RESEARCH PAPER

Investigation of the Hepatoprotective Potential of *Ziziphus nummularia* through Pharmacological Studies

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Abstract: Liver disorders represent a major health concern, and the search for safe, effective, and economically viable hepatoprotective agents has increased interest in medicinal plants as potential therapeutic alternatives. The present study was undertaken to evaluate the hepatoprotective and anti-inflammatory potential of selected medicinal plant whole-root extracts against carbon tetrachloride (CCl_4)-induced hepatic injury. The study was designed to investigate the protective effects of the selected plant extracts and to assess their influence on biochemical and histopathological alterations associated with chemically induced liver damage. The anti-inflammatory activity of the whole-root extracts was also evaluated to determine their potential role in attenuating hepatic inflammation. Furthermore, the study aimed to identify and isolate the active fraction responsible for the observed hepatoprotective and anti-inflammatory effects, followed by its characterization to establish a possible phytochemical basis for the pharmacological activities. The hepatoprotective efficacy of the extracts and active fraction was assessed through relevant serum biochemical parameters indicative of liver function, along with histopathological examination of hepatic tissues to determine structural and cellular changes following treatment. The overall findings are expected to provide scientific evidence supporting the traditional and therapeutic relevance of the selected medicinal plants in the management of liver injury. The study may further contribute to the identification of promising bioactive phytoconstituents and provide a basis for subsequent investigations involving mechanism of action, toxicity evaluation, and formulation development. Thus, the research is anticipated to support the development of safe, effective, and affordable plant-based hepatoprotective agents and enhance the understanding of natural therapeutic approaches for liver disorders.

Keywords: Hepatoprotective activity; Carbon tetrachloride; Liver injury; Medicinal plants; Anti-inflammatory activity; Phytoconstituents.
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Introduction:

The liver is the largest internal organ of the human body and performs a wide range of essential physiological functions, including regulation of metabolism, detoxification and biotransformation of xenobiotics, synthesis of plasma proteins, storage of glycogen and vitamins, secretion of bile, and maintenance of systemic homeostasis. Because of its continuous exposure to endogenous metabolites and exogenous chemicals, the liver is particularly vulnerable to toxic and pathological insults. Various factors, including prolonged exposure to drugs, alcohol, environmental pollutants, viral infections, poor dietary habits, and metabolic abnormalities, can impair hepatic function and lead to progressive liver damage. Hepatic disorders such as viral hepatitis, metabolic dysfunction-associated steatotic liver disease, fatty liver, fibrosis, cirrhosis, and hepatocellular carcinoma represent important causes of morbidity and mortality worldwide [1]. Although several pharmacological agents are available for the management of liver disorders, their therapeutic efficacy may be limited, while prolonged administration can be associated with adverse effects, drug interactions, and considerable treatment costs. Therefore, the search for safer, affordable, and effective hepatoprotective agents has increased considerably. Medicinal plants have been used for centuries in traditional systems of medicine, including Ayurveda, Siddha, Unani, and Traditional Chinese Medicine, for the prevention and treatment of hepatic disorders. Their therapeutic potential is largely associated with the presence of a diverse array of secondary metabolites, including flavonoids, phenolic acids, alkaloids, tannins, saponins, terpenoids, glycosides, and steroids [2]. Unlike many synthetic agents that act predominantly through a single pharmacological pathway, plant-derived preparations may exert hepatoprotective effects through multiple complementary mechanisms.

These mechanisms include scavenging of reactive oxygen species (ROS), inhibition of lipid peroxidation, enhancement of endogenous antioxidant defense, stabilization of hepatocyte membranes, suppression of inflammatory responses, inhibition of apoptosis, and stimulation of hepatocellular regeneration [3]. Consequently, medicinal plants continue to attract considerable scientific attention as potential sources of novel hepatoprotective phytoconstituents.

Among medicinal plants, *Silybum marianum* L. has been extensively investigated and is widely regarded as an important reference herb for hepatoprotective research. Its principal bioactive complex, silymarin, consists predominantly of flavonolignans such as silibinin, silychristin, and silydianin. Experimental and clinical evidence indicates that silymarin can protect hepatocytes by scavenging free radicals, reducing oxidative stress and lipid peroxidation, maintaining intracellular glutathione levels, modulating inflammatory responses, and supporting hepatic protein synthesis and regeneration [4]. Owing to these pharmacological properties, silymarin is frequently used as a standard/reference hepatoprotective agent in experimental studies evaluating medicinal plants and herbal formulations.

Several other medicinal plants have also demonstrated promising hepatoprotective properties in experimental investigations. *Phyllanthus niruri* has been reported to possess antioxidant, antiviral, anti-inflammatory, and hepatoprotective activities and has traditionally been associated with the management of hepatic disorders, particularly viral hepatitis [5]. *Picrorhiza kurroa*, a well-known medicinal plant used in traditional medicine, contains iridoid glycosides such as picroside I and picroside II, which have demonstrated antioxidant and anti-inflammatory properties and protective effects against experimentally induced hepatic injury [6]. Similarly, *Curcuma longa* and its principal constituent curcumin exhibit antioxidant, anti-inflammatory, and antifibrotic activities through modulation of oxidative stress and several intracellular signaling pathways [7]. Other medicinal plants, including *Tinospora cordifolia*, *Glycyrrhiza glabra*, *Boerhaavia diffusa*, and *Andrographis paniculata*, have also shown significant hepatoprotective effects in various preclinical models [8]. The increasing scientific interest in herbal hepatoprotective agents has also encouraged the development of novel formulations designed to improve the therapeutic performance of plant extracts and phytoconstituents. Conventional herbal dosage forms, including tablets, capsules, syrups, and suspensions, are widely used; however, several phytoconstituents suffer from poor aqueous solubility, inadequate intestinal absorption, instability, rapid metabolism, and low systemic bioavailability. To overcome these limitations, advanced drug delivery approaches such as phytosomes, liposomes, nanoparticles, nanoemulsions, and solid lipid nanoparticles have been investigated for herbal drug delivery [8]. These systems can improve the solubility, stability, dissolution, absorption, and bioavailability of poorly water-soluble phytoconstituents and may consequently enhance their pharmacological effectiveness. Furthermore, appropriate formulation strategies can improve dose uniformity, patient acceptability, stability, and therapeutic consistency of herbal preparations. Standardization and quality control are critical aspects of herbal hepatoprotective formulations because the chemical composition and biological activity of plant materials can vary according to geographical origin, cultivation conditions, harvesting period, processing, extraction procedures, and storage conditions. Phytochemical profiling, chromatographic fingerprinting, marker-compound estimation, and appropriate physicochemical evaluation are therefore essential to establish identity, purity, quality, batch-to-batch consistency, safety, and reproducibility of herbal products [9]. Modern analytical techniques, including high-performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC-MS/MS), gas chromatography-mass spectrometry (GC-MS), Fourier-transform infrared spectroscopy (FTIR), nuclear magnetic resonance (NMR), and mass spectrometry, have greatly facilitated the identification and characterization of bioactive phytoconstituents present in medicinal plants [10]. Recent research has increasingly focused on identifying the specific phytoconstituents responsible for hepatoprotective effects and understanding their molecular mechanisms. Isolation and structural characterization of flavonoids, phenolic compounds, terpenoids, alkaloids, and other secondary metabolites have contributed substantially to the identification of potential lead molecules for hepatic disorders [11]. At the molecular level, several plant-derived compounds have been reported to influence important signaling pathways, including nuclear

factor erythroid 2-related factor 2/antioxidant response element (Nrf2/ARE), NF-κB, mitogen-activated protein kinase (MAPK), phosphatidylinositol-3-kinase/protein kinase B (PI3K/Akt), and transforming growth factor-beta (TGF-β) pathways. Modulation of these pathways may reduce oxidative stress, inflammatory signaling, apoptosis, and extracellular-matrix deposition, thereby contributing to the prevention or attenuation of hepatic fibrosis and other chronic liver injuries [12]. The proposed study aims to investigate the hepatoprotective potential of the selected medicinal plant extract against carbon tetrachloride (CCl_4)-induced liver injury in experimental rats. Liver diseases remain a significant global health concern, and the search for safer, effective, and affordable therapeutic agents derived from natural sources has gained considerable attention. This study is designed to provide scientific evidence supporting the traditional use of medicinal plants in the management of hepatic disorders. *Ziziphus nummularia* exhibits diverse pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, antidiabetic, hepatoprotective, and analgesic effects. Its leaves, fruits, bark, and roots contain bioactive phytoconstituents such as flavonoids, phenolics, saponins, and triterpenoids, supporting its traditional use in managing inflammation, infections, metabolic disorders, and oxidative stress.

Material And Methods

Collection and Identification of Plant Material: Phytochemistry is an important interdisciplinary branch of science that integrates concepts of plant biochemistry and natural product chemistry. This discipline focuses on the isolation, characterization, biosynthesis, metabolism, distribution, and biological significance of secondary metabolites synthesized by plants. The plant materials selected for the present investigation included *Ziziphus nummularia* (Burm.f) were procured from the local herbal market of Bhopal. Botanical authentication was carried out in the Department of Botany, Government College. After authentication, the collected samples were shade-dried to preserve their phytochemical constituents, pulverized into coarse powder, and passed through sieve No. 10 before storage in airtight containers for further experimental work.

Microscopic Evaluation: Powder microscopy was performed to establish the diagnostic characteristics of the powdered plant materials of *Ziziphus nummularia*, *Ziziphus xylopyrus*, and *Achyranthes aspera*. Small quantities of the powdered samples were mounted on clean glass slides using a freshly prepared phloroglucinol–hydrochloric acid staining reagent (1:1). The prepared slides were examined under a compound microscope, and the characteristic microscopic features of the plant tissues were recorded for pharmacognostic identification [13].

Physicochemical Evaluation: Physicochemical parameters were determined according to standard pharmacopoeial procedures to evaluate the quality, purity, and identity of the crude plant materials. Total, water-soluble, and sulphated ash values were determined by controlled incineration of powdered plant material. Alcohol- and water-soluble extractive values were estimated by maceration followed by evaporation and weighing of residues. Loss on drying was determined by oven-drying the powdered samples to constant weight and calculating percentage weight loss [14].

Extraction of Plant Material

Maceration Method: Dried powdered plant materials (250 g each) were separately macerated with 2.5 L of 70% ethanol for 72 h at room temperature with intermittent shaking. Extracts were filtered, re-macerated twice, pooled, concentrated under reduced pressure below 45°C, vacuum-dried, weighed, and stored at 4°C.

Soxhlet Extraction Method: Dried powdered plant materials (100 g each) were separately extracted using Soxhlet apparatus with approximately 1 L of 95% ethanol for 6–8 h. Extracts were filtered and concentrated under reduced pressure below 45°C, vacuum-dried, weighed for yield determination, and stored in airtight amber containers at 4°C.

Microwave-Assisted Extraction (MAE): Powdered plant materials (20 g each) were mixed separately with 200 mL of 70% ethanol and subjected to microwave extraction at 400–600 W for 5–10 min using intermittent irradiation. Extracts were cooled, filtered, concentrated below 45°C, vacuum-dried, weighed, and stored at 4°C for further evaluation.

Qualitative Analysis of Plant Extracts: The prepared extracts were subjected to qualitative phytochemical analysis to identify the major classes of secondary metabolites using standard phytochemical procedures as described by Khandelwal (2007) and Harborne (1984). The screening was carried out to detect the presence of alkaloids, glycosides, saponins, carbohydrates,

phytosterols, tannins, phenolic compounds, flavonoids, proteins, and free amino acids [15].

Fractionation and Isolation of Active Constituents: Dried hydroalcoholic extract of *Ziziphus nummularia* leaves (20 g) was fractionated using silica gel (60–120 mesh) column chromatography. The extract was eluted successively with n-hexane, n-hexane acetate, and ethyl acetate mixtures of increasing polarity. Fractions of 20–25 mL were collected and monitored by TLC on silica gel GF254 plates using toluene acetate acid (5:4:1, v/v/v). Spots were detected under UV light and after anisaldehyde-sulfuric acid spraying. Similar fractions were pooled, concentrated, and further purified by column chromatography and preparative TLC. The isolated compounds were characterized using UV, FTIR, MS, ^{1}H-NMR, and ^{13}C-NMR spectroscopy [16].

Hepatoprotective effect of extracts and fractions of the Ziziphus nummularia (Burm.f): Excessive use of analgesics, antipyretics, prescription drugs, and alcohol can cause severe liver toxicity, particularly centrilobular hepatic necrosis. Although these substances are generally safe within therapeutic limits, prolonged or excessive consumption may induce hepatocellular injury through oxidative stress, reactive oxygen species generation, lipid peroxidation, glutathione depletion, and toxic metabolite formation such as NAPQI. The present study evaluated the hepatoprotective potential of extracts of *Ziziphus nummularia* (Burm.f) against paracetamol-induced hepatotoxicity in Wistar rats. The findings demonstrated the protective effects of these plant extracts against chemically induced hepatic damage.

Grouping of animal and treatment administered

Group I Normal control (Normal Saline 1ml/kg BW)

Group II Disease control (Carbon tetra chloride (CCl4) 1000 mg/kg p.o. for 7 days)

Group III Standard (silymarin treated) + Carbon tetra chloride (CCl4) (1000 mg/kg p.o.)

Group IV SZN Soxhlet extract of Ziziphus nummularia (Burm.f.) (200 mg/kg)

Group V MZN Microwave extract of Ziziphus nummularia (Burm.f.) (200 mg/kg)

Group VI ZN1 Isolated comp of Ziziphus nummularia (Burm.f.) (10 mg/kg)

Biochemical Analysis: Blood samples were collected from overnight-fasted rats through the retro-orbital plexus using capillary tubes and transferred into heparinized Eppendorf tubes. Samples were centrifuged at 3000 rpm for 10 min, and plasma was separated carefully for biochemical analysis. Liver function parameters were estimated using an automated biochemical analyzer (ERBA Diagnostic Mannheim GmbH, CHEM-7). The evaluated parameters included alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and bilirubin. These biochemical markers were selected to assess hepatic function and possible liver injury. Increased AST, ALT, ALP, and bilirubin levels may indicate hepatocellular damage, hepatobiliary dysfunction, or impaired hepatic metabolic activity.

Histopathology Evaluations: Histopathological examination of liver tissues was performed to assess paracetamol-induced structural alterations and the hepatoprotective effects of extracts of *Ziziphus nummularia* (Burm.f) and fraction of plant compared with silymarin. Liver samples were collected, weighed, excised, and thoroughly rinsed before fixation in 10% neutral buffered formalin for 24 h. The tissues were dehydrated through graded alcohol, cleared with xylene, and infiltrated with paraffin wax for approximately 5 h using an automated tissue processor (Leica ASP). Paraffin blocks were prepared and sectioned at 5 µm using a Leica motorized rotary microtome. Sections were stained with hematoxylin and eosin (H&E) using a Leica autostainer.

Result And Discussion

Macroscopic evaluation: The organoleptic evaluation of *Ziziphus nummularia* (Burm.f.) revealed dried leaf material with a greenish-brown colour, characteristic mild herbal odour, and slightly bitter, astringent taste. The leaves consisted of small to medium-sized, irregular ovate fragments with a rough and slightly wrinkled surface, confirming their characteristic macroscopic features.

Figure 1: Plant photograph of *Ziziphus nummularia (Burm.f.),*

Table 1: Organoleptic identification of *Ziziphus nummularia (Burm.f.)*

S. No.	Parameter	*Ziziphus nummularia (Burm.f.)*
1	Condition	Dried leaf/plant material
2	Colour	Greenish-brown
3	Odour	Characteristic mild herbal odour
4	Taste	Slightly bitter, astringent
5	Size	Small to medium leaf pieces
6	Shape	Irregular, ovate fragments
7	Surface	Rough, slightly wrinkled

Microscopic evaluation: The powder microscopy of *Ziziphus nummularia* revealed polygonal, thick-walled epidermal cells, unicellular non-glandular trichomes, fibrous tissue fragments, cluster-type calcium oxalate crystals, and vessels showing reticulate and pitted thickening. These characteristic microscopical features provide valuable diagnostic markers for the pharmacognostic identification, authentication, and quality control of *Ziziphus nummularia* powder.

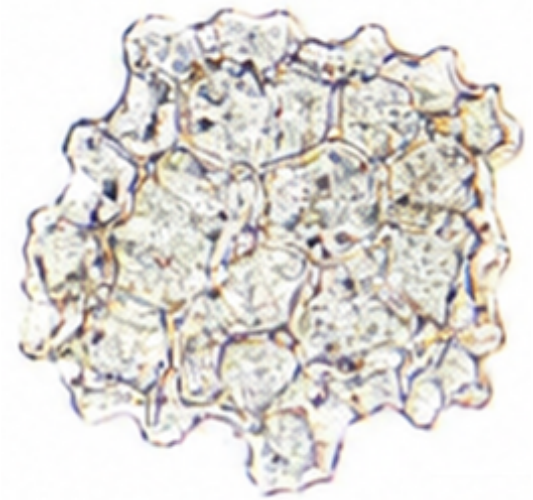

Figure 2: Powder microscopy of *Ziziphus nummularia (Burm.f)*

Physicochemical evaluation: The physicochemical evaluation of *Ziziphus nummularia* (Burm.f.) revealed characteristic values for the analyzed parameters. The total ash content was found to be 9.85% w/w, while water-soluble ash and acid-insoluble ash were 4.20% w/w and 1.35% w/w, respectively. The loss on drying was 7.80% w/w, indicating the moisture content of the crude drug. The alcohol-soluble extractive value was 11.60% w/w, whereas the water-soluble extractive value was comparatively higher at 15.40% w/w, suggesting the presence of appreciable water-soluble constituents. Foreign organic matter was found to be 0.90% w/w, indicating satisfactory purity of the plant material.

Table 2: Physicochemical parameters of *Ziziphus nummularia (Burm.f.)*

S. No.	Physicochemical parameter values (% w/w)	*Ziziphus nummularia* (Burm.f.)
1	Total ash	9.85
2	Water soluble ash	4.2
3	Acid insoluble ash	1.35
4	Loss on drying	7.8
5	Alcohol soluble extractive	11.6
6	Water soluble extractive	15.4
7	Foreign organic matter determination	0.9

Preliminary phytochemical screening

Extraction of plant material: The percentage yield of different extracts of *Ziziphus nummularia* (Burm.f.) varied considerably depending on the solvent used for extraction. Among the tested solvents, methanol produced the highest extraction yield of 12.80%, indicating its greater efficiency in extracting soluble phytoconstituents from the plant material. The aqueous extract showed the second-highest yield of 9.60%. Chloroform extraction resulted in a moderate yield of 3.50%, whereas petroleum

ether produced the lowest yield of 2.10%. Overall, the results demonstrated that polar solvents, particularly methanol, were more effective than non-polar solvents for extracting constituents from *Ziziphus nummularia*.

Table 3: Percentage yield of various extracts of *Ziziphus nummularia (Burm.f.)*

Solvents used for extraction	*Ziziphus nummularia* (Burm.f.) (% yield)
Petroleum ether	2.10%
Chloroform	3.50%
Methanol	12.80%
Water	9.60%

Phytochemical screening: Preliminary phytochemical screening of methanolic (MZML) and aqueous (AZML) extracts of *Ziziphus nummularia* (Burm.f.) revealed the presence of several important phytoconstituents. Both extracts showed positive tests for carbohydrates, proteins and amino acids, acidic compounds, alkaloids, glycosides, flavonoids, phenolic compounds, terpenoids, and saponins. Mucilage was detected only in the aqueous extract, whereas fixed oil and sterols/steroids were present only in the methanolic extract. Anthraquinones were absent in both extracts. Overall, the methanolic extract exhibited a broader phytochemical profile, while the aqueous extract also demonstrated considerable diversity. The presence of flavonoids, phenolics, alkaloids, terpenoids, and saponins suggests potential pharmacological activities.

Table 4: Preliminary phytochemical screening *Ziziphus nummularia (Burm.f.)*

S. No.	Phytochemical Tests for phytoconstituents	*Ziziphus nummularia* (Burm.f.)	
		MZML	AZML
1	Carbohydrates	+	+
2	Proteins and Amino Acids	+	+
3	Acidic Compounds	+	+
4	Mucilage	−	+
5	Fixed Oil	+	−
6	Alkaloids	+	+
7	Glycosides	+	+
8	Sterols and Steroids	+	−
9	Anthraquinones	−	−
10	Flavonoids	+	+
11	Phenolic Compounds	+	+
12	Terpenoids	+	+
13	Saponins	+	+

+ indicate present; - indicate absent

Fractionation of Extract and Isolation of Active Constituents of *Ziziphus nummularia:* The isolated constituent obtained from Ziziphus nummularia was subjected to spectroscopic characterization using ^1H NMR, mass spectrometry, and IR spectroscopy. The ^1H NMR spectrum (Figure 3) exhibited characteristic proton signals attributable to the aromatic and hydroxyl-substituted regions of quercetin, supporting the presence of a flavonoid skeleton. The mass spectrum (Figure 4) showed a molecular ion peak consistent with the expected molecular mass of quercetin (302 Da), providing evidence for its molecular identity. The IR spectrum (Figure 5) displayed characteristic absorption bands corresponding to hydroxyl (–OH), carbonyl (C=O), aromatic C=C, and C–O stretching vibrations, which are consistent with the functional groups present in quercetin. Based on the combined spectroscopic findings, the isolated compound was identified as quercetin. The proposed chemical structure is presented in Figure 6. Overall, the spectral data were in good agreement with reported characteristics of quercetin, confirming successful isolation and preliminary structural elucidation of the constituent from *Ziziphus nummularia* (ZN1).

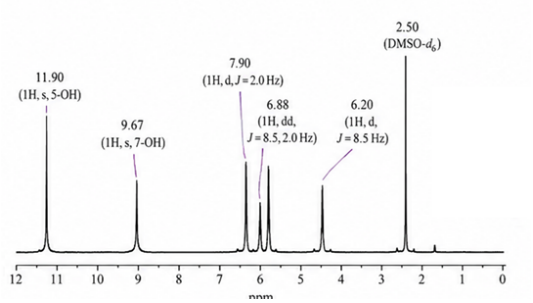

Figure 3: 1H NMR spectra of Structure of isolated Constituents from *Ziziphus nummularia* (quercetin)

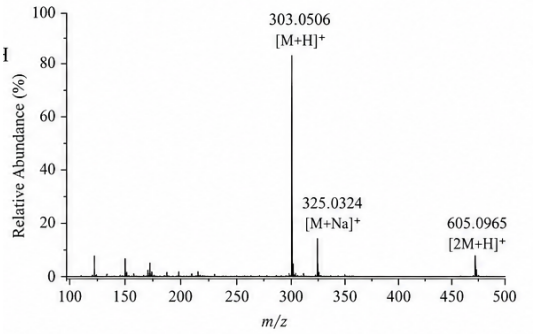

Figure 4: Mass spectra of Structure of isolated Constituents from *Ziziphus nummularia* (quercetin)

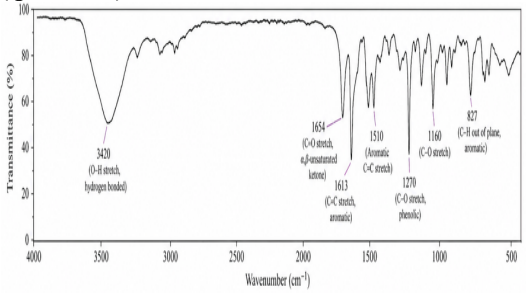

Figure 5: IR spectra of isolated Constituents from *Ziziphus nummularia* (quercetin)

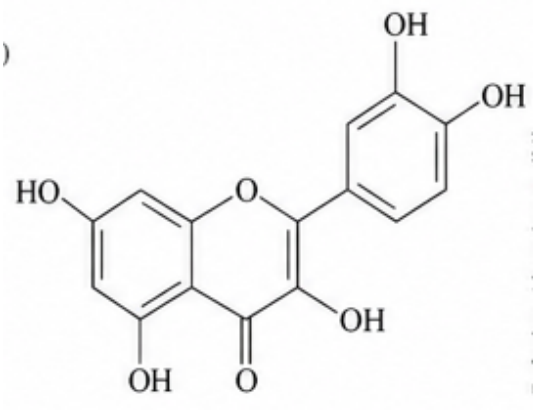

Figure 6: Structure of isolated Constituents from *Ziziphus nummularia* (quercetin)

In vivo animal study of extract and fractions: The hepatoprotective activity of *Ziziphus nummularia* (Burm.f.) was evaluated against carbon tetrachloride (CCl_4)-induced hepatic injury by assessing body weight gain, serum biochemical markers, liver weight, oxidative stress parameters, and histopathological changes. The results demonstrated that CCl_4 administration produced marked hepatic damage, whereas treatment with *Z. nummularia* extracts and the isolated fraction produced varying degrees of protection.

Effect on Body Weight Gain: The normal control group showed a body weight gain of 33.12 ± 0.29 g, whereas CCl_4 administration markedly reduced body weight gain to 2.25 ± 0.12 g, indicating considerable systemic toxicity and impaired metabolic function. Silymarin treatment substantially restored body weight gain to 30.10 ± 0.27 g, approaching the normal control value. Among the test preparations, the microwave extract (MZN) produced the greatest improvement, with body weight gain of 28.21 ± 0.87 g, followed by the Soxhlet extract (SZN) at 26.79 ± 0.68 g. The isolated fraction ZN1 produced a comparatively lower increase of 20.24 ± 0.46 g. These findings suggest that the extracts, particularly MZN, were more effective in restoring general physiological status than the isolated fraction.

Effect on AST: The AST level increased markedly from 110.76 ± 8.38 IU/L in the normal control to 207.34 ± 9.04 IU/L in the toxic control, confirming substantial hepatocellular damage. Silymarin reduced AST to 114.13 ± 10.19 IU/L. Treatment with SZN and MZN reduced AST to 141.96 ± 9.23 and 130.96 ± 13.78 IU/L, respectively, while ZN1 reduced it to 167.08 ± 10.65 IU/L. Thus, the order of hepatoprotective effectiveness based on AST was MZN > SZN > ZN1. The greater activity of MZN may be associated with improved extraction of thermally stable or moderately polar hepatoprotective phytoconstituents.

Effect on ALT: ALT showed a similar pattern. The enzyme level increased from 60.25 ± 1.87 IU/L in normal animals to 122.64 ± 5.36 IU/L after CCl_4 exposure. Silymarin markedly restored ALT to 69.92 ± 7.65 IU/L. MZN decreased ALT to 78.96 ± 7.78 IU/L, whereas SZN reduced it to 94.34 ± 5.27 IU/L. ZN1 showed comparatively weaker protection, with ALT of 104.44 ± 6.76 IU/L. The results again indicated that MZN was the most effective extract, followed by SZN and ZN1.

Effect on ALP: ALP increased from 235.8 ± 8.97 IU/L in the normal control to 407.62 ± 7.65 IU/L in the CCl_4-treated group. Silymarin decreased ALP to 271.14 ± 9.79 IU/L. SZN and MZN reduced ALP to 305.71 ± 12.85 and 286.48 ± 10.83 IU/L, respectively, whereas ZN1 resulted in 340.16 ± 14.67 IU/L. The stronger reduction produced by MZN suggests better preservation of hepatobiliary function.

Effect on Bilirubin: Total bilirubin increased from 0.34 ± 0.012 mg/dL in normal animals to 0.69 ± 0.032 mg/dL following CCl_4 exposure. Silymarin reduced bilirubin to 0.39 ± 0.012 mg/dL. MZN reduced the value to 0.46 ± 0.019 mg/dL, followed by SZN (0.51 ± 0.014 mg/dL) and ZN1 (0.61 ± 0.024 mg/dL). The reduction in bilirubin indicates improved hepatic processing and excretion following treatment, with MZN demonstrating the most pronounced effect among the test preparations.

Effect on Liver Weight: The toxic control showed increased liver weight (8.914 ± 0.36 g) compared with the normal control (7.992 ± 0.19 g), which may reflect hepatic enlargement associated with inflammation, cellular injury, and pathological accumulation. Silymarin reduced liver weight to 7.849 ± 0.15 g. SZN and MZN produced values of 8.459 ± 0.11 and 8.389 ± 0.14 g, respectively, whereas ZN1 showed 8.659 ± 0.14 g. The comparatively greater normalization produced by MZN suggests improved protection against CCl_4-induced hepatic enlargement.

Effect on MDA: MDA, an indicator of lipid peroxidation, was markedly increased in the toxic control (379.21 nmol/g tissue) compared with the normal control (168.59 nmol/g tissue). Silymarin reduced MDA to 197.49 nmol/g tissue. MZN reduced MDA to 229.18 nmol/g tissue, while SZN and ZN1 produced values of 252.42 and 313.71 nmol/g tissue, respectively. Thus, the microwave extract showed substantially greater inhibition of lipid peroxidation than the Soxhlet extract and isolated fraction. The findings indicate that the antioxidant constituents extracted by microwave treatment may effectively reduce CCl_4-induced oxidative damage to hepatic cell membranes.

Effect on SOD Activity: SOD activity decreased considerably from 75.14 ± 1.06 µM in normal animals to 42.98 ± 1.52 µM in the toxic control, demonstrating depletion of endogenous antioxidant defense. Silymarin restored SOD activity to 65.12 ± 1.25 µM. MZN increased SOD to 58.82 ± 2.02 µM, whereas SZN produced 50.82 ± 1.94 µM and ZN1 produced 43.82 ± 2.10 µM. The results indicate that MZN was more effective in restoring antioxidant defense than SZN and ZN1.

Effect on Catalase Activity: Catalase activity was markedly decreased in the toxic control (65.70 ± 1.78) compared with the normal control (133.92 ± 5.83), indicating impaired enzymatic antioxidant protection. Silymarin restored catalase activity to 119.56 ± 3.23. Treatment with MZN increased catalase activity to 105.56 ± 3.21, while SZN and ZN1 resulted in 98.56 ± 3.67 and 85.56 ± 3.78, respectively. Thus, MZN again exhibited the strongest antioxidant effect among the investigated *Z. nummularia* preparations.

Comparative Effect of Different Extraction Methods: The results clearly demonstrated that the method of extraction influenced the hepatoprotective potential of *Z. nummularia*. The microwave extract (MZN) consistently produced better responses than the Soxhlet extract (SZN) across almost all biochemical and oxidative stress parameters. MZN produced lower AST, ALT, ALP, bilirubin, liver weight, and MDA values and higher SOD and catalase activities than SZN.

The superior activity of MZN may be attributed to more efficient disruption of plant cellular structures and enhanced release of bioactive phytoconstituents during microwave-assisted extraction. In contrast, prolonged Soxhlet extraction may cause degradation or loss of certain thermolabile or oxidation-sensitive constituents. Nevertheless, SZN also demonstrated considerable hepatoprotective activity compared with the toxic control.

The isolated fraction ZN1 (10 mg/kg) showed a protective effect but was generally less effective than both crude extracts. This finding suggests that the hepatoprotective activity of *Z. nummularia* may depend on the combined or synergistic action of several phytoconstituents rather than a single isolated component. The relatively low dose of ZN1 compared with the extracts may also contribute to its lower overall response.

Overall, the relative hepatoprotective activity based on the majority of parameters was:

Silymarin > MZN > SZN > ZN1 > CCl_4 toxic control.

However, the test preparations were not equivalent in dose or chemical composition, so this ranking should be interpreted as a comparison of the observed treatment responses rather than a direct potency comparison.

Histopathological Results and Discussion: Histopathological examination further supported the biochemical findings. The normal control showed normal hepatic architecture without hepatocyte necrosis or inflammatory-cell infiltration in the periportal zone. The centrilobular region also showed no appreciable necrosis, inflammation, or sinusoidal dilation.

In contrast, the toxic control exhibited marked centrizonal hepatocyte necrosis (+++), inflammatory-cell infiltration (+++), and sinusoidal dilation (+++). These pathological changes are characteristic of CCl_4-induced hepatic injury and correspond well with the elevated AST, ALT, ALP, bilirubin, and MDA levels observed in the biochemical analysis.

The standard silymarin-treated group showed substantial preservation of hepatic architecture, with no detectable periportal hepatocyte necrosis or inflammatory-cell infiltration and no apparent centrilobular necrosis or inflammation. Only mild sinusoidal dilation (++) was observed. This finding confirms the protective effect of silymarin against CCl_4-induced hepatic damage.

The ZN1-treated group showed no periportal necrosis or inflammatory-cell infiltration. In the centrilobular region, only mild necrosis (+), mild inflammation (+), and mild sinusoidal dilation (+) were observed. Thus, ZN1 considerably reduced the severe histological alterations produced by CCl_4, although it did not restore the liver completely to normal architecture.

Collectively, the biochemical, antioxidant, and histopathological findings demonstrate that *Ziziphus nummularia* possesses significant hepatoprotective potential against CCl_4-induced hepatic injury. CCl_4 exposure caused substantial hepatic damage, characterized by reduced body weight gain, elevation of AST, ALT, ALP and bilirubin, increased liver weight and MDA, and depletion of SOD and catalase activities. Treatment reversed these changes to varying degrees.

Among the test preparations, MZN (microwave extract, 200 mg/kg) showed the most consistent hepatoprotective and antioxidant effect, followed by SZN (Soxhlet extract, 200 mg/kg). ZN1 (isolated fraction, 10 mg/kg) also demonstrated protection but was comparatively less effective. The superior response of MZN suggests that microwave-assisted extraction may be a more efficient approach for recovering the hepatoprotective phytoconstituents of *Z. nummularia*. The findings were further supported by histopathology, where treatment markedly reduced CCl_4-associated necrosis, inflammatory infiltration, and sinusoidal dilation.

Table 5: Effect of *Ziziphus nummularia (Burm.f.)*

Groups	Treatment	Body weight gain (g)	AST(IU/L)	ALT(IU/L)	ALP(IU/L)	Bilirubin level (mg/dL)	Liver weight (g)	MDA (nmol/g tissue)	SOD (µM of H2O2 produced/min/g of tissue)	SOD (µM) catalase activity
Group I	Normal control (Normal Saline 1 ml/kg BW)	33.12±0.29	110.76±8.38	60.25±1.87	235.8 ± 8.97	0.34 ±0.012	7.992 ±0.19	168.59	75.14±1.06	133.92 ±5.83
Group II	Disease control (Carbon tetrachloride (CCl_4) 1000 mg/kg p.o. for	2.25±0.12	207.34±9.04	122.64 ±5.36	407.62 ± 7.65	0.69± 0.032	8.914 ±0.36	379.21	42.98±1.52	65.70± 1.78

	7 days)									
Group III	Standard (Silymarin treated) + CCl_4 (1000 mg/kg p.o.)	30.10±0.27	114.13±10.19	69.92±7.65	271.14±9.79	0.39±0.012	7.849±0.15	197.49	65.12±1.25	119.56±3.23
Group IV	SZN Soxhlet extract of Ziziphus nummularia (Burm. f.) (200 mg/kg)	26.79±0.68	141.96±09.23	94.34±5.27	305.71±12.85	0.51±0.014	8.459±0.11	252.42	50.82±1.94	98.56±3.67
Group V	MZN Microwave extract of Ziziphus nummularia (Burm. f.) (200 mg/kg)	28.21±0.8	130.96±13.78	78.96±7.78	286.48±10.83	0.46±0.019	8.389±0.14	229.18	58.82±2.02	105.56±3.21
	7 days)									
Group VI	ZN1 Isolated comp of Ziziphus nummularia (Burm. f.) (10 mg/kg)	20.24±0.46	167.08±10.65	104.44±6.76	340.16±14.67	0.61±0.024	8.659±0.14	313.71	43.82±2.10	85.56±3.78

Histopathology:

Table 6: Observation of histopathological studies

S. No.	Experimental Group	Periportal Zone		Centrizonal Area		
		Hepatocyte necrosis	Inflammation cell	Necrosis	Inflammation	Sinusoidal dilation
1	Normal Contrl	Nil	Nil	-	-	-

2	Toxic Control	Nil	Nil	+++	+++	+++
3	Standard Control	Nil	Nil	-	-	++
4	ZN1	Nil	Nil	+	+	+

Summary: The present study established the pharmacognostic characteristics, physicochemical properties, phytochemical profile, isolation, and hepatoprotective potential of *Ziziphus nummularia* (Burm.f.). Macroscopic and microscopic evaluation revealed characteristic diagnostic features, including greenish-brown leaf material, thick-walled epidermal cells, non-glandular trichomes, fibrous tissues, calcium oxalate crystals, and reticulate and pitted vessels. Physicochemical analysis showed acceptable ash values, extractive values, moisture content, and low foreign organic matter. Among the investigated solvents, methanol exhibited the highest extraction yield (12.80%). Preliminary phytochemical screening confirmed the presence of carbohydrates, proteins, alkaloids, glycosides, flavonoids, phenolics, terpenoids, and saponins. The isolated constituent ZN1 was identified as quercetin based on ^1H NMR, mass spectrometry, and IR spectral characteristics. In the CCl_4-induced hepatotoxicity model, both Soxhlet and microwave extracts significantly improved body weight gain, serum AST, ALT, ALP, bilirubin, liver weight, MDA, SOD, and catalase levels compared with the toxic control. The microwave extract (MZN, 200 mg/kg) demonstrated the most pronounced protective effect among the test preparations, followed by SZN and ZN1. Histopathological findings further confirmed reduced hepatocellular necrosis, inflammation, and sinusoidal dilation. Overall, *Z. nummularia*, particularly its microwave extract, demonstrated promising hepatoprotective and antioxidant potential.

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