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RESEARCH PAPER

Antioxidant, Antibacterial and Anti-Proliferative Activities of *Opuntia Ficus-Indica L.* Nano-Emulsion Against Breast Cancer: An *In Vivo* and *In Vitro* Study

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ABSTRACT

Breast cancer accounts for 30% of new cancers in women and is known as the most common malignant cancer in women in the world. The present study focused on the antioxidant, antibacterial and anti-proliferative activities of *Opuntia ficus-indica* L. nano-emulsion (NE-OFI) on MCF-7 cell line and determined effect of NE-OFI in an in vivo study in breast cancer animal model. Treatment with NE-OFI (230 µg/mL) significantly upregulated pro-apoptotic genes (*Bax*, *Caspase-3*) while downregulating *Bcl2* in MCF-7 cells. It also induced oxidative stress, increasing ROS and reducing antioxidant enzymes. Antibacterial assays showed MICs of 80 µg/mL (*E. coli*) and 160 µg/mL (*P. aeruginosa*). In vivo, NE-OFI (400 mg/kg) reduced tumor size and enhanced apoptosis in breast cancer models. Histopathological analysis confirmed increased tumor cell death. These findings suggest NE-OFI's potential as a natural therapeutic agent against breast cancer, combining antioxidant properties with antibacterial effects against Gram-positive and Gram-negative bacteria.

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INTRODUCTION

Breast cancer is the most common neoplastic malignancy in women worldwide and represents the leading cause of cancer-related deaths among women [1,2]. Although its global incidence continues to rise, prevalence rates vary significantly across different countries [3]. While developed nations report the highest frequency of breast cancer, studies indicate a sharp increase in prevalence in developing countries [4].

One of the primary drivers of cancer development is exposure to environmental factors, which can trigger mutations and genetic alterations, ultimately leading to malignancy [5]. Notably, research suggests that dietary choices play a crucial role in cancer prevention, as certain antioxidant-rich foods may help reduce cancer risk [6].The cactus (*Opuntia ficus-indica* L.) is widely used as both a food source and medicinal plant in the Americas, with at least 84 of the approximately 200 known Opuntia species found in Mexico [7,8]. The sweet edible fruit of this cactus, commonly called prickly pear (*Cactaceae* family) [9], has been traditionally used as a remedy [10] and contains

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an extensive array of bioactive compounds including sugars, ascorbic acid, betalains, fat-soluble pigments, polyphenolic compounds, and flavonoids [11]. Modern research demonstrates that cactus pear fruits serve as a rich source of phytonutrients with multiple health benefits, exhibiting antioxidant properties, neuroprotective and cardioprotective effects, anti-inflammatory activity, antidiabetic potential [12], as well as anti-chromosomal aberration [13] and anti-mutation effects [14]. Furthermore, the fruit demonstrates protective effects on red blood cell membranes [15] and against acute gastric lesions [16], while also improving platelet function [17] and showing cancer-preventive properties [8].Given the rising prevalence of breast cancer in Iraq, its increasing incidence over the past two decades, and the high mortality rate among patients—coupled with the accessibility, affordability, and widespread consumption of herbal products—this study aimed to evaluate the therapeutic potential of *Opuntia* spp. against breast cancer cells. The MCF-7 cell line, a well-established model for in vitro breast cancer research, was derived in 1970 from the breast tissue of a 69-year-old woman with breast cancer [18] and serves as an ideal system for such investigations.

This study focused on the antioxidant, antibacterial, and anti-proliferative properties of NE-OFI (nanoparticle-enhanced *Opuntia ficus-indica* extract) against the MCF-7 cell line, while also conducting a comparative analysis of its in vivo and in vitro efficacy in a breast cancer animal model.

MATERIALS AND METHODS

Herbal extraction protocol

Fresh *Opuntia ficus-indica* (OFI) fruits (500 g) were thoroughly washed with tap water to remove surface impurities. Following washing, the outer skin was partially removed, and the fruits were sliced into thin pieces. The samples were then dried at 60°C for 24 hours in a light-protected environment using a drying oven (Shimiazma, Iran). The dried material was ground into a fine powder, after which a hydroalcoholic extract was prepared by soaking 100 g of OFI powder in 500 mL of absolute ethanol for 72 hours at room temperature. The mixture was periodically agitated to enhance extraction efficiency. After 3 days, the extract was filtered through Whatman No. 1 filter paper to remove particulate matter, followed by concentration under reduced pressure using a rotary evaporator (40–50°C).

Preparation of Opunita Ficus-Indica extract Nanoemulsions

To prepare the nanoemulsion, 1% (w/w) herbal extract was combined with 1% (w/w) soybean oil and 40% (w/w) Tween 80 in deionized water. The aqueous phase was gradually added to the oil phase at a rate of 2 mL/min under homogenization (28 ± 2 °C) using a conventional ultrasonic device, maintaining a 45:55 (aqueous:oil) phase ratio. After complete addition, homogenization continued for an additional 15 minutes to ensure uniform dispersion and stable nanoemulsion formation.

Characterization of nanoparticles

Dynamic Light Scattering (DLS)

The particle size distribution of NE-OFI was characterized using dynamic light scattering (DLS) with a Horiba SZ-100 nanoparticle analyzer (Kyoto, Japan).

Field Emission Scanning Electron Microscopy (FE-SEM) analyses

The size and morphology of NE-OFI were characterized using FE-SEM (TESCAN, Brno, Czech Republic). Prior to analysis, NE-OFI was centrifuged at 10,000 rpm for 15 min at 25°C. The pellet was washed three times with ultrapure water and air-dried overnight. For FE-SEM imaging, the sample was deposited onto a carbon-coated copper grid and sputter-coated with platinum to enhance conductivity.

Determination of the main bioactive compounds of Opunita Ficus-Indica extract

Bioactive compounds in the OFI extract were identified after clarification (12,000 × g, 15 min, room temperature). Total flavonoid content was quantified using a colorimetric method [19].

DPPH Radical Scavenging Assay

A 0.0789 mM DPPH solution was prepared by dissolving 7.89 mg DPPH in 100 mL of 99.5% ethanol, followed by 2-hour incubation in the dark. For the assay, 800 µL Tris-HCl buffer (pH 7.4) was mixed with 1,000 µL DPPH solution, and 200 µL NE-OFI was added. The mixture was incubated at 25°C for 30 min, and absorbance was measured at 517 nm using a spectrophotometer. A blank control (800 µL Tris-HCl + 1,200 µL ethanol) was

used for baseline correction. Radical scavenging activity (%) was calculated as:

Inhibition ratio (%) = (A1 - A2) × 100/A1

where A1 shows the absorbance of blank sample and A2 shows the absorbance of NE-OFI solution.

MIC/MBC, Disk Diffusion

The antibacterial properties of NE-OFI were evaluated against *Escherichia coli* (*ATCC 25922*) and *Pseudomonas aeruginosa* (*ATCC 27853*) using disk diffusion and MIC/MBC assays. Bacterial suspensions were adjusted to 0.5 McFarland standard (10^8 CFU/mL) and diluted 1:100. Then, 15 mL of Mueller-Hinton agar and 2 mL of bacterial suspension were plated per petri dish. After 5 minutes of incubation, excess liquid was removed, and 6 mm sterile filter paper discs (Whatman No. 1, Oxoid) impregnated with 15 µL of NE-OFI were placed on the inoculated plates. The plates were refrigerated at 4°C for 2 hours to allow diffusion, followed by incubation at 37°C for 24 hours. The diameter of inhibition zones was measured in millimeters.

Preparation and proliferation of MCF-7 (Human breast cancer cell line)

The MCF-7 cell line (ATCC: HTB-22) was obtained from the Pasteur Institute of Iran Cell Bank. Cells were cultured in DMEM (Gibco™, Cat. No. 41965039) supplemented with 10% FBS (Gibco, Cat. No. 10082147, USA) and maintained in a humidified incubator at 37°C with 5% CO_2 until reaching 80-90% confluency. The medium was replaced every three days. For cell counting, trypsinized cells were centrifuged at 300 × g for 5 minutes, and the resulting pellet was resuspended in 1 mL of fresh DMEM. A 1:9 dilution was prepared by mixing 10 µL of cell suspension with 80 µL of DMEM. Subsequently, 10 µL of the diluted suspension was loaded into a hemocytometer, and viable cells were counted in four corner quadrants using a light microscope. Cell density (cells/mL) and viability were calculated based on these counts.

Cell Viability assay; MTT protocol

MTT powder (Sigma, UK) was mixed at a concentration of 5 mg/ml in PBS (Merck, Germany) as a stock solution. The cells were seeded in each well of a 96-well plate and treated with NE-OFI (50, 100, 200, 400, 800 µg/ml) in triplicate, with approximately 10,000 cells per well. After treatment, the plate was placed in an incubator at 37°C for 72 hours to assess the toxicity of the different concentrations. 100 µl of MTT working solution with 0.5 mg/ml concentration was added to each well. After 4 hours incubation; formazan crystals were produced. Tetrazolium salts were disolved in 100 µL of DMSO (Merck, Germany) and changed to purpale color. The optical density of the treated cells was assessed using a spectrophotometer at 570 nm wavelength.

Apoptotic assay by flow cytometry

For apoptosis analysis by flow cytometry, approximately 100,000 cells were aliquoted into a microtube and resuspended in 500 µL of 1× Binding Buffer from the apoptosis detection kit. An unstained cell sample served as the negative control for instrument calibration. The cell suspension was then stained with 5 µL of Annexin V-FITC (BD Biosciences, USA) and incubated at 4°C in the dark for 15 minutes. After incubation, 1 mL of 1× Binding Buffer was added to each tube, followed by centrifugation at 1,500 rpm for 5 minutes. The supernatant was carefully aspirated, and the cell pellet was resuspended in 500 µL of fresh 1× Binding Buffer. Immediately prior to analysis, 3 µL of propidium iodide (PI) was added to each sample. Flow cytometry was performed using a BD FACSCalibur flow cytometer, and data were acquired using the manufacturer's software. Data analysis was conducted using FlowJo software (version X.X, Tree Star) for graphical representation and Prism 5 (GraphPad Software) for statistical analysis.

Antioxidant enzymes assay and lipid peroxidation level analysis

Assessment of Lipid Peroxidation via Malondialdehyde (MDA) Quantification

Lipid peroxidation levels in NE-OFI-treated MCF-7 cells (IC50 dose) were quantified by measuring malondialdehyde (MDA) concentration using a ZellBio MDA assay kit (ZellBio GmbH, Germany). Briefly, 125 µL of cell homogenate was vigorously mixed with 50 µL of Tris-buffered saline (TBS) and 125 µL of trichloroacetic acid (TCA) containing 0.01% butylated hydroxytoluene (BHT) for protein precipitation. After centrifugation (1,000 × g, 10 min, 4°C), the supernatant was reacted with 40 µL of 0.6 M HCl and 160 µL of thiobarbituric acid (TBA) solution (prepared in Tris buffer) and heated

at 80°C for 10 min. MDA-TBA adduct formation was quantified spectrophotometrically at 532 nm. A standard curve was generated using five MDA concentrations (0–20 µM). MDA concentrations in samples were calculated using a molar extinction coefficient of 156 $mM^{-1}cm^{-1}$ and expressed as nmol MDA per mg protein.

Analysis of Antioxidant Enzyme Activities

The activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were analyzed using commercial assay kits (ZellBio GmbH, Germany). SOD activity was measured via the ZellBio SOD assay kit, while CAT activity was determined colorimetrically (ZellBio CAT assay kit) by monitoring H_2O_2 decomposition at 240 nm. GPx activity was quantified as µmol oxidized GSH/min/mg protein at 25°C using the ZellBio GPx assay kit. Total protein concentration in cell lysates was determined by the Lowry method [20] with bovine serum albumin (BSA) as the standard.

Gene expression analysis

Total RNA was isolated from NE-OFI-treated MCF-7 cells using TRIzol™ reagent (Thermo Fisher Scientific, MA, USA) and quantified using a NanoDrop spectrophotometer. RNA integrity was verified (A260/A280 ratio ≥1.8) prior to storage at −80°C. Complementary DNA (cDNA) was synthesized from 1 µg RNA using a reverse transcription kit (manufacturer's protocol). Quantitative real-time PCR (qPCR) was performed on an ABI StepOnePlus™ system with SYBR® Green Master Mix (Takara Bio, USA) to analyze expression of apoptosis-related genes (*Bax*, *Bcl2*, *Caspase3*) normalized to Gapdh (housekeeping gene). Gene-specific primers (Table 1), designed using GeneRunner and validated by NCBI BLAST, were employed. The thermal profile consisted of: (1) initial denaturation (95°C, 5 min); (2) 40 cycles of denaturation (95°C, 30 s), primer annealing (58°C, 30 s), and extension (72°C, 10 s). All reactions were performed in quintuplicate. Relative gene expression was calculated via the 2−ΔΔCt method using REST software.

Animal keeping conditions

All animal experiments were conducted under controlled environmental conditions (23 ± 3°C, 50 ± 10% humidity) with ad libitum access to food and water and a standardized 12-hour light/dark cycle. Twelve male BALB/c mice (20 ± 2 g body weight) obtained from the Pasteur Institute Laboratory Animal Center were randomly divided into six groups: (1) tumor model group receiving a subcutaneous injection of 5×10^5 4T1 breast cancer cells in 50 µL PBS; (2) NE-OFI low-dose treatment group (200 mg/kg/day, oral gavage, 4 weeks); (3) NE-OFI high-dose treatment group (400 mg/kg/day, oral gavage, 4 weeks); and (4) positive control group receiving doxorubicin (1.5 mg/kg, intraperitoneal injection). Animals was cared for in accordance with the University of Babylon-College Science for Woman code of practice for the care and use of animals for scientific purpose (Approval No: IR-UU-AEC-1401-001).

Sample collection

Four weeks following NE-OFI treatment, mice were humanely euthanized by cervical dislocation. Tumor tissues were promptly excised, with portions fixed in 10% neutral buffered formaldehyde for histopathological analysis and remaining aliquots flash-frozen at -80°C for molecular studies.

Histopathological examination

Fixed tumor tissues underwent standard dehydration through a graded ethanol series (70%, 80%, 90%, and 100%) with 50-minute immersions at each concentration. Tissues were then cleared

Table 1. Primer sequence for qPCR assessment

Gene name	Primer sequence
h-GAPDH-F	CTTTGGTATCGTGGAAGGAC
h-GAPDH-R	GCAGGGATGATGTTCTGG
h-BAX-F	CGCCCTTTTCTACTTTGACA
h-BAX-R	GTGACGAGGCTTGAGGAG
h-BCL2-F	TGGTCTTCTTTGAGTTCCGG
h-BCL2-R	GGCTGTACAGTTCCACAA
h-Caspase3-F	GGAAGCGAATCAATGGACTCTGG
h-Caspase3-R	GCATCGACATCTGTACCAGACC

in xylene (Merck, Cat. #108298) through two changes (50 minutes each) and embedded in paraffin using two successive baths (50 minutes each). Following processing, 5 µm sections were cut using a rotary microtome, mounted on glass slides, and prepared for hematoxylin and eosin (H&E) staining and subsequent microscopic evaluation.

Statistical analysis

This randomized controlled trial was conducted with three independent experimental replicates. All statistical analyses were performed using GraphPad Prism (version 8.4.3). Normality of data distribution was verified using the Shapiro-Wilk test. One-way ANOVA followed by LSD post hoc testing was employed for group comparisons, with P-values < 0.05 considered statistically significant.

RESULTS AND DISCUSSION

Phytochemical contents analysis of OFI fruit extract

The results of the phytochemical analysis of the hydroalcoholic extract of OFI fruit confirmed the presence of various bioactive compounds, including phenolic compounds, sugar derivatives, flavonoids, proteins, fiber, and fats (Fig. 1). As shown in Fig. 1, the extract contained several antioxidant compounds, such as quercetin, gallic acid, and vanillic acid. Additionally, other phenolic acids, including p-coumaric acid, caffeic acid, and cinnamic acid, were identified as components of the extract. Benzoic acid and ferulic acid were also detected during the phytochemical analysis of OFI. The remaining constituents of the OFI extract included chlorogenic acid and m-coumaric acid. Trace amounts of sinapic acid and syringic acid were also identified in the extract.

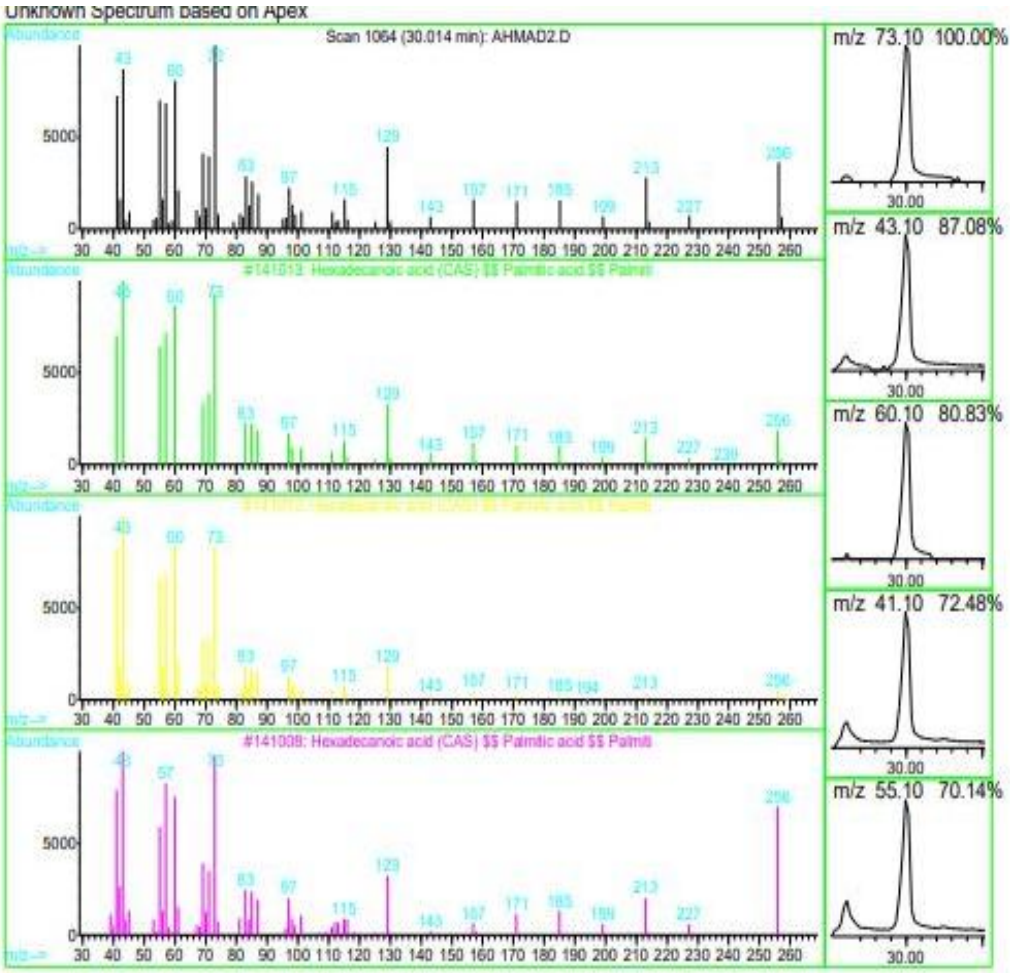

Fig. 1. OFI fruit extract chromatographic analysis

DPPH assay

The antioxidant potential of the OFI fruit extract was assessed using the DPPH radical scavenging assay (Fig. 2). The results demonstrated that the extract exhibited significant antioxidant activity, with an inhibition rate of 92.68 ± 1.62% in vitro. This finding aligns closely with the observations of Roghelia and Panchal [21], who reported comparable free radical scavenging activity in OFI extracts.

Characteristics of Ginger Nano-emulsion

The characterization results of the optimized nano-emulsion formulation (NE-OFI) are presented in Fig. 3. All NE-OFI formulations met the required organoleptic standards for nano-emulsions, including color, odor, clarity, and viscosity. Furthermore, the particle size of all NE-OFI formulations fell within the specified range of 90–100 nm, confirming the formation of a nano-emulsion with uniform particle size and a narrow size distribution (Fig. 3).

Antimicrobial effect of NE-OFI on Escherichia coli

As illustrated in Fig. 4 and Fig. 5, the minimum inhibitory concentration (MIC) of NE-OFI against

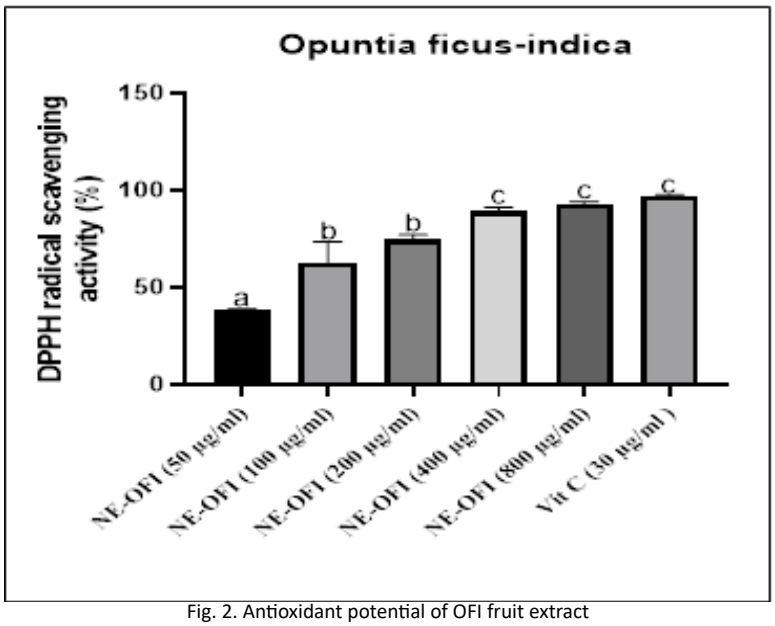

Fig. 2. Antioxidant potential of OFI fruit extract

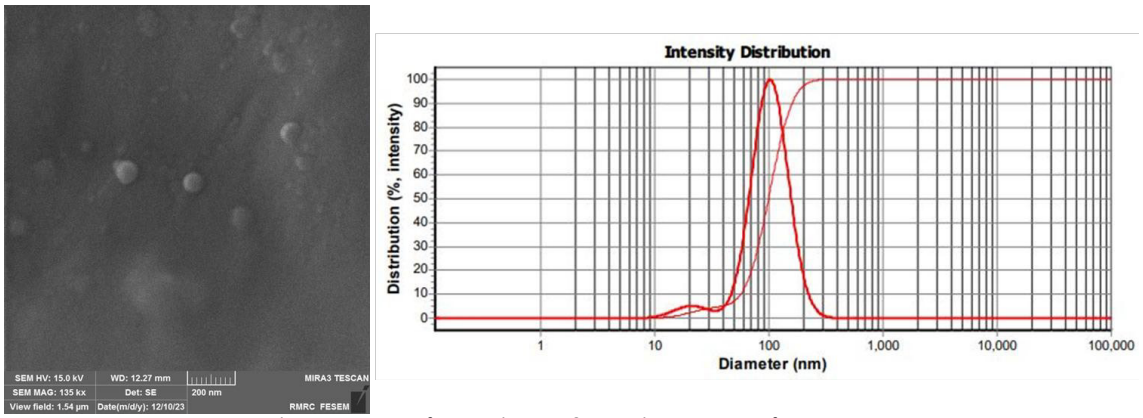

Fig. 3. Characterization of *NE-OFI* by SEM & DLS. The mean size of *NE-OFI* is 100 nm

E. coli and *S. aureus* was determined to be 80 µg/mL and 160 µg/mL, respectively. The largest zone of inhibition was observed for *E. coli,* while the smallest inhibition zone corresponded to *the S. aureus strain* (Table 2).

Anticancer effect of NE-OFI on MCF7

Evaluation of cell viability using the MTT assay revealed that NE-OFI exhibited a dose- and time-dependent effect on MCF-7 cell proliferation. As demonstrated in Fig. 6, lower concentrations

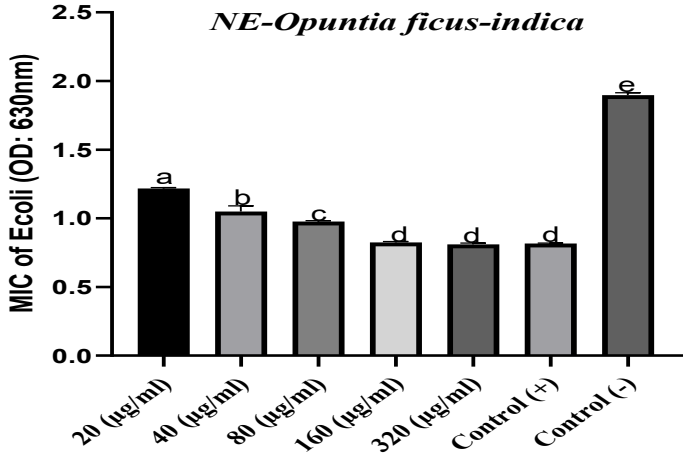

Fig. 4. Minimal inhibitory Concentrations of *NE-OFI on E.coli was* expressed in (80 µg mL^{-1}). The different signs show significant difference with other concentration

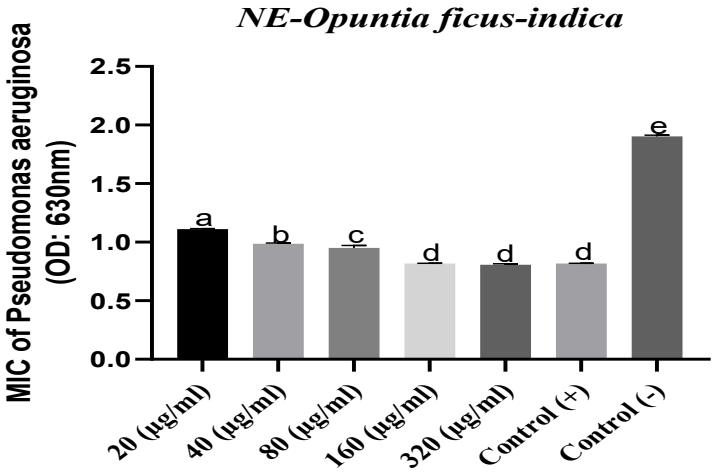

Fig. 5. Minimal inhibitory Concentrations of *NE-OFI on S. aureus* sp. *was* expressed in (160 µg mL^{-1}). The different signs show significant difference with other concentration

Table 2. Inhibition zones obtained with MIC concentration of *NE-OFI* on *E.coli & S. aureus* sp

BACTERIA	*NE-OFI*
Escherichia coli	4 mm
Pseudomonas aeruginosa	2 mm

(50–100 µg/mL) had minimal impact on cell survival, whereas higher doses (200–800 µg/mL) significantly reduced viability, inducing cell death. The half-maximal inhibitory concentration (IC50) was determined to be 230 µg/mL after 72 hours of treatment.

Effect of NE-OFI on apoptosis in MCF-7 cell

Flow cytometric analysis using the Annexin V-FITC/propidium iodide (PI) assay revealed a significant alteration in the cellular profile of MCF-7 cells following treatment with 230 µg/mL NE-OFI for 72 hours (Fig. 7). Both NE-OFI and doxorubicin (Dox) at their IC50 concentrations induced a statistically significant increase ($P < 0.05$) in total apoptosis compared to the control group. Notably, treatment with the IC50 concentration of NE-OFI resulted in 68% of cells undergoing apoptosis (Fig. 7).

Determination of antioxidant activity and lipid peroxidation of NE-OFI

Fig. 8 presents the comparative levels of antioxidant enzymes (CAT, SOD, and GPx) among the control group and cell groups treated with NE-OFI (230 µg/mL) and doxorubicin (5 µM). The untreated control group demonstrated significantly higher ($p \leq 0.05$) CAT, SOD, and GPx activities compared to treated groups (Fig. 8A-C). Conversely, lipid peroxidation analysis revealed

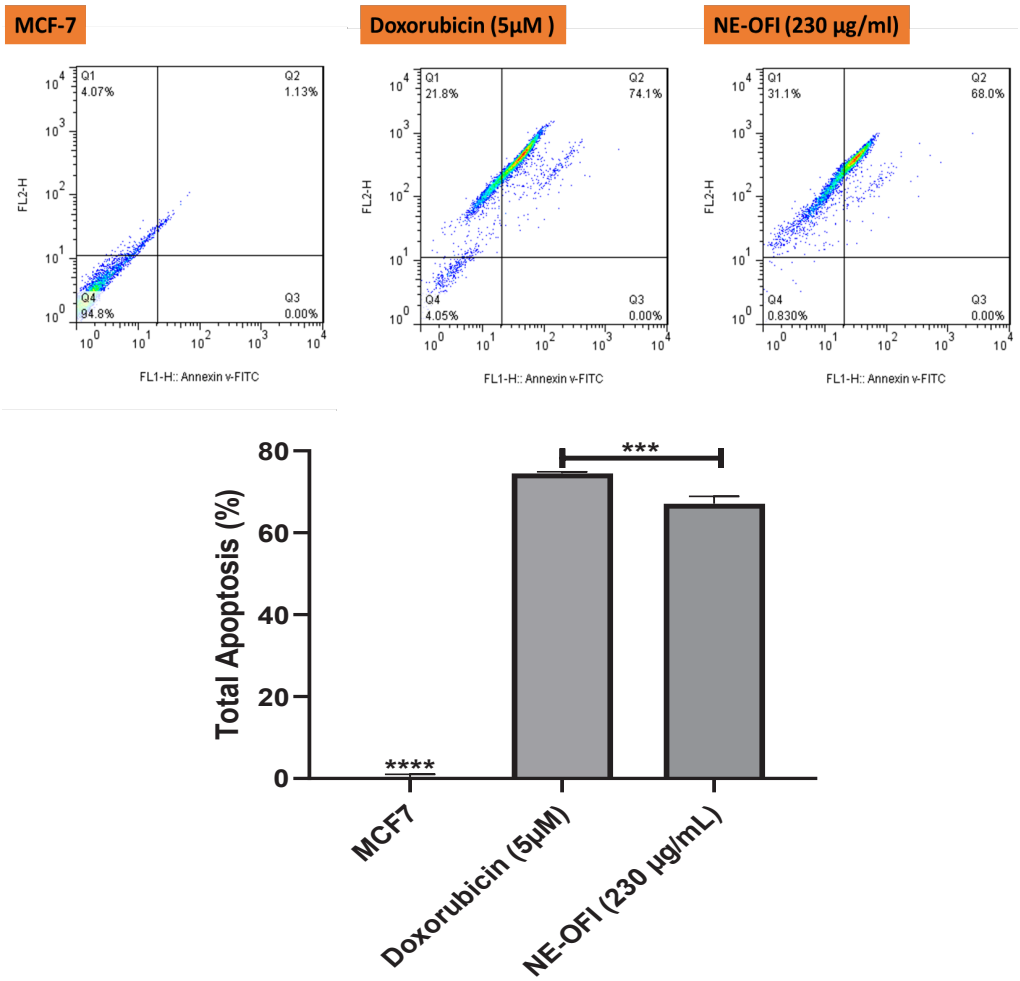

Fig. 7. *NE-OFI* Induced apoptosis in MCF-7 Cells. (A) flow cytometry profile of MCF-7 cells treated with *NE-OFI & DOX* (IC50 concentration) for 72 h and stained with annexin V/propidium iodide. (B) Total apoptosis analysis included late and early apoptosis in the treated MCF7 cell line. ****: significant difference of Control group with Dox and NE-OFI cell treatment ($p \leq 0.0001$); ***: significant difference of Dox and NE-OFI cell treatment with each other ($p \leq 0.001$)

a significant increase ($p \leq 0.05$) in MDA levels in MCF-7 cells following 72-hour treatment with either NE-OFI (230 µg/mL) or doxorubicin (5 µM) (Fig. 8D).

Bax, Caspase3 and Bcl2 mRNA expression in NE-OFI treated MCF7 cell lines

Quantitative PCR analysis demonstrated that NE-OFI treatment significantly modulated apoptosis-related gene expression in MCF-7 cells, with a 5.56 ± 0.74-fold upregulation ($p < 0.05$) of pro-apoptotic *Bax* mRNA and a 2.34 ± 0.06-fold increase ($p < 0.05$) in *Caspase 3* mRNA compared to untreated controls, while simultaneously causing significant downregulation of anti-apoptotic Bcl-2 expression (Fig. 9A-C), collectively indicating NE-

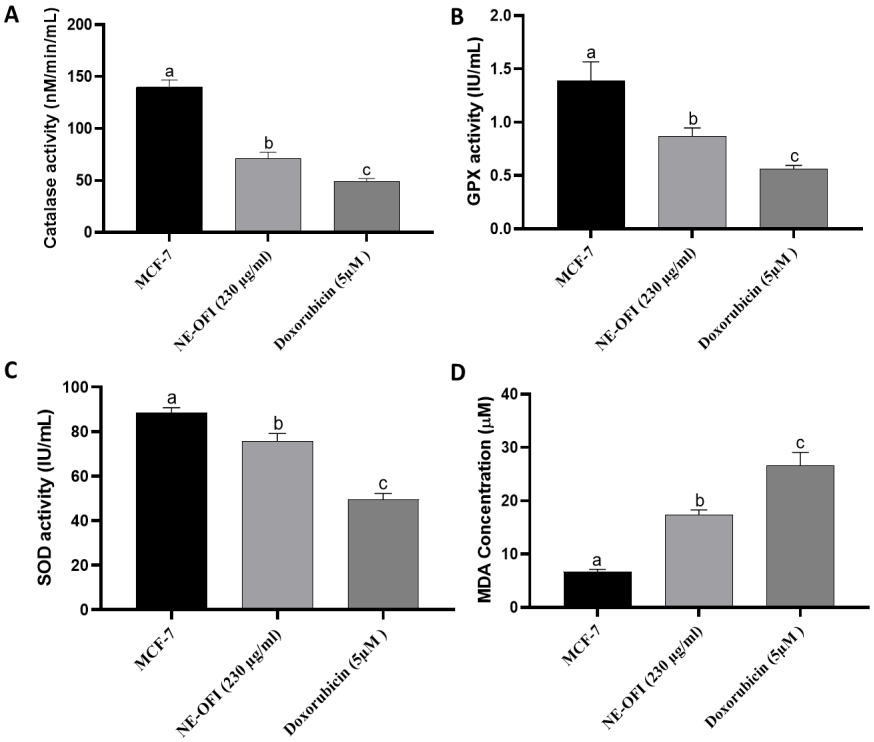

Fig. 8. Results of analysis of enzymatic antioxidant levels in different experimental groups of human breast cancer cell line MCF7 and comparison between groups (A-C). MDA concentration after MCF7 treatment with NE-OFI and Dox in IC50 doses. The different signs show significant difference with other concentration

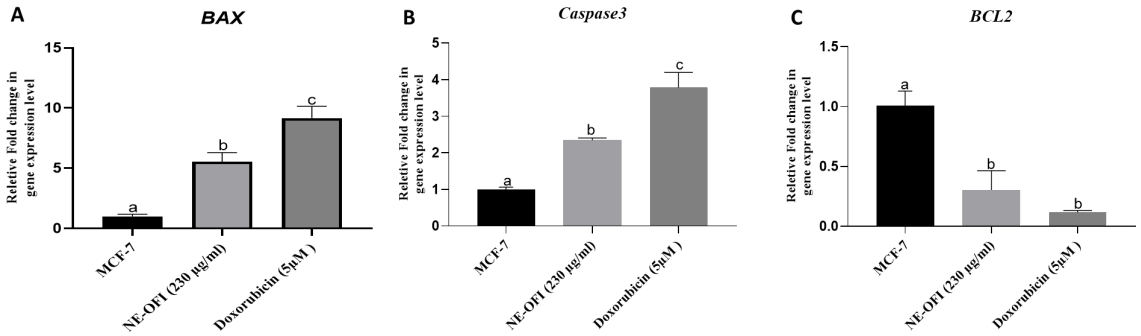

Fig. 9. Levels of apoptotic and proapoptotic gene expression ratio to untreated MCF7 cell in different experimental groups (A-C). Apoptotic gene expression elevated after MCF7 treatment with NE-OFI and Dox in IC50 doses (A & B). The different signs show significant difference with other concentration

OFI's potent activation of apoptotic pathways.

Tumor growth inhibition after 28 days treatment with NE-OFI treatment

Longitudinal monitoring of tumor dimensions at 7-day intervals revealed that both NE-OFI (400 mg/kg) and doxorubicin (DOX) monotherapy induced maximal tumor growth inhibition (TGI) of 33%, while combination treatment demonstrated significantly enhanced efficacy (40% TGI, $p < 0.05$ versus control) (Figs. 10-11).

Effect of NE-OFI on tumor tissue cell death by histopathological assessment

Histopathological analysis revealed that tumors from mice treated with NE-OFI (400 mg/kg)

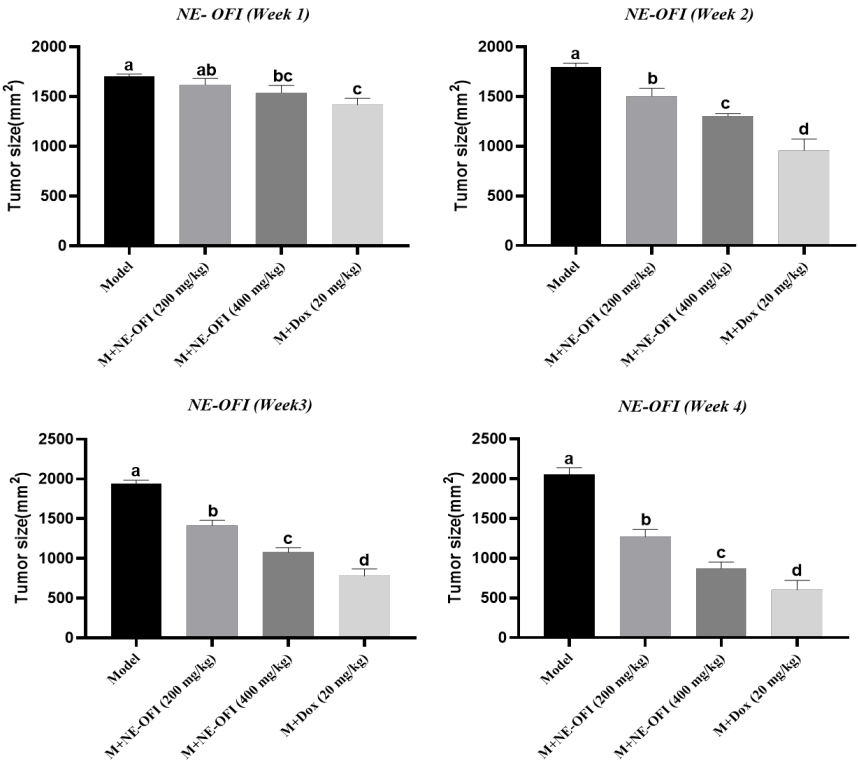

Fig. 10. Tumor growth in Balb/c mice. 7, 14, 21 and 28 days after tumor induction in different experimental groups. Tumor area [mm²] was analyzed in different experimental groups. Data presented in the control and treated group with *NE-OFI* and *DOX*. The different signs show significant difference with other concentration

Fig. 11. Tumor growth in Balb/c mice. 28th days after treatment of animal with *NE-OFI*. The tumor growth inhibited after 4 weeks of treatment with 200 & 400 mg/kg of *NE-OFI*

showed reduced inflammatory cell infiltration and decreased necrotic areas compared to the control group, which exhibited increased infiltration of granulocytes (neutrophils) (Figs. 12-13). Additionally, the destructive nuclear fragmentation process, known as karyorrhexis—a hallmark of late apoptosis, senescence, or cell death—was observed following NE-OFI (400 mg/kg) and DOX treatment. Microscopic examination also revealed the presence of vacuoles and nuclear pigments, as well as altered mitotic properties, in the NE-OFI-treated group. Furthermore, both NE-OFI and DOX significantly reduced cell proliferation potential relative to other groups (Figs. 12-13).

Breast cancer remains one of the most prevalent malignancies affecting women worldwide [22]. Given the established link between tumor progression, inflammation, and oxidative stress, compounds with anti-inflammatory or antioxidant properties may exhibit anticarcinogenic effects through pharmacological and biochemical mechanisms. These compounds—particularly those with antioxidant and anti-inflammatory activities—appear to play a role in anticarcinogenic and anti-mutagenic processes [23]. Numerous studies have demonstrated that bioactive

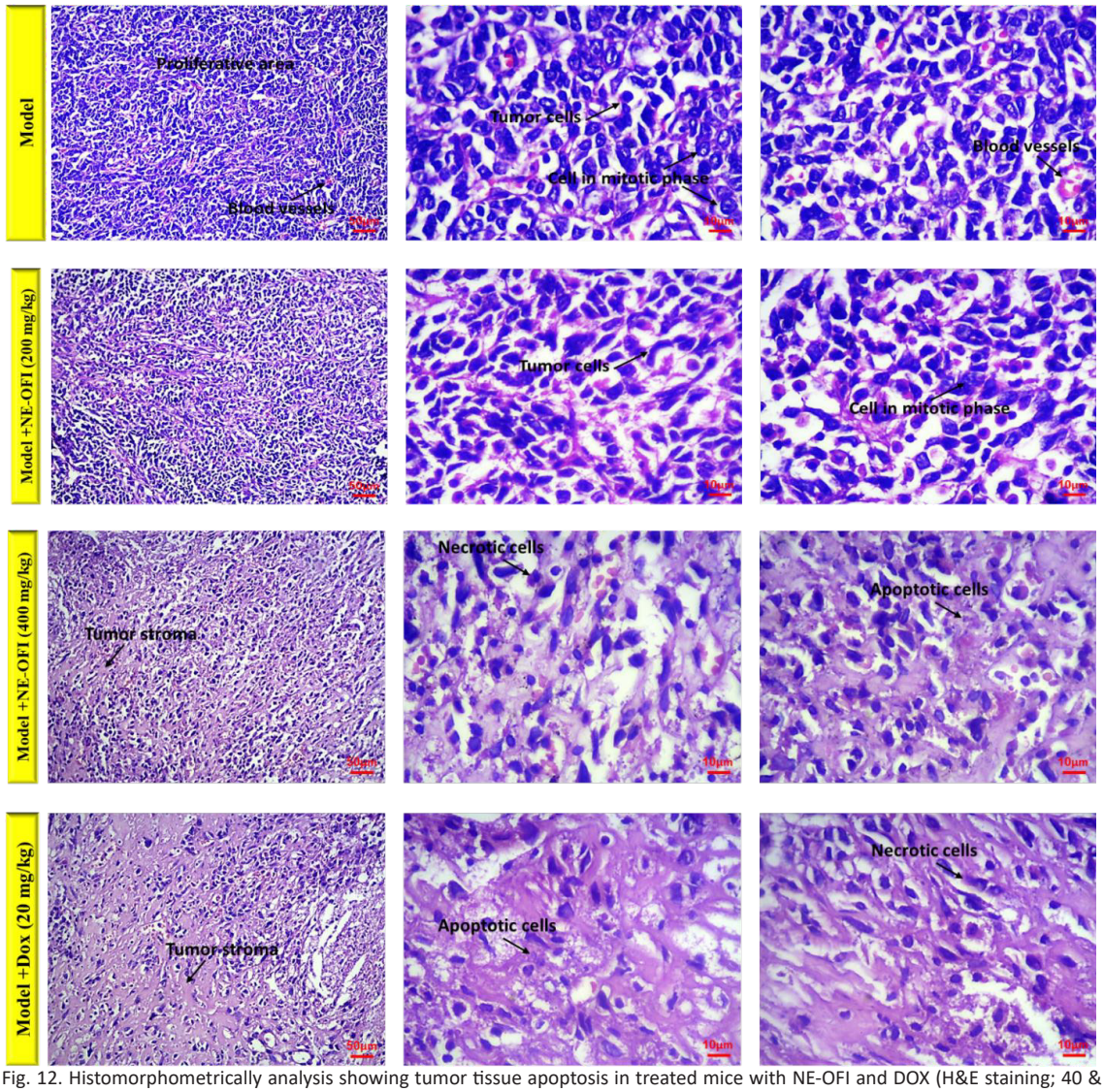

Fig. 12. Histomorphometrically analysis showing tumor tissue apoptosis in treated mice with NE-OFI and DOX (H&E staining; 40 & 400X); negative untreated group (Model); positive control group (Model + Dox); Treatment I group (Model + NE-OFI 200 mg/kg); Treatment II group (Model + NE-OFI 400 mg/kg)

components derived from OFI extract can inhibit cancer cell proliferation and induce apoptosis [24].

Research on various parts of *Opuntia*—including prickly pear fruits, seeds, peels, stems, cladodes, and roots—has shown that these plant components promote cell death in malignant cell lines [16]. For instance, Antunes-Ricardo et al. (2014) demonstrated the cytotoxic effects of purified isorhamnetin glycosides and cladode flour extracts from *O. ficus-indica* (var. Jalpa), which induced apoptosis in human colon cancer cells (Caco-2 and HT-29). Notably, these extracts exhibited selective anti-apoptotic effects against drug-resistant cancer cell lines while showing no toxicity toward normal fibroblast cells (NIH 3T3).These researchers demonstrated that the glycosylation pattern of purified isorhamnetin glycosides and cladode flour extract exhibited greater cytotoxic effects on Caco-2 and HT-29 cells compared to normal controls. The induction of apoptosis via Bax and *Caspase-3* gene activation plays a critical role in the cytotoxic effects of O. *ficus-indica* on cancer cells [25] examined the impact of *O. ficus-indica* fruit aqueous extract on the proliferation of Caco-2 human colon cancer cells, revealing its epigenomic effect on the $p16^{INK4A}$ tumor suppressor through promoter demethylation and subsequent $p16^{INK4A}$ upregulation. Similarly, Sreekanth et al. [26] reported that betanin from *O. ficus-indica* fruits suppresses K562 cell proliferation by activating the intrinsic apoptotic pathway.

This study represents the first in vitro and in vivo evaluation of NE-OFI for anticancer activity. MCF-7 cells treated with NE-OFI for 72 hours exhibited dose-dependent anti-proliferative effects, with the highest phenolic and kaempferol content correlating with maximal efficacy. Notably, organic acids derived from *O. ficus-indica*—including malic, quinic, and aconitic acids—are predominantly concentrated in peels and cladodes. Further research is needed to compare phenolic levels across flowers and seeds.

Quinic acid ($C_7H_{12}O_6$), a cyclohexanecarboxylic

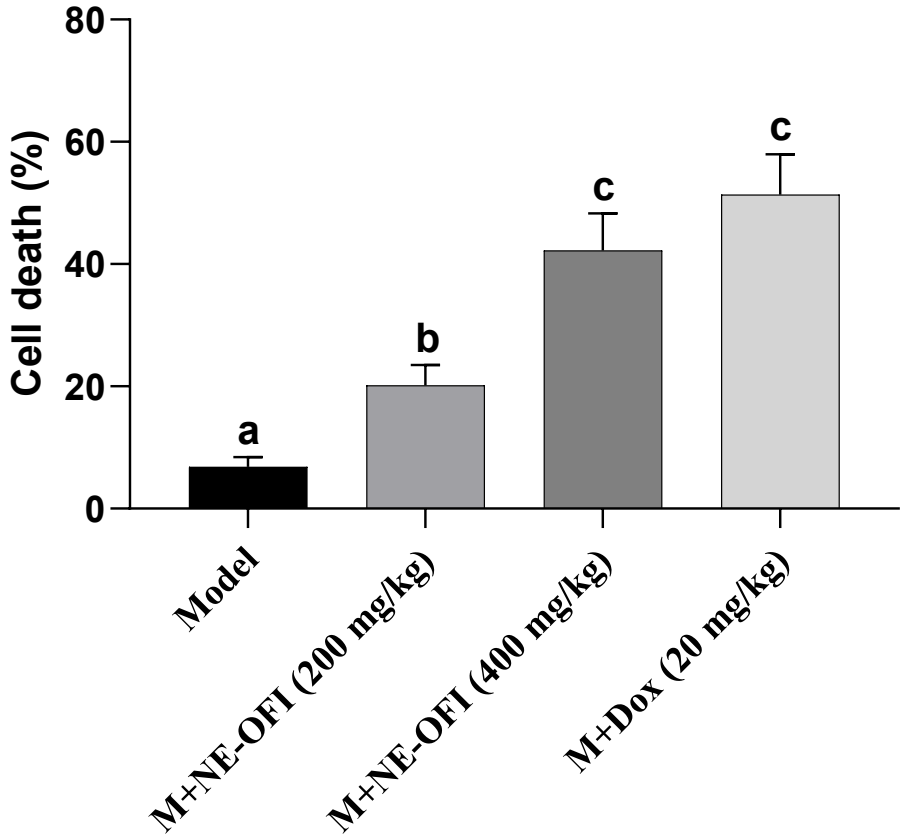

Fig. 13. Cell death percent of tumor tissue 4 weeks after treatment with NE-OFI 200 & 400 mg/kg. The different signs show significant difference with other concentration

acid derivative, is a key metabolite in the biosynthesis of aromatic compounds (e.g., flavonoids, phenolic acids) in plants and mammals [27]. Beyond antibacterial effects, D-(-)-quinic acid exhibits antioxidant, immunomodulatory, and cytotoxic properties [28]. Phenolic compounds have recently gained significant attention as potent anticancer agents due to their ability to modulate apoptosis, suppress cell proliferation, and influence critical carcinogenesis processes such as angiogenesis, differentiation, and cell invasion [29]. These metabolites include hydroxybenzoic acids (derived from benzoic acid) and hydroxycinnamic acids (characterized by aromatic ring substitutions), both of which are subclasses of phenolic acids [30]. Among these, hydroxycinnamic acid derivatives—such as cinnamic acid and p-coumaric acid—are notable polyphenolic constituents. OFI also contains simple esters of quinic acid or glucose, with cinnamic, chlorogenic, coumaric, and ferulic acids being the most abundant hydroxycinnamic acids. Minor constituents include caffeic, sinapic, and dimethoxycinnamic acids [27].

Nanomedicines, defined as nanoscale drug carriers, enable targeted delivery of therapeutic agents to specific tissues while facilitating controlled release over extended periods [31]. These systems enhance drug solubility and bioavailability. Nanoemulsions, thermodynamically stable and homogeneous, consist of aqueous phases, surfactants, and oil phases [32]. Typically, 20–200 nm in size, they are classified as oil-in-water (o/w) or water-in-oil (w/o) systems, with particle size dependent on synthesis and homogenization methods.

The present study demonstrated that NE-OFI exhibits antibacterial activity against E. coli and Pseudomonas aeruginosa, along with antiproliferative and pro-apoptotic effects on MCF-7 breast cancer cells. Notably, NE-OFI showed comparable efficacy to Dox in inhibiting tumor growth after 28 days of oral administration. These effects may be attributed to bioactive phenolics in OFI, which are increasingly recognized for their anticancer potential. Future studies should evaluate NE-OFI against other cancer cell lines and in vivo models to further assess its tumor growth inhibition potential.

CONCLUSION

This pioneering study demonstrates the anticancer potential of NE-OFI through both in vitro and in vivo investigations. At 800 µg/mL, NE-OFI exhibited 92.68 ± 1.62% antioxidant activity, while its minimum inhibitory concentrations were determined to be 80 µg/mL for E. coli and 160 µg/mL for *Pseudomonas aeruginosa*. Treatment with 230 µg/mL NE-OFI significantly upregulated pro-apoptotic genes (Bax and *Caspase-3*) while downregulating *BCL-2* expression in MCF-7 cells compared to untreated controls. Furthermore, this concentration induced oxidative stress characterized by increased reactive oxygen species and decreased antioxidant enzymes. Most notably, NE-OFI promoted significant apoptotic cell death at both 230 µg/mL (in vitro) and 400 mg/kg (in vivo), confirming its dose-dependent antitumor efficacy against breast cancer cells.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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