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Original Article

Pomegranate seed oil combined with low-frequency electromagnetic field enhances apoptosis in HT29 colon cancer cells via modulating caspase-3, caspase-9, Bax, and Bcl-2 expression

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Abstract

Colon cancer is one of the most prevalent cancers globally, characterized by the abnormal growth of cells in the intestines. Numerous studies have explored the effects of pomegranate-derived products, such as pomegranate seed oil (PSO), as anti-proliferative, anti-invasive, and pro-apoptotic agents against various cancer cell lines. Additionally, previous research has highlighted the anti-cancer properties of low-frequency electromagnetic fields (LF-EMF). In the present study, we investigated the combined effects of these two factors on the expression of the Caspase 3, Caspase 9, BAX, and Bcl2 genes. Human colon cancer cells of the HT29 line were sourced from the Pasteur Institute of Iran cell bank and maintained in a complete culture medium. The cells were categorized into four groups: control, PSO, EMF, and PSO+EMF. To assess cell viability and the type of cell death, MTT and Annexin V-FITC assays were employed. Changes in the expression levels of Caspase 3, Caspase 9, BAX, and Bcl2 were analyzed using Real-Time PCR. The results from the MTT and Annexin V-FITC assays indicated that both PSO and EMF reduced cell viability and promoted apoptosis in colon cancer cells. The Real-Time PCR results showed an upregulation of Caspase 3, Caspase 9, and BAX genes, along with a downregulation of Bcl2 expression in the treatment groups compared to the control group. This study demonstrated that the combination of PSO and EMF enhances apoptotic gene expression, thereby diminishing the proliferation and viability of cancer cells. Based on these findings, both PSO and LF-EMF exhibit cytotoxic effects on colon cancer cells, suggesting their potential as candidates for future research in the field of colon cancer.

Keywords: Colon cancer, Pomegranate seed oil, Low-frequency electromagnetic fields, Apoptotic genes

1. Introduction

Cancer is a multidimensional disease distinguished by the uncontrolled and abnormal multiplication of cells, with the potential to spread to other regions of the body via the bloodstream or lymphatic system[1, 2]. This syndrome typically results from disturbances in the cell cycle and defects in apoptosis, often caused by the activation of proto-oncogenes and the inactivation of tumor suppressor genes[3]. These molecular alterations contribute to uncontrolled cell proliferation and are considered key events in cancer initiation and progression[4]. Colon cancer especially entails the malignant proliferation of cells in the colon or rectum, which are segments of the large intestine. This condition arises from the atypical proliferation of cells capable of invading neighboring tissues and metastasizing[5]. While advancements have been made in the field of chemotherapy, there are still notable limitations, such as effective drug delivery to tumor sites, managing toxicity levels, resistance from patients to certain therapies, and collateral damage to healthy cells alongside cancerous ones. Therefore, it is crucial to develop innovative treatment strategies that are effective, biocompatible, and cost-effective in combating cancer[6]. Currently, numerous therapeutic strategies are directed towards inducing programmed cell death, known as apoptosis, in cancer cells[7]. Various molecules are involved in apoptosis pathways, with caspases being among the most recognized [8]. Caspase 3 serves a crucial function as a signaling molecule in various cell death pathways and is regarded as the ultimate executor of apoptosis in cancer cells[3]. Clinical studies have demonstrated that compounds derived from pomegranate can inhibit the growth of cancer cells in the skin, breast, and prostate. Additionally, several laboratory studies have highlighted the therapeutic effects of pomegranate extract on different cancer cell lines, including breast cancer (MBMDA and MCF-7), oral cancer (CAL-27 and KB),

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colon cancer (HT-29), and lung cancer. One notable compound extracted from pomegranate seeds is pomegranate seed oil (PSO), which constitutes 12-20% of the seed's weight. This oil is primarily composed of approximately 80% conjugated fatty acid punicic acid[9]. Numerous past studies have explored the anti-cancer properties of PSO, revealing its significant impact on the expression of Bcl2, BAX, and caspases in cancer cells[10, 11]. Electromagnetic fields can profoundly influence numerous growth and developmental processes, impacting cells, tissues, and various biological functions of animals[12]. These fields, which consist of electric fields generated by different voltage levels and their associated magnetic fields, are collectively known as electromagnetic fields[13]. Research has indicated that exposure to electromagnetic fields may lead to considerable alterations in membrane potential and cellular responses, primarily by impacting the cell membrane and altering the activity of various ion channels, including calcium channels. Additionally, electromagnetic fields can influence gene expression by affecting transcription factors or modifying other proteins and enzymes that are critical within the transcription process[14]. The alteration of the apoptotic process is notably noteworthy among the cellular mechanisms potentially associated with cancer, given its crucial function in preserving cellular homeostasis[15]. Prior studies have shown that low-frequency electromagnetic fields can decrease the viability of cancer cells and promote apoptosis [16, 17]. This study aims to investigate the effects of pomegranate seed oil (PSO) and low-frequency electromagnetic fields (LF-EMF) on the expression levels of apoptotic marker genes (caspase 3, caspase 9, Bax, Bcl2) in HT29 cancer cells.

2. Materials and Methods

2.1. Cell Culture

The HT29 cell line (human colon cancer cells) was obtained from the Animal Developmental Applied Biology Research Center of Iran. The cells were cultured in RPMI 1640 medium (Bio-Idea, Iran) supplemented with 10% Fetal Bovine Serum (FBS) (Gibco, USA) and 1% penicillin-streptomycin (Gibco, USA). The cell suspension was then transferred into cell culture flasks. The flasks containing the cells were placed in an incubator with the following conditions: 5% CO2, 95% humidity, and a temperature of 37°C.

2.2. MTT Assay

To determine cell viability, the MTT assay was employed. Initially, 3×10^3 HT29 cells were seeded into a 96-well plate. After allowing sufficient time for cell adhesion, the cells were treated with various concentrations of PSO and exposed to EMF at an intensity of 2 mT. The concentrations used for PSO were 0.25, 0.75, 1, 1.5, 2, 4, 6, 10, 20, 40, and 60 µg/mL. Untreated cells served as the control group, while cells exposed to the EMF with the field turned off were used as the sham group. After 24 hours of treatment, the culture medium was replaced with a fresh medium containing MTT solution at 1 mg/mL. The cells were incubated for 3 to 4 hours under 5% CO2 and 37°C. Following incubation, the medium was carefully removed, and dimethyl sulfoxide (DMSO) was added to dissolve the formazan crystals formed. The plate was incubated for an additional 10 minutes, and the absorbance was measured at 570 nm using a spectrophotometer (Epoch, USA).

2.3. Treatment Groups

In the control group, HT29 cells were untreated. In the PSO group, cells were treated with 10 µg/ml of PSO (IC50 obtained from the MTT assay). In the EMF group, cells were exposed to LF-EMF with an intensity of 2 mT. In the PSO+EMF group, cells were simultaneously treated with LF-EMF and 6 µg/ml of PSO.

2.4. Low-Frequency Electromagnetic Field(LF-EMF)

The Animal Developmental Applied Biology Research Center utilized an electromagnetic field generator system to produce LF-EMF for experiments. This system is capable of generating an electromagnetic field with a low frequency of 50 Hz and an intensity range between 1 and 60 millitesla (10 to 600 Gauss). In summary, the electromagnetic system comprises a power supply with a voltage range of 0 to 300 volts, a capacitor, an ammeter, and an incubation system. The magnetic intensity was calculated and adjusted by varying the electric voltage according to the equation B=µn, where BB represents the electromagnetic field intensity in Tesla, µ is $4\pi\times10^{-7}$, n, nn is the number of turns per unit length, and II is the current intensity. To ensure the accuracy of the electromagnetic field intensity, a teslameter was used for monitoring and calibration. Throughout the experiment, the temperature was maintained at 37 ± 0.3°C degrees Celsius[18].

2.5. Annexin V-FITC Assay

To observe the effect of PSO and LF-EMF on apoptosis in HT29 cancer cells, the Annexin V-FITC/PI apoptosis detection kit (Abcam, UK) was used (47). According to the manufacturer's instructions, after culturing and treating the cells, they were collected by centrifugation at 3000 g and resuspended in a binding buffer. Subsequently, 2 µL of Annexin V-FITC and 1 µL of PI were added for flow cytometry analysis.

2.6. Analysis of Gene Expression Changes

The Real-Time PCR technique was used to examine the changes in the expression of Caspase 3, Caspase 9, Bax, and Bcl2 genes. RNA extraction from cells treated with PSO and LF-EMF was performed under DNase- and RNase-free conditions using the Pars Tous kit, according to the manufacturer's instructions. The concentration of extracted RNA was quantitatively measured. cDNA synthesis was carried out by reverse transcription of RNA using the Pars Tous kit. Additionally, a pair of primers for each gene under study was designed and synthesized. The sequences of the primers used are listed in Table 1. Gene expression changes were analyzed using the Real-Time PCR technique with the Bio-Rad CFX96 RT-PCR system and the Bio-Rad CFX Manager software. The expression levels of all target genes were normalized to the housekeeping gene GAPDH.

2.7. Statistical Analysis

In all experiments, differences between means were analyzed using one-way analysis of variance (One-way ANOVA). Statistical analysis was performed using Prism version 8 software, with the one-way ANOVA test applied to compare data, and significance was considered at $P<0.001$. All graphs were plotted using Excel software. Each test was repeated three times.

Table 1. Primer Sequences of Genes Used for qRT-PCR (GAPDH was used as the housekeeping gene).

Gene	Sens	Antisense
GAPDH	5′ TGACTTCAACAGCGACACC 3′	5′ TTG CTG TAG CCA AAT TCG TT 3′
Caspase 3	5′ AGACAGACAGTGGTGTTGATG 3′	5′ GTTCATCCAGTCGCTTTGTGC 3′
Caspase 9	5′ CCAGAGATTCGCAAACCAGAG 3′	5′ CAATGTGAACTTCTGCCGTGA 3′
Bax	5′TTTGCTTCAGGGTTTCATCCA 3′	5′CTCCATGTTACTGTCCAGTTCGT 3′
Bcl2	5′TGTGTGGAGAGCGTCAACCG 3′	5′GGATAACGGAGGCTTGGGATGC 3′

3. Results

3.1. MTT Assay

The MTT assay was conducted to evaluate the viability of HT29 cells treated with PSO and EMF. Cells were treated with various concentrations of PSO (0.25, 0.75, 1, 1.5, 2, 4, 6, 10, 20, 40, and 60 µg/ml). After 24 hours, a significant decrease in cell viability was observed in the groups treated with different concentrations of PSO compared to the control group ($P < 0.001$). Additionally, the 10 µg/ml concentration was considered the IC50 (Figure 1A). Cell viability was also assessed in groups treated with LF-EMF and the field in the off-state (sham control). The results showed a significant decrease in cell viability in the EMF group compared to the control group ($P < 0.001$) (Figure 1B). Furthermore, statistical analysis revealed a significant decrease in the viability of HT29 cells in the group treated simultaneously with PSO (6 µg/ml) and LF-EMF (2 mT) compared to the control group ($P < 0.001$) (Figure 1C).

3.2. Analysis of Cell Death Using Annexin V/PI

HT29 cells were treated with PSO and LF-EMF. Based on the flow cytometry histograms, it was observed that in the control group, 84.9% of the cells remained viable (Figure 2A). In the PSO group, 59% of the cells were viable, while 38% underwent apoptosis (Figure 2B). In the LF-EMF group, 73% of the cells were viable, and 25% underwent apoptosis (Figure 2C). In the combined treatment group, 55% of the cells remained viable, and 44% underwent apoptosis (Figure 2D).

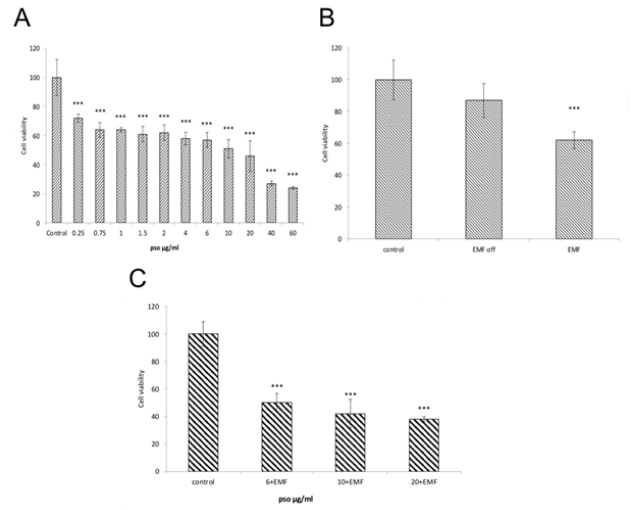


Fig. 1. Evaluation of the viability of HT29 colon cancer cells by MTT assay 24 hours after treatment with different concentrations of PSO (0.25, 0.75, 1, 1.5, 2, 4, 6, 10, 20, 40, and 60 µg/ml) (A). Treatment with LF-EMF (B). Synergistic effect of PSO at concentrations of 6, 10, and 20 µg/ml combined with LF-EMF (C). (***$P < 0.001$ indicates a significant difference compared to the control group).

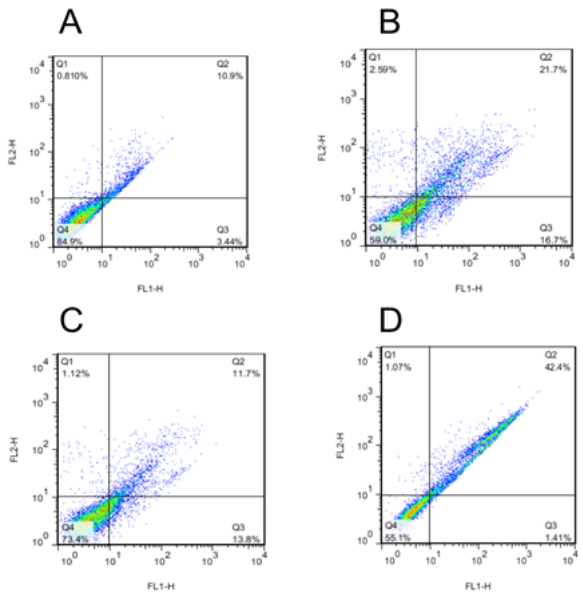


Fig. 2. Apoptosis Percentage Assessed by Annexin V-FITC/PI Staining. Approximately 85% of the cells are viable in the Control group(A). 38% of the cells underwent apoptosis in the PSO treatment group(B). 25% of the cells underwent apoptosis in the LF-EMF treatment group. (C).44% of the cells underwent apoptosis in PSO + EMF treatment groups(D).

3.3. Gene Expression Analysis Using Real-Time PCR

The expression levels of Caspase 3, 9, Bax, and Bcl2 genes in response to treatment with PSO and LF-EMF were measured using Real-Time PCR. The expression of the Caspase 3 gene significantly increased in the PSO, EMF, and combined treatment groups compared to the control group ($P < 0.001$) (Figure 3A). The expression of the Caspase 9 gene also significantly increased in the PSO, EMF, and combined treatment groups compared to the control group ($P < 0.001$) (Figure 3B). The expression of the Bax gene significantly increased in the PSO and combined treatment groups compared to the control group ($P < 0.001$), but no significant increase was observed in the EMF group (Figure 3C). The expression of the Bcl2 gene significantly decreased in the PSO, EMF, and combined treatment groups compared to the control group ($P < 0.001$) (Figure 3D).

4. Discussion

In recent years, numerous techniques have been developed for cancer treatment. However, a notable problem related to these treatments is their non-selective mechanism, which may result in the unintended harm of healthy cells in addition to cancer cells. The primary aim of cancer

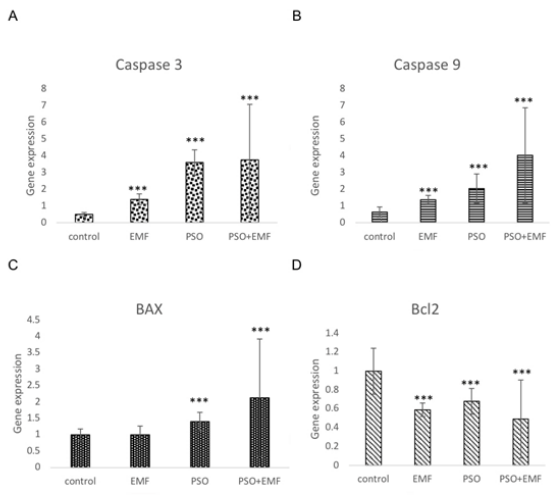


Fig. 3. Expression Patterns of Caspase 3, Caspase 9, Bax, and Bcl2 Genes in Cells Treated with PSO and Electromagnetic Field (A, B, C, and D). The value ***P < 0.001 indicates a significant difference compared to the control group.

therapy is to precisely target and eradicate cancer cells while safeguarding healthy cells to minimize side effects and improve therapeutic efficacy(18). This study examined the synergistic effects of pomegranate seed oil (PSO) and low-frequency electromagnetic fields (LF-EMF) on the expression of apoptotic genes, namely caspase-3, caspase-9, BAX, and Bcl-2, in HT29 colorectal cancer cells. Our findings demonstrated that the integration of PSO and LF-EMF resulted in a more significant suppression of cancer cell proliferation. The dysregulation of apoptosis is a hallmark of cancer, and nearly all non-surgical approaches aimed at targeting tumor cells rely on the induction of apoptosis. Moreover, exposure to electromagnetic fields, especially in conjunction with stressors such as chemotherapeutic drugs or ionizing radiation, has been demonstrated to augment apoptosis[19]. Previous studies have demonstrated that pomegranate derivatives exhibit anti-carcinogenic effects in multiple gastrointestinal cancer cell lines, including those derived from gastric and liver cancers[20]. In 2010, a study conducted by Zielińska et al. focused on pomegranate seed oil (PSO), which is known to contain numerous beneficial components. Their findings indicated that PSO is a superior source of punicic acid (PA), comprising more than 70% of its total fatty acid content by weight[21]. Further, a 2019 study by Li et al. examined the anti-cancer properties of key components of pomegranate (PC), such as punicalagin (PPPs) and ellagic acid (EA), specifically in HepG2 cells, while also exploring their mechanisms of action. This investigation assessed aspects of cell proliferation, morphology, the cell cycle, and apoptosis. The results showed that both PC and EA prompted cell cycle arrest in the S and G0/G1 phases, ultimately leading to apoptosis in HepG2 cells. The study concluded that both PC and EA are capable of inhibiting cell growth and inducing apoptosis in HepG2 cells[22]. In 2020, Lydia et al. produced gold nanoparticles (AuNPs) with pomegranate seed oil (Punica granatum L.) and created a functional yogurt to exploit its antioxidant and anticancer characteristics. Their research revealed that gold nanoparticles coated with pomegranate seed oil showed considerable anti-cancer properties against lung and colon cancer, highlighting their potential to improve cell viability[23]. In 2023, Moghimi Pour et al. conducted a study on Solid Lipid Nanoparticles, which shown significant anti-cancer benefits on the HT29 cell line[24]. In 2019, Amri et al. conducted a study examining the growth-inhibitory and pro-apoptotic effects of pomegranate seed oil (PSO) on human prostate cancer cells (Du145). Their research indicated that the oil effectively induced apoptosis in these cancer cells. The study employed the MTT assay to assess the anti-proliferative effects of pomegranate seed oil extract on the DU145 cell line, revealing that certain doses of the extract resulted in decreased cell viability[24]. In 2021, Zare et al. reported on the inhibitory effects of PSO on esophageal cancer cells, noting that the MTT assay results validated PSO's cytotoxic and growth-inhibitory capabilities on these cancer cells[9]. Furthermore, a study by Vazirijavid et al. examined the cytotoxic effects of punicic acid sourced from PSO, demonstrating a dose-dependent cytotoxicity on a leukemia cell line. Their results demonstrated that punicic acid had maximum cytotoxicity at an LC50 of 50 µg/mL[25]. Our investigation demonstrated that a concentration of 10 µg/mL of pomegranate seed oil was ideal for the treatment of HT29 cancer cells, corroborating earlier research, and drastically reduced the viability of HT29 cells. In 2018, Đurđević et al. evaluated the antioxidant and cytotoxic properties of PSO in many cancer cell lines. The MTT experiment findings revealed that PSO had the most significant cytotoxic effect against HeLa cell lines, with an IC50 value of 49.51 µg/mL[26]. Our study assessed the cytotoxic effects of pomegranate seed oil (PSO) on HT29 cells, revealing that the viability of treated cells was markedly diminished compared to untreated cells. In 2010, Kasimsetty et al. employed Annexin staining to illustrate that pomegranate-derived compounds can impede the proliferation of colorectal cancer cells by inducing cell cycle arrest and apoptosis[27]. In 2023, Moradi Haghighi et al. utilized Annexin PI labeling to examine apoptotic cell populations. Their findings indicated a greater rate of apoptosis in cells treated with the natural component Cramen Brom compared to those administered chemotherapeutic medications[5]. In the present work, we evaluated apoptosis levels in cells via Annexin staining and discovered that treatment with pomegranate seed oil (PSO) led to an increased percentage of apoptotic cells compared to the untreated groups. The Bax gene, recognized as a transcriptional target of p53, can be regulated in response to various p53-dependent apoptotic signals. The activation of p53 results in the upregulation of Bax expression and simultaneously decreases the levels of the anti-apoptotic protein Bcl-2. The alteration in the Bax/Bcl-2 ratio leads to mitochondrial malfunction, resulting in the release of cytochrome c into the cytoplasm, which activates caspase-9 and subsequently triggers the activation of caspase-3 and/or caspase-7[26]. A 2023 study by Mohamed et al. examined the impact of PSO nanoemulsion on tumor growth inhibition and apoptosis induction in mice with solid carcinoma. The administration of the nanoemulsion significantly elevated the expression of pro-apoptotic genes, including p53, Bax, and caspases, while concurrently diminishing the expression of the anti-apoptotic gene Bcl-2 in the tumor tissues of mice treated with PSO nanoemulsions[11]. Research by

Deng et al. in 2017 demonstrated that pomegranate peel extract induces apoptosis and inhibits metastasis in prostate cancer cells. Their western blot analysis revealed an elevation in the Bax/Bcl-2 expression ratio and the activation of the apoptotic executor caspase-3[28]. A study by Sayed et al. in 2022 revealed that pomegranate peel extract significantly reduces inflammatory blood markers, including interleukin-1 beta (IL-1β) and IL-6. The extract also increased levels of cyclooxygenase-2 (COX-2), transforming growth factor-beta 1 (TGF-β1), and caspase-3, while concurrently decreasing Bcl-2 levels. These findings suggest that pomegranate peel exhibits strong anti-inflammatory, pro-apoptotic, free radical scavenging, and antioxidant properties[29]. A 2023 study by Nasr et al. examined the anti-cancer properties of ethanolic extracts from pomegranate seeds and peels, demonstrating a significant upregulation of apoptosis-related genes, including Caspase-3, and a downregulation of the anti-apoptotic gene Bcl2[30]. Research conducted by Bishayee et al. in 2016 demonstrated that pomegranate derivatives impede breast carcinogenesis by diminishing cell proliferation and enhancing apoptosis. Immunohistochemical investigations revealed that pomegranate peel extract (PE) elevated intratumoral levels of Bax and reduced Bcl2 expression. Furthermore, gene expression analyses indicated that PE enhanced the levels of Caspase-3, Caspase-7, and Caspase-9 in breast cancers. Consequently, PE demonstrates chemopreventive effects against breast cancer by suppressing cell proliferation and promoting apoptosis via the overexpression of Bax and downregulation of Bcl2, alongside the activation of the caspase cascade[31]. Our study observed a notable elevation in the expression of the caspase-3, caspase-9, and Bax genes following treatment with pomegranate seed oil (PSO) relative to the untreated group. In contrast, the expression of the Bcl2 gene significantly diminished in the PSO-treated groups compared to the control group, corroborating previous research findings. The prevailing theory elucidating the anti-cancer effects of electromagnetic fields (EMF) centers on the induction of apoptosis via the modulation of intracellular reactive oxygen species (ROS), a mechanism corroborated by numerous experimental studies. Research has shown that a 24-hour exposure to a 60 Hz, 5 mT electromagnetic field can augment H2O2-induced apoptosis in HL-60 leukemia cells[19]. Furthermore, a 2024 study by Temiz et al. demonstrated that electromagnetic fields can trigger apoptosis in glioblastoma cells by elevating levels of miR-126-5p and intracellular Ca2+ concentrations. Their findings demonstrated that EMF exposure induces apoptosis in the A172 glioblastoma cell line through the upregulation of miR-129-5p, a known tumor suppressor[32]. Additionally, Siasi et al. conducted a study in 2021 examining the impact of electromagnetic fields (EMF) on gastric cancer cell lines, revealing that exposure to low-frequency electromagnetic fields (LF-EMF) resulted in heightened expression of miR-21 and miR-29, which function as either oncogenes or tumor suppressors in multiple cancers[14]. Additionally, Li et al. studied the apoptotic effects of 1800 MHz electromagnetic radiation on NIH/3T3 cell lines in 2020. Their investigation demonstrated a considerable decrease in cell viability in the exposed groups relative to controls, accompanied by a significant rise in apoptosis after 48 hours of exposure[16]. In 2024, Colciago et al. examined the impact of electromagnetic fields (EMF) on Schwann cells (SCs), which are associated with the onset of malignancies originating from these cells. Their laboratory results demonstrated a reduction in the proliferation, viability, and migration of stem cells after exposure to electromagnetic fields[33]. A study by Bertuccio et al. in 2023 employed the MTT test and demonstrated that the viability of neuroblastoma cells markedly decreased after 24 to 48 hours of exposure to electromagnetic radiation[34]. In the current investigation, both the MTT assay and Annexin staining results indicated that exposure to low-frequency electromagnetic fields (EMF) resulted in reduced viability of HT29 cells alongside an increase in apoptosis, consistent with earlier studies. For instance, Moori et al. (2020) reported that breast cancer cell lines exposed to a 100 mT, 1 Hz EMF for five days (2 hours each day) showed elevated levels of Bax mRNA and decreased Bcl2 expression in MDA-MB231 cells relative to control exposures. This change led to a significant increase in the Bax/Bcl2 ratio, facilitating the induction of apoptosis[35]. Bertuccio et al. (2023) observed an elevated Bax/Bcl2 ratio after exposure to electromagnetic radiation, hence substantiating the pro-apoptotic effect of EMF[34]. Our study evaluated the expression levels of caspase-3, caspase-9, Bax, and Bcl2 genes, revealing that EMF exposure led to increased expression of caspase-3, caspase-9, and Bax, while Bcl2 expression diminished in HT29 cells, supporting previous research findings. on 2024, Woo et al. investigated the influence of pulsed electromagnetic fields (PEMF) on the anticancer properties of caffeic acid phenethyl ester (CAPE) on MCF-7 breast cancer cells. Their findings demonstrated that PEMF stimulation enhanced CAPE-induced cell death by activating p53, caspases, and pro-apoptotic proteins such as Bax, while concurrently decreasing levels of anti-apoptotic proteins like Bcl-2. The study found that PEMF may serve as an additional treatment to enhance CAPE's effects on breast cancer cells by altering apoptosis-related molecules and activating the caspase-dependent apoptotic pathway[36]. In 2022, Woo et al. investigated the impact of pulsed electromagnetic fields (PEMF) in conjunction with etoposide, a chemotherapeutic drug, on apoptosis in MCF-7 breast cancer cells. Their findings demonstrated that the combination of etoposide with PEMFs resulted in a more pronounced decrease in cell viability than treatment with etoposide alone[17]. Furthermore, in 2024, the same cohort of researchers established that PEMF amplifies the efficacy of doxorubicin, another chemotherapeutic agent, in reducing the viability of MCF-7 breast cancer cells. Their research highlighted that PEMF can enhance the efficacy of several anticancer therapies. The researchers examined the processes affected by PEMF in doxorubicin-treated MCF-7 cells and discovered that the combined therapy significantly reduced cell viability compared to doxorubicin alone. PEMF induced the activation of caspases 8, 9, and 7, along with poly (ADP-ribose) polymerase (PARP) in MCF-7 cells. Consequently, PEMF amplifies the anticancer efficacy of doxorubicin by facilitating G1 cell cycle arrest and caspase-dependent apoptosis, underscoring the potential of PEMF as an adjunctive therapy in doxorubicin-based chemotherapy for breast cancer[37]. In 2020, Anwer et al. investigated the anticancer efficacy of electromagnetic fields (EMF), allicin, and their combination on hepatocellular carcinoma (HepG2) cells. Their research demonstrated that the synergy of EMF and allicin was more efficacious than either treat-

ment individually. The combined therapy markedly enhanced the expression of pro-apoptotic genes (Bax and P53) and diminished the expression of the anti-apoptotic gene (BCL-2), indicating that this combination may induce apoptosis and suppress cell proliferation in HepG2 cells[38]. A 2022 study by Mohamed et al. evaluated the anticancer potential of short-term exposure to extremely low-frequency electromagnetic fields (ELF-EMF) and silver nanoparticles (AgNPs), both separately and in together. They exhibited significant induction of apoptosis in MCF-7 cells after exposure to ELF-EMF or AgNPs, with the combined treatment yielding even greater levels of apoptosis[39]. Our work demonstrated that the synergistic effect of pomegranate seed oil (PSO) and low-frequency electromagnetic fields (LF-EMF) resulted in enhanced apoptosis and reduced cell proliferation in the HT29 cell line. Gene expression analysis revealed increased levels of Bax, caspase-3, and caspase-9 in groups subjected to both PSO and EMF relative to the control group, with further amplification noted in the combination treatment group. Additionally, we observed a reduction in Bcl2 gene expression in the combination treatment group compared to the other groups. Our findings indicate that the combined use of pomegranate seed oil and low-frequency electromagnetic fields significantly enhances their efficacy in cancer treatment. Consequently, the synergistic effects of these two treatments necessitate additional investigation from multiple viewpoints in future research.

5. Conclusion

This study revealed that the conjunction of pomegranate seed oil and low-frequency electromagnetic fields markedly suppresses proliferation and viability while enhancing apoptosis in colorectal cancer cells. The noted decline in cell proliferation is chiefly associated with the increased production of pro-apoptotic genes, such as caspase-3, caspase-9, and BAX, alongside a diminished expression of the anti-apoptotic gene Bcl-2 in these cells. However, the prospective use of pomegranate seed oil as an innovative therapeutic alternative or as a supplementary treatment in conjunction with low-frequency electromagnetic fields for colorectal cancer necessitates additional comprehensive clinical studies to validate these findings.

Human and animal rights

In this investigation, no people were used. The eighth version of the National Academy of Sciences' Guide for the Care and Use of Laboratory Animals (issued by The National Academies Press, Washington, D.C.) was followed in all animal research operations.

Consent for publication

Not applicable

Availability of data and materials

Not applicable

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Conflict of interest

The authors declare that they have no conflict of interest.

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Author's Contributions

Neda Ghorbani: Formal analysis, Writing - original draft, Analysis and interpretation of data, Drafting of the manuscript, Critical revision of the manuscript for important intellectual content, Statistical analysis.

Javad Baharara: Formal analysis, Writing - original draft, Study concept and design, Analysis and interpretation of data, Drafting of the manuscript, Critical revision of the manuscript for important intellectual content, Statistical analysis.

Maryam Lotfi: Formal analysis, Writing - original draft, Analysis and interpretation of data, Drafting of the manuscript, Critical revision of the manuscript for important intellectual content, Statistical analysis.

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