Research Article

Physiological Alterations due to Hepatotoxicity and the Protective Role of Cleome viscosa Linn Seed Extract in Experimental Animals

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Liver ailment is a key public health menace, principally in developing nations. Quite a lot of medicinal flora have been identified to have liver shielding activities. The current study was designed to assess in vitro antioxidant and in vivo hepatoprotective activities of seed extracts of Cleome viscosa Linn. (Capparaceae). Phytochemical screening of C. viscosa seed ethanol extract was carried out. Free radical scavenging activity of crude seed extract of the plant was conducted using the DPPH assay method. DNA damage protection potential of the crude seed extract was carried out using extract of the genomic DNA nicking assay. Hepatoprotective activity of the crude seed extract of the plant was carried out based on CCl4-induced liver damage in Wister albino rats. Serum biomarkers (aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), and total protein (TP)) were evaluated to find out the effect. Histopathological scrutiny was also carried out for all groups of rats to further confirm the discoveries. The phytochemical screening was positive for alkaloids, flavonoids, saponins, steroids, terpenes, tannins, and phenolic compounds in the seed extract. The antioxidant assay revealed that the ethanol crude extract of C. viscosa exhibited free radical scavenging activity with IC50 value of 17.82 ± 0.32 μg/mL, and this was further confirmed by the DNA damage protection activity. Pretreatment of the rats with the crude extract of C. viscosa significantly reduced ALP (p < 0.05). The hepatoprotective activity of the seed extract was confirmed by histopathological studies. From this study, it can be concluded that the crude seed extract revealed antioxidant and hepatoprotective activities. For that reason, in the future, oral intake of C. viscosa seed extract as an adjunct natural therapy may be worthwhile to protect against liver failure-mediated inhibitory effects on reproductive function.

1. Introduction

Industrial advancement has noxious consequences, and one such example is generation of carbon tetrachloride (CCl4) which is a prime potent ecological pollutant. Humans are exposed to carbon tetrachloride through oral, inhalation, and dermal routes [1, 2]. Being intoxicated with carbon tetrachloride is believed to be linked with excess free radical production in numerous organs, comprising the liver and kidney [3]. Carbon tetrachloride binds to liver cytochrome
P$_{450}$ to produce trichloromethyl free radicals (CCl$_3$) resulting in initiation of membrane lipid peroxidation [4]. Trichloromethylperoxy radical (OCCl$_3$), a secondary metabolic radical of CCl$_4$, reacts with lipids or proteins and as a result changes the permeability of the mitochondria, endoplasmic reticulum, and plasma membrane, leading to cell injury [5]. CCl$_4$-induced impairment correspondingly includes modification of the endogenous antioxidants in tissues which is demonstrated by histopathological lesions [6]. Research reports have indicated that the administration of carbon tetrachloride initiated male genotoxic effects in mouse bone marrow and germ cells [7] and elevated cholesterol, triglycerides, and free fatty acids in the liver as well as kidney of rats [8].

The liver, one of the vital organs in the human body, is responsible for metabolism and detoxification of potentially harmful xenobiotics. Its continuous exposure to toxicants, drugs, alcohol, and/or viruses lead to varying acute and chronic liver disorders with symptoms such as nausea, loss of appetite, tiredness, and jaundice [9]. Approximately, 2 million deaths are globally recorded each year as a result of liver diseases [10].

It is established that oxidative stress and free radicals play an important role in the etiology of liver disorders [11]. Oxidative stress is the state of an imbalance between reactive oxygen species (ROS) production and the ability of cells to neutralize them [12–15]. ROS are various forms of activated oxygen, which are generated as metabolic-by-products of biological processes including radicals such as superoxide anion (O$_2^-$), hydroxyl (OH$^-$), hydroperoxy (OH$_2^-$), peroxyl (ROO$^-$), alkoxyl (RO$^-$) and nonradicals such as singlet oxygen ($\cdot$O$_2$), hydrogen peroxide ($\cdot$H$_2$O$_2$), and lipid hydro peroxide (LOOH) [14, 16, 17]. ROS cause damage to biomolecules such as lipids, proteins, lipoproteins, membranes, and DNA. They are implicated in the pathogenesis of a host of degenerative and nondegenerative diseases including liver diseases. Exposure to chemicals such as carbon tetrachloride, ethanol, acetaminophen, benzene bromide, polycyclic aromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs) has been linked to hepatotoxicity and liver injury. Other commercially produced chemicals and environmental pollutants that are associated with hepatotoxicity in rodent models are reported in the literature [18–20]. Advances in orthodox medicine have fallen short to offer effective drugs for curing or protecting the liver of diseases, especially in less endowed countries where majority of the population cannot afford these drugs. In addition to this, most of the orthodox drugs have adverse effects such as insomnia, nausea, constipation, depression, and anxiety [21, 22]. Synthetic antioxidants such as tocopherol, tert-butylhydroquinone (TBHQ), butylated hydroxytoluene (BHT), and butylated hydroxyanisole (BHA) have been used as food supplements, but their toxicity, carcinogenicity, and other adverse effects have been cited [23, 24].

It is widely acknowledged that wild edible plants are important in terms of their pharmacological properties apart from food. Recently, several plants have attracted attention in terms of their pharmacology such as antioxidant, anticancer, antimicrobial, anti-diabetic, and anti-inflammatory properties. In addition to these, there are reports that edible or medicinal plants serve to prevent liver-related illnesses and improve liver health [25, 26]. For these reasons, medicinal plants have gained much attention as the foundation for the treatment or prevention of liver disorders because they offer rich sources of antioxidants and other phytoprinciples.

*Cleome viscosa* Linn. (family Capparaceae), commonly known as “wild mustard” or “dog mustard” in English, is an indigenous medicinal plant found as common weed and widely distributed in Africa and the tropics [27]. Traditionally, the plant is documented for the treatment of boils, earache, headache, ulcers, hemlinthic infections, fever, diarrhea, skin diseases [28], jaundice [29], malarial fevers, fever due to indigestion, leprosy, blood diseases, uterine complaints, and wounds [30]. Reported pharmacological properties exhibited by extracts and/or fractions of the various plant parts of *C. viscosa* include antipyretic, anti diarrheal, antimalarial, antinociceptive, anaesthetic, gastroprotective, analgesic, antiemetic, anthelmintic, antiscorbutic, anti-inflammatory, immunomodulatory, hepatoprotective, cytoxic, antimicrobial, mutagenic, and psychopharmacological [28, 30, 31]. It is established that antioxidants disrupt the ROS attack and radical mediated oxidative reactions, therefore have an ameliorating effect on the liver. In the present paper, we report the hepatoprotective activity of *C. viscosa* ethanol seed extract against CCl$_4$-induced liver injury in rats.

## 2. Materials and Methods

### 2.1. Collection and Extraction of Plant Samples.

The whole plant sample and seeds of *C. viscosa* were collected from Tono (10°52′13″N, 1°8′59″W), Upper East Region, Ghana, between October and December 2021. The plant was identified and authenticated by a plant taxonomist at the herbariums of Ghana Herbaria, Northern Savannah Biodiversity, and Savannah Herbarium. The voucher specimen (number SH 720) was deposited with the herbarium. The seeds were washed with distilled water and air-dried under shade for 2 weeks. They were ground into uniform powder.

### 2.2. Phytochemical Screening.

Phytochemical screening of the *C. viscosa* seed ethanol extract (CVE) for tannins and phenolic compounds, saponins, terpenes, reducing sugars, flavonoids, alkaloids, steroids, and anthraquinones was performed using modified standard protocols [32, 33].
2.3. Antioxidant Activity Assay. The 2,2-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay described by [33] with slight modification was used for the determination of antioxidant activity in vitro. DPPH (2 mL, 135 μM) in methanol (80%, v/v) was mixed with CVE (2 mL) at varying concentrations (6.25–200 μg/mL) prepared in methanol (80%, v/v). The mixture obtained was vortexed thoroughly and left to stand in the dark at room temperature. The absorbance was read using a spectrophotometer (SM 22PC Spectrophotometer, Surgifield Instrument, England) at 517 nm after 30 min. The blank was 80% (v/v) methanol. Ascorbic acid (AA) was used as the standard. The other control contained CVE in methanol at the same concentrations with no DPPH. The analysis was repeated three times on different days. The DPPH· scavenging effect was calculated using the following equation:

\[
\% \text{DPPH Radical Scavenging Activity} = \left( \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100\ %, \tag{1}
\]

where \(A_{\text{sample}}\) = absorbance of DPPH + CVE or AA at 517 nm; \(A_{\text{control}}\) = absorbance of DPPH + methanol at 517 nm.

GraphPad Prism® Version 8.02 (GraphPad Software Inc., USA) was employed to determine the \(IC_{50}\) value. The nonlinear regression (curve fit), log(inhibitor) vs. normalized response (variable slope) model was used.

2.4. DNA Nicking Induced by Hydroxyl Radical. DNA damage protection activity of CVE was carried out using the supercoiled Genomic DNA nicking assay described elsewhere [34] with little modifications. The reaction mixture consisted of 3 μL genomic DNA, 3 μL of CVE of varying concentrations (50, 100, 500, and 1000 μg/mL), 10 μL Fenton’s reagent (30 mM H₂O₂, and 50 mM ascorbic acid (AA) and 80 mM FeCl₃) and topped up with distilled water to make a final volume of 20 μL. The mixture was incubated for 30 min at 37°C. DNA was analysed on 1% agarose gel using ethidium bromide staining and photographed in the gel image system. Quercetin (50 μM) was used as a positive control.

2.5. Experimental Animals. Healthy albino rats (Wister strain) of both sexes, weighing between 110 and 177 g body weight (b.w.), were obtained from the Animal Husbandry of the Pharmacology Department, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana. All the animals were made to acclimatize to environmental conditions (normal ambient conditions of temperature and relative humidity with day/light 12/12 h cycle) for one week before commencement of the experiment. They were fed with standard pellet diet and fresh water ad libitum.

All processes with animals in the study were conducted in accord with national and institutional guiding principles for the maintenance and use of animals and ethical standards and practice. The animal studies were based on the guidelines of the University of Ghana Institutional Animal Care and Use Committee (UG-IACUC) and the National Agricultural Library (USDA) Animal Welfare Act Quick Reference Guides under the strict supervision of the KNUST veterinarian.

2.6. Safety Evaluation Study. A safety study to evaluate acute toxicity was carried out following acclimatization as described earlier [22]. Briefly, eight Wister albino rats were randomly selected and grouped into four \((n = 2\) per group), followed by administration of CVE of doses 10, 100, 1000, and 2000 mg/kg b.w., respectively. They were observed continuously for the first 6 h for behavioural changes, physiological changes, or death and then periodically for 72 h.

2.7. Evaluation of Hepatoprotective Activity. Wister albino rats were randomly selected and grouped into five groups (Groups I–V; \(n = 5\) in each group). Group I served as normal control and received only vehicles, that is, 0.9% normal saline (1 ml/kg b.w.) intragastrically and olive oil (3 ml/kg b.w.) intraperitoneally twice a week for 21 days. Group II was administered with CCl₄ (25% in olive oil) at a dose of 2 mg/kg b.w. intraperitoneally twice a week for 21 days. Group III received CCl₄ (25% in olive oil) at a dose of 2 mg/kg b.w. intraperitoneally on the first and fourth day in every week and then silymarin (100 mg/kg b.w.) intragastrically for the other five days in every week for 21 days. Groups IV and V were treated with CCl₄ (25% in olive oil) at a dose of 2 mg/kg b.w. intraperitoneally on the first and fourth day in every week and then CVE (200 mg/kg b.w. and 400 mg/kg b.w., respectively) intragastrically for the other five days in every week for 21 days. The animals were fed with standard pellet diet and fresh water ad libitum throughout the treatment period.

On the 22nd day, each group of animals was anesthetized by an intravenous dose of ketamine (50 mg/kg b.w.) and xylazine (5 mg/kg b.w.). Blood samples were collected from retro orbital plexus into red coded blood tubes for serum biochemical marker enzyme determination. The animals were then humanely sacrificed by cervical dislocation, following which the liver was carefully dissected, cleaned of extraneous tissue, and fixed in 10% formalin for histopathological analysis.

2.8. Serum Biochemical Marker Parameter Determination. Blood samples were allowed to stand for 30 min at room temperature and then centrifuged (Centro 8, J. P. Selecta,
Spain) at 4000 rpm for 15 min to obtain the sera. The sera were used for estimation of aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), and total protein (TP) using the Clinical Chemistry Analyser (Cobas C111, Roche Diagnostics, Germany).

2.9. Histopathological Assessment. Paraffin sections were prepared (automatic tissue processor, auto technique) and cut into 5 μm thick sections, using a rotary microtome. The sections were stained with haematoxylin and eosin (H&E). Mounted slides were examined for histopathological changes in the liver, and their micrographs were taken.

2.10. Statistical Analysis. Data were expressed as the mean ± standard deviation (SD). Statistical analyses were performed using ANOVA followed by Tukey’s post hoc test for multiple comparisons between the groups; p < 0.5 was considered statistically significant. Data were processed using GraphPad Prism® Version 8.02 (GraphPad Software Inc., USA).

3. Results

3.1. Phytochemical Screening. Qualitative phytochemical screening of *C. viscosa* seed ethanol extract (CVE) revealed the presence of alkaloids, flavonoids, steroids, saponins, terpenes, tannins, and phenolic compounds. Anthraquinones and reducing sugars tested negative (Table 1).

3.2. In vitro Antioxidant Activity. The antioxidant activity of CVE was evaluated in vitro using the DPPH radical scavenging assay. The scavenging activity (%) was dose-dependent, increasing as the concentration of CVE increases. The extract showed the highest antioxidant activity (90.08 ± 0.08%) at 200 μg/ml, though this was significantly lower (p < 0.05) compared with the reference drug, ascorbic acid (AA) (96.16 ± 0.29%). Similarly, the antioxidant activity of CVE was significantly lower than that of AA at all concentrations. The IC₅₀ was 17.82 ± 0.32 μg/ml for CVE and 4.53 ± 0.12 μg/ml for AA (Figure 1).

3.3. Protective Effects of Different Concentrations of CVE on Genomic DNA Damage. The hydroxyl radical is the most reactive among reactive oxygen species (ROS) and has the shortest half-life compared with similar ROS. It is considered to be responsible for much of the biological damage in free-radical pathology. The radical has the capacity to cause strand breakage in DNA, which contributes to carcinogenesis, mutagenesis, and cytotoxicity [35, 36]. The DNA protective effect of ethanol seed extract of *C. viscosa* was assessed against Fenton’s induced DNA damage of genomic DNA. The protection offered against DNA damage by CVE (250–1000 μg/μL) was concentration-dependent (Figure 2). At concentration of 1000 μg/mL, CVE protection was more effective and slightly close to that of 50 μM of quercetin experimented. Genomic DNA revealed the three forms, circular (single stranded), supercoiled, and linear, whereas DNA + Fenton’s reagent with the concentrations of 250 and 500 μg/mL of CVE exhibited complete degradation of DNA. The highest concentration showed very good protective activity and retained all three forms of DNA. The current results indicate that seed extract of *C. viscosa* effectively mitigates the oxidative stresses on predisposed biomolecules, such as DNA.

### Table 1: Qualitative phytochemical profile of *Cleome viscosa* L. seed ethanol extract (CVE).

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>CVE</th>
</tr>
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<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>–</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Reducing sugars</td>
<td>–</td>
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<tr>
<td>Saponins</td>
<td>+</td>
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<tr>
<td>Steroids</td>
<td>+</td>
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<tr>
<td>Tannins and phenolic compounds</td>
<td>+</td>
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<tr>
<td>Terpenes</td>
<td>+</td>
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+Detected; – no detection.

3.4. Effects of CVE on CCl₄-Induced Hepatotoxicity. At the end of the treatment period, it was observed that there was a significant elevation (p < 0.05) of the serum enzymes ALT, AST, and ALP in rats administered with only 2 mg/kg b.w. CCl₄ (Group II) compared with the normal control group (Group I). However, treatment of rats initially intoxicated with CCl₄ followed by the reference drug, 100 mg/kg b.w. silymarin (Group III) or 200 mg/kg b.w. and 400 mg/kg b.w. CVE (Groups IV and V, respectively), showed significantly reduced levels of the enzymes (p < 0.05) compared with the Group II animals (Figure 3). It was also noticed that the reduction of the levels of ALP in Groups III and V, AST in Group V, and ALT in Group V were significantly at par compared with the normal control group (Group I) (p > 0.05). In the case of AST, its level was significantly lower in the group treated with 400 mg/kg b.w. (Group V) compared with those treated with the reference drug, 100 mg/kg b.w. silymarin (Group III). Similarly, there was a significant reduction (p < 0.05) in the serum total protein (TP) in the group administered with 2 mg/kg b.w. CCl₄ (Group II) compared with the normal control (Group I). It was noticed that groups treated with silymarin (100 mg/kg b.w., Group III) and CVE (200 and 400 mg/kg b.w., Groups IV and V, respectively) showed significantly higher TP (p < 0.05) when compared with those in Group II. On the other hand, the level of TP in Groups III to V was significantly the same when compared with control Group I (p > 0.05) (Figure 4). It also observed that the level of TP in Group V was significantly higher (p < 0.05) compared with those administered with the reference drug silymarin (Group III).

3.5. Histopathological Evaluation of the Effect of CVE on CCl₄-Induced Liver Injury. Histopathological examinations by H&E Liver Injury. Histopathological examinations by H&E showed significant elevation in macrovesicular and microvesicular steatosis in Group II and Group III.
However, only microvesicular steatosis was found in Group IV and Group V. In addition to steatosis, fibrosis consistent with cirrhosis was found in Group II and Group III after CCl4 treatment. Contrary to the CCl4-induced group and silymarin supplementation group, the 200mg/kg b.w. and 400mg/kg b.w. groups had significant reduction in steatosis and fibrosis ($p < 0.05$). (Figures 5(a)–5(e)).

### 4. Discussion

The phytochemical screening process was positive for alkaloids, flavonoids, saponins, steroids, terpenes, tannins, and phenolic compounds in CVE. The presence of these compounds corroborates, in part, the report of [37, 38] who revealed the presence of alkaloids, saponins, flavonoids, tannins, and phenolic compounds in petroleum ether and ethyl acetate seed extracts. In contrast to this study, [37] reported anthraquinones to be present in the petroleum ether extract. Steroids, flavonoids, and tannins were also found present in the methanol seed extract [39]. Earlier studies elsewhere have also documented the presence of phytochemicals including those screened in the current work in various solvent extracts of the whole plant and other plant parts of *C. viscosa* [30, 40–42].

The fact that anthraquinones and reducing sugars gave a negative result in our study does not necessarily mean they are completely absent in the plant seed. It could be explained that their concentrations were below the detection limits of the test reagents used, resulting in the negative result observed. In addition, variations in phytochemical screening may be linked to agro-ecological conditions, varieties, extraction methods, plant parts, and solvents used. Two flavones (viscocic and viscosin), coumarinolignoids (cleomiscosins A, B, and C), lupeol, and other compounds have been isolated from the seed [28, 38, 43].

It is suggested that phytochemicals are effective in treating various diseases owing to their antioxidant and anti-inflammatory activities, and this could explain the traditional usage and pharmacological properties of the seed and other plant parts of *C. viscosa*. For example, alkaloids, one of the largest group of compounds in plants, provoke both toxic and desirable pharmacological response in vertebrates. Cytotoxicity, antimicrobial, anti-inflammatory, antimalarial, antipyretic, cardioprotective, immunoregulative, antioxidant, cerebroprotective, antimitogenic, and analgesic

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**Figure 1:** DPPH free radical scavenging activity of CVE. Each value is expressed as mean ± SD ($n = 3$). *Significantly different in each concentration group ($p < 0.05$). CVE = *Cleome viscosa* L. seed ethanol extract; AA = ascorbic acid.

**Figure 2:** Effect of CVE on the protection of genomic DNA against H2O2 induced hydroxyl radical. Lane 1: genomic DNA alone; lane 2: genomic DNA + Fenton’s; lane 3: genomic DNA + Fenton’s + quercetin; lane 4: genomic DNA + Fenton’s + 250 µg/mL CVE; lane 5: genomic DNA + Fenton’s + 500 µg/mL CVE; lane 6: genomic DNA + Fenton’s + 1000 µg/mL CVE.
Activities of alkaloids are well documented in the literature. Phenolic compounds, tannins, terpenes, and flavonoids are believed to act against free radicals, free radical mediated cellular signaling, inflammation, allergies, platelet aggregation, and hepatotoxins [44]. Plant steroids are suggested to stimulate the immune system and decrease cholesterol levels. They are also known to possess antitumor, immunosuppressive, hepatoprotective, cardiotonic, antiviral, and anti-inflammatory properties [45].

The present study has revealed that free radical scavenging activity of CVE increases in a dose-dependent manner (Figure 1). A similar trend was observed in the standard drug ascorbic acid (AA). The fact that the IC50 (17.82 ± 0.32 μg/mL) of CVE was higher than that of the standard drug AA (4.53 ± 0.12 μg/mL), an indication that the free radical scavenging effect of AA is significantly (p < 0.05) better than that of CVE; AA being a pure compound compared to CVE should be taken into consideration. The DPPH free radical scavenging assay used in this study is one of the most frequently utilized in vitro models for evaluating antioxidant activity because it is simple, rapid, sensitive, reproducible [46], and less costly compared to in vivo assays [17].

Gupta et al. [47] reported that methanol (70%, v/v) extracts of C. viscosa leaf and stem parts showed radical scavenging activities in DPPH with IC50 values of 373.18 and 511.10 μg/mL, respectively. In a similar work on the aerial part extracts of C. viscosa, the IC50 value was found to be in the order methanol (101.25 μg/mL) > ethyl acetate (105.63 μg/mL) > chloroform (115.46 μg/mL) > hexane (111.51 μg/mL) [41]. The presence of phenolic

Figure 3: Effect of CVE on serum biochemical maker enzymes. Data are expressed as mean ± SD (n = 5 in each group); group I = normal control (0.9% N-saline, 1 mg/kg b.w.); group II = CCl4 (2 mg/kg b.w.); group III = CCl4 + silymarin (100 mg/kg b.w.); group IV = CCl4 + CVE (200 mg/kg b.w.); group V = CCl4 + CVE (400 mg/kg b.w.); * significantly different as compared with Group I (p < 0.05); # significantly different as compared with Group II (p < 0.05); ¥ significantly different as compared with Group III (p < 0.05). ALT = alanine transaminase; AST = aspartate transaminase; ALP = alkaline phosphatase.

Figure 4: Effect of CVE on serum total protein. Data are expressed as mean ± SD (n = 5 in each group); group I = normal control (0.9% N-saline, 1 mg/kg b.w.); group II = CCl4 (2 mg/kg b.w.); group III = CCl4 + silymarin (100 mg/kg b.w.); group IV = CCl4 + CVE (200 mg/kg b.w.); group V = CCl4 + CVE (400 mg/kg b.w.); * significantly different as compared with Group I (p < 0.05); # significantly different as compared with Group II (p < 0.05); ¥ significantly different as compared with Group III (p < 0.05).
compounds and flavonoids in CVE could be responsible for the observed antioxidant activity. This activity is believed to be a redox process, where polyphenolic compounds scavenge on the stable free radical DPPH by acting as reducing agents, hydrogen donors, or electron donors (Scheme 1) [17, 48].

It is established that \( \cdot \)OH radicals damage DNA by reacting with base pairs of DNA producing base radicals and sugar radicals in a variety of mechanisms [17, 34]. In this work, the ability of CVE of varying concentrations (1000, 500, 100, and 50 \( \mu \)g/mL) to shield DNA from damage was studied in vitro. Genomic DNA damage was induced by Fenton’s reagent, which generated the \( \cdot \)OH radicals. Gallic acid was used as the reference antioxidant. It was observed that the CVE at the highest concentration (1000 \( \mu \)g/mL) was able to protect DNA from damaging, shown as a bright band in lane 1. The CVE at the other concentrations (lanes 2–4), however, did not show any DNA protection activity (Figure 2). DNA protection could be due to antioxidant compounds that act as electron donors to reduce \( \mathrm{H}_2\mathrm{O}_2 \) to \( \mathrm{H}_2\mathrm{O} \) [46], thus preventing the formation of \( \cdot \)OH radicals to attack DNA. The presence of phenolic compounds and flavonoids in CVE from our phytochemical analysis (Table 1) may be responsible for the observed DNA protection. Another mechanism relates that flavonoids prevent the production of ROS by forming complexes with cations (eg. Cu\(^{2+}\) and Fe\(^{2+}\)) that participate in \( \cdot \)OH radical formation [34]. Literature search indicates that this is the first time \( \textit{C. viscosa} \) seed extract has been evaluated for its in vitro protective activity against DNA damage. This notwithstanding, numerous studies have been carried out on the protection potential of plant extracts on DNA damage in vitro [34, 46, 49–51].

Carbon tetrachloride (CCl\(_4\)) is widely used in animal models to study chemically induced hepatotoxicity. The hepatotoxicity of CCl\(_4\) involves reductive dechlorination to a trichloromethyl free radical (\( \cdot \)CCl\(_3\)) and then to

**Figure 5:** Photomicrographs of liver cells under (H&E x 100). (a) Normal (Group I); (b) CCl\(_4\) intoxicated group (Group II); (c) CCl\(_4\) + silymarin (Group III); (d) CCl\(_4\) + CVE (200 mg/kg b.w.) (Group IV); (e) CCl\(_4\) + CVE (400 mg/kg b.w.) (Group V). Yellow arrows represent macrovascular steatosis, red arrows represent microvascular steatosis and black arrows represent fibrosis.
trichloromethyl peroxy radical (·OOCCl$_3$), which binds to protein and initiates lipid peroxidation by abstracting a hydrogen atom from polyunsaturated fatty acid (PUFA) of a phospholipid. The metabolic activation of CCl$_4$ is catalyzed by CYP2E1 [52].

CCl$_4$-induced liver injury results in steatosis, fibrosis, and cirrhosis leading to the leaching of various cellular contents into the blood and loss of functional integrity of the liver. For example, increase in aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) in the serum is an indicative of hepatic injury. In this study, it was observed that there was a significant elevation ($p < 0.05$) of these enzymes in the rats intoxicated with CCl$_4$ compared to the normal group, suggesting liver damage resulting from membrane lipid peroxidation possibly by ·CCl$_3$ and ·OOCCl$_3$ free radicals, formed during bioactivation of CCl$_4$. However, treatment of the rats with CVE reversed the liver damage, shown by a significant reduction ($p < 0.05$) in the serum biomarkers compared with the CCl$_4$ treatment group. The restoration of the levels of these enzymes by CVE was comparable to the standard drug silymarin ($p > 0.05$). Interestingly, CVE at a higher concentration (400mg/kg b.w.) was able to reduce the AST level significantly ($p < 0.05$) than silymarin (Figure 3).

ALT and AST are considered hepatic enzymes due to their high concentrations in the hepatocytes. However, ALT is highly selective and regarded as the most liver-specific enzyme compared to AST since the former is found only in cytosol of hepatocytes, while the latter also occurs in the skeletal muscle, cardiac cells, and kidney cells [53]. The main source of ALP is the liver, but it is also found in the intestine, pancreas, kidney, and bones. An increase in serum ALP, which serves as a biomarker of hepatobiliary injury, is thought to arise from an increased accumulation of bile acids in the hepatocytes, hence causing ALP to be released into the blood [52].

A significant decrease ($p < 0.05$) of serum TP observed in the CCl$_4$-treated group when compared to the normal control group suggests a disruption and dissociation of polyribosomes from the endoplasmic reticulum following CCl$_4$ exposure. This confirms the elevation of ALP in this group of rats since this enzyme is believed to help in the breakdown of proteins. However, treatment of rats with CVE increased the serum TP levels significantly ($p < 0.05$) compared to the CCl$_4$-induced toxicant group. In a similar fashion to what was observed in the serum marker enzymes, the activity of CVE at 400 mg/kg b.w. against serum TP was significantly higher when compared to the standard drug silymarin ($p < 0.05$) (Figure 4). The increase in TP could be attributed to stimulation of protein synthesis by the phytochemicals present in CVE, thus stabilizing endoplasmic reticulum causing the acceleration of the regeneration process of hepatocytes.

The previous study by [54] established that the ethanol leaf extract of C. viscosa has hepatoprotective activity against CCl$_4$-induced liver injured male albino rats of Druckrey strain, thus justifying the use of the plant to treat jaundice. Mobiya et al. [55] demonstrated that ethanol extract of C. viscosa seed was able to reinstate liver function in paracetamol-induced hepatotoxic albino rats. In addition to free radical scavenging activity, other pharmacological properties such as anti-inflammatory, anticholesterol, and collagen inhibitory activities attributed to the observed phytochemicals in CVE may have contributed to the restoration of the liver integrity after exposure to CCl$_4$.

Histopathological studies revealed a significant elevation in macrovesicular and microvesicular steatosis in Group II and Group III, which decreased with 200 mg/kg b.w. and 400 mg/kg b.w. CVE supplementation evidenced by the absence of macrovesicular steatosis in the latter two groups. Of note, after exposure to the hepatotoxic xenobiotic, CCl$_4$ leads to severe liver necrosis and steatosis [1, 56]. Metabolism of CCl$_4$ in hepatocytes catalyzed by CYP2E1 culminates in the production of highly unstable and reactive free radicals, trichloromethyl (·CCl$_3$) and trichloromethyl peroxy (·OOCCl$_3$) metabolites which play a crucial role in lipid peroxidation and oxidative stress. These free radicals covalently bond to fatty acids of membrane phospholipids and a number of cellular macromolecules hence inducing cell membrane lipid peroxidation through disruption of a number of double and triple bonds in polyunsaturated fatty acids within these cell membranes leading to the initiation and propagation of free radical chain reactions [1]. However, increasing doses of CVE supplementation after CCl$_4$-induced hepatotoxicity moderately ameliorated further lipid peroxidation leading to the elimination of macrovesicular steatosis. It can be inferred that although the antioxidant activity of CVE was slightly lower than the reference drug, ascorbic acid, it was able to adequately scavenge free radicals hence regulating lipid peroxidation. It is noteworthy that establishing an appropriate

![DPPH free radical](image1.png)

**Scheme 1:** Reaction of DPPH free radical with phenolic compound.
dose of CVE with an antioxidant activity higher than ascorbic acid in a future study will totally remove both microvesicular and macrovesicular steatosis.

In addition to steatosis, CCl₄ hepatic fibrosis consistent with cirrhosis was found in Group II and Group III after CCl₄ treatment (Figures 5(b) and 5(c)). Contrary to the CCl₄-induced group and silymarin supplementation group (Figures 5(b) and 5(c)), the CVE-treated groups (Groups IV and V; 200 mg/kg b.w. and 400 mg/kg b.w., respectively) had significant reduction in fibrosis ($p < 0.05$) (Figures 5(d) and 5(e)). Meanwhile, the normal control group (Group I) which received only vehicles, 0.9% normal saline (1 ml/kg b.w.), did not exhibit any breach in liver integrity (Figure 5(a)).

Liver fibrosis is the genesis of liver cirrhosis. It is as a result of continuous injury and repair (necrosis and apoptosis) mechanisms of hepatocytes caused by viral hepatitis, drugs, metabolic liver disease, fatty liver disease, and autoimmune liver disease [57]. CCl₄ directly damages hepatocytes and liver parenchyma cells in the hepatic portal vein region leading to an alteration in lysosomal and mitochondrial membrane permeability [58]. Central lobular necrosis is formed by highly active free radical metabolites through CYP2E1 [59]. In addition, collagen expression is found to be significantly increased after CCl₄ injury [60]. Our reference drug, silymarin, was not able to reverse fibrosis after CCl₄-induced hepatotoxicity, whereas there was an increasing trend of CVE amelioration of fibrosis in CCl₄ hepatotoxicity. This suggests that CVE provides a sufficient antioxidant capacity (activity) in repairing the damage caused by CCl₄ compared to silymarin. This can further be evidenced by an improvement in clinical chemistry parameters such as TP, ALT, AST, and ALP post-CVE treatment after CCl₄-induced hepatotoxicity.

Our findings indicate CCl₄ induced steatosis and fibrosis in our albino rat model. Silymarin could not reverse steatosis and fibrosis; however, there was a trend in improvement of histology of CCl₄-treated rats’ livers due to CVE supplementation for inhibition of steatosis, fibrosis, and hepatotoxicity. Hence, our study provides a baseline upon which further studies on the hepatoprotective effect of CVE can be referenced.

5. Conclusion

In the present study, the results show that the crude seed extract of C. viscosa demonstrated antioxidant and hepatoprotective activities. The hepatoprotective activities of the plant extract could be due to their free radical scavenging and antioxidant activities, resulting from the presence of some phytochemicals including phenolic compounds, flavonoids, saponins, and alkaloids. Our findings indicate that CCl₄ induced steatosis and fibrosis in our albino rats’ model. Silymarin could not reverse steatosis and fibrosis; however, there was a trend in improvement of histology of CCl₄-treated rat livers due to CVE supplementation for inhibition of steatosis, fibrosis, and hepatotoxicity. Hence, our study provides a baseline upon which further studies on the hepatoprotective effect of CVE can be referenced. In addition, the exact phytoconstituents and their mechanism of hepatoprotection is the next study in our laboratory. Furthermore, these findings validate the traditional use of the seed extract of Cleome viscosa in liver disorders and propose the potential exploitation of the seeds of the plant as a source of novel compounds for hepatoprotective activity. Nugraha et al. [61] have reported many epiphytic medicinal plants as having promising pharmacological activities including anti-inflammatory, antimicrobial, and anticancer activities.

Data Availability

The data used and/or analysed during the current study are available upon reasonable request from the corresponding author.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors’ Contributions

AMD, KAAK, and EA conceived and designed the study; KAAK and EA collected the plant samples and performed the experiments; BA analysed and interpreted the histopathology results; AMD and MND performed statistical/data analysis and drafted the initial manuscript; AMD, MND, BA, KAAK, EA, and RM edited and revised the final version of the manuscript.

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