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RESEARCH PAPER

3-aminopropyltrimethoxysilane-modified magnetic quadruple nanocomplex induces apoptosis in MCF7 cell line

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ABSTRACT

Objective(s): Natural compounds, such as Oleuropein, Quercetin, Coumarin, and Valproic acid, play a vital role in preventing the spread and progression of cancer. Oleuropein increases the expression of certain caspases, Quercetin reduces the activity of the PI3K/Akt/IKK-/NF-κB pathway, Coumarin inhibits aromatase, and Valproic acid acts as an inhibitor of histone deacetylases. This study aimed to produce a quadruple magnetic nanocomplex with high bioavailability and to examine whether this nanocomplex can induce apoptosis in MCF7 breast cancer cell lines.

Materials and Methods: A silicon bridge (Sio-N-) was built using nanomagnetic iron and methoxysilane to create a magnetic nanocomplex that incorporated the four natural substances. This quadruple nanocomplex was then analyzed using various spectroscopic techniques and measurements. The researchers assessed the inhibitory impact of the nanocomplex on apoptotic genes in the MCF7 breast cancer cell line using the MTT assay, Hoechst staining, flow cytometry, and real-time PCR analysis.

Results: The magnetic nanocomplex exhibited a greater level of toxicity and reduced the number of cancer cells compared to any of the individual natural compounds or the quadruple combination without nanoparticles. The quadruple magnetic nanocomplex induced overexpression of the pro-apoptotic genes P53, Bim, and Bak, while reducing the expression of the anti-apoptotic gene Bcl2. Additionally, the nanocomplex treatment increased the expression level of genes involved in apoptosis by up to two-fold.

Conclusion: The combination of plant-derived natural compounds and magnetic nanoparticles can enhance the toxicity and concentration of the materials against breast cancer cells. This approach may provide synergistic effects through the modulation of various molecular pathways, leading to the inhibition of cancer cell proliferation and the induction of apoptosis.

Keywords: *Apoptosis, Coumarin, Magnetic nanoparticles, Oleuropein, Quercetin, Valproic acid*

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INTRODUCTION

Breast cancer is the most frequent cancer among women and it is one of the most important causes of death worldwide [1]. However, current therapeutic modalities for cancer treatment face several drawbacks and limitations [2]. Drugs have faced challenges due to severe side effects, substantial toxicity of chemotherapeutic agents on normal cells, and the development of drug resistance or acquired resistance in cancer cells [3]. Breast cancer tumors are remarkably diverse and heterogeneous in nature [4]. The disruption of the balance between cell proliferation and apoptosis leads to the activation of anti-apoptotic signaling pathways, leading to uncontrolled growth of cancer cells and treatment resistance [5, 6]. Bcl2 gene family, P53 protein, and caspases 3, 6, 8, and 9 are involved in apoptosis [7].

The multifunctionality of natural compounds and their synergistic action against various diseases, including breast cancer, along with the unique properties of magnetic nanoparticles,

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suggests the potential for targeted therapy. Phenolic compounds are among the naturally occurring substances that are effective in regulating the interactions between growth factor receptors and cell signaling cascades like PI3K, Akt, mTOR, RAS, MAPK, and the expression of genes involved in cell cycle arrest [8]. Consuming polyphenols is useful in preventing breast cancer [9, 10]. Nanotechnology increases the bioavailability and bioactivity of herbal medicines by lowering the size of the particle, altering the surface, and entrapping the drug [11]. Nanoformulation of natural polyphenols, characterized by better-targeted therapy, improved pro-apoptotic activity against tumor cells, higher intracellular concentration, and slow release, results in more effective cancer prevention and treatment [12]. Magnetic nanoparticles, formed of iron oxide nanoparticles from magnetite and maghemitite, are being utilized more often in targeted therapeutics owing to their high bioavailability and low toxicity. These particles are ideal for magnetic field-based distribution to target specific regions [12, 13].

Oleuropein, a naturally occurring polyphenol, functions by blocking cells in the G2/M phase of the cell cycle and through epigenetic mechanisms, including the inhibition of HDAC, which helps slow tumor growth. In its nano-magnetic form, Oleuropein has demonstrated the ability to suppress the growth of MCF7 cells, significantly increase death induction, and promote apoptosis in the AGS cell line [12]. Coumarin, another polyphenolic substance with established anticancer properties, affects multiple cancer pathways, including cell cycle arrest, angiogenesis inhibition, kinase inhibition, aromatase inhibition, telomerase inhibition, and sulfatase inhibition [13, 14]. Coumarins' caspase-dependent induction of apoptosis exerts an anti-proliferative impact by suppressing the expression of Bcl2 in multiple organs and tissues [15]. NanoCoumarin is more effective and powerful than synthetic Coumarin in reducing the production of p53, Cyclin D1, Survivin, and STAT-3 [16].

Quercetin (3, 3, 4, 5, 7-pentahydroxy flavon) is a flavonoid compound that has been shown to induce apoptosis through the mitochondrial pathway, associated with the downregulation of the PI3K/Akt/NF-κB signaling pathway [17, 18]. Quercetin's high encapsulation efficiency, lengthy circulation duration, tumor targeting, and inhibitory actions are all enhanced by its incorporation into nanoparticles [19, 20]. One of the histone deacetylase inhibitors, Valproic acid, has been demonstrated to have antitumor effects on a variety of solid tumors, including breast cancer [21] and cause G0/G1 arrest, which results in apoptotic cell death in MDA-MB-231 TNBC cells [22]. This study aimed to evaluate the impact of transferring a synthetic quadruple magnetic anticancer nanocomplex, containing Oleuropein, Quercetin, Coumarin, and Valproic acid on the expression of apoptotic and anti-apoptotic genes in MCF7 cancer cells.

MATERIALS AND METHODS

Synthesis of nanocomplex

In this research, the first step involved coupling silicon oxide onto nano iron oxide in three stages, where the silicon oxide was connected to the iron backbone through oxygen and silicon oxide bridge materials (polyphenols). In this experiment, the concentrations of the four substances (Oleuropein, Quercetin, Coumarin, and Valproic acid) were synchronized based on their respective molecular weights. After the connection was made, the resulting impact was evaluated comparatively. Additionally, the rate of release of each material over time from the synthesized nano-construct within the culture medium was determined by spectrophotometry and regression analysis (though not reported in the current text). Furthermore, the binding of each substance was verified using the IR method.

Cell culture

MCF7 cells were bought from the Pasteur Institute cell bank in Tehran, Iran, for the purpose of this experimental work. The cells were maintained in an incubator at 37 °C, 5% CO_2, and 95% humidity while being grown in PRMI1640 culture media with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin.

Toxicity of magnetic quadruple nanocomplex

The effect of the quadruple nanocomplex on MCF7 cancer cells was evaluated using the colorimetric MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. MCF7 cells were first cultured in 96-well plates, with each well containing 100 µL of cells. The cells were then treated with various concentrations of the nanocomplex (0, 12.5, 25, 50, 100, 250,

500, and 1,000 µg/mL) and incubated for 24 hr. After the incubation, 5 mg/mL of the MTT solution was added to each well, and the plates were incubated for an additional 4 hr. Following this, DMSO solution was added, and the light absorption was measured at a wavelength of 570 nm using an ELISA reader. The percentage of cell survival in the negative control group was considered 100%, and the survival percentage of cells treated with different concentrations of the nanocomplex was calculated by dividing the absorbance of the treated wells by the absorbance of the negative control, and then multiplying by 100. The concentration of the tested compounds that reduced the percentage of cell viability by 50% (IC_{50}) was determined using the AAT Bioquest online system.

RNA extraction and cDNA synthesis

The SINA kit (RNAXPLUS Cat No: RN7713C) and its methodology were used to extract the RNA from the treated and control cells, and the SINAclon cDNA synthesis kit)Cat No: RT5201 (and reverse transcriptase enzyme were prepared in accordance with the manufacturer's instructions. NanoDrop (Thermo) was used to determine RNA purity. RNA integrity and the success of the reverse transcription reaction were monitored by PCR amplification of glyceraldehyde-3-phosphate dehydrogenase transcripts and denaturing agarose gel 2% [23]. Briefly, 2 µL of 5X PCR buffer, one microliter each of dNTP and oligo-dT, 0.5 µL of reverse transcriptase enzyme, and 100 ng RNA was used for cDNA synthesis.

Real-time PCR

The StepOnePlusTM Real-Time PCR System from Corbett Company was used to carry out the Real-Time PCR experiment. In this work, the expression of the apoptosis-related genes, including P53, Bcl2, Bak, Bim, caspase 9, and caspase 8, was examined. GAPDH2 was utilized as an internal reference gene. For primer design, the sequences of the examined genes were first chosen from the website http://www.ncbi.nlm.nih.gov, and then particular forward and reverse primers for each gene were generated using oligo primer design software. Table 1 displays each gene's sequence. After designing forward and reverse primers with Primer 3 software, BLAST was performed using the NCBI website. The reaction was carried out in triplicate in a volume of 10 µL. It contained 2 µLof RNase-free water, 1 µL of cDNA, 5 µL of Master Mixe SYBR Green, and 0.5 µL of each primer. Cycling program was as follows: primary denaturation 5 min at 95 °C for the activation of the polymerase, followed by 40 cycles of 15 sec at 95 °C, and 60 sec at 60 °C [24].

Statistical analyses

Statistical analyses were carried out with SPSS version 23.0 (SPSS Inc., Chicago, Illinois, USA). ANOVA was performed to determine the significant difference in cell viability between the treatments and the control and the mean comparison was done AAT Bioquest's online program ($P \leq 0.05$). Also, LinReg and REST software were used to analyze the relative gene expression in MCF7 cells ($P \leq 0.05$).

Hoechst staining for apoptosis analysis

In this study, apoptosis was examined using a fluorescence microscope and Hoechst 33285 staining. For this, MCF7 cells were exposed for 24 hr to a quadruple magnetic nanocomplex at a determined IC_{50} concentration. After the incubation period, the culture media was removed, and the cell layer was washed with FBS buffer. The layer of the cell was fixed using paraformaldehyde. The cell layer was then coated with a diluted solution

Table 1. Sequence of primers used to perform qRT-PCR

Gene	Primer Sequence (5'-3')	PCR product length (bp)
Caspase 8 forward	AAGTGCCCTTCCCTTGCTG	153
Caspase 8 reverse	GCAGAAAGTCAGCCTCATCC	
Caspase 9 forward	AAAGTTGTCGAAGCCAACCC	158
Caspase 9 reverse	GACTCACGGCAGAAGTTCAC	
Bcl2 forward	TGTGGCCTTCTTTGAGTTCG	162
Bcl2 reverse	CCTACCCAGCCTCCGTTATC	
P53 forward	AGGTTGGCTCTGACTGTACC	162
P53 reverse	GATTCTCTTCCTCTGTGCGC	
Bak forward	CAATGTCCTCCCTGCTGTTG	167
Bak reverse	AGAACCACACCCAGAACCAC	
Bim forward	AGTTCTGAGTGTGACCGAGA	156
Bim reverse	AGGAGGACTTGGGGTTTGTG	

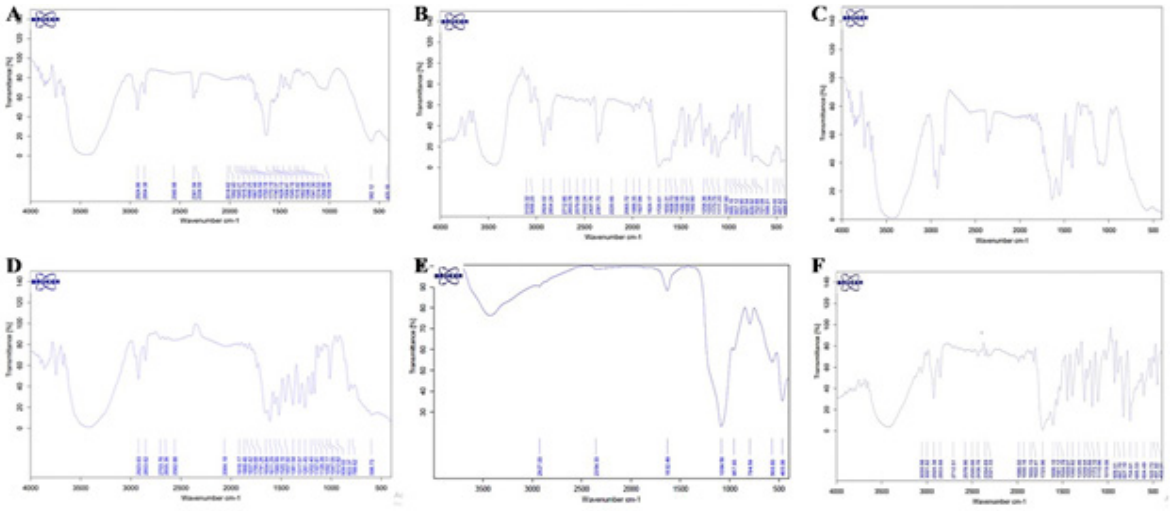

Fig. 1. FT-IR spectrum of nanoparticles Oleuropein (A), Coumarin nanoparticles (B), Valproic acid nanoparticles (C), Quercetin nanoparticles (D) nanoparticles attached to magnetite particles modified with 3 aminopropyltri methoxysilane (E) attached to the quadruple compound (F)

of Hoechst dye 33258 after being washed one more with FBS buffer. It was put on a microscope slide as a drop. The cells covered on the slide were examined using a fluorescent microscope after the smear preparation.

RESULTS

Examining the synthesized complex's mechanism

Fig. 1 shows the composition of the 3-aminopropyltrimethoxysilane-modified magnetite nanoparticles in the state where they were attached to the quadruple compound, which includes nanoOleuropein, nanoCoumarin, nanoValproic acid, and nanoQuercetin. Methoxysilane is shown individually. The development of the quadruple complex and the production of the quadruple magnetic nanocomplex of Oleuropein, Coumarin, Quercetin, and Valproic acid were demonstrated by the presence of unique peaks of each substance.

The crystallinity of the nanoparticles and the synthesized complex confirms that the magnetic nanoparticles modified with 3-aminopropyltrimethoxysilane coupled with Valproic acid, Oleuropein, Quercetin, and Coumarin conform to the given data of standard XRD patterns (JCPDS card No. 86-2267) (Fig. 2A). The saturation magnetization

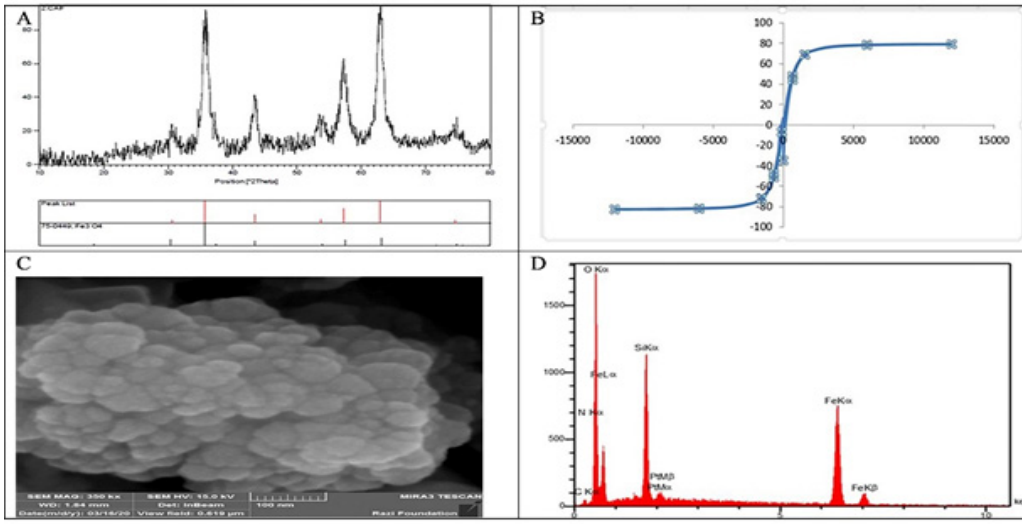

Fig. 2. XRD pattern(A) the VSM magnetization curve(B) SEM images at a magnification of 100 nm (C) EDAX images (D) associated with modified magnetite nanoparticles connected to compound 4 by 3-amino propyl trimethoxysilane

of magnetite iron oxide nanoparticles modified with 3-aminopropyltrimethoxysilane in the state connected to the quadruple complex is shown by the VSM magnetization curve in terms of the field, which exhibits the presence of a single-domain magnetic material without a residual loop—approximately 80 emu/g. The material encasing the magnetic core is causing the new lowering shift in the hysteresis loop, as seen in Fig. 2B.

By applying the Debye-Scherr formula to the width of the longest peak at half height in an X-ray diffraction analysis, the average particle size was determined 23.45 nm for magnetite nanoparticles without modification and 35.45 nm for those modified with 3-aminopropyltrimethoxysilane attached to compound 4. The SEM and EDAX pictures of magnetite nanoparticles modified with 3-aminopropyltrimethoxysilane connected to Oleuropein, Coumarin, Valproic acid, and Quercetin at a 100 nm magnification are shown in Fig. 2C-D, respectively. Spherical nanoparticles are properly distributed and uniformly distributed in EDAX and SEM images.

Cell viability assay (MTT)

The MTT test was used to analyze the effects of various doses of the compounds on MCF-7 cells Fig. 3). Comparing the treatments, the vitality of MCF7 cells varied significantly (P-value = 0.01). Using AAT Bioquest's online program, the IC_{50} for quadruple nanocomplex and quadruple physical combinations was calculated (Fig. 4 A-B). The IC_{50} concentration of quadruple compound was

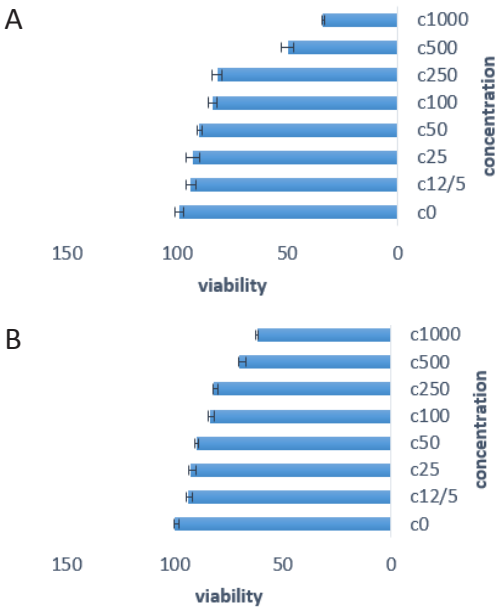

Fig. 3. Results of the MTT test on MCF-7 cells for various concentrations of the investigated materials
(A) magnetic nanocomplex (B) nanomagnetic iron

644.654 µg/mL, whereas the IC_{50} concentration of quadruple nanocomplex was 264.6226 µg/mL.

The relative expression of genes in treated cells by Real-time PCR

The relative expression levels of the examined genes in comparison to the housekeeping gene GAPDH2 were ascertained by using the Real-time PCR technique. P53, Bcl2, Bak, Bim, and caspase 8 genes showed a significant difference

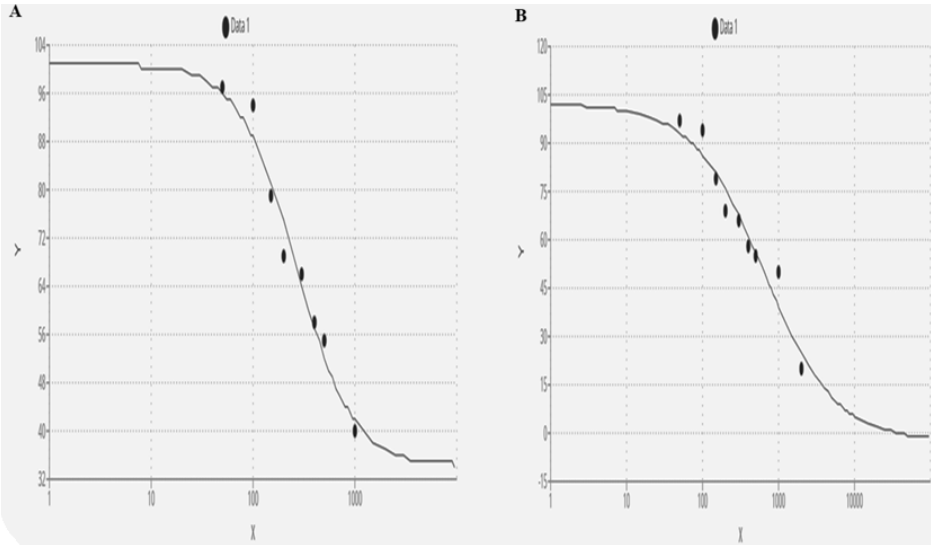

Fig. 4. IC_{50} curve for the quadruple (A) magnetic nanocomposite (B) physical composition

between the treated cells and control samples in terms of gene expression at a probability level of less than 1% (P<0. 01), while the caspase 9 gene showed the same expression in both cells (Fig. 5). Oleuropein, Coumarin, and Quercetin-treated cells showed the same expression. Only the Bcl2 gene showed a decrease in expression level at mRNA level, and this was most pronounced when Valproic acid and triple nanocomplex were used as nanomagnets. The rise in expression was detected in the P53, Bak, Bim, caspase 8, and caspase 9 genes (Fig. 5). When the substances under study were treated as formulated nanomagnets, the p53 gene experienced the highest increase in expression, which was 2.76 times higher and statistically significant compared to the control. The same result was observed for the Bak gene (Fig. 5). The treatment with the quadruple magnetic nanocomplex and healthy cells showed the greatest increase in expression, whereas the substantial treatment with Coumarin at IC_{50} showed a lesser rise in expression than the control treatment. The simultaneous application of a nanocomplex comprising four magnetic materials dramatically enhanced the expression of the Bim gene by 1.76 times, with the healthy control showing the greatest expression (2.63 times) (Fig. 5). Caspase 8 expression was most strongly induced by Oleuropein treatment, and it was significantly increased by 1.94 times when four substances were administered simultaneously in treatment. The highest increase was observed when the substances under study were added together to serve as nanomagnets, which resulted in the highest induction of expression. In comparison to the control, its expression was 1.98 times more significant (Fig. 5). Oleuropein, quadruple magnetic nanocomplex, and Valproic acid treatment, among the four different treatments examined, all successfully inhibited MCF7 cancer cells by inducing internal apoptosis through caspase 9 and external apoptosis through procaspase 9. As a result, it can be claimed that the expression level of apoptotic genes was greater and their IC_{50} inhibitory concentration was lower in the form of nanoformulation.

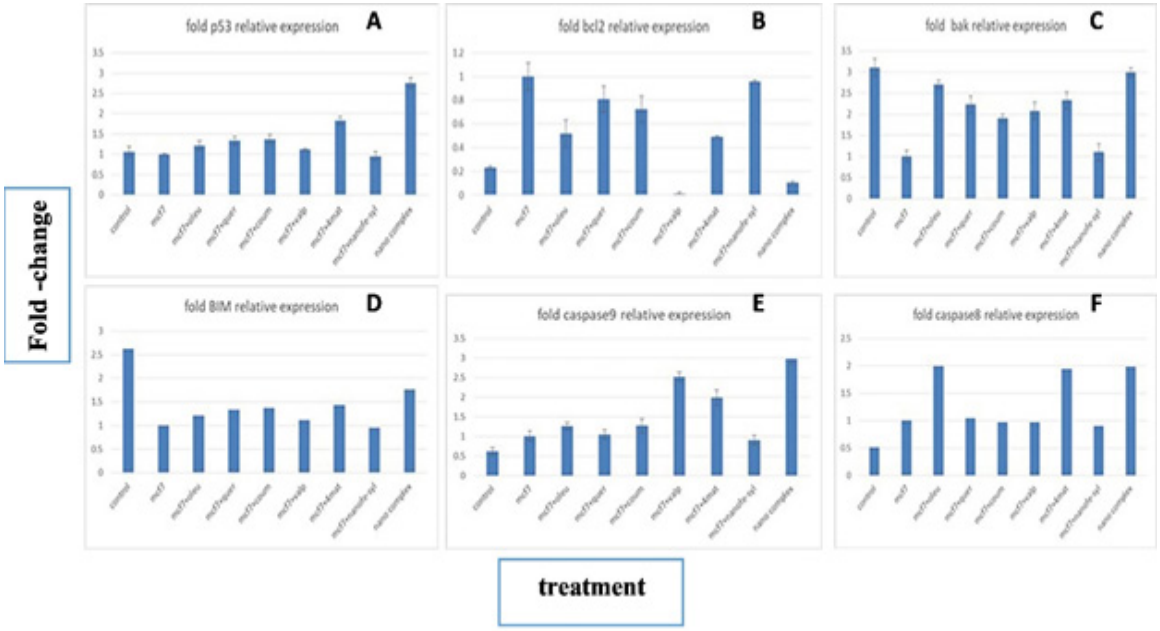

Fig. 5. The relative expression level of)A) P53 (B) Bcl2 (C) Bak (D) Bim (E) Caspase 9 (F) Caspase 8 in treated cells. A. The highest increase in p53 gene expression during treatment with the quadruple magnetic nanocomplex. B. The highest decrease in Bcl2 gene expression during treatment with valproic acid and the quadruple magnetic nanocomplex. C. The highest increase in BAK gene expression in healthy cells and during treatment with the quadruple magnetic nanocomplex. D. The highest increase in Bim gene expression in healthy cells and then during treatment with the quadruple magnetic nanocomplex. E. The highest increase in caspase 9 gene expression during treatment with the quadruple magnetic nanocomplex and then with valproic acid. F. The highest increase in caspase 8 gene expression during treatment with oleuropein and simultaneous use of 4 substances, and during treatment with the quadruple magnetic nanocomplex

DISCUSSION

Due to the medicinal characteristics of cells, steady cellular and molecular changes, aberrant expression of cancer cell surface chemicals, limited cell permeability, low concentration in cells, and the destruction of healthy cells, chemotherapy medications were unable to effectively treat cancer. Due to their anti-cancer and antioxidant characteristics, as well as their minimal negative effects on healthy cells, natural chemicals, particularly polyphenolic compounds, constitute one of the most varied classes of plant secondary metabolites and have caught the interest of researchers working on cancer treatments. However, their limited bioavailability makes it difficult to treat tumors. By passively targeting cancer cells, increasing anti-neoplastic activity, and improving bioavailability, nanofabrication has been demonstrated to increase the anti-cancer properties of natural polyphenols [18]. Researchers are interested in nanomagnets as a targeted carrier with a magnetic field, but they are also concerned about the health hazards posed by their toxicity, which may be reduced by altering the ambient conditions during the synthesis of nanomaterials [25]. The fundamental mechanism of nanomaterials' anti-tumor activities is based on interactions between magneto-mechanical and magneto-chemical systems, which affects the structure of loaded agents and alter the redox state of tumors by producing ROS [26].

In this study, four substances, including Oleuropein, Coumarin, Quercetin, and Valproic acid, were attached to nano-magnetic iron concurrently by an NH silica bridge, and the synthesis was verified by a TEM picture and EDAX compliance with the standard of connected substances. The same technique, congruent with the findings of this study, has been utilized to synthesise magnetic nano Oleuropein [27], curcumin [28], and other natural compounds. Observations revealed that doxorubicin might penetrate nano iron oxide coated with tetraheptylammonium more deeply into cancer cells through internal endocytosis mechanisms, increasing the amount of drug absorbed by the corresponding K562 leukemia cells significantly [29]. Furthermore, a research has demonstrated that magnetic iron oxide nanoparticles suppress lung cancer cells by triggering death by raising caspase 3 activity [30]. This study demonstrated that the quadruple magnetic nanocomposite significantly reduced the concentration required to inhibit cancer cells compared to any other material or material combination. The inhibition of 50% of the nanostructure in Valproic acid, or roughly twice as much, resulted in the greatest concentration reduction. The expression of Caspase 9 increased by Valproic acid; however, Caspase 8 did not. This indicates that the internal mitochondrial route was adopted to induce apoptosis. This finding was anticipated since the acetylation of histones and subsequent production of caspase are two processes that contribute to the induction of apoptosis [31].

Oleuropein has been found to affect the expression of most genes, including those for cytokines, transcription factors, adhesion molecules, chemokines, growth factor receptors, and inflammatory enzymes [32]. Oleuropein was recently reported to control the NF-B activation cascade, which results in the onset of apoptosis in breast cancer cells [33]. According to a study by Barzegar et al., magnetic nano Oleuropein increased the expression of the Kras gene, which in turn caused apoptosis and necrosis in AGS gastric cancer cells. Overexpression of the Kras gene led to the overexpression of the p53 gene, which balanced the expression of the Kras genes and inhibited the proliferation of cancer cells [27].

According to the results, Quercetin did not significantly affect the induction of death by caspases 8 and 9, but the rise in the Bim and Bak genes and the severe drop in Bcl2 support the use of Quercetin in a different manner to suppress cancer cells. While nanoQuercetin is non-cytotoxic to keratinocytes at concentrations lower than 250 ng/mL, it is known to be toxic to normal cells [34]. In comparison to bulk Quercetin at all dosages, nanoQuercetin exhibited the effects of apoptosis, mitochondrial malfunction, caspase activation, and G2 phase cell cycle arrest [35]. This suggests that nanoQuercetin may have therapeutic potential for cervical and breast cancer. A number of active mechanisms, including the induction of apoptosis through PI3K and concurrent caspase-mediated reduction of p-Akt, have been investigated in previous studies to demonstrate the inhibitory impact of Quercetin in cervical and breast cancer [36]. However, further examination needs to be conducted. Successful therapeutic development depends on understanding fundamental signaling pathways and how these nanoQuercetins affect

other cancer-related disorders through clinical research. In this work, the plant polyphenol Coumarin caused apoptosis by increasing caspase 9, which was supported by increased levels of Bim, p53, and Bak and decreased levels of Bcl2. Coumarin's ability to inhibit the MCF7 cell line was accomplished via upregulating procaspase 9.

This route was reinforced by the decline in Bcl2 expression and the rise in Bak, Bim, and P53. After Coumarin therapy, there was a substantial drop in Bcl-2 gene expression and an increase in Bax gene expression, indicative of a decrease in the Bcl2-/Bax ratio, which can result in the activation of the apoptotic protein caspase [37]. According to the reports, aromatase inhibitors enhance signaling for cell death and suppress cell growth [38]. Altogether, our research showed that Coumarin specifically suppresses the growth of MCF-7 breast cancer cells and causes cell cycle arrest, which activates apoptosis. At a dosage of 664 µg/mL, the triple compound killed 50% of MCF7 cancer cells *in vitro*. If its nanostructure was able to block 50% of the MCF7 cell line at a dose of 264 µg/mL. As evidenced by the fact that consumption was decreased by 2.5 times the inhibitory concentration of 50%, this study demonstrates the critical function that reduction in consumption plays in the manufacture of the examined pharmaceuticals in nano form. The increasing interaction between these materials in the inhibition and the capacity of the nanostructure to enter the cells from one side and transfer values may be the reason for this. Since nanoparticles have more surface area, the surface area is also high. This result was consistent with the previous studies [33, 34].

Two lines of research were carried out to examine the inhibitory mechanism of MCF7 cancer cells. Hoechst staining was employed to demonstrate that the first aspect of the analysis—cell apoptosis—was correct (Fig. 6). Oleuropein promotes apoptosis by caspase 8 according to the results of analyzing the expression of genes in various treatments, supported by the overexpression of P53, Bim, Bak, and the decrease in Bcl2 expression. Additionally, Oleuropein administration resulted in Caspase-9-mediated apoptosis activation.

This pattern was also present in the quadruple compound and quadruple magnetic nanocomposite treatments; however, the level of apoptosis inducer expression was stronger in the nano treatment. It was indicated in the procaspase 8 and 9 (internal and external routes) triple combination therapy, which demonstrates the additive and cumulative effects of drugs with various qualities. However, the induction of apoptosis at a lower dose, the overexpression of caspases, and the support of other genes active in apoptosis were enhanced when this chemical was manufactured in a nanoform. As a result, after nanotreatment, the expression level of overexpressed genes in apoptosis can be found to rise by up to twice. This study had some limitations. The study was conducted using the MCF7 breast cancer cell line (*in vitro*) only. Evaluating the effects of the quadruple nanocomplex on a wider range of breast cancer cell lines, or animal models, or patient-derived samples would provide a more comprehensive understanding of its efficacy. Furthermore, the study did not extensively evaluate the long-term stability and potential toxicity of the quadruple nanocomplex. Exploring different formulation parameters, such as the ratio of the natural compounds or alternative synthesis approaches, may help further optimize the nanocomplex's performance and therapeutic

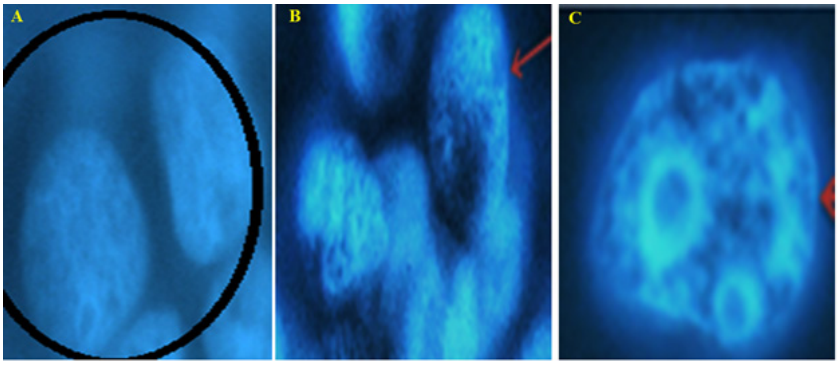

Fig. 6. The results of Hoechst staining of apoptotic cells - A - untreated MCF7 cells B - apoptotic MCF7 cells treated with quadruple magnetite nanocomplex and C - treated with Oleuropein

potential.

CONCLUSION

Considering that each of the cancer cells in a tissue may be in the different stages of the cell cycle, therefore, a type of drug with one or more limited mechanisms cannot be inhibitory. Therefore, the use of the combination of drugs with inhibitory mechanisms, under the condition of increasing their effect on the one hand and obtaining results *in vivo* conditions on the other hand, can create a new perspective in the development of new drugs with a wide range of action mechanisms. This study demonstrates the inhibitory and apoptotic properties of natural compounds and their synergistic effect in treating cancer cells, particularly in the form of their nanoformula. This is made possible by the ability of the nanostructure to penetrate the cells on the one hand and the transfer of large amounts due to the greater surface area of the nanoparticle on the other.

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CONFLICT OF INTERESTS

The authors proclaim that they have no conflicts of interest.

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