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Original Article

Hepatoprotective Potential of *Coffea arabica* Extract Against Carbon Tetrachloride Induced Liver Damage: Ultrasonographic and Cytokine Evaluation

Desi Novianti[1]*, I Nyoman Ehrich Lister[2], Ali Napiah Nasution[2]

ABSTRACT

Carbon tetrachloride (CCl_4) is a hepatotoxic agent widely used to model liver injury in experimental animals, and coffee (*Coffea arabica*) extract is known to contain various bioactive compounds with antioxidant and anti-inflammatory potential. This study aimed to evaluate the hepatoprotective activity of *Coffea arabica* extract in male Wistar rats induced with CCl_4 through phytochemical analysis, liver biochemical parameters (albumin, bilirubin, SGOT, SGPT), inflammatory cytokines (TNF-α, IL-6, CRP), and liver morphology observations using ultrasonography and histopathology. The extract contained alkaloids, flavonoids, triterpenoids, saponins, and glycosides, with total phenolic and flavonoid contents of 51.32 mg GAE/g and 1.25 mg QE/g extract, respectively, and moderate antioxidant activity (IC_{50} = 124.36 ppm). Administration of the extract at 300 mg/kg BW significantly improved liver structure, reduced TNF-α (120.62 ± 21.78 pg/mL), IL-6 (68 ± 14.44 pg/mL), CRP (199.0 ± 41.5 pg/mL), SGOT (147.0 ± 34.28 g/dL), and SGPT (69.6 ± 9.94 g/dL) levels, while increasing albumin (2.94 ± 0.34 g/dL) and reducing bilirubin (0.09 ± 0.04 mg/dL), approaching normal conditions. The 300 mg/kg BW dose showed the most optimal protective effect compared to other treatment groups, as evidenced by improvements in biochemical parameters, liver morphology, and hepatic histology, indicating that *Coffea arabica* extract has strong potential as an effective hepatoprotective agent against CCl_4-induced liver damage.

Keywords: *Coffea arabica*, hepatotoxicity, CCl_4, cytokines, liver protection, Ultrasonographic.

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INTRODUCTION

Liver is one of the most metabolically active organs in the human body, playing a central role in detoxification, biochemical synthesis, and regulation of homeostasis[1]. It is responsible for carbohydrate, protein, and lipid metabolism, bile production, and the biotransformation of xenobiotics[2]. Because of this wide array of functions, the liver is continuously exposed to toxic insults from environmental chemicals, drugs, alcohol, and metabolic by-products. Prolonged exposure to hepatotoxic substances can lead to oxidative stress, inflammation, fibrosis, and ultimately cirrhosis or hepatic failure[3]. Globally, liver diseases account for nearly two million deaths annually, highlighting the urgent need for effective preventive and therapeutic interventions[4]. Despite significant advances in modern pharmacotherapy, the search for safe and natural hepatoprotective agents remains a major research focus in hepatology and pharmacognosy.

Among hepatotoxins, carbon tetrachloride (CCl_4) has been widely used as a model compound to induce experimental liver injury. Its metabolism by cytochrome P450 enzymes generates trichloromethyl ($CCl_3\bullet$) and trichloromethyl peroxy ($CCl_3OO\bullet$) radicals, which initiate lipid peroxidation and damage cellular membranes[5]. This oxidative cascade disrupts mitochondrial integrity, promotes hepatocyte necrosis, and triggers the release of intracellular enzymes such as serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT)[6]. Concurrently, the inflammatory process is activated through increased secretion of pro-inflammatory cytokines, including tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and C-reactive protein (CRP)[7]. Persistent elevation of these mediators amplifies tissue injury and contributes to the pathogenesis of chronic liver disorders. Because the mechanisms of CCl_4 toxicity closely resemble those of human hepatotoxicity, this model remains one of the most reliable experimental tools to investigate hepatoprotective compounds.

A study has highlighted the pivotal role of oxidative stress and inflammation as interconnected processes in liver pathology[8]. Excessive reactive oxygen species (ROS) not only attack membrane lipids and proteins but also activate nuclear factor-kappa B (NF-κB) signaling, which regulates the transcription of inflammatory cytokines[9]. Therefore, natural compounds with strong antioxidant and anti-inflammatory properties can interrupt this vicious cycle and prevent the progression of hepatic damage[10]. Several plant-derived bioactives such as curcumin from *Curcuma longa*, and glycyrrhizin from *Glycyrrhiza glabra* have demonstrated promising hepatoprotective effects[11,12]. However, exploring new and locally available botanical resources remains essential to expand the pharmacological repertoire of natural liver protectants.

One such potential source is coffee (*Coffea arabica*), a globally consumed beverage that has gained attention for its diverse pharmacological benefits beyond its stimulating effect. Coffee beans are rich in polyphenolic compounds such as chlorogenic

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acid, caffeic acid, ferulic acid, trigonelline, and caffeine, which have demonstrated antioxidant, anti-inflammatory, and antifibrotic properties in various in vitro and in vivo studies[13]. Chlorogenic acid, for instance, scavenges free radicals and enhances endogenous antioxidant defenses by upregulating enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPx)[14]. Caffeine has been reported to modulate lipid metabolism and inhibit the activation of hepatic stellate cells, thereby reducing fibrosis[15]. Epidemiological studies further suggest that moderate coffee consumption correlates with a lower incidence of chronic liver diseases, non-alcoholic fatty liver disease, and hepatocellular carcinoma. Nonetheless, experimental validation of these findings through mechanistic and imaging-based analyses remains limited.

Traditional biochemical and histopathological assessments have long been used to evaluate liver injury. However, these methods alone cannot fully represent the structural and functional dynamics of the organ. The integration of ultrasonographic (USG) analysis offers a non-invasive, real-time imaging technique to assess hepatic morphology, echotexture, and vascular changes[16]. In experimental hepatotoxicity, ultrasonography provides a valuable adjunct to histopathology by enabling longitudinal observation of liver size, surface smoothness, and parenchymal echogenicity. When combined with cytokine profiling and biochemical assays, USG evaluation allows for a comprehensive assessment of hepatoprotection, bridging laboratory research with potential clinical translation.

Given these scientific premises, exploring the hepatoprotective potential of *Coffea arabica* extract through multimodal evaluation could provide new insight into its therapeutic applications. While previous studies have reported coffee's antioxidant and anti-inflammatory activities, few have examined its hepatoprotective efficacy using integrated cytokine and ultrasonographic analyses in an established CCl_4-induced liver injury model. Such a multidisciplinary approach is crucial to validate not only biochemical recovery but also structural regeneration of hepatic tissue.

Therefore, the present study aims to evaluate the hepatoprotective potential of *Coffea arabica* extract against carbon tetrachloride–induced liver damage in Wistar rats. The study investigates multiple parameters, including serum liver enzymes (SGOT, SGPT), albumin, bilirubin, and inflammatory cytokines (TNF-α, IL-6, CRP), alongside ultrasonographic and histopathological assessments. By integrating molecular and imaging evidence, this research seeks to elucidate the underlying protective mechanisms of coffee extract and establish its role as a potential natural hepatoprotective agent. The findings are expected to contribute to the growing body of evidence supporting the pharmacological and therapeutic significance of *Coffea arabica* in the management of chemically induced hepatic disorders.

MATERIALS AND METHODS

Study Design and Location

This research was an in vivo experimental study conducted at the Research Laboratory, Faculty of Medicine, Universitas Prima Indonesia, from April to June 2025. The purpose was to evaluate the hepatoprotective potential of *Coffea arabica* extract against carbon tetrachloride (CCl_4)-induced liver injury in Wistar rats through biochemical, cytokine, ultrasonographic, and histopathological evaluations.

Preparation of Extract

Arabica Gayo coffee beans (*Coffea arabica* L.) were obtained from a certified supplier in Medan, Indonesia. Three hundred grams of dried coffee powder were macerated three times using 70% ethanol (70:30, v/v) at 25°C for 24 hours each with continuous stirring. The filtrate was centrifuged at 3500 rpm for 10 minutes and concentrated at 38°C to yield a hydroethanolic extract[17].

Phytochemical and Antioxidant Analysis

The extract was analyzed for polyphenols, flavonoids, alkaloids, saponins, and tannins using standard phytochemical screening and LC-HRMS was used to determine the phytochemical contain in extract. LC–HRMS analysis was conducted using a Vanquish™ Horizon UHPLC system coupled to an Orbitrap Exploris™ 240 mass spectrometer (Thermo Scientific, USA) with a Hypersil GOLD C18 column (100 × 2.1 mm, 1.9 µm). The mobile phase consisted of water and acetonitrile (each containing 0.1% formic acid) at a flow rate of 0.3 mL/min. Data were acquired in both positive and negative ESI modes over a mass range of m/z 100–1500 with an injection volume of 5 µL[18]. Total phenolic content of the Coffea arabica extract was determined using the Folin–Ciocalteu method with gallic acid as the reference standard, and the results were expressed as mg gallic acid equivalents (GAE) per gram of extract. Total flavonoid content was quantified using the aluminum chloride colorimetric method with quercetin as the calibration standard and expressed as mg quercetin equivalents (QE) per gram of extract. Calibration curves were constructed for both assays using serial dilutions of the reference compounds, and all measurements were performed in triplicate to ensure analytical accuracy[19]. Antioxidant capacity was assessed by the DPPH assay, and IC_{50} values were calculated to represent 50% radical inhibition[20].

Animals

Twenty-five healthy male Wistar rats (12–16 weeks old, 150–200 g) were used. The rats were acclimatized for seven days under controlled conditions (temperature 22–24°C, humidity 50–60%, and 12-hour light/dark cycle) and provided a standard diet and water ad libitum.

Experimental Design

The rats were randomly divided into five groups (n = 5) based on Federer's:

1. Normal control (no treatment)
2. CCl_4 control (3 mL/kg BW intraperitoneally twice weekly for 28 days)
3. CCl_4 + *Coffea arabica* extract 100 mg/kg BW orally
4. CCl_4 + *Coffea arabica* extract 200 mg/kg BW orally
5. CCl_4 + *Coffea arabica* extract 300 mg/kg BW orally

On day 29, blood was collected from the orbital sinus for biochemical and cytokine analysis, and liver imaging was performed.

Ultrasonographic Examination

Liver ultrasonography was performed weekly (days 0, 7, 14, 21, and 28) using a Chison ECO 2 Portable ultrasound device with a mini-convex 10 MHz probe. The rats were anesthetized with ketamine, and the abdominal fur was shaved. The liver was scanned from the subcostal region to observe echogenicity, parenchymal homogeneity, and surface smoothness[21]. Assessments of ultrasonographic analysis were performed by observers who were blinded to the experimental group assignments to reduce observer bias.

Biochemical and Cytokine Assays

Serum was separated by centrifugation at 3000 rpm for 15 minutes. Albumin, bilirubin, SGOT, and SGPT were measured spectrophotometrically using standard methods[22]. TNF-α, IL-6, and CRP levels were determined using ELISA kits (Solarbio®, China).

These biomarkers were used to assess hepatic function and systemic inflammation.

Histopathological Evaluation

Liver tissues were fixed in 10% neutral buffered formalin for 24 hours, dehydrated through graded ethanol, cleared with xylene, and embedded in paraffin. Sections of 5 µm were stained with hematoxylin and eosin (H&E) and examined for necrosis, fatty degeneration, sinusoidal dilation, and inflammatory infiltration[23]. Histopathological examination was performed by a certified pathologist who was blinded to the treatment groups.

Statistical Analysis

Data were expressed as mean ± standard deviation (SD) and analyzed using one-way ANOVA followed by Tukey's post hoc test. A p-value < 0.05 was considered statistically significant.

Ethical Clearance

All experimental procedures were approved by the Animal Research Ethics Committee of Universitas Prima Indonesia under approval number 052/KEPK/UNPRI/III/2025. All protocols adhered to institutional and international ethical standards for the care and use of laboratory animals, and efforts were made to minimize animal discomfort.

RESULTS AND DISCUSSION

Phytochemical Composition and LC–HRMS Profile

Phytochemical screening of *Coffea arabica* extract (Table 1) revealed the presence of alkaloids, flavonoids, triterpenoids/steroids, glycosides, and saponins, while tannins were absent. These classes of secondary metabolites are widely associated with antioxidant and anti-inflammatory activities that contribute to hepatoprotection. Alkaloids and flavonoids play crucial roles in maintaining cellular integrity through free-radical scavenging and modulation of inflammatory mediators[24]. Triterpenoids strengthen hepatocyte membranes and promote tissue regeneration, while glycosides and saponins enhance antioxidant defenses and facilitate cellular repair[25].

The LC–HRMS analysis (Table 2) identified 121 bioactive compounds, including chlorogenic, caffeic, ferulic, *p*-coumaric, and gallic acids phenolic constituents previously reported to suppress lipid peroxidation and oxidative stress in hepatocytes. Similarly, abundant flavonoids such as quercetin, rutin, luteolin, and kaempferol have been shown to downregulate proinflammatory cytokines (TNF-α, IL-6) and stabilize hepatocyte membranes, consistent with findings in earlier studies on *Coffea arabica* and other polyphenol-rich plants[26]. Caffeine, theobromine, and trigonelline major alkaloids identified contribute antioxidant and regenerative effects[27], while volatile terpenoids like β-caryophyllene strengthen anti-inflammatory activity via NF-κB pathway inhibition[28].

Table 1. Phytochemical compound group of *Coffea arabica* 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

Quantitative phytochemical assays revealed that *Coffea arabica* extract contained a total phenolic content of 51.32 mg GAE/g and a total flavonoid content of 1.25 mg QE/g extract, with a DPPH IC_{50} value of 124.36 ppm, classified as moderate antioxidant activity (Table 3), while Quercetin showed a strong antioxidant activity with an IC_{50} value of 6.42 ppm, confirming the reliability and sensitivity of the assay. Phenolic and flavonoid compounds are key bioactive constituents responsible for neutralizing reactive oxygen species (ROS), chelating transition metals, and enhancing endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx)[29]. The moderate IC_{50} value suggests that the extract's antioxidant potential arises from the synergistic interactions among multiple polyphenolic constituents rather than from a single dominant compound.

The observed antioxidant activity is consistent with earlier studies on coffee-derived polyphenols. Chlorogenic acid, caffeic acid, and ferulic acid major phenolic acids identified in *Coffea arabica* have been shown to inhibit lipid peroxidation and scavenge hydroxyl and superoxide radicals, thereby reducing oxidative burden in hepatocytes[30]. Similarly, flavonoids such as quercetin, rutin, and luteolin exert both direct and indirect antioxidant effects by modulating the Nrf2 signaling pathway, leading to upregulation of antioxidant response element (ARE)-driven genes.

Ultrasonographic Evaluation

Ultrasonographic examination of rat livers demonstrated a clear and progressive hepatoprotective response following treatment with *Coffea arabica* extract against CCl_4-induced liver injury (Figure 1). The normal control group (A) consistently exhibited homogeneous hepatic parenchyma with smooth, well-defined margins and uniform echotexture throughout the study period, indicating normal hepatic morphology. In contrast, the CCl_4 control group (B) showed coarse and heterogeneous parenchyma with irregular margins and multiple nodular hyperechoic areas, which are characteristic of fibrosis and early cirrhotic transformation. These alterations confirm the successful induction of chronic hepatocellular damage by CCl_4, a known hepatotoxin that generates free radicals, disrupts membrane lipids, and promotes necroinflammatory changes in hepatic tissue.

Treatment with *Coffea arabica* extract showed marked improvement in liver architecture in a dose-dependent manner. In the 100 mg/kg BW group (C), a mild increase in parenchymal echogenicity was observed on day 7, indicating ongoing hepatic repair; by day 21, the echotexture had normalized, suggesting partial restoration of hepatocyte integrity. The 200 mg/kg BW group (D) exhibited the most substantial and consistent improvement, maintaining homogeneous parenchyma and smooth hepatic contours by day 28, closely resembling the normal control group. Interestingly, the 300 mg/kg BW group (E) displayed transient mild heterogeneity on day 21, which returned to a uniform appearance by day 28, suggesting a hormetic response at higher concentrations an adaptive physiological adjustment before full recovery.

These ultrasonographic findings are consistent with the biochemical and histopathological results reported in this study, where *Coffea arabica* extract significantly reduced serum SGOT, SGPT, bilirubin, and inflammatory cytokines (TNF-α, IL-6, CRP), while restoring albumin levels. Previous studies also support these outcomes, indicating that phenolic and flavonoid compounds in *Coffea arabica*, particularly chlorogenic and caffeic acids, attenuate hepatic fibrosis by suppressing oxidative stress and downregulating the NF-κB and TGF-β1 pathways[31]. Similar ultrasonographic improvements were documented in experimental models using polyphenol-rich extracts

Table 2. LC–HRMS Identification of Phytochemical Compounds in *Coffea arabica* Extract

No.	Compounds	Molecular Formula	Retention Time (min)	Reference Ion
1	Epicatechin	C7H34O9	7.5	[M+H]
2	Chlorogenic acid	C12H36O12	15.02	[M−H]
3	Sinapic acid	C8H26O3	8.76	[M−H]
4	Caffeic acid	C25H10O11	12.2	[M+H]
5	Naringenin	C26H20O11	6.57	[M−H]
6	Quercetin	C18H16O9	7.41	[M+H]
7	Theobromine	C8H12O7	20.95	[M+H]
8	Caffeine	C20H21O5	17.94	[M+H]
9	Quinic acid	C29H25O10	18	[M+H]
10	Catechin	C26H38O2	15.14	[M−H]
11	Vanillic acid	C24H33O8	6.71	[M−H]
12	Rutin	C8H32O5	18.48	[M−H]
13	Gallic acid	C10H15O12	5.35	[M+H]
14	Theophylline	C29H17O4	19.65	[M+H]
15	Kaempferol	C16H21O7	4.94	[M−H]
16	p-Coumaric acid	C14H20O9	18.59	[M−H]
17	Ferulic acid	C13H11O5	15.28	[M−H]
18	Trigonelline	C24H13O5	3.36	[M+H]
19	Syringic acid	C19H31O2	16.67	[M+H]
20	5-Caffeoylquinic acid	C27H17O7	18.14	[M−H]
21	5-Feruloylquinic acid	C8H23O6	10.48	[M+H]
22	3-Caffeoylquinic acid	C20H28O9	20.63	[M−H]
23	4-Caffeoylquinic acid	C19H18O8	16.07	[M+H]
24	3.5-Dicaffeoylquinic acid	C7H9O2	9.09	[M−H]
25	4.5-Dicaffeoylquinic acid	C12H34O10	5.88	[M−H]
26	Neochlorogenic acid	C13H20O2	8.82	[M+H]
27	Isochlorogenic acid A	C11H8O11	6.16	[M−H]
28	Isochlorogenic acid B	C26H22O8	3.29	[M+H]
29	Isochlorogenic acid C	C13H27O6	2.76	[M+H]
30	Protocatechuic acid	C19H8O4	9.59	[M−H]
31	Dihydrocaffeic acid	C11H8O2	11.63	[M−H]
32	p-Hydroxybenzoic acid	C14H32O10	19.21	[M+H]
33	1.3-Dicaffeoylquinic acid	C30H24O10	16.14	[M+H]
34	3.4-Dicaffeoylquinic acid	C7H39O2	10.12	[M+H]
35	1-Caffeoylquinic acid	C11H19O8	13.27	[M−H]
36	3-Feruloylquinic acid	C7H35O3	11.39	[M−H]
37	4-Feruloylquinic acid	C11H32O10	4.82	[M+H]
38	1-Feruloylquinic acid	C26H26O3	7.94	[M−H]
39	3.4-Di-O-caffeoylquinic acid	C9H20O6	6.44	[M+H]
40	Caffeoylshikimic acid	C13H24O6	6.55	[M+H]
41	Scopoletin	C19H31O8	19.3	[M+H]
42	Luteolin	C8H18O8	5.37	[M−H]
43	Apigenin	C20H32O11	10.5	[M+H]
44	Hesperetin	C25H16O3	16.96	[M−H]
45	Spathulenol	C7H22O10	3.33	[M+H]
46	β-Caryophyllene	C14H33O7	16.8	[M−H]
47	α-Humulene	C13H18O12	9.09	[M−H]
48	1.8-Cineole	C29H28O2	20.96	[M+H]
49	Camphor	C20H9O6	4.35	[M+H]
50	Linalool	C22H20O9	4.39	[M−H]
51	α-Terpineol	C8H18O12	20.66	[M+H]
52	Germacrene D	C25H23O5	16.06	[M−H]
53	Hexanal	C28H13O9	7.49	[M−H]
54	Nonanal	C14H24O5	11.88	[M+H]
55	Octanal	C9H39O9	4.21	[M+H]
56	Dodecane	C14H17O5	2.72	[M−H]
57	Tridecane	C13H14O10	7.46	[M+H]
58	Tetradecane	C17H10O12	5.16	[M−H]
59	Pentadecane	C22H14O12	3.34	[M−H]
60	Heptadecane	C14H10O11	19.11	[M+H]

61	Phytol	C24H38O3	9.05	[M−H]
62	Linoleic acid	C10H20O6	19.88	[M−H]
63	Oleic acid	C27H19O12	20.5	[M−H]
64	Palmitic acid	C10H21O10	7.75	[M+H]
65	Stearic acid	C11H22O3	12.92	[M−H]
66	Lauric acid	C10H20O5	3.09	[M−H]
67	Myristic acid	C20H33O3	13.25	[M−H]
68	Capric acid	C30H32O4	14.32	[M+H]
69	Caprylic acid	C12H21O10	16.28	[M+H]
70	Acetophenone	C26H25O11	15.56	[M+H]
71	Benzaldehyde	C28H22O11	7.19	[M−H]
72	Phenylacetaldehyde	C24H13O11	12.5	[M+H]
73	Methyl salicylate	C8H33O5	17.07	[M+H]
74	Salicylic acid	C9H9O11	9.4	[M−H]
75	Hydroxytyrosol	C26H36O4	5.6	[M−H]
76	Tyrosol	C28H15O5	19.46	[M+H]
77	Vanillin	C12H23O5	4.62	[M−H]
78	Syringaldehyde	C22H39O2	3.72	[M−H]
79	Guaiacol	C14H29O5	16.53	[M+H]
80	Eugenol	C16H11O5	12.42	[M+H]
81	Isoeugenol	C17H12O7	11.43	[M+H]
82	Cinnamic acid	C25H17O9	9.3	[M+H]
83	Cinnamyl alcohol	C10H15O4	11.9	[M+H]
84	Anethole	C16H21O7	14.38	[M−H]
85	Estragole	C8H29O12	11.54	[M+H]
86	Furfural	C30H37O11	19.14	[M−H]
87	2-Furylmethanol	C7H9O11	6.9	[M−H]
88	Chavicol	C22H40O12	7.37	[M+H]
89	Thymol	C24H20O6	12.75	[M+H]
90	Carvacrol	C28H13O10	2.84	[M+H]
91	Methyleugenol	C16H23O2	5.51	[M−H]
92	Rosmarinic acid	C25H18O11	3.26	[M−H]
93	Verbascoside	C17H15O9	16.69	[M+H]
94	Acteoside	C15H21O9	11.39	[M−H]
95	Geniposidic acid	C17H32O6	3.42	[M−H]
96	Loganin	C23H34O3	12.85	[M−H]
97	Secologanin	C29H17O12	3.17	[M+H]
98	Swertiamarin	C10H31O7	15.56	[M−H]
99	Oleuropein	C29H32O12	12.5	[M+H]
100	Verbascoside	C18H38O10	8.19	[M+H]
101	Linarin	C7H24O2	18.71	[M+H]
102	Vitexin	C11H19O4	10.2	[M+H]
103	Isovitexin	C23H39O2	8.9	[M+H]
104	Orientin	C30H10O8	5.05	[M−H]
105	Isoorientin	C14H9O8	11.12	[M+H]
106	Apigenin-7-O-glucoside	C9H32O5	2.57	[M+H]
107	Luteolin-7-O-glucoside	C25H12O10	18.35	[M+H]
108	Isoschaftoside	C14H36O10	7.49	[M−H]
109	Schaftoside	C23H13O9	17.66	[M−H]
110	Mangiferin	C16H18O2	7.36	[M+H]
111	Neomangiferin	C21H15O8	15.88	[M−H]
112	Harpagide	C28H25O6	6.55	[M−H]
113	Gentiopicroside	C23H26O12	9.08	[M−H]
114	Gentiopicrin	C25H10O6	14.09	[M−H]
115	Cynaroside	C23H11O9	9.19	[M+H]
116	Chrysin	C27H37O5	13.96	[M+H]
117	Acacetin	C26H23O4	13.77	[M−H]
118	Baicalein	C24H12O11	9.02	[M+H]
119	Wogonin	C27H22O9	20.25	[M+H]
120	Umbelliferone	C18H19O4	18.19	[M+H]
121	Esculetin	C14H19O3	4.02	[M−H]

Table 3. Total Phenolic, Flavonoid, and Antioxidant Content of Coffea arabica Extract

Parameter	Result
Total Phenolic Content	51.32 mg GAE/g extract
Total Flavonoid Content	1.25 mg QE/g extract
Antioxidant Activity (IC_{50})	124.36 ppm (Moderate)
Antioxidant Activity (IC_{50}) Quercetin Standard	6.42 ppm

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

Table 4. Effect of *Coffea arabica* Extract on Serum Cytokine Levels in CCl_4-Induced Rats

Group	TNF-α (pg/mL)	IL-6 (pg/mL)	CRP (pg/mL)
Normal control	72,12 ± 27,34 *	61,2 ± 23,21*	163,0 ± 95,2 *
CCl_4 control	182,87±18,23	259,6 ± 35,49	377,0 ± 94,5
Coffea arabica 100 mg/kgBW	141,87± 45,65	138,4 ± 78,63*	345,0 ± 123,0
Coffea arabica 200 mg/kgBW	158,12± 44,13	68,4 ± 12,53*	261,0 ± 69,6
Coffea arabica 300 mg/kgBW	120,62± 21,78*	68 ± 14.44*	199,0 ± 41,5*

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 Coffea arabica 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	2,98 ± 0,36*	0,11 ± 0,02*	144,4 ± 45,89*	67,8 ± 14,22*
CCl_4 control	2,36 ± 0,17	0,196 ± 0,05	202,2 ± 9,73	93,4 ± 9,74
Coffea arabica 100 mg/kgBW	2,86 ± 0,17*	0,148 ± 0,05	129,2 ± 25,07*	70,0 ± 21,46
Coffea arabica 200 mg/kgBW	3,00 ± 0,25*	0,128 ± 0,03	149,6 ± 21,77*	67,6 ± 11,15*
Coffea arabica 300 mg/kgBW	2,94 ± 0,34*	0,09 ± 0,04*	147,0 ± 34,28*	69,6 ± 9,94*

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. Representative ultrasonographic images of rat livers. Group A = Normal control; Group B = CCl_4 control; Group C = Coffea arabica 100 mg/kg BW; Group D = Coffea arabica 200 mg/kg BW; Group E = Coffea arabica 300 mg/kg BW.

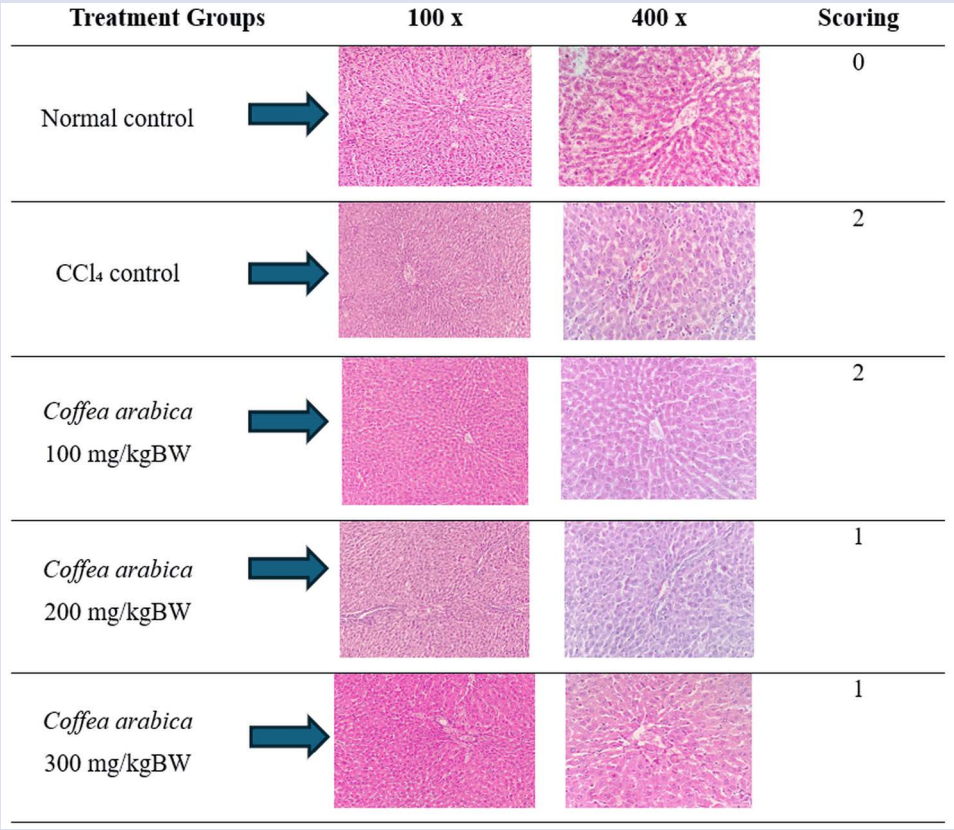

Figure 2. Histological Analysis of Liver Tissue in CCl_4-Induced Rats Treated with *Coffea arabica* Extract. Representative micrographs were captured at ×100 and ×400 magnifications. Score 1 = mild hepatocellular degeneration and minimal inflammatory infiltration; Score 2 = moderate necrosis with inflammatory cell infiltration; and Score 3 = severe necrosis with extensive inflammation and architectural distortion.

such as *Camellia sinensis* and *Curcuma longa*, where reductions in hepatic echogenicity correlated with improved cellular antioxidant status and reduced collagen deposition.

Serum Cytokine Levels

The administration of *Coffea arabica* extract resulted in significant modulation of inflammatory cytokines, as presented in Table 4. The CCl_4 control group exhibited markedly elevated levels of TNF-α (182.87 ± 18.23 pg/mL), IL-6 (259.6 ± 35.49 pg/mL), and CRP (377.0 ± 94.5 pg/mL), confirming that CCl_4 exposure triggers strong oxidative and inflammatory responses leading to hepatocellular injury. These findings align with earlier reports that CCl_4 generates trichloromethyl radicals, initiating lipid peroxidation and stimulating Kupffer cells to release pro-inflammatory cytokines such as TNF-α and IL-6, which subsequently elevate CRP synthesis in hepatocytes[32].

Treatment with *Coffea arabica* extract significantly attenuated these inflammatory responses. The 200 mg/kg BW and 300 mg/kg BW doses notably decreased IL-6 and CRP concentrations ($p \leq 0.05$), while the 300 mg/kg BW group also produced a significant reduction in TNF-α levels compared with the CCl_4 control. These results demonstrate the dose-dependent anti-inflammatory action of *Coffea arabica*, which can be attributed to its abundant phenolic compounds particularly chlorogenic acid, caffeic acid, and quercetin that inhibit NF-κB and MAPK activation, thereby reducing transcription of pro-inflammatory mediators.

The downregulation of TNF-α and IL-6 observed in this study corroborates the hepatoprotective mechanisms reported by Frost-Meyer et al. (2012), who wrote that daily coffee consumption in humans reduced systemic inflammatory markers, including IL-6 and CRP[33]. Similarly, experimental studies by Mohamed et al. (2025) demonstrated that *Coffea arabica* extract modulates cytokine production and enhances antioxidant defense enzymes such as SOD and catalase in hepatotoxic rat models. Furthermore, the decline in CRP concentration reflects systemic recovery and normalization of hepatic function. Previous research indicates that polyphenols in coffee inhibit the acute-phase response by blocking hepatic CRP gene expression and reducing circulating inflammatory proteins[35]. These combined effects reinforce the synergistic role of phenolic acids, flavonoids, and alkaloids in *Coffea arabica* that act through antioxidant and immunomodulatory pathways to protect hepatocytes from CCl_4-induced injury.

Liver Function Biomarkers

The biochemical evaluation of liver function markers revealed significant improvements following treatment with *Coffea arabica* extract in CCl_4-induced hepatotoxic rats (Table 5). The CCl_4 control group demonstrated a typical hepatotoxic profile, characterized by decreased albumin (2.36 ± 0.17 g/dL) and elevated bilirubin (0.196 ± 0.05 mg/dL), SGOT (202.2 ± 9.73 U/L), and SGPT (93.4 ± 9.74 U/L) levels, reflecting hepatocellular necrosis and impaired protein synthesis. These findings are consistent with the well-established mechanism of CCl_4-induced hepatic injury, in which trichloromethyl radicals (CCl_3•) cause lipid peroxidation, mitochondrial dysfunction, and leakage of cytosolic enzymes into the bloodstream[32].

Administration of *Coffea arabica* extract at all tested doses restored these parameters toward normal levels. The 200 mg/kg BW and 300

mg/kg BW groups showed the most significant hepatoprotective effects, with albumin levels rising to 3.00 ± 0.25 g/dL and 2.94 ± 0.34 g/dL, respectively, while bilirubin levels decreased to 0.128 ± 0.03 mg/dL and 0.09 ± 0.04 mg/dL. Correspondingly, SGOT and SGPT activities declined substantially, suggesting stabilization of hepatocyte membranes and prevention of enzyme leakage. This biochemical normalization indicates improved hepatic function and structural integrity, correlating with the ultrasonographic and histopathological observations.

These improvements can be attributed to the rich composition of *Coffea arabica* extract, which contains polyphenols (chlorogenic acid, caffeic acid, and ferulic acid), flavonoids (quercetin and rutin), and alkaloids (caffeine and trigonelline) known for their potent antioxidant and hepatoprotective actions[31]. Phenolic compounds scavenge reactive oxygen species (ROS) and enhance endogenous antioxidant defenses such as superoxide dismutase (SOD) and glutathione (GSH), thereby reducing hepatocyte membrane damage[36]. Quercetin and chlorogenic acid also inhibit cytochrome P450-mediated CCl_4 bioactivation, suppressing the formation of CCl_3• radicals and attenuating oxidative stress[37].

Furthermore, the observed increase in serum albumin levels reflects improved hepatic protein synthesis, suggesting that *Coffea arabica* not only prevents hepatocellular damage but also supports regenerative processes. Similar biochemical recovery patterns have been reported in studies using coffee polyphenols in ethanol- and acetaminophen-induced hepatotoxicity models[38,39], confirming the consistency of the hepatoprotective mechanism across different hepatic injury models.

Histopathological Findings

Histopathological evaluation of liver tissue provided strong morphological evidence supporting the biochemical and ultrasonographic findings (Figure 2). In the CCl_4 control group, extensive hepatic tissue damage was observed, characterized by diffuse hepatocellular degeneration, sinusoidal dilation, and infiltration of mononuclear inflammatory cells surrounding the central vein. Areas of focal necrosis and cytoplasmic vacuolization were also evident, indicating oxidative damage and inflammatory insult consistent with hepatocellular necrosis and fibrosis. The mean histopathological damage score for this group was 2 (moderate injury), confirming the successful induction of hepatotoxicity.

In contrast, the 100 mg/kg BW *Coffea arabica* treatment group showed partial improvement, with reduced necrosis and less pronounced inflammatory infiltration, suggesting the onset of hepatic repair. The 200 mg/kg BW and 300 mg/kg BW groups displayed near-normal hepatic architecture, with well-arranged polygonal hepatocytes, centrally located nuclei, intact sinusoidal spaces, and minimal inflammatory cell presence. These structural restorations corresponded to a damage score of 1 (mild injury), demonstrating substantial recovery of hepatic tissue integrity.

The histological normalization observed in the higher-dose groups aligns with the improvements in biochemical parameters (Table 5) and decreased cytokine levels (Table 4). The preservation of hepatocyte morphology and reduction in necrosis indicate that *Coffea arabica* extract effectively suppressed oxidative stress and inflammatory cascades triggered by CCl_4 exposure. This effect can be attributed to its rich polyphenolic and flavonoid constituents particularly chlorogenic acid, caffeic acid, quercetin, and rutin which are known to inhibit lipid peroxidation and reduce TNF-α–mediated hepatocellular apoptosis Previous similarly reported that coffee phenolics exert hepatoprotective activity through dual mechanisms: direct free-radical scavenging and modulation of inflammatory gene expression, particularly by downregulating NF-κB and TGF-β1 pathways[31]. These actions contribute to reduced collagen deposition and prevention of fibrosis. Moreover, the presence of caffeine and trigonelline enhances mitochondrial function and promotes hepatocyte regeneration, accelerating structural recovery of the liver.

CONCLUSION

The present study demonstrated that *Coffea arabica* extract exhibits significant hepatoprotective activity against CCl_4-induced liver injury in Wistar rats. The extract contains active compounds such as alkaloids, flavonoids, triterpenoids, saponins, and glycosides, contributing to its antioxidant and anti-inflammatory properties. Administration of *Coffea arabica* extract, particularly at doses of 200–300 mg/kg BW, improved liver structure as observed through ultrasonography and histopathology, restored biochemical markers (albumin, bilirubin, SGOT, SGPT), and reduced inflammatory cytokines (TNF-α, IL-6, CRP). Among the tested doses, 300 mg/kg BW showed the most pronounced hepatoprotective effect, indicating its strong potential as a natural therapeutic agent for preventing or mitigating liver damage.

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