Hindawi

Research Article

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

Addai-Mensah Donkor [ID],[1,2] **Martin Ntiamoah Donkor** [ID],[3] **Benjamin Ahenkorah,**[4] **Kwadwo Asante Asare-Konadu,**[5] **Emmanuel Asiedu,**[5] **and Richard Mosobil**[6]

[1]*Department of Pharmaceutics, School of Pharmacy and Pharmaceutical Sciences, University for Development Studies, Tamale, Ghana*
[2]*Department of Pharmaceutical Chemistry, School of Pharmacy and Pharmaceutical Sciences, University for Development Studies, Tamale, Ghana*
[3]*Department of Biochemistry and Forensic Sciences, School of Chemical and Biochemical Sciences, C. K. Tedam University of Technology and Applied Sciences, Navrongo, Ghana*
[4]*Department of Medical Laboratory Science, Bolgatanga Technical University, Bolgatanga, Ghana*
[5]*Department of Biochemistry, Faculty of Applied Sciences, University for Development Studies, Tamale, Ghana*
[6]*Department of Applied Chemistry, School of Chemical and Biochemical Sciences, C. K. Tedam University of Technology and Applied Sciences, Navrongo, Ghana*

Correspondence should be addressed to Addai-Mensah Donkor; dr.adonkor@uds.edu.gh and Martin Ntiamoah Donkor; mdonkor@cktutas.edu.gh

Liver ailment is a key public health menace, principally in developing nations. Quite a lot of medicinal florae 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 CCl_4-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 IC_{50} value of $17.82 \pm 0.32\,\mu$g/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 (CCl_4) 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 ($\cdot CCl_3$) resulting in initiation of membrane lipid peroxidation [4]. Trichloromethylperoxy radical ($\cdot OOCCl_3$), 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 ($\cdot O_2^-$), hydroxyl ($\cdot OH$), hydroperoxyl ($\cdot O_2H$), peroxyl ($ROO\cdot$), alkoxyl ($RO\cdot$) and nonradicals such as singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_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 pathogeneses 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, antidiabetic, 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, helminthic 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, antidiarrheal, antimalarial, antinociceptive, anaesthetic, gastroprotective, analgesic, antiemetic, anthelmintic, antiscorbutic, anti-inflammatory, immunomodulatory, hepatoprotective, cytotoxic, 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. The powdered plant sample (100 g) was then macerated with ethanol (70% v/v, 1 L) at room temperature for 72 h. The extract was filtered through the Whatman No 1 filter paper and concentrated using the rotary evaporator (Laborota 4001 Efficient, Heidolph Instruments GMBH, Germany), warmed on water bath at 70°C to obtain a semisolid product. It was kept in the refrigerator at 4°C until further use. Genomic DNA of cocoa (*Theobroma cacao* L.) leaves was obtained from the Molecular and Microbiology Laboratory of the Cocoa Research Institute of Ghana (CRIG), New Akyem Tafo, Eastern Region, Ghana.

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, Surgienfield 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:

$$\% \text{ DPPH Radical Scanvenging Activity} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100\,\%, \tag{1}$$

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 H_2O_2, and 50 mM ascorbic acid (AA) and 80 mM $FeCl_3$) 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 CCl_4 (25% in olive oil) at a dose of 2 mg/kg b.w. intraperitoneally twice a week for 21 days. Group III received CCl_4 (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 CCl_4 (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 IC$_{50}$ 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

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

Phytochemicals	CVE
Alkaloids	+
Anthraquinones	−
Flavonoids	+
Reducing sugars	−
Saponins	+
Steroids	+
Tannins and phenolic compounds	+
Terpenes	+

+Detected; −no detection.

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.

3.4. Effects of CVE on CCl$_4$-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. CCl$_4$ (Group II) compared with the normal control group (Group I). However, treatment of rats initially intoxicated with CCl$_4$ 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. CCl$_4$ (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 CCl$_4$-Induced Liver Injury. Histopathological examinations by H&E stain showed significant elevation in macrovesicular and microvesicular steatosis in Group II and Group III.
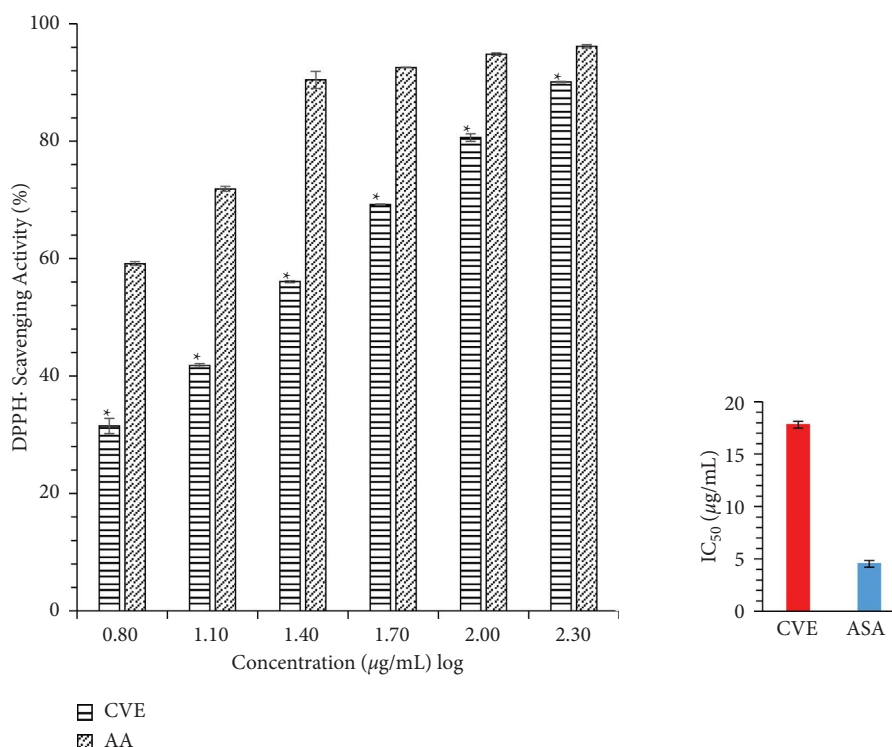
FIGURE 1: DPPH free radical scavenging activity of CVE. Each value is expressed as mean $\pm$ SD ($n = 3$). *Significantly different in each concentration group ($p < 0.05$). CVE = *Cleome viscosa* L. seed ethanol extract; AA = ascorbic acid.
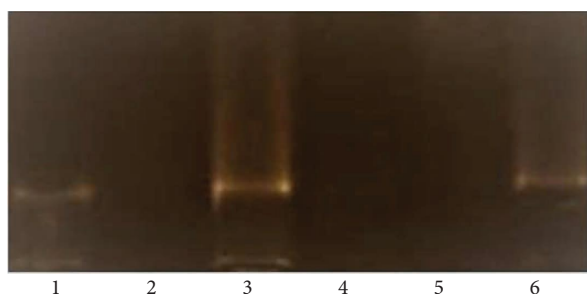

FIGURE 2: Effect of CVE on the protection of genomic DNA against H_2O_2 induced hydroxyl radical. Lane 1: genomic DNA alone; lane 2: genomic DNA + Fenton's; lane 3: genomic DNA + Fenton's + quercentin; lane 4: genomic DNA + Fenton's + 250 μg/mL CVE; lane 5: genomic DNA + Fenton's + 500 μg/mL CVE; lane 6: genomic DNA + Fenton's + 1000 μg/mL CVE.

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 CCl_4 treatment. Contrary to the CCl_4-induced group and silymarin supplementation group, the 200 mg/kg b.w. and 400 mg/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, antimutagenic, and analgesic
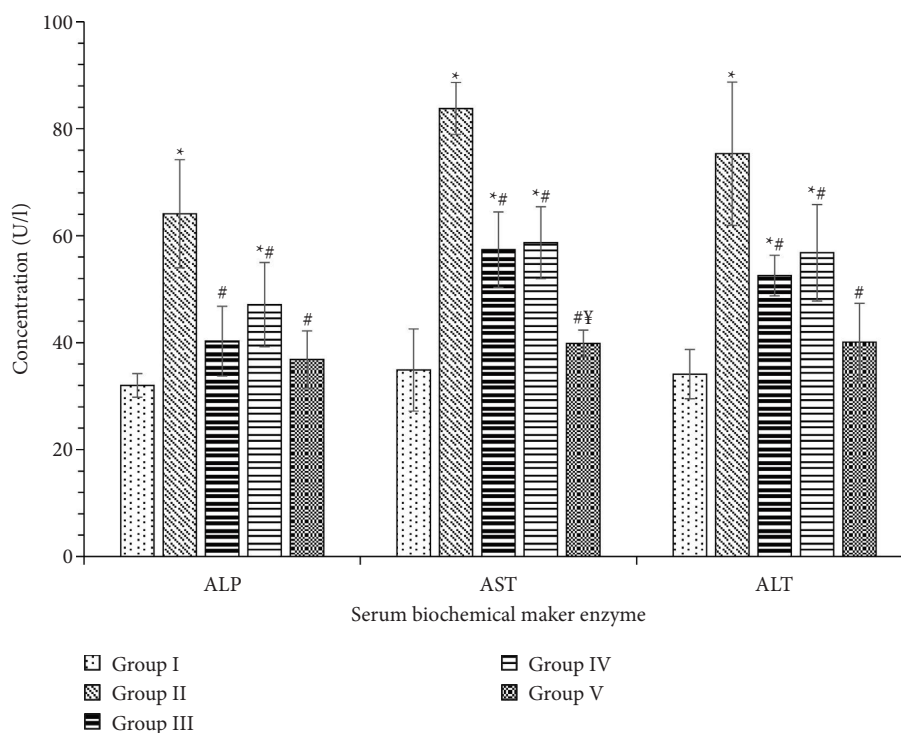
FIGURE 3: Effect of CVE on serum biochemical maker enzymes. Data are expressed as mean $\pm$ SD ($n = 5$ in each group); group I = normal control (0.9% N-saline, 1 mg/kg b.w.); group II = CCl$_4$ (2 mg/kg b.w.); group III = CCl$_4$ + silymarin (100 mg/kg b.w.); group IV = CCl$_4$ + CVE (200 mg/kg b.w.); Group V = CCl$_4$ + 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.
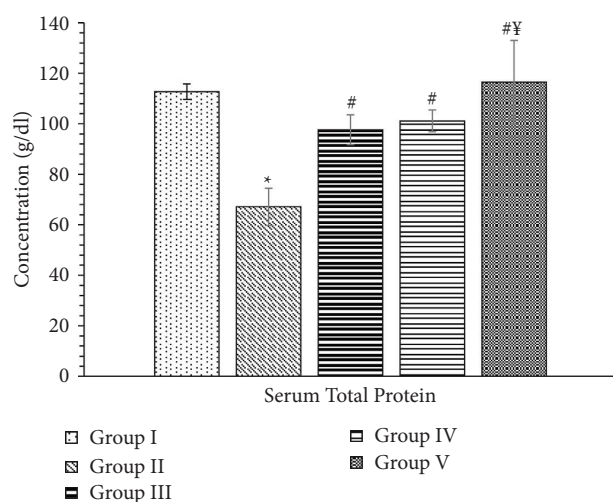

FIGURE 4: Effect of CVE on serum total protein. Data are expressed as mean $\pm$ SD ($n = 5$ in each group); Group I = normal control (0.9% N-saline, 1 mg/kg b.w.); Group II = CCl$_4$ (2 mg/kg b.w); Group III = CCl$_4$ + Silymarin (100 mg/kg b.w.); Group IV = CCl$_4$ + CVE (200 mg/kg b.w.); Group V = CCl$_4$ + 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 ($p < 0.05$).

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 IC$_{50}$ (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 IC$_{50}$ values of 373.18 and 511.10 μg/mL, respectively. In a similar work on the aerial part extracts of *C. viscosa*, the IC$_{50}$ 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
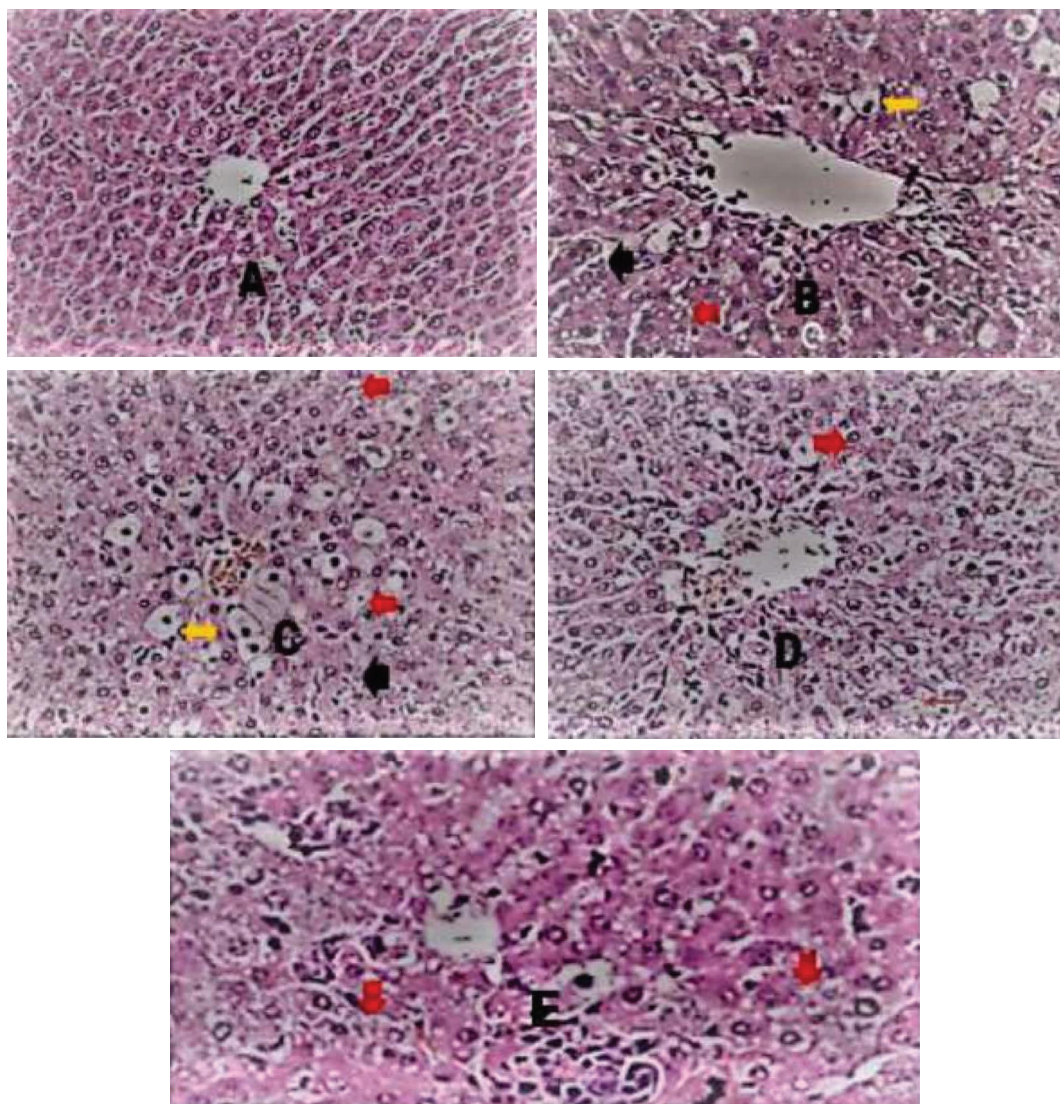
FIGURE 5: Photomicrographs of liver cells under (H&E x 100). (a) Normal (Group I); (b) CCl$_4$ intoxicated group (Group II); (c) CCl$_4$ + silymarin (Group III); (d) CCl$_4$ + CVE (200 mg/kg b.w.) (Group IV); (e) CCl$_4$ + CVE (400 mg/kg b.w.) (Group V). Yellow arrows represent macrovascular steatosis, red arrows represent microvascular steatosis and black arrows represent fibrosis.

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 ·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 µg/mL) to shield DNA from damage was studied in vitro. Genomic DNA damage was induced by Fenton's reagent, which generated the ·OH radicals. Gallic acid was used as the reference antioxidant. It was observed that the CVE at the highest concentration (1000 µg/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 H$_2$O$_2$ to H$_2$O [46], thus preventing the formation of ·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 ·OH radical formation [34]. Literature search indicates that this is the first time 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 (·CCl$_3$) and then to
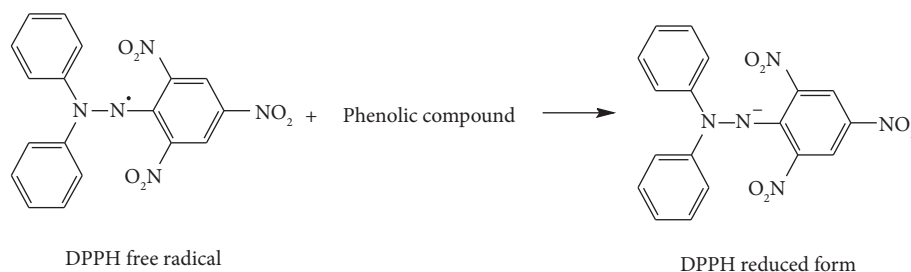
Scheme 1: Reaction of DPPH free radical with phenolic compound.

trichloromethyl peroxyl radical ($\cdot OOCCl_3$), 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 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 $\cdot CCl_3$ and $\cdot OOCCl_3$ 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 (400 mg/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 disassociation 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 ($\cdot CCl_3$) and trichloromethylperoxy ($\cdot OOCCl_3$) 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

dose of CVE with an antioxidant activity higher than ascorbic acid in a future study will totally remove both microvesicular and microvesicular steatosis.

In addition to steatosis, CCl_4 hepatic fibrosis consistent with cirrhosis was found in Group II and Group III after CCl_4 treatment (Figures 5(b) and 5(c)). Contrary to the CCl_4-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]. CCl_4 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 CCl_4 injury [60]. Our reference drug, silymarin, was not able to reverse fibrosis after CCl_4-induced hepatotoxicity, whereas there was an increasing trend of CVE amelioration of fibrosis in CCl_4 hepatotoxicity. This suggests that CVE provides a sufficient antioxidant capacity (activity) in repairing the damage caused by CCl_4 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 CCl_4-induced hepatotoxicity.

Our findings indicate CCl_4 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 CCl_4-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 CCl_4 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 CCl_4-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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References

[1] M. K. Manibusan, M. Odin, and D. A. Eastmond, "Postulated carbon tetrachloride mode of action: a review," *Journal of Environmental Science and Health, Part C*, vol. 25, no. 3, pp. 185–209, 2007.

[2] H. Elsawy, G. M. Badr, A. Sedky, B. M. Abdallah, A. M. Alzahrani, and A. M. Abdel-Moneim, "Rutin ameliorates carbon tetrachloride (CCl_4)-induced hepatorenal toxicity and hypogonadism in male rats," *PeerJ*, vol. 7, no. 7, Article ID e7011, 2019.

[3] K. C. Preethi and R. Kuttan, "Hepato and reno protective action of Calendula officinalis L. Flower extract," *Indian Journal of Experimental Biology*, vol. 47, no. 3, pp. 163–168, 2009.

[4] M. S. Abdel-Kader, A. T. Abulhamd, A. M. Hamad, A. H. Alanazi, R. Ali, and S. I. Alqasoumi, "Evaluation of the hepatoprotective effect of combination between hinokiflavone and Glycyrrhizin against CCl_4 induced toxicity in rats," *Saudi Pharmaceutical Journal*, vol. 26, no. 4, pp. 496–503, 2018.

[5] M. M. Rahman, A. Y. Muse, D. I. O. Khan et al., "Apocynin prevented inflammation and oxidative stress in carbon tetrachloride induced hepatic dysfunction in rats," *Biomedicine & Pharmacotherapy*, vol. 92, pp. 421–428, 2017.

[6] G. M. Alshammari, A. Balakrishnan, and T. Chinnasamy, "2-Hydroxy-4-methoxy benzoic acid attenuates the carbon tetrachloride-induced hepatotoxicity and its lipid

abnormalities in rats via anti-inflammatory and antioxidant mechanism," *Inflammation Research*, vol. 66, no. 9, pp. 753–763, 2017.

[7] K. A. Diab, M. A. Fahmy, Z. M. Hassan, E. M. Hassan, A. B. Salama, and E. A. Omara, "Genotoxicity of carbon tetrachloride and the protective role of essential oil of Salvia officinalis L. in mice using chromosomal aberration, micronuclei formation, and comet assay," *Environmental Science & Pollution Research*, vol. 25, no. 2, pp. 1621–1636, 2018.

[8] S. Marimuthu, R. S. Adluri, R. Rajagopalan, and V. P. Menon, "Protective role of ferulic acid on carbon tetrachloride-induced hyperlipidemia and histological alterations in experimental rats," *Journal of Basic and Clinical Physiology and Pharmacology*, vol. 24, no. 1, pp. 59–66, 2013.

[9] Y. C. Lin, K. M. Cheng, H. Y. Huang et al., "Hepatoprotective activity of Chhit-Chan-Than extract powder against carbon tetrachloride- induced liver injury in rats," *Journal of Food and Drug Analysis*, vol. 22, no. 2, pp. 220–229, 2014.

[10] S. K. Asrani, H. Devarbhavi, J. Eaton, and P. S. Kamath, "Burden of liver diseases in the world," *Journal of Hepatology*, vol. 70, no. 1, pp. 151–171, 2019.

[11] M. Sevindik, H. Akgul, M. Pehlivan, and Z. Selamoglu, "Determination of therapeutic potential of *Mentha longifolia* Ssp," *Longifolia. Fresenius Environ Bull*, vol. 26, no. 7, pp. 4757–4763, 2017.

[12] O. Unal, E. C. Eraslan, I. Uysal, F. S. M. M. Sevindik, and H. Akgul, "Biological activities and phenolic contents of *Rumex scutatus* collected from Turkey," *Fresenius Environmental Bulletin*, vol. 31, no. 7, pp. 7341–7346, 2022.

[13] F. S. Mohammed, E. Kına, M. Sevindik, M. Doğan, and M. Pehlivan, "Antioxidant and antimicrobial activities of ethanol extract of *Helianthemum salicifolium* (Cistaceae)," *Indian J Nat Prod Resour*, vol. 12, no. 2, pp. 459–462, 2021.

[14] G. Pizzino, N. Irrera, M. Cucinotta et al., "Oxidative stress: harms and benefits for human health," *Oxidative Medicine and Cellular Longevity*, vol. 2017 Article ID 8416763, 2017.

[15] B. Jalali Ghassam, H. Ghaffari, H. S. Prakash, and K. R. Kini, "Antioxidant and hepatoprotective effects of *Solanum xanthocarpum* leaf extracts against CCl$_4$-induced liver injury in rats," *Pharmaceutical Biology*, vol. 52, no. 8, pp. 1060–1068, 2014.

[16] F. S. Mohammed, N. Korkmaz, M. Doğan, A. E. Şabik, and M. Sevindik, "Some medicinal properties of *Glycyrrhiza glabra* (Licorice)," *Ankara Universitesi Eczacilik Fakultesi Dergisi*, vol. 45, no. 3, pp. 524–534, 2021.

[17] S. B. Nimse and D. Pal, "Free radicals, natural antioxidants, and their reaction mechanisms," *RSC Advances*, vol. 5, no. 35, Article ID 27986, 2015.

[18] L. Al-Eryani, B. Wahlang, K. C. Falkner et al., "Identification of environmental chemicals associated with the development of toxicant-associated fatty liver disease in rodents," *Toxicologic Pathology*, vol. 43, no. 4, pp. 482–497, 2015.

[19] F. S. Mohammed, S. Gunal, M. Pehlivan, M. Dogan, M. Sevindik, and H. Akgul, "Phenolic content, antioxidant and antimicrobial potential of endemic *Ferulago platycarpa*," *Gazi Univ J Sci*, vol. 33, no. 4, pp. 670–677, 2020.

[20] A. Dayangaç, N. Korkmaz, and M. Sevindik, "Antioxidant, antimicrobial and antiproliferative activities of *Galium aparine*," *Ankara Universitesi Eczacilik Fakultesi Dergisi*, vol. 45, no. 3, pp. 554–564, 2021.

[21] N. D. Mahmood, S. S. Mamat, F. H. Kamisan et al., "Amelioration of paracetamol-induced hepatotoxicity in rat by the administration of methanol extract of Muntingia calabura

[22] M. N. Donkor, S. Y. Ayikanle, and S. Adoesomdonkor, "Effect of ethanol stem bark extract of *Berlina grandiflora* Hutch and Dalz on marker liver enzymes in rats treated with," *International Journal of Pharmacy and Pharmaceutical Sciences*, vol. 10, pp. 69–73, 2018.

[23] F. S. Mohammed, M. Karakas, H. Akgul, and M. Sevindik, "Medicinal properties of *Allium calocephalum* collected from gara mountain (Iraq)," *Fresenius Environmental Bulletin*, vol. 28, no. 10, pp. 7419–7426, 2019.

[24] Y. Jiang, W. Han, T. Shen, and M. H. Wang, "Antioxidant activity and protection from DNA damage by water extract from pine (*Pinus densiflora*) bark," *Preventive Nutrition and Food Science*, vol. 17, no. 2, pp. 116–121, 2012.

[25] C. E. Ugwu and S. M. Suru, "Medicinal plants with hepatoprotective potentials against carbon tetrachloride-induced toxicity: a review," *Egyptian Liver Journal*, vol. 11, no. 1, 88 pages, 2021.

[26] B. A. Venmathi Maran, M. Iqbal, P. Gangadaran, B.-C. Ahn, P. V. Rao, and M. D. Shah, "Hepatoprotective potential of Malaysian medicinal plants: a review on phytochemicals, oxidative stress, and antioxidant mechanisms," *Molecules*, vol. 27, no. 5, 1533 pages, 2022.

[27] JSTOR, "Global Plants," 2021, https://plants.jstor.org/stable/10.5555/al.ap.upwta.1_642.

[28] R. G. Mali, "*Cleome viscosa* (wild mustard): a review on ethnobotany, phytochemistry, and pharmacology," *Pharmaceutical Biology*, vol. 48, no. 1, pp. 105–112, 2010.

[29] G. Lakshmanan, A. Sathiyaseelan, P. T. Kalaichelvan, and K. Murugesan, "Plant-mediated synthesis of silver nanoparticles using fruit extract of *Cleome viscosa* L.: assessment of their antibacterial and anticancer activity," *Karbala International Journal of Modern Science*, vol. 4, no. 1, pp. 61–68, 2018.

[30] U. Bose, V. Bala, T. N. Ghosh, K. Gunasekaran, and A. A. Rahman, "Antinociceptive, cytotoxic and antibacterial activities of *Cleome viscosa* leaves," *Rev Bras Farmacogn*, vol. 21, no. 1, pp. 165–169, 2011.

[31] S. Ahmed, M. Sultana, M. Mohtasheem, U. Hasan, and I. Azhar, "Analgesic and antiemetic activity of *Cleome viscosa* L," *Pakistan Journal of Botany*, vol. 43, pp. 119–122, 2011.

[32] J. Harborne, *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*, Chapman & Hall, New York, NY, USA, 3 edition, 1998.

[33] O. A. Aiyegoro and A. I. Okoh, "Preliminary phytochemical screening and in vitro antioxidant activities of the aqueous extract of *Helichrysum longifolium* DC," *BMC Complementary and Alternative Medicine*, vol. 10, no. 1, 21 pages, 2010.

[34] U. Golla and S. S. R. Bhimathati, "Evaluation of antioxidant and DNA damage protection activity of the hydroalcoholic extract of *Desmostachya bipinnata* L," *Stapf The Scientific World Journal*, vol. 2014, Article ID 215084, 8 pages, 2014.

[35] P. Prieto, M. Pineda, and M. Aguilar, "Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E," *Analytical Biochemistry*, vol. 269, no. 2, pp. 337–341, 1999.

[36] F. S. Mohammed, H. Akgul, M. Sevindik, and T. Kbm, "Phenolic content and biological activities of *Rhus coriaria* var. zebaria," *Fresenius Environmental Bulletin*, vol. 27, no. 8, pp. 5694–5702, 2018.

[37] H. Singh, A. Mishra, and A. K Mishra, "Phytochemical screening, in vivo anthelmintic and anticonvulsant activity of

Cleome viscosa Linn seeds extract," *The Natural Products Journal*, vol. 6, no. 3, pp. 213–218, 2016.

[38] H. Singh, S. S. Ali, N. A. Khan, A. Mishra, and A. K. Mishra, "Wound healing potential of *Cleome viscosa* Linn . seeds extract and isolation of active constituent," *South African Journal of Botany*, vol. 112, pp. 460–465, 2017.

[39] B. Parimaladevi, R. Boominathan, and S. Mandal, "Studies on analgesic activity of *Cleome viscosa* in mice," *Fitoterapia*, vol. 74, no. 3, pp. 262–266, 2003.

[40] A. Rajani, E. Sunitha, and K. Shailaja, "Analysis of phytochemical constituents in leaf extract of *Cleome viscosa* L," *World Journal of Pharmaceutical Research*, vol. 3, no. 6, pp. 1008–1013, 2014.

[41] G. Lakshmanan, C. Sivaraj, A. Sathiyaseelan, P. Kalaichelvan, and K. Murugesan, "Phytochemical analysis and in vitro antioxidant activities of *Cleome viscosa* L," *Eur J Biomed*, vol. 5, no. 1, pp. 609–616, 2018.

[42] A. J. Uraku and O. H. Uraku, "Quantitative assessment of phytochemicals and nutritional potential of leaves and seeds of *Cleome viscosa* from Abakaliki, Nigeria," *Journal Of Bioscience And Biotechnology Discovery*, vol. 3, no. 1, pp. 25–29, 2018.

[43] R. K. Upadhyay, "*Cleome viscosa* Linn : a natural source of pharmaceuticals and pesticides," *International Journal of Green Pharmacy*, vol. 9, no. 2, pp. 71–85, 2015.

[44] M. Thakur, K. Singh, and R. Khedkar, "Phytochemicals: extraction process, safety assessment, toxicological evaluations, and regulatory issues," in *Functional and Preservative Properties of Phytochemicals*, pp. 341–361, Elsevier B.V, Amsterdam, Netherlands, 2020.

[45] S. S. Patel and J. K. Savjani, "Systematic review of plant steroids as potential antiinflammatory agents: current status and future perspectives," *The Journal of Phytopharmacology*, vol. 4, no. 2, pp. 121–125, 2015.

[46] M. Kumar, S. Kumar, and S. Kaur, "Investigations on DNA protective and antioxidant potential of chloroform and ethyl acetate fractions of *Koelreuteria paniculata* Laxm," *African Journal of Pharmacy and Pharmacology*, vol. 5, no. 3, pp. 421–427, 2011.

[47] V. P. Gupta, P. Gupta, and R. Gupta, "Comparison of the antioxidant activity and total phenolic, flavonoid content of aerial part of *Cleome viscosa* L," *American Journal of Ophthalmology*, vol. 151, no. 2, pp. 386-387, 2011.

[48] M. Irshad, M. Zafaryab, M. Singh, and M. M. A. Rizvi, "Comparative analysis of the antioxidant activity of *Cassia fistula* extracts," *International Journal of Medicinal Chemistry*, vol. 2012, Article ID 157125, 6 pages, 2012.

[49] A. Kumar and S. Chattopadhyay, "DNA damage protecting activity and antioxidant potential of pudina extract," *Food Chemistry*, vol. 100, no. 4, pp. 1377–1384, 2007.

[50] İ Meydan, G. Kizil, H. Demir, B. Ceken Toptanci, and M. Kizil, "In vitro DNA damage, protein oxidation protective activity and antioxidant potentials of almond fruit (*Amygdalus trichamygdalus*) parts (hull and drupe) using soxhlet ethanol extraction," *Advances in Traditional Medicine*, vol. 20, no. 4, pp. 571–579, 2020.

[51] M. D. Kalim, D. Bhattacharyya, A. Banerjee, and S. Chattopadhyay, "Oxidative DNA damage preventive activity and antioxidant potential of plants used in Unani system of medicine," *BMC Complementary and Alternative Medicine*, vol. 10, no. 1, 77 pages, 2010.

[52] C. Klaassen, *Casarett and Doull's Toxicology: The Basic Science of Poisons*, McGraw-Hill, New York, NY, USA, 9 edition, 2019.

[53] S. Sookoian and C. J. Pirola, "Liver enzymes, metabolomics and genome-wide association studies: from systems biology to the personalized medicine," *World Journal of Gastroenterology*, vol. 21, no. 3, p. 711, 2015.

[54] V. Dixit and N. Gupta, "Evaluation of hepatoprotective activity of *Cleome viscosa* Linn," *Indian Journal of Pharmacology*, vol. 41, no. 1, pp. 36–40, 2009.

[55] A. K. Mobiya, A. K. Patidar, G. Selvam, and M. Jeyakandan, "Hepatoprotective effect of *Cleome viscosa* L . Seeds in paracetamol induced hepatotoxic rats," *International Journal of Pharmaceutical & Biological Archive*, vol. 1, no. 4, pp. 399–403, 2010.

[56] N. Brautbar and J. Williams II, "Industrial solvents and liver toxicity: risk assessment, risk factors and mechanisms," *International Journal of Hygiene and Environmental Health*, vol. 205, no. 6, pp. 479–491, 2002.

[57] M. W. Robinson, C. Harmon, and C. O'Farrelly, "Liver immunology and its role in inflammation and homeostasis," *Cellular and Molecular Immunology*, vol. 13, no. 3, pp. 267–276, 2016.

[58] L. W. D. Weber, M. Boll, and A. Stampfl, "Hepatotoxicity and mechanism of action of haloalkanes: carbon tetrachloride as a toxicological model," *Critical Reviews in Toxicology*, vol. 33, no. 2, pp. 105–136, 2003.

[59] R. C. Zangar, J. M. Benson, V. L. Burnett, and D. L. Springer, "Cytochrome P450 2E1 is the primary enzyme responsible for low-dose carbon tetrachloride metabolism in human liver microsomes," *Chemico-Biological Interactions*, vol. 125, no. 3, pp. 233–243, 2000.

[60] K. N. M. Man, S. Philipsen, and K. C. Tan-Un, "Localization and expression pattern of cytoglobin in carbon tetrachloride-induced liver fibrosis," *Toxicology Letters*, vol. 183, no. 1–3, pp. 36–44, 2008.

[61] A. S. Nugraha, B. Triatmoko, P. Wangchuk, and P. A. Keller, "Vascular epiphytic medicinal plants as sources of therapeutic agents: their ethnopharmacological uses, chemical composition, and biological activities," *Biomolecules*, vol. 10, no. 2, 181 pages, 2020.