Pharmacogn J. 2025; 17(6): 760-769
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com

Original Article

Protective Role of *Centella asiatica* Extract Against Carbon Tetrachloride–Induced Hepatic Damage: A Biochemical and Ultrasonographic Study

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ABSTRACT

This study aimed to evaluate the hepatoprotective activity of *Centella asiatica* extract on Wistar rats induced with carbon tetrachloride (CCl_4). The extract is known to contain active compounds such as flavonoids, phenolics, and triterpenoids, which contribute to its antioxidant and anti-inflammatory effects. The total phenolic and flavonoid contents were 70.31 mg GAE/g and 13.49 mg QE/g, respectively, with very strong antioxidant activity (IC_{50} = 48.45 ppm). Evaluation through ultrasonography and histopathology revealed structural improvement in the liver of treated groups, particularly at doses of 200 and 300 mg/kgBW, marked by reduced abnormal echogenicity and improved liver parenchyma, along with a decrease in histopathological score from 2 to 1. The administration of the extract also significantly reduced pro-inflammatory cytokines TNF-α and IL-6 ($P \leq 0.05$), as well as CRP levels, indicating strong anti-inflammatory potential. In addition, liver function showed meaningful recovery, with the highest albumin level recorded at 200 mg/kgBW (3.00 ± 0.52 g/dL), and a significant reduction in bilirubin level at 300 mg/kgBW to 0.102 ± 0.040 mg/dL. Significant decreases were also observed in SGOT and SGPT enzyme levels in the treatment groups, especially at 300 mg/kgBW, indicating protection of hepatocyte integrity. In conclusion, this study demonstrated that Centella asiatica extract possesses hepatoprotective effects through anti-inflammatory, antioxidant, and liver function-restorative mechanisms. These findings support the potential development of pegagan as a phytopharmaceutical agent for adjunct therapy in liver disorders and highlight the need for further studies on its active compounds and long-term safety.

Keywords: *Centella asiatica,* Hepatoprotective, Antioxidant, Anti-inflammatory, Cytokines, Ultrasonography

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History

- Submission Date: 17-10-2025;
- Review completed: 04-11-2025;
- Accepted Date: 19-11-2025.

DOI : 10.5530/pj.2025.17.95

Article Available online

http://www.phcogj.com/v17/i6

INTRODUCTION

The liver plays a vital role in metabolism, detoxification, and homeostasis, making it one of the most essential organs in maintaining physiological balance[1]. However, due to its central role in the biotransformation of xenobiotics, the liver is highly susceptible to injury from various hepatotoxic substances such as industrial chemicals, drugs, alcohol, and environmental pollutants[2]. Continuous exposure to these toxic agents can lead to oxidative stress, inflammation, and structural degeneration of hepatic tissue, resulting in acute or chronic liver disease[3]. Among the numerous hepatotoxins, carbon tetrachloride (CCl_4) remains one of the most extensively used compounds in experimental models due to its well-established mechanism of hepatotoxicity[4]. It induces liver injury through the formation of trichloromethyl (CCl_3•) and trichloromethyl peroxy (CCl_3OO•) radicals, which initiate lipid peroxidation, mitochondrial dysfunction, and hepatocellular necrosis[5]. These events elevate serum transaminase levels and impair liver function, simulating the pathological conditions seen in human liver disorders.

Although several synthetic hepatoprotective agents, such as silymarin, N-acetylcysteine, and corticosteroids, have been used to treat liver damage, their effectiveness remains inconsistent, and long-term use can cause undesirable side effects[6]. Consequently, there is a growing interest in natural plant-based therapies that offer multi-target protection through antioxidant, anti-inflammatory, and regenerative mechanisms.

Centella asiatica (L.) Urban, known as pegagan in Indonesia or gotu kola in other regions, is a perennial medicinal herb of the Apiaceae family widely recognized in traditional medicine across Asia. It has been used for centuries to treat various ailments, including wound healing, neurological disorders, and liver dysfunction[7,8]. The therapeutic potential of *C. asiatica* is primarily attributed to its bioactive compounds triterpenoids (asiatic acid, madecassic acid, asiaticoside), flavonoids, phenolics, and sterols[9]. These compounds exhibit potent antioxidant and anti-inflammatory properties capable of scavenging free radicals, inhibiting lipid peroxidation, and suppressing the production of pro-inflammatory mediators such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6)[10,11].

Oxidative stress and inflammation are central to the pathogenesis of hepatic injury induced by toxic substances, including alcohol and drugs such as acetaminophen and isoniazid. Excessive reactive oxygen species (ROS) generation damages cellular proteins, lipids, and DNA, while Kupffer cell activation leads to the release of cytokines that perpetuate necroinflammation and fibrosis[12]. *C. asiatica* and its phytochemicals have been reported to counteract these processes by activating the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway enhancing endogenous antioxidant defense enzymes and by inhibiting nuclear factor-kappa B (NF-κB) signaling, thereby reducing oxidative stress and inflammation[13].

Cite this article: Ica Y P, Ermi G, Yolanda E P L. Protective Role of *Centella asiatica* Extract Against Carbon Tetrachloride–Induced Hepatic Damage: A Biochemical and Ultrasonographic Study. Pharmacogn J. 2025;17(6): 760-769.

Recent advances in hepatoprotective research have incorporated non-invasive imaging modalities such as ultrasonography (USG) to assess liver parenchymal integrity and echogenicity[14]. When combined with biochemical and molecular parameters, including liver enzymes (SGOT, SGPT), serum albumin, bilirubin, and inflammatory cytokines (TNF-α, IL-6, and CRP), USG provides a comprehensive evaluation of liver function and structural recovery. Previous studies using plant-based antioxidants such as curcumin and green tea catechins, have demonstrated significant improvements in both biochemical of toxin-induced liver damage[15,16]

Considering these findings, the present study was designed to evaluate the hepatoprotective potential of *Centella asiatica* extract in Wistar rats subjected to carbon tetrachloride–induced liver damage. The investigation employed biochemical assays, cytokine profiling, and ultrasonographic imaging to elucidate the extract's protective and restorative effects. This study hypothesizes that *Centella asiatica* extract exerts dose-dependent hepatoprotective effects against CCl_4-induced liver injury, as evidenced by improvements in liver function markers, histopathological features, and inflammatory biomarkers including TNF-α, IL-6, and CRP.

MATERIALS AND METHODS

Study Design and Location

This study employed an in vivo experimental design and was carried out at the Research Laboratory, Faculty of Medicine, Universitas Prima Indonesia, between April and June 2025. The experiment aimed to investigate the hepatoprotective efficacy of *Centella asiatica* extract in Wistar rats subjected to liver injury induced by carbon tetrachloride. The evaluation encompassed biochemical, cytokine, ultrasonographic, and histopathological analyses to comprehensively assess the extract's protective mechanisms and therapeutic potential against hepatotoxic damage.

Preparation of Extract

The *Centella asiatica* herb used in this study was sourced from local farmers in Bandar Baru Village, Karo Regency. A total of 300 grams of dried plant powder was subjected to maceration using 70% ethanol (70:30, v/v) at 25°C. The mixture was stirred continuously to ensure optimal extraction and allowed to stand for 24 hours before filtration. This procedure was repeated three times to maximize yield. All filtrates were pooled and then centrifuged at 3500 rpm for 10 minutes to remove residual plant particles. The resulting supernatant was subsequently evaporated at 38°C to obtain a concentrated hydroethanolic extract of *C. asiatica*, which was stored at 4°C until further use[17].

Phytochemical and Antioxidant Analysis

Qualitative phytochemical analysis was carried out using standard screening methods. Alkaloids were detected using Bouchardat's, Mayer's, and Dragendorff's reagents. Flavonoids were identified using the Mg–HCl reduction test followed by the addition of concentrated H_2SO_4. The presence of triterpenoids and steroids was assessed using the Liebermann–Burchard reaction. Glycosides were detected by the Molisch test combined with sulfuric acid. Saponins were identified using the foam test with distilled water, while tannins were detected using ferric chloride ($FeCl_3$) reagent. While LC–HRMS analysis was performed to identify and confirm the chemical constituents within the extract[18]. The total phenolic content was determined using the Folin–Ciocalteu method, with results expressed as milligrams of gallic acid equivalents (mg GAE/g extract). Meanwhile, the total flavonoid content was measured through the aluminum chloride colorimetric method and expressed as milligrams of quercetin equivalents (mg QE/g extract)[19]. The antioxidant activity of the extract was evaluated using the DPPH radical scavenging assay, and the IC_{50} value representing the concentration required to inhibit 50% of free radicals was calculated to quantify antioxidant strength[20].

Animals

A total of twenty-five healthy male Wistar rats aged 12–16 weeks and weighing 150–200 g were used in this experiment. Prior to treatment, all animals underwent a seven-day acclimatization period under controlled environmental conditions, including a temperature range of 22–24°C, relative humidity of 50–60%, and a 12-hour light/dark cycle. Throughout the study, the rats were maintained on a standard laboratory diet and provided with water ad libitum to ensure optimal physiological stability before experimental induction.

Animal model of CCl_4-induced liver injury

Carbon tetrachloride (CCl_4) was diluted in olive oil at a ratio of 1:1 (v/v) prior to administration. Liver injury was induced by intraperitoneal injection of CCl_4 at a dose of 3 mL/kg body weight, administered twice weekly for 28 days[20]. This dosing regimen was selected based on established protocols for inducing reproducible chronic hepatic injury while avoiding excessive acute toxicity.

Experimental Design

The twenty-five Wistar rats were randomly assigned into five experimental groups (n = 5) following Federer's formula to ensure adequate statistical power. The groups were organized as follows:

1. Normal control: received no treatment.
2. Carbon tetrachloride control: administered carbon tetrachloride at 3 mL/kg body weight intraperitoneally, twice a week for 28 days.
3. Carbon tetrachloride + *Centella asiatica* extract (100 mg/kg BW): extract given orally once daily.
4. Carbon tetrachloride + *Centella asiatica* extract (200 mg/kg BW): extract administered orally once daily.
5. Carbon tetrachloride + *Centella asiatica* extract (300 mg/kg BW): extract administered orally once daily.

On day 29, blood samples were collected from the orbital sinus under light anesthesia for biochemical and cytokine analyses, followed by liver ultrasonography to assess morphological and structural changes in hepatic tissue.

Ultrasonographic Examination

Liver ultrasonography was conducted at weekly intervals on days 0, 7, 14, 21, and 28 using a Chison ECO 2 portable ultrasound system equipped with a 10 MHz mini-convex probe. Prior to imaging, each rat was anesthetized with ketamine, and the abdominal hair was carefully shaved to ensure optimal probe contact and image clarity. The liver was scanned from the subcostal region, and parameters such as echogenicity, parenchymal homogeneity, and surface contour were systematically evaluated to monitor structural changes and recovery during treatment[21].

Biochemical and Cytokine Assays

Serum samples were obtained by centrifuging blood at 3000 rpm for 15 minutes, after which the supernatant was collected for biochemical and cytokine analyses. The concentrations of albumin, bilirubin, SGOT (serum glutamic oxaloacetic transaminase), and SGPT (serum glutamic pyruvic transaminase) were determined spectrophotometrically following standard clinical chemistry protocols[22]. Levels of TNF-α, IL-6, and CRP were quantified using commercial ELISA kits (Solarbio®, China) according to the manufacturer's instructions. These parameters

served as key indicators of hepatic function and systemic inflammatory response, allowing comprehensive evaluation of the hepatoprotective effects of *Centella asiatica* extract.

Histopathological Evaluation

Liver tissue samples were fixed in 10% neutral-buffered formalin for 24 hours to preserve cellular integrity. The samples were then dehydrated through a graded ethanol series, cleared using xylene, and subsequently embedded in paraffin wax. Thin tissue sections of approximately 5 µm thickness were prepared using a microtome and stained with hematoxylin and eosin (H&E) for microscopic examination[23]. The slides were evaluated for histopathological alterations, including hepatocellular necrosis, fatty degeneration, sinusoidal dilation, and inflammatory cell infiltration, to assess the extent of hepatic injury and recovery following treatment[24].

Statistical Analysis

Statistical analysis was performed using GraphPad Prism version 9.0 (GraphPad Software, San Diego, CA, USA). Data normality was assessed using the Shapiro–Wilk test, and homogeneity of variances was evaluated using Levene's test before applying one-way analysis of variance (ANOVA). Between-group comparisons were conducted using Tukey's post hoc test. Differences were considered statistically significant at $p \leq 0.05$.

Ethical Clearance

All experimental procedures were conducted in accordance with ethical standards and were approved by the Animal Research Ethics Committee of Universitas Prima Indonesia under approval number 057/KEPK/UNPRI/III/2025. All protocols followed both institutional and international guidelines for the care and use of laboratory animals, ensuring humane treatment throughout the study. Appropriate measures were taken to minimize animal stress and discomfort during handling, treatment, and sample collection.

RESULTS AND DISCUSSION

Phytochemical Composition and LC–HRMS Profile

Phytochemical analysis of *Centella asiatica* extract (Table 1) confirmed the presence of alkaloids, flavonoids, triterpenoids, glycosides, saponins, and tannins, all of which are well-recognized for their pharmacological activities. These compounds are known to act synergistically to provide antioxidant, anti-inflammatory, and hepatoprotective effects. The triterpenoids particularly asiatic acid, madecassic acid, and asiaticoside play a major role in stabilizing hepatocyte membranes, enhancing tissue regeneration, and scavenging free radicals generated during oxidative stress[25]. The presence of flavonoids and phenolic compounds further contributes to the extract's ability to inhibit lipid peroxidation, regulate cellular redox balance, and suppress inflammatory mediators such as TNF-α and IL-6, which are elevated in toxin-induced hepatic injury[26].

Moreover, the LC–HRMS profile of *C. asiatica* extract (Table 2) revealed more than one hundred bioactive compounds, including chlorogenic acid, quercetin, luteolin, isoferulic acid, and asiaticoside derivatives. These phytochemicals have been widely reported to modulate oxidative stress and inflammatory pathways through activation of the Nrf2/ARE signaling cascade and inhibition of NF-κB translocation[27]. In particular, quercetin and luteolin are potent inhibitors of pro-inflammatory cytokines and protect hepatocytes against necrosis by maintaining mitochondrial function[27]. Moreover, isoferulic acid also identified in the extract, are also known to prevent lipid peroxidation and improve hepatic enzyme levels in carbon tetrachloride–induced models[28].

Table 1. Phytochemical compound group of *Centella asiatica* extract

Parameter	Reagent	Result
Alkaloids	Bouchardat	+
	Meyer	+
	Dragendorff	+
Flavonoids	Mg-HCl + H_2SO_4	+
Triterpenoids/Steroids	Liebermann–Burchard	+
Glycosides	Molisch + H_2SO_4	+
Saponins	Distilled water (foam test)	+
Tannins	$FeCl_3$	+

Total Phenolic, Flavonoid, and Antioxidant Activity

The quantitative analysis of *Centella asiatica* extract demonstrated high levels of total phenolic and flavonoid contents, measured at 70.31 mg GAE/g and 13.49 mg QE/g extract, respectively (Table 3). These values indicate a rich presence of polyphenolic compounds, which are key contributors to the plant's antioxidant and hepatoprotective properties. The extract also exhibited strong antioxidant activity, with an IC_{50} value of 48.45 ppm, suggesting potent radical-scavenging potential.

Phenolic and flavonoid compounds play a crucial role in neutralizing free radicals and preventing lipid peroxidation in hepatocytes exposed to toxic agents such as carbon tetrachloride. The presence of compounds such as chlorogenic acid, quercetin, and luteolinas identified in LC–HRMS profiling may synergistically enhance antioxidant capacity through hydrogen donation and metal ion chelation[29].

Previous studies have reported comparable antioxidant activity of *C. asiatica* extract, where high phenolic and flavonoid content was associated with elevated superoxide dismutase (SOD) and catalase (CAT) activities in hepatotoxic models[30,31]. The strong antioxidant effect observed in this study suggests that *C. asiatica* effectively counteracts reactive oxygen species (ROS) induced oxidative stress, thereby preserving hepatocellular integrity and improving overall liver function.

Ultrasonographic Evaluation

Ultrasonographic observations revealed distinct patterns of hepatic recovery among the experimental groups following carbon tetrachloride administration. As shown in Figure 1, the normal control group (A) consistently exhibited homogeneous liver parenchyma with smooth margins across all observation days, confirming normal hepatic morphology. In contrast, the carbon tetrachloride–induced control group (B) showed progressive structural deterioration characterized by heterogeneous and coarse hepatic parenchyma, irregular margins, and multiple hepatic nodules by day 14, which persisted through day 28 hallmarks of early fibrosis and inflammatory injury.

Treatment with *Centella asiatica* extract led to notable structural improvements. The 100 mg/kg group (C) exhibited coarse hyperechoic parenchyma in both hepatic lobes on day 0, with visible nodules that became faint by day 14 and absent by day 28. The 200 mg/kg group (D) showed a similar pattern of recovery with regular hepatic margins and complete disappearance of nodules by day 28, indicating near-restoration of normal hepatic echotexture. The 300 mg/kg group (E) demonstrated the most significant regeneration, maintaining coarse but uniform echogenicity with no visible nodules by day 28.

These findings align with previous studies showing that *Centella asiatica*'s triterpenoids particularly asiaticoside and madecassoside stimulate hepatocyte proliferation and collagen remodeling[32], thereby reducing fibrotic progression and improving hepatic echogenicity[33]. The USG results correspond well with the biochemical improvements in

Table 2. LC–HRMS Identification of Phytochemical Compounds in *Centella asiatica* extract

No.	Compounds	Molecular Formula	Retention Time (min)	Reference Ion
1	2",6"-Di-O-Acetyl isovitexin	C25 H24 O12	4.211	[M-H]-1
2	Citric acid	C6 H8 O7	0.727	[M-H]-1
3	Choline	C5 H13 N O	0.717	[M+H]+1
4	[FAhydroxy(4:1/2:0)]2-hydroxy-2-butenedioicacid	C4 H4 O5	1.044	[M-H+HAc]-1
5	1-phenylpropane-1_2-dione	C9 H8 O2	1.436	[M+NH4]+1
6	[FAtrihydroxy(18:0)]9_10_13-trihydroxy-11-octadecenoicacid	C18 H34 O5	7.285	[M-H]-1
7	Chlorogenic acid	C16 H18 O9	2.719	[M-H]-1
8	Aureusidin 6-glucuronide	C21 H18 O12	4.557	[M-H]-1
9	2-(beta-D-Glucosyl)-sn-glycerol	C9 H18 O8	0.735	[M+Na]+1
10	1-Linoleoylglycerophosphocholine	C26 H50 N O7 P	10.596	[M+H]+1
11	3-Mercaptolactate	C3 H6 O3 S	11.042	[M-H]-1
12	α,α-Trehalose	C12 H22 O11	0.757	[M+Cl]-1
13	9,12,15-Octadecatrien-1-ol	C18 H32 O	13.221	[M+NH4]+1
14	(2R_3S)-2_3-Dimethylmalate	C6 H10 O5	0.726	[2M+NH4]+1
15	(+/-)9,10-dihydroxy-12Z-octadecenoic acid	C18 H34 O4	9.583	[M-H]-1
16	Indoline	C8 H9 N	1.44	[M+H]+1
17	Pyromyxone A	C19 H23 N O2	11.484	[M+H]+1
18	(2S) -5,7,3',4'-Tetrahydroxyflavanone 7- (6-galloylglucoside)	C28 H26 O15	4.837	[M-H]-1
19	Luteolin	C15 H10 O6	6.651	[M-H]-1
20	Miquelianin	C21 H18 O13	4.103	[M-H]-1
21	2-[(1S,2S,4aR,8aS)-1-hydroxy-4a-methyl-8-methylidene-decahydronaphthalen-2-yl]prop-2-enoic acid	C15 H22 O3	4.631	[M+H]+1
22	4-Oxoproline	C5 H7 N O3	1.045	[M-H]-1
23	Fumaric acid	C4 H4 O4	0.728	[M-H]-1
24	trans-3-Indoleacrylic acid	C11 H9 N O2	2.157	[M+H]+1
25	(S)-2-Acetolactate	C5 H8 O4	0.761	[M-H+HAc]-1
26	NP-003535	C30 H48 O6	8.117	[M+FA-H]-1
27	NP-022394	C15 H22 O3	4.862	[M+H]+1
28	Boc-Asp-OH	C9 H15 N O6	0.72	[M+H+MeOH]+1
29	NP-017664	C22 H36 O12	3.452	[M-H]-1
30	Hirsutatin A	C34 H52 N4 O10	9.488	[M+FA-H]-1
31	Isoferulic acid	C10 H10 O4	5.134	[M-H]-1
32	(3beta,5xi,9xi)-3-[(6-Deoxy-beta-D-glucopyranosyl)oxy]urs-12-ene-27,28-dioic acid	C36 H56 O9	5.444	[M+H]+1
33	NP-019748	C30 H48 O5	5.86	[M+H]+1
34	Dichloroacetic acid	C2 H2 Cl2 O2	0.037	[M+H]+1
35	Butoxytriglycol	C10 H22 O4	4.989	[M+Na]+1
36	PG(16:0/0:0)	C22 H45 O9 P	10.2	[M-H]-1
37	1,2-di-O-methyl-4-[(2R)-2,4-dihydrobutyramido]-4,6-dideoxy-α-D-mannopyranoside	C12 H23 N O7	1.076	[M+H]+1
38	3-Oxoglycyrrhetinate	C30 H44 O4	8.136	[M+H-H2O]+1
39	4,6-dideoxy-4-(3-deoxy-L-glycero-tetronamido)-2-O-methyl-α-D-mannopyranose	C11 H21 N O7	0.803	[M+H]+1
40	Sesbanimide A	C15 H21 N O7	1.404	[M+H]+1
41	Icosanamide	C20 H41 N O	15.252	[M+H]+1
42	Tilidine	C17 H23 N O2	11.482	[M+H-NH3]+1
43	N-Methyl-(R_S)-tetrahydrobenzylisoquinoline	C17 H19 N	11.484	[M+H]+1
44	1-O-Sinapoyl-beta-D-glucose	C17 H22 O10	3.628	[M-H-H2O]-1
45	D-(-)-Quinic acid	C7 H12 O6	2.72	[M-H]-1
46	Ceriporic acid C	C21 H36 O4	10.358	[M+H]+1
47	Leucylproline	C11 H20 N2 O3	2.027	[M+H]+1
48	Pipericine	C22 H41 N O	14.66	[M+H]+1
49	Biphenyl	C12 H10	11.484	[M+H]+1
50	alpha-Curcumene	C15 H22	10.556	[M+H]+1
51	NP-015114	C11 H19 N O6	0.775	[M+H+MeOH]+1
52	Penidienone	C14 H18 O	5.426	[M+H+MeOH]+1
53	NP-016437	C15 H24 O5	3.477	[M+H]+1
54	(+)-ar-Turmerone	C15 H20 O	13.339	[M+H]+1

55	trans-Aconitic acid	C6 H6 O6	0.747	[M-H]-1
56	D-Pipecolicacid	C6 H11 N O2	0.813	[M+H]+1
57	PG(16:1(9Z)/0:0)	C22 H43 O9 P	10.024	[M-H]-1
58	trans-beta-damascenone	C13 H18 O	4.686	[M+H]+1
59	(24ξ)-Stigmasta-3,5-diene	C29 H48	17.148	[M+H]+1
60	Trifolin	C21 H20 O11	4.439	[M-H]-1
61	N-Methyl-(R_S)-tetrahydrobenzylisoquinoline	C17 H19 N	9.635	[M+H]+1
62	(1r,3R,4s,5S)-4-{[(2E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy}-1,3,5-trihydroxycyclohexane-1-carboxylic acid	C16 H18 O9	4.702	[M+H]+1
63	Quercetin	C15 H10 O7	5.805	[M-H]-1
64	4-hydroxy-3-methoxyphenyl 4-O-methyl-β-glucopyranoside	C14 H20 O8	2.905	[M-H]-1
65	Cyprodenate	C13 H25 N O2	8.041	[M+H]+1
66	Adenosine	C10 H13 N5 O4	0.799	[M+H]+1
67	4-hydroxy-6-[2-(2-methyl-1,2,4a,5,6,7,8,8a-octahydronaphthalen-1-yl)ethyl] oxan-2-one	C18 H28 O3	10.805	[M+H]+1
68	cis-Aconiticacid	C6 H6 O6	1.045	[M-H]-1
69	NP-021030	C9 H8 O4	3.084	[M-H]-1
70	iso-Debromo-laurinterol	C15 H20 O	11.669	[M+H]+1
71	(E)-4-phenyl-3-(pyridine-2-yl)but-2-en-1-ol	C15 H15 N O	8.853	[M+H]+1
72	Sporovexin C	C15 H19 N O6	1.392	[M+H]+1
73	N-Acetylglucosaminitol	C8 H17 N O6	0.72	[M+H]+1
74	1-palmitoylglycerol;MAG(16:0)	C19 H38 O4	12.857	[M+H-H2O]+1
75	dodecanamide	C12 H25 N O	10.184	[M+H]+1
76	Epilumaflavanone B	C30 H32 O6	13.456	[M+H]+1
77	Okanin 4'-O- (4",6"-di-O-acetylglucoside)	C25 H26 O13	2.899	[M-H]-1
78	beta-Ionone	C13 H20 O	10.744	[M+H]+1
79	2′-α-mannosyl-L-tryptophan	C17 H22 N2 O7	2.04	[M+H]+1
80	dasc#1	C26 H46 O11	9.896	[M-H]-1
81	Orphenadrine	C18 H23 N O	11.255	[M+H]+1
82	(1S,3aS,3bS,9aR,9bS,11aS)-9a,11a-Dimethyl-1-(2,4,6-triisopropylbenzoyl)-1,2,3,3a,3b,4,5,8,9,9a,9b,10,11,11a-tetradecahydro-7H-cyclopenta[i]phenanthridin-7-one	C34 H49 N O2	12.212	[M+H]+1
83	Bisphenol C	C17 H20 O2	13.044	[M+H]+1
84	Makomotine C	C17 H30 O8	6.366	[M-H]-1
85	2E,4E,6E-Nonatrienal	C9 H12 O	11.864	[2M+H]+1
86	3,5,7-Trimethyl-2E,4E,6E,8E-undecatetraene	C14 H22	9.612	[M+H]+1
87	2R-amino-4S-hydroxy-5-hexynoic acid	C6 H9 N O3	1.667	[M+H]+1
88	Octylamine	C8 H19 N	2.696	[M+H]+1
89	(-)-alpha-Cedrene	C15 H24	11.54	[M+H]+1
90	Methyl 3-O-α-D-arabinofuranosyl-α-D-arabinofuranoside	C11 H20 O9	1.174	[M-H]-1
91	NP-019992	C10 H12 O4	3.531	[M+H-H2O]+1
92	NP-006255	C17 H26 O4	9.635	[M-H]-1
93	4E,6E,10Z-Hexadecatrien-1-ol	C16 H28 O	12.154	[M+H]+1
94	(2S,4aS,6R,8aS)-6-[2-(β-D-Glucopyranosyloxy)-2-propanyl]-8a-methyl-4-methylenedecahydro-2-naphthalenyl 6-O-[(2R,3R,4R)-3,4-dihydroxy-4-(hydroxymethyl)tetrahydro-2-furanyl]-β-D-glucopyranoside	C32 H54 O16	8.631	[M-H]-1
95	5,7-Dihydroxy-2-(4-hydroxyphenyl)-4-oxo-4H-chromen-3-yl 2,3,4-tri-O-acetyl-6-deoxy-α-L-mannopyranoside	C27 H26 O13	5.307	[M-H]-1
96	(1R,2R,5S,8R,10R,14R)-20-hydroxy-1,2,14,18,18-pentamethyl-17-oxo-8-(prop-1-en-2-yl)pentacyclo[11.8.0.0Â?,Âaâ⍰°.0â µ,â a.0Âaâ ´,Âaâ a]henicosane-5-carboxylic acid	C30 H46 O4	6.68	[M+H]+1
97	Pyrrolidine Linoleamide	C22 H39 N O	14.146	[M+H]+1
98	cinitapride	C21 H30 N4 O4	13.156	[M+Na]+1
99	Farfugin A	C15 H18 O	9.144	[M+H]+1
100	3-Hydroxybenzoic acid	C7 H6 O3	1.345	[M-H]-1
101	[FAhydroxy(4:0)]N-(3S-hydroxy-butanoyl)-homoserinelactone	C8 H13 N O4	0.754	[M+H]+1
102	1-O-coumaroyl-β-D-glucose	C15 H18 O8	2.787	[M-H]-1
103	3'-hydroxyacetophenone;3-ACETYLPHENOL	C8 H8 O2	9.818	[M+H-H2O]+1
104	Bacillamidin G	C17 H35 N O	13.52	[M+H]+1
105	Lucidulactone A	C12 H14 O6	5.865	[M+H]+1
106	9,10,13-trihydroxy-Octadecanoic acid	C18 H36 O5	7.015	[M-H]-1
107	13-tetradecen-2,4-diyn-1-ol	C14 H20 O	9.012	[M+H]+1

108	Methohexital	C14 H18 N2 O3	3.111	[M+H]+1
109	2-Formylglutarate	C6 H8 O5	0.754	[M+FA-H]-1
110	Apigenin 7- (6"-crotonylglucoside)	C25 H24 O11	5.25	[M-H]-1
111	Scopolin	C16 H20 O9	3.104	[M-H]-1
112	Isoliquiritigenin 4,4'-dimethyl ether	C17 H16 O4	6.835	[M-H]-1
113	Phenolicsteroid	C18 H24 O	12.506	[M+H]+1
114	Ethyl 3-O-beta-D-glucopyranosyl-butanoate	C12 H22 O8	1.934	[M-H]-1
115	1-O-(8R-hydroxy-8-methyl-3Z,9-decadienoyl)-beta-D-glucopyranose	C17 H28 O8	6.062	[M-H]-1
116	Formicolide B	C33 H48 O7	9.006	[M-H]-1
117	Porphobilinogen	C10 H14 N2 O4	0.776	[M+H]+1
118	C12E4	C20 H42 O5	11.528	[M+H]+1
119	Vitexin 2"-O-p-coumarate	C30 H26 O12	6.744	[M-H]-1
120	(Z)-2-Decene-4,6,8-triyn-1-ol	C10 H8 O	11.484	[M+H]+1
121	Trimethyl 6-hydroxy-6-(2-methoxy-2-oxoethyl)-5-(2-methyl-1-propenyl)-3-oxo-1,2,4-cyclohexanetricarboxylate	C19 H26 O10	5.533	[M-H]-1
122	6,3'-Dihydroxy-4,4'-dimethoxy-5-methylaurone	C18 H16 O6	4.754	[M-H]-1
123	L-Tyrosine methyl ester	C10 H13 N O3	9.719	[M-H]-1
124	3-Biphenylyl(difluoro)methanesulfonic acid	C13 H10 F2 O3 S	6.658	[M-H]-1
125	(3R,4R,5S)-4-Acetamido-5-({[2-(3-chlorobenzyl)hydrazino]carbonyl}amino)-3-(3-pentanyloxy)-1-cyclohexene-1-carboxylic acid	C22 H31 Cl N4 O5	4.814	[M-H]-1
126	Daumone-3	C15 H26 O6	5.742	[M-H+HAc]-1
127	(2S)-1-{[3-(3-Chlorophenyl)-1-methyl-1H-pyrazol-4-yl]methyl}-2-isopropyl-4-methylpiperazine	C19 H27 Cl N4	9.42	[M-H]-1
128	Surugapyrrole A	C9 H12 N2 O4	1.28	[M-H]-1
129	Diphenol glucuronide	C12 H14 O8	2.552	[M-H]-1
130	F-11334-B1	C11 H14 O3	6.102	[M+H]+1
131	(–)-4-O-(4-O-β-D-glucopyranosylcaffeoyl)quinic acid	C22 H28 O14	2.388	[M-H]-1
132	Methyl [3-(2-methyl-1,3-dioxolan-2-yl)-4-oxo-2-azetidinyl]acetate	C10 H15 N O5	1.059	[M+NH4]+1
133	(±)-Isoalternatine A	C14 H17 N O3	6.108	[M+H]+1
134	(6RS,10RS)-6,10-dimethylbicyclo[4.4.0]dec-1-en-3-one	C12 H18 O	3.298	[M+H-H2O]+1
135	Esculin	C15 H16 O9	2.369	[M-H]-1
136	Molybdenite	Mo S2	0.906	[M-H]-1
137	Carbofuran, 3OH-	C12 H15 N O4	1.417	[M-H]-1
138	Octadecyl hydrogen sulfate	C18 H38 O4 S	12.358	[M-H]-1
139	Phenyl (1R,2S,4R)-5,6-bis(4-hydroxyphenyl)-7-oxabicyclo[2.2.1]hept-5-ene-2-sulfonate	C24 H20 O6 S	3.628	[M-H]-1
140	Diethyl (3-oxopropyl)phosphonate	C7 H15 O4 P	4.114	[M+H]+1
141	OOB-PE	C27 H50 N O9 P	10.062	[M-H]-1
142	Pantothenic acid	C9 H17 N O5	1.641	[M-H]-1
143	ditrimethylolpropane	C12 H26 O5	4.315	[M+Na]+1

Table 3. Total Phenolic, Flavonoid, and Antioxidant Content of *Centella asiatica* extract

Parameter	Result
Total Phenolic Content	70.3063 mg GAE/g extract
Total Flavonoid Content	13.4891 mg QE/g extract
Antioxidant Activity (IC_{50})	48.45 ppm (Strong)

Note: GAE = Gallic Acid Equivalent; QE = Quercetin Equivalent.

Table 4. Effect of *Centella asiatica* extract on Serum Cytokine Levels in CCl_4-Induced Rats

Group	TNF-α (pg/mL)	IL-6 (pg/mL)	CRP (pg/mL)
Normal control	57.38 ± 15.34*	64.00 ± 35.51*	275.0 ± 95.0
CCl_4 control	131.38 ± 47.16	313.60 ± 157.04	418.0 ± 94.3
Centella asiatica 100 mg/kgBW	81.12 ± 59.87	208.00 ± 82.21	323.0 ± 52.6
Centella asiatica 200 mg/kgBW	42.12 ± 13.75*	127.60 ± 97.99*	261.0 ± 113.4*
Centella asiatica 300 mg/kgBW	52.88 ± 15.02*	121.60 ± 70.07*	261.0 ± 95.7*

Data are expressed as mean ± SD (n = 5). *Significant differences compared with the CCl_4 control group were observed at $p \leq 0.05$ (ANOVA followed by Tukey's post hoc test).

Table 5. Effect of Centella asiatica extract on Liver Function Biomarkers in CCl_4-Induced Rats

Group	Albumin (g/dL)	Bilirubin (mg/dL)	SGOT (U/L)	SGPT (U/L)
Normal control	2.96 ± 0.43*	0.094 ± 0.023*	116.0 ± 31.75*	72.4 ± 3.77*
CCl_4 control	2.20 ± 0.30	0.198 ± 0.033	166.8 ± 14.53	90.4 ± 16.54
Centella asiatica 100 mg/kgBW	2.80 ± 0.30	0.156 ± 0.026	137.6 ± 13.99	71.2 ± 12.99*
Centella asiatica 200 mg/kgBW	3.00 ± 0.52*	0.124 ± 0.021*	143.0 ± 9.92	66.6 ± 12.5*
Centella asiatica 300 mg/kgBW	2.90 ± 0.50*	0.102 ± 0.040*	122.4 ± 29.35*	69.75 ± 6.85*

Data are expressed as mean ± SD (n = 5). *Significant differences compared with the CCl_4 control group were observed at $p \leq 0.05$ (ANOVA followed by Tukey's post hoc test).

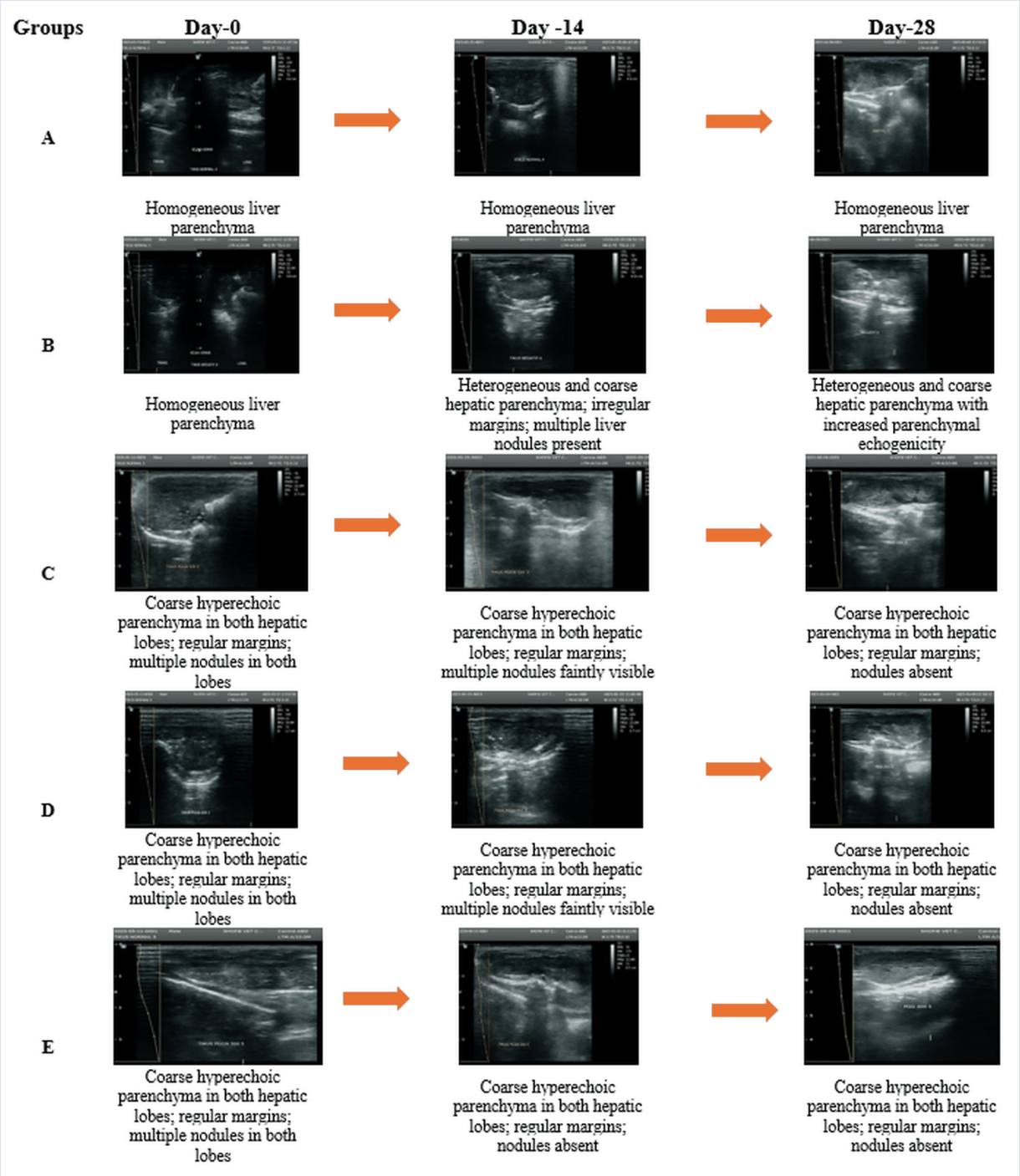

Figure 1. Ultrasonographic Evaluation. Group A = Normal control; Group B = Carbon tetrachloride control; Group C = *C. asiatica* 100 mg/kg; Group D = *C. asiatica* 200 mg/kg; Group E = *C. asiatica* 300 mg/kg.

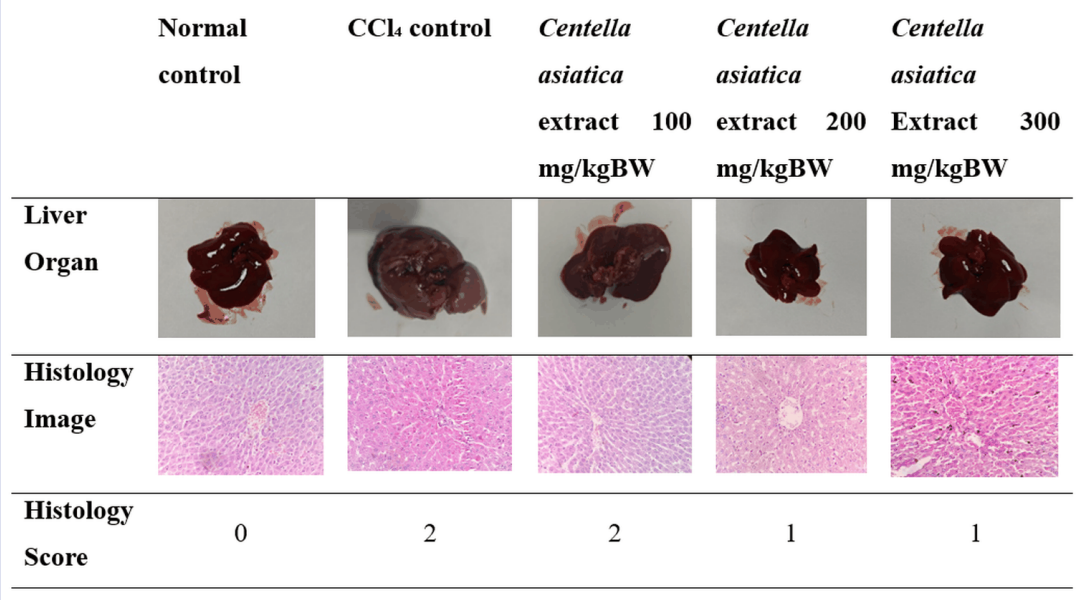

Figure 2. Histological Analysis of Liver Tissue in CCl_4-Induced Rats Treated with *Centella asiatica* extract

liver enzyme and cytokine levels observed in treated groups, confirming the extract's hepatoprotective potential through antioxidative and anti-inflammatory pathways.

Serum Cytokine Levels

Analysis of serum cytokine profiles provided insight into the anti-inflammatory potential of *Centella asiatica* extract against carbon tetrachloride–induced hepatic injury. As shown in Table 4, administration of carbon tetrachloride resulted in a significant increase in tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and C-reactive protein (CRP) levels compared with the normal control group, indicating marked hepatic inflammation and systemic inflammatory response. Elevated TNF-α and IL-6 levels are typical indicators of hepatocellular damage and cytokine-mediated oxidative stress, while CRP acts as a downstream acute-phase protein reflecting inflammation severity[34].

Treatment with *C. asiatica* extract produced a dose-dependent reduction in inflammatory cytokines. The 200 mg/kg and 300 mg/kg groups showed significant decreases ($p \leq 0.05$) in TNF-α (42.12 ± 13.75 pg/mL and 52.88 ± 15.02 pg/mL, respectively) and IL-6 (127.60 ± 97.99 pg/mL and 121.60 ± 70.07 pg/mL) compared to the carbon tetrachloride control group (131.38 ± 47.16 pg/mL and 313.60 ± 157.04 pg/mL, respectively). CRP levels also declined notably, supporting the systemic anti-inflammatory effect of the extract. The cytokine suppression observed in this study indicates that *C. asiatica* mitigates hepatic inflammation by modulating pro-inflammatory signaling pathways, particularly by inhibiting the activation of NF-κB and downregulating the release of pro-inflammatory mediators[35].

These results are consistent with earlier findings by Choi et al. (2023), who reported that triterpenoids such as madecassoside in *C. asiatica* inhibit cytokine overexpression and protect hepatocytes from oxidative and inflammatory injury[36]. Moreover, Lv et al. (2017) demonstrated that *C. asiatica* extracts decreased TNF-α and IL-6 levels while restoring antioxidant enzyme activity in toxin-induced liver damage[37]. The dual antioxidant and anti-inflammatory actions of the extract likely contribute synergistically to its hepatoprotective efficacy observed in biochemical and ultrasonographic analyses. Furthermore,the hepatoprotective effects observed in this study are consistent with previous reports showing that *Centella asiatica* reduces oxidative stress and inflammation in experimental liver injury models. Mechanistically, these effects may be explained by activation of the Nrf2 signaling pathway, which enhances cellular antioxidant defense through increased expression of detoxifying and antioxidant enzymes[38]. In parallel, inhibition of the NF-κB pathway may suppress the excessive inflammatory response associated with CCl_4-induced hepatotoxicity, thereby reducing cytokine production and tissue damage. These mechanisms together provide a plausible explanation for the biochemical, ultrasonographic, and histopathological improvements observed in the present study.

Liver Function Biomarkers

The assessment of serum biochemical parameters further substantiated the hepatoprotective role of *Centella asiatica* extract in carbon tetrachloride–induced hepatotoxicity. As shown in Table 5, carbon tetrachloride administration caused a marked decline in serum albumin levels (2.20 ± 0.30 g/dL) and a significant increase in bilirubin, serum glutamic oxaloacetic transaminase (SGOT), and serum glutamic pyruvic transaminase (SGPT) levels compared to the normal control group. These alterations indicate impaired liver function, hepatocellular leakage, and reduced protein synthesis capacity, all of which are typical outcomes of oxidative and inflammatory injury to hepatic cells.

Treatment with *C. asiatica* extract significantly restored biochemical parameters toward normal values in a dose-dependent manner. The 200 mg/kg and 300 mg/kg groups exhibited the most substantial hepatoprotective effects, as evidenced by a significant increase in albumin levels (3.00 ± 0.52 g/dL and 2.90 ± 0.50 g/dL, respectively) and a reduction in bilirubin levels (0.124 ± 0.021 mg/dL and 0.102 ± 0.040 mg/dL, respectively). Additionally, both doses produced notable decreases in SGOT (143.0 ± 9.92 U/L and 122.4 ± 29.35 U/L) and SGPT (66.6 ± 12.5 U/L and 69.75 ± 6.85 U/L), indicating reduced hepatocellular enzyme leakage and improved membrane integrity.

These biochemical improvements correspond with the ultrasonographic and histopathological findings, which demonstrated structural recovery of the liver parenchyma. The observed hepatoprotective effect can be attributed to the triterpenoid and flavonoid constituents of *C. asiatica*, particularly asiaticoside, madecassoside, and quercetin,

which stabilize cell membranes, enhance antioxidant enzyme activity, and suppress lipid peroxidation and it significantly decreased serum transaminase levels and improved liver function markers in toxin-induced hepatotoxic models[36].

Histopathological Findings

Histopathological examination further confirmed the hepatoprotective effects of *Centella asiatica* extract in rats exposed to carbon tetrachloride–induced liver injury. As illustrated in Figure 2, the normal control group displayed a typical hepatic architecture with polygonal hepatocytes, well-defined nuclei, and intact sinusoidal structures, corresponding to a histology score of 0.

The normal control group exhibited a well-preserved hepatic architecture characterized by uniform polygonal hepatocytes, distinct nuclei, and intact sinusoidal spaces, indicating normal liver function (histology score = 0). In contrast, the carbon tetrachloride control group showed marked structural alterations, including hepatocellular vacuolization, ballooning degeneration, and focal necrosis, accompanied by dense infiltration of inflammatory cells around the central vein. These findings (histology score = 2) are typical manifestations of oxidative stress–induced hepatotoxicity, resulting from the metabolic activation of carbon tetrachloride into reactive free radicals that cause lipid peroxidation and membrane disruption.

Administration of *Centella asiatica* extract significantly ameliorated these histopathological lesions in a dose-dependent manner. At 100 mg/kg BW, mild hepatocellular degeneration and limited inflammatory infiltration persisted (score = 2). However, treatment with 200 and 300 mg/kg BW resulted in near-normal lobular architecture, characterized by restored hepatic cords, uniform cytoplasm, and minimal inflammatory reaction (score = 1). The presence of healthy hepatocytes with prominent nuclei and clear cytoplasmic boundaries suggests active hepatocyte regeneration and recovery of functional liver tissue.

These observations align with the biochemical and cytokine results (Tables 4 and 5), which demonstrated reduced serum transaminases, bilirubin, and inflammatory cytokines (TNF-α, IL-6, CRP) in *C. asiatica*–treated groups. The hepatoprotective effects can be attributed to the synergistic action of triterpenoids such as asiatic acid, madecassoside, and asiaticoside, which are known to enhance antioxidant enzyme activity, stabilize cellular membranes, and inhibit NF-κB–mediated inflammatory pathways. Choi et al. (2016) demonstrated its potential in restoring hepatic histoarchitecture through enhanced tissue repair and decreased lipid peroxidation[38].

CONCLUSION

This study demonstrated that *Centella asiatica* herb extract possesses strong hepatoprotective potential against carbon tetrachloride–induced liver injury in Wistar rats. The extract contains bioactive compounds such as flavonoids, phenolics, and triterpenoids, contributing to its potent antioxidant (IC_{50} = 48.45 ppm) and anti-inflammatory activities. Ultrasonographic and histopathological analyses confirmed significant hepatic improvement at 200 and 300 mg/kg BW, marked by reduced echogenicity, disappearance of nodules, and lower histopathological scores. These effects were accompanied by decreased pro-inflammatory cytokines (TNF-α, IL-6, CRP) and improvements in liver function biomarkers, including increased albumin and decreased bilirubin, SGOT, and SGPT levels. Overall, *Centella asiatica* extract effectively protected and restored hepatic structure and function, indicating its potential as a natural hepatoprotective agent for mitigating oxidative and inflammatory liver damage.

ACKNOWLEDGMENTS

The authors express gratitude to the Faculty of Medicine, Dentistry, and Health Sciences, Universitas Prima Indonesia, for providing research facilities and laboratory support.

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Cite this article: Nishamol K S, Sundarrajan T. Integration of Analytical and Computational Techniques for Bioactive Profiling of *Celtis timorensis* Span Bark. Pharmacogn J. 2025;17(6): 760-769.