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Original Article

Ultrasonographic and Biochemical Evaluation of the Hepatoprotective Effect of *Cinnamomum burmannii* Bark Extract in Carbon Tetrachloride–Induced Liver Injury

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ABSTRACT

This study aimed to evaluate the hepatoprotective activity of ethanolic extract of cinnamon (*Cinnamomum burmannii*) in male Wistar rats induced with carbon tetrachloride (CCl_4). Cinnamon extract is known to contain bioactive compounds such as flavonoids and polyphenols, which play significant roles in antioxidant and anti-inflammatory mechanisms. Phytochemical analysis revealed that the extract contained total phenolic content of 71.55 mg GAE/g and flavonoid content of 0.41 mg QE/g, with a potent antioxidant activity indicated by an IC_{50} value of 18.19 ppm. Administration of the extract for 28 days at a dose of 300 mg/kg body weight resulted in a significant reduction ($P<0.05$) in pro-inflammatory cytokines TNF-α, IL-6, and CRP levels compared to the negative control group. The 300 mg/kg dose showed the highest efficacy, with TNF-α levels approaching those of the normal group. Furthermore, liver function parameters improved, as evidenced by significant reductions in SGOT and SGPT enzyme levels, an increase in serum albumin (2.96 ± 0.52 g/dL), and a decrease in serum bilirubin to 0.102 ± 0.040 mg/dL. Ultrasonographic examination showed improved liver parenchymal homogeneity and a reduction in the number of nodules. Histopathological findings revealed a decrease in liver tissue damage score from moderate to mild. These findings suggest that *Cinnamomum burmannii* extract has potential hepatoprotective effects through anti-inflammatory, antioxidant, and hepatocellular recovery mechanisms. Therefore, this extract holds promise as a phytopharmaceutical candidate for complementary therapy in liver function disorders; however, further studies are required to isolate the active compounds and evaluate long-term toxicity.

Keywords: *Cinnamon,* hepatoprotective, anti-inflammatory, antioxidant, histopathology, cytokine

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INTRODUCTION

Liver disease remains a major global health burden, contributing significantly to morbidity and mortality worldwide. According to the World Health Organization (WHO), liver-related disorders account for more than 2 million deaths annually, representing approximately 4% of all global deaths[1]. The most common forms include chronic hepatitis, alcoholic liver disease, non-alcoholic fatty liver disease (NAFLD), and cirrhosis, with rising prevalence due to increasing alcohol consumption, sedentary lifestyles, obesity, and exposure to environmental toxins[2]. In Southeast Asia, including Indonesia, the incidence of liver dysfunction continues to grow, often linked to poor dietary habits, hepatotoxic drug exposure, and limited access to early diagnostic interventions[3]. The progression from mild hepatic injury to advanced fibrosis and cirrhosis poses a serious clinical challenge, as most cases are diagnosed at late stages when treatment options are limited and irreversible damage has occurred[4]. Therefore, the development of effective preventive and therapeutic strategies particularly, those that mitigate oxidative stress and inflammation has become a crucial focus in hepatology research.

The liver is a vital organ responsible for the metabolism, detoxification, and homeostatic regulation of various physiological processes[5]. It plays a central role in converting nutrients, eliminating xenobiotics, and maintaining systemic biochemical balance[6]. However, continuous exposure to hepatotoxic agents including chemical compounds, pharmaceuticals, alcohol, and industrial pollutants can induce progressive hepatic injury and impair cellular integrity. Among these hepatotoxins, carbon tetrachloride (CCl_4) is widely utilized in experimental studies as a classic model for inducing liver injury due to its well-defined mechanism of toxicity[7]. This compound undergoes bioactivation via cytochrome P450 2E1, generating trichloromethyl (CCl_3•) and trichloromethyl peroxy (CCl_3OO•) radicals that initiate lipid peroxidation, disrupt cellular membranes, and promote necrosis and apoptosis of hepatocytes[8].

The pathological progression of carbon tetrachloride–induced hepatotoxicity involves oxidative stress and inflammatory cascades, which collectively exacerbate cellular injury[9]. Free radical formation leads to depletion of endogenous antioxidants such as superoxide dismutase and catalase, while activation of Kupffer cells triggers the release of pro-inflammatory cytokines, including tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and C-reactive protein (CRP[10]. These mediators further contribute to hepatic necroinflammation and fibrotic remodeling[11]. Consequently, therapeutic interventions that attenuate oxidative stress, modulate cytokine activity, and restore hepatocellular function have garnered increasing attention as viable strategies for hepatoprotection.

Plant-based therapies are recognized as a promising approach due to their phytochemical diversity and pharmacological safety profile. Among these,

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Cinnamomum burmannii (Indonesian cinnamon) has gained interest for its potent antioxidant and anti-inflammatory properties. Its bark contains a rich composition of cinnamaldehyde, eugenol, coumarins, flavonoids, and phenolic acids, which collectively scavenge free radicals, inhibit nuclear factor-kappa B (NF-κB) signaling, and stabilize hepatocyte membranes[12,13]. Previous studies have shown that *C. burmannii* extract mitigates oxidative stress and lowers serum hepatic enzyme levels in High fat-induced liver injury models, indicating its potential as a natural hepatoprotective agent[14].

While conventional biochemical markers such as serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), albumin, and bilirubin remain critical for evaluating hepatic function, they provide limited insight into the structural and morphological recovery of liver tissue[15]. In this context, ultrasonography (USG) has emerged as a valuable non-invasive imaging tool that enables real-time evaluation of hepatic echogenicity, parenchymal texture, and nodularity, providing complementary evidence of tissue regeneration during therapeutic interventions[16].

Although *Cinnamomum burmannii* is known for its antioxidant properties, its hepatoprotective potential has not been comprehensively evaluated using combined ultrasonographic, cytokine, and histopathological analyses in a carbon tetrachloride–induced model. This study provides the first integrated assessment linking liver morphology, inflammatory signaling, and biochemical recovery following treatment with *C. burmannii* extract.

MATERIALS AND METHODS

Study Design and Location

This research adopted an in vivo experimental approach conducted at the Research Laboratory of the Faculty of Medicine, Universitas Prima Indonesia, from April to June 2025. The primary objective was to evaluate the hepatoprotective potential of *Cinnamomum burmannii* bark extract in Wistar rats exposed to carbon tetrachloride–induced hepatic injury. To elucidate the extract's protective and therapeutic actions, a comprehensive assessment was performed through biochemical profiling, cytokine quantification, ultrasonographic examination, and histopathological evaluation.

Preparation of Extract

The *Cinnamomum burmannii* samples utilized in this investigation were obtained from local cultivators in Telagah Village, Langkat Regency, Indonesia. A total of 300 grams of dried plant powder underwent maceration with 70% ethanol (70:30, v/v) at 25°C under continuous agitation to facilitate efficient extraction. The mixture was allowed to stand for 24 hours and subsequently filtered, with the process repeated three times to optimize the extraction yield. All filtrates were then combined and centrifuged at 3500 rpm for 10 minutes to eliminate residual plant debris. The supernatant was carefully evaporated at 38°C, yielding a concentrated hydroethanolic extract of C. burmannii, which was stored at 4°C until use[17].

Phytochemical and Antioxidant Analysis

The extract was subjected to phytochemical screening to detect the presence of polyphenols, flavonoids, alkaloids, saponins, and tannins, following standard qualitative procedures[18]. To further elucidate its chemical composition, liquid chromatography–high-resolution mass spectrometry (LC–HRMS) analysis was conducted for identification and confirmation of the extract's bioactive constituents. The total phenolic content was quantified using the Folin–Ciocalteu method, and results were expressed as milligrams of gallic acid equivalents (mg GAE/g extract). Similarly, the total flavonoid content was determined through the aluminum chloride colorimetric method, expressed as milligrams of quercetin equivalents (mg QE/g extract)[19]. The antioxidant potential of the extract was assessed using the DPPH radical scavenging assay, with the IC_{50} value representing the concentration required to inhibit 50% of DPPH radicals calculated to indicate its free radical–neutralizing capacity[20].

Animals

A total of twenty-five healthy male Wistar rats, aged 12–16 weeks and weighing between 150–200 g, were employed in this study. Prior to the initiation of experimental procedures, all animals were acclimatized for seven days under controlled laboratory conditions, maintaining a temperature of 22–24°C, relative humidity of 50–60%, and a 12-hour light/dark photoperiod. During the acclimatization and treatment phases, the rats were provided with a standard laboratory chow and unrestricted access to water (ad libitum) to preserve normal physiological conditions before the induction of liver injury.

Experimental Design

The twenty-five Wistar rats were randomly allocated into five experimental groups (n = 5) in accordance with Federer's formula, ensuring sufficient statistical validity. The experimental design was structured as follows:

1. Normal control group: received no treatment.
2. Carbon tetrachloride (CCl_4) control group: administered CCl_4 at a dose of 3 mL/kg body weight via intraperitoneal injection, twice weekly for 28 days.
3. CCl_4 + *Cinnamomum burmannii* extract (100 mg/kg BW): extract administered orally once daily.
4. CCl_4 + *Cinnamomum burmannii* extract (200 mg/kg BW): extract administered orally once daily.
5. CCl_4 + *Cinnamomum burmannii* extract (300 mg/kg BW): extract administered orally once daily.

Administration of the *Cinnamomum burmannii* extract was initiated concurrently with carbon tetrachloride (CCl_4) exposure and continued orally once daily for 28 consecutive days. On day 29, blood samples were collected from the orbital sinus under light anesthesia for biochemical and cytokine analyses. Ultrasonographic examination of the liver was subsequently performed to assess morphological and structural alterations in hepatic tissue.

Ultrasonographic Examination

Liver ultrasonography was performed weekly on days 0, 7, 14, 21, and 28 using a Chison ECO 2 portable ultrasound system fitted with a 10 MHz mini-convex transducer. Prior to imaging, each rat was anesthetized with ketamine, and the abdominal area was shaved to facilitate optimal probe contact and enhance image resolution. The liver was visualized through the subcostal approach, and parameters including echogenicity, parenchymal homogeneity, and surface contour were systematically assessed to observe hepatic structural alterations and the extent of recovery throughout the treatment period[21]. All ultrasonographic examinations and image interpretations were performed by the same experienced operator who was blinded to the treatment groups. This procedure was applied to minimize observer bias and to ensure consistency of image acquisition and interpretation throughout the study.

Biochemical and Cytokine Assays

Serum samples were prepared by centrifuging whole blood at 3000 rpm for 15 minutes, after which the supernatant was carefully collected for subsequent biochemical and cytokine analyses. The concentrations of albumin, total bilirubin, SGOT (serum glutamic oxaloacetic

transaminase), and SGPT (serum glutamic pyruvic transaminase) were measured spectrophotometrically in accordance with standard clinical chemistry procedures[22]. The levels of tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and C-reactive protein (CRP) were determined using commercial ELISA kits (Solarbio®, China), following the manufacturer's protocols. These biomarkers served as critical indicators of hepatic integrity and systemic inflammation, enabling a comprehensive assessment of the hepatoprotective potential of the *Cinnamomum burmannii* extract.

Histopathological Evaluation

Liver specimens were fixed in 10% neutral-buffered formalin for 24 hours to preserve cellular and tissue architecture. The fixed samples were then dehydrated through a graded series of ethanol, cleared with xylene, and embedded in paraffin wax. Tissue sections approximately 5 µm thick were obtained using a rotary microtome and subsequently stained with hematoxylin and eosin (H&E) for histological observation[23]. The prepared slides were microscopically examined to identify histopathological alterations, such as hepatocellular necrosis, fatty degeneration, sinusoidal dilation, and inflammatory cell infiltration, thereby determining the severity of hepatic damage and the degree of tissue recovery following treatment[24].

Statistical Analysis

All quantitative data were expressed as mean ± standard deviation (SD). Statistical analyses were conducted using a one-way analysis of variance (ANOVA) to evaluate differences among experimental groups, followed by Tukey's post hoc test for pairwise comparisons. A p-value of less than 0.05 ($p < 0.05$) was considered statistically significant, denoting a meaningful difference between the treatment and control groups.

Ethical Clearance

All experimental procedures were carried out in strict accordance with ethical standards and received approval from the Animal Research Ethics Committee of Universitas Prima Indonesia (Approval No. 061/KEPK/UNPRI/III/2025). The study adhered to both institutional and international guidelines for the care and use of laboratory animals, ensuring the humane and ethical treatment of all subjects throughout the experimental period. Appropriate measures were implemented to minimize stress, pain, and discomfort during animal handling, administration of treatments, and sample collection.

RESULTS AND DISCUSSION

Phytochemical Composition and LC–HRMS Profile

As presented in Table 1, the ethanolic extract of *Cinnamomum burmannii* bark demonstrated the presence of several major classes of bioactive compounds, including alkaloids, flavonoids, triterpenoids/steroids, glycosides, saponins, and tannins. The detection of these phytochemical groups confirms that the extract contains multiple secondary metabolites responsible for its broad pharmacological properties, particularly antioxidant and hepatoprotective activities.

Table 1. Phytochemical compound group of *Cinnamomum burmannii* 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	$FeCl_3$	+

The presence of flavonoids and polyphenols is particularly important, as they inhibit lipid peroxidation and enhance antioxidant enzymes such as SOD and catalase[25]. Similar findings were reported by Niknezhad et al. (2019), who demonstrated that *Cinnamomum* genus extracts reduced oxidative stress and improved liver histology[26]. Overall, the diverse phytochemical profile supports the strong antioxidant and hepatoprotective potential of *C. burmannii* observed in biochemical and histopathological evaluations.

Additionally, as shown in Table 2, LC–HRMS profiling revealed that *Cinnamomum burmannii* bark extract contains a diverse range of phytochemicals, including flavonoids (quercitrin, catechin, epicatechin, rutin, kaempferol), phenolic acids (caffeic, ferulic, syringic, chlorogenic), and aromatic aldehydes and terpenoids (cinnamaldehyde, eugenol acetate, beta-caryophyllene, 1,8-cineole). These compounds are crucial for hepatoprotection due to their synergistic antioxidant and anti-inflammatory properties[27,28].

For instance, catechin and epicatechin enhance the activities of superoxide dismutase (SOD) and catalase, thereby mitigating oxidative stress and lipid peroxidation in hepatocytes[29]. Cinnamaldehyde, a major volatile compound, exhibits membrane-stabilizing and detoxifying effects, reducing hepatocyte necrosis and inflammation. Similarly, chlorogenic and caffeic acids have been shown to suppress TNF-α and IL-6 expression, preventing cytokine-mediated injury[30]. These results align with Zhang et al. (2022), who reported that cinnamon polyphenols restored hepatic enzyme balance and reduced histopathological damage in paracetamol-induced rats[31].

Total Phenolic, Flavonoid, and Antioxidant Activity

The antioxidant profile summarized in Table 3 confirms that the *Cinnamomum burmannii* extract exhibits a high phenolic content (71.55 mg GAE/g) and moderate flavonoid concentration (0.41 mg QE/g), accompanied by a very strong antioxidant activity (IC_{50} = 18.19 ppm). These findings suggest that phenolic compounds play a predominant role in the extract's antioxidant capacity, functioning as hydrogen donors and free radical scavengers. Similar trends were reported by Zhao et al. (2021), who found that high polyphenol levels in Cinnamomum species correlated strongly with inhibition of lipid peroxidation and protection against inflammation[32]. Thus, the potent antioxidant potential of *C. burmannii* supports its proposed hepatoprotective mechanism through oxidative stress modulation.

Ultrasonographic Evaluation

The ultrasonographic findings, as presented in Figure 1 and described in Table 4, clearly demonstrate the hepatoprotective efficacy of *Cinnamomum burmannii* bark extract in carbon tetrachloride–induced liver injury. In the negative control group, progressive hepatic damage was evident, characterized by inhomogeneous parenchymal texture, increased echogenicity, rough and irregular margins, and the appearance of multiple hyperechoic nodules. These sonographic features are consistent with fatty degeneration, fibrosis, and hepatocellular necrosis resulting from oxidative stress and inflammation induced by carbon tetrachloride metabolism via cytochrome P450 enzymes[33].

Conversely, the groups treated with *C. burmannii* extract, particularly at 200 mg/kg BW and 300 mg/kg BW, showed significant improvement in liver structure. The parenchymal texture became more homogeneous, margins appeared smoother, and the number and thickness of nodules decreased markedly. By the 28th day, rats receiving 300 mg/kg BW exhibited nearly normal echogenicity and hepatic architecture, indicating regeneration of hepatocytes and restoration of tissue integrity.

Table 2. LC–HRMS Identification of Phytochemical Compounds in *Cinnamomum burmannii* extract

No.	Compounds	Molecular Formula	Retention Time (min)	Reference Ion
1	Procyanidin dimer. A type	$C_{30}H_{24}O_{12}$	11.13	[M−H]
2	Beta-caryophyllene	C26H9O3	5.84	[M−H]
3	Quercitrin (quercetin-3-rhamnoside)	$C_{21}H_{20}O_{11}$	20.87	[M−H]
4	Procyanidin dimer. A type	$C_{30}H_{24}O_{12}$	7.48	[M−H]
5	1.8-Cineole	C7H28O3	12.82	[M+H]
6	Syringic acid	$C_9H_{10}O_5$	7.68	[M−H]
7	3.4-Dihydroxybenzaldehyde (Protocatechualdehyde)	$C_7H_6O_3$	4.08	[M−H]
8	Procyanidin dimer. A type	$C_{30}H_{24}O_{12}$	8.74	[M−H]
9	Cinnamaldehyde derivative	C23H9O11	4.21	[M−H]
10	Caryophyllene oxide	C26H28O10	18.62	[M+H]
11	Pinene derivative	C15H13O8	5.42	[M−H]
12	Protocatechuic acid	$C_7H_6O_4$	3.66	[M−H]
13	Alpha-terpineol	C11H29O3	4.68	[M−H]
14	Cinnamic acid glucoside	C23H11O7	6.56	[M+H]
15	Rutin	$C_{27}H_{30}O_{16}$	19.73	[M−H]
16	Epicatechin	$C_{15}H_{14}O_6$	8.96	[M−H]
17	Coumarin	$C_9H_6O_2$	14.69	[M+H]
18	Camphor derivative	C27H24O4	13.1	[M−H]
19	Catechin	$C_{15}H_{14}O_6$	6.64	[M−H]
20	Safrole derivative	C25H34O12	18.16	[M+H]
21	Procyanidin B2	$C_{30}H_{26}O_{12}$	9.05	[M−H]
22	Cinnamic acid	$C_9H_8O_2$	17	[M−H]
23	Methyl cinnamate	C21H11O6	6.19	[M+H]
24	Eugenol acetate	C8H22O9	7.07	[M+H]
25	Cinnamyl alcohol	C25H39O9	13.91	[M−H]
26	Coumarin derivative	C10H8O7	17.46	[M−H]
27	Caffeic acid derivative	C10H23O11	7.94	[M+H]
28	Linalool oxide	C27H15O5	11.23	[M+H]
29	Bornyl acetate	C10H39O10	12.85	[M−H]
30	Vanillin derivative	C7H30O10	20.94	[M+H]
31	Ferulic acid	C6H36O1	7.44	[M+H]
32	Kaempferol-3-O-rutinoside	C11H8O11	5.72	[M−H]
33	Quercetin-3-O-glucoside	C13H19O6	12.5	[M+H]
34	Gallic acid derivative	C16H10O5	3.59	[M+H]
35	Apigenin derivative	C13H23O1	17.05	[M−H]
36	Rosmarinic acid	C24H7O2	11.07	[M−H]
37	Catechin gallate	C25H21O6	4.04	[M−H]
38	Epicatechin	C17H20O12	11.84	[M−H]
39	Procyanidin B1	C26H37O6	19.98	[M+H]
40	Procyanidin C1	C13H40O4	5.93	[M+H]
41	Rutin	C30H14O7	9.89	[M+H]
42	Naringenin	C27H35O3	19.94	[M−H]
43	Taxifolin	C27H23O5	5.35	[M−H]
44	Chlorogenic acid	C9H16O12	6.93	[M+H]
45	Myricetin	C6H23O5	15.36	[M−H]
46	Isorhamnetin	C8H31O12	8.35	[M+H]
47	Hesperidin	C13H24O5	10.45	[M−H]
48	Diosmin	C8H7O1	11.64	[M+H]
49	Luteolin	C17H33O5	10.08	[M+H]
50	Genistein	C29H34O11	15.66	[M−H]
51	Formononetin	C11H7O3	16.56	[M+H]
52	Glycitein	C7H19O10	20.37	[M−H]
53	Pinoresinol	C10H22O1	4.22	[M+H]
54	Lariciresinol	C6H29O10	5.6	[M−H]
55	Secoisolariciresinol	C22H11O11	18.16	[M+H]
56	Matairesinol	C29H6O7	12.32	[M−H]
57	Cinnamoyl glucose	C16H39O4	9	[M−H]
58	Cinnamoyl quinic acid	C8H25O9	19.72	[M+H]
59	Coumaroyl shikimate	C27H35O12	6.61	[M−H]
60	Sinapic acid	C15H28O4	4.96	[M−H]
61	Syringic acid derivative	C18H9O7	16.92	[M+H]
62	Ellagic acid	C18H23O8	10.04	[M+H]
63	Hydroxycinnamic acid	C19H6O7	12.7	[M+H]

Table 3. Total Phenolic, Flavonoid, and Antioxidant Content of *Cinnamomum burmannii* extract

Parameter	Result
Total Phenolic Content	71.5530 mg GAE/g extract
Total Flavonoid Content	0.4097 mg QE/g extract
Antioxidant Activity (IC_{50})	18.19 ppm (Very Strong)

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

Table 4. Description for Ultrasonographic Evaluation

Group	Day-0	Day-7	Day-14	Day-21	Day-28
Normal	Homogeneous parenchymal texture, smooth surface, no visible nodules.	Homogeneous parenchymal texture, smooth surface, no visible nodules.	Homogeneous parenchymal texture, smooth surface, no visible nodules.	Homogeneous parenchymal texture, smooth surface, no visible nodules.	Homogeneous parenchymal texture, smooth surface, no visible nodules.
Negative Control	Homogeneous parenchymal texture, smooth surface, no visible nodules.	Inhomogeneous parenchymal texture, rough surface, multiple hyperechoic nodules visible.	Inhomogeneous parenchymal texture, rough surface, irregular margin, multiple hyperechoic nodules visible.	Inhomogeneous parenchymal texture, rough surface, irregular margin, multiple hyperechoic nodules visible.	Inhomogeneous parenchymal texture, rough surface, irregular margin, increased parenchymal echogenicity, multiple hyperechoic nodules visible.
Cinnamon Extract 100 mg/kg BW	Homogeneous parenchymal texture, smooth surface, no visible nodules.	Inhomogeneous parenchymal texture, rough surface, increased parenchymal echogenicity, multiple nodules visible.	Inhomogeneous parenchymal texture, rough surface, increased parenchymal echogenicity, multiple nodules visible.	Inhomogeneous parenchymal texture, rough surface, increased echogenicity, multiple nodules visible.	Inhomogeneous parenchymal texture, rough surface, increased parenchymal echogenicity, multiple nodules visible.
Cinnamon Extract 200 mg/kg BW	Homogeneous parenchymal texture, smooth surface, no visible nodules.	Inhomogeneous parenchymal texture, rough surface, increased parenchymal echogenicity, multiple nodules visible.	Inhomogeneous parenchymal texture, rough surface, increased parenchymal echogenicity, multiple nodules visible.	Inhomogeneous parenchymal texture, rough surface, increased parenchymal echogenicity, multiple nodules visible.	Inhomogeneous parenchymal texture, rough surface, increased parenchymal echogenicity, multiple nodules visible.
Cinnamon Extract 300 mg/kg BW	Homogeneous parenchymal texture, smooth surface, no visible nodules.	Inhomogeneous parenchymal texture, rough surface, increased parenchymal echogenicity, multiple hyperechoic nodules visible.	Inhomogeneous parenchymal texture, rough surface, increased parenchymal echogenicity, multiple hyperechoic nodules visible.	Inhomogeneous parenchymal texture, relatively smooth surface, increased parenchymal echogenicity, multiple hyperechoic nodules visible.	Inhomogeneous parenchymal texture, relatively smooth surface, increased parenchymal echogenicity, thin hyperechoic nodules visible.

Table 5. Effect of *Cinnamomum burmannii* extract on Serum Cytokine Levels in CCl_4-Induced Rats

Group	TNF-α (pg/mL)	IL-6 (pg/mL)	CRP (pg/mL)
Normal control	44.88 ± 8.81*	60.4 ± 20.77*	209.0 ± 38.3*
CCl_4 control	166.13 ± 17.88	334.8 ± 75.28	497.0 ± 124.2
Cinnamomum burmannii 100 mg/kgBW	150.63 ± 29.31	180.4 ± 86.45*	377.0 ± 140.3
Cinnamomum burmannii 200 mg/kgBW	152.38 ± 25.47	166.4 ± 39.79*	291.0 ± 74.2*
Cinnamomum burmannii 300 mg/kgBW	68.38 ± 16.70*	186.4 ± 72.02*	251.0 ± 66.0*

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 6. Effect of *Cinnamomum burmannii* 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	3.06 ± 0.42*	0.15 ± 0.03*	128.4 ± 23.86*	65.2 ± 11.52*
CCl_4 control	1.68 ± 0.15	0.18 ± 0.05	233.6 ± 22.51	123.0 ± 29.56
Cinnamomum burmannii 100 mg/kgBW	2.62 ± 0.50*	0.172 ± 0.05	167.4 ± 21.00*	86.0 ± 32.27
Cinnamomum burmannii 200 mg/kgBW	1.98 ± 0.46	0.178 ± 0.10*	177.2 ± 31.30*	88.4 ± 19.71
Cinnamomum burmannii 300 mg/kgBW	2.64 ± 0.42*	0.092 ± 0.04*	150.6 ± 62.74*	63.6 ± 24.49*

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).

These results are in agreement with Cao et al. (2023) and George et al, (2013), who demonstrated that the polyphenolic constituents of *Cinnamomum* species, such as cinnamaldehyde, catechin, and procyanidin, play crucial roles in reducing lipid peroxidation, stabilizing cell membranes, and attenuating inflammation[34,35]. Therefore, the observed ultrasonographic recovery supports the hepatoprotective potential of *C. burmannii*, attributed to its potent antioxidant and anti-inflammatory bioactive compounds.

Serum Cytokine Levels

The serum cytokine profile presented in Table 5 and Figure 2 demonstrates a clear anti-inflammatory effect of *Cinnamomum burmannii* extract in rats exposed to carbon tetrachloride–induced hepatotoxicity. The CCl_4 control group exhibited a marked elevation in pro-inflammatory cytokines TNF-α (166.13 ± 17.88 pg/mL), IL-6 (334.8 ± 75.28 pg/mL), and CRP (497.0 ± 124.2 pg/mL) reflecting severe

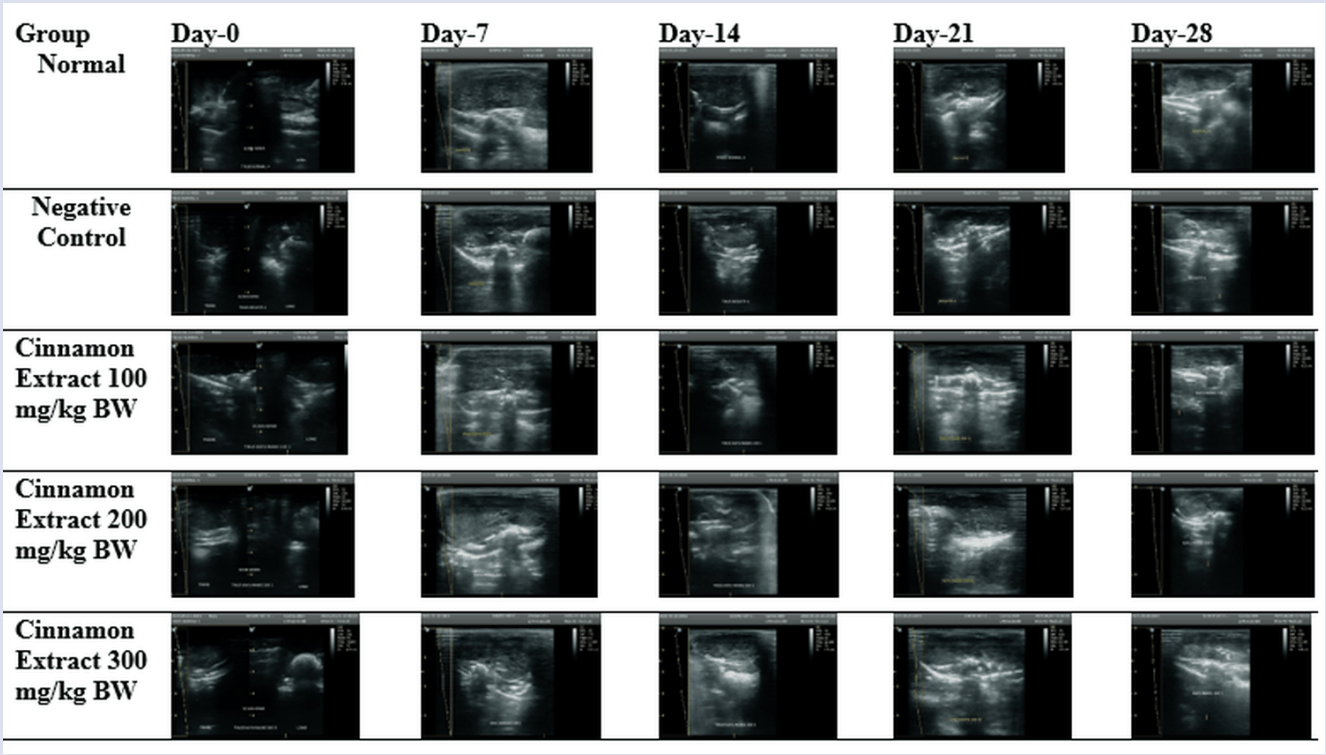

Figure 1. Ultrasonographic Evaluation. Group A = Normal control; Group B = Carbon tetrachloride control; Group C = *C. asiatica* 100 mg/kg; Group D = *C. asiatica* 200 mg/kg; Group E = *C. asiatica* 300 mg/kg.

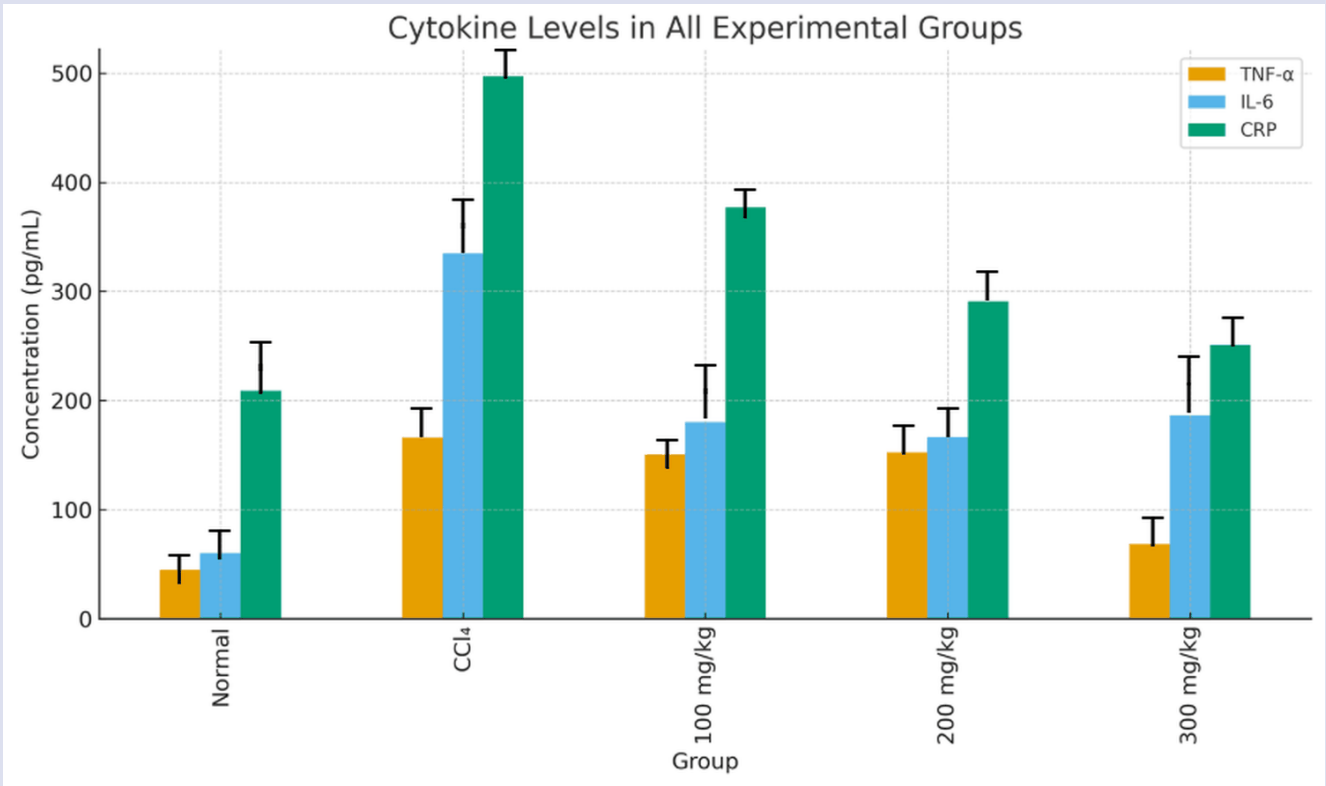

Figure 2. Cytokine levels in all experimental groups

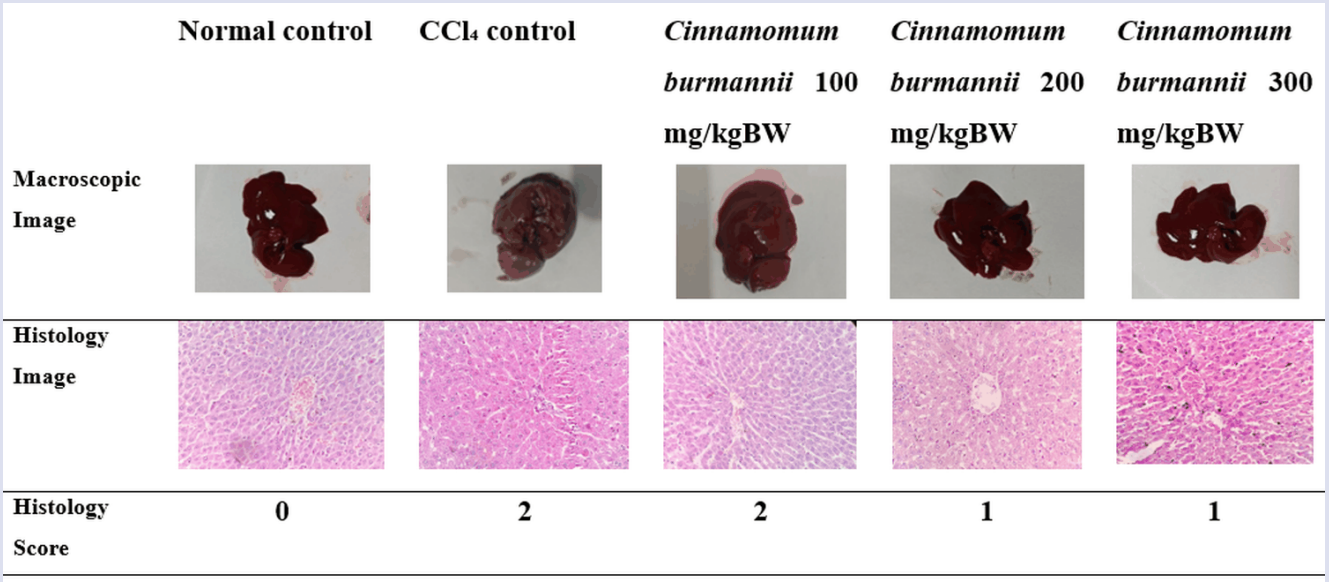

Figure 3. Histological Analysis of Liver Tissue in CCl_4-Induced Rats Treated with *Cinnamomum burmannii* extract

hepatic inflammation and oxidative stress. In contrast, treatment with C. burmannii extract significantly reduced these cytokines in a dose-dependent manner, with the 300 mg/kg BW group showing the most pronounced decrease, approaching values of the normal control.

This attenuation of cytokine expression suggests that *C. burmannii* exerts its anti-inflammatory effects primarily through inhibition of the NF-κB signaling pathway and suppression of reactive oxygen species (ROS)-mediated hepatocyte injury. By downregulating TNF-α and IL-6, the extract helps restore the balance between pro- and anti-inflammatory mediators, thereby preventing hepatocellular apoptosis and necrosis. These effects are consistent with previous findings by Alahdal et al. (2025), who reported that cinnamaldehyde, eugenol, and polyphenols derived from *Cinnamomum* species significantly reduce cytokine production and protect hepatic tissues from toxin-induced injury[36].

Liver Function Biomarkers

As summarized in Table 6, treatment with *Cinnamomum burmannii* extract resulted in a remarkable improvement in liver function biomarkers in rats subjected to carbon tetrachloride (CCl_4)–induced hepatotoxicity. The CCl_4 control group exhibited significant biochemical disturbances, characterized by decreased serum albumin and increased bilirubin, SGOT, and SGPT levels findings consistent with extensive hepatocellular injury and reduced synthetic capacity of the liver. These alterations are classical indicators of hepatic dysfunction caused by oxidative stress and lipid peroxidation induced by CCl_4 metabolism[37].

Rats treated with *C. burmannii* extract showed dose-dependent restoration of these biomarkers. The 300 mg/kg BW group demonstrated the most notable improvements, with albumin levels increasing to 2.64 ± 0.42 g/dL, while bilirubin levels declined to 0.092 ± 0.04 mg/dL. Similarly, serum SGOT and SGPT levels decreased to 150.6 ± 62.74 U/L and 63.6 ± 24.49 U/L, respectively, approaching the normal control values. In term of lowering percentage compared with the CCl_4 control group, *Cinnamomum burmannii* extract produced marked percentage improvements in liver function biomarkers in a dose-dependent manner. At 100 mg/kg BW, serum albumin increased by 56.0%, while SGOT and SGPT decreased by 28.3% and 30.1%, respectively. The 200 mg/kg BW group exhibited a modest increase in albumin (17.9%) with reductions of 24.2% in SGOT and 28.2% in SGPT. The most pronounced effects were observed at 300 mg/kg BW, where albumin increased by 57.1%, while bilirubin decreased by 48.9%, SGOT by 35.5%, and SGPT by 48.3% relative to the CCl_4 group. These findings indicate substantial restoration of hepatic synthetic function and membrane integrity, particularly at the highest dose.

The recovery in albumin and transaminase levels implies enhanced hepatocyte integrity and improved protein synthesis, both of which are essential markers of functional liver recovery. The strong antioxidant potential of *C. burmannii*, attributed to its rich content of cinnamaldehyde, eugenol, and polyphenols, likely plays a critical role in neutralizing reactive oxygen species (ROS) and preventing further hepatocyte necrosis[38]. Together, these results affirm that *C. burmannii* possesses significant hepatoprotective activity by stabilizing cellular membranes, restoring enzymatic balance, and supporting hepatic tissue regeneration through antioxidant and anti-inflammatory pathways.

Histopathological Findings

As shown in Figure 3, histopathological examination supported the biochemical findings, demonstrating significant protective effects of *Cinnamomum burmannii* extract against carbon tetrachloride (CCl_4)–induced hepatic injury. The normal control group exhibited well-preserved hepatic lobular architecture, with polygonal hepatocytes, distinct nuclei, and intact sinusoidal spaces, corresponding to a histological score of 0.

In contrast, the CCl_4 control group showed extensive hepatocellular degeneration, vacuolation, fatty infiltration, and focal necrosis, particularly around the central vein, consistent with a histological score of 2, indicating moderate to severe hepatic damage. These changes reflect oxidative stress–induced cytotoxicity and inflammation triggered by the metabolic activation of CCl_4 into trichloromethyl radicals.

Rats treated with C. burmannii extract exhibited dose-dependent histological recovery. The 100 mg/kg BW group displayed mild hepatocellular degeneration (score 2), while the 200 mg/kg and 300 mg/kg BW groups showed near-normal hepatic morphology with minimal inflammatory infiltration and preserved hepatic cords (score 1). The reduction in necrotic and degenerative areas suggests enhanced cellular regeneration and membrane stabilization.

The observed hepatocellular regeneration is likely associated with the antioxidant and anti-inflammatory properties of *C. burmannii*, particularly due to compounds such as cinnamaldehyde, eugenol, catechins, and procyanidins. These bioactive constituents neutralize reactive oxygen species (ROS), inhibit lipid peroxidation, and stabilize hepatocyte membranes, facilitating tissue repair. Similar findings were reported by Gao et al. (2025) who demonstrated that *Cinnamaldehyde* promote hepatocyte regeneration and normalize histoarchitecture in toxin-induced liver injury[39]. Thus, the combination of morphological recovery and improved cellular integrity confirms the strong hepatoprotective efficacy of *C. burmannii* extract.

CONCLUSION

The ethanolic extract of *Cinnamomum burmannii* bark demonstrated strong hepatoprotective effects against carbon tetrachloride–induced liver injury in rats. The extract contained high levels of phenolics (71.55 mg GAE/g) and flavonoids (0.41 mg QE/g) with potent antioxidant activity (IC_{50} = 18.19 ppm). Treatment, especially at 300 mg/kg, significantly reduced TNF-α, IL-6, CRP, SGOT, and SGPT levels while increasing albumin and decreasing bilirubin, indicating restored liver function. Ultrasonographic and histopathological findings showed improved hepatic architecture, reduced nodularity, and mild tissue damage. These results suggest that *C. burmannii* possesses strong antioxidant and anti-inflammatory properties and may serve as a promising natural hepatoprotective agent for preventing and managing liver dysfunction.

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