

RESEARCH ARTICLE

In Vitro and *In Vivo* Anticancer, Anti-inflammatory, and Antioxidant Activity of Dhimran (*Ocimum forsskaolii benth*) Extract and Essential Oil on Carbon Tetrachloride-Induced Hepatotoxicity in Mice

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Abstract

Medicinal plants are rich in bioactive components, which exert various biological activities that are beneficial for living. This study evaluated the antioxidant, antitumor, and anti-inflammatory activities of Dhimran (*Ocimum forsskaolii benth*) in mice. The main volatile compounds in Dhimran essential oil (DEO) were endo-fenchol, tau-cadinol, and β -atlantol, while Dhimran extract (DE) was rich in caffeic acid and quercetin. These active compounds in DE and DEO possess antioxidant activity, scavenging 93% of DPPH radicals, antibacterial activity against MDR bacteria, and anticancer activity against HepG2 cancer cell line. These activities influenced the mice's health. A total of 180 mice were divided into six treatment groups for 30 days as follows: T1 was fed a basal diet; T2 received CCl₄ (187 mg/kg BW); T3 received DEO (4 mL/kg); T4 received DE (4 mL/kg); T5 received DEO+CCl₄; and T6 received DE+CCl₄. The oral administration of CCl₄ to mice resulted in an increase in absolute liver weight (ALW) and relative organ weight (ROW) and a significant decrease in body weight gain and feed conversion ratio (FCR). Additionally, there was a notable reduction in levels of red blood cells (RBCs), hematocrit (Ht), hemoglobin (Hb), and platelets; however, there was an increase in white blood cells (WBCs). The administration of CCl₄ in mice lowered total protein content; however, it raised the activity of AST, ALT, ALP, and LDH in mice liver homogenate compared to the control (P<0.05). CCl₄ increased inflammation cytokines (TNF- α and IL-6). The coadministration of DEO or DE with CCl₄ significantly corrected all biomarkers to control levels, with a preference for DE. Liver sections from the control, DE, and DEO groups revealed a normal structure. It is concluded that Dhimran extract or essential oil has antioxidant and anti-inflammatory properties that can be used as hepatoprotective agents against CCl₄ toxicity.

Keywords: Anticancer, Antioxidant, Anti-inflammatory, CCl₄ toxicity, Essential oils, Hepatoprotective, *Ocimum forsskaolii benth*

INTRODUCTION

The nutritional, pharmacological, and antioxidant properties of numerous *Ocimum* species plants provide them with substantial commercial and therapeutic benefits [1]. The sweet basil "*Ocimum basilicum*" is the most nutritious. It is widely dispersed and employed in the perfumery and dietary supplement industries [2]. Sharmin et al. [3] conducted numerous studies on extracts of essential oils (EOs) and *Ocimum* species, demonstrating powerful antitumor and antimicrobial properties. The apoptosis of HeLa cells was significantly increased by caffeic acid, isolated from *Ocimum gratissimum* L. It had apoptotic properties on human cervical cancer cells (HeLa) through activating numerous caspases [4]. Ursolic acid

also affected microtubules and F-actin [5]. *Ocimum* species are rich in volatile organic components, i.e., phenylpropanoids, monoterpenes, and sesquiterpenes, which exhibit substantial antimicrobial activity against microbes [6].

Dhimran (*Ocimum forsskaolii benth*) is an aromatic medicinal plant belonging to the family *Lamiaceae* that grows in Saudi Arabia and is represented by 26 genera and about 70 species in Saudi Arabia [7]. Native Saudi Arabians have long used it to enhance the flavor of butter and tea and its therapeutic benefits [8]. Traditionally, it is employed as an insect repellent in Eretria, as a fever-reducing agent in Yemen, and for treating eye infections in Rwanda [8]. This plant has exhibited several biological



actions, including local anesthetic^[9], antiepileptic, anti-ulcer, and anti-inflammatory properties^[10]. Furthermore, it has antibacterial, antioxidant, and cytotoxic properties^[8].

The chemical composition of *Ocimum forsskaolii* essential oil varies depending on its geographical origin. The main constituents are estragole (59.4-65.2%) and linalool (25.0-28.1%)^[11]. The Ethiopian sample was found to have high levels of (E)-methyl cinnamate or myrcene and methyl chavicol^[12]. Sharmin et al.^[3] found that the aqueous extract of *Ocimum forsskaolii* leaves contained silver nanoparticles that exhibited antibacterial properties. The primary categories of chemicals in the *Ocimum* include phenylpropanoids, monoterpenes, and sesquiterpenes^[13].

A recent *in vitro* study revealed that *Ocimum forskolin* essential oils exhibited *in vitro* anticancer and antibacterial properties. This plant inhibits the growth of cancer cells by conducting tests to measure cytotoxicity. Furthermore, the study validated the oil's impact on specific molecular targets using advanced techniques. Furthermore, the antimicrobial activity of the *Ocimum forsskaolii* essential oil was evaluated against both Gram-positive and Gram-negative bacteria, i.e., *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia*^[14].

Additionally, the previous studies stated that essential oil derived from *O. basilicum* seeds exhibited significant anticancer effects against Hep3B and MCF-7 with IC₅₀ ranging between 56.23 and 80.35 µg/mL cells, surpassing the efficacy of the positive control, Doxorubicin. Furthermore, the essential oil had strong antibacterial effects against *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus aureus*, *Proteus mirabilis*, and *Pseudomonas aeruginosa*. It also shows potent antifungal activity against *Candida albicans*. The oil had an intense antioxidant action^[15].

Carbon tetrachloride (CCl₄) is an artificial chemical compound that has found primary application in the synthesis of chlorofluorocarbons, albeit also being utilized as a pesticide and cleansing agent. As a result of its toxic effects, its production is currently restricted. Carbon tetrachloride's high volatility and environmental persistence lead to its build-up in the atmosphere and groundwater. The general population might be exposed to harmful substances mainly by drinking polluted water and inhaling contaminated air. Elevated substance levels can harm the respiratory, hepatic, central nervous, and renal systems. Tetrachloride carbon is considered potentially carcinogenic to humans^[16].

Furthermore, research has shown that basil possesses a diverse range of pharmacological properties that can be applied to several conditions, such as brain injury^[17],

liver fibrosis, Type 2 diabetes allergies, and anemia^[18]. Furthermore, the simultaneous administration of deltamethrin and *Ocimum basilicum* protects against oxidative stress caused by Hg and Cd in an *in vivo* model of acetaminophen-induced liver injury in Wistar rats^[19]. Teofilović et al.^[20] demonstrated the effect of basil extract on the markers of oxidative stress, biochemical biomarkers, and morphology of liver injury. The extract improved the efficacy of antioxidant enzymes and decreased the oxidation of macromolecules. The animals treated with basil extract had significantly reduced levels of AST, ALP, and ALT in their blood compared to the control group. There are no studies on the *in vivo* effect of Dhimran extract or essential oils on the CCl₄ toxicity on rats; Hence, this study evaluated the *in vitro* and *in vivo* antioxidant, anti-inflammatory, and anticancer activities of Dhimran (*Ocimum forsskaolii* benth) essential oils and extracts in CCl₄-induced hepatotoxicity in mice.

MATERIALS AND METHODS

Ethical Approval

The Ethical Committee of King Abdulaziz University, Faculty of Medicine, accepted the study with ethical approval reference No. 516-23.

Plant Materials and Preparation of Dhimran Extract (DE)

The dried Dhimran (*Ocimum forsskaolii* benth) plant was obtained from a local herb market in Taif, Saudi Arabia, while the Dhimran essential oil (DEO) was purchased from Aldousiah Farm, Al Bahah, Saudia Arabia.

The dried Dhimran leaves were ground into a fine powder. A 10 g of the flour was dispersed in ethanol (70%) and stirred overnight (200 rpm, 25°C). The ethanol was separated by rotary evaporation, and the residues were freeze-dried, and 1 gm was obtained to prepare the different concentrations^[21].

GC-MS Analysis

The GC-MS analysis was performed using a Trace GC Ultra/ISQ Single Quadrupole MS and TGSMS Fused Silica Capillary Column (30 m, 0.25 mm, 0.1 mm Film thickness) manufactured by Thermo Scientific, USA. An electron ionization apparatus with a constant flow rate of 1 mL/min and an ionization energy of 70 eV served as the carrier gas for GC-MS detection. The MS injector and transfer line were maintained at a constant temperature of 280°C.

The oven was set to increase its temperature from an initial value of 40°C and gradually raised to 280°C. A percentage relative peak area was utilized to investigate the identified components. The volatile compounds were tentatively

identified by comparing their respective retention periods and mass spectra with those of different Library data of the GC/MS system [22].

Phenolic Compounds Content

The total phenolic compounds were in DEO and DE estimated using Folin-Ciocalteu method as Abd Elkader et al. [23]. The phenolic compounds and flavonoids were detected using an HPLC Shimadzu series (Shimadzu, Japan) and a UV-Vis DAD for HPLC analysis. Polyphenols were separated using a separation Gemini column (C18, 150x4.6 mm 3 μ m), (Phenomenex, USA) at 0.5 mL/min flow rate and 5°C temperature. The DE was dissolved in a methanol-water mixture (50:50) and injected into the autosampler. All chemicals used in this investigation were of HPLC grade; phosphoric acid (mobile phase B) and acetonitrile (mobile phase A) were combined with Water (Carlo Erba, Germany) to obtain a pH of 2. In addition to a sixty-min total runtime, the gradient elution percentage was altered as follows: a) Initial 5% A and 95% B; b) 35% A and 65% B for 15 min; c) 35% A and 65% B for 20 min; d) 40% A and 60% B for 30 min; e) 40% A and 60% B for 35 min; f) 50% A and 50% B for 40 min; g) 70% A and 30% B for 52 min; and h) 60 min. Examining the UV-Vis spectra of various phenolic standards, 210, 280, and 360 nm were chosen for subsequent HPLC-DAD analysis in the present investigation [24].

The Biological Activities

The Scavenging Activity: The scavenging power of DEO and DE was measured according to Jia et al. [25]. The essential oils and extract at concentrations of (50, 100, and 200 μ g/mL) were mixed with DPPH (Sigma, USA) solution and incubated for 30 min at room temperature in the dark. The resultant mixture's absorbance was subsequently quantified at 517 nm. % AA was calculated using the following equation:

$$\% \text{ Scavenging activity} = \frac{\text{OD control} - \text{OD sample}}{\text{OD control}} \times 100 \quad (1)$$

Antibacterial Activity: The antimicrobial activity of DEO and DE was assessed using the disc diffusion assay, following the methodology of Singh et al. [26], with specific modifications for basil EOs. The McFarland standard solution (0.05) was made by mixing (0.5 mL) BaCl₂ and (99.5 mL) H₂SO₄ to obtain a solution with equivalent turbidity to 1.5 \times 10⁸ (CFU mL⁻¹) cell density. 200 μ L of each bacterial and fungal culture (*L. monocytogenes*, *S. aureus*, *C. jejuni*, and *S. typhi*) was spread on nutrient agar plates. Six mm discs impregnated with varying DEO and DE concentrations were added to plates. The antibacterial activity was quantified by measuring the width of the inhibitory area during a 24-h incubation at 37°C.

Cytotoxic Impact on HepG2 Cells: Cell viability was assessed by measuring their ability to convert the yellow

dye MTT to a purple formazan, a process dependent on healthy mitochondria. HepG2 cells were suspended in RPMI 1640 media with 1% antibiotic antimycotic combination (10.000 U/mL potassium penicillin, 10.000 μ g/mL streptomycin sulfate, and 25 μ g/mL amphotericin B), as well as 1% L-glutamine at 37°C under 5% CO₂ using a water-jacketed carbon dioxide incubator (Sheldon, TC2323, Cornelius, OR, USA).

Around 10.000 liver cancer cells were seeded per well in fresh media (2 mL) on 96-well plates. The microtiter plate was incubated for a day at 37°C and CO₂. The medium was replaced with a fresh one supplemented with FBS and varying concentrations of DE or DEO (50, 100, and 200 μ g/mL), then incubated for two days [27]. The medium was aspirated after 48 h of incubation. Then, 40 μ L of MTT salt (2.5 μ g/mL) was added to each well, and the cells were further incubated for 4 h at 37°C with 5% CO₂. To end the reaction and dissolve crystals, 200 μ L of 10% SDS in deionized water was added to each well and incubated overnight at 37°C. A known cytotoxic natural substance (100 μ g/mL) was a positive control, causing complete cell death under the same conditions. The cells were collected using a trypsin-EDTA buffer and treated with trypan blue to distinguish viable cells. The live cell count was determined, and the findings were presented as the percentage of inhibition of liver cancer cell lines [28]. A microplate reader (Bio-Rad, USA) was used to measure the absorbance at 595 nm to evaluate the effects of DEO in DMSO on cell viability.

$$\% \text{ viability} = \frac{\text{Reading of extract}}{\text{Reading of negative control}} \times 100 \quad (2)$$

Animal and Experimental Design

A total of 180 mice weighted (30-42 g) were obtained from the breeding animal house, adapted, and kept under full hygienic conditions. The plastic boxes were subjected to a 12-h dark-light cycle, 40-60% relative humidity, and a temperature of 23.2°C. They delivered water and rats' diet at their discretion throughout the experiment [29]. The mice were given two weeks to acclimate to the experimental animal laboratory setting. The accommodation and administration of the animals and the experimental protocols were conducted per the principles delineated in the Guide for the Care and Use of Lab Animals following the National Committee of Bioethics (NCBE 2023). After the accommodation period, the animals were weighed. Randomly, ten mice were allocated to six groups: The first group was fed a basal diet, the second group was delivered carbon tetrachloride (CCl₄, 187 mg/kg BW, 1/10 LD50), the third group delivered dhimran essential oil (DEO, 4 mL/kg), the fourth group received dhimran extract (DE, 4 mL/kg), the fifth group received DEO+CCl₄, and the sixth

group delivered DE+CCl₄. The mice received their daily oral dosing for one month.

Determination of Weight Gain and Organ Weight

The growth performance parameters of mice groups were calculated following Zhou et al.^[30]. At the end of the experiment, the animals were decapitated from the cervical region. Following the dissection of the heart, brain, liver, kidney, lung, and spleen, excess fat was eliminated, and the percentages of the relative weight of organs were calculated.

Blood Biochemistry

Sample Collection and Preparation: At the end of 28 days, the mice were fasted overnight, then were slaughtered via jugular vein severance, and two blood samples were obtained. The first 0.5 mL sample was gathered in an EDTA tube for hematological examination. The other part (2 mL) was collected in EDTA-free tubes and centrifugated at 3000 ×g for 10 min to collect the serum.

Hematology: The blood sample was applied for the evaluation of the total count of red blood cells (RBCs), white blood cells (WBCs), and hemoglobin (Hb). The WBCs, RBCs lymphocytes, and platelets were determined using an automated cell counter (HOSPITEX analyzer, Italy)^[31].

Serum Biochemical Parameters: A colorimetric approach was utilized to evaluate the levels of aspartate transaminase (AST, 260 001, Spectrum, Cairo, Egypt) and alanine aminotransferase (ALT, 265 001 Spectrum, Cairo, Egypt), while alkaline phosphatase (ALP) was determined in the serum following Zhou et al.^[30]. The serum total protein concentrations were determined^[32], whereas the quantities of albumin were tested using the technique developed by Zhou et al.^[30]. The difference between total protein and albumin calculated the globulin content. The concentration of glucose was measured using the^[33] method. The creatinine and urea in serum were determined following^[30]. The microplate reader (Infinite M Nano, manufactured by TECAN, Austria) was used in colorimetric measurements.

Antioxidant Enzymes: Malondialdehyde (MDA, cat no. 230001, Spectrum, Cairo, Egypt), the lipid peroxidation marker, was determined following the instructions given using Spectrum kits. The total non-enzymatic antioxidant capacity (TAC) was assessed using the BioDignostic kits following^[34]. The measurements were conducted *via* a microplate reader at the respected wavelength (Infinite M Nano, TECAN, Austria).

Histopathological Examination

The liver tissues were picked, preserved in formalin, and processed by an automated processor. An initial phase was fixed and then dehydrated. The fixation was conducted

by immersing the tissue for 48 h in 10% formalin, after which the fixation solution was removed using distilled water for 30 min. The tissues were subsequently dehydrated by immersing in elevating levels of alcohol (70, 90, and 100%) for 120, 90, and 90 min, respectively. The dehydration was subsequently cleared using multiple cycles of xylene. The procedure involved submerging the tissue for one h in a solution of 50% xylene & 50% alcohol and then for an additional 1.5 h in pure xylene. The specimens were then saturated with melted paraffin wax, encased, and sealed. Hematoxylin & eosin were used for 4-5 μm paraffin cut sections. Blood circulation disruptions, irritation, degenerations, apoptosis, necrosis & additional histopathological alterations in the tissues were monitored.

Analysis of Inflammatory Cytokines via Real-time PCR

The liver's inflammatory cytokines (TNF-α, IL-6) were evaluated using qPCR. The RNA was isolated using a commercial kit (Thermo Scientific, USA), and then its concentration was estimated by the Quawell Nanodrop instrument (USA). Reverse transcription was utilized to produce cDNA using a RevertAid commercial kit (Thermo Scientific, USA). The qPCR analysis was conducted utilizing an Applied Biosystem StepOnePlus real-time PCR system (USA) in combination with a mix of cDNA and 2X Maxima SYBR Green Master Mix (USA) and primers specific to each gene. As an internal control, the β-actin gene was employed to compute the change in the target genes using reference genes.

Statistical Analysis

The experiments were conducted using an entirely random design (CRD) with triplicates. The triplicate data means were analyzed using one-way ANOVA via Microsoft Excel (v 2108, 2021). The means were compared with the LSD at P<0.05.

RESULTS

Essential Oil Content

The results of the GC-MS analysis of the oils are shown for dhimran (*Ocimum forsskaolii* benth) in *Table 1*, where the eluents are provided in the order of the HP5MS column elution. The primary components identified in the EO of *Ocimum forsskaolii* benth included were endo-Fenchol, tau-cadinol, α-eudesmol, α-terpineol, α-Bulnesene, and β-atlantol. The contents of these volatile compounds were 18.89, 11.6, 8.3, 7.2, 5.2, 10.9%, respectively. Endo-fenchol, tau-cadinol, and β-atlantol volatile compounds represented 52% of total VOCs. Medium contents of VOCs represented in germacrene B, endo-borneol, isoshyobunone, β-Thujone, and γ-eudesmol, while other compounds are in low contents

Table 1. GC/MS profile of essential oils detected in Dhimran (*Ocimum forsskaolii benth*)

Detected Compounds	% Area
endo-Fenchol	18.89±0.9a
tau-cadinol	11.6±1.1b
germacrene B	3.6±0.2e
endo-borneol	2.6±0.3f
isoshyobunone	2.5±0.2f
Bornylene	0.33±0.01j
Germacrene D	0.25±0.03j
Borneol L	0.61±0.01h
delta-Guaiene	0.68±0.02h
α-Pinene	0.5±0.01i
α-eudesmol	8.3±0.6c
α-terpineol	7.2±0.3cd
α-Bulnesene	5.2±0.5d
β-atlantol	10.9±0.8b
β-Pinene	1.5±0.2g
β-Myrcene	0.62±0.02h
β-Thujone	2.2±0.1f
γ-eudesmol	2.4±0.2f
P value	<0.0001

Different Lowercase letters in the same column indicate significant differences at P<0.05

Phenolic Content and Antioxidant Activity

The scavenging power of DEO and DE is shown in *Table 2*. Dhimran extract had the highest total phenolic and flavonoid content, i.e., 53 and 32 mg/g DW, with a relative increase of 28 and 24% of DEO. Accordingly, the antioxidant activity of DE recorded the highest values, 93%, followed by DEO, 89%. DE showed the lowest IC₅₀ of 50 µg/mL, followed by DEO.

The polyphenolic profile detected by HPLC is shown in *Table 3*. Phenolic acids and flavonoids were the main

Table 2. The antioxidant content of dhimran essential oil and ethanolic extract

Parameters	Dhimran (<i>Ocimum forsskaolii benth</i>)		P Value
	DEO	DE	
TPC (mg/g)	42±1.2b	53±3.2a	<0.0001
TFC (mg/g)	27±2.3b	32±1.2a	<0.0001
AA (%)	89±2.2b	93±3.6a	<0.0001
IC50 (µg/mL)	100±1.2a	50±0.9b	<0.0001

Different Lowercase letters in the same row indicate significant differences at P<0.05. Dhimran essential oil (DEO), dhimran extract (DE). Total phenolic compounds (TPC); total flavonoids compound (TFC); Antioxidant activity (AA); Inhibitory concentration (IC)

Table 3. Phenolic compounds profile of dhimran extract (DE) detected by HPLC

Phenolic Compounds	Value in DE (mg/g)
Gallic	6.5±0.2b
Caffeic	8.6±0.8a
Ferulic	5.9±0.3bc
Sinapic	3.6±0.1c
Synergic	2.5±0.6d
Quercetin	3.8±0.7c
Luteolin	1.8±0.2e
Rutin	2.1±0.3d
Apigenin	2.9±0.3d
Kaempferol	2.2±0.1d
P value	<0.0001

Different Lowercase letters in the same column indicate significant differences at P<0.05

constituents of DE. Caffeic acid was the primary phenolic acid (8.6 mg/g), followed by gallic and ferulic acids. The main flavonoid was quercetin (3.8 mg/g), followed by Rutin, Apigenin, and Kaempferol.

Antibacterial Activity of DEO and DE

The summary of the antibacterial activity of DEO and DE against a variety of four bacterial species is presented in *Table 4*. The DEOs and DE were tested for their ability

Table 4. Antibacterial activity of dhimran essential oil (DEO) and dhimran extract (DE)

Test Bacteria	Dhimran Concentration (µg/mL)						P Value
	DE			DEO			
	50	100	200	50	100 Cardamom	200	
<i>S. typhi</i>	11±0.2c	14±0.2b	18±0.1a	9±0.2d	11±0.6c	15±0.3b	<0.0001
<i>C. jejuni</i>	9±0.1d	11±0.5c	15±0.3a	0±0.0	9±0.2d	12±0.2b	<0.0001
<i>L. monocytogenes</i>	11±0.3c	16±0.3b	22±0.4a	9±0.1d	12±0.5c	17±0.3b	<0.0001
<i>S. aureus</i>	15±0.8d	18±0.5c	25±0.7a	12±0.3e	16±0.1d	21±0.5b	<0.0001

Different Lowercase letters in the same row indicate significant differences at P<0.05. *Salmonella typhi*, *Campylobacter jejuni*, *Listeria monocytogenes*, and *Staphylococcus aureus*. Dhimran essential oil (DEO), dhimran extract (DE)

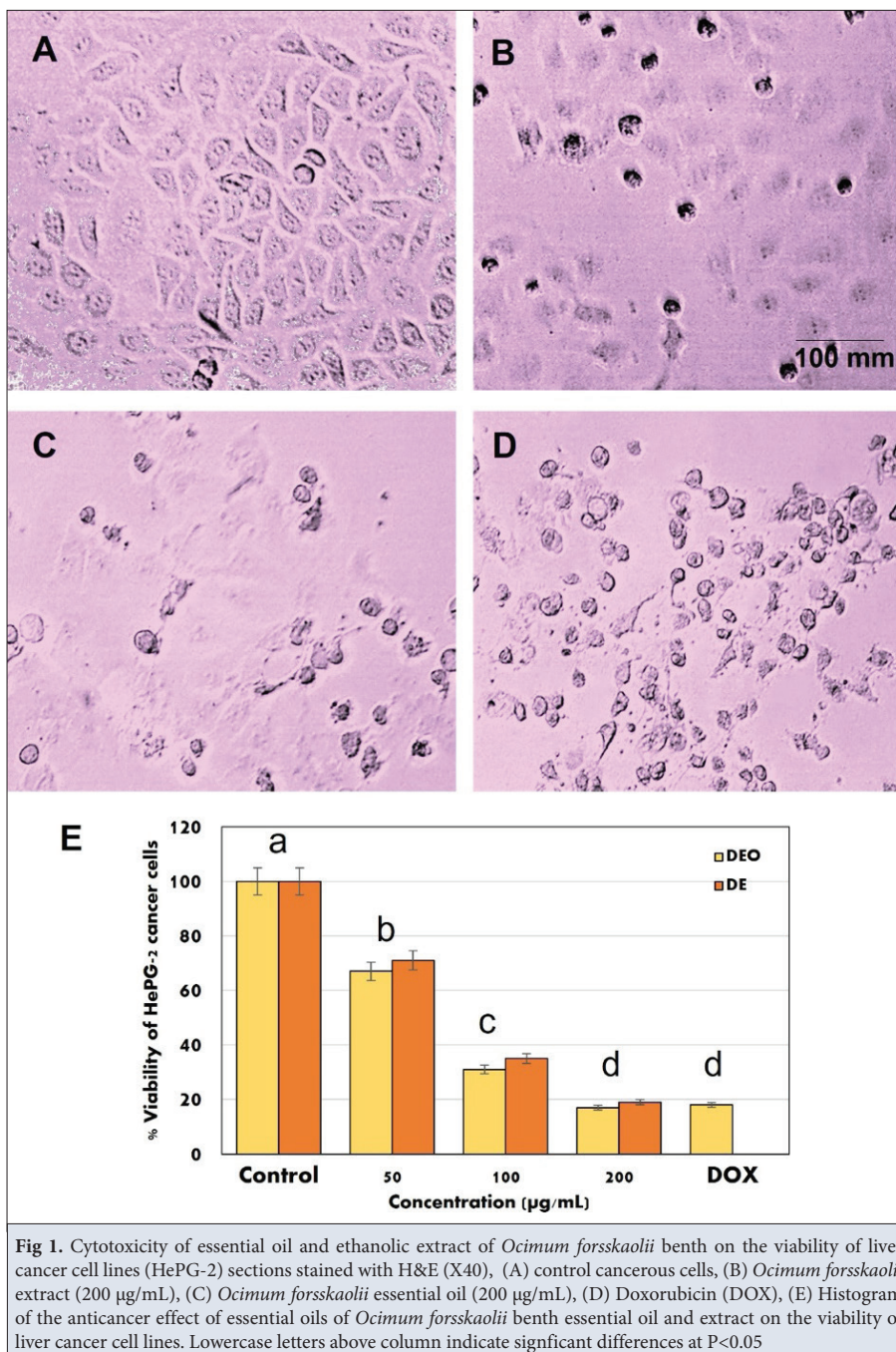


Fig 1. Cytotoxicity of essential oil and ethanolic extract of *Ocimum forsskaolii* benth on the viability of liver cancer cell lines (HepG-2) sections stained with H&E (X40), (A) control cancerous cells, (B) *Ocimum forsskaolii* extract (200 µg/mL), (C) *Ocimum forsskaolii* essential oil (200 µg/mL), (D) Doxorubicin (DOX), (E) Histogram of the anticancer effect of essential oils of *Ocimum forsskaolii* benth essential oil and extract on the viability of liver cancer cell lines. Lowercase letters above column indicate significant differences at $P < 0.05$

to inhibit gram-positive growth, i.e., *L. monocytogenes* and *S. aureus* and gram-negative bacteria, including *S. typhi* and *C. jejuni*. The ethanolic extract of dhimarn (200 µg/mL) recorded the highest antibacterial activity against *S. aureus* (25 mm) and *S. typhi* (18 mm), with relative increases of 20 and 26% compared to DEO. DE exhibited an inhibition area ranging from 9 to 25 mm against all the pathogens tested. The DEO recorded inhibition zone diameters against the tested bacteria in the 9-21 mm range. DEO (50 µg/mL) has not antibacterial activity against *Campylobacter jejuni*. *C. jejuni* was the most resistant bacteria to DE and DEOs; however,

S. aureus was the most sensitive to EOs and extract of dhimarn.

Cytotoxic Activity of Dhimarn EOs Against Hepatic Cancer Cell Lines

Fig. 1 shows that each essential oil and ethanolic extract of *Ocimum forsskaolii* benth (dhimarn) has cytotoxicity on human liver cancer cells (HepG2). *Ocimum forsskaolii* benth extract reduces the viability of HepG2 cancer cells by 86%. *Ocimum forsskaolii* benth EO inhibited 82% of cancerous cell viability; all results compared with Doxorubicin reduced cancerous cell proliferation by 85%. The results correlated with microscopic images. On the

Table 5. The effect of DEO and DE administration on growth parameters of CCl₄-induced liver cancer

Parameters	Treatments						P Value
	Control	DE	DEO	CCl ₄	DE+CCl ₄	DEO+CCl ₄	
IBW	30.5±0.2	31.2±0.1	31.6±0.2	30.8±0.5	31±0.2	31±0.6	0.9
FBW	98.5±1.2b	106±2.2a	101.6±3.1ab	73.8±2.1d	90±1.6bc	86±2.2c	<0.0001
BWG	68±1.3c	75±0.9a	70±0.8b	43±1.2e	59±0.9d	55±0.9de	<0.0001
FCR	1.45±0.2b	1.6±0.2a	1.55±0.1ab	1.2±0.2c	1.39±0.1b	1.35±0.2bc	0.0041
SR	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	0.98
ALW	5.1±0.2c	5.3±0.3c	5.2±0.4c	7.9±0.5a	6.1±0.2b	6.0±0.1b	0.0033
ROW	2.5±0.1c	2.56±0.1c	2.7±0.3c	3.8±0.7a	2.9±0.1b	3.1±0.2b	0.00235

Lowercase letters in the same row indicate significant differences at $p<0.05$. Absolute liver weight (ALW), Relative organ weight (ROW), survival rate (SR), initial body weight (IBW), final body weight (FBW), and feed conversion ratio (FCR). Dhimran essential oil (DEO), dhimran extract (DE)

Table 6. The effect of DEO and DE administration on the hematology of CCl₄ rats-induced liver cancer

Hematology	Treatments						P Value
	Control	DE	DEO	CCl ₄	DE+CCl ₄	DEO+CCl ₄	
RBCs × 10 ⁶ /l	7.5±0.2ab	7.9±0.2a	7.7±0.5ab	4.8±0.2c	6.5±0.2b	6.0±0.2b	0.00021
WBCs × 10 ⁶ /l	5.2±0.6c	5.5±0.1c	5.1±0.2c	17.2±1.2a	7.2±1.1b	6.9±0.4b	0.0023
Ht %	43.68±2.2a	42.9±3.1ab	42.0±1.1ab	22.5±2.1c	39.7±0.9b	38.2±1.3b	0.00154
Hb g/dl	14.3±0.3a	14±0.2a	14.2±0.3a	8.2±0.5c	13.1±0.7ab	12.8±0.9b	0.031
Platelets × 10 ⁶ /l	820±6.3b	835±3.3a	822±4.1b	350±3.2d	790±2.3c	750±3.6c	0.0042

Different lowercase letters in the same row indicate significant differences at $P<0.05$. Dhimran essential oil (DEO), dhimran extract (DE). Red blood cells (RBCs); white blood cells (WBCs); hematocrit (Ht); Hemoglobin (Hb)

Table 7. The effect of DEO and DE administration on the liver markers of CCl₄ rats-induced liver cancer

Liver Markers	Treatments						P value
	Control	DE	DEO	CCl ₄	DE+CCl ₄	DEO+CCl ₄	
AST	123±2.1a	115±1.1b	113±2.1b	85±1.1d	105±0.9c	100±0.0c	0.0014
ALP	355±3.6a	320±2.1c	318±3.1c	260±2.1d	340±3.3b	338±3.2b	0.0051
ALT	165±1.2a	152±2.3b	150±1.2b	120±1.3d	144±2.1c	140±1.1c	0.0085
LDH	789±2.2d	820±6.1c	817±6.2c	989±7.0a	856±8.3b	850±8.8b	0.0041
TP	175±1.1b	189±3.2a	180±1.2ab	122±1.1d	160±1.1c	156±2.1c	0.0011

Lowercase letters in the same row indicate significant differences at $p<0.05$. Dhimran essential oil (DEO), dhimran extract (DE)

other hand, the IC₅₀ of treatments inversely correlated with the inhibition percentage, where the IC₅₀ of DE was lowest after DOX (20 µg/mL), followed by DEO (31 µg/mL).

In vivo Experiment of Hepatoprotective Impact of DEO and DE on CCl₄ Toxicity

Growth Parameters: Clinical poisoning manifestations were observed in the CCl₄ mice, such as salivation, digging, trembling, squirming, and convulsions; however, there were no mortalities. Compared with the control group, an increase in relative liver weights (ALW and ROW) and considerable reduction in weight gain and FCR were observed in CCl₄ mice. These parameters were significantly reduced when treating the mice with DEO or

DE with a preference for DE. No significant differences were observed in the single application of DEO, DE, and control (Table 5).

Hematology: The oral administration of CCl₄ to mice resulted in notable reductions in levels of red blood cells (RBCs), hematocrit (Ht), hemoglobin (Hb), and platelets; however, a raise in white blood cells (WBCs). The single application of DEO or DE did not affect the hematological parameters ($P>0.05$). However, when the combined application DEO+CCl₄ or DE+CCl₄ significantly $P<0.05$ restored all levels to the control levels compared to the CCl₄ group (Table 6).

Liver Enzymes: The liver (ALP, AST, ALT, and LDH)

enzymes are of interest as potential hepatotoxicity biomarkers. The administration of CCl₄ in mice lowered total protein content; however, it raised the activity of AST, ALT, ALP, and LDH in mice liver homogenate compared to the control (P<0.05). The combined oral application of DEO+CCL₄ or DE+CCL₄ significantly P<0.05 reduced the activity of liver enzymes and increased the total protein content. The single application of DE or DEO substantially altered the studied parameters (Table 7).

Lipid and Protein Oxidation Markers: A notable increase was observed in the concentrations of lipid hydroperoxides (LOOH) after the administration of CCl₄ compared to the control group. However, mice that were administered DEO+CCL₄ or DE+ CCL₄ exhibited a substantial reduction in the concentrations of LOOH in comparison to the CCl₄-treated group (Fig. 2-A). Furthermore, the hepatic levels of protein oxidation indicator, advanced oxidized protein products (AOPP), were significantly elevated in the CCl₄-intoxicated group. The oral application of DEO or DE reduced the high concentration of AOPP. The levels of lipid peroxidation and protein oxidation in the DEO-mice or DE-mice differed significantly compared to the control group (Fig. 2-B).

Antioxidant Status: The liver homogenates of CCl₄-mice show a significant decrease in the non-enzymatic defense, glutathione (GSH), as well as lower activity of the antioxidant system (SOD, CAT, and GPx) when compared

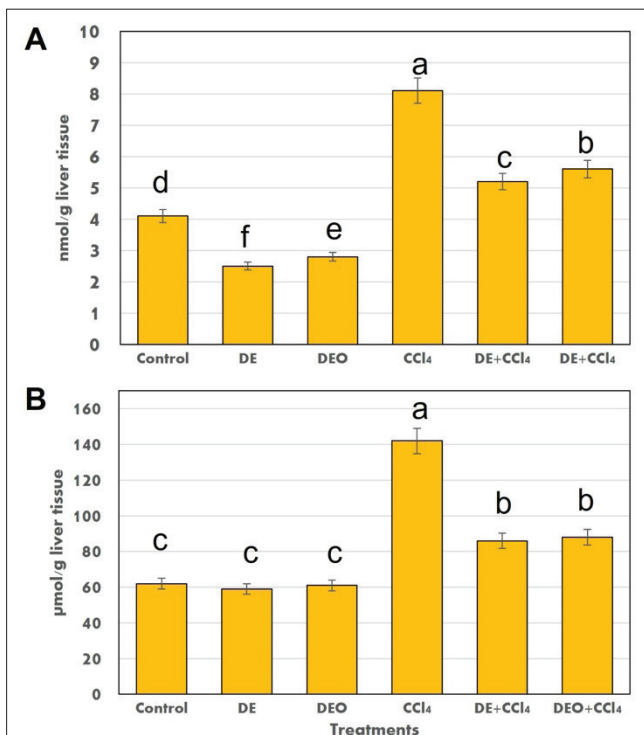


Fig 2. The effect of DEO and DE on mitigating oxidative stress induced by hepatocytes CCl₄ administration, (A) LOOH; lipid hydroperoxide, (B) AOPP; advanced oxidized protein product. The significant differences between treatments are indicated by letters above columns (P<0.05)

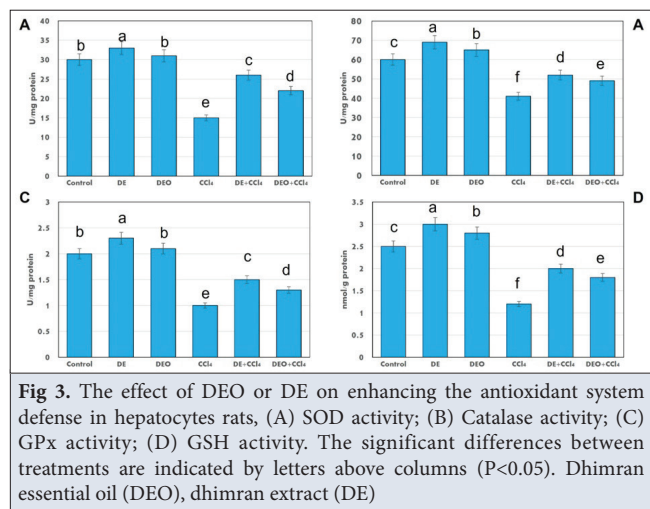


Fig 3. The effect of DEO or DE on enhancing the antioxidant system defense in hepatocytes rats, (A) SOD activity; (B) Catalase activity; (C) GPx activity; (D) GSH activity. The significant differences between treatments are indicated by letters above columns (P<0.05). Dhimran essential oil (DEO), dhimran extract (DE)

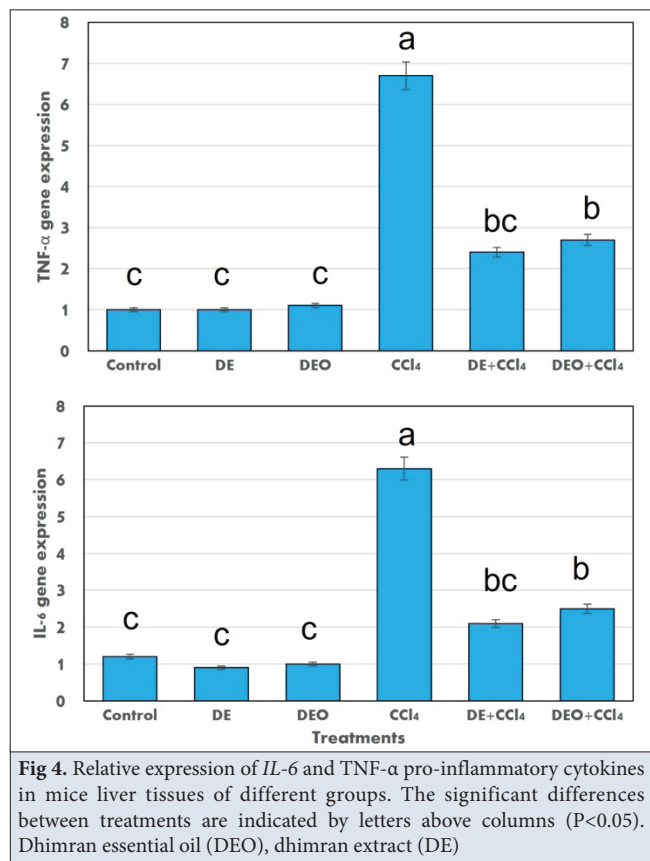


Fig 4. Relative expression of IL-6 and TNF-α pro-inflammatory cytokines in mice liver tissues of different groups. The significant differences between treatments are indicated by letters above columns (P<0.05). Dhimran essential oil (DEO), dhimran extract (DE)

to the control group. However, there was a notable increase in these parameters in the mice treated with combined application of DE+CCL₄ or DEO and CCl₄, compared to CCl₄. The indices are substantially enhanced (P<0.05) by the sole application of DEO or DE (Fig. 3).

Inflammatory Cytokines: The liver tissue of mice administered CCl₄ exhibited notable elevations in the mRNA levels of TNF-α and IL-6-related inflammation, as determined by qPCR, compared to the control group. When mice were orally pretreated with DEO or DE and subsequently intoxicated with CCl₄, the examined

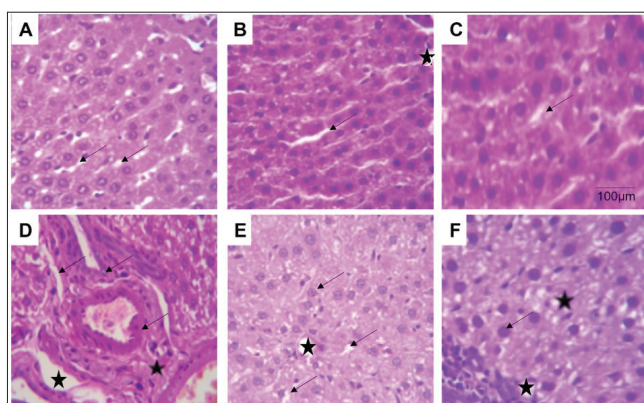


Fig 5. Photographs liver sections stained by H&E (X40) showing a) normal hepatic central vein and hepatic cords in control group; b) DE group, typical histological structure of hepatic lobule; c) DEO group showed standard histological structure; d) The CCl_4 group showed perivascular collagen fiber deposits adjacent to severe acute cell swelling; e) DE+ CCl_4 mild peribiliary lymphocytic infiltrations adjacent and recovery of normal tissues; f) DEO+ CCl_4 mild peribiliary lymphocytic infiltrations adjacent and recovery of normal tissues

inflammation cytokines exhibited significant ($P < 0.05$) downregulation (Fig. 4).

Histological Studies

Liver section tissue in control, DE, and DEO groups showed maintained liver cords, portal triad's structures, biliary system, vascular tributaries, sinusoids, Von Kuepfer's cells, and supporting stroma (Fig. 5-A,B,C). The liver section of 1/10 CCl_4 LD₅₀ treated groups showed portal biliary proliferative reactions. The portal blood vessels appear moderately to markedly dilate with occasional portal edema and infiltration of round cells (lymph-plasmacytes). The hepatic sinusoids are mild to moderately dilated, sometimes with atrophy of the surrounding hepatocytes (Fig. 5-D). Hepatic Sections from 1/10 CCl_4 LD₅₀ co-treated with DEO or DE revealed mild to moderate vascular dilatations, round cell aggregations, and recovery to the normal liver tissues (Fig. 5-E,F).

DISCUSSION

A growing need for alternative therapeutic agents devoid of severe adverse effects has necessitated the widespread application of natural plant compounds to treat various diseases. The global market has witnessed a surge in the demand for research about natural products owing to their challenging therapeutic attributes [35]. Furthermore, research has indicated that the escalating demand is significantly influenced by the therapeutic chemical composition and biological activity of natural products, such as essential oils (EOs), extracts, and other plant-based products, which possess surplus medicinal values [36].

In this study, we extracted the essential oils of *Ocimum forsskaolii* benth; the content of the essential oil profile

consisted of Bader et al. [14], who found the primary constituents of DEO were methyl eugenol, eugenol, linalool, germacrene D and β -caryophyllene with contents between (2.57-55.65%) %.

Also, Elansary and Mahmoud [37] found the main compounds in the *O. basilicum* oil were methyl cinnamate (43.8%) and chavicol methyl ether (39.1%).

The considerable content of EOs in basil cultivars possess various biological activities where the obtained results were consistent with other findings Nguyen et al. [38] stated that the volatile oil extracted from *Ocimum basilicum* by distillation steam and a Clevenger-type device, that have antioxidant activity determined by DPPH and linoleic acid peroxidation. The findings demonstrated that essential oils have antioxidant characteristics and revealed that the basil leaf extract exhibited a substantial antioxidant capacity, as evidenced by its IC₅₀ of 285.36 $\mu\text{g}/\text{mL}$.

Furthermore, Tshilanda et al. [39] determined the antioxidant potential of the basil plant using the DPPH free radical scavenging activity method. The essential oil had antioxidant properties, with an IC₅₀ of 1180 $\mu\text{g}/\text{mL}$. The methanolic and ethyl acetate extracts showed higher antioxidant activity than essential oil, with IC₅₀ values of 25 and 85 $\mu\text{g}/\text{mL}$, respectively. The essential oil was less active than methanol and ethyl acetate extracts regarding IC₅₀ values. The most active extract was methanol crude extract. The ability of non-polar extracts to scavenge radicals was minimal; hence, IC₅₀ values could not be determined. Also, Qasem et al. [6] estimated the antioxidant properties of basil (*Ocimum basilicum* L.) extracts that inhibit DPPH radicals with IC₅₀ 20 $\mu\text{g}/\text{mL}$; this activity because of the considerable content of the total flavonoids from 40 mg/g and total phenolic of 65 mg/g.

The antimicrobial activity of dhimran essential oil affects the development of three different kinds of bacteria. According to the survey findings, essential oils have a strong antibacterial effect on all Gram (+) and Gram (-) bacteria strains and the fungus *C. albicans*. The capacity of an essential oil constituent to permeate the cell walls of a bacteria or fungus is directly correlated with how soluble they are in water. Therefore, the solubility of essential oils in the phospholipid bilayer of cell membranes accounts for their antibacterial activity [40]. Consistent with the current study, the essential oil showed superior antibacterial activity against gram-positive bacteria (*S. aureus*) and moderate activity against gram-negative bacteria (*E. coli*), as evaluated by the agar diffusion method. The growth of bacteria was only faintly suppressed by essential oil at low concentrations (5 μL). Numerous literature studies have shown that several *Ocimum basilicum* essential oil components have antibacterial properties [41].

The essential oils and extract of dhimran possess anti-

cancer activity against liver cancer cell lines, as the obtained results stated; in this regard, Eid et al.^[15] indicated basil essential oil demonstrated potent anticancer activity against Hep3B (IC₅₀ 56.23 µg/mL) and MCF-7 (80.35 µg/mL) compared to Doxorubicin. The research indicates that the extract of *Ocimum basilicum* has an anti-proliferative effect on liver cancer cells due to the presence of phytochemicals, specifically phenolic compounds^[42].

This research investigates the impact of CCl₄ exposure on protein oxidation products and lipid peroxidation, including AOPP and LOOH^[43]. The observed elevation in free radical concentrations and compromised antioxidant defenses indicate that CCl₄ may induce cellular harm by inhibiting membrane mobility.

Liver fibrosis, distinguished by the accumulation of collagen in the liver, is affected by the detected elevation in hydroxyproline (HYP), the principal constituent of collagen^[44].

Glutathione is a vital constituent of the antioxidant defense system, aiding in maintaining cellular redox equilibrium and protecting against free radicals^[45]. Increased lipid and protein oxidation (LPO) levels, which indicate heightened ROS production, may account for the observed decrease in GSH levels and the activities of GPx and GR in rats treated with CCl₄^[5]. It is believed that enzymatic antioxidants (SOD and CAT) serve as the initial line of defense against the detrimental impacts of reactive oxygen species (ROS) produced by CCl₄ on biological macromolecules^[46].

The liver enzymes (ALT, AST, ALP, and LDH) significantly changed in CCl₄ mice because of the disruption of cellular membrane integrity caused by LPO^[5]. This disruption permits the release of cytoplasmic enzymes into the bloodstream after hepatocellular injury.

The present investigation examines the hepatic impacts of CCl₄, explicitly focusing on inflammation and the expression of TNF-α and IL-6 genes. Additionally, the function of reactive oxygen species (ROS) in producing pro-inflammatory cytokines such as IL-12, INF-α, and TNF-α is investigated. Reactive oxygen species (ROS) have a solid ability to stimulate the NF-κB transcription factor, an elemental component of inflammation and innate immunity^[47]. Elevated levels of IL-6 and TNF-α correlate with increased expression of pro-apoptotic genes IL-1β, IL-10, NF-κβ, Bax, and P53^[48].

Depletion of hepatic proteins signifies a breach in the integrity of the cell membrane, which permits the entry of toxicants into the cells^[48]. Reducing hepatic protein levels can impact genetics by stimulating hepatic cell growth through alterations in the cell cycle.

CCl₄ accumulates in mice's liver tissues, which induces a surge in the production of free radicals, oxidative stress, and apoptosis^[49]. It was demonstrated that

Ocimum gratissimum (OG), *O. basilicum* (OB), and *O. tenuiflorum* (OT) extracts possess more anti-inflammatory characteristics in their ability to scavenge nitric oxide and inhibit lipoxygenase^[50].

In this finding, the oral administration of DEO and DE decreased LPO and increased antioxidant status due to the oil's diverse phenolic components and natural products^[51]. DEO's decreased radical scavenging activity was almost certainly attributable to its principal component, estragole^[20]. DEO and DE supplementation alleviated the fluctuation of the antioxidant defense system and liver biomarkers induced by CCl₄^[52]. Due to its high concentration of phenolic compounds, including flavonoids and phenolic acids, basil possesses redox properties and can scavenge free radicals^[53]. With a substantial reduction in hydroxyproline deposition in hepatocytes, basil is an effective treatment for hepatic fibrosis^[54]. The study observed fibrotic changes in the liver and found that it stimulates liver regeneration in mice with fibrosis, which was alleviated by basil^[55]. Because of its strong ability to remove ROS and reduce inflammation, the essential oil of basil leaves might mitigate the cytotoxic impact of CCl₄ when utilized as a phytochemical drug.

CCl₄-induced oxidative stress disrupted liver function and antioxidant status biomarkers in mice liver tissue. A pre-treatment with DEO or DE restored the efficiency of enzymes and biomarkers. DEO and DE demonstrated substantial antioxidant, antibacterial, and anticancer effects attributed to their volatile molecules and phenolic constituents, which are scavengers for free radicals. Furthermore, the hepatotoxicity generated by CCl₄ in mice, including cell cycle arrest, inflammation, oxidative stress, and DNA damage, can be alleviated by DEO and DE's anti-inflammatory and antioxidant capabilities. Therefore, dhimran essential oil or ethanolic extract is a commonly accessible and affordable botanical treatment with significant antioxidant activity.

DECLARATIONS

Availability of Data and Materials: The datasets used and/or analyzed during the current study are available from the corresponding author (A. A. Alharbi) on reasonable request.

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Competing Interests: The authors declared that there is no conflict of interest.

Declaration of Generative Artificial Intelligence (AI): The author declare that the article tables and figures were not written or created by AI and AI-assisted technologies.

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