Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergikafkas.org
E-ISSN: 1309-2251

Kafkas Univ Vet Fak Derg
30 (5): 729-740, 2024
DOI: 10.9775/kvfd.2024.32444

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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How to cite this article?

Alharbi AA: *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. *Kafkas Univ Vet Fak Derg,* 30 (5): 729-740, 2024.
DOI: 10.9775/kvfd.2024.32444

Article ID: KVFD-2024-32444
Received: 05.06.2024
Accepted: 09.08.2024
Published Online: 10.09.2024

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 CCl_4 (187 mg/kg BW); T3 received DEO (4 mL/kg); T4 received DE (4 mL/kg); T5 received DEO+CCl_4; and T6 received DE+CCl_4. The oral administration of CCl_4 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 CCL_4 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 ($P<0.05$). CCl_4 increased inflammation cytokines (TNF-α and IL-6). The coadministration of DEO or DE with CCl_4 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 CCl_4 toxicity.

Keywords: Anticancer, Antioxidant, Anti-inflammatory, CCl_4 Toxicity, Essential oil, 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].

Ocimum forsskaolii benth, which is named locally in Saudi Arabia as Dhimran 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 IC_{50} 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 (CCl_4) 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 CCl_4 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 CCl_4-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)

Ocimum forsskaolii benth plant was collected at Al-Shafa area in Taif City, Saudi Arabia, in June 2023. The plant was identified and authenticated by Dr. Faraj Al-Ghamdi (taxonomist at King Abdulaziz University Herbarium). A plant was deposited at King Abdulaziz University Herbarium, Faculty of Sciences, King Abdulaziz University, Kingdom of Saudi Arabia; the reference specimen is (A. Harbi, 708). The Dhimran essential oil (DEO) was purchased from Aldousiah Farm, Al Bahah, Saudia Arabia.

The Dhimran leaves were dried in the shade and 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 in DEO and DE were 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 µm), (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 µg/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:

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

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) $BaCl_2$ and (99.5 mL) H_2SO_4 to obtain a solution with equivalent turbidity to 1.5×10^8 (CFU mL^{-1}) cell density. 200 µL 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 µg/mL streptomycin sulfate, and 25 µg/mL amphotericin B), as well as 1% L-glutamine at 37°C under 5% CO_2 using a water-jacketed carbondioxide 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 CO_2. The medium was replaced with a fresh one supplemented with FBS and varying concentrations of DE or DEO (50, 100, and 200 µg/mL), then incubated for two days [27]. The medium was aspirated after 48 h of incubation. Then, 40 µL of MTT salt (2.5 µg/mL) was added to each well, and the cells were further incubated for 4 hat 37°C with 5% CO_2. To end the reaction and dissolve crystals, 200 µL of 10% SDS in deionized water was added to each well and incubated overnight at 37°C. A known cytotoxic natural substance (100 µg/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.

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

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 (CCl_4, 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+CCl_4, and the sixth group delivered DE+CCl_4. 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

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	

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

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 IC_{50} of 50 µg/mL, followed by DEO.

Table 2. The antioxidant content of dhimran essential oil and ethanolic extract			
Parameters	**Dhimran (*Ocimum forsskaolii* benth)**		**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 raw 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	

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

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**	
S. typhi	11±0.2c	14±0.2b	18±0.1a	9±0.2d	11±0.6c	15±0.3b	<0.0001
C. jejuni	9±0.1d	11±0.5c	15±0.3a	0±0.0	9±0.2d	12±0.2b	<0.0001
L. monocytogenes	11±0.3c	16±0.3b	22±0.4a	9±0.1d	12±0.5c	17±0.3b	<0.0001
S. aureus	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 raw indicate significant differences at P<0.05. Salmonella typhi, Campylobacter jejuni, Listeria monocytogenes, Staphylococcus aureus. Dhimran essential oil (DEO), Dhimran extract (DE)							

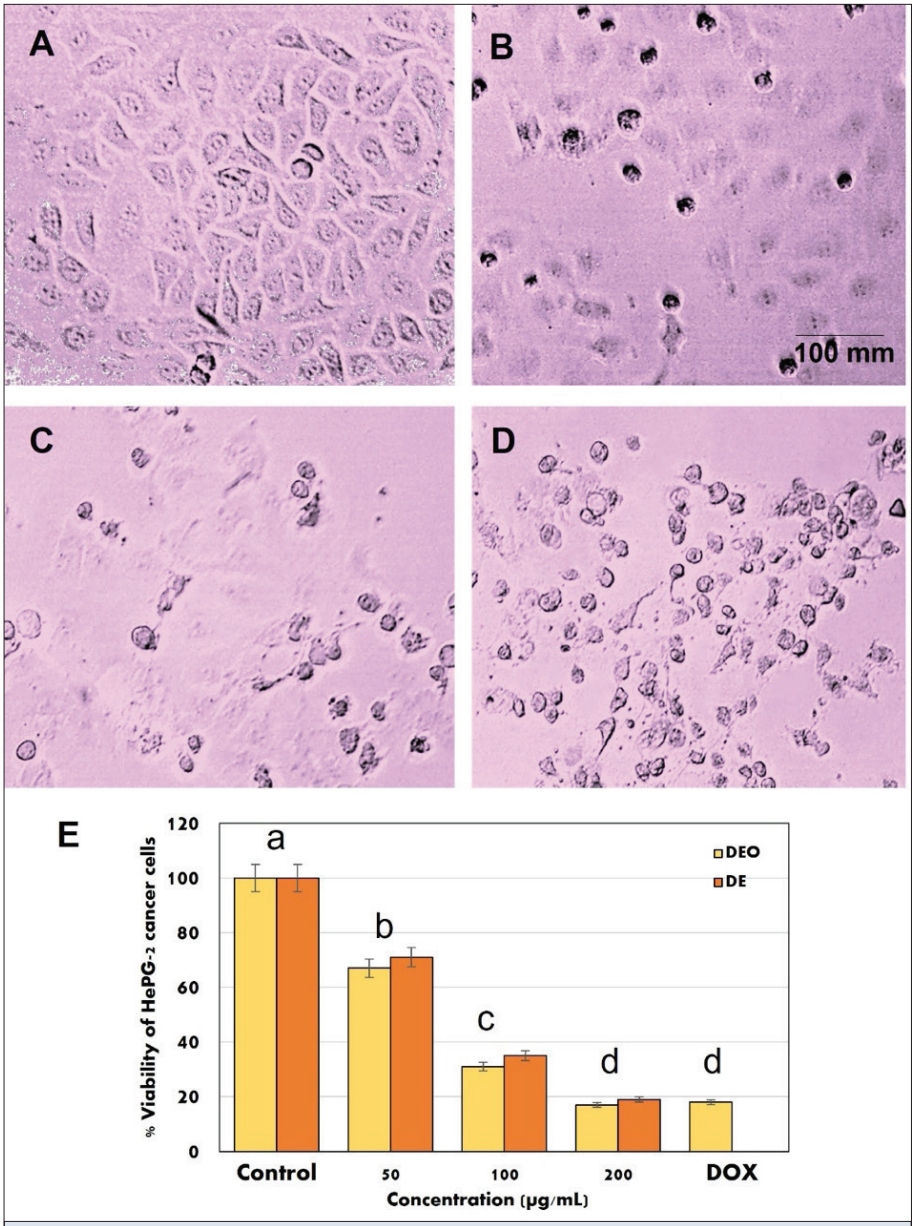

Fig 1. Cytotoxicity of essential oil and ethanolic extract of *Ocimum forsskaolii* benth on the viability of liver cancer cell lines (HePG2) sections stained with H&E (X40), (A) control cancerous cells, (B) *Ocimum forsskaolii* extract (200 µg/mL), (C) *Ocimum forsskaolii* essential oil (200 µg/mL), (D) Doxorubicin (DOX), (E) Histogram of the anticancer effect of *Ocimum forsskaolii* benth essential oil and extract on the viability of HepG2 cell line. Lowercase letters above column indicate signficant differences at P<0.05

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 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

Table 5. *The effect of DEO and DE administration on growth parameters of CCl_4-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 raw 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 CCl_4 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 raw 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 CCl_4 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 raw indicate significant differences at p<0.05. Dhimran essential oil (DEO), Dhimran extract (DE)

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 other hand, the IC_{50} of treatments inversely correlated with the inhibition percentage, where the IC_{50} 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 CCl_4 Toxicity

Growth Parameters: Clinical poisoning manifestations were observed in the CCl_4 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 CCl_4 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 CCl_4 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+CCl_4 or DE+CCl_4 significantly P<0.05

restored all levels to the control levels compared to the CCl_4 group *(Table 6)*.

Liver Enzymes: The liver (ALP, AST, ALT, and LDH) enzymes are of interest as potential hepatotoxicity biomarkers. The administration of CCL_4 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+CCl_4 or DE+CCl_4 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 CCl_4 compared to the control group. However, mice that were administered DEO+CCl_4 or DE+ CCl_4 exhibited a substantial reduction in the concentrations of LOOH in comparison to the CCl_4-treated group *(Fig. 2-A)*. Furthermore, the hepatic levels of protein oxidation indicator, advanced oxidized protein products (AOPP), were significantly elevated in the CCl_4-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)*.

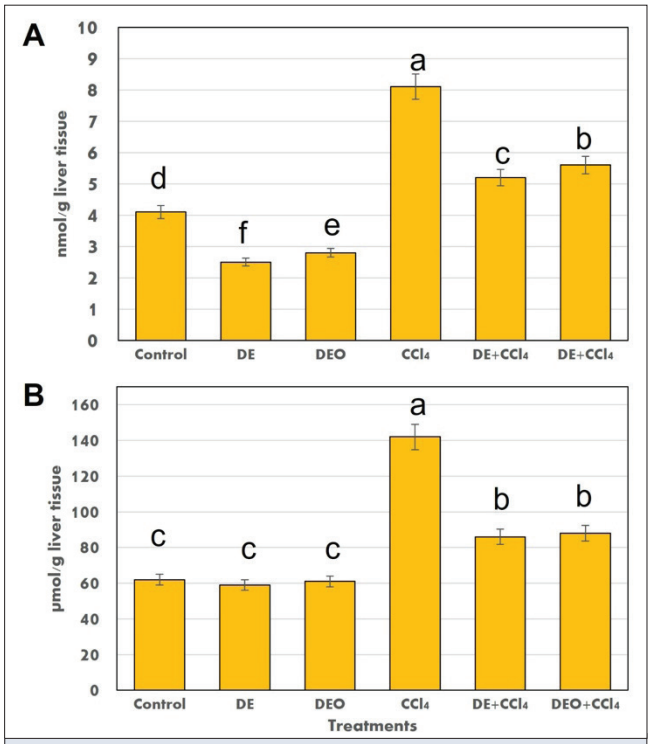

Fig 2. The effect of DEO and DE on mitigating oxidative stress induced by hepatocytes CCl_4 administration, (A) Lipid hydroperoxide (LOOH), (B) Advanced oxidized protein product (AOPP). The significant differences between treatments are indicated by letters above columns ($P<0.05$)

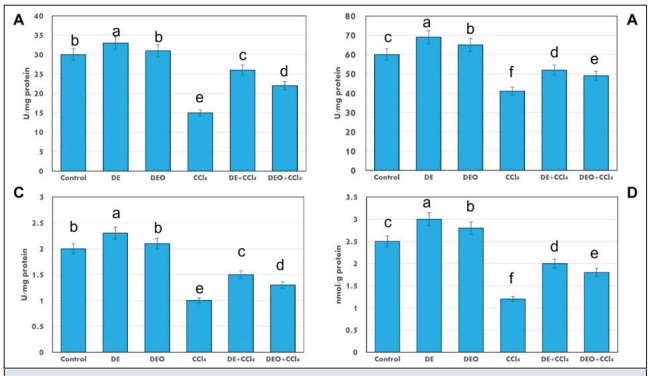

Fig 3. The effect of DEO or DE on enhancing the antioxidant system defense in hepatocytes rats, (A) SOD activity; (B) Catalase activity; (C) GPx activity; (D) GSH activity. The significant differences between treatments are indicated by letters above columns ($P<0.05$). Dhimran essential oil (DEO), Dhimran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

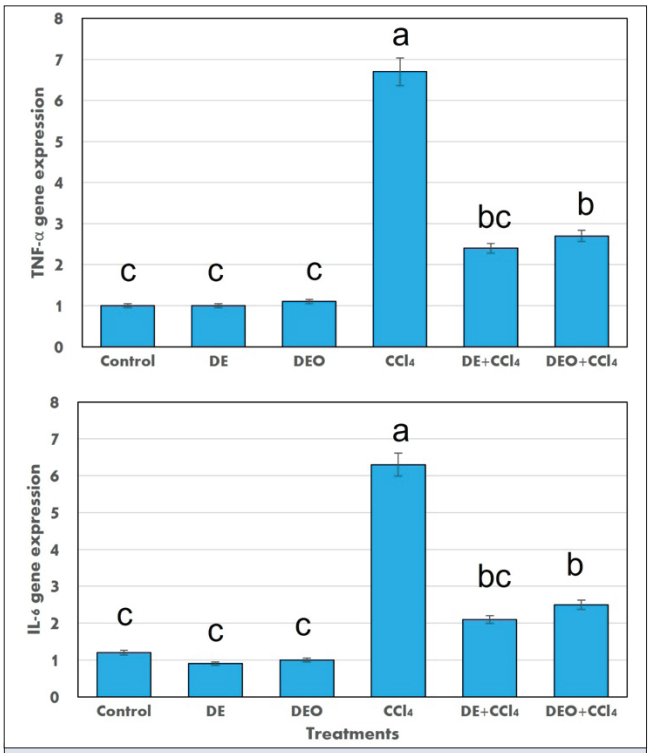

Fig 4. Relative expression of *IL-6* and TNF-α pro-inflammatory cytokines in mice liver tissues of different groups. The significant differences between treatments are indicated by letters above columns ($P<0.05$). Dhimran essential oil (DEO), Dhimran extract (DE)

Antioxidant Status: The liver homogenates of CCl_4-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 to the control group. However, there was a notable increase in these parameters in the mice treated with combined application of DE+CCL_4 or DEO and CCl_4, compared to CCL_4. 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 CCl_4 exhibited notable elevations in the

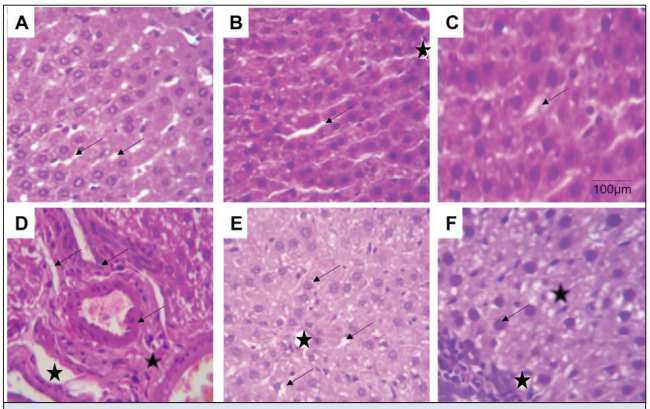

Fig 5. Photographs liver sections stained by H&E (X40) showing a) normal hepatic central vein and hepatic cords in control group; b) DE group, typical histological structure of hepatic lobule; c) DEO group showed standard histological structure; d) The CCl_4 group showed perivascular collagen fiber deposits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

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 CCL_4, the examined 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 LD_{50} 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 LD_{50} 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

Plant compounds are commonly used as alternative medicinal agents due to their reduced adverse side effects. 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, that possess surplus medicinal values [36].

In this study, the essential oils of *Ocimum forsskaolii* benth was analysed. Similiarly, in a study by Bader et al.[14], the essential oil of Ocimum forsskaolii benth was found to contain methyl eugenol, eugenol, linalool, germacrene D, and β-caryophyllene as its primary constituents. These compounds comprised between 2.57% and 55.65% of the oil.

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 µg/mL.

Furthermore, Tshilanda et al.[39] determined the anti-oxidant potential of the basil plant using the DPPH free radical scavenging activity method. The essential oil had antioxidant properties, with an IC_{50} of 1180 µg/mL. The methanolic and ethyl acetate extracts showed higher antioxidant activity than essential oil, with IC_{50} values of 25 and 85 µg/mL, respectively. The essential oil was less active than methanol and ethyl acetate extracts regarding IC_{50} values. The most active extract was methanol crude extract. The ability of non-polar extracts to scavenge radicals was minimal; hence, IC_{50} 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 IC_{50} 20 µg/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 (IC_{50} 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 CCl_4 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 CCl_4 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 CCl_4 [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 CCl_4 on biological macromolecules [46].

The liver enzymes (ALT, AST, ALP, and LDH) significantly changed in CCl_4 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 CCl_4, 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-kB 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-kβ, 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.

CCl_4 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 CCl_4 [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 CCl_4 when utilized as a phytochemical drug.

CCl_4-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 CCl_4 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.

Acknowledgments: This project was funded by the Deanship of Scientific Research (DSR) at King Abdulaziz University, Jeddah, Saudi Arabia, under grant no. (GPIP-533-247-2024). The authors, therefore,acknowledge with thanks DSR for technical and financial support.

Funding: This project was funded by the Deanship of Scientific Research (DSR) at King Abdulaziz University, Jeddah, Saudi Arabia,

under grant no. (GPIP-533-247-2024). The authors, therefore, acknowledge with thanks DSR for technical and financial support.

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.

References

1. Martiz RM, Patil SM, Abdulaziz M, Babalghith A, Al-Areefi M, Al-Ghorbani M, Mallappa Kumar J, Prasad A, Mysore Nagalingaswamy NP, Ramu R: Defining the role of isoeugenol from *Ocimum tenuiflorum* against diabetes mellitus-linked Alzheimer's disease through network pharmacology and computational methods. *Molecules,* 27:2398, 2022. DOI: 10.3390/molecules27082398

2. Nadeem HR, Akhtar S, Ismail T, Qamar M, Sestili P, Saeed W, Azeem M, Esatbeyoglu T: Antioxidant effect of *Ocimum basilicum* essential oil and its effect on cooking qualities of supplemented chicken nuggets. *Antioxidants,* 11:1882, 2022. DOI: 10.3390/antiox11101882

3. Sharmin E, Kafyah MT, Alzaydi AA, Fatani AA, Hazazzi FA, Babgi SK, Alqarhi NM, Sindi AAH, Akram D, Alam M, Alam J, Al-Madboly LA, Shoeib NA, Alqahtani AM, Mojally M: Synthesis and characterization of polyvinyl alcohol/corn starch/linseed polyol-based hydrogel loaded with biosynthesized silver nanoparticles. *Int J Biol Macromol,* 163, 2236-2247, 2020. DOI: 10.1016/j.ijbiomac.2020.09.044

4. Arshad Qamar K, Dar AS, Siddiqui B, Kabir N, Aslam H, Ahmed S, Erum S, Habib S, Begum S: Anticancer activity of *Ocimum basilicum* and the effect of ursolic acid on the cytoskeleton of MCF-7 human breast cancer cells. *J Lett Drug Design,* 7, 726-736, 2010. DOI: 10.2174/1570180811007010726

5. Kumar A, Siddiqi NJ, Alrashood ST, Khan HA, Dubey A, Sharma B: Protective effect of eugenol on hepatic inflammation and oxidative stress induced by cadmium in male rats. *Biomed Pharmacother,* 139:111588, 2021. DOI: 10.1016/j.biopha.2021.111588

6. Qasem A, Assaggaf H, Mrabti HN, Minshawi F, Rajab BS, Attar AA, Alyamani RA, Hamed M, Mrabti NN, Baaboua AE, Omari NE, Alshahrani MM, Awadh AAA, Sheikh RA, Ming LC, Goh KW, Bouyahya A: Determination of chemical composition and investigation of biological activities of *Ocimum basilicum* L. *Molecules*, 28 (2):614, 2023. DOI: 10.3390/molecules28020614

7. Ali NAA, Chhetri BK, Dosoky NS, Shari K, Al-Fahad AJA, Wessjohann L, Setzer WN: Pollen morphology of certain species of the family Lamiaceae in Saudi Arabia. *Saudi J Biol Sci,* 25, 354-360, 2018. DOI: 10.1016/j.sjbs.2017.03.001

8. Ali NAA, Chhetri BK, Dosoky NS, Shari K, Al-Fahad AJ, Wessjohann L, Setzer WN: Antimicrobial, antioxidant, and cytotoxic activities of *Ocimum forskolei* and *Teucrium yemense* (Lamiaceae) essential oils. *J Medicines*, 4 (2):17, 2017. DOI: 10.3390/medicines4020017

9. Elsayed A, Elkomy A, Alkafafy M, Elkammar R, Fadl SE, Abdelhiee EY, Abdeen A, Youssef G, Shaheen H, Soliman A, Aboubakr M: Ameliorating effect of lycopene and N-acetylcysteine against cisplatin-induced cardiac injury in rats. *Pak Vet J,* 42, 107-111, 2022. DOI: 10.29261/pakvetj/2021.035

10. Aboubakr M, Elmahdy AM, Taima S, Emam MA, Farag A, Alkafafy M, Said AM, Soliman A: Protective effects of N acetylcysteine and vitamin E against acrylamide-induced neurotoxicity in rats. *Pak Vet J,* 43, 262-268, 2023. DOI: 10.29261/pakvetj/2023.027

11. Fatope MO, Marwah RG, Al Hadhrami NM, Onifade AK, Williams JR: Identification of the chemotypes of *Ocimum forskolei* and *Ocimum basilicum* by NMR spectroscopy. *Chemistry,* 5, 2457-2463, 2008. DOI: 10.1002/cbdv.200890211

12. Odalo JO, Omolo MO, Malebo H, Angira J, Njeru PM, Ndiege IO, Hassanali A: Repellency of essential oils of some plants from the Kenyan coast against *Anopheles gambiae*. *Acta Tropica,* 95, 210-218, 2005. DOI: 10.1016/j.actatropica.2005.06.007

13. Stanojevic LP, Marjanovic-Balaban ZR, Kalaba VD, Stanojevic JS, Cvetkovic DJ, Cakic MD: Chemical composition, antioxidant and antimicrobial activity of basil *(Ocimum basilicum* L.) essential oil. *J Essent Oil-Bear Plants,* 20, 1557-1569, 2017. DOI: 10.1080/0972060X.2017.1401963

14. Bader A, Abdalla AN, Obaid NA, Youssef L, Naffadi HM, Elzubier ME, Almaimani RA, Flamini G, Pieracci Y, El-Readi MZ: *In vitro* anticancer and antibacterial activities of the essential oil of forsskal's basil growing in extreme environmental conditions. *Life,* 13 (3):651, 2023. DOI: 10.3390/life13030651

15. Eid AM, Jaradat N, Shraim N, Hawash M, Issa L, Shakhsher M, Nawahda N, Hanbali A, Barahmeh N, Taha B, Mousa A: Assessment of anticancer, antimicrobial, antidiabetic, anti-obesity and antioxidant activity of *Ocimum basilicum* seeds essential oil from Palestine. *BMC Complement Med Ther,* 23:221, 2023. DOI: 10.1186/s12906-023-04058-w

16. Fareed MM, Khalid H, Khalid S, Shityakov S: Deciphering molecular mechanisms of carbon tetrachloride-induced hepatotoxicity (fibrosis): A brief systematic review. *Curr Mol Med,* 24 (9): 1124-1134, 2024. DOI: 10.2174/0115665240257603230919103539

17. Seyed MA, Ayesha S, Azmi N, Al-Rabae FM, Al-Alawy AI, Al-Zahrani OR, Hawsawi Y: The neuroprotective attribution of *Ocimum basilicum*: A review on the prevention and management of neurodegenerative disorders. *Futur J Pharm Sci,* 7:139, 2021. DOI: 10.1186/s43094-021-00295-3

18. Dhama K,Sharun K, Gugjoo MB, Tiwari R, Alagawany M, Iqbal Yatoo M, Thakur P, Iqbal HM, Chaicumpa W, Michalak I: A comprehensive review on chemical profile and pharmacological activities of *Ocimum basilicum*. *Food Rev Int,* 39, 119-147, 2023. DOI: 10.1080/87559129.2021.1900230

19. Sun Y, Liu Y, Ma X, Hu H: The influence of cell cycle regulation on chemotherapy. *Int J Mol Sci,* 22:6923, 2021. DOI: 10.3390/ijms22136923

20. Teofilović B, Grujić-Letić N, Gligorić E, Rašković A, Igić R, Vastag G, Gadžurić S: Experimental and computational evaluation of extraction procedure and scavenging capacity of sweet basil extracts *(Ocimum basilicum* L.). *Plant Foods Hum Nutr,* 76, 240-247, 2021. DOI: 10.1007/s11130-021-00902-x

21. Saad AM, Sitohy MZ, Ahmed AI, Rabie NA, Amin SA, Aboelenin SM, Soliman MM, El-Saadony MT: Biochemical and functional characterization of kidney bean protein alcalase-hydrolysates and their preservative action on stored chicken meat. *Molecules,* 26:4690, 2021. DOI: 10.3390/molecules26154690

22. Zukowska G, Durczynska Z: Properties and applications of essential oils: A review. *J Ecol Eng,* 25 (2): 333-340, 2024. DOI: 10.12911/22998993/177404

23. Abd Elkader AM, Labib S, Taha TF, Althobaiti F, Aldhahrani A, Salem HM, Saad A, Ibrahim FM: Phytogenic compounds from avocado *(Persea americana* L.) extracts; antioxidant activity, amylase inhibitory activity, therapeutic potential of type 2 diabetes. *Saudi J Biol Sci,* 29, 1428-1433, 2022. DOI: 10.1016/j.sjbs.2021.11.031

24. El-Saadony MT, Khalil OS, Osman A, Alshilawi MS, Taha AE, Aboelenin SM, Shukry M, Saad AM: Bioactive peptides supplemented raw buffalo milk: Biological activity, shelf life and quality properties during cold preservation. *Saudi J Biol Sci,* 28, 4581-4591, 2021. DOI: 10.1016/j.sjbs.2021.04.065

25. Jia N, Kong B, Liu Q, Diao X, Xia X: Antioxidant activity of black currant *(Ribes nigrum* L.) extract and its inhibitory effect on lipid and protein oxidation of pork patties during chilled storage. *Meat Sci,* 91, 533-539, 2012. DOI: 10.1016/j.meatsci.2012.03.010

26. Singh G, Passsari AK, Leo VV, Mishra VK, Subbarayan S, Singh BP, Kumar B, Kumar S, Gupta VK, Lalhlenmawia H, Nachimuthu SK: Evaluation of phenolic content variability along with antioxidant, antimicrobial, and cytotoxic potential of selected traditional medicinal plants from India. *Front Plant Sci,* 7:407, 2016. DOI: 10.3389/fpls.2016.00407

27. Mosmann T: Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J Immunol Methods,* 65, 55-63, 1983. DOI: 10.1016/0022-1759(83)90303-4

28. Chunarkar-Patil P, Kaleem M, Mishra R, Ray S, Ahmad A, Verma D, Bhayye S, Dubey R, Singh HN, Kumar S: Anticancer drug discovery based on natural products: From computational approaches to clinical studies. *Biomedicines,* 12 (1):201, 2024. DOI: 10.3390/biomedicines12010201

29. National Research Council: Nutrient Requirements of Poultry: Ninth Revised Edition, 1994. Washington, DC: The National Academies Press, 1994. DOI: 10.17226/2114

30. Zhou L, Abouelezz K, Momenah MA, Bajaber MA, Baazaoui N, Taha TF, Awad AE, Alamoudi SA, Beyari EA, Alanazi YF, Allohibi A, Saad AM: Dietary *Paenibacillus polymyxa* AM20 as a new probiotic: Improving effects on IR broiler growth performance, hepatosomatic index, thyroid hormones, lipid profile, immune response, antioxidant parameters, and caecal microorganisms. *Poult Sci,* 103 (2):103239, 2024. DOI: 10.1016/j.psj.2023.103239

31. Alghamdi MA, Reda FM, Mahmoud HK, Bahshwan SM, Salem HM, Alhazmi WA, Soror AFS, Mostafa NG, Attia S, Mohamed MDA, Saad AM, El-Tarabity KA, Abdelgeliel SA: The potential of *Spirulina platensis* to substitute antibiotics in Japanese quail diets: Impacts on growth, carcass traits, antioxidant status, blood biochemical parameters, and cecal microorganisms. *Poult Sci,* 103:103350, 2024. DOI: 10.1016/j.psj.2023.103350

32. Saad AM, Elmassry RA,Wahdan KM, Ramadan FM: Chickpea *(Cicer arietinum)* steep liquor as a leavening agent: Effect on dough rheology and sensory properties of bread. *Acta Period Technol,* 46 (46): 91-102. DOI: 10.2298/APT1546091S

33. Trinder PJ: Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. *Ann Clin Biochem,* 6, 24-27, 1969. DOI: 10.1177/00045632690060010

34. El-Saadony MT, Saad AM, Elakkad HA, El-Tahan AM, Alshahrani OA, Alshilawi MS, El-Sayed H, Amin SA, Ahmed AI: Flavoring and extending the shelf life of cucumber juice with aroma compounds-rich herbal extracts at 4°C through controlling chemical and microbial fluctuations. *Saudi J Biol Sci,* 29, 346-354, 2022. DOI: 10.1016/j.sjbs.2021.08.092

35. Piras A, Gonçalves MJ, Alves J, Falconieri D, Porcedda S, Maxia A, Salgueiro L: *Ocimum tenuiflorum* L. and *Ocimum basilicum* L., two spices of Lamiaceae family with bioactive essential oils. *Ind Crops Prod,* 113, 89-97, 2018. DOI: 10.1016/j.indcrop.2018.01.024

36. Sakkas H, Papadopoulou C: Antimicrobial activity of basil, oregano, and thyme essential oils. *J Microbiol Biotechnol,* 27, 429-438, 2017. DOI: 10.4014/jmb.1608.08024

37. Elansary HO, Mahmoud EA: Basil cultivar identification using chemotyping still favored over genotyping using core barcodes and possible resources of antioxidants. *J Essent Oil Res,* 27, 82-87, 2015. DOI: 10.1080/10412905.2014.982874

38. Nguyen V, Nguyen N, Thi N, Thi C, Truc T, Nghi P: Studies on chemical, polyphenol content, flavonoid content, and antioxidant activity of sweet basil leaves *(Ocimum basilicum* L.). *IOP Conference Series: Mater Sci Eng,* 1092 (1):012083, 2021. DOI: 10.1088/1757-899X/1092/1/012083

39. Tshilanda DD, Mutwale PK, DOnyamboko DVN, Babady PB, Tsalu PV, Tshibangu DST, Ngombe NK, Frederich M, Ngbolua KN, Mpiana PT: Chemical fingerprint and anti-sickling activity of rosmarinic acid and methanolic extracts from three species of ocimum from DR Congo. *J Biosci Med,* 4, 2016. DOI: 10.4236/jbm.2016.41008

40. Knobloch K, Pauli A, Iberl B, Weigand H, Weis N: Antibacterial and antifungal properties of essential oil components. *J Essent Oil Res,* 1, 119-128, 1989. DOI: 10.1080/10412905.1989.9697767

41. Zhakipbekov K, Turgumbayeva A, Akhelova S, Bekmuratova K, Blinova O, Utegenova G, Shertaeva K, Sadykov N, Tastambek K, Saginbazarova A, Urazgaliyev K, Tulegenova G, Zhalimova Z, Karasova Z: Antimicrobial and other pharmacological properties of *Ocimum basilicum*, Lamiaceae. *Molecules,* 29 (2):388, 2024. DOI: 10.3390/molecules29020388

42. Hazrati S, Ebadi MT, Mollaei S, Khurizadeh S: Evaluation of volatile and phenolic compounds, and antioxidant activity of different parts of *Ferulago angulata* (schlecht.) Boiss. *Ind Crops Prod,* 140:111589, 2019. DOI: 10.1016/j.indcrop.2019.111589

43. Iqbal Z, Aftab A, Swar SO, Hussain R, Mohiuddin M: Determination of lipid lowering effect of corn flour in hyperlipidemic albino mice. *Pak Vet J,* 42 (1): 112-116, 2022. DOI: 10.29261/pakvetj/2021.075

44. Huss M, Elger T, Loibl J, Kandulski A, Binder B, Stoeckert P, Patricia Mester P, Müller M, Buechler C, Tews HC: Urinary hydroxyproline as an inflammation-independent biomarker of inflammatory bowel disease. *Gastroenterol Insights,* 15 (2): 486-497, 2024. DOI: 10.3390/gastroent15020035

45. Vairetti M, Di Pasqua LG, Cagna M, Richelmi P, Ferrigno A, Berardo C: Changes in glutathione content in liver diseases: An update. *Antioxidants,* 10:364, 2021. DOI: 10.3390/antiox10030364

46. Hashem AS: Defensive impact of propolis against CCl_4 actuated rats' testicular damage. *J Adv Vet Anim Res,* 8 (1): 70-77, 2021. DOI: 10.5455/javar.2021.h487

47. Maaruf NA, Najjar SM, Ali SA: Anatomical and histopathological comparative studies between right and left mammary tumors in a rat model induced by DMBA. *Pak Vet J,* 43 (2): 232-240, 2023. DOI: 10.29261/pakvetj/2023.037

48. Tang SP, Mao XL, Chen YH, Yan LL, Ye LP, Li SW: Reactive oxygen species induce fatty liver and ischemia-reperfusion injury by promoting inflammation and cell death. *Front Immunol,* 13:870239, 2022. DOI: 10.3389/fimmu.2022.870239

49. Fatima M, Khan MR, Al-Keridis LA, Alshammari N, Patel M, Adnan M, Sahreen S: *Pleurospermum candollei* methanolic extract ameliorates CCl_4-Induced liver injury by modulating oxidative stress, inflammatory, and apoptotic markers in rats. *ACS Omega,* 8, 25999-26011, 2023. DOI: 10.1021/acsomega.3c02031

50. Hamid S, Oukil NF, Moussa H, Djihad N, Mróz M, Kusznierewicz B, Attia A, Djenadi K, Mahdjoub MM, Bouhenna MM, Chebrouk F: Chemical and biological characterization of *Ocimum basilicum* L. phenolic extract and essential oil derived through ultrasound and microwave-assisted extraction techniques. *Food Biosci,* 60:104359, 2024. DOI: 10.1016/j.fbio.2024.104359

51. Sindi RA, Alam S, Rizwan M, Ullah MI, Ijaz N, Iqbal Z, Muzafar R, Akram R, Nazar MW, Hussain R: Investigations of hemato-biochemical, histopathological, oxidative stress and reproductive effects of thiram in albino rats. *Pak Vet J,* 43 (2): 255-261, 2023. DOI: 10.29261/pakvetj/2023.031

52. Renovaldi D, Adam AK: Potential of sweet basil *(Ocimum basilicum)* as a hepatoprotector agent for liver injury related to drugs. *Muhammadiyah Med J,* 1, 63-68, 2020. DOI: 10.24853/mmj.1.2.21-26

53. Rezzoug M, Bakchiche B, Gherib A, Roberta A, Guido F, Kilinçarslan Ö, Mammadov R, Bardaweel SK: Chemical composition and bioactivity of essential oils and ethanolic extracts of *Ocimum basilicum* L. and *Thymus algeriensis* Boiss. & Reut. from the Algerian Saharan Atlas. *BMC Complement Med Ther,* 19, 1-10, 2019. DOI: 10.1186/s12906-019-2556-y

54. Téllez-Morales JA, Rodríguez-Miranda J, Aguilar-Garay R: Review of the influence of hot air frying on food quality. *Measurement: Food,* 14:100153, 2024. DOI: 10.1016/j.meafoo.2024.100153

55. El-Wakeil NHM, Salem ZAW: Possible protective effect of basil seeds and their oil against zinc oxide nanoparticle-induced nephrotoxicity in rats. *Egypt J Nutr,* 39 (1): 163-184. 2024.