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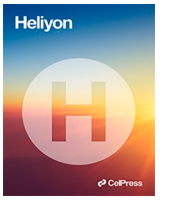

Research article

Hepato-renal protective impact of nanocapsulated *Petroselinum crispum* and *Anethum graveolens* essential oils added in fermented milk against some food additives via antioxidant and anti-inflammatory effects: *In silico* and in vivo studies

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ABSTRACT

The study assessed the efficacy of parsley and dill essential oils (EOs) nanocapsules incorporated into fermented milk in hepato-renal protection against specific food additives. A molecular docking assay was conducted between parsley and dill EOs bioactive molecules and inflammatory cytokines. Freeze-dried parsley and dill EOs nanocapsules were developed, characterized for their morphological structure, particle size, zeta potential, polydispersity index and encapsulation efficiency and assessed in fast green dye and sodium benzoate (SB) combination-treated rats. The docking results revealed that the primary constituents of parsley and dill EOs (apiol, myristicin, α-pinene, (−)-carvone, and d-limonene) interacted with the active sites of TNF-α, IL-1β and TGF-1β cytokines with hydrophobic and hydrogen bond interactions. D-limonene had the highest binding affinity (6.4 kcal/mol) for the TNF-α. Apiol and myristicin had the highest binding affinity (5.1, 5.0, 5.0 and 5.0 kcal/mol, respectively) for the IL-1β and TGF-β1 receptors. Biochemically and histopathologically, the excessive co-administration of fast green and SB revealed adverse effects on the liver and the kidney. Whereas the treatment with parsley and dill EOs nanocapsules afford hepato-renal protective effects as manifested by suppression the elevated liver and kidney functions. Parsley and dill EOs nanocapsules showed a significant reduction of the liver (64.08 and 80.5 pg/g, respectively) and kidney (59.3 and 83.6 pg/g, respectively) ROS. Moreover, parsley and dill EOs nanocapsules down-regulated the liver and the kidney inflammatory cytokines (IL-6, TNF-α, IL-1β and TGF-1β) and lipid peroxidation and up-regulated the antioxidant enzymes. In conclusion, the data suggest a potential hepato-renal protective effects of parsley and dill EOs nanocapsules.

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1. Introduction

Food additives have enabled the production of diverse and appealing foods and beverages for a growing population. They make significant contributions to food processing and preservation [1]. Nonetheless, despite their obvious benefits, it is important to remember that many permitted food additives are synthetic, and when used in excess, they can cause a range of negative consequences, toxicity, and other unpleasant reactions, including cancer. Food additives are subject to severe review by regulatory organizations worldwide because to potential health risks such as gastrointestinal, respiratory, dermatological, and neurological issues [2]. Even when used within permissible limits, certain food additives may appear to be harmless. When analyzing the safety of these chemicals, however, the interactions between many of them, which constitute their normal form in foods, are not taken into account [3]. These food additives are xenobiotics, metabolized in the liver and excreted by the kidneys [4]. Pro-inflammatory cytokines are produced by renal tubule cells in response to pollutants, xenobiotics, and infections [5]. These pro-inflammatory cytokines attempt a fundamental role in the course of renal failure [6]. Furthermore, certain food additives have a negative impact on liver and renal function. The increase in oxidative stress, inflammatory reactions, and cytokines release may assist these negative effects [7,8].

Essential oils (EO) are widely recognized for their biological and aromatic properties. The biological actions of EO's terpenes and terpenoids include anti-inflammatory, anti-cancer, anti-ulcerogenic, antioxidant, and permeation-promoting qualities [9]. Parsley (*Petroselinum crispum*; Apiaceae family) is a food enhancer as well as an edible medicinal plant. The essential oil is primarily responsible for the flavor of parsley leaf. It has anti-inflammatory, antioxidant, and antiapoptotic effects in a variety of tissues [10]. The primary constituent of parsley EO include myristicin, apiol, pinene, myrcene, 1, 3, 8-p-menthatriene, and β-phellandrene, to which the pharmacological effects of parsley EO can be attributed [11].

Dill (*Anethum graveolens* L.; Umbeliferaa family) is an annual aromatic plant used in food and medication and possesses high levels of essential oil. The demand for its ethnopharmacological features and medical benefits, such as its potential antioxidant, anticancer, antibacterial, and anti-inflammatory has increased recently [12]. Parsley, dill and their essential oils have been generally recognized as safe [13]. According to the findings of Shafaei et al. [12], in different organs of mice exposed to oxidative stress caused by cadmium, the dill seed's oil nanoemulsion decreased lipid peroxidation, cellular antioxidant redox potential and inflammation. Consequently, the goal of the current study was to assess the adverse effects of fast green FCF and sodium benzoate combination on the liver and the kidney and the protective impact of administrating parsley and dill essential oils nanocapsules incorporated into the fermented milk.

2. Materials and methods

2.1. Raw materials and chemicals

Parsley and dill seeds were acquired at a local market (Haraz, Cairo, Egypt) and validated by Dr. Abdelmiged Ali Abedelmiged, Professor of Flora and Plant Taxonomy, Egypt Agriculture Research Center. Fast Green FCF (C37H34N2Na2O10S3, E143) was imported from Oxford Lab Chemical Co. in India. Sodium benzoate (SB, C7H5O2Na, E211) was imported from S.D. Fine-Chem Ltd. in India. DPPH (1, 1-diphenyl-2-picrylhydrazyl) and 2, 20 -Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) were imported from Sigma (Sigma-Aldrich, St. Louis, MO, USA). Arabic gum and maltodextrin were imported from Loba Chemie in Mumbai, India. Low-heat skimmed milk powder (USA, 34 % protein, 51 %, fat 8.2 %, lactose, 1.2 % ash and 4 % moisture) was purchased from a local market in Egypt. Starter strains (*Streptococcus thermophilus* and *Lactobacillus bulgaricus)* were provided from dairy lab, NRC, Egypt. Fine analytical grade reagents and chemicals were used in this investigation.

2.2. Experimental animals

Twenty four adult male Wister rats weighing an average of 134.3 ± 6.0 g were obtained from the animal house of the National Research Centre in Cairo, Egypt. The rats were four weeks old. Each rat was housed in a stainless steel cage. The feeding environment was maintained at a steady temperature of 23 ± 2 °C, relative humidity of 45–50 %, and a 12-h light and dark cycle. The rats had full access to food and water.

2.3. Methods

2.3.1. Extraction of parsley and dill essential oils

Clevenger's apparatus was used to hydrodistill the seed samples. Water impurities were removed from the separated essential oils by passing them through anhydrous Na_2SO_4. The essential oils were collected and stored at 4 °C in a clean, dark glass vial for later analysis.

2.3.2. Identification of chemical composition of parsley and dill EOs using gas chromatography-mass spectrometry (GC-MS)

A gas chromatography system (Agilent 8890 GC System) coupled to an HP-5MS fused silica capillary column (30 m, 0.25 mm i.d., 0.25 mm film thickness) and a mass spectrometer (Agilent 5977B GC/MSD) were utilized for the analysis. First, the oven was supposed to reach 50 °C. After that, it was supposed to climb to 220 °C at a pace of 5 °C/min, 280 °C at a rate of 15 °C/min, and finally stay at 280 °C for 7 min. The carrier gas used was helium, flowing at a rate of 1.1 mL per minute. After diluting the essential oil (30 μL essential oil/mL diethyl ether), 1 μL of the solution—with a split ratio of 1:50—was injected into the GC. 230 °C was the injection temperature. At 70 eV, mass spectra were obtained in the electron impact mode (EI), with scan *m/z* spanning from 39 to 500 amu. By cross-

referencing the separated peaks with mass spectra data from the National Institute of Standards and Technology NIST, they were identified.

2.3.3. Determination of antioxidant activity of parsley and dill EOs

2.3.3.1. DPPH free radical scavenging activity assay. Oil concentrations (10–50 μg/ml) were added to a 5 mL methanol–0.004 % DPPH solution. Using a UV–vis spectrophotometer (Jasco V-730, serial No. 112361798, Tokyo, Japan), the absorbance at 517 nm was measured after 30 min of incubation at room temperature in comparison to a blank. The experiment was run in triplicate. A graph that plotted percent inhibition against concentration was used to calculate the IC_{50} concentration, which is the concentration at which 50 % of DPPH radicals were neutralized. Using equation (1), the percentage of inhibition was determined.

$$\text{DPPH scavenging activity (\%)} = (A_{blank} - A_{sample}) \times 100 / A_{blank} \quad (1)$$

Where, A_{blank} is the absorbance of the control (without oil) and A_{sample} is the absorbance of the tested oil [14].

2.3.3.2. ABTS radical scavenging activity assay. The ABTS radical cation decolorization test, developed by Arnao et al. [15], was used to assess the parsley and dill EOs' capacity to scavenge free radicals. Before being used, 7. mM ABTS in water and 2.45 mM potassium persulfate (1:1) were reacted for 12–16 h at room temperature to create the ABTS cation radical. Methanol was added to the ABTS solution to dilute it until the absorbance at 734 nm was 0.700. Thirty minutes after mixing, 3.995 ml of diluted ABTS solution was mixed with 10–50 μg/ml of oil, and the absorbance at 734 nm was measured with a UV–vis spectrophotometer (Jasco V-730, serial No. 112361798, Tokyo, Japan). The experiment was run in triplicate. Equation (2) was utilized to compute the scavenging effect of ABTS.

$$\text{ABTS scavenging activity (\%)} = (A_{blank} - A_{sample}) \times 100 / A_{blank} \quad (2)$$

Where, A_{blank} is the absorbance of the control (without oil) and A_{sample} is the absorbance of the tested oil.

2.3.4. In silico predictions of the anti-inflammatory effect of parsley and dill EOs through docking assay

The binding affinity score of parsley and dill EOs bioactive molecules on inflammatory cytokines namely, tumor necrosis factor (TNF-α) (PDB: 2AZ5), transforming growth factor 1 beta (TGF 1-β) (PDB: 1KLC) and human interleukin 1 beta (IL-1β) (PDB: 1HIB) was studied using PyRx software. The 3D chemical structures of three bioactive molecules apiol, myristicin, and (−)-Carvone (SMILE code) were created using the chem draw tool and the pubchem database, which can be accessed at https://pubchem.ncbi.nlm.nih.gov/. After loading the target proteins and ligands into the PyRx program, the GRID parameters were maximized, the target proteins and ligands were minimized and converted to PDBQT [16,17]. A docking study was then carried out. PyMOl and Discovery Studio were used to visualize protein and ligand complexes.

2.3.5. Nanoencapsulation of parsley and dill EOs

Nanoemulsions of parsley and dill EOs were prepared according to Mahdi et al. [18]. Arabic gum (15.0 % w/v) was hydrated in deionized water at 4 °C overnight, and then maltodextrin (15.0 % w/v) was added to the gum solution. Tween-80® (1.0 % w/v, based on water) was added. To the aqueous solution, a precise volume of oil (10 ml) was added drop by drop. A high-speed homogenizer (Ingenieurbüro CAT, Germany) was used to homogenize the mixture for 5 min at 10,000 rpm. Using a sonicator (vibra cell, Sonics & Materials, Inc., Newtown, CT, USA), the nanoencapsulated dill and parsley were prepared. A homogenous suspension was obtained after sonication for 1 s and then rest for 1 s in an ice bath for 4 min. The suspensions were immediately freeze-dried at −52 °C for 24 h using freeze-dryer (ALPHA 1–4 LSC). The dried nanoencapsulated parsley and dill EOs were stored at 4 °C for further analysis.

2.3.6. Determination of the nanomulsions particle size, polydispersity index and zeta potential

The particle size distribution, polydispersity index (PDI) and zeta potential were estimated via Zetasizer ver. 7.04 (Malvern Instruments Ltd, UK) at 25 ± 0.1 °C. The nanoemulsion sample was diluted with distilled water at a ratio of 1:20 v/v. After that, the mixture was lightly sonicated. The measurements were taken with 1 mL of the diluted sample after it had been moved to a disposable PVC clear cuvette.

2.3.7. Determination of encapsulation efficiency (EE)

According to Dwivedy et al. [14], EE was determined using UV–Vis spectrophotometric method. The amount of oil left inside the nanocapsules was calculated by extracting the oil from 10 mg of the freeze-dried nanocapsules using 3 ml of ethyl acetate and comparing the absorbance to a standard curve made for the essential oils at 259 nm. The EE was calculated from equation (3):

$$\text{EE (\%)} = \text{total amount of oil in nanocapsules} / \text{initial amount of the oil X } 100 \quad (3)$$

2.3.8. Scanning electron microscope (SEM) of nanoencapsulated parsley and dill EOs

A high resolution scanning electron microscope (TESCAN VEGA 3 with field emission gun, Czech Republic) was utilized to investigate the morphological structure of the freeze-dried EOs nanocapsules. The samples were coated with a gold layer using Quorum (Q 150 ES, United Kingdom) for 60 s.

2.3.9. Preparation of fermented milk loaded with parsley and dill nanocapsules

Fermented milk was prepared by heating the reconstituted skim milk powder (14 % total solids) to 90 °C for 10 min. The milk was cooled to 45 °C. Following the inoculation of the starting culture (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) at a rate of 3 g/100 ml of milk, the milk was transferred into 100 ml plastic cups. The incubation process lasted approximately 3 h at 45 ± 1 °C. Set fermented milk cups were placed in a refrigerator at 4 °C once the required consistency was reached. Before administrating to animal, the fermented milk was loaded with the freeze-dried parsley and dill nanocapsules.

2.3.10. Design of the animal experiment

Twenty-four rats were acclimatized for one week then allotted into 4 groups (n = 6 per group) of control normal (CN) and other 3 groups fed maintenance AIN-93 balanced diet (58.5 % maize starch, 5 % fiber, 3.5 %, 10 % corn oil, 10 % sucrose, 12 % casein-supplemented protein, 3.5 % AIN-93 salt mixture, and 1 % AIN-93 vitamins mixture) which prepared according to Reeves et al. [19]. The normal control group was orally administered 2 ml of distilled water, the second group (food additives combine, FAC) was considered as positive control and orally administered an amalgam of fast green dye (125 mg/kg body weight) and sodium benzoate (10 mg/kg body weight) dissolved in 2 ml distilled water. These doses were selected according the outcome of previous studies [20, 21]. The third group (encapsulated parsley oil, EPO) was orally co-administered 1 ml distilled water containing an amalgam of fast green dye and sodium benzoate and 1 ml of fermented milk containing an amount (200 mg) of freeze-dried encapsulated parsley oil containing the equivalent of 0.5 ml of oil per kg of body weight. The fourth group (encapsulated dill oil, EDO) was orally co-administered 1 ml distilled water containing an amalgam of fast green dye and sodium benzoate and 1 ml of fermented milk containing an amount (200 mg) of freeze-dried encapsulated dill oil containing the equivalent of 0.5 ml of oil per kg of body weight according to Refs. [11,22]. The experimental period lasted 30 consecutive days. Food intake was recorded daily. Body weight gain or loss was measured by the following equation: [body weight gain or loss = body weight at the end of the experiment - body weight at the start of the experiment].

2.3.11. Blood collection

Rats were euthanized by decapitation and their blood was gathered from the body trunk in EDTA-free tubes, and the serum was obtained by centrifuging (3000 rpm; 10 min). All serum samples were maintained at −20 °C until analysis.

2.3.12. Tissues collection

Parts of the liver and the kidney were immersed in formalin-saline (10 %) to the histopathological examination. To make homogenates (10 % w/v) in a cold homogenization buffer (100 mM potassium phosphate buffer, pH 7.4), liver and kidney sections were removed from each rat. After centrifuging the homogenates, the biochemical tests were carried out using the supernatants.

2.3.13. Biochemical analysis of the serum and tissues

The serum of each rat was assessed for total protein and the activities of gamma-GT (γ- GT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), aspartate transaminase (AST) and alanine transaminase (ALT) using the colorimetric methods described by Rheinhold and Seligron [23], Szasz [24], Bessey et al. [25], Zimmerman and Weinstein [26], and Reitman and Frankel [27], respectively. The levels of total and direct bilirubin as well as creatinine, urea, and albumin were determined using the colorimetric methods described by Balistreri and Shaw [28], Larsen [29], Fawcett and Scott [30], and Doumas et al. [31], respectively. The liver and the kidney malondialdehyde (MDA), glutathione peroxidase (Gpx), superoxide dismutase (SOD), nitric oxide (NO), and catalase (CAT) levels were assessed using the colorimetric methods described by Ohkawa et al. [32], Paglia and Valentine [33] and Nishikimi

Table 1
Chemical constituents of parsley and dill EOs.

Parsley EO				Dill EO			
Compound	RI[a]	RI[b]	%	Compound	RI[a]	RI[b]	%
β-Thujene	957	923	0.22	1,3,8-p-Menthatriene	1031	1010	0.2
α-Pinene	964	933	13.61	p-Cymene	1051	1022	0.53
Sabinene	1000	978	0.74	D-Limonene	1055	1030	12.61
β-Pinene	1004	979	10.02	γ-Terpinene	1086	1075	0.13
β-Myrcene	1016	992	0.63	p-(1-Propenyl)-toluene	1119	1095	0.25
p-Cymene	1051	1022	0.57	Cis-Limonene oxide	1166	1140	0.17
D-Limonene	1055	1030	6.46	Trans-Dihydrocarvone	1235	1200	14.11
γ-Terpinene	1086	1075	1.17	Dihydrocarvone	1243	1203	9.7
p-(1-Propenyl)-toluene	1118	1095	0.31	1,6-Dihydrocarveol	1254	1211	0.24
5-Pentylcyclohexa-1,3-diene	1191	1161	0.29	(−)-cis-Carvyl Acetate	1258	1229	0.28
Myrtenal	1234	1197	1.21	Dihydrocarveol	1269	1236	0.61
Myristicin	1517	1521	25.18	(−)-Carvone	1286	1247	40.37
Elemicine	1591	1531	3.11	Thymol	1290	1298	1.1
Apiol	1665	1677	33.45	Myristicin	1517	1521	0.41
trans-Sedanolide	1789	1723	0.86	Apiol	1665	1677	18.54
Hexanedioic acid, bis(2-ethylhexyl) ester	1973	1956	2.17	Hexanedioic acid, dioctyl ester	1973	1956	0.75

RI[a], Calculated retention index; RI[b], Reported retention index from the literature.

et al. [34], Montgomery and Dymock [35], and Beers and Sizer [36], respectively. The liver and the kidney tumor necrosis factor-alpha (TNF-α), interleukin 6 (IL-6), transforming growth factor 1-beta (TGF 1-β), reactive oxygen species (ROS) and interleukin 1-betta (IL-1β) were determined using ELISA kits (Sunlong Co., Ltd. China) as described in the manufacturer's instructions.

2.3.14. Histopathological examination

The kidney and liver samples were cleaned in xylol, dehydrated in an increasing series of ethanol, and then processed for the creation of paraffin blocks. Using a microtome, paraffin slices measuring 4–6 μm were created. They were then stained with hematoxylin and eosin (H&E) stain and inspected under a light microscope (CX21; Olympus, Tokyo, Japan).

2.3.15. Statistical analysis

The SPSS software, version 21, was used to conduct the statistical analysis. The results were statistically evaluated using the Duncan test and a one-way analysis of variance (ANOVA). The results were reported as mean ± standard error (SE). When $P < 0.05$, a difference was deemed statistically significant.

3. Results and discussion

3.1. Identification of the chemical composition of parsley and dill EOs using GC-MS

The chemical profile of EOs varies based on geography, climate, and sample quality. As a result, the chemical composition of parsley and dill EOs was determined prior to assessing their potential bioactivity. The hydro-distillation of parsley seeds yielded 1.2 % (w/w), whereas dill seeds yielded 1 % of clear yellowish liquid oil. The GC-MS analysis of parsley and dill oils (Table 1 and Fig. 1A and B) identified 16 distinct components for each oil. The main elements of parsley EO were apiol (33.45 %), myristicin (25.18 %), α-pinene (13.61 %), β-pinene (10.02 %), and d-limonene (6.46 %). The major components of dill EO were (−)-carvone (40.37 %), apiol (18.54 %), trans-dihydrocarvone (14.11 %), and d-limonene (12.61 %). Marín et al. [37] identified myristicin (36.15 %), apiol (20.97 %), α-pinene (15.47 %), and β-pinene (10.43 %) as the main ingredients of Spanish parsley EO. According to Badr et al. [11], the predominant components in Egyptian parsley EO from leaves were 1,3,8-menthatriene (34.48 %), myristicin (21.04 %), and apiol (18.08 %). According to Hao et al. [38], the primary components of dill EO are carvone (45.78 %) and apiol (23.83 %). Ghafarifarsani et al. [39] found 13 components in Iranian dill essential oil, including α-phellandrene (29.6 %), apiol (16.58 %), limonene (15.6 %), carvone (14.66 %), and dill ether (13.2 %).

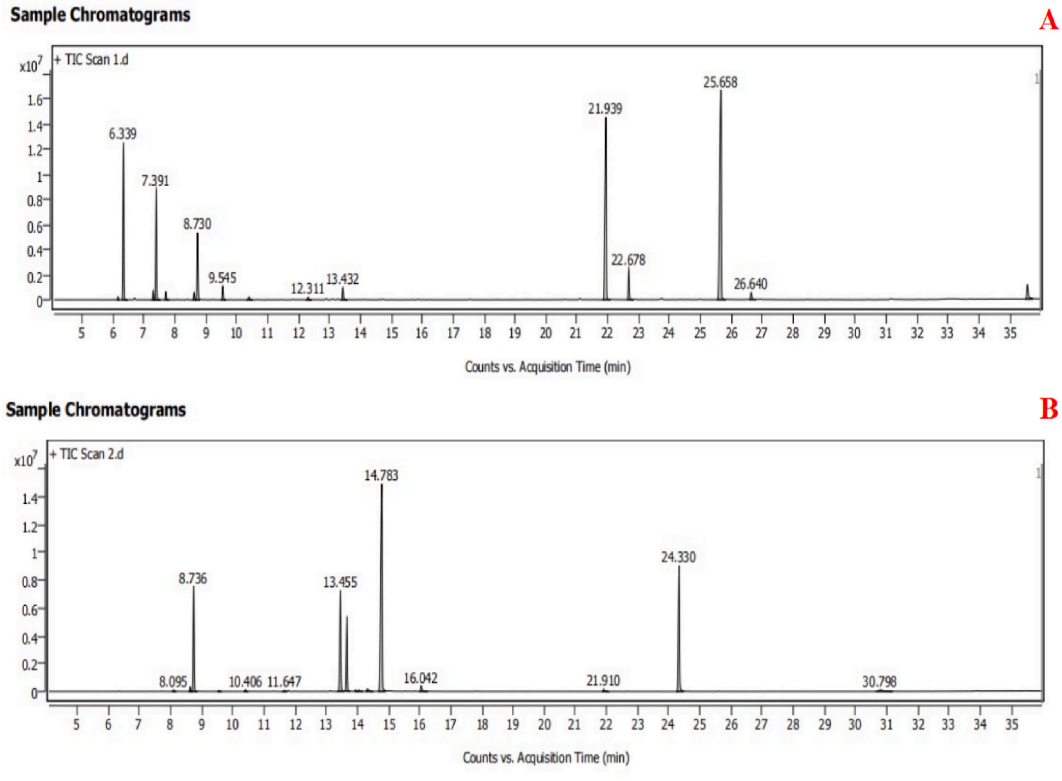


Fig. 1. GC-MS chromatogram of parsley EO (A) and dill EO (B).

3.2. The radical scavenging activity of parsley and dill EOs

Natural products' antioxidant activity is primarily determined by their hydrogen donating ability, or radical scavenging activity, as assessed using the stable radical DPPH. As hydrogen is converted to DPPH, the colour fades. The capacity to gift hydrogen intensifies the bleaching activity and lowers the IC50 [37]. As a result, the DPPH radical scavenging capacity and ABTS cation of parsley and dill EOs were determined in the current study. Butylated hydroxytoluene (BHT) acted as a positive control. Table 2 shows the antioxidant activity of parsley and dill EOs, with IC_{50} values of 24 μg/ml and 28 μg/ml for DPPH. The major components of parsley EO include myristicin (phenylpropene), apiol (phenylpropanoid), and α- and β-pinene isomers of pinene are responsible for its antioxidant activity [40,41]. The presence of aromatic rings, as well as the quantity and configuration of hydroxyl groups in parsley and dill EOs, may all contribute to the oil's antioxidant properties [42]. Ghafarifarsani et al. [39] revealed that the hydrocarbon (limonene) and oxygenated (apiol and carvone) monoterpenes, as well as a larger hydrogen donating ability, are responsible for dill EO's expanded antioxidant effects.

3.3. Predicted anti-inflammatory effect of parsley and dill EOs through molecular docking

Molecular docking can be utilized to investigate the interactions between EO molecules and reference proteins, revealing information about their potential health advantages. Molecular docking is a computer approach that foretell ligand location in a target interaction site. Docking is commonly used to describe and anticipate potential interactions, appraise performance, and investigate links between chemical construction and biological activity [43].

TNF-α, IL-1β, and IL-6 are among the most common upstream pro-inflammatory cytokines. TNF-α, IL-1β are the most important inducers of IL-6 expression [44]. Acute kidney damage triggers the activation of TGF-β1, which can regulate cellular responses to the toxin in a positive or negative way. Any prolonged kidney injury raises TGF-β1, which promotes renal fibrosis [45]. Keeping this in view, a molecular docking assay was performed to look into the interactions between parsley and dill EO molecules and TNF-α, IL-1β and TGF-β1 as target proteins. The docking results (Table 3) show that apiol, myristicin, α-pinene, (−)-carvone and d-limonene blocked the active sites of TNF-α with hydrophobic bonds at different residues. Apiol blocked the active sites of TGF-β1 with 5 hydrophobic interactions and conventional hydrogen bonds at the GLY 46 residue. Myristicin blocked the active sites of TGF-β1 with 2 conventional hydrogen bonds at the LYS 110 residues. Apiol blocked the active sites of IL-1β with 3 hydrophobic bonds, 2 carbon-hydrogen bonds and a conventional hydrogen bond at the GLY 64 residue. Myristicin blocked the active sites of IL-1β with hydrophobic bond, carbon-hydrogen bond and 2 conventional hydrogen bonds at the LYS 80 and LYS 134 residues. (−)-Carvone blocked the active sites of IL-1β with 4 hydrophobic interactions and 3 conventional hydrogen bonds at the GLU 64, LYS 65 and ASN 66 residues. According to Zia et al. [46], substances with reinforced hydrophobic contacts and hydrogen bonds that dock with cytokines may be involved in inflammation inhibition. When the binding affinity values are compared, d-limonene appears to have the highest binding affinity of the ligands for the TNF-α receptor. Apiol and myristicin appear to have the highest binding affinity of the ligands for the IL-1β and TGF-β1 receptors. In addition to the interaction through the hydrogen and hydrophobic bonds, the higher percentage of apiol and myristicin in parsley oil suggests the higher potential to inhibit IL-1β and TGF-β1 cytokines.

3.4. Physical properties of parsley and dill EOs nanoparticles

Nanoencapsulation technology improves the physical stability, boosts the bioactivity of EOs and food constituents and shields them from the other components and processes. Nanoencapsulation technology can also enable the use of naturally occurring bioactive compounds at lower dosages [47]. Therefore, in this work, nanoencapsulation was employed for developing parsley and dill EO capsules.

According to Baranauskaite et al. [48], emulsion parameters such as z-average, zeta potential, and polydispersity index (PDI) are all directly related to encapsulation efficiency (EE). The data (Table 4) show the particle size (z-average hydrodynamic diameter), zeta potential, PDI and EE of parsley and dill EOs nanocapsules. Z-average, zeta potential and PDI are important indicators of emulsion physical stability during storage. The zeta potential expounds the variation in electric potential between the particle's surface and the medium. Therefore, zeta potential values (negative or positive) near 0 are related to unstable systems that sediment at a specific period. A higher zeta potential, positive or negative, suggests increased emulsion stability. The zeta potential values of parsley and dill,

Table 2
Antioxidant activity of parsley and dill EOs.

		Concentration (μg/ml)				
		10	20	30	40	50
DPPH (%)	Parsley	29.13 ± 0.81	41.33 ± 0.85	59.37 ± 0.57	64.9 ± 0.70	70.33 ± 0.61
	Dill	26.93 ± 0.78	38.50 ± 0.46	53.30 ± 0.66	61.6 ± 0.36	67.66 ± 0.59
	BHT	77.63 ± 0.04	79.22 ± 0.02	83.37 ± 0.11	85.4 ± 0.03	88.24 ± 0.06
ABTS (%)	Parsley	16.52 ± 0.25	19.72 ± 0.41	27.33 ± 0.21	35.12 ± 1.20	38.41 ± 1.04
	Dill	11.14 ± 0.46	14.07 ± 1.32	26.27 ± 0.62	28.52 ± 0.50	32.41 ± 0.36
	BHT	64.57 ± 0.23	66.87 ± 0.62	69.40 ± 0.07	72.21 ± 0.26	74.22 ± 0.08

Values are presented as mean ± SD.

Table 3
Molecular docking and interactions between parsley and dill EOs molecules and target proteins.

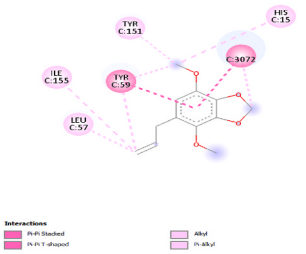
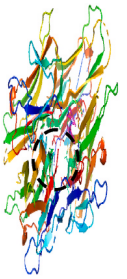
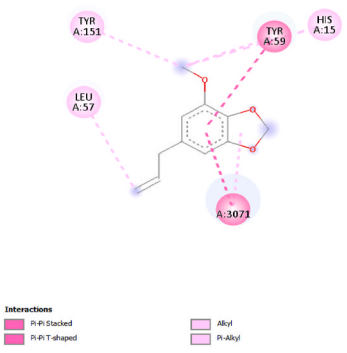
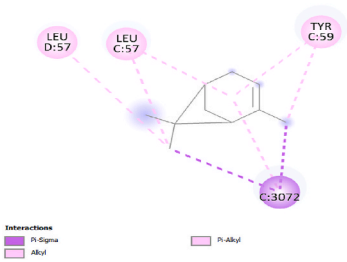
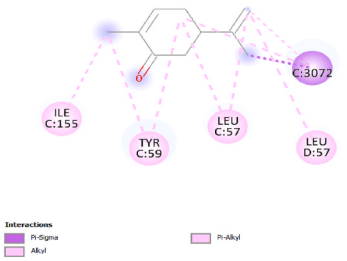
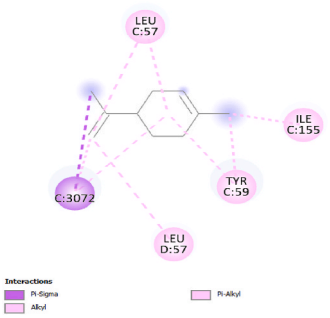
Protein	Ligand	Binding affinity (kcal/mol)	The 3D complex of the binding	2D Protein-ligand complex
TNF-α (2AZ5)	Apiol	−6.2		
	Myristicin	−6.3		
	α-Pinene	−6.3		
	(−)-Carvone	−6.3		
	D-Limonene	−6.4		

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Table 3 (*continued*)

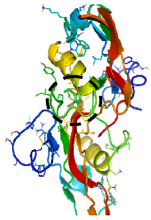
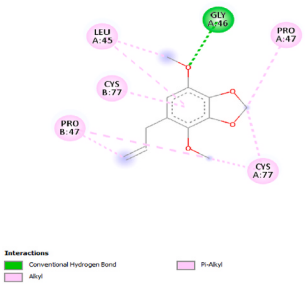
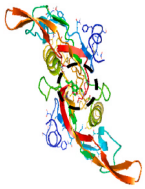
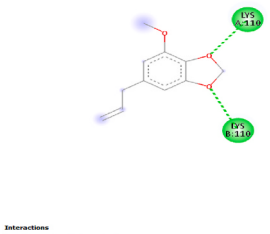
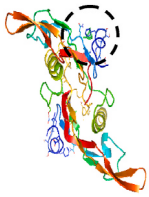
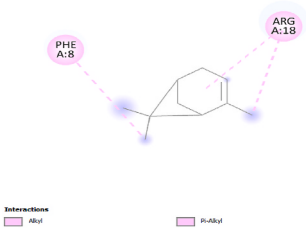
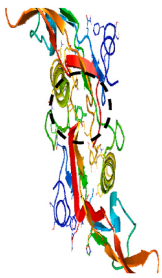
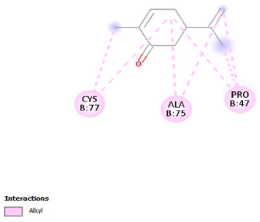
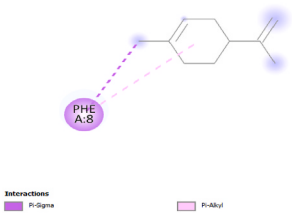
Protein	Ligand	Binding affinity (kcal/mol)	The 3D complex of the binding	2D Protein-ligand complex
TGF 1-β (1 kL C)	Apiol	−5.0		
	Myristicin	−5.0		
	α-Pinene	−4.3		
	(−)-Carvone	−4.3		
	D-Limonene	−4.1		

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Table 3 (*continued*)

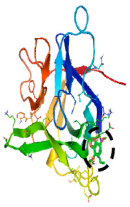
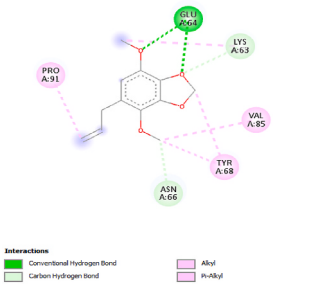
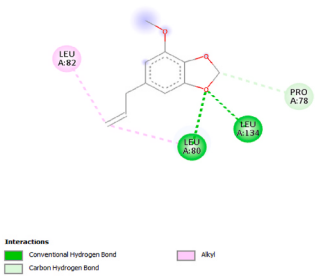
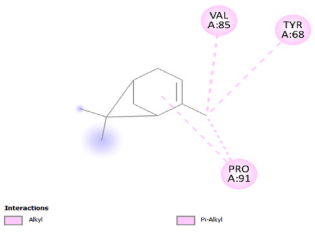
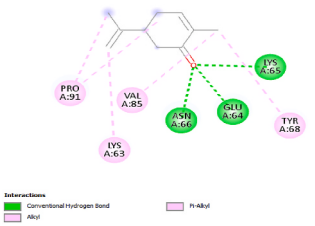
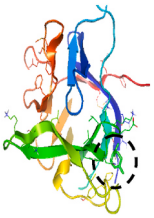
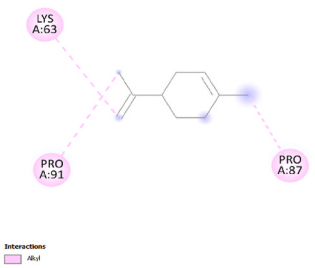
Protein	Ligand	Binding affinity (kcal/mol)	The 3D complex of the binding	2D Protein-ligand complex
IL-1β (1HIB)	Apiol	−5.1		
	Myristicin	−5.0		
	α-Pinene	−4.7		
	(−)-Carvone	−4.6		
	D-Limonene	−4.0		

Table 4
Characteristics of parsley and dill EOs nanoparticles.

	Particle size (nm)	Zeta potential (mV)	PDI	EE (%)
Parsley	184.0 ± 65.87	−33.1 ± 4.65	0.189 ± 0.00	88.21 ± 1.66
Dill	152.8 ± 44.17	−7.56 ± 3.40	0.138 ± 0.00	84.47 ± 1.53

particularly parsley, nanoemulsions referred to good colloidal stability. The PDI value represents the width of the droplet size distribution and shows the homogeneity of the generated nanodroplets in nanoemulsions. The PDI values range from 0 to 1, with the closer to zero number pointing a more unvarying distribution [48]. The low PDI values of parsley and dill nanoemulsions referred to the homogeneous distribution of the particles. The EE % of parsley EO was higher than that of dill EO, implying higher oil retention in the polymeric parsley nanoparticles.

The morphology of the nanocapsules loaded with parsley and dill oil was determined using scanning electron microscopy (SEM). The size and shape of the freeze-dried nanocapsules could be determined using a SEM analysis. Fig. 2 A, B, C, and D depict oil capsules embedded in the structure of Arabic gum and maltodextrin polymers. SEM nano-images revealed that the essential oil-loaded nanocapsules have a spherical surface shape with a mean size of less than 200 nm.

3.5. *Effects of fermented milk fortified with parsley and dill EOs nanocapsules on the growth performance*

Despite the widespread use of food additives in the production of food and pharmaceuticals, these chemicals are classified as xenobiotics [49]. Xenobiotics are metabolized in the liver and eliminated by the kidneys. Continuous consumption of these chemicals strains and damages the liver and the kidney [50]. Fast Green FCF is the least popular credentialed artificial dye in the United States, and it is illegal in the EU and certain other nations [51]. FCF may have mutagenic and tumorigenic effects on experimental animals [52]. However, FCF is used as a green dye in beverages, salad dressings, candies, cereals, and yoghurt [53]. Sodium benzoate (SB) is regularly employed in food and beverage preservation as it keeps fruit juices, jams, salad dressings, carbonated beverages, and liquid medications fresh. As it prevents bacterial and fungal growth in the acidic environs of carbonated beverages, it is a well-known soft drink preservative. However, the function of the liver and the kidney is adversely affected by SB. The increment in oxidative stress, inflammatory responses, and cytokines production may facilitate these adverse effects [8]. Both substances (FCF and SB) are commonly found together in a variety of food products. Therefore, the research was conducted on their combination. In addition to their pleasant aromatic flavor, the antioxidant and anti-inflammatory characteristics of parsley and dill EOs motivated investigation to look into their role in mitigating oxidative stress and elevated cytokines following extended consumption of FCF and SB combination. Fermented milk is not only a popular product, but it is also a handy way to give numerous fortifiers with health advantages [54]. Vitalini et al. [55] fortified cheese with parsley EO and revealed the antimicrobial effect of parsley EO with the panelist's appreciation for this cheese. Hence, fermented milk was selected for this study, as a food product, to be fortified with parsley and dill EOs nanocapsules.

The acceptable daily intake (the quantity that may be consumed daily for a lifetime without causing any health problems in humans) of SB (0–5 mg/kg) was shown to be surpassed in several countries owed to the allowed high amount of SB (250 mg/kg) in soft drinks [56]. In the current study, treatment with FCF (125 mg/kg) and SB (10 mg/kg) brought about a significant decrease in body weight gain (Table 5) in comparison to the CN group, indicating that they had detrimental consequences. Meanwhile, rats of EPO and EDO groups gained significantly ($P < 0.05$) more body weight than the FAC group. It should be noted that there was a considerable

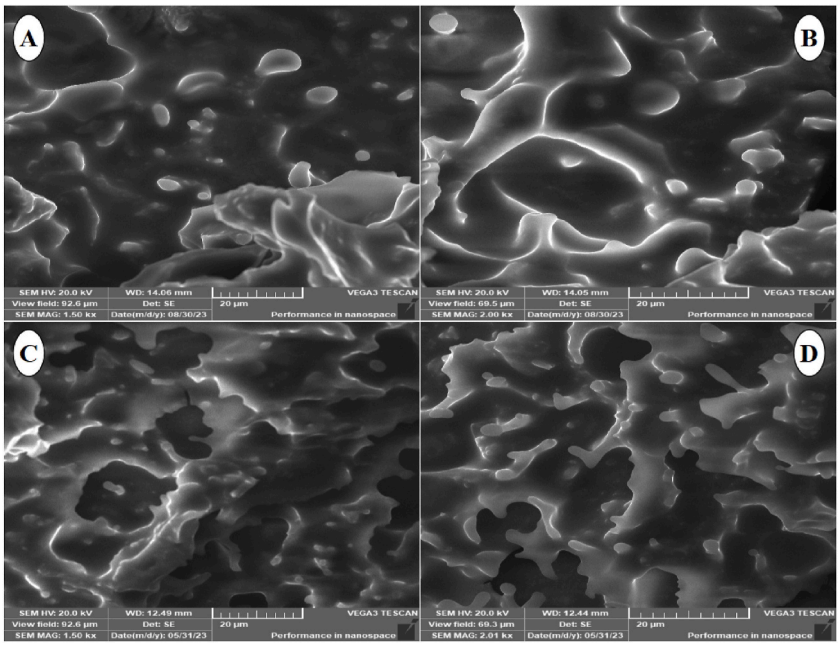


Fig. 2. Scanning electron microscopy (SEM) figures of the freeze-dried parsley and dill EOs nanocapsules. A and B; SEM figures of the freeze-dried parsley EO nanocapsules with magnification 1.5 and 2 kx, respectively. C and D; SEM figures of the freeze-dried dill EO nanocapsules with magnification 1.5 and 2 kx, respectively.

Table 5
Growth performance of CN, FAC, EPO and EDO groups.

	CN	FAC	EPO	EDO
Initial body weight (g)	$134.33^{a}\pm1.67$	$134.17^{a}\pm2.61$	$134.33^{a}\pm2.90$	$134.50^{a}\pm3.11$
Final body weight (g)	$215.33^{c}\pm2.33$	$173.33^{a}\pm3.62$	$200.67^{b}\pm2.60$	$193.17^{b}\pm3.54$
Body weight gain (g)	$81.00^{c}\pm3.57$	$39.17^{a}\pm5.27$	$66.33^{b}\pm3.37$	$58.67^{b}\pm5.17$
Food intake/day (g)	$19.05^{c}\pm0.25$	$16.11^{a}\pm0.15$	$17.05^{b}\pm0.21$	$17.18^{b}\pm0.25$
Food efficiency ratio	$0.14^{c}\pm0.01$	$0.08^{a}\pm0.01$	$0.13^{bc}\pm0.01$	$0.11^{b}\pm0.01$
Relative liver weight (%)	$3.12^{a}\pm0.05$	$3.22^{a}\pm0.22$	$3.15^{a}\pm0.11$	$3.22^{a}\pm0.24$
Relative kidney weight (%)	$0.68^{a}\pm0.03$	$0.73^{a}\pm0.06$	$0.69^{a}\pm0.02$	$0.70^{a}\pm0.04$

The mean ± SE (n = 6) is used to express the data. Each row's distinct superscript differ significantly from one another ($P < 0.05$).

difference between the EPO and the EDO groups in terms of maintaining body weight growth; the EPO had a larger, although not statistically significant ($P > 0.05$), body weight gain than the EDO.

3.6. *Effects of fermented milk fortified with parsley and dill EOs nanocapsules on the oxidative makers*

The underlying mechanism of various adverse effects of food additives has been proposed as oxidative stress [3]. In this study, when compared to the CN group, the FAC group showed an increment in MDA, NO and ROS levels and a decrease in GPx, CAT and SOD activities in the liver and kidney tissues (Fig. 3 A, B, C, D, E and F). The depletion of the antioxidant enzymes could be attributed to their overuse in inhibition of the free radicals induced by the studied food additives [3]. It's reported that FCF is not absorbed by the body, but it accumulates in the liver and the kidney, where its molecules infiltrate the membrane's phospholipids. FCF attaches to proteins, causing them to degrade and modify their functions [20]. Radwan et al. [57] reported that SB (200 mg/kg BW for 8 weeks) caused nephrotoxicity, promoted oxidative stress, increased lipid peroxidation as well as tumor suppressor gene p53 and reduced antioxidant enzymes in the rats' kidney. Another study reported that SOD, CAT, and reduced glutathione levels in rats' liver and kidneys significantly reduced after 90 days of treatment with 0.9 mg/kg SB [8]. Meanwhile, rats administered parsley or dill oil nanocapsules had significantly ($P < 0.05$) lower MDA, NO and ROS levels as well as higher GPx, CAT and SOD activities than the positive control group (Fig. 4). The EPO was more efficient than The EDO in lowering ROS and MDA levels while increasing SOD, GPx, and CAT activity in the liver and kidney tissues. According to previous reports [40,41], the antioxidant issue of parsley and dill EOs nanocapsules may be strongly attributed to their major constituents (apiol, myristicin, α-pinene, (−)-carvone and d-limonene). The present study's results are agreeable with those of Khalil et al. [22] study, which disclosed that treatment with parsley oil exhibited

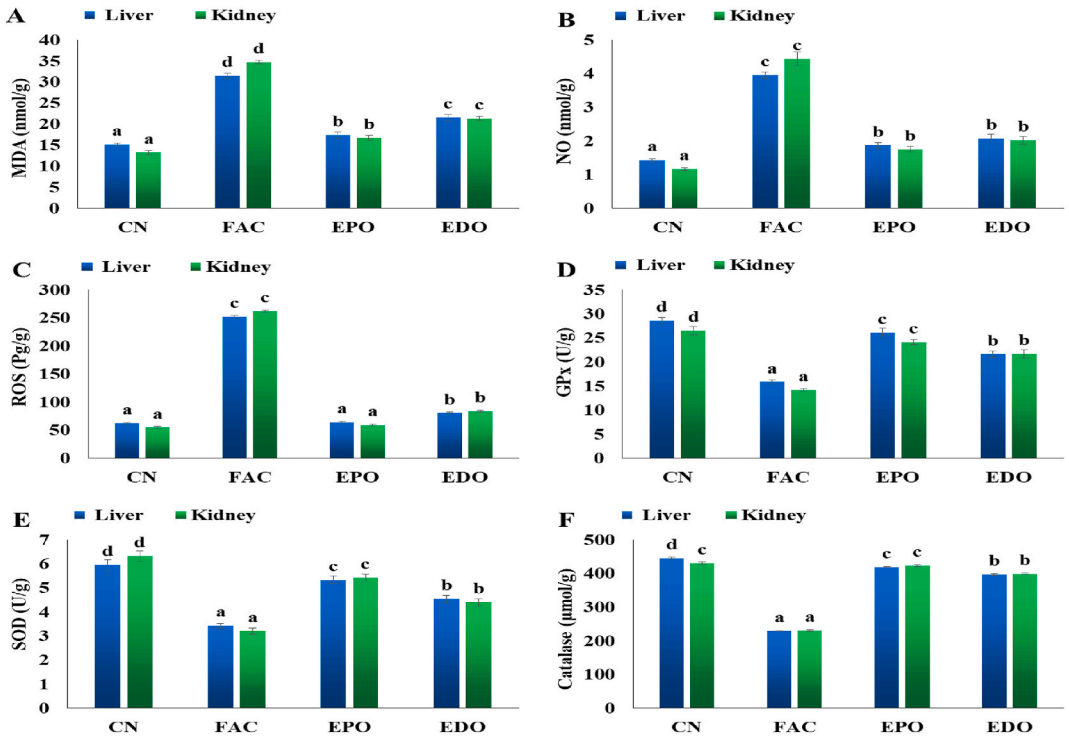


Fig. 3. The hepatic and renal oxidative makers of CN, FAC, EPO and EDO groups. The mean ± SE (n = 6) is used to express the data. Each par's distinct superscript differ significantly from one another ($P < 0.05$).

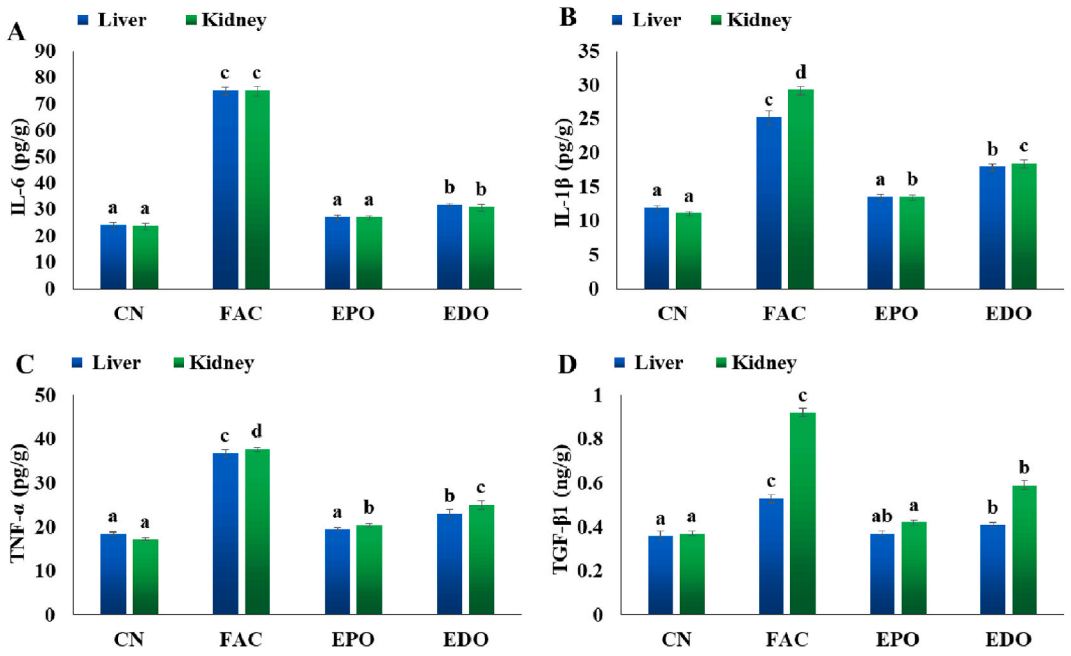


Fig. 4. The hepatic and renal inflammatory cytokines of CN, FAC, EPO and EDO groups. The mean ± SE (n = 6) is used to express the data. Each par's distinct superscript differ significantly from one another ($P < 0.05$).

potent antioxidants and restored the hepatotoxic effects caused by carbon tetra-chloride with a notable rise in SOD and reduced glutathione levels as well as a diminution in hepatic MDA. The findings of the Shafaei et al. [12] investigation demonstrated that treatment with dill nanoemulsion improved lipid peroxidation, and the expression of the GPx and iNOS genes in different tissues of mice subjected to oxidative stress caused by cadmium.

3.7. Effects of fermented milk fortified with parsley and dill EOs nanocapsules on the inflammatory cytokines

TNF-α, IL-1β, and IL-6 are important participants in diseases associated with inflammation throughout the body. Specific inhibitors that block these crucial mediators of inflammation may aid in lowering inflammatory response in various organs [58]. TGF 1-β is the primary fibrotic agent that promotes renal cell dysfunction by increasing extracellular matrix protein deposition, hypertrophy, renal tubular injury, and increases water, glucose, albumin, and electrolyte output [59]. Consequently, the co-administration of FCF and SB resulted in an increment in IL-16, IL-1β, TNF-α and TGF 1-β in the liver and kidney tissues compared to the CN group (Fig. 4 A, B, C and

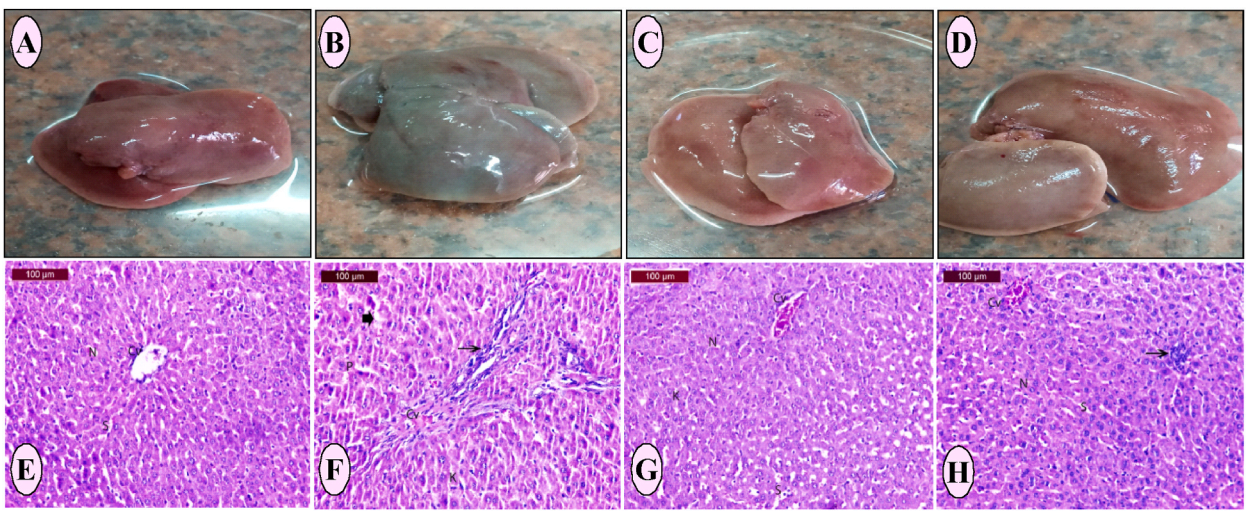


Fig. 5. Photographs and Photomicrographs of rat liver section (stained with H&E ×100): (A and D) control group, (B and F) FAC group, (C and G) rats treated with EPO and (E and H) rats treated with EDO.

Table 6
The hepatic and renal functions of CN, FAC, EPO and EDO groups.

	CN	FAC	EPO	EDO
AST (U/L)	40.00^{a}±1.06	72.00^{c}±1.24	41.30^{a}±0.91	48.83^{b} ± 0.79
ALT (U/L)	24.67^{a}±1.05	50.33^{c}±1.05	25.75^{a}±0.73	29.00^{b} ± 0.73
ALP (U/L)	118.50^{a}±0.99	217.45^{d} ± 1.12	125.50^{b} ± 1.82	129.67^{c}±1.17
LDH (U/l)	251.83^{a}±2.30	359.97^{d} ± 2.09	261.83^{b} ± 2.31	273.33^{c}±1.45
Total bilirubin (mg/dl)	2.60^{a}±0.10	4.26^{c}±0.13	2.76^{ab} ± 0.12	3.08^{b} ± 0.10
Direct bilirubin (mg/dl)	1.83^{a}±0.04	2.87^{b} ± 0.18	1.90^{a}±0.06	2.10^{a}±0.07
γ-GT (U/l)	13.73^{a}±0.37	30.63^{c}±0.73	15.38^{a}±0.73	21.08^{b} ± 0.71
Albumin (g/dl)	4.45^{c}±0.15	2.57^{a}±0.16	4.17^{bc}±0.12	3.82^{b} ± 0.12
Creatinine (mg/dl)	0.38^{a}±0.01	0.86^{d} ± 0.04	0.46^{b} ± 0.02	0.61^{c}±0.02
Urea (mg/dl)	27.50^{a}±0.42	36.98^{d} ± 0.88	30.17^{b} ± 0.70	33.00^{c}±0.73

The mean ± SE (n = 6) is used to express the data. Each row's distinct superscript differ significantly from one another ($P < 0.05$).

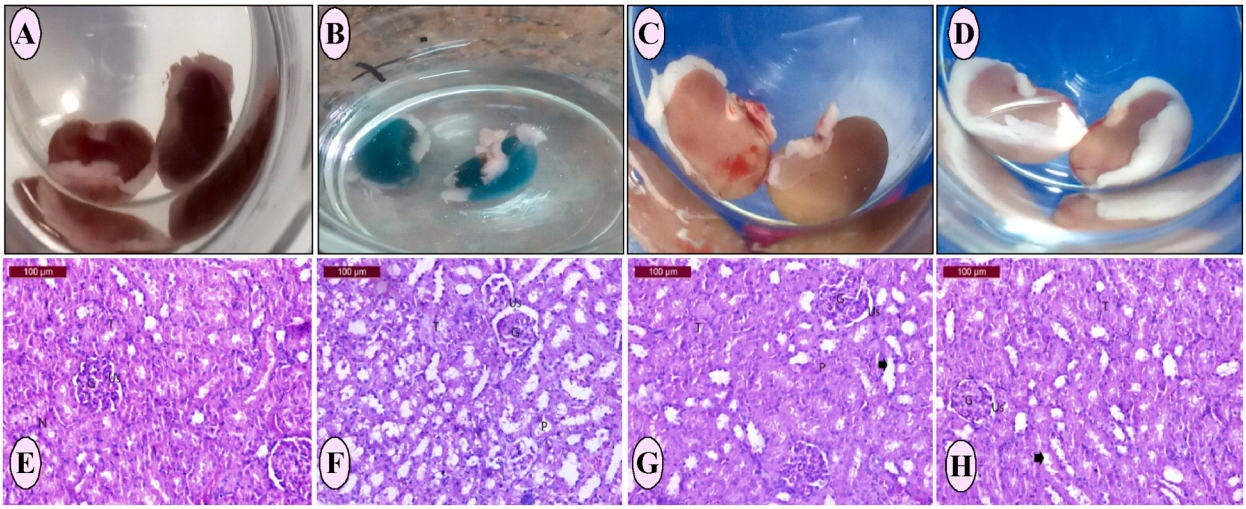


Fig. 6. Photographs and Photomicrographs of rat kidney section (stained with H&E ×100): (A and D) control group, (B and F) FAC group, (C and G) rats treated with EPO and (E and H) rats treated with EDO.

D). These outcomes are in concordant with Oladele et al. [60] report which demonstrated that SB induced noticeable disturbance in cellular architecture and initiated an inflammatory response in the liver and kidney of the exposed rats. In the same context, Akintoye et al. [61] recorded elevation of serum IL-1β after 3 weeks of treatment with SB (200 mg/kg BW). Our results (Fig. 5) showed that rats of EPO and EDO groups exhibited significant ($P < 0.05$) lower hepatic and renal IL-16, IL-1β, TNF-α and TGF 1-β levels than the FAC group. Hepatic and renal TNF-α, IL-16, TGF 1-β and IL-1β levels were significantly ($P < 0.05$) higher in the EPO group than the EDO group. The anti-inflammatory effects of parsley and dill EOs nanocapsules may be attributed to apiol, myristicin, α-pinene, (−)-carvone and d-limonene, which inhibit the inflammatory signaling pathways and decrease the inflammatory mediators [40,41,62].

3.8. Effects of fermented milk fortified with parsley and dill EOs nanocapsules on the liver and the kidney functions

Since the liver is the primary organ where xenobiotics are metabolized, it is extremely vulnerable to their negative effects [63]. The circulation is exposed to cytosolic enzymes upon disruption of the hepatocyte membrane. The production of free radicals may also account for the elevated liver functions. These free radicals cause damage to the mitochondria and plasma membrane as well as enzyme leakage when they interact with polyunsaturated fatty acids in the cell membrane [64]. Because the kidney serves as the primary excretory pathway for the majority of xenobiotics and poisons, it is extremely vulnerable to acquiring a variety of injuries [8]. The liver and kidney functions (Table 6) including serum γ- GT, ALP, ALT, AST, LDH, total and direct bilirubin as well as creatinine and urea increased while albumin level decreased post the co-administration of FCF and SB compared to the CN group. Similar results have been disclosed by Abo Raya et al. [65] in FCF and SB-treated rats for 30 days. Additionally, the detrimental effects of SB, alone or in combination with other food additives, on liver and kidney functions were reported by several studies [8,20]. Results (Table 4) demonstrated that the rats of EPO and EDO groups exhibited significant ($p < 0.05$) lower serum content of γ- GT, ALP, LDH, AST and ALT, total and direct bilirubin as well as creatinine and urea and higher albumin level than the FAC group. The EPO outperformed EDO in restoring liver and kidney functions. The EOs of parsley and dill, which contain monoterpene compounds with higher capacity for both hydrogen donation and antioxidant effects, may have a greater ability to safeguard hepatocytes through the stabilization of cell membranes or the application of advantageous antioxidant effects [39]. In previous studies, the hepato-renal protective effect of

parsley and dill EOs against other harmful substances (cadmium and carbon tetra-chloride) was reported as manifested by reduction in the liver and kidney functions and amelioration in the histopathological changes [12,22].

3.9. Effects of fermented milk fortified with parsley and dill EOs nanocapsules on the morphological and histopathological of the liver and the kidney

Regarding the morphological and histopathological changes of the liver tissues (Fig. 5), the liver section in the CN group shows a normal morphological and histological image, central vein lies at the center of the lobule surrounded by the hepatocytes between the strands of hepatocytes the hepatic sinusoids are evidenced and distinct nuclei are displayed as shown in. The liver of the FAC group shows a morphological change represented by a change in color as an outcome of the accumulation of green pigment, which indicates the inability of the kidneys to get rid of the pigment, which may be due to the increased injury of the kidneys and their inability to perform their function efficiently. The liver also shows histopathological changes represented by moderately thin fibrous connective tissue with inflammatory cells (arrow), congestion of the central vein, mild degenerated hepatocyte (arrowhead) with pyknotic nuclei and increased Kupfer cells. The liver section of the EPO group shows the nearly normal morphological image and reappearance of hepatocytes and sinusoids, mild congestion of the central vein and slightly increased Kupfer cells. The liver section of the EDO group shows an almost normal morphological image and structure and sinusoids with few inflammatory cell infiltration (arrow), and pyknotic nuclei.

Fig. 6 illustrates the morphological and histopathological changes of the kidney tissues. The light microscopic of renal tissues from the CN group shows normal morphological image and architecture of glomeruli and normal urinary space in between, with normal tubular structures. The green color, due to the accumulation of the fast green pigment is more visible in the kidney tissue. The kidney section of the FAC group shows shrunken glomeruli, dilated urinary space, and noticeable degenerative renal tubules (arrowhead). The green color does not appear in the kidney tissues of the EPO and the EDO groups, which suggests the ability of the parsley and dill oils nanocapsules to reverse the damage occurring in the kidneys and thus increase their efficiency in performing their function of eliminating colored substances. The kidney sections of the EPO and the EDO groups show normal histological architecture with normal glomerulus and mild dilated urinary space, intact renal tubules, but few renal tubules show mild degenerative (arrowhead).

4. Conclusion

In conclusion, the molecular docking results demonstrated that the primary constituents of parsley and dill EOs (apiol, myristicin, α-pinene, (−)-carvone, and d-limonene) interacted with the active sites of TNF-α, IL-1β and TGF-1β cytokines with hydrophobic and hydrogen bond interactions, which gives a prediction of inhibitory effect of parsley and dill EOs for the inflammatory cytokines. The excessive co-administration of fast green dye and sodium benzoate induced pronounced hazardous effects in the liver and the kidney as evidenced by disturbances in their functions, morphological and histological alterations, oxidative indicators, and elevated inflammatory cytokines. Treatment with fermented milk containing either parsley or dill EO nanocapsules reduced and alleviated the harmful impacts of fast green and sodium benzoate on the majority of the tested parameters, which may be imputed to the antioxidant and anti-inflammatory properties of parsley or dill EO nanocapsules.

Data availability statement

Data included in article are referenced in the article.

Ethical approval

All experiments were carried out according to the research protocols approved by the Medical Research Ethics Committee (MREC) at the National Research Centre, which is in accordance with the provisions of the relevant Egyptian laws and with Helsinki Declaration as well as the institutional Animal Care and Use Committee (IACUC) guidelines and recommendations and WHO rules regarding the ethics of scientific research. Ethical Approval Certificate No. 13050212–1.

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CRediT authorship contribution statement

Rasha S. Mohamed: Writing – review & editing, Writing – original draft, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Karem Fouda:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Amany S. Maghraby:** Writing – review & editing, Supervision, Investigation. **Fayza M. Assem:** Writing – review & editing, Supervision, Investigation. **Medhat M. Menshawy:** Writing – review & editing, Methodology. **Ahmed H. Zaghloul:** Writing – review & editing, Supervision, Investigation. **Ahmed M. Abdel-Salam:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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