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RESEARCH ARTICLE

Biochemical Characterization of Some Basil Cultivars Extracts with Biological Activities on Physiological, Immunological, Antioxidant Properties, and Modulation of Defense Genes in Carbon Tetrachloride-Induced Rats

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ABSTRACT

Global trends favor plant-derived bioactive compounds as eco-friendly alternatives to synthetic drugs. This is driven by consumer demand for natural solutions that provide effective treatments with potential environmental safety, thus supporting sustainable healthcare. In this study, the active compounds and the antioxidant, antimicrobial, cytotoxic, and hepatoprotective properties of essential oils (EOs) derived from three basil cultivars were evaluated, with a focus on lemon basil essential oil (LEO) in the context of CCl_4-induced hepatotoxicity in rats. The main volatile compound in LEO was methyl cinnamate (42.02%). LEO significantly inhibited 93% of DPPH and had broad-spectrum antimicrobial effects against pathogenic microbial isolates in the animal body, specifically gut, skin, and dental tissues, causing inflammation and tissue destruction (*Listeria monocytogenes, Bacillus cereus, Staphylococcus aureus, Campylobacter jejuni, Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa, Porphyromonas gingivalis, Enterococcus faecalis, Candida tropicalis,* and *Candida albicans*), with MIC values (0.125–0.5 mg/mL). It also exhibited potent cytotoxicity against HepG2 liver cancer cells (86% inhibition at an IC_{50} of 20 µg/mL). *In vivo*, CCl_4 administration induced significant hepatotoxicity, evidenced by elevated liver enzymes, increased oxidative stress markers (LOOH, AOPP), reduced antioxidant defenses (GSH, SOD, CAT, GPx), upregulated inflammatory markers (TNF-α, IL-6), and severe histopathological alterations. Importantly, LEO pretreatment significantly mitigated physiological distress, effectively reducing body weight loss and suppressing hepatomegaly, confirming its role in stabilizing key physiological parameters without adverse effects. It also upregulated the antioxidant defense genes (Nrf2, HO-1, SOD, CAT) and downregulated pro-inflammatory genes (TNF-α, IL-6). LEO also exerted protective effects against CCl_4-induced hepatotoxicity. *In vivo* evidence demonstrates that LEO activates Nrf2-mediated antioxidant defense and modulates the gut microbiome, thereby establishing a liver-gut protective synergy. It concluded that LEO offers a sustainable, multi-target nutraceutical alternative to synthetic liver drugs by dual-regulating oxidative and inflammatory genes.

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INTRODUCTION

Basil (*Ocimum* spp.) is a member of the mint family, Lamiaceae, and has been extensively used in traditional medicine in past centuries. The plant comes from tropical areas of Southeast Asia and Central Africa. It has just recently been grown and used around the world (Lee *et al.*, 2005). It is rich in bioactive compounds, especially essential oils and polyphenols (Nadeem *et al.*, 2022). These phytochemicals give basil its featured sensory properties, i.e., taste and scent, as well as its pharmacological efficacy. Besides, these compounds show considerable antioxidant, antimicrobial, anti-inflammatory, and anticarcinogenic activities (Takeuchi *et al.*, 2020; Perna *et al.*, 2022).

Basil extracts have long been used in traditional medicine to treat respiratory diseases, skin problems, and digestive disorders (Kunnumakkara *et al.*, 2018). Recent scientific evidence also suggests that the plant has great potential in the prevention and clinical treatment of chronic diseases such as cancer, diabetes and cardiovascular diseases (Mahmoud *et al.*, 2016; Widjaja and Savira, 2019).

There are many species of the genus *Ocimum* which are rich in volatile essential oils, usually ranging from 0.1 to 0.7%. Current phytochemical literature has documented over 200 unique chemical constituents within these oils, most notably 1,8-cineole, linalool, and methyl chavicol (estragole). While these aromatic compounds are industrially indispensable for their flavoring and olfactory properties, they also serve as potent antimicrobial and antioxidant agents (Filip *et al.*, 2016; Muráriková *et al.*, 2017; Sharmeen *et al.*, 2021).

On the other hand, carbon tetrachloride (CCl_4) is a synthetic halocarbon that was used in the past for the synthesis of chlorofluorocarbons, as well as for pesticide and industrial solvent applications. Its production is now strictly controlled due to its severe toxicological profile. The compound's high vapor pressure and environmental persistence lead to its bioaccumulation in the atmosphere and groundwater. The main routes of exposure for the general population include inhalation of contaminated air and ingestion of contaminated water. High level exposure results in multi-organ failure, specifically affecting the hepatic, renal, pulmonary and central nervous systems, and the compound is classified as a probable human carcinogen (Fareed *et al.*, 2024).

Basil is widely known as one of the most effective herbal medicines for various symptoms (Patel *et al.*, 2018). It exhibits various biological activities including antitumor, antioxidant, antibacterial, anti-inflammatory, and anti-genotoxic activities (Teofilović *et al.*, 2021). Several empirical studies have investigated the complex pharmacological profile of basil, which exhibits therapeutic versatility across a wide range of clinical pathologies. Evidence suggests that its bioactive components exhibit neuroprotective effects in traumatic brain injury and antifibrotic activity in liver tissue. In addition, studies indicate that the herb may modulate metabolic dysfunction, particularly in the management of Type 2 diabetes mellitus. Apart from its effects on metabolic health, basil has been shown to decrease hypersensitivity reactions and to counteract hematological disturbances, including anemia, and thus is an important prospective candidate for integrative medicinal applications (Eftekhar *et al.*, 2019; Widjaja and Savira, 2019; Zangeneh *et al.*, 2019). The co-administration of *O. basilicum* and deltamethrin also provided protection against oxidative stress caused by mercury chloride and cadmium (Sakr and Al-Amoudi, 2012; Sakr *et al.,* 2013). Although the pharmacological properties of basil have been studied intensively, there are limited comparative studies on the differential protective effects of essential oils isolated from different basil cultivars against CCl_4-induced hepatotoxicity. Therefore, this study systematically investigated the association between the phytochemical profiles of different cultivars and their efficacy in attenuating liver injury, inflammation, and gut dysbiosis.

Some previous studies have shown the potential prebiotic effects of plant-derived bioactive compounds in protecting against various chemical stressors (Hack et al., 2022; Al-Quwaie *et al.,* 2023; El-Saadony *et al.,* 2025). However, the present comparative study was designed to evaluate the protective properties of *Ocimum basilicum* essential oils (Sweet, Lemon, and Italian) against CCl_4-induced hepatotoxicity and inflammation in Wistar rats.

Specifically, this study aimed to characterize the cultivar-specific phytochemical profiles using GC-MS analysis; quantify total phenolic and flavonoid contents and evaluate *in vitro* antioxidant potential; assess broad-spectrum antimicrobial activity against clinically relevant pathogens; evaluate in vitro cytotoxic potential against HepG-2 hepatocellular carcinoma cells; investigate the in vivo hepatoprotective effects of the most promising cultivar (Lemon basil essential oil) against CCl_4-induced liver injury through comprehensive assessment of growth parameters, hematological indices, liver function biomarkers, lipid peroxidation and protein oxidation markers, enzymatic and non-enzymatic antioxidant defenses, inflammatory gene expression, and hepatic histoarchitecture; elucidate the modulatory effects on gut microbial community composition and diversity; and establish correlations between cultivar-specific phytochemical profiles and their corresponding biological activities to identify the most promising chemotype for potential therapeutic applications in liver disorders and gut health management.

MATERIALS AND METHODS

Essential oil extraction: The basil seeds (Sweet, Lemon, and Italian) used in the present studies were randomly selected from each cultivar, synchronized with the onset of anthesis (the flowering stage). To preserve the integrity of the volatile constituents, the harvested biomass was subjected to a systematic desiccation protocol for 14 days, conducted in a shaded environment at ambient room temperature. Subsequently, essential oils (EOs) were isolated by hydro distillation using a Radnor Clevenger apparatus (PA, USA) to ensure standardized extraction conditions. For 3 hours, 50g of dried matter from the aerial portion was submerged in water. It was desiccated by passing the gathered essential oil through anhydrous sodium sulfate. Following recovery, the oil was stored at cold conditions for analysis (El-Saadony *et al.*, 2022).

GC-MS analysis: Volatile constituents were analyzed by GC-MS with a Shimadzu QP2010 Ultra system equipped with an Rtx-5MS column (30m × 0.25mm, 0.25µm film thickness). Helium was used as the carrier gas at a flow rate of 1.0mL/min. The mass spectrometer was operated at 70 eV in electron-impact ionization mode. The MS transfer line and injector manifold temperatures were strictly maintained at 280°C to minimize sample volatilization and to prevent fluid condensation during analysis.

The oven temperature profile was initiated at 40°C, followed by an isothermal hold for 3 minutes. Subsequently, the temperature was increased to a final setpoint of 280°C at 5°C/min, followed by a 5-minute terminal hold. Individual component abundances were calculated by regulating the peak area percentages of the individual components. Tentative identification of the isolated volatile compounds was based on comparison of their mass spectral signatures and relative retention indices with those reported in the NIST08s, WILLY8 and Adams libraries (Adams, 2001, 2012; Ghareeb *et al.*, 2016).

Phenolic compound content: The total polyphenols in basil essential oils were estimated using the Folin-Ciocalteu colorimetric assay, following Singleton and Rossi (1965), with gallic acid used to generate the standard calibration curve. Concurrently, the total flavonoid content (TFC) of the basil essential oils (EOs) was determined through the aluminum chloride-based spectrophotometric assay described by Chang *et al.* (2002). For the determination of flavonoid concentration, quercetin served as the reference analytical standard.

Biological activities

Antioxidant activity: The radical scavenging potential of essential oils derived from Italian, Lemon, and sweet basil cultivars was evaluated following the methodology described by Jia *et al.* (2012). Aliquots of the essential oils at three concentrations of 50, 100 and 200µg/mL were treated with a DPPH solution. The mixtures were incubated for 30 minutes at room temperature in the dark to prevent photochemical degradation and allow the radical-antioxidant reaction. After incubation, the absorbance of the samples was measured at 517nm, and the corresponding percentage of antioxidant activity (% AA) was calculated using the following formula:

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

Antimicrobial activity: The antimicrobial susceptibility of EOs from Italian, Lemon and Sweet basil cultivars was tested using the disc diffusion technique, following the experimental design proposed by Singh *et al*. (2016) with some modifications. To guarantee the homogeneity of the inocula, a standardized turbidity solution was prepared. 0.5 McFarland standard was prepared by adding 0.5mL of barium chloride ($BaCl_2$) to 99.5mL of sulfuric acid (H_2SO_4). This preparation produced a suspension with an optical density equivalent to a microbial concentration of 1.5×10^8 colony-forming units per milliliter (CFU/mL). 100µL of each bacterial and fungal culture *(Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Listeria monocytogenes*, *Campylobacter jejuni*, *Enterococcus faecalis*, *Bacillus cereus*, *Salmonella Typhi*, *Porphyromonas gingivalis*, *Candida tropicalis*, and *Candida albicans*) was spread on specific agar plates (nutrient agar and potato dextrose agar, respectively) for bacterial or fungal strains. Eight mm discs were impregnated with different concentrations of Sweet, Lemon, and Italian EOs and placed into the Petri plates. The diameters of the inhibition zones were measured to determine the total antimicrobial potential of the treatments, including MIC, MBC, and MFC, after the optimum incubation time and temperature (24 h at 37ºC for bacteria and 5 days at 25ºC for fungi).

Cytotoxic impact on HePG2 Cells

Cell culture and maintenance: Aseptic procedures were performed strictly within a sterilized laminar-flow workstation (Baker, SG403INT, Sanford, USA). Human hepatocarcinoma (HepG2) cell lines were cultivated in RPMI 1640 medium supplemented with 1% L-glutamine and a 1% antibiotic-antimycotic solution (10,000U/mL Penicillin G, 10,000µg/mL Streptomycin sulfate, and 25 µg/mL Amphotericin B). Environmental conditions were maintained at 37ºC in a humidified atmosphere containing 5% CO_2 using a water-jacketed incubator (Sheldon, TC2323, Cornelius, OR, USA).

MTT viability assay: The cytotoxic potential of the plant oils was evaluated via the colorimetric MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. This method assesses metabolic competence based on the capacity of mitochondrial succinate dehydrogenase in viable cells to reduce the yellow tetrazolium salt into insoluble purple formazan crystals (Mosmann, 1983).

After 10 days of batch cultivation, cells were collected and distributed into 96-well microplates, maintaining a seeding density $1x10^4$ of cells per well. Upon achieving attachment, the original medium was aspirated and replaced with serum-free RPMI containing serial dilutions of the experimental samples. Negative control was performed using cells incubated in medium alone, and positive control was performed using a known cytotoxic natural agent (100µg/mL) (Thabrew *et al*., 1997; El-Menshawi *et al.,* 2010).

Quantification and data analysis: Following a 48-hour incubation period, the supernatant was aspirated, and 40µL of MTT reagent (2.5µg/mL) was added to each well. The plates were incubated for an additional 4 hours at 37ºC under 5% CO_2. To quench the reaction and solubilize the formazan crystals, 200µg/mL 10% sodium dodecyl sulfate (SDS) in deionized water was added to each well, followed by overnight incubation at 37°C. The essential oils were prepared in DMSO, ensuring that the final solvent concentration in the wells remained below 0.2% (v/v) to prevent vehicle-induced toxicity. Optical density was measured at 595nm (reference wavelength 620nm) using a Bio-Rad microplate reader. The resulting data were subjected to statistical analysis to identify significant variances in percentage viability across treatments.

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

Animal and experimental design

Experimental subjects and housing: Forty-eight male Wistar Albino rats (*Rattus norvegicus*), with an initial body weight ranging from 180-190g, were procured from the institutional animal breeding facility. The animals were maintained in sterilized polycarbonate cages under strictly controlled hygienic conditions. The macro-environment was regulated to provide a consistent 12 h light/dark photoperiod, a relative humidity of 40-60%, and a stabilized ambient temperature of 23.2ºC.

Nutritional regimen and ethical compliance: The rats were provided with water ad libitum and standard laboratory diet throughout the study period. All animal handling, housing conditions, and surgical or treatment procedures were conducted in strict accordance with the principles delineated in the Guide for the Care and Use of Laboratory Animals. Following the stabilization period, baseline body weights were recorded for each subject to facilitate subsequent experimental comparisons. The rats were randomly divided into four experimental groups: Control group received normal saline (oral gavage) for 28 consecutive days, LEO group received lemon basil essential oil (200mg/kg body weight) dissolved in 0.5% Tween 80 via oral gavage once daily for 28 days. CCl_4 group received a single intraperitoneal injection of carbon tetrachloride (1mL/kg body weight, mixed 1:1 with olive oil) on the 28th day only, and LEO + CCl_4 group received Lemon basil essential oil (200mg/kg body weight, oral gavage) for 28 consecutive days, followed by a single intraperitoneal injection of CCl_4 (1mL/kg body weight) on the 28th day, administered 2 hours after the final LEO dose (Fig. 1).

Growth performance parameters: At the end of the experimental period, the animals were euthanized via cervical decapitation. The carcasses were promptly excised, and any extraneous adipose tissue was meticulously trimmed from the harvested organs to ensure measurement accuracy. Subsequently, the relative organ weights were calculated and expressed as a percentage of the final total body mass for each specimen (Zhou *et al.*, 2024).

Blood biochemistry

Sample collection and preparation: At the end of the 28-day experimental period, the rats were fasted overnight to minimize metabolic interference. Euthanasia was performed via exsanguination through jugular vein severance, during which two distinct blood fractions were harvested from each subject. An initial aliquot of 0.5mL was collected into ethylenediaminetetraacetic acid (EDTA) tubes for immediate hematological profiling (Kureshi *et al.,* 2025) . A secondary 2mL volume was collected into additive-free tubes to facilitate serum isolation. This second fraction was subsequently centrifuged at 5000rpm for 10 min. The resulting serum supernatant was aspirated and cryopreserved at -20ºC for subsequent biochemical characterization (Saad *et al.*, 2022).

Hematology: The initial blood fraction was used to characterize the primary hematological profile, specifically to quantify hemoglobin (Hb, g/dL) concentration and total erythrocyte (RBC, $x10^6$/L) and leukocyte (WBC, $x10^6$/L) counts. A comprehensive automated cellular analysis was performed using a HOSPITEX hematology analyzer (Italy) to determine the levels of circulating lymphocytes and thrombocytes (platelets, $x10^6$/L), and to provide definitive counts of total WBCs and RBCs (Alghamdi *et al.*, 2024).

Serum biochemical parameters

Serum enzyme quantitation: The liver enzymes ALT and AST were assessed calorimetrically following the method that described in Zhou *et al*. (2024) study, Concurrently, alkaline phosphatase (ALP) levels were determined in accordance with the experimental method of Belfield and Goldberg (1971).

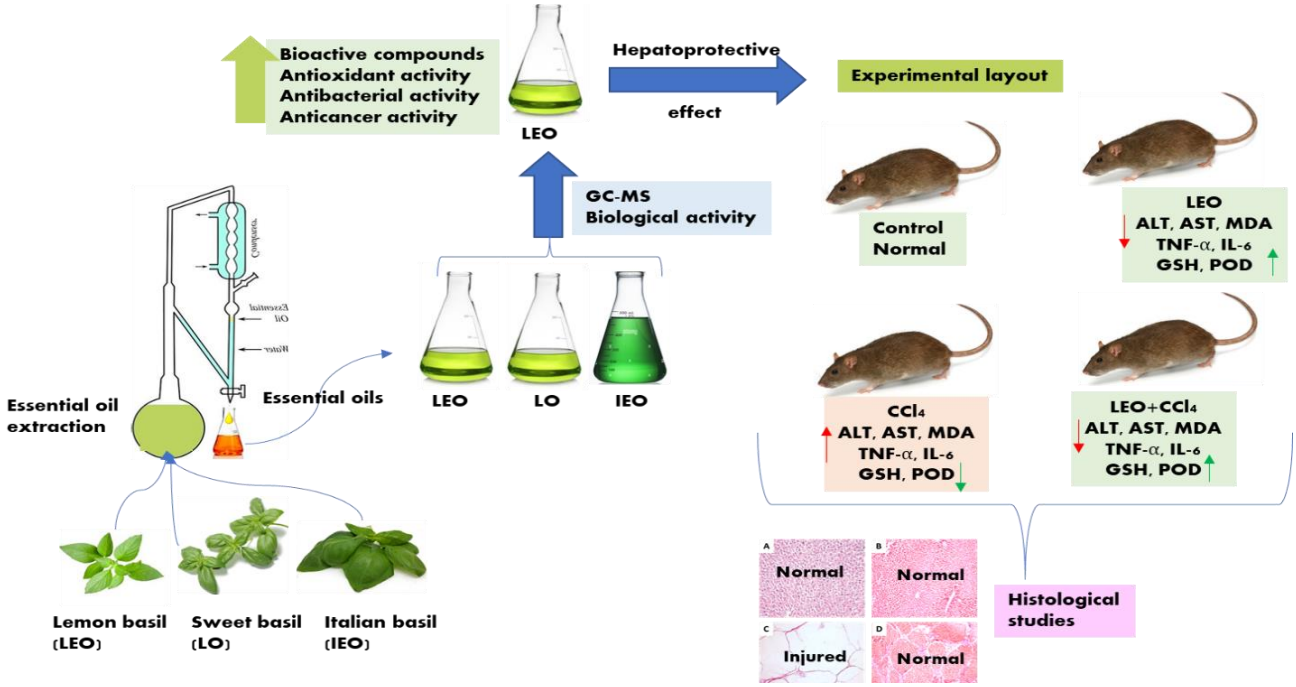

Fig. 1: Experimental design of the effect of basil essential oil on CCl_4-challenged rats.

Protein and metabolite profiling: The total serum protein concentration was estimated according to the methods of Grant (1987) and Saad *et al*. (2015), and the albumin fraction was determined by the analytical methodology of Westgard and Poquette (1972). The globulin concentration was calculated by the difference between total protein and albumin. In addition, the glycemic status was assessed by quantifying glucose using the Trinder method (1969).

Renal markers and instrumentation: The levels of urea and creatinine were evaluated to assess renal metabolic status using the colorimetric methods of Fawcett and Scott (1960) and Bartels *et al*. (1972), respectively. All spectrophotometric and colorimetric determinations were performed using the Infinite M Nano microplate reader (TECAN, Austria) to measure standard optical density.

Antioxidant enzymes: Malondialdehyde (MDA) accumulation was measured as an index of lipid peroxidation following the experimental procedure described by Ohkawa *et al*. (1979) using BioDiagnostic kits. Non-enzymatic total antioxidant capacity (TAC) was assessed following the protocols of Koracevic *et al*. (2001) using BioDiagnostic commercial kits. An Infinite M Nano microplate reader (TECAN, Austria) was employed for all spectrophotometric measurements at the respective wavelengths.

Histopathological examination

Tissue processing and paraffin embedding: Liver tissues were promptly placed in 10% neutral buffered formalin solution upon removal and kept for 48 hours for stabilization. Following fixation, the samples were rinsed in distilled water for 30 min to remove residual fixative, then processed using an automated tissue processor. The dehydration sequence involved an ascending ethanol series to prevent tissue distortion: Dehydration was accomplished by sequential immersion in increasing concentrations of ethanol: 70% alcohol for 120 minutes, 90% alcohol for 90 minutes, and absolute ethanol (100%). Clearing was achieved through sequential xylene baths, beginning with a 1:1 xylene-ethanol mixture for 1h, followed by a final 1.5 h immersion in pure xylene.

Sectioning, staining, and evaluation: Serial sections of 4–5 µm thickness were prepared from paraffin-embedded tissues utilizing a rotary microtome. Following sectioning, the tissue slices were mounted on glass slides and subjected to routine Hematoxylin and Eosin (H&E) staining. The stained slides were qualitatively evaluated under light microscopy to identify histopathological alternations, including circulatory disturbances, inflammatory infiltration, degenerative changes, and cellular death (apoptosis and necrosis).

Analysis of molecules via real-time PCR: Hepatic mRNA expression of the pro-inflammatory cytokines (TNF-α and IL-6) was quantified by quantitative real-time PCR. Total RNA specimens were isolated from the liver using a specific RNA Purification Kit (Thermo Scientific, USA) following the automated protocol. RNA quantities were checked using a Quawell Nanodrop spectrophotometer (USA). Reverse transcription was then performed to synthesize first-strand cDNA using RevertAid H Minus Reverse Transcriptase (Thermo Scientific, USA) according to the provided instructions. Amplification by qPCR was carried out on a StepOnePlus instrument (Applied Biosystems, USA), with each reaction well containing the cDNA template, 2X Maxima SYBR Green Master Mix (Applied Biosystems, USA), and forward- and reverse-gene-specific primers (primer details are given in Table 1). The expression values were normalized to β-actin as the internal control transcript. Relative quantification was calculated using the 2^−ΔΔCt method, which normalizes the target gene threshold cycle (Ct) values to the reference gene Ct values under both control and experimental conditions (Amsen *et al.*, 2009).

Gut microbiome

Sample collection: Twenty-four hours following the last treatments, all rats were euthanized by carbon dioxide inhalation followed by cervical dislocation. The abdominal cavity was opened aseptically and the entire cecum and colon were immediately excised. Fecal contents were collected by gentle squeezing into sterile pre-weighed tubes, placed on ice and processed within 2h to maintain microbial viability.

Microbial DNA extraction: Total genomic DNA was isolated from approximately 200mg of fecal material using the QIAamp PowerFecal Pro DNA Kit (Qiagen, Germany) according to the manufacturer's recommended procedure. DNA yield and purity were determined using a NanoDrop spectrophotometer (Thermo Scientific, USA), with quality evaluated based on the A260/A280 and A260/A230 absorbance ratios. The integrity of the DNA samples was checked by separation on 1% (w/v) agarose gel. The purified DNA samples were stored at −80°C until further analysis.

Quantitative Real-Time PCR (qPCR) for bacterial quantification: Total bacteria and specific groups of bacteria were quantified using species-specific primers that target the 16S rRNA gene (Table 1). Quantitative real-time PCR (qPCR) analysis was conducted on a StepOnePlus Real-Time PCR System (Applied Biosystems, USA). Amplification reactions (20µL) contained 10µL of 2× SYBR Green Master Mix, 0.4µL of each primer (10µM), 2µL of genomic DNA template (10ng/µL), and nuclease-free water to the final reaction volume. The cycling conditions included an initial enzyme activation and DNA denaturation step at 95°C for 10 min, followed by 40 cycles of denaturation (95°C, 15s), primer-specific annealing (30s), and extension (72°C, 30s). The specificity of amplified products was confirmed by post-amplification melting curve analysis. Standard curves were generated using serial ten-fold dilutions of plasmid DNA containing the target gene sequences. Bacterial counts were expressed as $\log_{10}$ colony-forming units per gram of fecal content ($\log_{10}$ CFU/g).

Bioinformatic analysis: The V3–V4 hypervariable regions (where "V" stands for variable region) of the 16S rRNA gene were amplified for comprehensive microbial community analysis using 341F and 806R primers. Following amplification, Purified PCR amplicons were

combined at equimolar amounts and sequenced on an Illumina MiSeq instrument through a commercial laboratory.

Raw sequencing data were processed using Quantitative Insights into Microbial Ecology (QIIME) version 2023.2. Demultiplexed sequences were denoised using DADA2 to generate amplicon sequence variants (ASVs), with chimeric sequences and singletons removed. Taxonomy assignment was performed using the SILVA reference database (version 138) with a confidence threshold of 0.8. Alpha diversity metrics, including the Shannon diversity index, were calculated after rarefying samples to a uniform depth of 50,000 reads per sample. The Firmicutes/Bacteroidetes ratio was calculated based on the relative abundances of these phyla.

Study statistics: All experimental treatments were arranged in a completely randomized design (CRD). Data management and preliminary organization were executed using Microsoft Excel. To evaluate the significance of the observed variances, the treatment means were determined and compared via Tukey's test at significance level ($P<0.05$).

RESULTS

GC-MS profile of basil cultivar essential oils: As shown in Table 1, the GC-MS analysis revealed distinct phytochemical profiles for each of the three *Ocimum basilicum* L. cultivars. The primary volatile components identified across all cultivars comprised methyl cinnamate, 1,8-cineole, eugenol, and linalool. However, significant quantitative and qualitative differences were observed among cultivars.

Table 1: Identification and quantification of volatile compounds in essential oils of three *Ocimum basilicum* L. cultivars using GC-MS technique

RT (min)	Components	Basil cultivars			P value
		Sweet	Lemon	Italian	
11.883	α-Pinene	0.3ab	0.45a	0.03b	0.026
15.817	β-Pinene	1.3b	1.42a	ND	0.011
19.318	β-Myrcene	0.55a	0.51ab	0.23d	0.00163
20.283	Bornylene	0.23b	0.31a	0.02e	0.0001
20.62	Eugenol	3.1a	3.08ab	2.2b	0.021
21.133	1.8-Cineole	5.3b	6.01a	4.1c	0.016
30.858	β-Thujone	2.9ab	3.12a	1.1c	0.019
36.750	Linalool	26.3b	33.5a	25.3b	0.0189
37.867	Germacrene D	0.12ab	0.15a	0.09b	0.036
38.350	α-Bergamotene	2.9b	3.22a	0.9c	0.020
42.435	Borneol L	0.5a	0.44ab	0.09c	0.0101
43.133	delta-Guaiene	0.41b	0.56a	0.16e	0.0001
56.533	Methyl cinnamate	33.5b	42.02a	29.3c	0.023
58.833	α-Cadinol	3.2b	4.1a	2.1c	0.019

Values within the same row, followed by different lowercase letters, differ significantly at $P<0.05$.

Lemon basil essential oil is characterized by a high content of methyl cinnamate (41.23%), followed by linalool (32.87%) and 1,8-cineole (6.42%). Sweet basil essential oil is characterized by methyl cinnamate (32.86%), linalool (25.94%), and 1,8-cineole (5.61%) as the major compounds. Italian basil has the highest methyl cinnamate content (28.74%), with linalool (21.38%) second. The BW cultivar has a linalool content of 22.15%, whereas the BI cultivar has a higher linalool content (24.87%). Eugenol was found in all essential oils, with sweet basil having the highest content (3.35%), followed by lemon basil (3.29%), and Italian basil the lowest (2.42%). Sweet basil and lemon basil contained significant amounts of β-pinene (2.87 and 2.64%, respectively), whereas Italian basil did not contain this constituent. Conversely, α-bergamotene levels were elevated in lemon and sweet basils at 3.41 and 3.06%, respectively, but remained minimal in Italian basil (1.12%). Lemon basil contained very high levels of α-cadinol (2.83%) and β-thujone (2.51%) compared to the other cultivars, in which these compounds were present in much lower concentrations or below detectable limits. All analyses were carried out in triplicate, and results were expressed as the mean relative percentage of the total composition of essential oils. The complete list of identified compounds, along with their retention indices and relative percentages for each cultivar, is presented in Table 1.

Table 2: Antioxidant content of essential oils of three basil cultivars

Parameters	Basil cultivars			p value
	Sweet	Lemon	Italian	
TPC (mg/g)	40b	51a	15c	<0.0001
TFC (mg/g)	25b	31a	9c	<0.0001
AA (%)	88b	93a	64c	<0.0001
IC50 (µg/mL)	100b	50c	200a	<0.0001

Values within the same row, followed by different lowercase letters, differ significantly at $P<0.05$.

Antioxidant activity of basil cultivar essential oils: The antioxidant activity of the essential oils obtained from the three basil cultivars is summarized in Table 2. Lemon basil showed the highest total phenolic content (TPC) (52.8±1.6mg GAE/g DW) and the total flavonoid content (TFC) (32.4mg QE/g DW), with relative increases of 27% and 23%, respectively, compared to sweet basil (41.6mg GAE/g DW and 26.3mg QE/g DW). Italian basil showed significantly lower TPC (29.7mg GAE/g DW) and TFC (18.5mg QE/g DW) than lemon and sweet cultivars ($P<0.05$). Consistent with its higher phenolic and flavonoid contents, lemon basil essential oil exhibited the strongest DPPH radical-scavenging activity, with 92.4% inhibition at the highest concentration tested, followed by sweet basil at 87.3%. Italian basil showed the lowest antioxidant activity (76.8% inhibition). The IC_{50} (half maximal inhibitory concentration) values for lemon basil were the lowest (48.6µg/mL), indicating the strongest antioxidant activity, followed by sweet basil (62.3µg/mL) and Italian basil (89.5µg/mL).

Antimicrobial activity of basil essential oils: The antimicrobial properties of essential oils from three basil cultivars, Sweet, Lemon, and Italian were characterized against 11 microbial strains, including Gram-negative and Gram-positive bacteria and fungi (Table 3). Analysis of inhibition zone diameters alongside MIC, MBC, and MFC values revealed cultivar-specific variations in antimicrobial activity. Across all tested microorganisms, the Lemon basil cultivar consistently exhibited the highest inhibitory activity, with zone diameters ranging from 15mm to 25 mm, followed by sweet basil, while Italian basil showed the weakest activity. This trend was statistically significant ($P < 0.0001$) for all strains.

Porphyromonas gingivalis is a prominent Gram-negative anaerobic bacterium recognized as a keystone pathogen in severe dental infections, particularly chronic periodontitis. By deploying an array of virulence factors, such as gingipains and fimbriae, it actively degrades periodontal tissues, evades host immune defenses, and drives the subgingival dysbiosis that ultimately leads to alveolar bone loss. Gram-positive bacteria, particularly *Staphylococcus aureus* and *Bacillus cereus*, were more susceptible to the essential oils, with larger inhibition zones and lower MIC values (0.125–0.25mg/mL) compared to Gram-negative bacteria such as *Pseudomonas aeruginosa*, *Porphyromonas gingivalis,* and *Escherichia coli*, which required higher concentrations (MIC: 0.5mg/mL) for inhibition. The fungicidal activity against *Candida* species is notable, with Lemon basil again demonstrating superior efficacy. The MBC and MFC values were typically twice the MIC values, indicating predominantly bactericidal and fungicidal effects rather than mere inhibition.

Table 3: Antibacterial and antifungal activity (zone of inhibition in mm), MIC, MBC, and MFC of essential oils from three basil cultivars

Microorganisms	Basil cultivars			MIC	MBC	MFC	P value
	Sweet	Lemon	Italian	(mg/mL)	(mg/mL)	(mg/mL)	
Salmonella typhi	15b	18a	11c	0.25	0.5	-	<0.0001
Campylobacter jejuni	12b	15a	9c	0.5	1.0	-	<0.0001
Escherichia coli	14b	17a	10c	0.5	1.0	-	<0.0001
Pseudomonas aeruginosa	13b	16a	10c	0.5	1.0	-	<0.0001
Staphylococcus aureus	21b	25a	15c	0.125	0.25	-	<0.0001
Listeria monocytogenes	17b	22a	12c	0.25	0.5	-	<0.0001
Bacillus cereus	18b	21a	13c	0.25	0.5	-	<0.0001
Enterococcus faecalis	16b	19a	11c	0.5	1.0	-	<0.0001
Candida albicans	16b	20a	11c	0.5	-	1.0	<0.0001
Porphyromonas gingivalis	15b	19a	10c	0.5	-	1.0	<0.0001
Candida tropicalis	14b	18a	9c	0.5	-	1.0	<0.0001

a-c lowercase letters in the same row indicate significant differences at <0.05. MIC: Minimum Inhibitory Concentration; MBC: Minimum Bactericidal Concentration; MFC: Minimum Fungicidal Concentration

Cytotoxicity: As shown in Fig. 2, all three basil essential oils exhibited concentration-dependent cytotoxic effects against HepG-2 liver cancer cells, with varying degrees of potency. Lemon basil essential oil exhibited the strongest anticancer activity, reducing HepG-2 cell viability by 85.3% at the highest concentration tested, followed closely by sweet basil with 79.6% inhibition. In contrast, Italian basil essential oil showed substantially weaker cytotoxic activity, achieving only 38.7% reduction in cancer cell viability. For comparison, the positive control doxorubicin (DOX) exhibited 84.2% inhibition of cancer cell proliferation under the same experimental conditions. These quantitative results were consistent with microscopic observations, in which treated cells exhibited characteristic morphological changes associated with apoptosis, including cell shrinkage, membrane blebbing, and reduced cell density relative to untreated controls.

The IC_{50} values of the essential oils inversely correlated with their inhibition percentages, further confirming the differential cytotoxic potency among cultivars. Lemon basil essential oil exhibited the lowest IC_{50} value (19.4 ± 1.2µg/mL), indicating the highest anticancer activity, followed by sweet basil (30.2 ± 1.5µg/mL), while Italian basil showed the highest IC_{50} (44.3 ± 1.9µg/mL), reflecting its weaker cytotoxicity. Doxorubicin, as expected, demonstrated the lowest IC_{50} value (8.7 ± 0.6µg/mL) among all treatments.

***In vivo* experiment**

Growth and physiological parameters: Some behavioral and physiological signs of toxicity were observed in the CCl_4 rats, including salivation, burrowing, tremors, writhing, and convulsions. All animals survived the duration of the study without lethal outcomes. Compared with the control group, rats exposed to CCl_4 showed an increase in relative liver weight and a significant reduction in body weight. These parameters were significantly reduced when comparing the rats exposed to CCl_4 to those given LEO and then treated with CCl_4. No significant changes were observed in rodents when LEO was administered alone (Table 4).

Hematology: Compared with the control group, CCl_4 administration in rats resulted in notable reductions in Hb, Ht, RBC, and platelets, but an increase in WBC. The hematological parameters showed slight alterations when LEO was administered in isolation, compared with the control group. However, when BEO was administered before CCl_4 intoxication, these parameters were substantially restored to the control levels compared to the challenged group (Table 5).

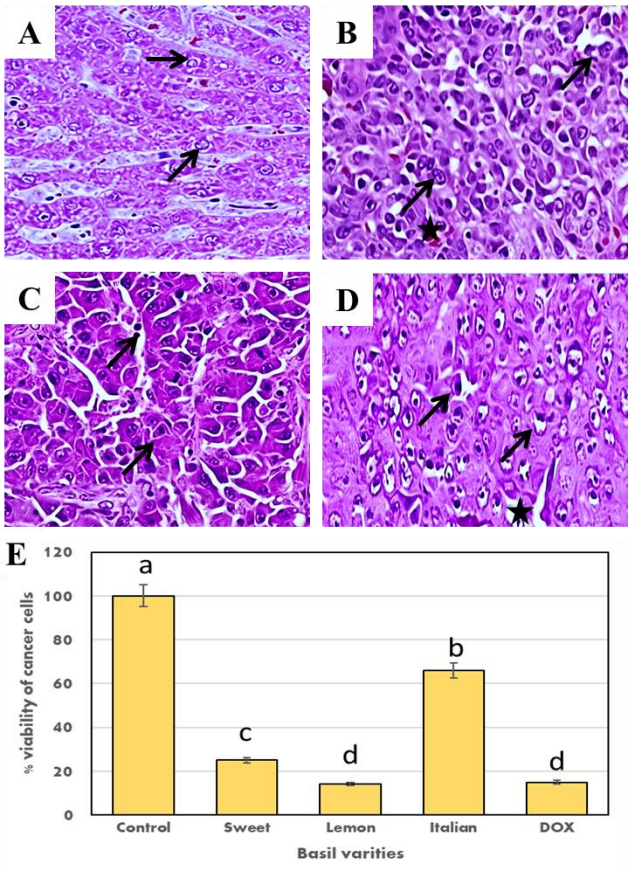

Fig. 2: Cytotoxicity of essential oils of three basil cultivars on the viability of liver cancer cell lines (A) control cancerous cells, (B) the cytotoxicity effect of sweet basil EO on cancerous cells, (C) the cytotoxicity effect of Lemon basil EO, (D) the cytotoxicity effect of Italian basil EO, (E) Histogram of the cytotoxicity effect of essential oils of three basil cultivars on the viability of liver cancer cell lines. Divergent lowercase letters atop columns indicate significant treatment differences at p<0.05.

Table 4: Assessment of growth parameters in CCl_4-induced hepatocellular carcinoma rats treated with lemon basil essential oil

Parameters	Treatments groups				P value
	Control	LEO	CCl_4	LEO+CCl_4	
IBW	152b	155a	154b	156a	0.69
FBW	220b	230a	197c	210bc	0.01
BWG	68b	75a	43d	54c	<0.0001
FCR	1.45b	1.6a	1.2d	1.35c	<0.0001
SR	100a	100a	89b	100a	0.04
ALW	5.1c	5.5c	7.9a	6.1b	0.03
ROW	2.5b	2.56b	3.8a	3ab	0.04

Row values labeled with divergent lowercase letters differ significantly at the 5% significance level at P<0.05.

Table 5: Hematological assessment of CCl_4-intoxicated rats with liver cancer receiving lemon basil essential oil

Hematology	Treatments groups				P value
	Control	LEO	CCl_4	LEO+CCl_4	
RBCx 10^6/L	7.5ab	7.9a	4.8c	6.5b	0.001
WBCx 10^6/L	5.2c	5.5c	17.2a	7.2b	0.01
HT %	43.68a	42.9a	22.5c	39.7b	0.02
Hb g/dL	14.3a	14a	8.2c	13.1b	0.015
Platelets x 10^6/L	820b	835a	350d	790c	<0.0001

Row values labeled with divergent lowercase letters differ significantly at the 5% significance level at p<0.05.

Liver biomarkers: Rats given CCl_4 showed changes in total protein content in liver homogenate and in ALT, ALP and AST activities (p<0.05). Additionally, LDH activity was significantly higher. Compared to the group treated with CCl_4 alone, enzyme activities and biochemical markers in rats exposed to LEO+CCl_4 changed considerably. The parameters studied were significantly altered when LEO was administered alone (Table 6).

Table 6: Changes in hepatic marker expression in CCl_4-induced liver cancer rats treated with lemon basil essential oil

Liver markers	Treatments groups				P value
	Control	LEO	CCl_4	LEO+CCl_4	
AST	123a	115b	85d	100c	<0.0001
ALP	355a	320c	260d	338b	<0.0001
ALT	165a	152b	120d	140c	<0.0001
LDH	789d	820c	989a	850b	<0.0001
TP	175b	189a	122d	156c	<0.0001

Row values labeled with divergent lowercase letters differ significantly at the 5% significance level at P<0.05.

Lipid and protein oxidation markers: Fig. 3 shows that CCl_4 administration significantly increased lipid peroxidation markers in liver tissue. LOOH levels in the CCl_4-only group (28.6±1.8µmol/mg protein) were significantly higher than in the healthy control group (12.4±0.9µmol/mg protein), representing a 2.3-fold increase (P<0.001). Pretreatment of rats with Lemon basil essential oil before CCl_4 intoxication (LEO + CCl_4 group) led to significantly lower LOOH levels (17.2µmol/mg protein) with a 39.9% decrease compared to the CCl_4 only group, showing significant protection against CCl_4-induced lipid peroxidation.

Similarly, hepatic levels of protein oxidation markers followed the same trend. The CCl_4-only group exhibited significantly higher AOPP levels (3.85nmol/mg protein) than the control group (1.62±0.11nmol/mg protein), indicating a 2.4-fold increase in protein oxidative damage (P<0.001). Remarkably, LEO pre-treatment effectively attenuated protein oxidation, with the LEO + CCl_4 group showing AOPP levels of 2.41nmol/mg protein, representing a 37.4% reduction compared to the CCl_4-only group. Notably, rats treated with LEO alone exhibited LOOH levels (11.8 µmol/mg protein) and AOPP levels (1.54nmol/mg protein) that were slightly lower than, but not statistically different from, the control group (P>0.05).

Antioxidant (enzymatic and non-enzymatic): In CCl_4 treated rats, hepatic tissue antioxidant status was compromised, as demonstrated by significant reduction in glutathione levels and activities of SOD, CAT and GPx compared to controls. However, the parameters mentioned above were significantly increased in the animals treated with LEO and CCl_4 when compared to animals treated solely with CCl_4. The indices were substantially enhanced when LEO was used in isolation (Fig. 4).

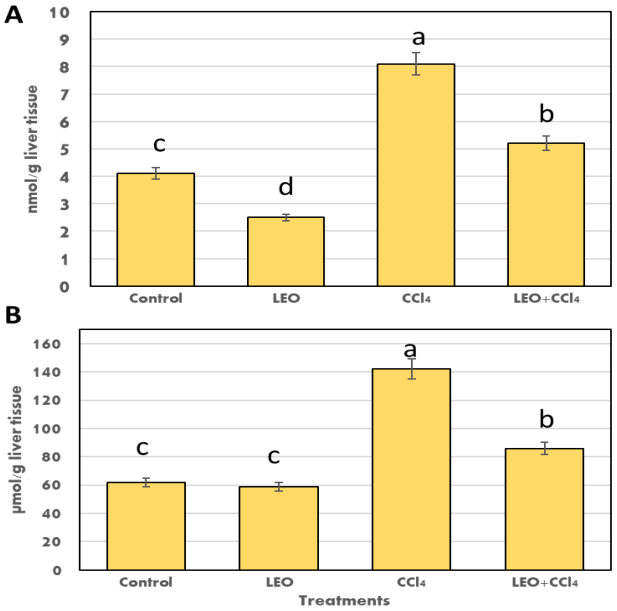

Fig. 3: Attenuation of CCl_4-induced oxidative damage in liver cells by lemon basil essential oil: (A) lipid hydroperoxide content, (B) advanced oxidized protein product levels. Divergent lowercase letters atop columns indicate significant treatment differences at p<0.05.

Inflammatory markers-related genes: CCl_4 administration caused a marked upregulation of pro-inflammatory cytokine genes in liver tissue, as shown in Fig. 5. In the CCl_4 only group, -α mRNA expression was significantly increased (5.8 fold) compared to the healthy control group (P<0.001). Similarly, CCl_4 administration caused a significant increase in gene expression of IL-6 (6.3 ± 0.5-fold) compared to the control group (P<0.001), revealing a strong inflammatory response caused by CCl_4 hepatotoxicity. On the other hand, the inflammatory gene expression was significantly suppressed in the rats pretreated with lemon basil essential oil prior to CCl4 intoxication (LEO + CCl_4 group). The mRNA level of TNF-α in the LEO + CCl_4 group was reduced to 2.4 ± 0.3-fold above the control level, representing a 58.6% decrease compared to the CCl_4 only group (P<0.01). Similarly, the IL-6 expression in the LEO + CCl_4 group was downregulated to 2.7-fold above the control level, which is a 57.1% decrease compared to the CCl_4 only group (P<0.01).

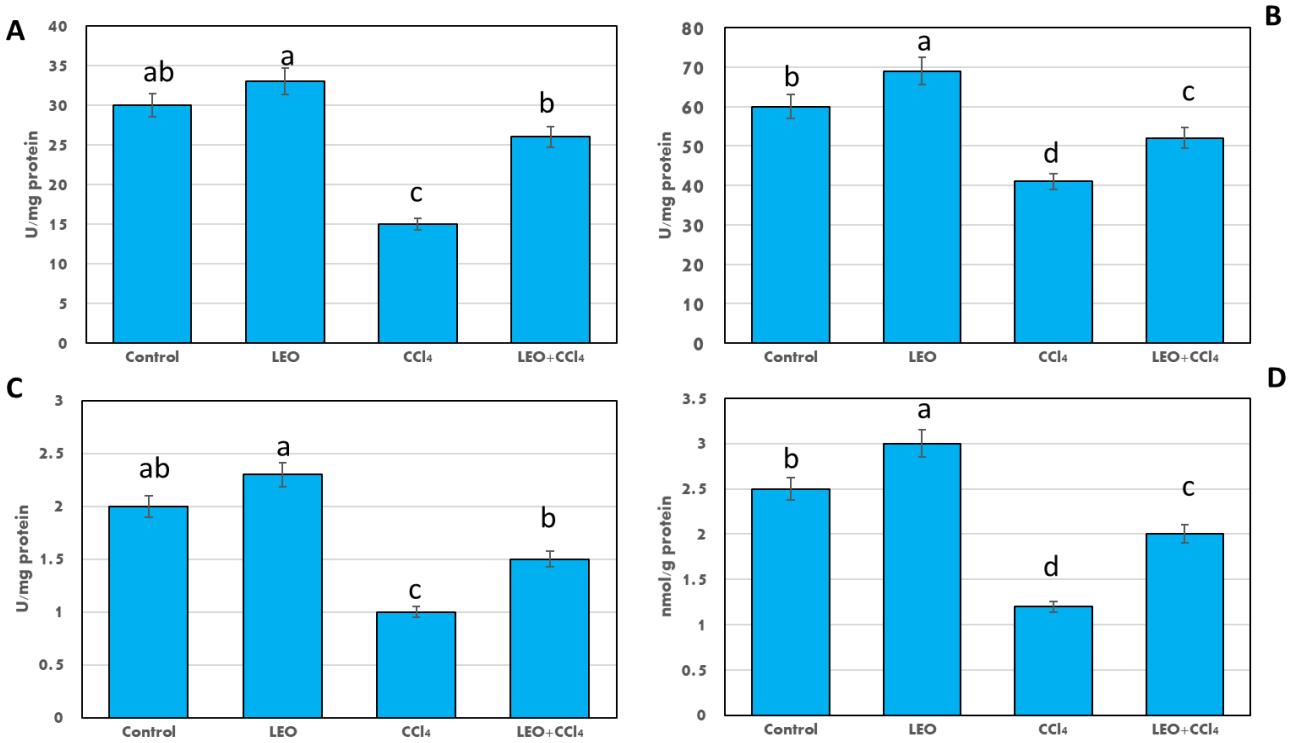

Fig. 4: Protective effect of lemon basil essential oil on the antioxidant system in rat liver Tissue: (A) superoxide dismutase activity, (B) catalase activity, (C) glutathione peroxidase activity, (D) reduced glutathione content. Divergent lowercase letters atop columns indicate significant treatment differences at $p<0.05$.

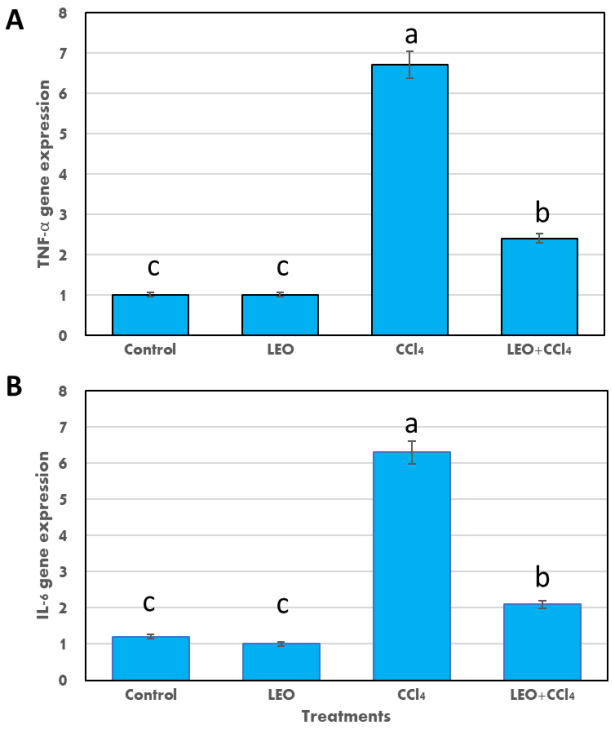

Fig. 5: Relative expression of *IL-6* and TNF-α genes in rats' liver tissues of different groups. Divergent lowercase letters atop columns indicate significant treatment differences at $P<0.05$.

Histopathological observations: As illustrated in Fig. 6, liver sections from the healthy control group (Fig. 6A) exhibited normal hepatic architecture with well-preserved hepatocytes arranged in radiating cords around the central vein. The portal triad structures (hepatic artery, portal vein, and bile duct) appeared normal, with an intact biliary system, vascular tributaries, and hepatic sinusoids. Kupffer cells (Von Kupffer's cells) were visible within sinusoidal lumina, and the supporting stroma showed no evidence of pathological alterations. Similarly, liver sections from rats treated with Lemon basil essential oil alone (LEO group, Fig. 6B) revealed tissue morphology comparable to control specimens, with intact hepatic cords, normal portal triad structures, unremarkable sinusoids, and no evidence of cellular injury or inflammatory cell accumulation, demonstrating the non-toxic nature of LEO administration.

In contrast, liver sections from rats treated with 1/10 LD_{50} of carbon tetrachloride (CCl_4 group, Fig. 6C) revealed pronounced histopathological alterations characteristic of hepatotoxicity. Moderate portal biliary proliferative reactions were observed, indicating bile duct hyperplasia. Marked dilation of portal blood vessels was noted, together with occasional periportal edema and focal inflammatory cell clusters dominated by lymphoplasmacytic elements. The hepatic sinusoids showed mild-to-moderate dilation, with some areas exhibiting atrophy of the surrounding hepatocytes. Hepatocellular degeneration, characterized by cytoplasmic vacuolization and pyknotic nuclei, was evident in centrilobular regions, reflecting the zone-specific susceptibility to CCl_4-induced injury. Notably, liver sections from rats pretreated with Lemon basil essential oil before CCl_4 intoxication (LEO + CCl_4 group, Fig. 6D) showed significant histological recovery compared with the CCl_4-only group. Only mild-to-moderate vascular dilations were observed, with a reduced frequency and severity of round-cell aggregations. The hepatic parenchyma showed preserved lobular architecture with minimal hepatocellular degeneration. The portal areas exhibited decreased

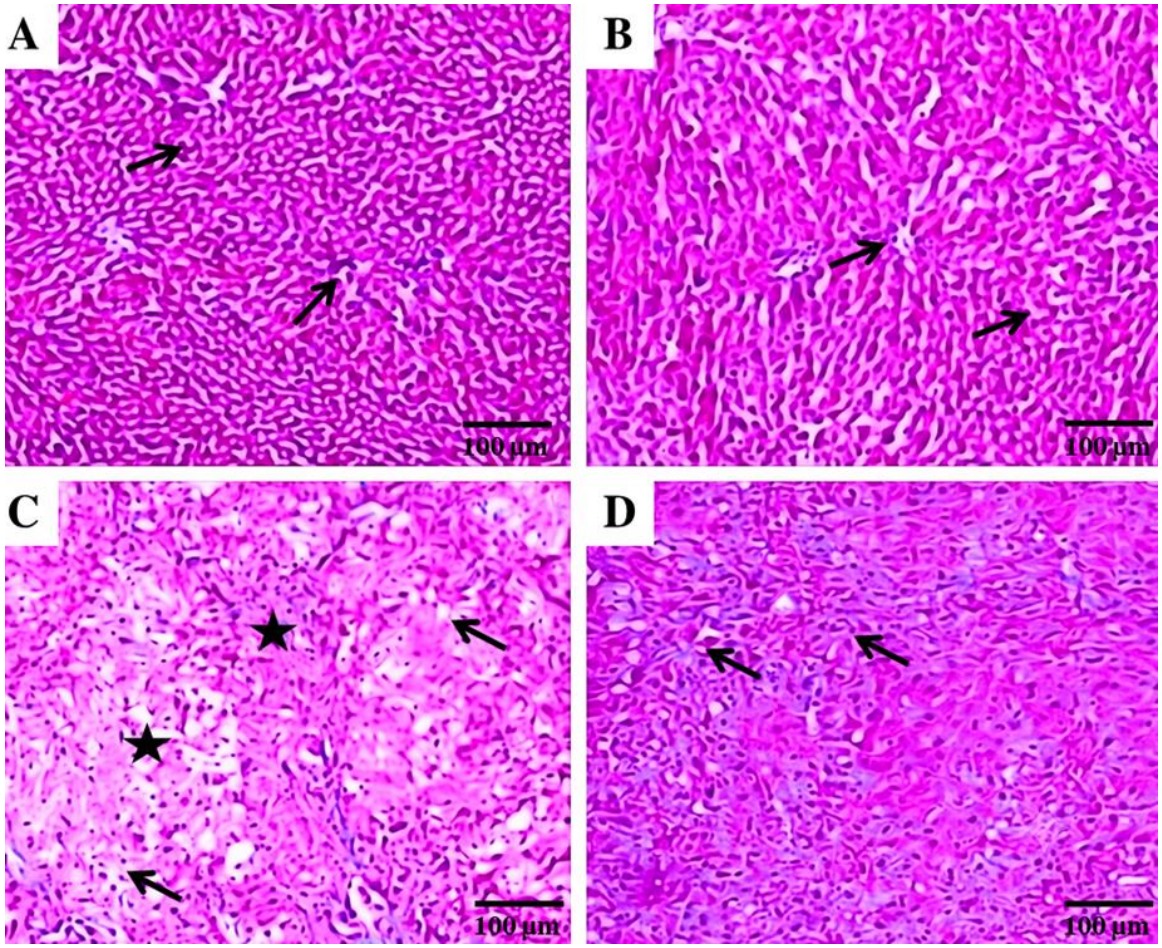

Fig. 6: Histological images about the effect of LEO on enhancing liver tissues from cancerous hepatocytes rats, (A) control; (B) LEO treatment; (C) CCl_4 induced cancer; (D) LEO treated tissues after the CCl_4 administration.

Table 7: Effects of Lemon basil essential oil (LEO) and carbon tetrachloride (CCl_4) on the gut microbial composition in rats

Microbial parameter	Treatment groups				P value
	Control	LEO	CCl_4	LEO + CCl_4	
Total bacterial count (log_{10} CFU/g)	8.42±0.31a	8.15±0.28a	6.23±0.42c	7.38±0.35b	<0.001
Lactobacillus spp. (log_{10} CFU/g)	7.56±0.33a	7.82±0.29a	4.87±0.51c	6.41±0.38b	<0.001
Bifidobacterium spp. (log_{10} CFU/g)	7.18±0.30a	7.41±0.26a	4.52±0.47c	6.12±0.34b	<0.001
Enterobacteriaceae (log_{10} CFU/g)	5.23±0.27b	4.85±0.24c	7.64±0.44a	5.98±0.32b	<0.001
Bacteroides spp. (log_{10} CFU/g)	6.89±0.28b	6.45±0.25c	8.12±0.46a	7.23±0.33b	<0.001
Firmicutes/Bacteroidetes ratio	1.28±0.11b	1.15±0.09c	2.46±0.18a	1.52±0.13b	<0.001
Shannon diversity index	3.42±0.15a	3.58±0.14a	2.31±0.21c	3.05±0.17b	<0.001

Values are expressed as mean± standard deviation (n=6 rats per group). a–c Lowercase letters in the same row indicate significant differences at $P<0.05$. LEO: Lemon basil essential oil (200mg/kg body weight, oral gavage); CCl_4: Carbon tetrachloride (1mL/kg body weight, intraperitoneal injection); Control: No treatment; LEO + CCl_4: Combined treatment with LEO and CCl_4.

inflammatory infiltration, and sinusoidal dilation was markedly attenuated. Evidence of tissue regeneration, including binucleated hepatocytes and restored cord arrangement, was observed in several fields.

Gut microbiome: Effect of lemon basil essential oil (LEO) and carbon tetrachloride (CCl_4) on rat gut microbiota in the four groups. Table 7 shows that the administration of CCl_4 alone resulted in significant dysbiosis, with decreased levels of beneficial bacteria (*Lactobacillus* and *Bifidobacterium* spp.) and total bacteria, and increased levels of Enterobacteriaceae and Bacteroides spp. ($P<0.001$). These changes were related to an increase of the Firmicutes/Bacteroidetes ratio and a decrease of the Shannon diversity index, showing reduced microbial diversity and a shift towards a proinflammatory state. Administration of LEO alone maintained microbial homeostasis compared to controls, with moderate increases in beneficial bacteria and diversity. In the group treated with the combination LEO + CCl_4, protective effects were seen with increased *Lactobacillus* and *Bifidobacterium* counts, reduced levels of pathogens and increased diversity compared to CCl_4 alone.

DISCUSSION

Essential oils (EOs) are the main metabolites of aromatic plants and have a dual role: they are involved in the plant's response to environmental stimuli and in protecting the plant from herbivores, insects, and microbial threats (Tharakan, 2021). In this study, we extracted essential oils from three basil cultivars. The essential oil profile was based on Elansary and Mahmoud (2015), who determined the chemical profile of essential oil from

Ocimum basilicum L analyzed by GC–MS. The primary essential oil contents of *O. basilicum* L. were methyl cinnamate (43.8%), and chavicol methyl ether (39.1 and 32.3%) in *O. basilicum* purple ruffle and anise, respectively, and linalool (30.9 and 30.6%) in *O. basilicum* Genovese and bush green, respectively. Both Eugenol and linalool, it was discovered to have the opposite effect, also, Ahmed *et al.* (2019) screened two varieties of basil (*Ocimum basilicum* L.) chemical components of essential oils identified by (IR) and (GC/MS).

The primary and prevalent constituents of oils derived from purple and green varieties were the following: linalool (44.37; 46.24%), 1,8-cineole (9.28; 3.28%), estragole (20.05; 13.26%), trans-methyl cinnamate (15.05; 0.45%), and epi-α-cardinal (1.38; 3.10%). Al Bakain *et al.* (2026) characterized the volatile constituents of basil essential oil obtained through hydrodistillation using gas chromatography-mass spectrometry (GC-MS). The main compounds were linalool and methyl chavicol in the EOs, representing two third of all active compounds.

The DPPH radical-scavenging and linoleic acid peroxidation assays used in these studies demonstrated notable antioxidant activity, consistent with the findings presented herein. In a comparative investigation, Tshilanda *et al.* (2016) found that basil essential oil had moderate antioxidant activity (IC 50 = 1.180mg/mL), while polar extracts such as methanol (IC 50 = 0.025mg/mL) and ethyl acetate (0.085mg/mL) had higher activity, whereas non-polar extracts had low activity and yields. Moreover, the cultivation source affects basil's phytochemical profile. Ahmed *et al*. (2019) reported different antioxidant activity in ethanolic extracts of *Ocimum basilicum* L., which may be due to environmental factors affecting the accumulation of secondary metabolites.

The polyphenol levels found in basil extracts surpassed that of essential oils in DPPH free radical scavenging. Among the samples, the basil extract exhibited the greatest activity, with a total polyphenol content of 82.45mg PE/g and an IC50 of 1.29mg/mL. Teofilović *et al.* (2021) assessed the free radical scavenging activity and phenolic content of *Ocimum basilicum* L. using the DPPH assay and spectrophotometric methods, reporting IC50 values ranging from 0.04 to 12.99µg/mL across various solvent extracts. Also, Nguyen *et al.* (2021) investigated ethanolic and aqueous extracts of sweet basil, revealing that the alcoholic extract contained 29.60mg GAE/g total phenolics and 19.58mg QE/g total flavonoids.

Basil essential oil has shown antimicrobial activity against a number of bacterial and fungal pathogens. Basil essential oil appears to have real antimicrobial and antibiofilm potential for oral infections involving caries, endodontic pathogens, denture-associated *Candida*, and possibly periodontal organisms. The main caveat is that the strongest evidence remains laboratory-based (Wiwattanarattanabut *et al.,* 2017; Makade *et al*., 2024), correlating with the current study. Also, the previous studies have indicated that essential oils from *Ocimum basilicum* have significant inhibitory effects on bacterial strains and yeast (Ramalingam *et al.,* 2020). Structure-activity relationship studies have revealed different levels of antimicrobial potency among essential oil components (Ramalingam *et al.,* 2020). The antimicrobial efficacy of essential oil components is directly influenced by their ability to penetrate microbial cell walls, which correlates positively with their aqueous solubility. Consequently, the capacity of essential oils to integrate into and traverse the phospholipid bilayer of microbial membranes underpins their antibacterial mechanism of action (He *et al*., 2022).

The Gram-positive bacterium (*S. aureus*) in this study exhibited superior antibiotic activity. In contrast, the Gram-negative bacterium (*E. coli*) showed only moderate antibacterial activity at the essential oil concentration. Minimal bacterial suppression was observed when the essential oil was applied at a concentration of 5 µL. Numerous published studies have reported the antimicrobial efficacy of individual components present in basil essential oil (Rezzoug *et al.*, 2019; Alkadi, 2021).

Cancer arises when healthy cells are transformed into malignant cells that can proliferate and metastasize to other organs and tissues (Abd-Rabou and Edris, 2022). The increasing demand for safer therapeutic alternatives with minimal adverse effects has driven renewed interest in plant-derived bioactive compounds for disease management. The current study provides scientific support for the traditional use of this medicinal species in folk medicine and alternative therapeutic approaches. Additionally, these results are in agreement with prior investigations that established the anticancer activity of basil leaf extracts against cancer cell lines (Aburjai *et al.*, 2020).

Carbon tetrachloride has the potential to induce cellular oxidative harm and generate peroxidation products, which are frequently employed as indicators of oxidative stress (Unsal *et al.*, 2021). The results showed a significant increase in these parameters of oxidative stress and a decrease of the antioxidant systems, suggesting that the CCl_4 toxicity could be mediated by alterations in the structural integrity of the membrane and decreased molecular mobility in the lipid bilayer (Wali *et al.*, 2021). The CCl_4 toxicity on tissues can be expressed as the oxidative damage caused by lipid peroxidation, which initiates after the conversion of CCl_4 to highly hazardous ($\bullet CCl_3$) and ($\bullet CCl_3O_2$) via cytochrome P450 enzyme. Oxidative damage is represented by the destruction of lipids (Unsal *et al.*, 2021).

The hepatic fibrosis characterized by excessive collagen deposition in the liver tissue is related to the elevation of hydroxyproline (HYP) levels, which is the main amino acid component of collagen. The observed elevation in HYP content confirms the development of fibrotic changes following CCl_4 exposure (Yan *et al.*, 2023). The antioxidant defense system relies heavily on glutathione to maintain cellular redox homeostasis and counteract the deleterious effects of free radical exposure (Raj *et al.*, 2021). Enhanced lipid peroxidation indicates oxidative stress, with elevated LPO levels corresponding to increased ROS production. This oxidative burden may explain the significant reduction in GSH content and the impaired function of GPx and GR enzymes in animals administered CCl_4. Superoxide dismutase and catalase function as the foremost protective barrier against reactive oxygen species-mediated injury to biological structures induced by carbon tetrachloride (Ben Hsouna *et al.*, 2022).

Hepatocellular membrane destabilization resulting from enhanced lipid peroxidation likely accounts for the elevated liver enzyme activities detected in CCl_4-treated

rats, with injured cells releasing cytoplasmic enzymes into the peripheral circulation (Usunobun *et al.*, 2020).

Additionally, protein is one of the most vulnerable macromolecules to ROS damage; The principal cause of protein content reduction is the excessive loss of proteins through nephrosis, proteolytic enzyme activity, or degradation (Martemucci *et al.*, 2022). The current study evaluates the hepatotoxic profile of carbon tetrachloride, with a specific emphasis on the induction of inflammatory responses and the transcriptional modulation of TNFα and IL-6 (Liu *et al.*, 2021). The role of reactive oxygen species in promoting the release of inflammatory markers, INF-γ, IL-12, and TNF-α was also assessed. ROS facilitates NF-κB activation, a pivotal event in inflammatory signaling cascades and innate immune regulation. Enhanced levels of IL-6 and TNF-α were accompanied by heightened expression of pro-apoptotic markers, specifically Bax, NF-κB, IL-1β, IL-10, and P53 (Moawadh *et al.*, 2023).

A parallel pattern appears in neuroinflammatory and stress models. *Ocimum basilicum* extract downregulated hippocampal TLR4, TNF-α, and IL-1β and reduced MDA while increasing total antioxidant capacity in a maternal-separation mouse model (Amini-Khoei *et al.,* 2025). During fish transport stress, *Ocimum basilicum* essential oil reduced cortisol and HSP70, increased liver antioxidant enzymes, and altered arachidonic acid, steroid hormone, and amino acid metabolism (Wang *et al.*, 2026). Diminished hepatic protein levels signify altered membrane permeability, a condition that facilitates the translocation of toxic substances across the cell membrane into hepatocytes (Ghosh *et al.*, 2020). The metabolic intermediates and toxicants in question have the potential to cause several complications at cellular level like the activation of hydrolytic enzymes that render cells more vulnerable to chromosomal damage and disruption of the cell cycle (Hussein *et al.*, 2016). Genetics may be affected by a reduction in hepatic protein, which induces hepatic cell proliferation via modifying the cell cycle (Omotuyi *et al.*, 2006). The detrimental impact of CCl_4 on DNA arises from its accumulation in hepatic tissue, causing a marked increase in reactive oxygen species production, oxidative stress, and cellular apoptosis (Dua *et al.*, 2023).

The reduction in lipid peroxidation and enhancement of antioxidant parameters observed after oral basil leaf oil treatment can be explained by the synergistic action of its polyphenolic constituents and secondary metabolic products (Touiss *et al.*, 2021). The variation in the CCl_4-induced antioxidant defense system as well as liver biomarkers was alleviated through LEO supplementation (Mazani *et al.*, 2022). The redox-modulating capacity of basil is attributable to its abundant phenolic constituents, particularly flavonoids and phenolic acids, which confer potent free radical scavenging activity. Furthermore, the marked reduction in hydroxyproline accumulation within hepatocytes demonstrates the therapeutic potential of basil against hepatic fibrosis (Sallam *et al.*, 2022). The study indicated that basil ameliorates fibrotic disturbances in the liver of fibrotic rats and stimulates liver regeneration. Due to its potent free radical scavenging and anti-inflammatory properties, the use of basil leaf essential oil as a botanical therapeutic agent could potentially diminish the cellular damage induced by carbon tetrachloride.

Conclusions: CCl_4 administration in rats induced profound hepatotoxicity and gut dysbiosis, evidenced by systemic oxidative stress, depletion of endogenous antioxidants, upregulation of pro-inflammatory cytokines, and marked histopathological damage. Lemon basil essential oil (LEO), owing to its rich phenolic and flavonoid content, particularly methyl cinnamate and linalool, demonstrated potent antioxidant, antimicrobial, and cytotoxic properties *in vitro*. More importantly, LEO pretreatment effectively enhanced the physiological parameters by reduced the body weight loss and mortality, while it mitigated CCl_4-induced liver injury in rats by activating Nrf2-mediated cytoprotective genes (HO-1, SOD, CAT), restoring antioxidant enzyme activities, suppressing TNF-α and IL-6 expression, and enhancing gut microbial homeostasis.

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