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Original Research Article

PHARMACOLOGICAL EVALUATION OF *Hamelia patens* LEAF EXTRACT AGAINST CCL_4-INDUCED TOXICITY IN MALE ALBINO RATS: HAEMATOLOGICAL, BIOCHEMICAL, OXIDATIVE AND INFLAMMATORY EVIDENCE

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ABSTRACT

Carbon tetrachloride (CCl_4) induces systemic toxicity associated with haematological, hepatic, renal, oxidative and inflammatory disturbances, necessitating investigation of protective agents. This study evaluated the protective effects of aqueous *Hamelia patens* leaf extract (HPLE) against CCl_4-induced toxicity in male albino rats. Thirty-five rats (140–160 g) were randomly assigned to seven groups (n=5): normal control, CCl_4 control, silymarin (50 mg/kg)+CCl_4, HPLE (200, 400 and 800 mg/kg)+CCl_4, and HPLE-only (800 mg/kg). Hepatotoxicity was induced with CCl_4 (0.5 mL/kg, intraperitoneally), followed by daily oral treatment for 28 days. Haematological, hepatic, renal, antioxidant and inflammatory parameters were assessed and analysed using one-way ANOVA at $P<0.05$. CCl_4 reduced RBC, PCV and Hb from 6.94±0.13 to 4.39±0.31 $\times10^6$/mm³, 44.33±0.58 to 33.33±2.89%, and 14.60±0.36 to 11.00±0.87 g/dL, respectively. HPLE (800 mg/kg) improved these to 5.78±0.37 $\times10^6$/mm³, 40.00±1.73% and 13.40±0.26 g/dL. ALT, AST and bilirubin increased from 27.00±2.65, 33.00±2.65 U/L and 0.60±0.04 mg/dL to 111.33±7.10, 122.00±13.00 U/L and 1.17±0.03 mg/dL, respectively, but declined to 76.67±2.52, 69.00±4.00 U/L and 0.83±0.05 mg/dL following HPLE (800 mg/kg). Urea and creatinine increased to 35.20±1.87 and 1.29±0.04 mg/dL, respectively, but decreased to 27.90±1.68 and 0.92±0.02 mg/dL. HPLE also improved antioxidant and inflammatory profiles. The findings indicate haematoprotective, hepatoprotective and renoprotective potential of HPLE. Further studies should establish its mechanisms, safety and therapeutic relevance.

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INTRODUCTION

Globally, hepatic disorders represent a leading cause of mortality [1]. These conditions account for roughly two million deaths annually and place a profound socioeconomic strain on healthcare systems internationally [2]. Statistical data indicates that liver cirrhosis and related long-term hepatic pathologies led to 1,472,011 global deaths in 2019, contrasting with 1,012,975 recorded fatalities back in 1990 [3]. Functioning as a highly intricate organ, the liver coordinates critical immune-regulatory, endocrine, and digestive processes. Consequently, it remains highly vulnerable to damage from an array of pathogenic factors, including toxic substances, viral infections, metabolic shifts, alcohol consumption, and chemical metabolites, all of which can trigger acute or chronic hepatic trauma culminating in organ failure [2]. As an increasing number of chemicals and drugs are recognized to be hepatotoxic, it is important to identify and validate compounds that can provide liver protection. Carbon tetrachloride (CCl_4) is a volatile,

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colourless liquid, and it has long been a cornerstone in experimental liver research, and a model to induce liver injury in laboratory animals. CCl_4 is metabolized primarily by cytochrome P450 enzymes in the liver, and is converted to the highly reactive trichloromethyl free radicals ($\bullet CCl_3$) by the cytochrome P450 family member known as CYP2E1 [5]. This free radical species rapidly binds with molecular oxygen, resulting in the formation of the trichloromethyl peroxy radical ($CCl_3OO\bullet$) [6]. Lipid peroxidation, calcium sequestration, oxidative stress, apoptosis and inflammatory response are the factors that regulate CCl_4 hepatotoxicity [6]. Scholten et al. [4] reported that the model accurately reflects hepatotoxicity, liver fibrosis and cirrhosis observed in several liver diseases in humans. The liver Kupffer cells are especially responsive to oxidative stress and respond to reactive oxygen species (ROS) by inducing production of tumour necrosis factor-α (TNF-α) that can lead to further inflammation or apoptosis [7]. These pathological cascades do not only occur in the liver, but are also extended to haematological profiles and renal function, which is why CCl_4 is used as an appropriate toxicant to assess toxicity of multiple organs. The extracts of the plant Silybum marianum have been well recognized as hepatoprotective due to their phyto-constituent, silymarin [8]. Experimental data compiled by Pradhan and Girish [9] confirmed the broad efficacy of silymarin across diverse models of induced hepatic disease. Their analysis indicated that the compound operates through an array of therapeutic actions, including immunomodulatory responses, membrane-stabilizing activity, tissue regeneration, anti-inflammatory defense, anti-lipid peroxidation, and antifibrotic capabilities. However, its poor water solubility and low oral bioavailability [10] hinder its clinical application. Such side effects have kept the search for alternative or complementary agents that are hepatoprotective and derived from natural sources ongoing.

Globally cultivated in warm, humid regions as an ornamental shrub, *Hamelia patens* Jacq. (Rubiaceae), frequently referred to as firebush, holds a prominent place in traditional medicine for treating gastrointestinal issues, skin infections, and inflammation [11, 12]. Ahmad et al. [13] indicated that Hamelia houses a diverse array of bioactive components, notably phenolic fractions, flavonoids, and oxindole alkaloids. Extraction with methanol concentrates specific constituents like epicatechin and chlorogenic acid, which endow the plant with profound antioxidant and blood-sugar-lowering capabilities [14].

A wide range of pharmacological trials have traced various therapeutic applications back to this species. These include wound-healing capacity, diuretic properties, antiurolithiac activity, liver protection, antidepressant features, anthelmintic action, anti-diarrhoeal tracking, pain relief, and anti-inflammatory properties [14]. Further laboratory work by Pérez-Meseguer et al. [15] screened the hepatoprotective and antioxidant properties of the plant's hexane, ethyl acetate, methanolic, and butanol fractions, confirming strong defensive traits in HepG2 cell cultures exposed to CCl_4-mediated toxicity in vitro. Toxicological evaluations tracking both acute and sub-acute exposure intervals verify the safety profile of *H. patens* [14].

Despite these encouraging preliminary data, no in vivo study has comprehensively evaluated the hepatoprotective potential of *H. patens* leaf extract against CCl_4-induced toxicity using an integrated assessment of antioxidant, anti-inflammatory, haematological, hepatic, and renal biochemical indices in male albino rats. The present study was therefore designed to bridge this gap by investigating the effects of graded doses of *H. patens* leaf extract on serum liver, kidney function markers and haematological parameters in CCl_4-intoxicated rats, with silymarin as the reference standard.

MATERIALS AND METHODS

Experimental animals

Male albino rats of apparently normal health and weighing between 140 and 160 g, ranging from 8 to 10 weeks old, were obtained from the Animal House, Department of Physiology, College of Medicine, University of Nigeria, Nsukka. Animals were maintained in clean cages, under normal laboratory conditions, for 14 days before the experiment was conducted. They were fed and watered ad libitum from commercial growers. All experimental procedures were carried out in accordance with the guidelines for the care and use of laboratory animals [16]. Animal Care and Use Committee of Michael Okpara University of Agriculture, Umudike (Approval No: CREC/005/26).

Plant collection and authentication

The fresh leaves of *Hamelia patens* Jacq. were harvested from its natural habitat at Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. The plant was authenticated by a taxonomist from the Department of Plant Science and Biotechnology, and a voucher specimen was

deposited at the departmental herbarium (MOUAU-PSB-2026-0847).

Preparation of aqueous *Hamelia patens* leaf extract

The leaves were washed thoroughly, air-dried at room temperature to constant weight, and pulverized into fine powder. One hundred grams (100 g) of the powdered leaves was macerated for 24h in 1000 mL of distilled water, with periodic stirring. The mixture was filtered successively through muslin cloth and then Whatman No. 1 filter paper. The filtrate was concentrated by evaporation on a water bath and stored under refrigeration until use, following the procedure of Daniel et al. [17], with slight modification.

Drugs and chemicals

Silymarin tablets (140 mg; Silybon-140®) were purchased from an authorized pharmacy in Umuahia, Abia State, Nigeria. Silymarin is manufactured by Micro Labs Limited, India, and is registered and distributed in Nigeria through its marketing arm, Micro Nova Pharmaceuticals. Carbon tetrachloride (CCl_4) was purchased from Sigma-Aldrich, USA. All other reagents and chemicals of analytical grade were obtained from reputable manufacturers, including Randox Laboratories, UK, and TECO Diagnostics, USA.

Preparation and administration of treatments

Silymarin tablets were freshly ground into powder, and the required amount was dissolved in distilled water. The aqueous *H. patens* extract was reconstituted to the required concentration, and individual doses were calculated according to each animal's body weight. Treatments were administered orally by gavage.

Experimental design

A total of thirty-five (35) rats were used in this study, with five (5) rats assigned to each of the seven (7) experimental groups, as follows: Group I – normal control; Group II – CCl_4-induced hepatotoxicity, untreated; Group III – CCl_4 + silymarin (50 mg/kg); Group IV – CCl_4 + HPLE (200 mg/kg); Group V – CCl_4 + HPLE (400 mg/kg); Group VI – CCl_4 + HPLE (800 mg/kg); Group VII – HPLE only (800 mg/kg).

Induction of hepatotoxicity

Hepatic injury was induced by intraperitoneal administration of CCl_4 at 0.5 mL/kg body weight [18]. Treatment with silymarin or *H. patens* leaf extract commenced 24h after CCl_4 administration.

Treatment of experimental animals

The respective treatments were administered orally once daily for 28 days following induction. Body weights were monitored throughout the experimental period. At the end of treatment, the animals were fasted overnight and humanely sacrificed under approved laboratory procedures.

Blood collection and serum preparation

Blood samples were collected by cardiac puncture. Samples intended for haematological analysis were transferred into EDTA-containing tubes, while blood for biochemical assays was collected into plain tubes. Blood in plain tubes was allowed to clot and centrifuged at approximately 3000 rpm for 10 min. The separated serum was collected into clean tubes and stored at −20 °C until analysis.

Haematological analysis

Haematological parameters, including red blood cell count (RBC), packed cell volume (PCV), haemoglobin concentration (Hb), white blood cell count (WBC), platelet count (PLT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC), were determined using an automated haematology analyser (Mindray BC-2800).

Assessment of liver function

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined according to the method of Reitman and Frankel [19], while alkaline phosphatase (ALP) activity was determined using the method of Ochei and Kolhatkar [20]. Serum total protein was estimated by the Biuret method of Weichselbaum [21], while albumin was determined using the bromocresol green method. Total bilirubin was determined by the Jendrassik and Grof method. All assays were performed according to the manufacturers' instructions for the respective commercial reagent kits.

Assessment of kidney function

Serum urea was determined using the urease method described by Weatherburn [22], while creatinine was estimated using the kinetic Jaffe method of Bowers and Wong [23]. Serum sodium, potassium, chloride and bicarbonate concentrations were determined using standard colorimetric/spectrophotometric methods and commercial diagnostic kits according to the manufacturers' instructions.

Determination of serum antioxidant parameters

Serum reduced glutathione (GSH), glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) were determined using established spectrophotometric methods. SOD activity was assayed based on the inhibition of epinephrine auto-oxidation according to the method of Misra and Fridovich [24]. Catalase activity was determined by measuring the decomposition of hydrogen peroxide using the method of Sinha [25]. Serum MDA, an index of lipid peroxidation, was determined using the thiobarbituric acid reactive substances method of Ohkawa et al. [26]. GSH concentration was determined using 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) according to Ellman [27]. GPx activity was determined using a commercial diagnostic assay kit following the manufacturer's instructions. Absorbance was measured at the appropriate wavelengths using a spectrophotometer, and results were expressed in the respective units indicated in the tables.

Determination of serum inflammatory cytokines

Serum concentrations of tumour necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β) and interleukin-6 (IL-6) were determined using rat-specific enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturers' instructions. Briefly, serum samples and standards were added to antibody-precoated microplates, followed by incubation, washing and addition of biotinylated detection antibodies and avidin-horseradish peroxidase conjugate. After subsequent washing and substrate development, the reaction was terminated with stop solution, and absorbance was measured at 450 nm using a microplate reader. Cytokine concentrations were determined from the respective standard curves and expressed as pg/mL. All samples were analysed in duplicate.

Statistical analysis

Data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by an appropriate post-hoc multiple comparison test. Differences between groups were considered statistically significant at $P < 0.05$.

RESULTS

Effects of HPLE on haematological parameters in CCl_4-induced toxicity

Table 1, showing the effects of *Hamelia patens* leaf extract (HPLE) on haematological parameters in CCl_4-induced toxicity, reveals that CCl_4 administration significantly reduced ($P < 0.05$) RBC, PCV, Hb and WBC, with a concurrent significant increase in platelet count and MCV, compared with the normal control; MCHC was not significantly affected. Treatment with silymarin and HPLE (200–800 mg/kg) significantly improved RBC, PCV, Hb and WBC while normalising platelet count and MCV toward control values, compared with the negative control. The HPLE-only (800 mg/kg) group recorded values comparable to or better than the normal control.

Table 1: Effects of *Hamelia patens* Leaf Extract (HPLE) on Haematological Parameters in CCl_4-Induced Toxicity in Rats

Treatment groups	RBC ($x10^6/mm^3$)	PCV (%)	Hb (g/dL)	WBC ($x10^3/mm^3$)	PLT ($x10^3/mm^3$)	MCV (fL)	MCH (pg)	MCHC (g/dL)
Normal control	6.94±0.13^{c}	44.33±0.58^{c}	14.60±0.36^{c}	8.13±0.20^{b}	504.33±25.79^{ab}	63.85±0.38^{a}	21.03±0.14^{a}	32.93±0.42^{a}
Neg. control (CCl4 only)	4.39±0.31^{a}	33.33±2.89^{a}	11.00±0.87^{a}	6.57±0.46^{a}	679.67±33.83^{d}	75.93±1.31^{c}	25.07±0.26^{c}	33.02±0.40^{a}
Silymarin (50 mg/kg) + CCl4	5.59±0.23^{b}	39.00±1.00^{b}	12.97±0.45^{b}	7.90±0.21^{b}	565.00±35.38^{c}	69.84±1.17^{b}	23.21±0.28^{b}	33.24±0.31^{a}
HPLE (200 mg/kg) + CCl4	5.39±0.40^{b}	38.33±1.53^{b}	12.83±0.42^{b}	7.68±0.41^{b}	591.33±28.75^{c}	71.19±2.62^{b}	23.84±1.00^{bc}	33.48±0.27^{a}
HPLE (400 mg/kg) + CCl4	5.68±0.28^{b}	39.33±1.16^{b}	13.27±0.25^{b}	8.11±0.18^{b}	605.00±20.88^{c}	69.34±1.55^{b}	23.40±0.75^{b}	33.74±0.48^{a}
HPLE (800 mg/kg) + CCl4	5.78±0.37^{b}	40.00±1.73^{b}	13.40±0.26^{b}	8.18±0.27^{b}	547.67±59.10^{abc}	69.31±1.56^{b}	23.25±1.25^{b}	33.53±1.15^{a}
HPLE only (800 mg/kg)	7.10±0.07^{c}	44.67±0.58^{c}	15.13±0.40^{c}	8.23±0.13^{b}	485.67±56.66^{a}	62.91±0.22^{a}	21.31±0.40^{a}	33.88±0.52^{a}

Values are presented as mean ± standard deviation (n = 5); values with different letter superscripts within a column are significantly (P < 0.05) different.

Effects of HPLE on liver function parameters in CCl_4-treated rats

Table 2, showing the effects of HPLE on liver function parameters in CCl_4-treated rats, reveals that the negative control had a significant decrease ($P < 0.05$) in total protein and albumin, with significantly elevated ($P < 0.05$) ALT, AST, ALP, and bilirubin levels compared with the normal control. Treatment with silymarin and HPLE (200–800 mg/kg) significantly reduced ALT, AST, ALP, and bilirubin levels while improving total protein and albumin compared with the negative control. The 800 mg/kg HPLE group showed the greatest improvement among the treated groups, while the HPLE-only (800 mg/kg) group recorded values comparable to the normal control.

Table 2: Effects of *Hamelia patens* Leaf Extract (HPLE) on Liver Function Parameters in CCl_4-Treated Rats

Treatment groups	Total protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)	ALT (U/L)	AST (U/L)	ALP (U/L)	Bilirubin (mg/dL)
Normal control	5.97±0.16^{d}	3.07±0.06^{b}	2.89±0.10^{b}	27.00±2.65^{a}	33.00±2.65^{a}	82.00±3.46^{a}	0.60±0.04^{a}
Neg. control (CCl4 only)	5.01±0.13^{a}	2.32±0.08^{a}	2.69±0.05^{ab}	111.33±7.10^{d}	122.00±13.00^{d}	162.00±9.17^{c}	1.17±0.03^{d}
Silymarin (50 mg/kg) + CCl4	5.43±0.13^{bc}	2.59±0.05^{a}	2.84±0.14^{ab}	64.67±4.51^{b}	69.67±5.51^{b}	129.67±3.51^{b}	0.89±0.02^{b}
HPLE (200 mg/kg) + CCl4	5.21±0.11^{ab}	2.58±0.10^{a}	2.63±0.12^{ab}	85.33±5.03^{c}	86.67±6.81^{bc}	128.00±8.89^{b}	0.97±0.06^{c}
HPLE (400 mg/kg) + CCl4	5.65±0.19^{cd}	3.08±0.07^{b}	2.57±0.25^{a}	79.33±7.51^{c}	80.67±6.66^{bc}	128.67±4.59^{b}	0.84±0.04^{b}
HPLE (800 mg/kg) + CCl4	5.69±0.32^{cd}	2.95±0.22^{b}	2.74±0.17^{ab}	76.67±2.52^{c}	69.00±4.00^{b}	124.67±4.16^{b}	0.83±0.05^{b}
HPLE only (800 mg/kg)	5.86±0.26^{d}	3.08±0.42^{b}	2.78±0.17^{ab}	27.67±1.53^{a}	32.33±2.52^{a}	79.00±3.61^{a}	0.56±0.07^{a}

Values are presented as mean ± standard deviation (n = 5); values with different letter superscripts within a column are significantly (P < 0.05) different.

Effects of HPLE on kidney function parameters in CCl_4-treated rats

Table 3 shows that the negative control exhibited a significant increase ($P < 0.05$) in urea and creatinine levels, with significantly reduced ($P < 0.05$) sodium, potassium, and chloride concentrations compared with the normal control, while bicarbonate levels were not significantly altered. Treatment with silymarin and HPLE (200–800 mg/kg) significantly reduced urea and creatinine levels and improved serum sodium and chloride concentrations compared with the negative control. The HPLE-only (800 mg/kg) group recorded kidney function values comparable to the normal control.

Table 3: Effects of *Hamelia patens* Leaf Extract (HPLE) on Kidney Function Parameters in CCl_4-Treated Rats

Treatment groups	Urea (mg/dL)	Creatinine (mg/dL)	Na^+ (mEq/L)	K^+ (mEq/L)	Cl^- (mEq/L)	HCO_3^- (mMol/L)
Normal control	21.07±2.99^{a}	0.66±0.04^{a}	135.10±1.27^{c}	4.94±0.19^{b}	90.43±0.75^{bc}	18.87±0.23^{ab}
Neg. control (CCl4 only)	35.20±1.87^{e}	1.29±0.04^{e}	126.27±1.51^{a}	4.22±0.22^{a}	86.70±1.18^{a}	19.33±0.12^{bc}
Silymarin (50 mg/kg) + CCl4	26.43±1.21^{b}	0.89±0.04^{b}	128.77±2.10^{ab}	4.33±0.07^{a}	89.03±1.19^{bc}	18.90±0.36^{ab}
HPLE (200 mg/kg) + CCl4	28.50±1.84^{b}	1.08±0.03^{d}	128.07±1.20^{b}	4.25±0.06^{a}	88.67±1.21^{abc}	19.10±0.26^{abc}
HPLE (400 mg/kg) + CCl4	26.37±1.33^{b}	0.98±0.04^{c}	129.03±0.45^{ab}	4.39±0.04^{a}	88.60±0.35^{ab}	19.40±0.20^{c}
HPLE (800 mg/kg) + CCl4	27.90±1.68^{b}	0.92±0.02^{bc}	130.20±1.87^{b}	4.41±0.37^{a}	89.43±1.50^{bc}	19.27±0.25^{abc}
HPLE only (800 mg/kg)	21.23±1.14^{a}	0.60±0.08^{a}	135.43±1.64^{c}	4.78±0.15^{b}	90.87±1.40^{c}	18.83±0.25^{a}

Values are presented as mean ± standard deviation (n = 5); values with different letter superscripts within a column are significantly (P < 0.05) different.

Effects of HPLE on serum antioxidant parameters in CCl_4-treated rats

Table 4, showing the effects of HPLE on serum antioxidant parameters in CCl_4-treated rats, reveals that the negative control exhibited a significant decrease ($P < 0.05$) in GSH, GPx, SOD, and CAT activities, with a significant increase ($P < 0.05$) in MDA levels compared with the normal control. Treatment with silymarin and HPLE (200–800 mg/kg) significantly increased GSH, GPx, SOD, and CAT activities while significantly reducing MDA levels compared with the negative control. The 800 mg/kg HPLE group showed the greatest improvement among the treated groups, whereas

the HPLE-only (800 mg/kg) group recorded antioxidant values comparable to or better than those of the normal control.

Table 4: Effects of *Hamelia patens* Leaf Extract (HPLE) on Serum Antioxidant Parameters in CCl_4-Treated Rats

Treatment groups	GSH (mg/dL)	GPx (U/L)	SOD (U/L)	CAT (U/L)	MDA (mg/dL)
Normal control	14.74±0.89^{d}	49.67±2.08^{d}	35.00±1.73^{de}	24.67±1.16^{d}	3.23±0.07^{c}
Neg. control (CCl4 only)	10.86±0.39^{a}	34.67±2.52^{a}	26.33±1.16^{a}	14.33±0.58^{a}	5.46±0.31^{d}
Silymarin (50 mg/kg) + CCl4	12.03±0.86^{bc}	38.00±1.73^{ab}	29.00±2.00^{ab}	18.33±1.53^{b}	3.87±0.06^{b}
HPLE (200 mg/kg) + CCl4	11.09±0.18^{ab}	40.00±2.00^{b}	30.67±2.08^{bc}	18.33±1.53^{b}	4.67±0.23^{c}
HPLE (400 mg/kg) + CCl4	12.70±0.40^{c}	41.67±2.08^{bc}	31.33±1.15^{bc}	19.33±1.16^{bc}	4.43±0.40^{c}
HPLE (800 mg/kg) + CCl4	12.79±0.54^{c}	44.67±1.58^{c}	32.33±2.08^{cd}	20.67±1.16^{c}	4.29±0.25^{c}
HPLE only (800 mg/kg)	16.02±0.42^{e}	51.00±3.61^{d}	37.67±1.53^{e}	25.67±1.16^{d}	2.85±0.08^{a}

Values are presented as mean ± standard deviation (n = 5); values with different letter superscripts within a column are significantly (P < 0.05) different.

Effects of HPLE on inflammatory parameters in CCl_4-treated rats

Table 5, showing the effects of HPLE on inflammatory parameters in CCl_4-treated rats, reveals that the negative control had significantly elevated (P < 0.05) serum levels of IL-1β, IL-6, and TNF-α compared with the normal control. The 400–800 mg/kg of silymarin and HPLE significantly reduced the levels of these inflammatory cytokines when compared with the negative control group, with the highest reduction in inflammatory cytokine levels being for the 800 mg/kg HPLE group. The levels of cytokines in the HPLE-only (800 mg/kg) group were similar to the normal control.

Table 5: Effects of *Hamelia patens* Leaf Extract (HPLE) on Inflammatory Parameters in CCl_4-Treated Rats

Treatment groups	IL-1β (pg/mL)	IL-6 (pg/mL)	TNF-α (pg/mL)
Normal control	0.17±0.04^{a}	7.30±0.37^{a}	41.42±2.68^{a}
Neg. control (CCl4 only)	2.04±0.15^{e}	21.12±2.91^{c}	69.82±3.55^{c}
Silymarin (50 mg/kg) + CCl4	1.25±0.07^{b}	13.77±0.64^{b}	53.99±3.06^{b}
HPLE (200 mg/kg) + CCl4	1.60±0.08^{d}	15.01±0.47^{b}	57.00±5.25^{b}
HPLE (400 mg/kg) + CCl4	1.52±0.04^{cd}	15.19±0.27^{b}	58.25±2.73^{b}
HPLE (800 mg/kg) + CCl4	1.43±0.06^{c}	13.89±0.57^{b}	58.14±2.37^{b}
HPLE only (800 mg/kg)	0.17±0.05^{a}	6.06±1.00^{a}	37.00±4.60^{a}

Values are presented as mean ± standard deviation (n = 5); values with different letter superscripts within a column are significantly (P < 0.05) different.

DISCUSSION

The present study aimed to assess the protective action of aqueous *Hamelia patens* leaf extract (HPLE) against carbon tetrachloride (CCl_4)-induced toxicity in male albino rats. Chronic exposure to CCl_4 resulted in marked oxidative stress, inflammatory activation, and subsequent liver, kidney and blood function abnormalities that were all improved by HPLE treatment, especially at the higher doses. These observations extend previous in vitro evidence of the hepatoprotective and antioxidant activity of *H. patens* to an in vivo setting of a chemical model of hepatotoxicity.

Oxidative stress was observed after administration of CCl_4, with a significant decrease in the activities of serum GSH, GPx, SOD and CAT, along with a raised level of MDA. These findings are consistent with the known mechanism of CCl_4 hepatotoxicity, which involves cytochrome P450 2E1-mediated biotransformation that produces trichloromethyl (CCl_3•) and trichloromethylperoxy radicals that trigger lipid peroxidation and deplete antioxidant reserves [4,28]. An increase in MDA concentration serves as a direct indicator of oxidative injury affecting membrane-bound polyunsaturated fatty acids. Simultaneously, the depletion of GSH underscores its primary function as an internal thiol defense mechanism responsible for neutralizing toxic metabolites [29, 30]. In support of the reliability of this oxidative stress model, Johra et al. [31] also confirmed that CCl_4-induced toxicity in rats leads to elevated MDA levels alongside a severe depletion of hepatic antioxidant enzymes.

The activities of GSH, GPx, SOD and CAT were significantly restored after treatment with HPLE (200–800 mg/kg), while the concentration of MDA was reduced in comparison with the CCl_4-only

group. Of all tested groups, the 800 mg/kg dose demonstrated the highest efficacy, bringing the data points nearly level with the results from the silymarin-treated rats. Based on these findings, HPLE can be concluded to possess antioxidant activity capable of attenuating CCl_4-induced oxidative imbalance.

A study by Pérez-Meseguer et al. [15] revealed that the butanolic, ethyl acetate, and methanolic fractions of *H. patens* possess robust DPPH radical-scavenging properties directly linked to their total polyphenol concentration. Remarkably, these extracts shielded HepG2 cells from CCl_4-mediated toxicity, with the butanolic fraction delivering a level of liver protection that actually surpassed the reference drug silibinin. Surana et al. [32] similarly reported substantial phenol and flavonoid content and in vitro antioxidant activity of *H. patens* stem extracts. The present in vivo findings provide functional evidence that this antioxidant capacity translates into measurable protection against chemically induced oxidative stress.

CCl_4 exposure also produced a pronounced inflammatory response, as evidenced by significant elevations in serum IL-1β, IL-6, and TNF-α. These pro-inflammatory cytokines are known mediators of hepatic injury in the CCl_4 model, arising primarily from activation of Kupffer cells in response to oxidative stress and hepatocyte damage [29,31]. Scholten et al. [4] reported that CCl_4 treatment significantly induced hepatic TNF and IL-1β expression, and that silymarin extract attenuated this inflammatory response in a dose-dependent manner. The present findings are consistent with this pattern and indicate that CCl_4-induced oxidative stress and inflammation occur concurrently in this model.

HPLE treatment significantly reduced the concentrations of all three cytokines compared with the negative control, with the 800 mg/kg dose showing the greatest reduction among the extract-treated groups. While the present study did not directly measure NF-κB activation or other signalling intermediates, the attenuation of pro-inflammatory cytokines suggests that HPLE may modulate inflammatory pathways, possibly secondary to its antioxidant effects. Reactive oxygen species are known activators of NF-κB, and by reducing oxidative burden, antioxidant agents may indirectly suppress cytokine transcription [4,31]. Previous studies have documented anti-inflammatory activity in *H. patens* extracts. Jiménez-Suárez et al. [12] reported that oral administration of a hexane extract of *H. patens* at 200–500 mg/kg significantly reduced carrageenan-induced paw oedema in mice, providing in vivo evidence of anti-inflammatory activity that supports the present observations. Ahmad et al. [13] noted that *H. patens* has been traditionally used to treat inflammatory conditions, and that crude extracts demonstrate anti-inflammatory effects.

The hepatotoxic effect of CCl_4 was clearly demonstrated by significant elevation of serum ALT, AST, ALP, and bilirubin, together with reduced total protein and albumin in the negative control group. These changes reflect hepatocellular membrane damage, impaired hepatic synthetic function, and cholestatic disturbance. Leakage of cytosolic aminotransferases into the circulation is a well-established marker of hepatocyte injury following CCl_4 exposure, while reduced albumin indicates compromised protein synthesis [28,31].

Treatment with HPLE significantly reduced ALT, AST, ALP, and bilirubin while improving total protein and albumin compared with the CCl_4-only group. The 800 mg/kg dose had the greatest improvement among the extract-treated groups, with only a slight decrease from the efficacy of silymarin. These data suggest that HPLE protects against CCl_4-induced hepatocellular injury. The observed antioxidant and anti-inflammatory effects may account for the hepatoprotection seen in this study, whereby HPLE could limit the production of inflammatory cytokines and lipid peroxidation and maintain the integrity of hepatocyte membranes and their synthetic function. Supporting this view, Pérez-Meseguer et al. [15] established a direct correlation between the antioxidant strength of *H. patens* extracts and their ability to protect HepG2 cells against CCl_4-induced hepatotoxicity. Silymarin, the reference hepatoprotectant used in this study, similarly exerts protection through combined antioxidant and anti-inflammatory mechanisms [4,34], and the comparable pattern of effects observed with 800 mg/kg HPLE suggests overlapping modes of action.

Although CCl_4 is primarily recognised as a hepatotoxin, the present study demonstrated concurrent nephrotoxicity, as indicated by significant elevation of serum urea and creatinine and reduction of sodium, potassium, and chloride concentrations in the negative control group. Interestingly, bicarbonate levels remained unaffected, suggesting that acid-base regulatory mechanisms were preserved at the employed CCl_4 dose. CCl_4-induced nephrotoxicity has been attributed to renal CYP2E1-mediated generation of reactive metabolites and secondary oxidative damage to tubular epithelium [35,36]. The electrolyte disturbances observed suggest impaired tubular reabsorptive function. HPLE treatment significantly reduced urea and creatinine levels and improved sodium and chloride concentrations compared with the negative control, indicating

attenuation of CCl_4-induced renal dysfunction. This renoprotective effect is plausibly related to the systemic antioxidant activity of the extract, which would reduce oxidative injury in renal tissue as well as the liver. While no previous studies have specifically examined the renoprotective effects of *H. patens*, the observed improvement is consistent with reports that plant extracts with antioxidant activity can ameliorate CCl_4-induced nephrotoxicity [35,36].

CCl_4 administration induced significant reductions in RBC, PCV, Hb, and WBC, with concurrent increases in platelet count, MCV, and MCH; MCHC was not significantly affected. These haematological alterations are consistent with oxidative damage to erythrocyte membranes leading to accelerated haemolysis, as well as possible bone marrow suppression. Erythrocytes are particularly susceptible to lipid peroxidation due to their high polyunsaturated fatty acid content, and oxidative haemolysis has been documented following CCl_4 exposure [37]. The elevated MCV and MCH may represent a compensatory change in response to the release of larger, younger erythrocytes, or interference with folate/vitamin B_{12} metabolism due to liver dysfunction. The thrombocytosis observed contrasts with the thrombocytopenia usually seen with chronic CCl_4-induced cirrhosis, and may represent an acute-phase reactive response. HPLE treatment was highly beneficial for RBC, PCV, Hb and WBC counts, and normalised the platelet count, MCV and MCH toward the control range. Alonso-Castro et al. [38], in a subacute toxicity study with *H. patens* extract in rats, reported no induction of toxicity, as the extract did not cause anaemia or alter most haematological parameters, suggesting that the plant does not cause haematotoxicity. The haematoprotective effects seen in the present study are therefore likely due to the ability of HPLE to reduce the oxidative damage caused by CCl_4, rather than to a direct haematopoietic effect.

Across the majority of evaluated markers, HPLE treatment yielded notable improvements across all three experimental doses (200, 400, and 800 mg/kg), with the highest concentration typically delivering the most pronounced therapeutic benefit. Although this trend points toward a dose-dependent effect, the relationship was not strictly linear across every parameter, so these outcomes should not be conclusively classified as a traditional dose-dependent response. Importantly, the group administered only the 800 mg/kg extract, without CCl_4, displayed health profiles that were either equal to or better than the normal control animals. This baseline stability spanned blood counts, hepatic and renal function profiles, antioxidant enzyme activity, and inflammatory cytokine levels, indicating that HPLE carries no inherent toxicity under these testing conditions. This safety profile aligns with the findings of Alonso-Castro et al. [38], who documented an oral LD_{50} above 5000 mg/kg for the extract in mouse models, alongside an absence of toxic side effects at doses up to 2500 mg/kg, thereby supporting the safety of the dosing framework used in the current study.

CONCLUSION

The data gathered from this investigation demonstrate that the aqueous extract derived from *Hamelia patens* leaves effectively mitigates CCl_4-mediated damage across several organ systems in male albino rats. The recovery of vital antioxidant enzyme pathways alongside a simultaneous drop in MDA levels points directly to an underlying antioxidant mode of action. In addition, the extract's capacity to suppress the inflammatory response is clearly demonstrated by the marked down-regulation of key signalling cytokines, specifically TNF-α, IL-6, and IL-1β. These effects appear to underlie the observed improvements in hepatic function (reduced transaminases and bilirubin, improved protein synthesis), renal function (reduced urea and creatinine, improved electrolytes), and haematological indices (improved erythrocyte and leucocyte parameters). The 800 mg/kg dose demonstrated the greatest protective effect, approaching the efficacy of silymarin across most parameters. These findings extend previous in vitro evidence of the hepatoprotective and antioxidant properties of *H. patens* to an in vivo model and support further investigation of this plant as a source of hepatoprotective agents.

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AUTHORS CONTRIBUTION

OSE and GSA designed the study. OSE carried out the research under the supervision of ODO and GSA. OSE drafted the original manuscript. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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