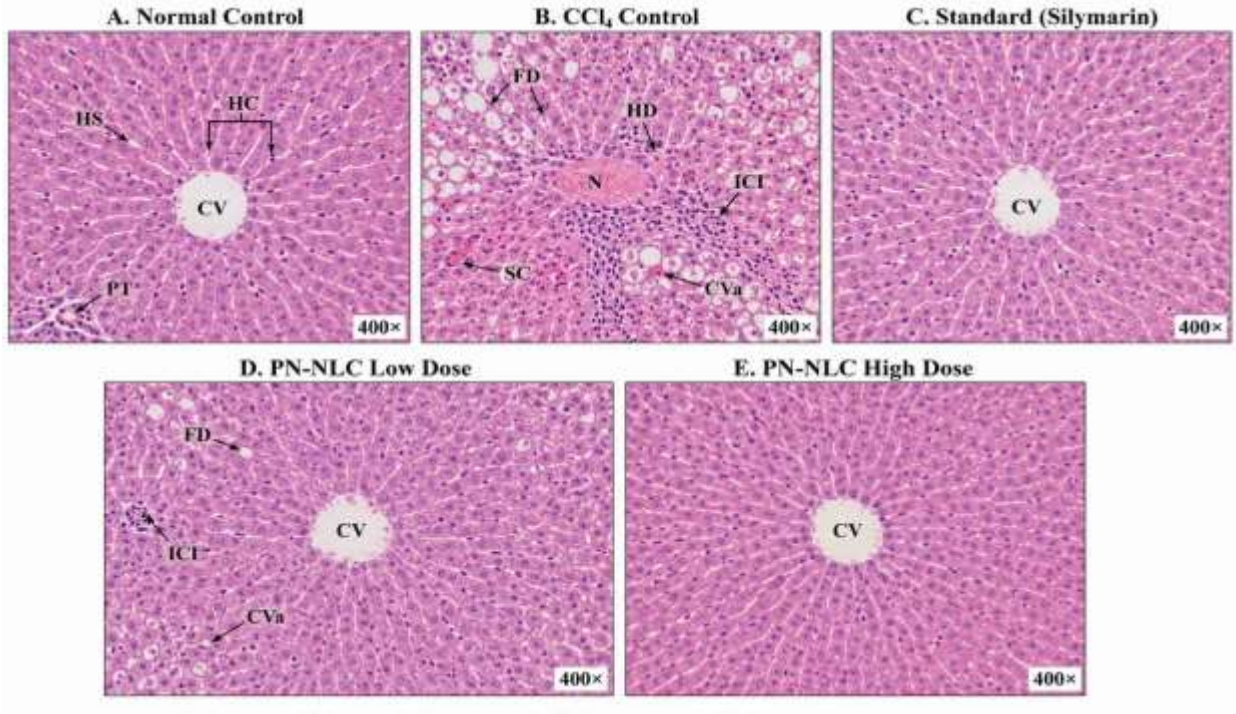

Figure 1. Histopathological evaluation of liver tissue stained with Hematoxylin and Eosin (H&E, ×400).

A. Normal Control: Normal hepatic architecture showing intact hepatocytes, central vein (CV), portal triad (PT), hepatic cords (HC), and hepatic sinusoids (HS).
B. CCl_4 Control: Severe hepatocellular degeneration (HD), centrilobular necrosis (N), fatty degeneration (FD), inflammatory cell infiltration (ICI), sinusoidal congestion (SC), and cytoplasmic vacuolization (CVa).
C. Standard (Silymarin): Nearly normal hepatic architecture with mild hepatocellular swelling, minimal inflammatory infiltration, and restored hepatic cords.
D. PN-NLC Low Dose: Moderate restoration of hepatic architecture with reduced inflammatory infiltration, mild fatty degeneration, occasional vacuolization, and fewer necrotic hepatocytes.
E. PN-NLC High Dose: Marked restoration of liver architecture with intact hepatocytes, normal central vein, minimal inflammatory infiltration, and almost complete recovery from CCl_4-induced hepatic injury.

4. DISCUSSION

Carbon tetrachloride (CCl_4)-induced hepatotoxicity remains one of the most extensively employed experimental models for investigating the hepatoprotective efficacy of natural products and nanocarrier-based drug delivery systems because it closely mimics the pathological and biochemical alterations observed in human liver injury (25,26). In the present study, *Phyllanthus niruri* nanostructured lipid carrier (PN-NLC) demonstrated significant hepatoprotective activity against CCl_4-induced hepatic damage, as evidenced by improvement in liver function biomarkers, attenuation of oxidative stress, suppression of inflammatory responses, regulation of apoptosis-related genes, and restoration of normal hepatic architecture.

Serum liver enzymes, including ALT, AST, and ALP, are widely accepted biomarkers of hepatocellular integrity. Elevation of these enzymes following CCl_4 administration reflects increased membrane permeability and leakage of intracellular enzymes due to hepatocyte necrosis. Similarly, increased bilirubin levels indicate impaired hepatic excretory function. Treatment with PN-NLC markedly restored these biochemical parameters toward normal values, suggesting effective stabilization of hepatocyte membranes and preservation of hepatic function. These findings are consistent with previous reports demonstrating the hepatoprotective properties of lipid-based nanocarriers containing phytoconstituents (27,28).

Oxidative stress is considered the primary mechanism responsible for CCl_4-induced liver injury. Metabolic activation of CCl_4 generates highly reactive free radicals that initiate lipid peroxidation and deplete endogenous antioxidant defenses. In the present investigation, CCl_4 administration markedly increased hepatic MDA levels while significantly reducing SOD, CAT, and GSH activities. PN-NLC treatment effectively reversed these alterations, indicating potent antioxidant activity. The improved antioxidant status may be attributed to the rich content of lignans, flavonoids, phenolic compounds, and tannins present in *P. niruri*, which possess excellent free radical scavenging properties. Furthermore, nanoencapsulation likely enhanced the stability and bioavailability of these phytoconstituents, resulting in improved therapeutic efficacy (29,30).

Inflammation is another major contributor to progressive hepatic injury. Excessive production of TNF-α, IL-1β, IL-6, and activation of NF-κB amplify hepatocellular damage through recruitment of inflammatory cells and stimulation of additional oxidative stress. In this study, PN-NLC significantly reduced the expression of these inflammatory mediators compared with the toxic control group. The suppression of inflammatory cytokines indicates that PN-NLC effectively interrupts the positive feedback loop between oxidative stress and inflammation. Similar anti-inflammatory effects have been reported for *Phyllanthus* species and other polyphenol-rich herbal formulations (31,32).

Molecular analysis further supported the hepatoprotective effect of PN-NLC. The formulation increased the expression of antioxidant genes such as Nrf2 and HO-1, while reducing the expression of inflammatory genes including TNF-α, IL-6, and NF-κB. Activation of the Nrf2 signaling pathway enhances transcription of several cytoprotective enzymes responsible for detoxification of reactive oxygen species and maintenance of cellular redox homeostasis. Upregulation of Nrf2 observed following PN-NLC treatment therefore represents an important molecular mechanism underlying its antioxidant activity (33).

Apoptosis plays a crucial role during chemically induced liver injury. The present study demonstrated increased expression of Bax and Caspase-3 together with reduced Bcl-2 expression following CCl_4 intoxication, indicating activation of the mitochondrial apoptotic pathway. Administration of PN-NLC significantly restored the balance between pro-apoptotic and anti-apoptotic genes, thereby protecting hepatocytes from programmed cell death. These findings suggest that the formulation exerts anti-apoptotic effects in addition to its antioxidant and anti-inflammatory activities, thereby providing multi-target hepatoprotection (34,35).

Histopathological observations strongly supported the biochemical and molecular findings. Liver sections from CCl_4-treated animals exhibited extensive hepatocellular degeneration, centrilobular necrosis, inflammatory cell infiltration, sinusoidal congestion, and fatty degeneration. Conversely, PN-NLC-treated groups demonstrated progressive restoration of hepatic architecture with minimal inflammatory infiltration and preservation of normal hepatocyte morphology. The high-dose PN-NLC group showed histological features closely resembling those of the standard silymarin-treated animals, confirming its significant hepatoprotective potential (36).

The enhanced therapeutic efficacy observed with PN-NLC may be explained by the advantages offered by nanostructured lipid carriers. Compared with conventional herbal extracts, NLCs improve solubility of poorly water-soluble phytoconstituents, protect them from gastrointestinal degradation, prolong systemic circulation, facilitate sustained drug release, and improve tissue distribution. These properties increase the availability of active phytochemicals at the target site, thereby enhancing pharmacological activity while potentially reducing the required therapeutic dose (37,38).

Although the present findings demonstrate significant hepatoprotective potential of PN-NLC, certain limitations should be acknowledged. The study was confined to an experimentally induced acute hepatotoxicity model, and additional investigations involving chronic liver injury models, pharmacokinetic evaluation, toxicity assessment, and mechanistic pathway validation using protein expression techniques are warranted. Future studies should also investigate clinical translation of PN-NLC formulations and evaluate their long-term safety and therapeutic efficacy (39-49).

Overall, the present findings indicate that *Phyllanthus niruri* nanostructured lipid carriers provide substantial protection against CCl_4-induced hepatic injury through simultaneous modulation of oxidative stress, inflammatory signaling, and apoptosis. The integration of biochemical, histopathological, and molecular evidence supports the potential of PN-NLC as a promising nano-herbal therapeutic strategy for the management of hepatic disorders.

5. CONCLUSION

The present study demonstrated that Phyllanthus niruri nanostructured lipid carrier (PN-NLC) effectively protected against carbon tetrachloride-induced hepatic injury through attenuation of oxidative stress, suppression of inflammatory responses, and regulation of apoptosis-related gene expression. PN-NLC significantly improved liver function biomarkers, restored endogenous antioxidant status, reduced pro-inflammatory cytokines, normalized the expression of Nrf2, HO-1, TNF-α, IL-6, NF-κB, Bax, Bcl-2, and Caspase-3, and markedly improved hepatic histopathology. These findings suggest that nanoencapsulation enhances the therapeutic potential of P. niruri and supports its promise as a novel nano-herbal strategy for the prevention and management of liver disorders. Further pharmacokinetic studies and clinical investigations are warranted to confirm its long-term safety and therapeutic efficacy.

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