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Faradarmani Consciousness Field Treatment Reduces MDA-MB-231 Cancer Cell Viability

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Abstract

Breast cancer is the most common form of malignancy among women worldwide and is a multifactorial disease, the development of which involves various factors. The Faradarmani Consciousness Field (FCF) was introduced by Mohammad Ali Taheri as one of many Consciousness Fields that are neither energy nor matter, nor are they quantifiable; thus they cannot be directly observed or measured. However, it is possible to evaluate their effects indirectly through controlled experiments in the laboratory. This study aimed to investigate the effect of FCF on breast cancer cells (MDA-MB-231) measured using MTT and flow cytometry methods at 24, 48, and 72 hours. First, the MDA-MB-231 cell line was cultured, then the effect of FCF was studied at 24, 48, and 72 hours. To determine the cell death in the sample treated with FCF and compare it with the control, cells were stained with Annexin-V and propidium iodide (PI). Our results showed that the measured survival in the MTT test, at 24 hours, showed a 16% decrease in the experimental sample compared to the control. At this time, the percentage of early and late apoptosis and total apoptosis and necrosis in the sample under the influence of the FCF compared to the control cell lines increased by 5.92%, 3.49%, 9.41% and 4.68% respectively. Finally, the rate of programmed death of cancer cells increased in time intervals of 24 (9.41 %), 48 (21.44 %) and 72 (23.04 %) hours under the influence of this field in the examined cell line.

Keywords: Breast cancer, Taheri Consciousness Fields, Faradarmani, MTT assay, Cancer cells

Introduction

Breast cancer is one of the most prevalent malignancies affecting women worldwide (Akorafas et al., 2002; Bener et al., 2017). According to the statistics of the World Health Organization, breast cancer accounts for about 30% of cancers among women. Approximately 1.2 million women are diagnosed with breast cancer annually. This type of cancer is reported to be the second leading cause of cancer-related deaths in women after lung cancer. It has been estimated that the prevalence of breast cancer will increase from two million patients in 2018 to more than three million patients in 2046, which represents an increase of 46% (Sung et al.,2021; Siegel et al., 2019). Furthermore, this type of cancer is known as one of the most costly diseases globally (Davari et al.,2013).

During recent decades, changes in lifestyle have contributed to an increased incidence and prevalence of breast cancer worldwide (Fredslund et al., 2013). Breast cancer is a disease in which malignant cells arise from breast tissue and proliferate uncontrollably. These cells typically originate from the epithelial cells lining the milk ducts or the lobules of the breast (Shah et al., 2014). Breast cancer is a multifactorial disease in which various risk factors are known to play a role. According to the studies conducted, these risk factors include family history, older age, age at menarche below 12 years, age at menopause after 54 years, first childbirth after age 30, nulliparity, high mammographic density, elevated sex hormone levels, childhood exposure to ionizing radiation, race, socioeconomic status, body mass index, and lifestyle factors such as diet, physical activity, smoking, and alcohol consumption (Moller et al., 2003; Kaminska et al., 2015; Sun et al., 2017).

The MDA-MB-231 cell line is derived from human breast cancer, and it has a spindle-shaped epithelial morphology. These undifferentiated cells are very aggressive and lack estrogen and progesterone receptors, as well as the human epidermal growth factor receptor 2 (HER2). Therefore, this cell line is classified as triple-negative breast cancer (TNBC). The invasive power of this cell line is mediated through the ability to proteolytically alter the extracellular matrix (Chavez et al., 2010; Lukasiewicz et al., 2021).

The nature of consciousness and its place in science has received much attention in the current century. Many philosophical and scientific theories have been proposed in this area. In the1980s, Mohammad Ali Taheri introduced novel fields with non-material/non-energetic nature (Taheri, 2013) named Taheri Consciousness Fields (TCFs).

In previous studies, the effects of TCFs on various cancer cell lines, as well as on animal and plant models, have been investigated (Taheri et al., 2020; Taheri et al., 2022; Torabi et al., 2023; Taheri et al., 2024). The aim of this study was to investigate the effect of the Faradarmani Consciousness Field on breast cancer cells (MDA-MB-231) using MTT and flow cytometry methods.

Materials and Methods

Application of Faradarmani Consciousness Field

TCFs were applied to the samples according to the protocols regulated by the COSMOintel research center (www.COSMOintel.com). A detailed explanation is provided in the general discussion of this issue. In this study, MDA-MB-231 cells were exposed to the Faradarmani Consciousness Field once at 24, 48, and 72 hours from the start of the study. MDA-MB-231 cells that were not exposed to the FCF were considered the control group.

Cell culture

In this research, the MDA-MB-231 breast cancer cell line, obtained from the cell bank of the Pasteur Institute of Iran, was used. The cells were cultured in DMEM (Dulbecco's Modified Eagle Medium) (Gibco, USA) supplemented

with 2 mM L-glutamine enriched, fetal bovine serum (FBS) (Gibco, USA) and 1% Penicillin/ streptomycin antibiotic solution (Biosera, France) under controlled conditions of 37°C and 5% CO_2. The cells grew as a monolayer in the flask. This culture medium was changed three times a week, and sterile trypsin-EDTA solution was used to harvest the cells.

Evaluation of cytotoxicity effect of FCF by MTT assay

The MTT test is a quantitative and colorimetric assay based on the reduction of a yellow, water-soluble salt (3-(4, 5-dimethylthiazol-2-yl) -2, 5-diphenyl tetrazolium bromide) (MTT) (Sigma-Aldrich, Germany) and the formation of dark blue and insoluble formazan crystals in water. The regeneration of MTT occurs by the mitochondrial enzyme succinate dehydrogenase and only in living cells. Formazan crystals are soluble in organic solvents such as isopropanol and DMSO (Merck, Darmstadt, Germany), and by measuring their light absorption, the metabolic activity level of living cells can be determined (Ghasemi et al., 2021). The procedure was carried out in such a way that 104 cells were cultured in each well of a 96-well plate and after 24 hours and upon reaching approximately 80% confluency, the supernatant was exposed to FCF at time intervals of 24, 48, and 72 hours. Other culture plates were used as controls for the MTT test at intervals of 24, 48, and 72 hours. After the desired times passed, the plates were removed from the incubator, the supernatant of the wells was removed and 100 microliters of DMEM medium without alpha naphthol along with 10 microliters of MTT (5 mg/mL) was added to each well. Then the plates were again transferred to the incubator and kept at 37°C for 3 to 4 hours. In the next step, the produced formazan product was dissolved by adding 50 microliters of dimethyl sulfoxide (DMSO) solvent, and color intensity was measured by an ELISA reader at a wavelength of 570 nm. The biological capacity of cells treated with FCF was determined as the ratio of percentage of absorbance in comparison to the absorbance of formazan in the control group.

Evaluation of apoptosis by flow cytometry

To determine the percentage of apoptotic cells in a cell population treated with FCF and compare it with the control cell population, cells were stained with Annexin-V and propidium iodide (PI) (Sigma-Aldrich, Germany). After treatment of the cells with FCF at 24, 48, and 72 hours, the cells were trypsinized and washed with sterile phosphate-buffered saline (PBS). 100 microliters of binding buffer were added to the sediment resulting from the centrifugation of the cells in a 1.5 ml microtube. Next, 10 microliters of PI and 5 microliters of Annexin-V were added to the tube. The contents were gently mixed by manually shaking the microtube to ensure the cell pellet was fully resuspended. In the next step, the samples were incubated at room temperature (25°C) for ten minutes in the darkness. Finally, cell analysis was performed by flow cytometry (BD Biosciences, San Diego, CA, USA). Data analysis was done by using the software of the device and dividing the points recorded in the two-dimensional curve into four regions Q1 to Q4. To assess the effect of FCF on apoptosis and necrosis, the percentage of cells in each quadrant was calculated and reported using the flow cytometry analysis software (FCS Express).

Statistical analysis

GraphPad Prism 9 and SPSS version 2016 were used for statistical calculations. The collected data were analyzed using one-way ANOVA followed by Tukey's post hoc test. Assays were repeated three times. A p-value of less than 0.05 was considered statistically significant.

Results

Examining the effect of FCF on cell survival using MTT assay

According to Figure 1, the only significant change was observed at 24 hours, with a 16% decrease in survival in the samples treated with FCF.

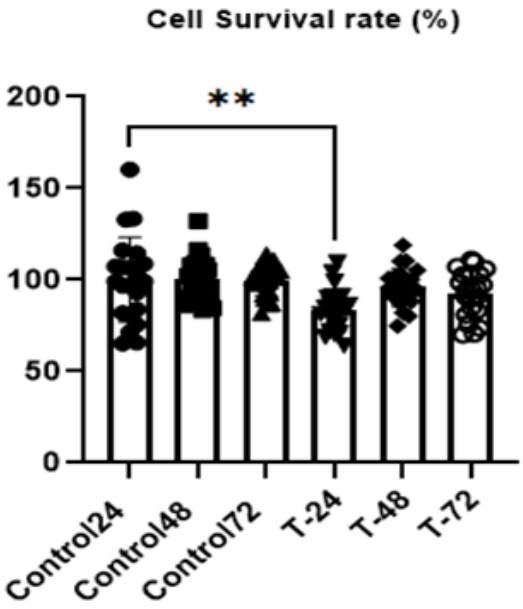

Figure.1. Survival changes based on the MTT test in this study; ** represents p-value=0.002. (T: Faradarmani Consciousness Field treatment). **: p-value< 0.01.

Assessment of the effect of FCF on apoptosis using flow cytometry

Figure 2 illustrates the apoptotic cells in three time periods of 24, 48, and 72 hours for both the treatment and control groups. As shown, the percentage of live cells (Q4 region) in the control group was 90.7%, 92.6%, and 74.6% at 24, 48, and 72 hours, respectively. In contrast, in the FCF-treated group, the corresponding percentages of live cells were 76.6%, 69%, and 49.6%, respectively.

The percentage of early apoptotic cells (Q3 region)—i.e., cells in the early stages of apoptosis— increased from 10.6% at 24-hour treatment period, and 20.2% at 48-hours to 33% at 72-hour. The percentage of late apoptotic or necrotic cells (Q2 area)—i.e., cells in the final stages of apoptosis—also increased with longer FCF exposure, rising from 4.42% at 24 hours to 6.69% at 48 hours and reaching 8.47% at 72 hours. Additionally, the percentage of necrotic cells (Q1 region) in the control group was 3.72%, 1.94%, and 6.91% at 24, 48, and 72 hours, respectively. In contrast, the corresponding percentages in the FCF-treated group were 8.40%, 4.07%, and 8.92%, respectively.

As shown in Figure 2, with the increase in the duration of the FCF effect, the lowest percentage of cells in the Q4 region was observed in the FCF-treated group at 72 hours, indicating the apoptotic effect of FCF treatment. According to the results of Table 1, a statistically significant difference was observed between the control group and the group under the effect of FCF treatment ($P<0.001$). In other analyzed hours, cases of statistically significant correlation were also seen. For example, based on the results of Figure 3, there was a statistically significant difference between the number of apoptotic and live cells in the treatment and control groups at 48 and 72-hour intervals under the effect of FCF ($P < 0.01$). The results showed that the percentage of early and late apoptosis and total apoptosis and were higher in FCF-treated cell lines compared to the control (Figure 3).

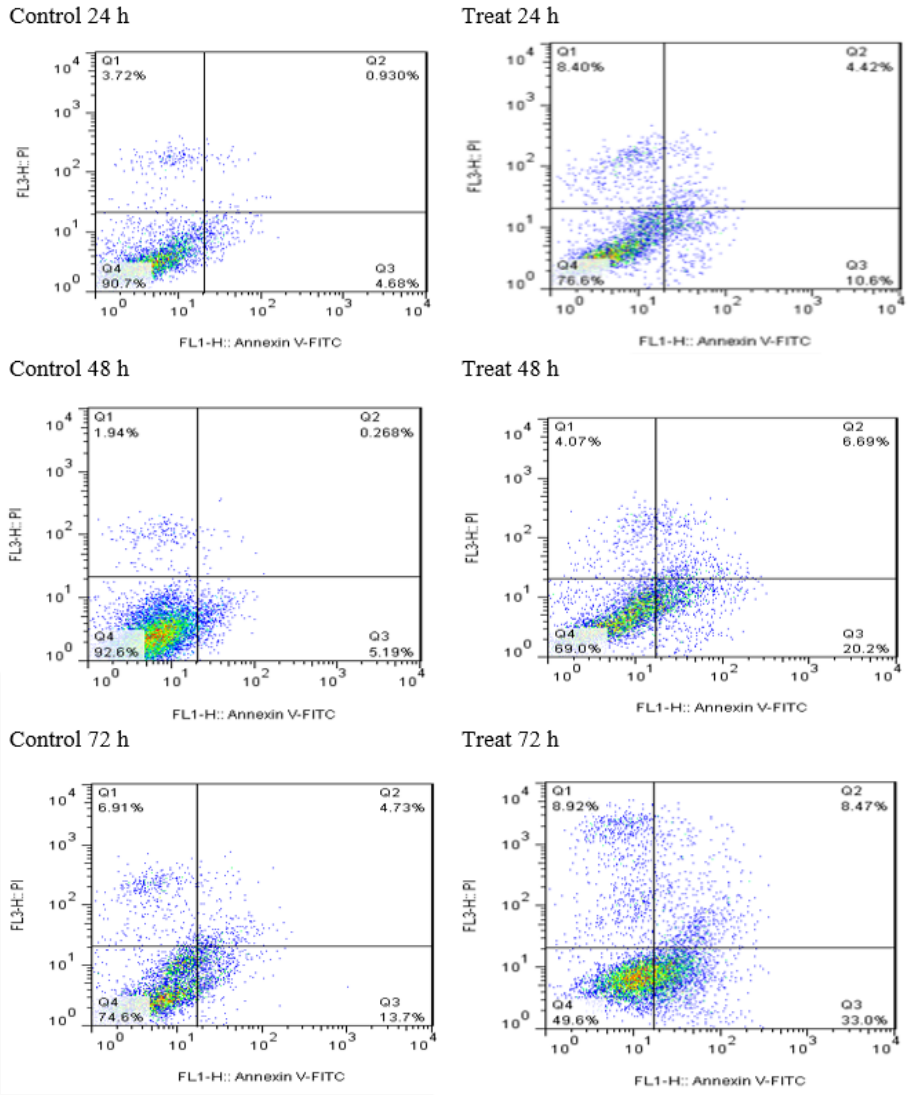

Figure.2: The effect of Faradarmani Consciousness Field (FCF) at time intervals of 24, 48 and 72 hours on apoptosis in MDA-MB-231 cells. Control group (cells without FCF treatment).

Q1: Necrotic cells (Annexin-V negative and PI positive), Q2: late apoptotic or necrotic cells (Annexin-V and PI positive), Q3: healthy cells: (Annexin-V and PI negative), Q4: early apoptotic cells (Annexin-V positive and PI negative)

Table 1. Percentage of cells at different stages of necrosis and apoptosis based on flow cytometry analysis, comparing the control and test groups treated with the Faradarmani Consciousness Field (FCF) in MDA-MB-231 cells over a 24-hour period.

	Q1	Q2	Q3	Q2+Q3	Q4
Control (-)	3.72%	0.93%	4.68%	5.61%	90.7%
FCF	8.40%	4.42%	10.6%	15.02%	76.6%
Difference of FCF from negative control	4.68%	3.49%	5.92%	9.41%	14.1%

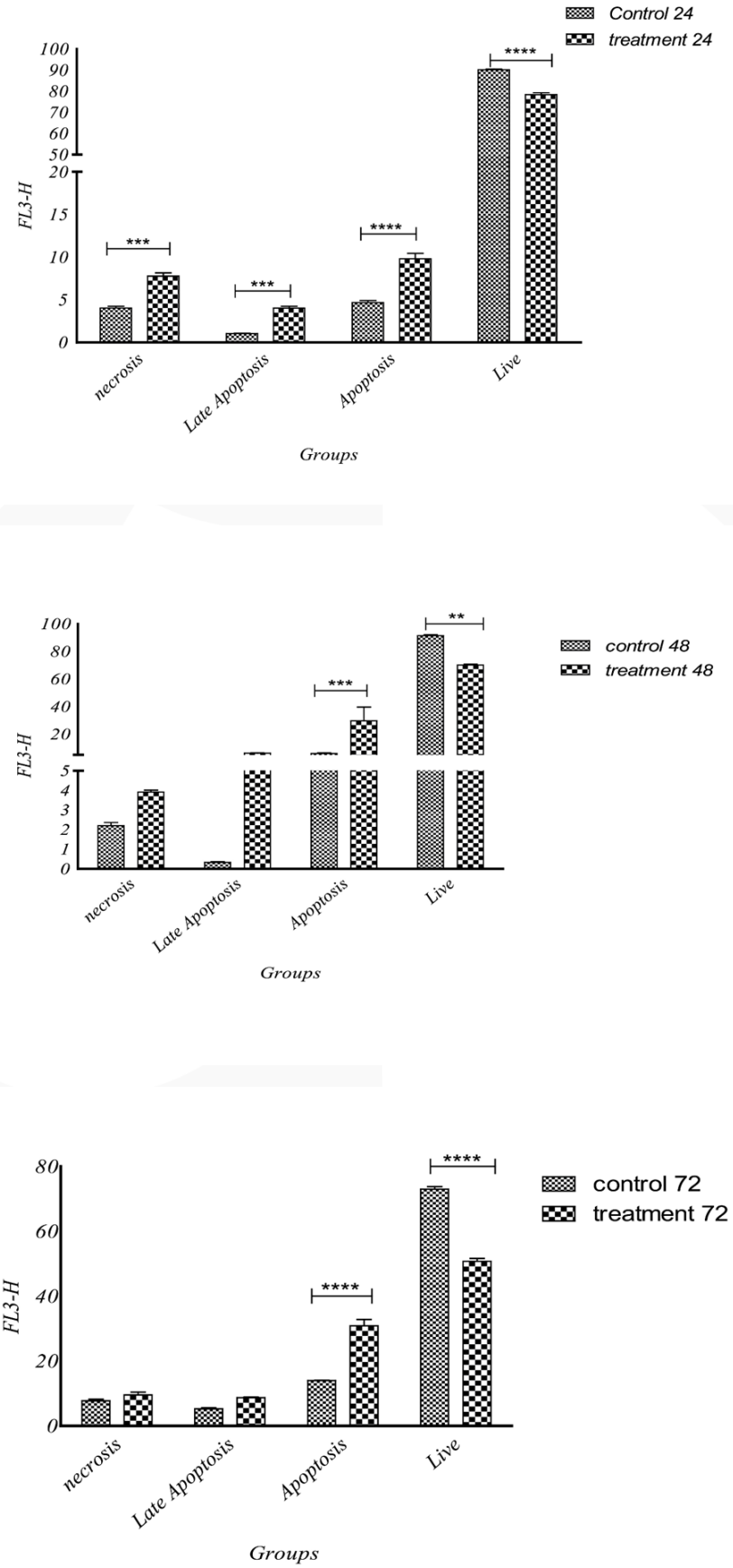

Figure 3. Chart showing changes in cell death at 24-, 48-, and 72-hour intervals in control and FCF-treated groups based on flow cytometry analysis. ****: Statistically significant ($p<0.0001$), ***, **: Statistically significant ($p<0.05$)

Discussion and conclusion

In recent years, the effects of TCFs have been investigated in a wide range of experiments. According to Taheri, these fields are neither matter nor energy, and it is not possible to measure them with conventional scientific tools, but it is possible to examine their effects on living organisms or non-living materials. One of the advantages of utilizing TCFs in the treatment of diseases like breast cancer is their drug-free approach which eliminates the risk of side effects and the need for any materials, equipment, or instruments to obtain the results. In addition, due to the nature of this filed, the application of TCFs is free of charge.

In the present study, the effect of Faradarmani Consciousness Field (FCF) on breast cancer cell line (MDA-MB-231) was investigated using MTT assay at 24, 48, and 72 hours. To determine the percentage of apoptotic and necrotic cells in a cell population treated with FCF and compare it to the cell population in the control group, cells were stained with Annexin-V and propidium iodide (PI). The results of MTT test showed that FCF caused a decrease in survival in the MDA-MB-231 cell line at 24 hours. These findings were confirmed by flow cytometry analysis. Flow cytometry analysis showed that a significant percentage of cells underwent programmed cell death (apoptosis) as a result of the treatment with FCF. The level of necrosis in treated cells was not significant compared to the percentage of apoptotic cells. There was a statistically significant relationship between the number of apoptotic cells and the number of live cells in both treatment and control groups at the 48- and 72-hour time points.

According to Taheri, the application of FCF is effective in repairing and modifying the system under study, restoring it to its optimal condition. From the perspective of Interuniversalism (Farakolnegari), proposed by Taheri, human existence consists of numerous hardware and software components. From this perspective, the part of the human being that forms the body is considered the "hardware" component that is operated by a large system of multiple software programs (the Operating System). In other words, behind the scenes of this hardware, there are a multitude of software programs that manage the physical body (Taheri 2011).

In conventional intervention methods, interventions affect the hardware. For example, in the treatment of various types of cancer, including breast cancer, a range of methods is used, such as surgery, radiotherapy and chemotherapy to specific and targeted therapies. Due to the biological and genetic differences in individuals, the conventional treatments in these patients have different responses and may be unsuccessful and carry a high risk of recurrence (Wayteck et al., 2014). But the application of Faradarmani Consciousness Field is a non-invasive method where the effects occur at the level of the software or infrastructure of the system under study, meaning FCF changes the behavior of the hardware by modifying the software (that runs the hardware) behind the scenes (Taheri et al., 2013). Previous studies have reported varying behaviors among different cell lines, suggesting that FCF exerts cell-specific effects (Taheri et al., 2023).

In conclusion, the findings of this study on the MDA-MB 231 cell line indicate that FCF can reduce cancer cell viability and promote programmed cell death (apoptosis). As a next step, we suggest that in future studies, the effect of FCF on the level of expression of apoptosis-inducing molecules such as Fas (CD95) be investigated. Further research is required to clarify the precise mechanisms through which FCF exerts its effects in biological systems.

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