

Original Research Article

Hepatoprotective effect of the methanol fraction of *Cuminum cyminum* against acetaminophen-induced toxicity in rats

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Abstract

Purpose: To evaluate the hepatoprotective activity of methanol fraction of *Cuminum cyminum* leaves (MFCCL) against acetaminophen (APAP)-induced liver injury in rats, and to characterize its bioactive polyphenolic constituents.

Methods: Dried leaves of *C. cyminum* were extracted with 80 % methanol and partitioned to obtain methanol fraction. Quantitative phytochemical analysis and Gas Chromatography-Flame Ionization Detector (GC-FID) profiling were performed. Acute toxicity was tested in mice ($n = 3$ per group) using standard methods. For hepatoprotective study, 48 male Wistar rats (150 – 200 g) were randomly assigned to six groups ($n = 8$). Groups 1 (normal control) and 2 (hepatotoxic control) received distilled water (2 mL/kg). Group 3 received silymarin (200 mg/kg). Groups 4 – 6 received MFCCL (100, 200, and 400 mg/kg, respectively). All treatments were administered orally once daily for seven days, 30 min before daily APAP administration (400 mg/kg, p.o.). On day 8, blood and liver samples were collected for biochemical and histopathological analyses.

Results: The MFCCL was rich in flavonoids (53.11 mg/100g) and tannins (3846.67 mg/100g). GC-FID identified isoflavones (25.02 %), flavonones (12.90 %), and galocatechin (11.86 %) as major polyphenols. Acute toxicity test showed no mortality up to 5000 mg/kg. The serum of APAP-only treated rats exhibited significant ($p < 0.05$) elevations in liver enzymes, total bilirubin, and hepatic malondialdehyde, with concomitant depletion of antioxidant enzymes and GSH. Pretreatment with MFCCL significantly ($p < 0.05$) reversed these alterations, restored antioxidant status, and preserved hepatic architecture comparable to silymarin.

Conclusion: Methanol fraction of *Cuminum cyminum* leaves exhibits significant hepatoprotective effect against APAP-induced liver injury, mediated through its polyphenolic constituents and antioxidant mechanisms, supporting its potential as a safe therapeutic agent for drug-induced hepatotoxicity.

Keywords: Hepatotoxicity, Medicinal plants, *Cuminum cyminum*, Antioxidants, Polyphenols, Phytochemicals

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INTRODUCTION

The liver plays a vital role in various biochemical and physiological processes, such as nutrient

metabolism, energy supply, homeostasis, immunity, and detoxification. Factors that increase the risk of liver diseases include industrial pollutants, medications, free radicals,

food additives, and alcohol consumption. Substances like acetaminophen (N-acetyl-para-aminophenol, APAP, or paracetamol), non-steroidal anti-inflammatory drugs (NSAIDs), statins, isoniazid, and antimicrobial agents can lead to hepatotoxicity [1]. Acetaminophen overdose-induced liver damage is commonly used as a model to assess the hepatoprotective potential of pharmaceuticals [1]. While APAP is safe at therapeutic doses, higher doses can cause centrilobular necrosis, resulting in liver failure.

Most APAP is conjugated with glucuronic acid or sulfate, with the remainder being converted into N-acetyl-p-benzoquinoneimine (NAPQI) by cytochrome P450 enzymes. Under normal conditions, NAPQI combines with reduced glutathione (GSH) to form mercapturic acid, which is excreted in urine. However, in cases of overdose, excess NAPQI depletes GSH and binds to hepatic cell proteins, causing mitochondrial dysfunction and oxidative stress, ultimately leading to hepatocyte necrosis [2]. Medicinal plants, containing synergistic phytochemicals, are potential hepatoprotective agents [3]. Dietary polyphenolic compounds found in fruits, seeds, legumes, and vegetables offer health benefits by restoring the balance between antioxidants and free radicals. They enhance natural antioxidant defenses, such as superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione-S-transferase (GST), and scavenge free radicals. Plant-derived polyphenols also possess anti-inflammatory properties [4].

Cuminum cyminum L. (Cumin), native to the East Mediterranean and South Asia, is an annual herbaceous flowering plant in the parsley family (Umbelliferae), classified as part of the Apiaceae family [5]. *Cuminum cyminum* is known as nature's Magical Seeds and Bitter cumin leaves. The word cumin is derived from Latin *cuminum* and Greek (*kyminon*), common names include: Arabic: Kamoun, Kamun; Chinese: Oushiluo, Ma qin, Xian hao, Xiang han qin, Zi ran; English: Cumin, Roman caraway; French: Cumin, Cumin de Malte, Cumin blanc, Cumin du Maroc, Faux anis; German: Kreuzkümmel, Römischer Kümmel, Weißer Kreuzkümmel; Greek: Kimino, Kiminon; India: Jiiraa, Zeera, afedziiraa, Safed jiiraa; Italian: Cumino; Japanese: Hime unikyoo, Kumin; Portuguese: Cominho; Russian: Kmin, Kminrimskii, Kmintminovyi; Spanish: Comino; Swedish: Spiskummin [6,7]. *Cuminum cyminum* leaves and seeds are used in traditional medicine [5]. Its medicinal values are derived from its phytochemical constituents such as

flavonoids, alkaloids, tannins, phenols, glycosides, and saponins, which produce pharmacological effects [5].

Studies have shown that *Cuminum cyminum* L. (*C. cyminum*) leaves and seeds exhibits immunomodulatory, anti-diabetic, anti-microbial, anti-fungal, analgesic, anti-osteoporotic, anti-oxidant, anti-inflammatory, anti-asthmatic, anti-stress, anti-infertility, dietary fiber, anti-cancer, blood platelet aggregation, anti-tussive, hypotensive, bronchodilatory, immunological, contraceptive, anti-amyloidogenic, anti-osteoporotic, aldose reductase, alpha-glucosidase and tyrosinase inhibitory, and ophthalmic effects [5].

This study aimed to determine the effects of *C. cyminum* leaves on acetaminophen-induced hepatotoxicity in rats and identify its bioactive constituents.

EXPERIMENTAL

Collection, identification and authentication of plant material

Fresh leaves of *Cuminum cyminum* were collected from New Haven (6.4602°N, 7.5220°E), Enugu North Local Government Area, Enugu State, Nigeria, in May 2023. The leaves were identified and authenticated by Mr. Alfred Ozioko, a taxonomist at the Bio-resources Development and Conservation Programme (BDPC) Research Center, University of Nigeria, Nsukka. A voucher specimen with herbarium number (Intercedd/16319) was assigned to it, and the sample was deposited in the herbarium for reference purposes. The identity and authenticity of the plant samples were further verified with those deposited in the databases <http://www.theplantlist.org/> and <http://www.ipni.org/>.

Study animals

Forty-eight male Wistar rats (150 – 200 g) were obtained from the animal house of the Faculty of Veterinary Medicine and kept in a metal cage for approximately one week in the animal house of the Department of Biochemistry, University of Nigeria, Nsukka, Nigeria, for acclimatization under standard environmental conditions of 12 h light and dark cycles. Animals were fed standard food pellets (Commercial Growers Mash Nig. Ltd.) and clean water *ad libitum*. The study was approved by the Institutional Ethics and Biosafety Committee of the Faculty of Biological Sciences, University of Nigeria, Nsukka (approval no. UNN/FBS/EC/1078).

Chemical, drugs and reagents

All chemicals and reagents used in this study were of analytical grade. Acetic acid, acetic anhydride, alkaline copper reagent, chloroform were products of Riedel Dettan (RDH), Germany, concentrated sulfuric acid, Dragendorff reagent, methanol and ethyl acetate (JHD China), ethyl acetate (Sigma-Aldrich, USA), ferric chloride and ferric sulphoxide (FeSO₄), Folin-Ciocalteu's reagent, sodium carbonate, formaldehyde, glacial acetic acid, hydrochloric acid, lead acetate, Mayer's reagent, methanol, Molisch's reagent, n-hexane, petroleum ether, phosphomolybdic acid reagent and sodium chloride (May and Barker, England), potassium ferricyanide (Burgoyne, India).

Concentrations and levels of vitamin C, α-tocopherol, Alkaline phosphatase (ALP), bilirubin, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were measured using the Accurex kit (Accurex Biomedical Pvt. Ltd, India). The drugs: acetaminophen (APAP) and silymarin were purchased from Evans Medical Plc. Enugu State, Nigeria.

Plant extraction

Cuminum cyminum leaves were air-dried at room temperature (25 - 30 °C) for one week with regular turning until crispy. The dried leaves were pulverized into a coarse form using a mechanical grinder and weighed using an electronic balance. A quantity (1200 g) of the pulverized leaves of *Cuminum cyminum* was extracted by maceration in 5 litres of 80 % methanol and allowed to stand for 72 h. This was filtered through a mesh, followed by Whatman No. 1 filter paper. The filtrate was concentrated using a rotary evaporator at 40°C to obtain the crude methanol extract, which was weighed and the percentage yield was determined.

The methanol extract of *Cuminum cyminum* leaves (MECCL) was subjected to solvent-solvent partitioning for fractionation using the modified method of Emran *et al* [8]. The crude methanol extract was partitioned using n-hexane, ethyl acetate, and methanol in the order of increasing polarity. The physical appearances of the fractions and their quantities after partitioning were noted. The resulting plant fractions were dried by evaporating the respective solvents using high vacuum on a rotary evaporator. The concentrated crude extract and fractions were stored at 4 °C in an airtight container until required.

Phytochemical analysis of MFCCL

Quantitative phytochemical analyses were performed to ascertain the quantities of plant secondary metabolites in the crude methanol extract and MFCCL. The determinations were performed using a standard protocol, as described by Trease and Evans [9]. The concentrations of the various phytochemical constituents were calculated using Eq 1.

$$\text{Concentration } \left(\frac{\text{mg}}{100\text{g}} \right) = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Dilution factor} \dots\dots\dots (1)$$

GC-FID analysis of MFCCL

Gas Chromatography-Flame Ionization Detection (GC-FID) analysis was employed to identify and quantify the polyphenolic aglycones in MFCCL. The sample underwent alkaline hydrolysis (saponification) to cleave the glycosidic bonds, thereby converting flavonoid glycosides and other conjugated polyphenols into their free aglycone forms. This step enhances the volatility and detectability of these compounds in GC analysis, allowing for a more accurate profiling of the core polyphenolic constituents responsible for bioactivity.

Preparation of sample

A one-gram sample was weighed into a test tube, and 15 mL of ethanol and 10 mL of 50 %m/v potassium hydroxide were added. The test tube was heated in a water bath at 60 °C for 60 min. After the reaction, the reaction product contained in the test tube was transferred to a separatory funnel. The tube was washed sequentially with 20 mL of ethanol, 10 mL of cold water, 10 mL of hot water, and 3 mL of hexane, which were transferred to a separating funnel. These extracts were combined and washed three times with 10 mL of 10 %v/v ethanol aqueous solution. The solution was dried with anhydrous sodium sulfate, and the solvent was evaporated. The sample was solubilized in 1000 µL pyridine, of which 200 µL was transferred to a vial for analysis.

Acute toxicity test

The median lethal dose (LD₅₀) of the fraction was determined using the method of Lorke, using eighteen (18) mice [10]. The test was divided into two phases. In the first phase, nine (9) mice were divided into three (3) groups of three mice each and were treated orally with the extract at doses of 10, 100, and 1000 mg/kg body weight. The mice were observed for 24 h to detect signs of toxicity. In the second phase, nine mice were

divided into three groups of three mice each and treated orally with the extract at doses of 1600, 2900, and 5000 mg/kg body weight, respectively. The administration was performed using an oral intubation. The mice were observed for 24 h for general behavioral, neurological, autonomic, and mortality profiles. The LD₅₀ of the extract was calculated using Eq 2.

$$LD_{50} = Dh * DI \dots\dots\dots (2)$$

Where Dh is the highest dose that produced no mortality, while DI is the lowest dose that produced mortality.

Induction of hepatotoxicity

After acclimatization, hepatotoxicity was induced in groups 2 - 6 by administering a single oral dose of 400 mg/kg bw APAP once daily for seven consecutive days. On the eighth day, two hours post-APAP administration, rats received an injection of thiopentone (50 mg/kg. bw i.p.) and blood was collected via cardiac puncture. Subsequently, the relative weights of the livers were determined.

Study design: A preventive study

Forty-eight male Wistar rats were randomly assigned to six groups, each consisting of eight rats. All treatments (MFCCL and silymarin) were administered orally once daily for seven days, 30 min prior to each daily dose of acetaminophen (APAP). Group 1, which served as the normal control, received 2 mL of distilled water daily. Hepatotoxicity was induced in Groups 2 - 6 by administering 400 mg/kg body weight of APAP once daily for seven days. Group 2 was administered APAP alone (untreated hepatotoxic control). Group 3 was administered silymarin (200 mg/kg body weight). Groups 4 – 6 were treated with 100, 200, and 400 mg/kg body weight of MFCCL, respectively. On the eighth day, two hours after the final APAP administration, the rats were anesthetized with thiopentone (50 mg/kg i.p.), and blood was collected via cardiac puncture.

Determination of biochemical parameters

Alkaline phosphatase (ALP), bilirubin, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were measured by an auto-analyzer using Accurex kits (Accurex Biomedical Pvt. Ltd, India) [11]. The total protein content was estimated [12] and malondialdehyde (MDA) levels in the liver were determined [13]. Reduced glutathione (GSH) levels and superoxide dismutase (SOD) activity were also

estimated. Catalase (CAT) and glutathione reductase (GR) activities were measured [14]. The GSH-S-transferase (GST) activity was measured spectrophotometrically [15]. Glutathione peroxidase (GPx) activity was calculated.

Histological assessment

Part of the liver was homogenized in phosphate-buffered saline (0.1 M PBS, pH 7.4). The homogenates were centrifuged at 4000 × g for 20 min at 4 °C and the supernatant was stored at -80 °C for biochemical estimation. The second part of the rat liver from different groups was fixed in 10 % neutral-buffered formalin for histological analysis. After fixation, liver samples were dehydrated in alcohol, cleared in xylene, and embedded in paraffin wax at 56 °C in a hot air oven for 24 h. Paraffin-embedded tissue blocks were prepared for sectioning at 5 mm thickness using a microtome. Tissue sections were collected on glass slides, deparaffinized, and stained with hematoxylin and eosin (H&E) for histological examination using a light microscope [16].

Statistical analysis

Data obtained were analyzed using one-way analysis of variance (ANOVA) in Statistical Package for the Social Sciences (SPSS) version 20.0. The results are presented as mean ± standard deviation (SD) of the triplicate measurements. Statistical significance was set at $P \leq 0.05$.

RESULTS

Percentage yield of MECCL and fractions

The extraction of 1200 g of powdered *Cuminum cyminum* leaves afforded 88.05 g of the extract, which represented 7.34 % of the original sample. The solvent partitioning of 40 g of the crude extract of *Cuminum cyminum* leaves with n-hexane, ethyl acetate, and methanol gave different weights and percentage yields of the fractions as follows: 4.97 g and 12.43 %, 2.75 g and 6.88 %, 5.84 g and 14.6 %, respectively. Several factors, such as the type and percentage of solvent used for extraction, solvent-to-solid ratio, particle size of the plant material, nature of the leaves, harvest time, percentage of acidification, drying temperature, storage conditions, temperature, and duration of extraction, could affect the extraction yield of plant extracts [17].

Preliminary phytochemical compositions of MECCL and its fractions

Table 1 shows the quantitative phytochemical profiling of MECCL and its fractions, which revealed the presence of phytochemical constituents in varied proportions. The concentration of saponins was higher in the methanol extract and lower in the ethyl acetate fraction. The methanol fraction had the highest amount of tannins, while n-hexane had the lowest. Flavonoid content was highest in the methanol fraction, while ethyl acetate had the lowest. The glycoside content of the methanol fraction was greater, with the methanol extract having the lowest content. Terpenoids were high in n-hexane, with the methanol fraction having the lowest content. The alkaloid content of the methanol fraction was higher than that of the other fractions, and the ethyl acetate fraction had the lowest. The ethyl acetate fraction was high in phenols compared to the rest of the fraction, with the methanol fraction having the lowest content. The steroid content was found to be highest in the ethyl acetate fraction, with the crude extract having the lowest content.

GC-FID profile of MFCCL

Figure 1 shows the GC-FID chromatogram of MFCCL depicting presence of polyphenolic (bioactive flavonoids) antioxidant phytochemical compounds at different retention time,

Anthocyanin (0.123 min), Chalcones (31.363 min), Isoflavones (5.793 min), Flavonones (12.450 min), Flavan-3-ol (14.040 min), Flavone (21.553 min), Aglycone (24.580 min), Galocatechin (34.120 min), Catechins (39.900 min), and Epicatechin (41.836 min).

Acute toxicity profile of MECCL

Table 2 presents the acute toxicity test results of MECCL. The data showed neither death nor signs of toxicity, behavioral changes, or body weight changes, even at the highest dose of 5000 mg/kg body weight. The results indicate that the plant species can be classified as non-toxic and safe for human and animal consumption, and this complements earlier studies.

Effect of MFCCL on body and organ weights

Table 3 shows the changes in body and organ weights of acetaminophen-induced hepatotoxic rats treated with MFCCL and the standard drug. The liver weight of group 2 (untreated hepatotoxic group) was significantly ($p < 0.05$) higher than that of the treated hepatotoxic groups (3 - 6). There were no significant ($p > 0.05$) differences in the spleen and lung weights among the groups. Kidney weight in group 2 was significantly ($p < 0.05$) higher than that in the study groups.

Table 1: Quantitative analysis of MECCL and its fractions

Sample	Saponins (%)	Tannins (mg/100g)	Flavonoids (mg/100g)	Glycosides (mg/100g)	Terpenoids (mg/100g)	Alkaloids (%)	Phenols (%)	Steroids (Mg/100g)
Methanol extract	46.02±1.25 ^a	905.00±3.60 ^b	32.64±0.91 ^b	51.73±2.06 ^a	433.76±3.22 ^b	18.35±0.250 ^c	12.74±0.31 ^c	0.60±0.08 ^a
n-Hexane fraction	13.51±.41 ^b	491.00±3.00 ^a	21.82±0.90 ^a	95.99±2.21 ^b	898.15±7.29 ^d	8.50±0.38 ^b	9.23±0.47 ^b	0.71±0.047 ^b
Ethyl acetate fraction	5.57±0.13 ^c	1151.67±3.06 ^c	20.94±1.69 ^a	192.32±1.24 ^c	497.75±2.08 ^c	2.27±0.14 ^a	17.60±0.33 ^d	1.21±0.08 ^c
Methanol fraction	30.94±0.62 ^d	3846.67±1.08 ^d	53.11±0.28 ^c	268.39±1.45 ^d	77.07±0.95 ^a	27.07±0.96 ^d	2.09±0.09 ^a	0.97±0.13 ^d

Note: Means within the same column followed by different superscript letters (a, b, c, and d) are significantly different at $p < 0.05$

Table 2: Acute toxicity study of the MECCL on albino mice

Group	Dose of extract (mg/kg/bw)	Mortality	Behavioral Changes	Body weight changes
Phase I				
Group 1	10	0/3	Nil	Not significant
Group 2	100	0/3	Nil	Not significant
Group 3	1000	0/3	Nil	Not significant
Phase II				
Group 1	1600	0/3	Nil	Not significant
Group 2	2900	0/3	Nil	Not significant
Group 3	5000	0/3	Nil	Not significant

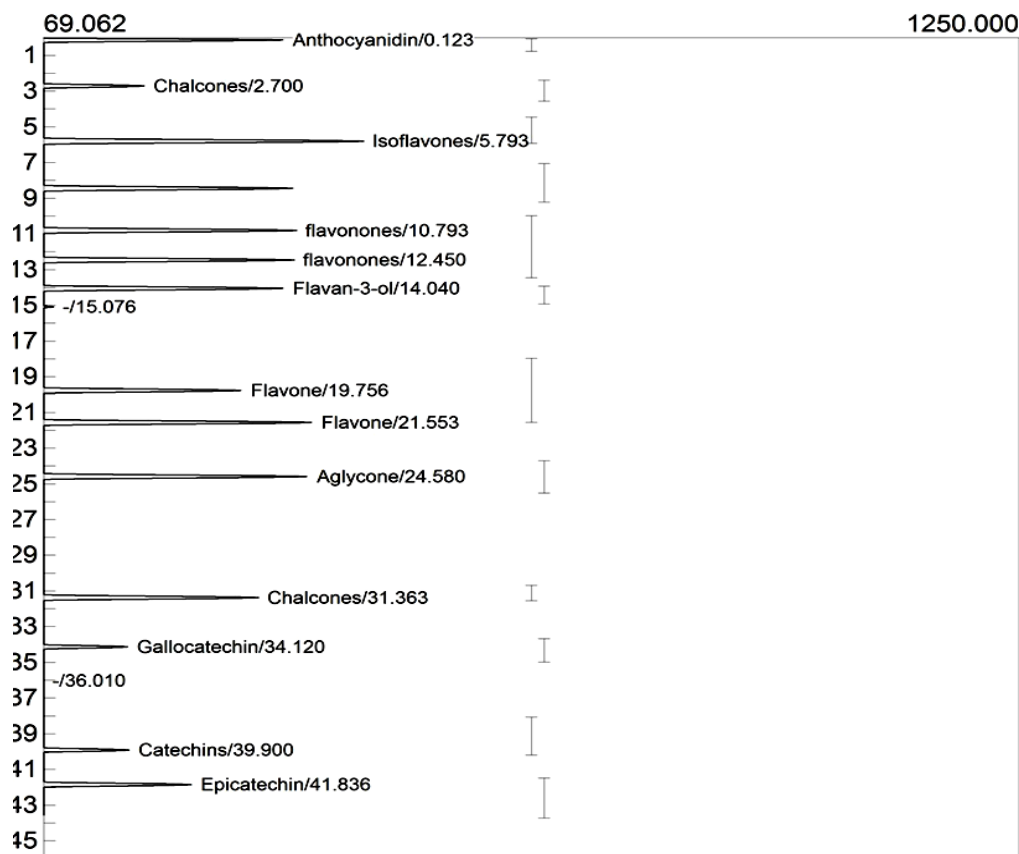


Figure 1: Representative GC-FID chromatogram of the methanol fraction of *Cuminum cyminum* leaves (MFCCL) extract

Peaks correspond to identified polyphenolic compounds

Component	Retention	Area	Height	External	Units
Anthocyanidin	0.123	8081.7916	360.197	11.5200	ug/ml
Chalcones	2.700	2436.2256	191.759	3.2387	ug/ml
Isoflavones	5.793	5819.4134	456.746	25.0192	ug/ml
flavonones	10.793	4775.5038	375.479	6.2905	ug/ml
flavonones	12.450	4743.7844	372.795	6.2487	ug/ml
Flavan-3-ol	14.040	4579.5053	360.005	7.6559	ug/ml
Flavone	19.756	3930.6483	309.146	3.8411	ug/ml
Flavone	21.553	5025.1523	394.848	3.8411	ug/ml
Aglycone	24.580	4953.9757	389.494	7.6800	ug/ml
Chalcones	31.363	4199.9884	329.743	2.3974	ug/ml
Galocatechin	34.120	2174.5382	171.250	11.5320	ug/ml
Catechins	39.900	2206.4649	173.478	2.9755	ppm
Epicatechin	41.836	3159.5558	248.559	4.9642	ug/ml
		56086.5477		97.2042	

Figure 2: Relative abundance of polyphenolic compounds identified in MFCCL by GC-FID analysis

Effect of MFCCL on lipid peroxidation and antioxidant status of acetaminophen-induced hepatotoxicity in rats

The data presented in Table 4 show the effect of MFCCL on MDA concentration and the antioxidant status of acetaminophen-induced

hepatotoxicity in rats. The MDA concentration in group 2 (untreated) was found to be significantly ($p < 0.05$) higher than that in the treatment groups, with a corresponding decrease in the activities of SOD, CAT, GPx, GST, GR, and GSH.

Table 3: Effect of MFCCL on body weights and organ weights of acetaminophen-induced hepatotoxicity in rats

Group	Body weight (g)	Liver (g)	Spleen (g)	Lung (g)	Kidney (g)
1	186.80±4.82 ^a	6.47±0.15 ^{ab}	0.95±0.03 ^a	2.69±0.05 ^b	1.68±0.03 ^c
2	191.80±3.49 ^{ab}	9.20±0.44 ^d	1.63±0.11 ^a	2.83±0.31 ^b	1.95±0.024 ^d
3	194.00±3.08 ^b	6.74±0.56 ^{abc}	0.96±0.03 ^a	2.73±0.20 ^b	1.54±0.07 ^a
4	192.20±4.15 ^{ab}	7.07±0.60 ^c	0.99±0.05 ^a	2.61±0.114 ^{ab}	1.59±0.06 ^{ab}
5	187.80±2.86 ^a	6.89±0.05 ^{bc}	0.93±0.01 ^a	2.35±0.214 ^a	1.63±0.04 ^{bc}
6	194.00±3.39 ^b	6.34±0.03 ^a	0.94±0.03 ^a	2.57±0.22 ^{ab}	1.60±0.09 ^{abc}

Results are expressed as the mean ± SD (n = 8). Mean values with different letters as superscripts in the column were considered significant at $p < 0.05$. (1) Normal controls (2 mL/kg. bw of distilled water). (2) Positive control (untreated hepatotoxic rats). (3) Standard control (hepatotoxic rats treated with 200 mg/kg) b.w. silymarin). (4) Hepatotoxic rats treated with 100 mg/kg. b.w. MFCCL. (5) Hepatotoxic rats treated with 200 mg/kg. b.w. MFCCL. (6) Hepatotoxic rats treated with 400 mg/kg. b.w. MFCCL

Table 4: Effect of MFCCL on lipid peroxidation and antioxidant status of acetaminophen-induced hepatotoxicity in rats

Group	MDA (mg/dl)	SOD (IU/L)	CAT (IU/L)	GPX (IU/L)	GSH (IU/L)	GST (IU/L)	GR (IU/L)
1	3.10±0.35 ^{bc}	9.38±0.41 ^d	0.83±0.06 ^c	10.53±0.37 ^d	0.33±0.11 ^{bc}	64.41±3.81 ^b	8.19±0.56 ^{bc}
2	9.91±0.71 ^f	3.13±0.831 ^a	0.30±0.12 ^a	4.18±0.68 ^a	0.13±0.0 ^a	39.21±2.93 ^a	4.08±0.44 ^a
3	4.02±0.41 ^{de}	8.11±0.43 ^{bc}	0.73±0.07 ^b	9.19±0.39 ^b	0.28±0.06 ^b	68.45±4.02 ^{bcd}	8.78±0.46 ^c
4	4.28±0.22 ^e	7.88±0.73 ^b	0.78±0.08 ^{bc}	9.47±0.44 ^{bc}	0.39±0.05 ^c	72.64±2.82 ^d	8.84±0.47 ^c
5	2.55±0.56 ^{ab}	8.84±0.29 ^{cd}	0.85±0.04 ^c	9.54±0.84 ^{bc}	0.31±0.05 ^{bc}	66.53±2.83 ^{bc}	7.59±1.20 ^b
6	2.25±0.21 ^a	9.12±0.59 ^d	0.86±0.03 ^c	10.14±0.59 ^{cd}	0.37±0.03 ^{bc}	64.38±3.01 ^b	8.13±1.17 ^{bc}

Results are expressed as mean ± SD (n = 8), and mean values with different letters as superscripts down the column are considered significant at $p < 0.05$. (1) Normal controls (2 ml/kg bw of distilled water). (2) Positive control (untreated hepatotoxic rats). (3) Standard control (hepatotoxic rats treated with 200 mg/kg bw silymarin). (4) Hepatotoxic rats treated with 100 mg/kg bw MFCCL. (5) Hepatotoxic rats treated with 200 mg/kg bw MFCCL. (6) Hepatotoxic rats treated with 400 mg/kg bw MFCCL

Table 5: Effects of MFCCL on liver biomarkers, Total protein and Total bilirubin of acetaminophen-induced hepatotoxicity in Rats

Group	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Total protein (mg/dL)	Total bilirubin (mg/dL)
1	19.60±2.70 ^a	29.20±1.30 ^a	43.20±2.28 ^a	6.71±0.34 ^a	4.96±0.73 ^{ab}
2	52.40±3.78 ^d	73.60±7.92 ^c	95.20±5.54 ^c	2.05±0.49 ^b	8.89±0.45 ^d
3	23.40±1.14 ^b	31.80±1.79 ^{ab}	42.60±3.57 ^a	6.34±1.18 ^b	5.99±0.72 ^c
4	26.60±1.67 ^c	35.40±2.19 ^b	47.60±1.52 ^b	6.68±0.557 ^b	5.59±0.44 ^{bc}
5	22.00±1.58 ^{ab}	33.00±1.58 ^{ab}	44.00±1.87 ^{ab}	6.26±0.678 ^b	5.67±0.47 ^{bc}
6	22.20±1.92 ^{ab}	32.40±2.30 ^{ab}	42.00±1.58 ^a	7.05±0.585 ^b	4.71±0.45 ^a

Results are expressed as the mean ± SD (n = 8). Mean values with different letters as superscripts in the column were considered significant at $p < 0.05$. (1) Normal controls (2 ml/kg bw of distilled water). (2) Positive control (untreated hepatotoxic rats). (3) Standard control (hepatotoxic rats treated with 200 mg/kg bw silymarin). (4) Hepatotoxic rats treated with 100 mg/kg bw of MFCCL. (5) Hepatotoxic rats treated with 200 mg/kg. b.w. MFCCL. (6) Hepatotoxic rats treated with 400 mg/kg bw of MFCCL

Effects of MFCCL on liver biomarkers, total protein and total bilirubin

Table 5 shows the effect of MFCCL on liver biomarker enzymes, Total Protein and Total Bilirubin in acetaminophen-induced liver damage in rats. The activities of AST, ALT, and ALP were significantly ($p < 0.05$) higher in group 2 (untreated) than in the treatment groups. The total protein content of group 2 (untreated) was significantly ($p < .05$) lower than that of the treatment groups. The total bilirubin level in group 2 (untreated) was significantly ($p < 0.05$) higher than that in the study groups.

Histological observations of liver tissues of treated and untreated rats

Histological observations of the liver showed that rats (groups 5 and 6) that received treatment with graded doses of MFCCL and silymarin (group 3) showed mild-to-moderate hepatic histo-architecture. Moderate hepatocytes arranged in interconnecting rods around the central veins (V) and normal structures of the portal area (hepatic artery, hepatic vein, bile duct), which appeared similar to normal rats in group 1 when compared to group 2 (untreated), which showed multifocal areas of hepatocellular degeneration and necrosis with marked infiltration of inflammatory leukocytes (arrow) and the central Vein (V).

Group 4 showed mild multifocal areas of hepatocellular degeneration and necrosis with mild infiltration of inflammatory leukocytes (arrow) and the central vein (V) when compared to group 2 (untreated).

DISCUSSION

This study highlights the significant hepatoprotective effect of the methanol fraction

of *Cuminum cyminum* leaves (MFCCL) against acetaminophen (APAP)-induced liver injury in rats. The protection observed is multifaceted, encompassing the amelioration of biochemical markers, enhancement of the endogenous antioxidant defense system, reduction of oxidative stress, and improvement of hepatic histo-architecture.

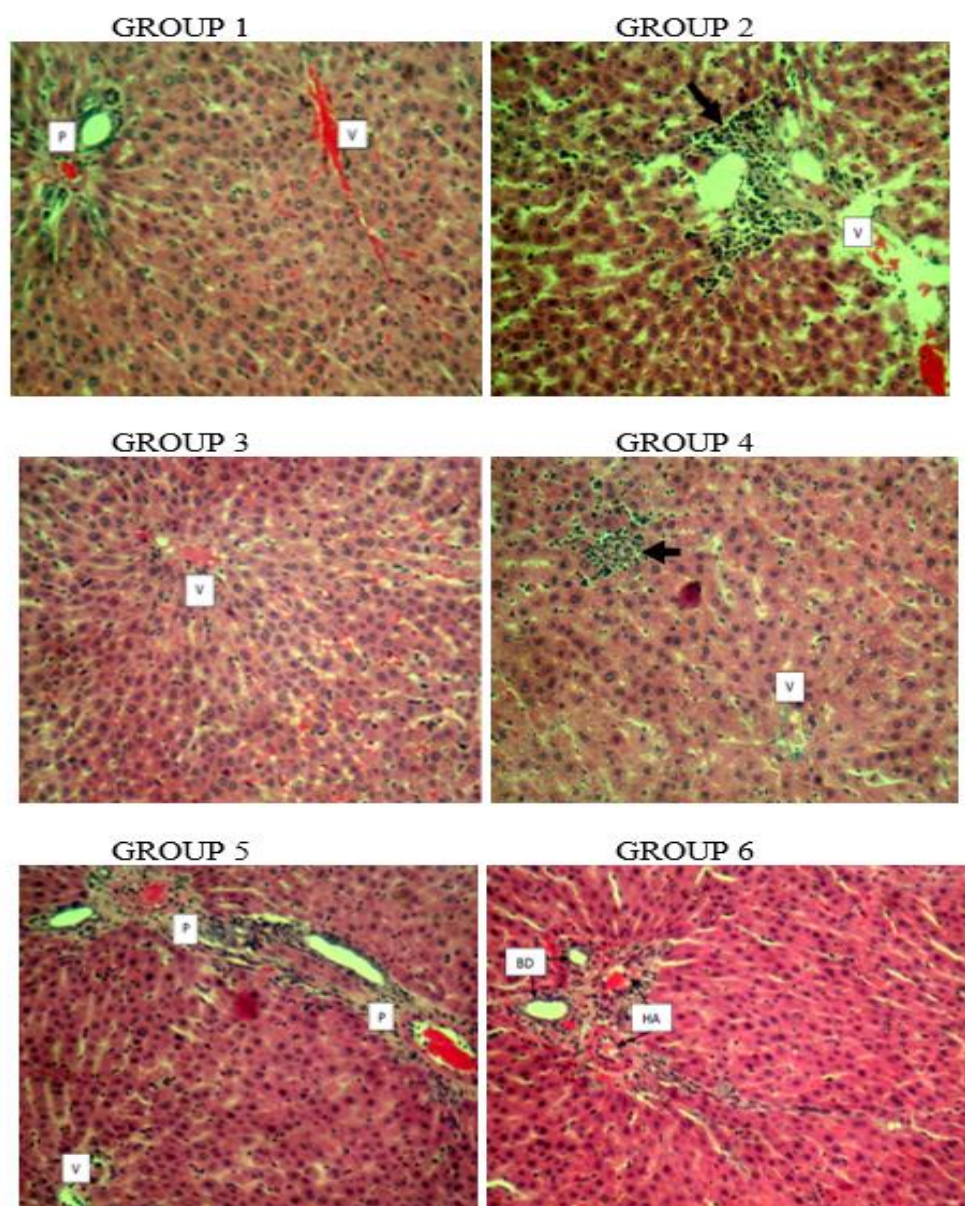


Figure 3: Histopathological examination of liver sections from study groups (H&E staining, 160× magnification). (A) Group 1 (Normal control): Normal hepatic architecture with intact hepatocytes radiating from the central vein (V) and well-defined portal areas (P). (B) Group 2 (APAP-only): Severe hepatocellular degeneration, necrosis, and marked inflammatory infiltrate (arrow). (C) Group 3 (Silymarin + APAP): Moderate preservation of hepatic architecture. (D) Group 4 (MFCCL 100 mg/kg + APAP): Mild hepatocellular degeneration and necrosis with mild inflammation (arrow). (E) Group 5 (MFCCL 200 mg/kg + APAP): Mild hepatic architecture with moderate hepatocyte arrangement. (F) Group 6 (MFCCL 400 mg/kg + APAP): Moderate hepatic architecture near normal. CV: central vein; HA: hepatic artery; BD: bile duct

These effects are closely tied to the rich and diverse phytochemical profile of MFCCL, as characterized in this study.

Quantitative phytochemical analysis revealed that MFCCL contains appreciable amounts of flavonoids, tannins, phenols, alkaloids, and glycosides. Notably, the methanol fraction (MFCCL) exhibited the highest concentrations of flavonoids and tannins among the tested fractions. This phytochemical profile aligns with previous reports on *C. cyminum*. Flavonoids and phenolic compounds are widely recognized for their potent antioxidant and free radical scavenging activities. The GC-FID analysis further detailed the specific polyphenolic composition of MFCCL, identifying compounds such as isoflavones (25.02 %), flavonones (12.90 %), gallic catechin (11.86 %), and anthocyanin (11.85 %). Many of these compounds, including flavonones, flavones, and gallic catechin, possess hepatoprotective, anti-inflammatory, and antioxidant properties [18]. Therefore, the therapeutic effects observed in this study may be attributed to the synergistic action of bioactive flavonoids and polyphenols present in MFCCL.

Acetaminophen-induced hepatotoxicity is primarily mediated by the toxic metabolite N-acetyl-p-benzoquinone imine (NAPQI), which depletes hepatic glutathione (GSH) and initiates oxidative stress, leading to lipid peroxidation and cellular necrosis [19]. These findings were consistent with this mechanism. The untreated hepatotoxic group (Group 2) exhibited a significant increase in lipid peroxidation, indicated by elevated malondialdehyde (MDA) levels, along with a marked depletion in GSH content and the activities of key antioxidant enzymes (SOD, CAT, GPx, GST, and GR). Treatment with MFCCL, particularly at doses of 200 and 400 mg/kg, effectively reversed these effects. It significantly reduced MDA levels and restored the activities of antioxidant enzymes and GSH content to near-normal levels, comparable to those of the standard drug silymarin. This indicates that MFCCL confers hepatoprotection partly by bolstering the hepatic antioxidant defense system and mitigating APAP-induced oxidative stress, a property associated with its polyphenolic constituents [20].

The hepatoprotective efficacy of MFCCL was further validated by its impact on serum biochemical markers. An overdose of APAP led to a significant increase in liver enzymes (AST, ALT, and ALP) and total bilirubin, along with a reduction in total protein. These changes are indicative of hepatic cellular damage and impaired synthetic function [20]. Pre-treatment

with MFCCL resulted in a dose-dependent mitigation of these alterations, significantly reducing the levels of AST, ALT, ALP, and bilirubin, while enhancing total protein. The normalization of these parameters suggests that MFCCL aids in maintaining the integrity of hepatocyte membranes and supports normal liver metabolic function. Macroscopic evidence of protection was corroborated by histopathological examination. Liver sections from the APAP-only group (group 2) exhibited severe hepatocellular degeneration, necrosis, and inflammatory cell infiltration. In contrast, sections from rats pretreated with MFCCL (200 and 400 mg/kg) showed markedly preserved hepatic architecture, with only mild to moderate histopathological changes, closely resembling the normal and silymarin-treated groups. This histological recovery highlights MFCCL's ability to prevent or significantly reduce structural damage to liver tissue induced by APAP. A significant finding of this study is the safety profile of extract. The acute toxicity test showed no mortality or signs of toxicity at doses up to 5000 mg/kg, indicating that MFCCL is practically nontoxic. This high safety margin enhances the potential therapeutic relevance of *C. cyminum* extract.

CONCLUSION

The methanol fraction of *Cuminum cyminum* leaves exhibits pronounced hepatoprotective activity against acetaminophen-induced liver damage in rats. The mechanism of action appears to be closely associated with its potent antioxidant properties, which are mediated by a rich array of polyphenolic compounds, particularly flavonoids. By scavenging free radicals, enhancing endogenous antioxidant defenses, and stabilizing hepatocyte membranes, MFCCL effectively counteracted the key pathological pathways of APAP hepatotoxicity. These findings, supported by biochemical, histological, and phytochemical data, validate the traditional use of *C. cyminum* and suggest its potential as a safe and effective natural adjuvant or supplement in the management of drug-induced hepatotoxicity. Further studies are warranted to isolate specific active compounds and elucidate their molecular targets.

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Ethical approval

The study was approved by the Institutional Ethics and Biosafety Committee of the Faculty of Biological Sciences, University of Nigeria, Nsukka (approval no. UNN/FBS/EC/1078).

Use of Artificial intelligence/Large language models

The authors confirm that AI-based tools were used solely for language editing and manuscript polishing purposes (e.g., grammar, phrasing, and clarity). No AI tools were used to generate content, interpret data, or influence study design, results, or conclusions. All intellectual contributions, study analyses, and interpretations reported in the manuscript are the original work of the authors.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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