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T-Consciousness Fields and Their Application in Cancer Research

Mohammad Ali Taheri
Originator of T-Consciousness Theory

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Editorial

T-Consciousness Fields and Their Application in Cancer Research

Mohammad Ali Taheri
Founder of T-Consciousness Theory



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Consciousness is one of the most complex subjects discussed in mainstream science, and many efforts have been made to understand its position, role, and function. Nevertheless, no single definition can fully capture this complex concept. In the collection of studies presented in this issue, a theory concerning the subject of Consciousness is introduced. To distinguish this theory from other theories, the term "T-Consciousness" has been used. In this approach, T-Consciousness is presented as the fundamental constituent of the universe, possessing a non-frequency nature and serving as the origin of matter, energy, and information. Furthermore, various T-Consciousness Fields (TCFs) with different functions are proposed as subsets of the "Cosmic Consciousness Network."

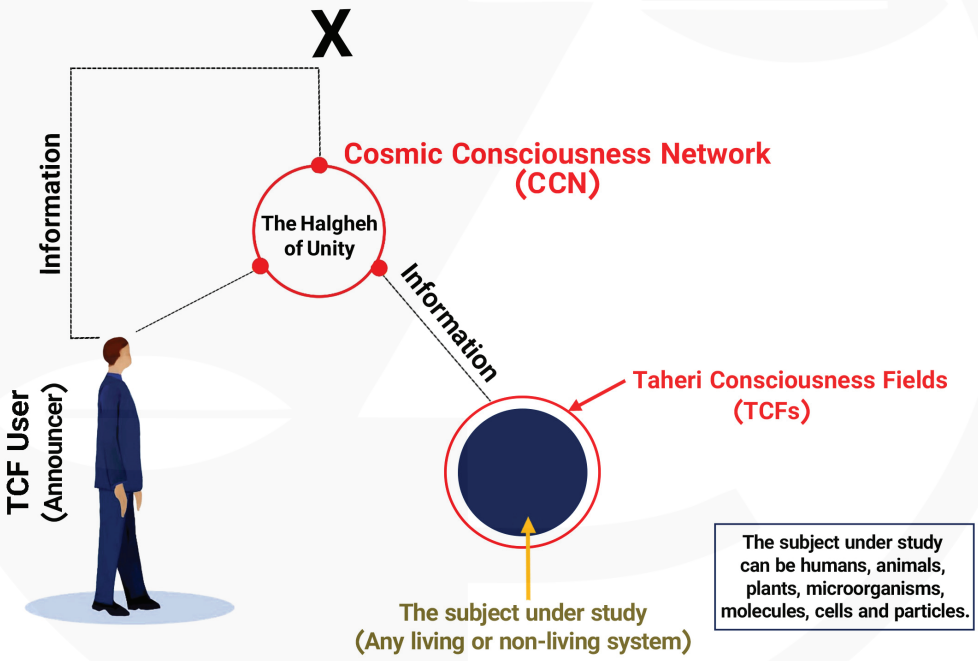
One of the most important challenges in consciousness studies is the possibility of operational experimentation, especially when consciousness is considered in a nonlocal form or beyond the brain and on the scale of the universe. The theory of T-Consciousness Fields TCFs, through the possibility of operational experimentation, provides researchers in this field with the opportunity to obtain practical evidence for the existence of consciousness and its position and role, thereby taking an important step toward understanding this complex concept.

To further explain this concept, the physical and observable aspects studied in mainstream science can be compared to a computer's hardware, which requires a software component with the capacity to process specific information in order to function properly. From the perspective of mainstream science, this software component, which is not directly observable, has remained hidden. For example, concepts such as mind, memory, information, consciousness, and similar phenomena cannot be regarded as hardware, nor can their properties be quantitatively measured and described using conventional instruments. The use of TCFs and the recording of their effects allow researchers to indirectly obtain practical evidence in this domain.

To evaluate the effects of TCFs, the experimental design compares changes in groups exposed to these fields with control groups or groups not exposed to TCFs. Using the methodology of mainstream science, the effects are recorded and, following statistical analyses, the results are reported. The proposed hypothesis is that information transmitted from TCFs may lead to changes in the samples exposed to the fields compared with the controls. In fact, this type of influence, which occurs without physical intervention and solely through a brief and instantaneous attention directed toward the subject under study, is referred to as a kind of "software effect."

It should be noted that the Faradarmani Consciousness Field has been introduced as a complementary medicine approach. This Consciousness Field is applied without any physical intervention and solely through a brief and instantaneous attention. It can be applied to a wide range of subjects, including living organisms as well as nonliving materials. The hypothesis proposes that information transmitted from TCFs induces changes in the samples under investigation.

In this issue, practical evidence supporting this hypothesis has been presented through the use of various cancer cell lines and experiments conducted under *in vivo, in vitro,* and *ex vivo* conditions. In addition, a study investigating the effects of the Faradarmani Consciousness Field on stem cells has been carried out. Further studies are recommended to better understand the effects of TCFs.



A schematic on applying T-Consciousness Fields (TCFs). The effect of TCFs begins with connecting to the Cosmic Consciousness Network (CCN) and through the Faradarmangar (announcer). Variable T-Consciousness Fields are a subset of CCN, and by applying each TCF, specific information is transmitted. In this way, the subject of study, which can be living or non-living creatures, is exposed to this information. It should be noted that TCFs and the information do not have a material or energetic nature; therefore, they cannot be measured directly and quantitatively. However, it is possible to record and examine their effects by designing different experiments. For this purpose, the behavior or indicators measured by the researchers in the subject under study after being exposed to the TCFs are compared with the control samples (without the effect of TCFs), and the results are reported after statistical analysis.

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Considerations of This Issue Introduction

1-1 T-Consciousness and the New Science of Sciencefact

In the past few decades, the nature of Consciousness and its place in science has received considerable attention. Many philosophical and scientific theories have been presented so far in this field. In the 1980s, Mohammad Ali Taheri introduced new fields of non-material and non-energy nature, known as T-Consciousness Fields (TCFs). In Taheri's view, T-Consciousness, along with matter and energy, are the three main constituents of the universe, with T-Consciousness being different from matter and energy. According to his theory, there are a wide variety of TCFs, with each having certain functionalities. TCFs are also considered a subset of "Cosmic Internet Network" in Taheri's theory, which is named the Cosmic Consciousness Network (CCN).

The main difference between the theory of TCFs and other concepts introduced so far for describing the nature of consciousness is the applicability and practicability of TCFs. In other words, these fields can be applied to all living organisms and non-living objects, such as plants, animals, microorganisms, materials, molecules, atoms, etc. In this respect, Mohammad Ali Taheri introduced "Sciencefact" in 2020 as one of the subgroups of the "Erfan-e-Keyhani-e-Halgheh" school, which he had previously founded. The name "Sciencefact" was chosen to confirm the existence of T-Consciousness as a "fact" scientific research method is utilized. Although common science merely considers the study of matter and energy, Sciencefact investigates the effects of TCFs (which are neither material nor energy) on matter and energy and all their manifestations (such as humans, animals, plants, microorganisms, cells, materials, molecules, atoms, etc.). By repeatably conducting laboratory research experiments in various fields of science and applying TCFs, Sciencefact has emerged as a common ground between science and TCFs and uses this capability to investigate T-Consciousness and T-Consciousness Fields resulting from it.

The influence of TCFs begins with the connection (Etesal) between the Cosmic Consciousness Network as the Whole Consciousness and the subject under study as a component. The connection is established by the mind of the Faradarmangar (a person who has been trained to assign TCFs). The human mind has the role of an intermediary (announcer) that acts with short and immediate attention to the subject under study, and the main achievement is obtained due to the effects of TCFs. These fields cannot be directly measured by science, but their effects on various subjects can be investigated through repeatable experiments

1-2 Methodology of T-Consciousness Fields Research

The research methodology followed in the study of T-Consciousness is based on *Assumption, Argument, and Proof*:

The basic *Assumption* is that the universe is formed by a third element, called T-Consciousness, and that is different from matter and energy.

The *Argument* is that the existence of TCFs can be shown through their effects on matter and energy (e.g., humans, animals, plants, microorganisms, cells, materials, molecules, atoms, etc.)

The *Proof* is the scientific verification of the TCFs' effects on matter and energy (according to the *Argument*) through various reproducible scientific experiments

1-3 Study phases in Sciencefact

To investigate and verify the existence, effects, and mechanisms of TCFs, the five following research phases (Phase 0 to Phase 4) and their objectives are outlined below:

In Phase 0 of the studies, the goal is to demonstrate the existence of TCFs by observing their influence on matter and energy. The nature of T-Consciousness

and what it is will not be addressed in this phase. Phase 1 is dedicated to exploring various effects of different TCFs. In Phase 2, one examines the reasons behind the effects of these fields. Then, during Phase 3, the mechanisms of TCFs' effects on matter and energy are investigated. Finally, the goal of Phase 4 is to draw conclusions, particularly with regard to the *mind and memory of matter* and their relation to T-Consciousness, etc.

1-4 Using T-Consciousness Fields

The samples under study were subjected to T-Consciousness Fields (TCFs) according to the specified protocol on the website of Research Management on T-Consciousness Fields (www.cosmointel.com). The request for *Etesal* (connection) to the Cosmic Consciousness Network to use TCF can be submitted through the Cosmointel website in the "Assign Announcement" section. This access is freely available to everyone. Researchers can register on this website anytime to experience TCFs and conduct research in this area. Detailed information about the experiment needs to be provided to the research center; for example, the number and name of samples and controls must be specified. These studies were conducted in a double-blind manner, where lab technicians were completely unaware of TCFs theory, and the Faradarmangar at the COSMOintel research center who established the consciousness bond was unaware of the details of the study. Double-blindness is a gold standard that is common in science experiments in the field of medicine and psychology, involving theoretical and practical testing.

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Effects of the Taheri Consciousness Fields on Rat C6 Glioma Cell Line

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Abstract

The most common primary spinal cord and brain tumors are gliomas, which represent 81% of malignant brain tumors. In neuro-oncology investigations, the rat C6 glioma cell line has been used most often as an experimental model. Taheri Consciousness Fields (TCFs), introduced by Mohammad Ali Taheri, are novel fields that are neither matter nor energy. Therefore, they are non-quantifiable and cannot be directly observed or measured. However, it is possible to demonstrate and measure the effects of these fields through standard scientific experiments. This study aimed to evaluate the effects of TCF-1 and TCF-2 on the C6 glioma cell line, compared with the untreated control group. The morphology and microscopic characteristics of the cells were evaluated. Also, trypsinized and detached cells were stained with trypan blue, and then dead cells were counted. To evaluate the effect of TCFs on cell viability, the MTT assay was used. The expression of *Bax* and *Bcl-2* genes was assessed using reverse transcription quantitative PCR (RT-qPCR). The results showed that TCFs treatment not only reduced the number of C6 glioma cells but also changed their morphology. Similarly, in the trypan blue staining method, more dead cells were counted in the TCF groups compared to the control group. In the MTT assay, both TCFs exhibited cytotoxic effects on C6 glioma cells at the incubation times tested ($p < 0.05$). *Bax/Bcl2* ratio increased 3.5-fold and 7-fold compared to the control via TCF-1 and TCF-2, respectively ($p < 0.05$). The findings suggest that TCF-1 and TCF-2 can induce apoptosis in rat C6 glioma cells. The mechanism of action of TCFs is still not well understood, and studies of the effects of TCFs *in vivo* and in clinical research could be useful to clarify their therapeutic effects.

Keywords: T-Consciousness Fields, C6 glioma cells, cell viability, cancer

Introduction

The most common tumors of the central nervous system (CNS) are gliomas, which originate from glial cells (Louis et al., 2016). The prevalence and mortality of gliomas are expected to increase, especially in developing countries (A Ghotme et al., 2017). The overall survival rate of patients with glioma is about 20 to 36 months, while the survival in the most malignant types is not more than 14 months. In recurrent cases of malignant glioma, the average time to tumor progression is only 8 weeks despite routine treatment (Ashby et al., 2004). Even with progress in combination therapy with chemotherapy, radiotherapy, and surgery, the glioma patient's prognosis is still extremely poor (Hanif et al., 2017). Given the poor outcomes and slowly developing technologies in radiotherapy and surgery, the innovation and application of new methods are crucial. Although several drugs have been developed to treat cancer in recent decades, few drugs have been approved by the U.S. Food and Drug Administration (FDA) to treat glioma. One reason for the lack of progress in glioma treatment is the blood-brain barrier, which limits the delivery of therapeutic agents to the brain. The unique CNS structure inhibits the entry of most anticancer drugs into the brain, posing challenges in the progress of antiglioma drugs (Ballabh et al., 2004; Oberoi et al., 2015). No single strategy is strong enough to make significant progress in treating glioma, so the use of novel strategies may lead to successful solutions.

Mohammad Ali Taheri introduced novel fields with a non-material/non-energetic nature named Taheri Consciousness Fields (TCFs). From this perspective, T-Consciousness is the main component of the universe, and its matter, energy, and information are derived from it. According to this theory, there are various TCFs with different functions that have a non-frequency nature. These fields cannot be directly measured using current scientific methods, but their effects can be investigated across various subjects through reproducible laboratory experiments. The effect of TCFs begins with the brief and immediate attention of the Faradarmangar (trained individual) to the subject of study (Taheri, 2013).

In previous research, we observed the effects of the TCFs on the MCF7 cancer cell line (Taheri et al., 2020a), spatial memory and avoidance behavior of a rat model of Alzheimer's disease (Taheri et al., 2021a), wheat plant (Torabi et al., 2020), bacterial population growth (Taheri et al., 2021b), and the electrical activity of the brain during Faradarmani in the Faradarmangars population (Taheri et al., 2020b).

This study aimed to evaluate the effect of T-Consciousness Fields (1 and 2) with in the C6 glioma cell line compared with the untreated control group by evaluating the number of dead cells, morphology and microscopic features in cell culture, then evaluating cell viability and, in the next stage, quantitatively evaluating the expression of two genes related to apoptosis.

Methods and Materials

Application of the Taheri Consciousness Fields

TCFs were applied to the samples according to the protocols regulated by the COSMOintel Research Center (www.COSMOintel.com). More details are provided in the Common Considerations section of this issue. Announcement programs in this study are shown in Table 1.

Table1. Announcement programs for treatment and control groups

Groups	Number of Announcements	Announcement intervals after seeding of cells			
TCF-1	3	20 min	24 hours	48 hours	
TCF-2	4	20 min	24 hours	48 hours	72 hours
Control	0	-	-	-	-

Cell culture and trypan blue staining assay

C6 glioma was prepared from the National Cell Bank of Iran. The cells were cultured in DMEM/F12 media supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin, and incubated at 37 °C, 90% humidity, and 5% CO2.

Trypan blue is a vital dye that leaves living cells unstained, while non-living cells appear blue under a microscope. Living cells have a healthy cell membrane and therefore do not take in the stain.

Cells were detached from 6-well plates using trypsin, and one part of the cell suspension and one part of 0.4% trypan blue were mixed and incubated at room temperature for 3 minutes. Then, dead and alive cells were counted using a hemocytometer, and the percentage of dead cells was determined. Three replications were considered for all groups. The formula for the calculation of the percentage (%) of dead cells stained with trypan blue (Strober, 2015):

$$\text{Dead cells (\%)} = (A_{\text{Dead cells}}/A_{\text{All counted cells}}) \times 100$$

MTT assay

To accurately evaluate the effects of TCF-1 and TCF-2 on cell viability relative to the untreated group (control), the MTT method was used. This method is a colorimetric method based on the reduction of yellow tetrazolium by the enzyme succinate dehydrogenase. First, cells were seeded into each well of the plate (10,000 cells per well). After 48 and 72 hours for the TCF-1 and TCF-2 groups, respectively, following microscopic examination of cells, the medium in wells was replaced with MTT solution (0.5 mg/ml MTT in fresh medium). Plates were incubated for 4 hours at 37 °C and 5% C. Then, the supernatant was removed, and 100 µL dimethyl sulfoxide (DMSO) was added. The plates were shaken for 10 minutes. The absorbance of wells was measured and reported at 570 nm with an ELISA reader (BDSL Immunoskan MS, Finland). In order to estimate the percentage of living cells, the following formula was used (Chueh et al., 2014), and all experiments were repeated three times:

$$\text{Cell Viability (\%)} = \frac{OD\ test - OD\ blank}{OD\ control - OD\ blank} \times 100$$

Evaluation of gene expression by the RT-qPCR method

Expression of *Bax* and *Bcl-2* genes was assessed in C6 glioma cancer cells using reverse transcription quantitative PCR (RT-qPCR). For this purpose, the Favor Prep total RNA Isolation Kit (Favorgen, Taiwan) was employed. Extraction steps were performed according to the kit instructions. After extracting RNA from each sample, the quantity and purity of the obtained RNA were evaluated using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific). For cDNA synthesis, we used the cDNA synthesis kit (Biotechrabbit GmbH, Germany) according to the manufacturer's instructions. The qPCR reaction was performed in a Mic real-time PCR cycler (BioMolecular Systems). The final volume of each reaction was 20 µL, containing 10 picomoles of reciprocating primers, 40 ng cDNA, and 10 µL SYBR green PCR master mix (Ampliqon, Denmark). In this study, the *GAPDH* gene was considered the reference gene. The primer sequences used in the present study are listed in Table 2.

Table 2. Name, length, annealing temperature, and sequence of primers used in qPCR

Target Gene	Sequence of primers	Length	Annealing Temperature
Bax	F: 5'-TTGCTTCAGGGTTTCATCCAG-3' R: 5'-AGCTTCTTGGTGGACGCATC-3'	101 bp	65
Bcl-2	F: 5'-TGTGGATGACTGAGTACCTGAACC-3' R: 5'-CAGGCAGGAGAAATCAAACAGAG-3'	122 bp	66
GAPDH	F: 5'-CGTCTGCCCTATCAACTTTCG-3' R: 5'-CGTTTCTCAGGCTCCCTCT-3'	74 bp	63

The following conditions were applied for the PCR reaction: first incubation step was 94°C for 12 min, followed by 40 cycles for amplification, each cycle, including a denaturation step for 15 s at 94°C, an annealing step for 15 s at 62- 67°C, and an extension step for 10 s at 72°C. To verify the specificity of the amplicons, the melting curve was analyzed. The comparative expression of genes was assessed using the standard $2^{-\Delta\Delta ct}$ method (Livak et al., 2001; Arocho et al., 2006).

Statistical analysis

In this study, all tests were repeated three times and the values were calculated as mean ± SD. One-way ANOVA was used to assess the effects of TCFs on cell viability and gene expression, and a p-value < 0.05 was considered significant.

Results

Evaluation of morphology and microscopic properties of cells

C6 glioma cell counts after 72 hours showed that the average cell counts for the control, TCF-1, and TCF-2 groups were 2.0×10^5 cells/mL, 1.5×10^5 cells/mL, and 1.4×10^5 cells/mL, respectively.

In addition to reducing the number of cells, notable effects of TCFs on cancer cells included changes in morphology, the appearance of the nucleus, and cell widening. In the TCFs groups, intercellular gaps remained visible, and empty spaces remained on the cell culture plate after 24, 48, and 72 h. Figure 1 shows microscopic evaluation of control C6 glioma cells and cells exposed to the effect of the TCF-2 at 24, 48, and 72 hours after culture.

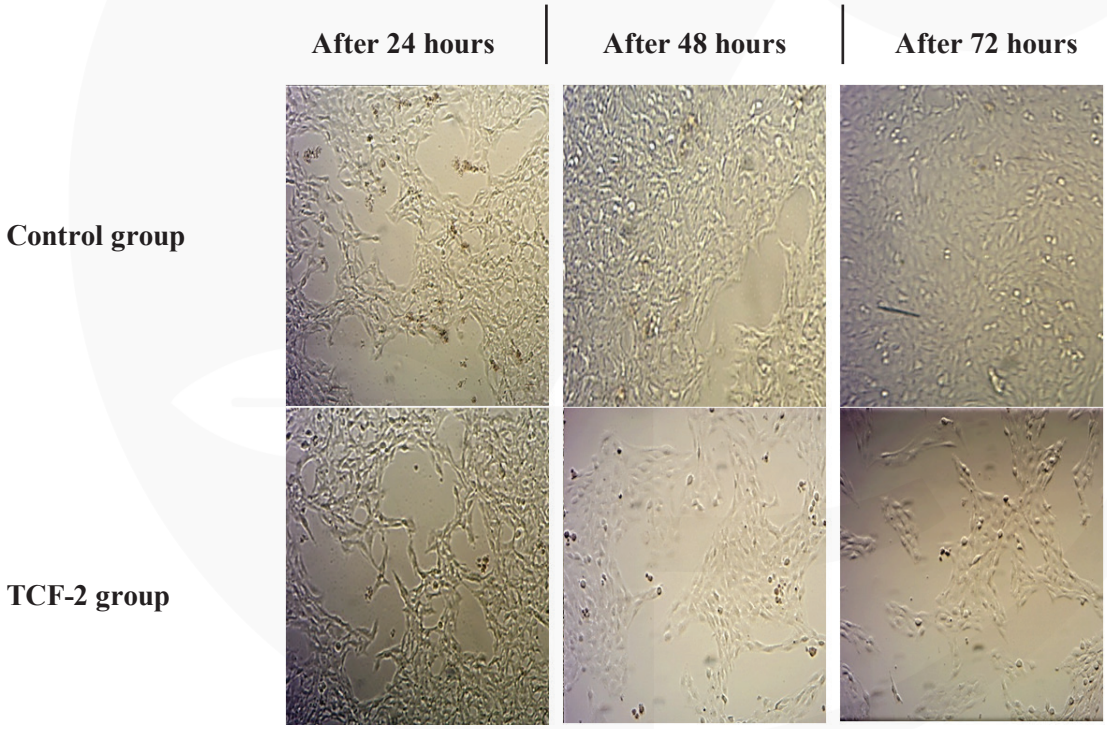


Figure 1. The microscopic evaluation of control and TCF-2 affected cells at 24, 48, and 72 hours after culture. In the TCF-2 group, cells did not fully occupy the culture surface compared to the control group, and intercellular gaps remained visible.

Trypan blue staining assay

On average, in the trypan blue dye count, about 12% and 9% more dead cells were counted in the TCF-2 and TCF-1 groups, respectively, than in the control. This shows TCF-induced effect on C6 glioma cell death.

MTT test results of the C6 glioma cell

In order to evaluate the viability of TCF-1 and TCF-2 treated cells, the MTT assay was used. Results showed that both TCFs significantly decreased cell viability compared with the control group ($p < 0.05$).

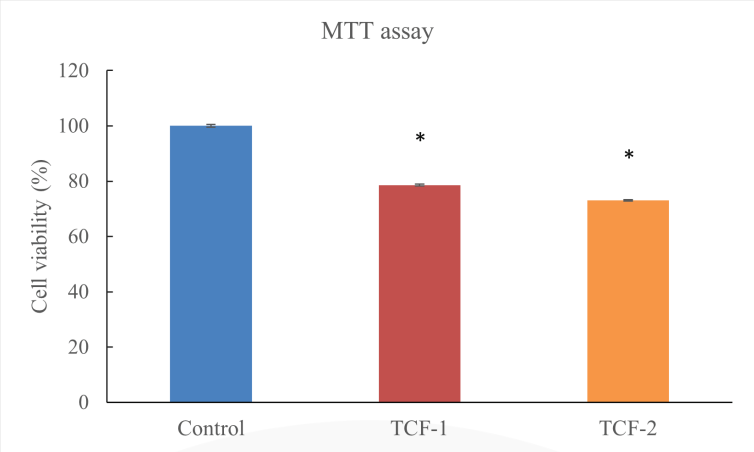
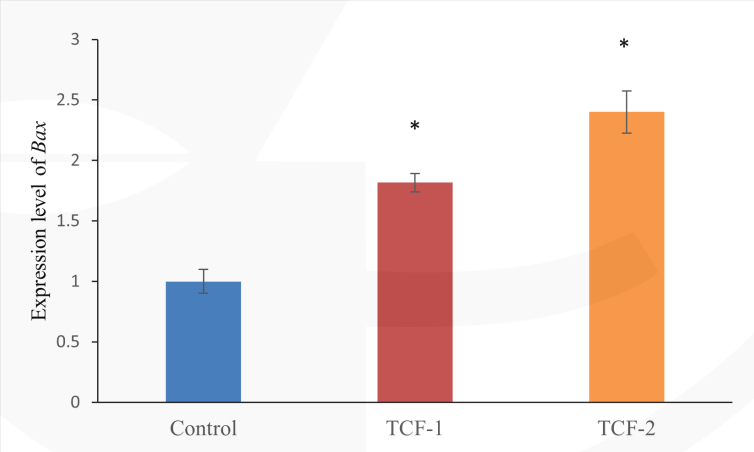
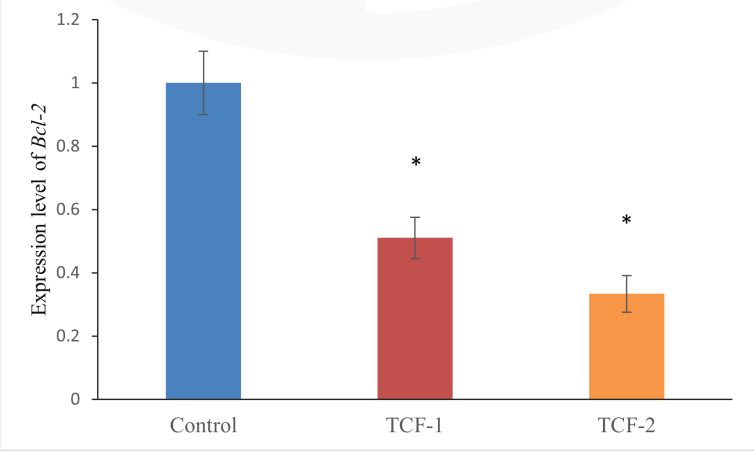


Figure 2. Viability of TCF-1 and TCF-2 treated cells compared with the control group. The asterisk (*) indicates a statistically significant difference ($p < 0.05$) between the experimental and control groups.

Bax and *Bcl-2* genes expression in TCF-1 and TCF-2 treated C6 glioma cells

The present study showed alterations in the expression of *Bcl-2* (anti-apoptotic) and *Bax* (pro-apoptotic) genes, as well as the *Bax/Bcl-2* ratio, after stimulating an apoptotic state via TCF-1 and TCF-2 in the C6 glioma cell line. The expression of the *Bax* gene level was increased in both TCF-1 and TCF-2 treated cells compared with the control (Figure 3a). The *Bcl-2* expression was decreased in both TCFs groups significantly ($p < 0.05$; Figure 3b). The ratio of *Bax/Bcl-2* is an indicator for determining cell susceptibility to apoptosis. *Bax/Bcl-2* ratio was significantly increased in the TCFs treated cells ($p < 0.05$; Figure 3c).





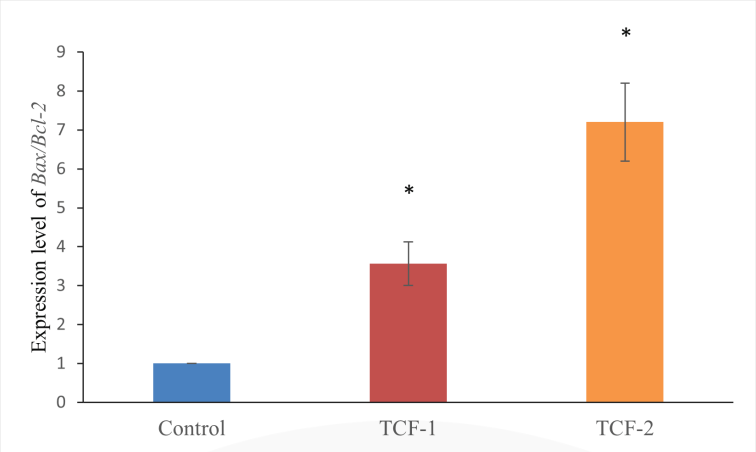


Figure 3. Expression of *Bax* (a) and *Bcl-2* (b) and the ratio of *Bax/Bcl-2* (c) in TCF-1 and TCF-2 treated cells compared with control. The asterisk (*) indicates a significant difference between the TCFs and control groups ($p < 0.05$).

Discussion

In the present study, the effects of two types of TCFs (1 and 2) on the number of dead cells, morphology, and microscopic characteristics in rat C6 glioma cell culture were evaluated. In addition, the expression of the *Bax* apoptotic and *Bcl-2* anti-apoptotic genes under the influence of TCFs was investigated. Using the MTT assay to evaluate cytotoxicity induced by TCF-1 and TCF-2, we found that both TCFs displayed cytotoxic effects at the specified incubation times in C6 glioma cells ($p < 0.05$). In the trypan blue dye count, more dead cells were counted in the TCF groups than in the control. This observation may be related to the inducing effect of the TCFs on the death of C6 glioma cells.

The Bcl-2 family members play a critical role in the regulation of the death or survival of the cells. Chemotherapeutic drugs partially control the expression of various members of the Bcl-2 family in cancerous cells. The members of this family contribute significantly to apoptosis via activation (Bax) or inhibition (Bcl-2) (Green et al., 1998; Rao et al., 1997). In the present study, *Bax* expression increased, and *Bcl-2* expression decreased in both the TCF-1 and TCF-2 groups compared with the control.

The *Bax/Bcl-2* ratio helps determine cell fate (Gross, 2001). *Bax/Bcl2* ratio increased 3.5 and 7-fold compared to control via TCF-1 and TCF-2, respectively ($p < 0.05$), which significantly increased the apoptosis induced by TCF-1 and TCF-2.

In the previous study, the effect of Faradarmani CF on MCF7 cells was evaluated. The Faradarmani CF was applied every hour until the end of the experiment groups, which were at 6, 18, and 24 hours from the initial start time. The MTT assay assessed cell viability, and the expression of *Bax* and *Bcl-2* in MCF-7 cells was measured by RT-qPCR. Faradarmani CF significantly increased MCF-7 cell proliferation (18%) compared to the control in a time-dependent manner. Moreover, the RT-qPCR results showed that in cells treated with Faradarmani CF, the *Bax/Bcl2* ratio decreased (1-fold) compared with control, suggesting higher MCF7 cell survival and resistance to death (Taheri et al., 2020a).

The effect of TCFs on cancer cells appears to vary depending on the type of cancer cells, the duration, and the frequency of announcements. The findings indicate that it is necessary to investigate other TCFs further and conduct laboratory tests across a variety of areas.

The mechanism of action of TCFs remains poorly understood, and this study suggests that *in vivo* and clinical research can help elucidate the effects of TCF treatment.

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Conflicts of Interest

The authors declare no conflict of interest.

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Effects of Taheri Consciousness Fields on the HT29 Human Colorectal Cancer Cells

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Abstract

Colorectal cancer accounts for 11% of all diagnosed cancers and is the second deadliest cancer worldwide. Surgery, chemotherapy and targeted therapy are generally used for the treatment of colorectal cancer. However, there are significant challenges, such as recurrence of the tumor and drug resistance, so the application of novel methods is required for the treatment of this cancer. T-Consciousness Fields (TCFs) were introduced by Mohammad Ali Taheri. These new fields are neither energy nor matter and cannot be measured directly. However, we can evaluate the effects of TCFs indirectly through various kinds of research in the laboratory. This study aimed to evaluate the effect of TCF-1 and TCF-2 on HT29 human colon cancer cells compared with the control group. The morphology and microscopic properties of cells were investigated in the TCF-1 and TCF-2 groups compared with the control, and the cells were detached and stained with trypan blue, and then the dead cells were counted. To evaluate the effect of the TCFs on cell viability, the MTT assay was used. The expression of two apoptosis-related genes (*Bax* and *Bcl-2*) was assessed using reverse transcription quantitative PCR (RT-qPCR). The results showed that TCFs decreased the number of cells and changed the morphology of HT29 cells. In the trypan blue staining method, more dead cells were counted in the TCF groups compared to the control group. In the MTT assay, both TCFs decreased the viability of cells during incubation times ($p < 0.05$). *Bax/Bcl2* ratio increased 4.9-fold and 7.6-fold in TCF-1 and TCF-2 treated cells, respectively ($p < 0.05$). Therefore, TCF-1 and TCF-2 induced apoptosis in HT29 cells. There are still many ambiguities and questions about the nature and effect of TCFs. To clarify this issue, more research is needed *in vitro, in vivo*, and clinically.

Keywords: Taheri Consciousness Fields, HT29 human colon cancer cells, cell viability

Introduction

The third most common type of cancer is colorectal cancer, and it is the second leading cause of cancer-related mortality rates worldwide. It is estimated that more than 1.8 million colorectal cancer cases and 881,000 related deaths occurred in the world in 2018 (Ferlay et al., 2019). Although the highest incidence of colorectal cancer is still seen in Western economically developed countries, it has recently increased rapidly in other parts of the world (Brenner et al., 2018). Although modern research has elucidated the pathogenesis of colorectal cancer and provided advanced screening strategies, the incidence of colorectal cancer continues to increase (Siegel et al., 2020). The current standard treatment of colorectal cancer is removing the tumor with surgery, then adjuvant chemotherapy or targeted therapy. However, tumor recurrence and drug resistance are important challenges in treating colorectal cancer (Wolpin et al., 2008; Gao et al., 2021). The median overall survival of patients with metastatic colorectal cancer is approximately 30 months, indicating a poor prognosis in these patients (Van Cutsem et al., 2016). For this reason, innovative and more effective approaches are essential for the treatment of metastatic disease.

According to Taheri's theory, T-Consciousness is the main component of the universe, and its matter, energy, and information are derived from it. According to this theory, there are various TCFs with different functions that have a non-frequency nature. These fields cannot be directly measured by science, but their effects can be investigated across various subjects through reproducible laboratory experiments. The effect of TCFs begins with the brief and immediate attention of the Faradarmangar (trained individual) to the subject of study (Taheri, 2013).

In previous research, we observed the effects of the TCFs on the MCF7 cancer cell line (Taheri et al., 2020a), spatial memory and avoidance behavior of a rat model of Alzheimer's disease (Taheri et al., 2021a), wheat plant (Torabi et al., 2020), bacterial population growth (Taheri et al., 2021b), and the electrical activity of the brain during Faradarmani in the Faradarmangars population (Taheri et al., 2020b).

This study aimed to evaluate the effect of T-Consciousness Fields (1 and 2) on HT29 human colon cancer cells compared with the untreated control group. The comparison was made by counting and evaluating the number of dead cells, morphology, and microscopic characteristics in cell culture. MTT test to compare cell viability in the treated and untreated groups was performed. Also, quantitative evaluation of *Bax* apoptotic and *Bcl-2* anti-apoptotic genes expression using reverse transcription quantitative PCR (RT-qPCR) was performed.

Methods and Materials

Application of the Taheri Consciousness Fields

TCFs were applied to the samples according to the protocols regulated by the COSMOintel research center (www.COSMOintel.com). More details are provided in the Common Considerations section of this issue. Announcement programs in this study are shown in Table 1.

Table 1. Announcement programs for the TCFs treatment and control groups.

Groups	Number of Announcements	Announcement intervals after seeding of cells		
TCF-1	2	20 min	24 hours	
TCF-2	3	20 min	24 hours	48 hours
Control	0	-	-	-

Cell culture and study of the morphology and microscopic properties of cells

The HT29 human colorectal cancer cells are not only utilized to investigate the biology of human colorectal cancers but also receive special interest

in studies of food bioavailability and digestion because of their capability to express properties of mature intestinal cells (Martínez-Maqueda et al., 2015). HT29 human colorectal cancer cells were obtained from the National Cell Bank of Iran. RPMI 1640 media (Gibco, Germany) with 10% fetal bovine serum (FBS) (Gibco, Germany) and 1% streptomycin/ penicillin (Sigma Aldrich, Germany) was utilized for cell culture, and the cells were incubated at 37 °C, 90% humidity, and 5% CO2. The morphology and microscopic properties of cells were evaluated in the TCFs treatment and control groups.

Trypan blue staining assay

Trypan blue is a vital stain that stains dead cells blue, leaving living cells unstained, as seen under a microscope. Living cells do not absorb stains because they have a healthy cell membrane (Ludford, 1929).

For the trypan blue staining assay, cells were detached from 6-well plates by trypsin, then an equal volume of cell suspension and trypan blue (0.4%) was mixed and incubated for three minutes at room temperature. Then, dead cells were counted using a hemocytometer, and the ratio of living to dead cells was calculated. Three replications were considered for all examinations. The formula for the calculation of the percentage (%) of dead cells stained with trypan blue (Strober, 2015):

$$\text{Dead cells (\%)} = (A_{\text{Dead cells}}/A_{\text{All counted cells}}) \times 100$$

MTT assay

MTT assay was used to determine the effect of the TCF-1 and TCF-2 on the viability of HT29 cells. First, the HT29 cells were seeded in 96-well microplates (10,000 cells per well). After 24 hours for the TCF-1 group and 48 hours for the TCF-2 group, the medium in the wells was replaced with MTT solution (0.5 mg/mL), and the microplates were incubated at 37 °C under 5% CO2 for 4 hours. Then, the MTT solution was replaced with 100 µL dimethyl sulfoxide (DMSO). A plate reader (BDSL Immunoskan MS, Finland) was used to measure the absorbance of the wells at 570 nm. All tests were repeated three times. To evaluate the cell viability, the following formula was utilized (Chueh et al., 2014):

$$\text{Cell Viability (\%)} = \frac{OD\ test - OD\ blank}{OD\ control - OD\ blank} \times 100$$

Evaluation of gene expression by the RT-qPCR method

The expression of *Bax* and *Bcl-2* in HT29 cells was assessed by RT-qPCR using the Favor Prep total RNA Isolation Kit (Favorgen, Taiwan). Extraction steps were performed according to the kit instructions. After extracting RNA from each sample, the quantity and purity of the obtained RNA were evaluated through the NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific).

The cDNA was synthesized using a cDNA synthesis kit (Biotechrabbit GmbH, Germany) following the manufacturer's instructions. The qPCR reaction was performed in a MIC real-time PCR system (BioMolecular Systems, London, UK). The final volume of each reaction was 20 µl, containing 10 picomoles of reciprocating primers for the *Bax* and *Bcl-2* genes, 40 ng cDNA, and 10 µL SYBR green PCR master mix (Ampliqon, Denmark). In this study, the *GAPDH* gene was considered the reference gene. The primer sequences used in the present study are listed in Table 2.

Table 2. Name and properties of primers used in qPCR.

Target Gene	Sequence of primers	Length	Annealing Temperature
Bax	F: 5'-TTGCTTCAGGGTTTCATCCAG-3' R: 5'-AGCTTCTTGGTGGACGCATC-3'	101 bp	65
Bcl-2	F: 5'-TGTGGATGACTGAGTACCTGAACC-3' R: 5'-CAGGCAGGAGAAATCAAACAGAG-3'	122 bp	66
GAPDH	F: 5'-CGTCTGCCCTATCAACTTTCG-3' R: 5'-CGTTTCTCAGGCTCCCTCT-3'	74 bp	63

For the PCR reaction, the following conditions were used: the first step was incubation at 94°C for 12 min, followed by 40 cycles for amplification, each cycle consists of a denaturation step for 15 seconds at 94°C, an annealing step for 15 seconds at 62-67°C, and an extension step for 10 seconds at 72°C. Melting curve analysis was performed to confirm the specificity of amplicons. The comparative gene expression was calculated with the standard $2^{-\Delta\Delta ct}$ (Livak et al., 2001; Arocho et al., 2006).

Statistical analysis

In this study, all tests were repeated three times and calculated as mean ± SD. One-way ANOVA was applied to evaluate the TCFs effects on cell viability, gene expression level examinations, and $p < 0.05$ was considered the significant level.

Results

Evaluation of morphology and microscopic properties of cells

In the microscopic examination of HT29 cells in the TCFs groups, the cell colonies were smaller, and the cells were rounder, and less accumulation was seen. Also, the time required for cells to detach after trypsinization from the plate, which is usually three to five minutes, was reduced to two minutes in the treatment group (this could be due to cell weakness and mortality). The cell confluency of the TCFs groups was significantly lower than that of the control group, and after incubation times, there were still empty spaces in the cell culture plate. Figure 1 shows microscopic evaluations of control HT29 cells and cells exposed to the TCF-2 at 24 and 48 hours after culture.

Trypan blue staining assay

The number of dead cancer cells of both TCFs groups in trypan blue dye was counted on average at least 10% more than the control group.

MTT test results of HT29 human colon cancer cells

MTT assay was used to evaluate the viability of TCF-1- and TCF-2 treated cells. Results showed that both TCFs significantly decreased cell viability compared with the control group (Figure 2) ($p < 0.05$).

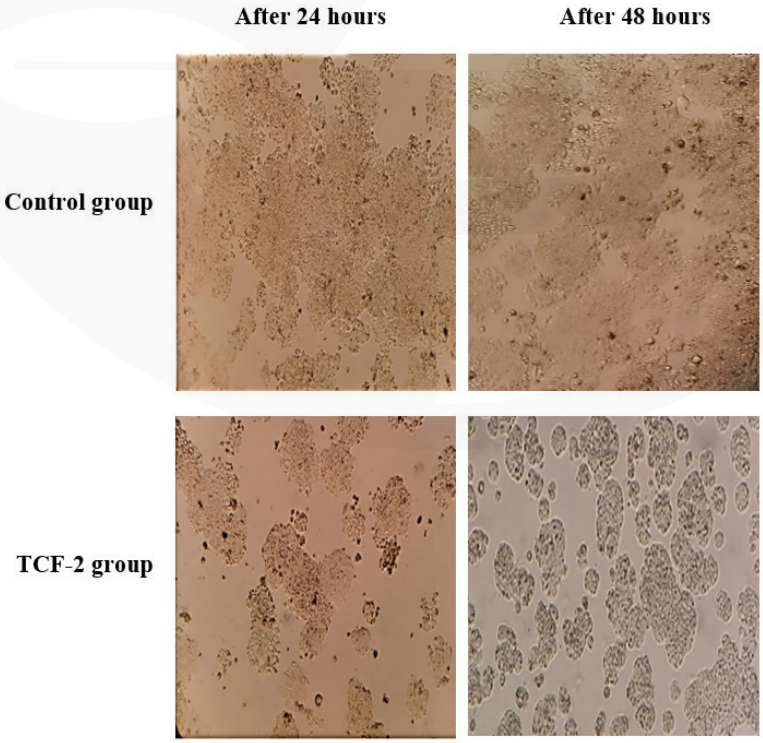


Figure 1. Microscopic evaluation of control HT29 cells and cells exposed to the TCF-2 at 24 and 48 hours after culture. There is more intercellular space in the TCF-2 group compared to the control group.

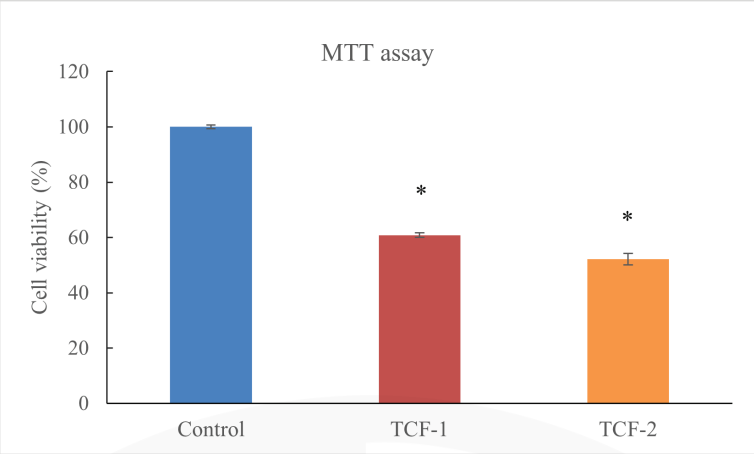
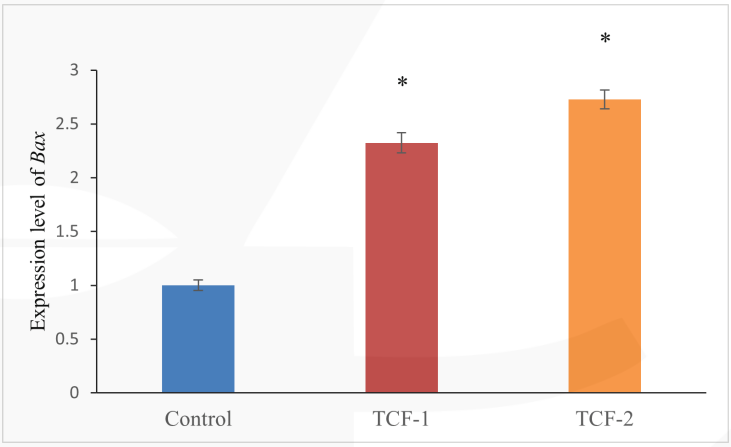
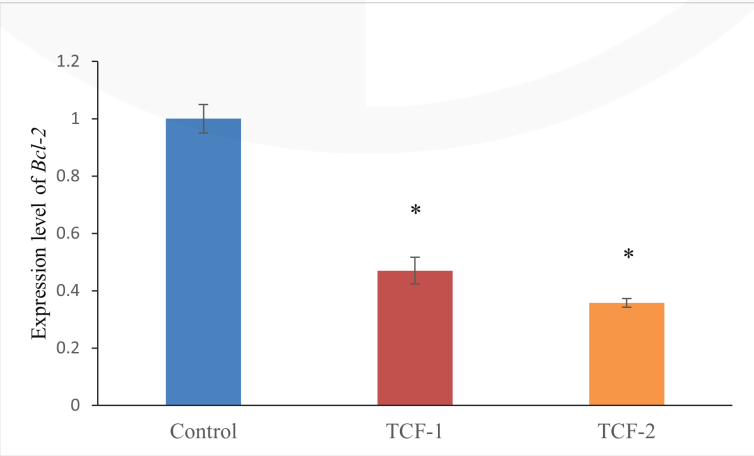


Figure 2. Cell viability of TCF-1 and TCF-2 treated groups compared with the control. The asterisk shows a statistically significant difference ($p < 0.05$) between the experimental and control groups.

Expression of *Bax* and *Bcl-2* genes in TCF-1 and TCF-2 treated HT29 cells

This study showed the change in the expression of *Bcl-2* and *Bax* genes and the ratio of *Bax/Bcl- 2* in TCF-1 and TCF-2 treated HT29 cells. The expression of the *Bax* gene was increased in both TCF-1, and TCF-2 treated cells compared with control (Figure 3a). The *Bcl-2* expression was significantly decreased in both TCFs groups ($p < 0.05$; Figure 3b). *Bax/Bcl-2* ratio was significantly increased in the TCFs treated cells ($p < 0.05$; Figure 3c).





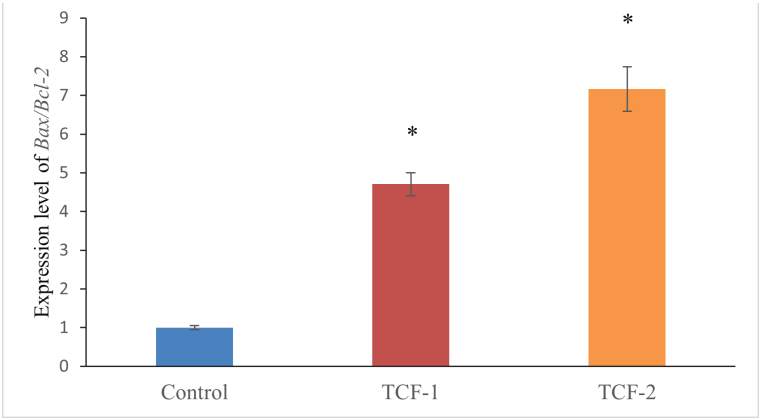


Figure 3. The expression of *Bax* (a) and *Bcl-2* (b) and the ratio of *Bax/Bcl-2* (c) in TCF-1 and TCF-2 treated cells compared with control. The asterisk displays a significant difference between the TCFs groups and the control ($p < 0.05$).

Discussion

In the present study, TCF-1 and TCF-2 considerably influenced the number of cells and the morphology of HT29 human colon cancer cells. In the trypan blue staining assay, more dead cells were counted in the TCFs groups compared with the control. This may indicate an inducing effect of the TCFs on HT29 cell death. In the MTT assay, both TCFs decreased cell viability at the specified incubation times ($p < 0.05$).

Bcl-2 family members play a critical role in regulating cell death or survival. Chemotherapeutic agents partially affect the expression of numerous members of the Bcl-2 family in cancerous cells. Members of the Bcl-2 family regulate apoptosis, with Bax acting as a pro-apoptotic factor and Bcl-2 acting as an anti-apoptotic factor.(Green et al., 1998; Rao et al., 1997).

The expression of *Bcl-2* and *Bax* genes and the *Bax/Bcl-2* ratio were evaluated in the present study. The results showed that *Bax* gene expression increased and *Bcl2* gene expression decreased in both TCF-1 and TCF-2 groups compared to the control. The ratio of *Bax/Bcl-2* is an indicator of cell susceptibility to apoptosis and helps determine cell fate (Gross, 2001). The *Bax/Bcl2* ratio increased 4.9-fold and 7.6-fold compared to control via TCF-1 and TCF-2, respectively ($p < 0.05$), indicating that apoptosis induced by both TCFs was significant.

The effect of Faradarmani CF on MCF7 cells was assessed in a previous study. The Faradarmani CF was announced every hour until the end of the experimentation at 6, 18, and 24 hours from the initial start time. Cell viability was assessed using the MTT assay. The expression of the *Bax* and *Bcl-2* genes was assessed using RT-qPCR in MCF-7 breast cancer cells. Faradarmani CF significantly increased MCF-7 cell proliferation (18%) compared to the control in a time-dependent manner. Furthermore, in cells treated with Faradarmani CF, the *Bax/Bcl2* ratio decreased (1-fold) compared with the control, suggesting higher MCF7 cell survival and resistance to death (Taheri et al., 2020a).

The influence of TCFs on cancer cells seemingly displays different results. Both of the TCFs showed a significant anticancer effect. It is necessary to further study TCFs and perform laboratory tests in a variety of areas. Therefore, extensive studies *in vitro* and *in vivo* are needed to evaluate the efficacy of TCFs on cancer, and further studies are recommended.

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Conflicts of Interest

The authors declare no conflict of interest.

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The Influence of Faradarmani Consciousness Field on the Survival and Death of MCF-7 Breast Cancer Cells: An Optimization Perspective

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Abstract

Mohammad Ali Taheri, the founder of the Faradarmani Consciousness Field (CF) maintains that, beyond matter and energy, there exist Taheri Consciousness Fields (TCFs) with a non-frequency nature. It is hypothesized that exposure to these fields may result in an optimization of a subject's structure and function. Apart from the fact that most studies in the field of cancer are aimed at the death of cancer cells, the purpose of this study was to investigate the MCF-7 cancer cell survival and death under the influence of the Faradarmani CF. For the purpose, cellular viability was evaluated by MTT assay for 6-24 h under the influence of the Faradarmani CF. Consequently, cell cycle analysis was performed for the evaluation of apoptotic cell death. Finally, the expression of *Bcl2* and *Bax* genes in MCF-7 cells was evaluated as a completion of the obtained cellular scale results. Our findings demonstrate that the Faradarmani CF treatment significantly induced cell proliferation in the MCF-7 cell culture mediated by increasing cellular viability by about 18% in comparison to the control in a time-dependent manner. Moreover, the S-phase in the cell cycle, as a sign of cell population in the sample treated with the Faradarmani CF, was increased by 56% (up to 24h) in comparison to the control. The real-time RT-PCR reaction results show that in cells treated with the Faradarmani CF, the *Bax/Bcl2* ratio decreased after 24 h (more than the 1-fold negative control (untreated)), suggesting a higher cell survival and resistance to death. Considering the concepts of the TCFs, according to Taheri, proliferation and survival appear to become optimized in MCF-7 cancer cell lines under the influence of the Faradarmani CF.

Keywords: *Bax/Bcl2*; Cell cycle; Taheri Consciousness Fields; Cosmic Consciousness Network; Faradarmani Consciousness Field; MCF-7; MTT assay; Survival

Introduction

The nature of consciousness, its location, and mechanism of action in humans are largely unmeasurable and unknown (Hameroff and Penrose, 2014). In fact, the concept of consciousness has different definitions and applications in different branches of research, from philosophy, clinical psychology, and neuroscience to cognitive science, quantum physics, and biology. Common fields of study explore the nature of consciousness (Crick and Koch 1998; Modestino 2016; Penfield 1938). For instance, the consciousness nature is studied in philosophy, and also experiences of consciousness at different levels of individual and social life are investigated in psychology. Similarly, the creation of consciousness during information processing at the network level of the nervous system, neurotransmitters and other processes of the cerebral cortex are explored in cognitive neurology.

Investigations of the connection and correlation of consciousness and bodily processes and cognitive disorders in psychoneuroimmunology (Moynihan et al., 2010; Torkamani et al., 2018), psychosomatic disease and processes (Montecucco 2015; Puente 1984) and biofeedback research (Bagdasaryan and Le Van Quyen 2013; Kannape and Blanke 2013) are also frequently reported. Explanation of consciousness by quantum physics keywords (Jahn 1993; Neppe and Close 2015) and its reduction in quantum physics and quantum biology (Hameroff and Penrose 1996, 2014) are more recent approaches in basic science toward understanding the concept of consciousness. In the Orch OR theory, introduced by Penrose and Hameroff (1996), events in the field of consciousness were analyzed in the form of electromagnetic fields caused by molecular structures of the nervous system, related to the fundamentals of quantum mechanics and space–time geometry. According to the concepts of this theory, there is a connection between the brain's biomolecular processes and the basic structure of the universe, relating the brain's structure and function to consciousness in the universe, as published in a review (Hameroff and Penrose, 2014).

According to Chalmers, explaining whether, why and how organisms have experiences of consciousness separate from objective material processes has been termed the hard problem of consciousness (Chalmers, 1995). Different studies tackle the problem of consciousness with different approaches. For example, in the pan-psychic approach, every material, however small, has an element of individual consciousness (Bruntrup and Jaskolla, 2016; Du Toit, 2016). Understanding the relationship between consciousness and the wider dimensions of the universe is covered in numerous studies. Moreover, Ervin Laszlo, in his connectivity hypothesis (Laszlo, 2010) introduces a new Integral science that merges the concepts of consciousness with quantum, cosmos, and life criteria by considering his own concept of "coherence in nature". This time- and space-invariant (nonlocally) coherence causes nonconventional connections between the parts that make up a system as well as between systems and their surrounding environment. He believes that nature comprises connected coherent systems in space, containing physically effective "in-formation", and is as fundamental as energy such that it is also conserved.

Approaches close to the hypothesis of a non-physical nature having consciousness attributions have also been reported (Bruntrup and Jaskolla 2016; Laszlo 2010; Nelson 2006; Radin 2007; Sheldrake 2013). A study in 2000 from the CIA reports the need for separation of consciousness from matter and energy in a "triangular structure" (Shuji, 2000). In this study, consciousness is quantified based on basic mathematical and physical equations.

In the 1980s, Mohammad Ali Taheri introduced novel fields with a non-material/non-energy nature named T-Consciousness Fields (TCFs). In this perspective, T-Consciousness is one of the three existing elements of the universe apart from matter and energy. The influence of these fields is initiated via a short and brief attention to the subject under the study. It is possible to record their effects through conducting laboratory experiments (Taheri, 2013).

Some descriptive (Taheri and Mizani 2013; Taheri 2014) and clinical (Taheri et al., 2011; Taheri et al., 2013a and b; Rahimabadi et al., 2017) studies have been performed to experience the Faradarmani CF applications. Apart from the theory and mechanism

of action of the introduced TCF, it seems that what must first be taken into account is the repetitive influence of TCF on the subjects under the conditions of empirical research, especially in basic sciences. Investigating the apoptotic behavior of cancer cells in their interactions with drugs is one of the common screening mechanisms for drug efficacy and specificity (Pilco-Ferreto and Calaf, 2016; Rahnamay et al., 2018).

On the other hand, considering cancer and its process and also its therapeutic resistance as an evolutionary process have been reviewed in more than 2000 research papers (Aktipis et al., 2011; Merlo et al., 2006). Moreover, studying the effects of the dimensions of consciousness on the growth of cancer cells and its clinical applications have been very limited (Brown 2000; Radin et al., 2015; Zachariae et al., 2005). Also, *in vitro* study of cancer cell line proliferation behavior to be optimized according to its nature has not been reported yet. For this purpose, in the present study, we explore the behavior of MCF-7 cancer cells in controlled growth conditions as well as under the influence of the Faradarmani CF. Finally, the effect of the Faradarmani CF treatment on changes in cell proliferation, cell cycles and apoptosis gene expression were analyzed with respect to the application of the Faradarmani CF and its action.

Materials and Methods

Application of Faradarmani CF

Faradarmani Consciousness Field was applied to the samples according to the protocol established by the Cosmointel research center. More details are presented in the general considerations of this issue.

In the present study, we requested an announcement for MCF-7 cancer cells in incubator B, with dishes on the 2nd level, and the first level was kept as the control group. We also specified the time conditions to announcer, i.e. 24, 48, and 72 hours from a set time point. The only relationship between the announcer and the researcher is contractual and related to the sample plate, control plate names and positions in the incubator; for example, the names of the plate A in the upper level of the incubator I and plate B in the lower level of incubator I.

Spacing condition: In this study, three spacing conditions were selected between the sample (affected by Faradarmani CF) and control groups.

No. 1- *The middle spacing test*: sample and control cell plates were cultured in the same incubator at 5% CO_2 and at 37° C but at different 96 well plate and at distinct separable distances, for example two different incubator levels.

No. 2- *The highest spacing test*: samples were placed in a 96-well plate in an incubator and the control groups were placed in another 96-well plate in a different incubator in different laboratory operating at the same conditions (i.e. 5% CO_2 and at 37° C).

No. 3- *The lowest spacing test*: sample and control groups were all in the same 96 well plate in an incubator.

Timing conditions: The Faradarmani CF was applied *every one hour* until the end of the experiment which was at 6, 18, and 24 hours from the initial start time. The Faradarmani CF was applied in the three aforementioned spacing test conditions and only to the sample plates. After the end of the testing time, MTT assay, cell cycle distribution, and real time RT-PCR experiments were performed, respectively.

Cytotoxicity evaluation by MTT assay

The cytotoxic effect of the Faradarmani CF was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay. Cells were seeded in 96-well plates (SPL, South Korea) at concentration of 5×10^3 cells/ml and were exposed to Faradarmani CF and Doxorubicin (Dox) (as a positive control) for 6, 18, and 24 hours. After 24 hours, 20 µl/well of MTT solution (5 mg/ml) was added and incubated at 37 °C for two hours. The supernatant was then discarded, formazan crystals were solubilized in 150 µl of DMSO/well, and their absorbance was evaluated by the micro-well plate reader (Bio-Tek, Elx 808, USA) at a wavelength of 570 nm.

Cell cycle distribution analysis

Flow cytometry was employed to examine the cell phase distribution by identifying the nuclear DNA content. MCF-7 cells (1.5×10^5 cells/ml) were seeded in 12-well plates and treated with the Faradarmani CF and Dox (as a positive control) for 12- 48 hours. Untreated cells were used as a negative control. Briefly, after expiring the incubation period, cells were collected and rinsed in cold 1xPBS, then stained with propidium iodide (20 µg/ml) for 1 hour in the dark. Consequently, cells were sorted in a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA, USA), and the cell cycle proportion was interpreted by FlowJo software (Ashland, OR, USA). Distributions of cells in the G0, G0/G1, S, and G2/M phases were determined as DNA histograms. Apoptotic cells were displayed as a hypodiploid (the sub-G1) peak.

RNA extraction, cDNA synthesis, and real-time RT-PCR

After the extraction steps, 1 to 2 µl of extracted RNA from each sample was transferred onto agarose gel to ensure its integrity. In this experiment, two bands related to S28 and S18 ribosomal RNAs are clearly visible due to their high concentration, which indicates the extracted RNAs are healthy and non-degradable during the extraction process.

The total RNAs from *announced/un-announced* MCF-7 cells were extracted after 12-24 hours of treatment using RNX-plus reagent (Cinnagen, Iran), as the manufacturer recommended. The quality and quantity of extracted RNAs were evaluated respectively by using the 1% agarose gel electrophoresis and Nanodrop (Thermo Scientific, USA).

For the synthesis of the complementary DNA (cDNA), 1 µg of total RNA was reverse transcribed using the Prime Script RT reagent kit (Takara Bio, USA) according to the manufacturer's instructions. Subsequently, qRT-PCRs were carried out on step one software using RealQ Plus 2x Master Mix Green High ROX™ (Amplicon, Denmark). The levels of *Bax* and *Bcl-2* were normalized against the housekeeping gene (β-actin) and were expressed relative to the control cells (un-announced). The expression levels of mRNAs were interpreted using the comparative ΔCt method. qPCR primers were synthesized by Oligo (Macrogen, Seoul, South Korea), and their sequences are shown in Table 1. qPCR was performed under the following protocol: one denaturation cycle of 95ºC for 5 min, followed by 40 amplification cycles of 95º C for 30 sec, 60-64ºC for 30 sec, 72ºC for 30 sec and a final extension of 72˚C for 5 min. Each individual run was followed by a melting curve analysis to evaluate the amplification specificity and lacking primer dimers. Additionally, the amplification efficiency for each primer was calculated by LinRegPCR 2017.9 version.

Table 1. Primer sequences used in real-time PCR.

Gene name	Forward and Reverse Primers (5′-3′)	Annealing Temp. (º C)	Amplicon size (bp)
β-actin	F: AGAGCTACGAGCTGCCTGAC R: AGCACTGTGTTGGCGTACAG	58	184
Bax	F: GCAAACTGGTGCTCAAGG R: ACTCCCGCCACAAAGA	64	187
Bcl2	F: TGGGAAGTTTCAAATCAGC R: GCATTCTTGGACGAGGG	64	297

F = Forward; R = Reverse

Statistical analysis

All values in the present study are expressed as mean ± SD, calculated from three independent experiments. Statistical analysis was done using GraphPad Prism with two-way analysis of variance (ANOVA), and Tukey multiple comparison tests and $p < 0.05$ are considered statistically significant.

Results

MTT assay

Images from MCF-7 cells in each group of the present study are in Fig 1. Fig 1.D shows the Faradarmani CF applied to MCF-7 cells, in comparison with the negative control group in the same conditions, at 18 and 24-hours treatment with a significant increase in cell viability. It can be noted that the cells in the positive control group treated with Dox, as a positive control, also show a significant decrease in cell viability at 24 hours. Furthermore, the changes in the cell viability of the treated cells with 10% FBS were not significant at either of the study times.

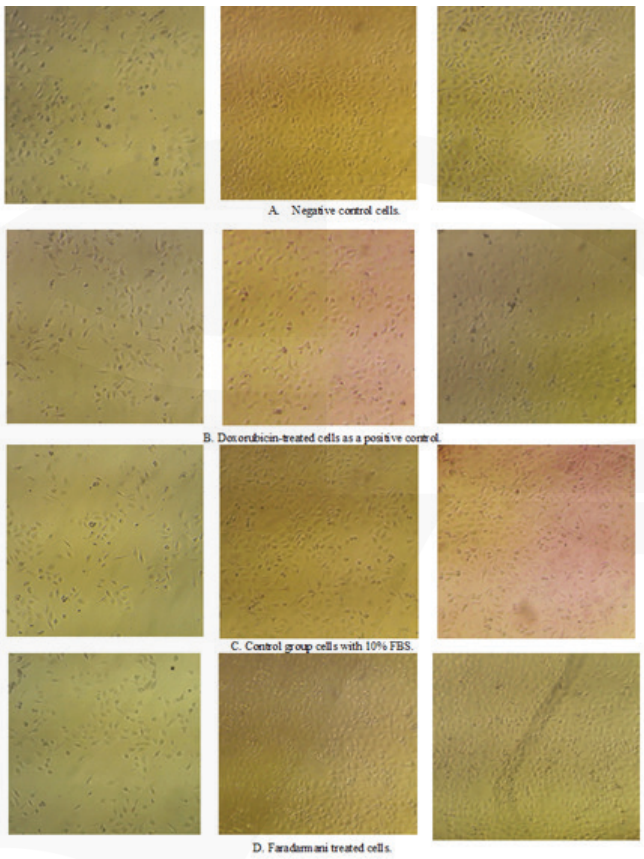


Figure 1. MCF-7 cells in each group at 6h (left), 18h (middle) and 24h (right) treatment. A: Negative control cells; B: Doxorubicin-treated positive control cells; C: 10% FBS control cells. D: Faradarmani CF treated cells.

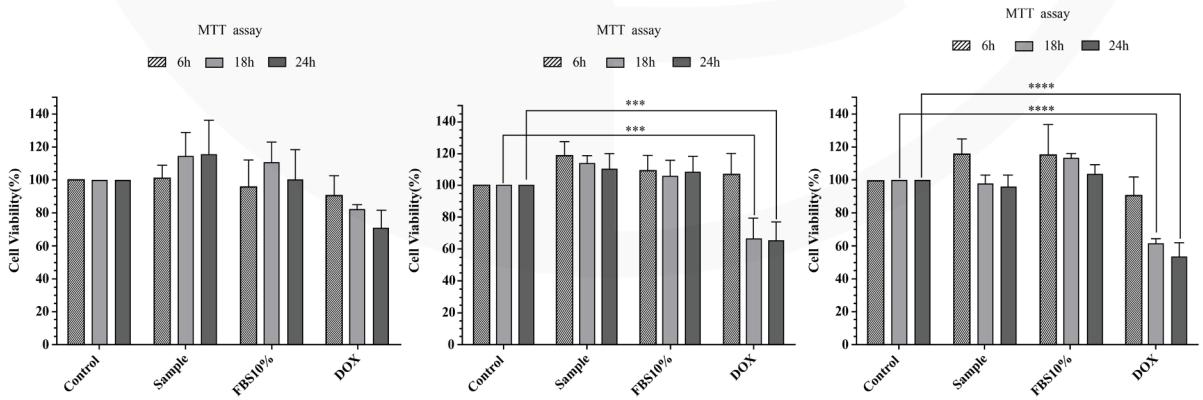


Figure 2. The diagram of samples treated with Faradarmani CF (sample), Dox (positive control), and dietary factor (FBS 10%) compared to the untreated or negative control (control), evaluated by MTT test at three treatment times of 6, 18, and 24 hours in the three spacing test conditions. Right: No.1 spacing test condition; middle: No.2 spacing test condition; left: No. 3 spacing test condition. The data are indicated as the mean ± SD of three individual trials. Stars show the level of significance from three independent samples t-test (*P < 0.05, **P < 0.01, ***P < 0.001, ns: not significant).

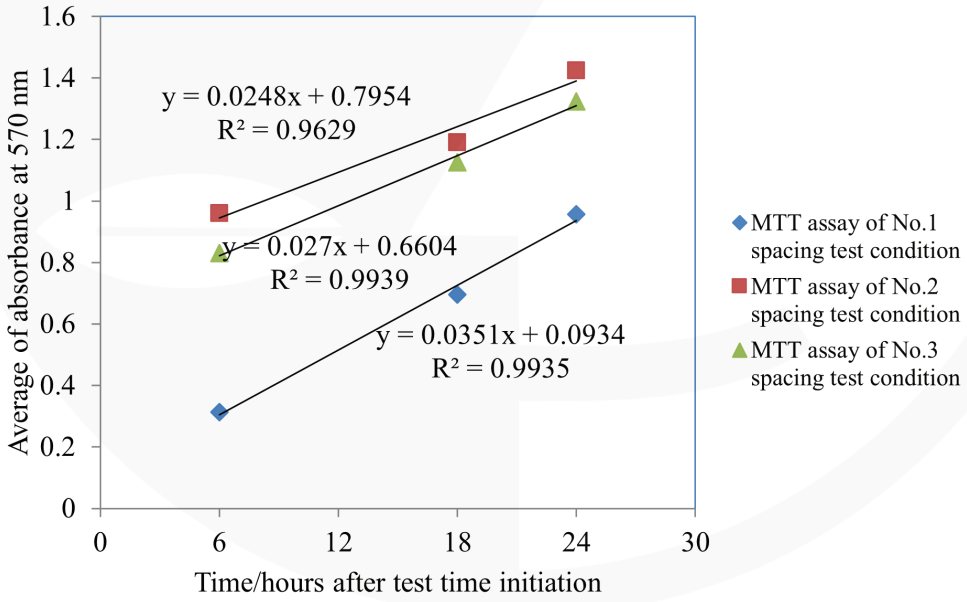
In the spacing test conditions of No. 2 and No. 3, none of the changes in cell viability is significant in the samples under Faradarmani CF and FBS 10% treatments. In contrast, there are decreases in cell viability of the positive control (Dox) at 18 and 24 hours in spacing test conditions No.2 and No.3, with p-values < 0.01 and < 0.001, respectively (Fig 2).

Population changes trends in all test groups during the first 24 hours after test initiation

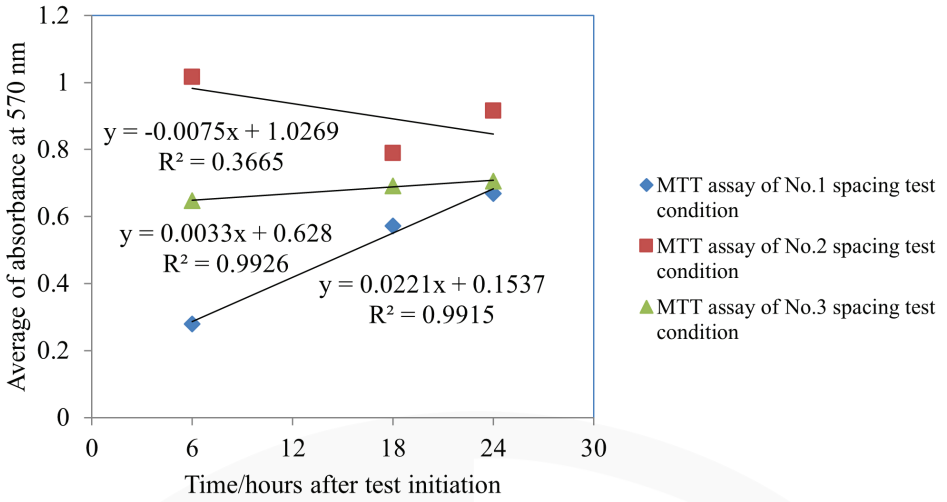
Figure 3 shows the analyses of the cell population viability changes in each study group (controls and sample) compared with one another in three spacing test conditions. As presented in Figure 3A, the slope of increase in negative control cell viability in spacing test condition No. 1 is approximately 32% and 30% more than the No. 2 and No. 3 spacing test conditions, respectively. Figure 3B shows that the slope of increase in population decreases at 37% in test condition No.1 compared with negative control. Moreover, this increase under treatment of Dox suggests the lowest drug efficacy in the No.1 spacing test condition in comparison with two other spacing test conditions. The highest efficacy of Dox treatment is observed in the No. 2 spacing test condition.

As indicated in Figure 3C, cell viability is increased in positive control groups in the No.1 spacing test condition at approximately 3.8% lower than the negative control, not a significant number. However, the increase is 31% and 92% higher than No.2 and No. 3 spacing test conditions, respectively.

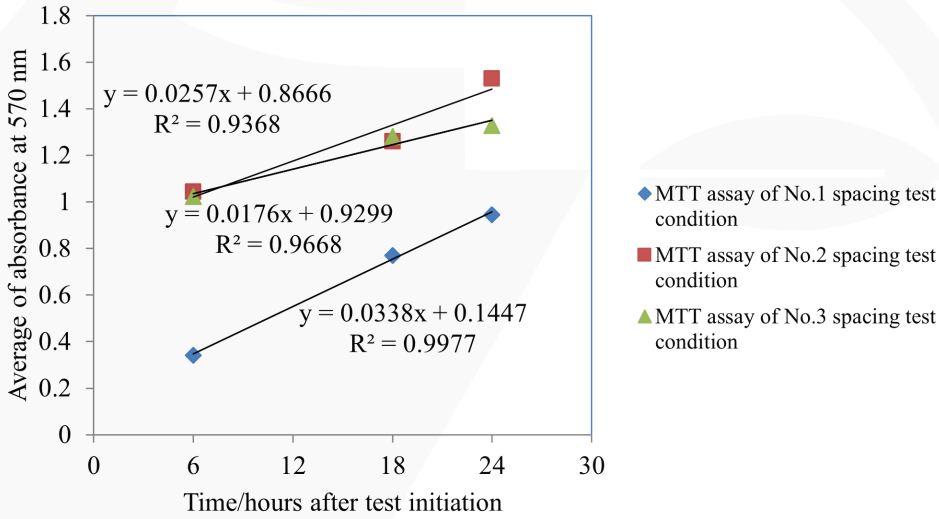
As shown in Fig 3D, the No.1 test condition with a 41% higher slope of the population is increased in comparison with negative control, showing the highest rate of increase in cell viability 24 hours after test initiation. While this increase in two other spacing test conditions is lower than according to the negative control.



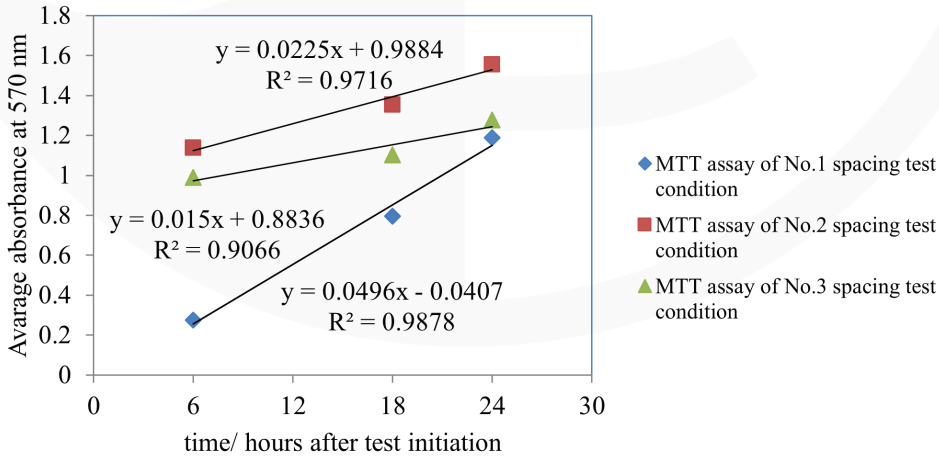
A. The MCF-7 cell viability increased in negative controls groups.



B. The MCF-7 cell viability changed under Doxorubicin (Dox) treatment (positive control).



C. The MCF-7 cell viability increased in FEB 10%.



D. The MCF-7 cell viability increased in the sample groups (with Faradarmani CF treatment).

Figure 3. Cell population viability changes in each study group (controls and sample) compared with one another in three spacing test conditions. A) Negative control cells; B) Doxorubicin-treated positive control cells; C) 10% FBS control cells. D) Faradarmani CF treated cells. Blue diamond: MTT assay of No. 1 spacing test. Red cube: MTT assay of No. 2 spacing test. Green triangle: MTT assay of No. 3 spacing test.

Flow cytometry

In the No.1 spacing test condition, MCF-7 cells are treated in order to investigate the effect of the desired treatment at different cell cycle stages. In the negative control, most of the population is in the G0/G1 phase (Fig 4A). After treatment with DOX-positive control cells, the cell population in the SubG1 phase increases significantly in a time-dependent manner compared to the control (Fig 5). The accumulation of cells in this region indicates the induction of apoptotic cell death in the cells.

However, there are no significant changes in the SubG1 phase cell population in cells treated with Faradarmani CF (Fig 4C) and in cells treated with more nutrients (FBS) (Fig 4B). In contrast, cells treated with Faradarmani CF show a 56% increase (up to 24h) in the S phase compared with control (Fig 5). These data are consistent with the data from the drug toxicity test (MTT assay), suggesting growth inhibition and cell death in Dox-treated cells and increased growth and viability in the Faradarmani CF cells.

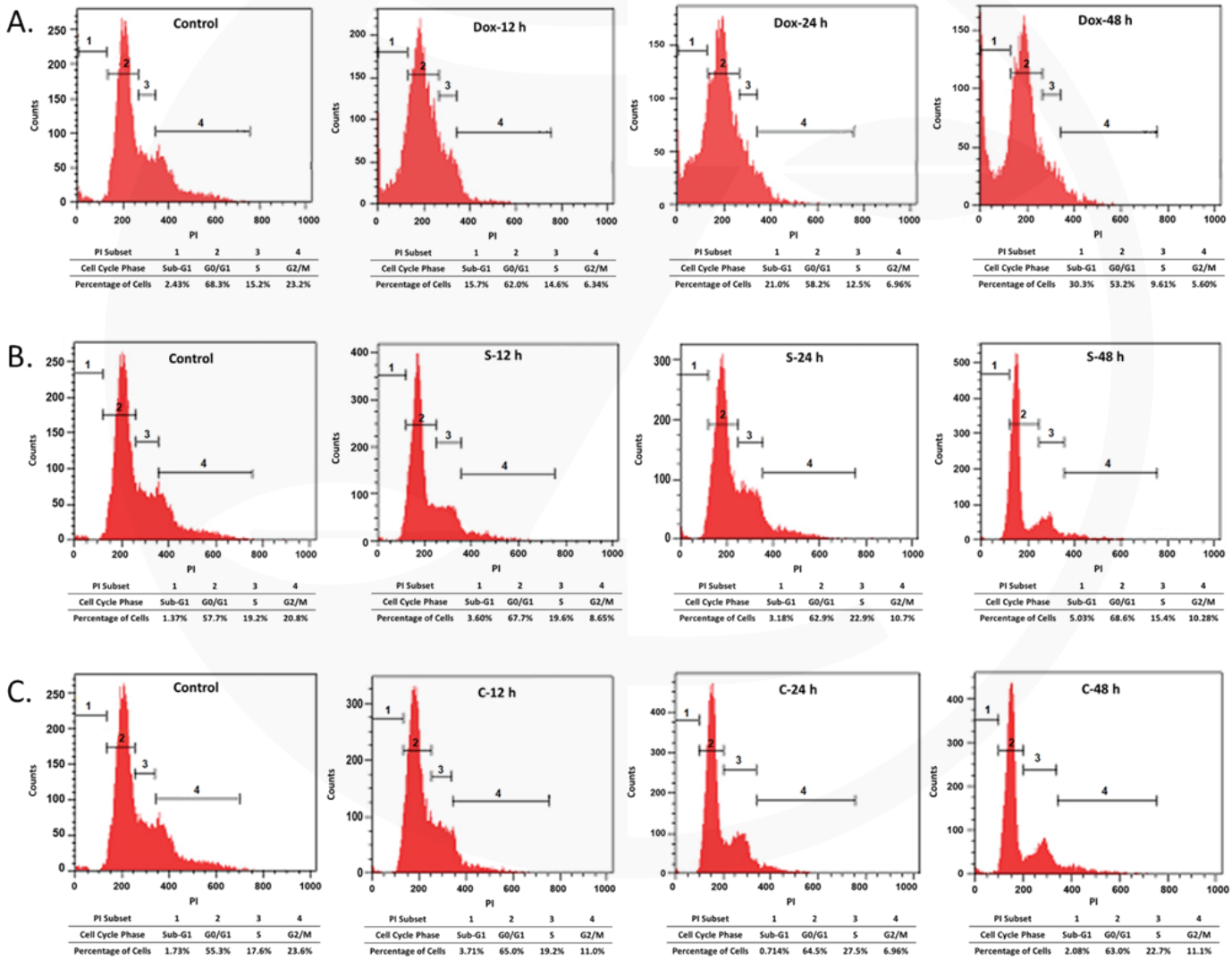


Figure 4. Cell cycle at different stages in each group of study; A. DOX-treated cells; B. FEB 10% -treated cells; C. Faradarmani CF-treated cells. All groups were compared with the negative control.

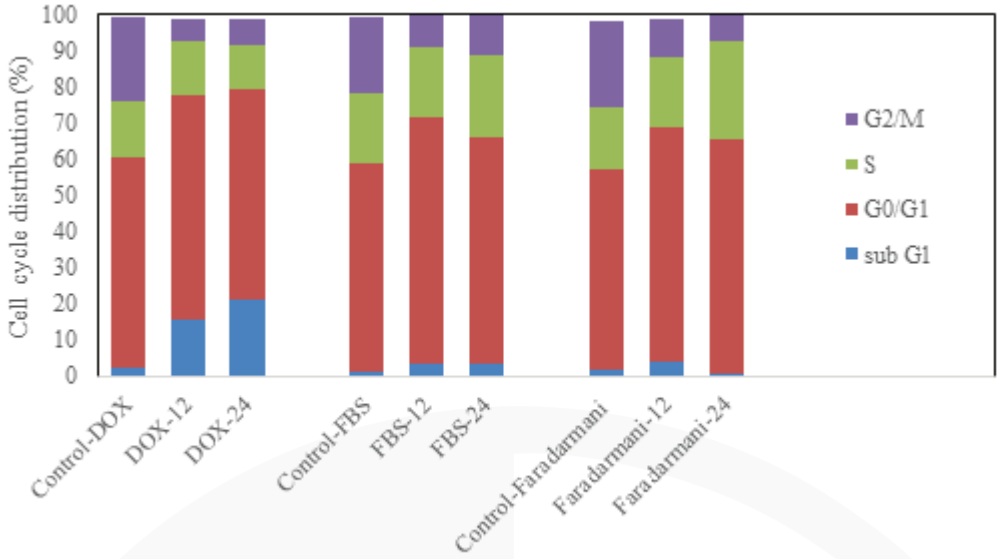


Figure 5. Cell cycle distribution of sample and controls up to 24h.

Gene expression analyses for *Bcl2 and Bax* genes

The expression of *Bax* and *Bcl-2* pro-and anti-apoptotic members of the *Bcl2* family were examined. The results of the real-time RT-PCR demonstrate that in the cells treated with Faradarmani CF in the No.1 spacing test condition, the *Bax/Bcl2* ratio was decreased after 24 hours (more than 1-fold as compared with the negative control), indicating improved cell survival and resistance to cell death. However, in cells treated with Dox as a positive control, the *Bax/Bcl2* ratio was significantly increased (more than 10-fold compared to the negative control), which induced apoptotic cell death (Fig 7). These data show that the Faradarmani CF results in a gradual time-dependent decrease in the ratio of *Bax/Bcl2* transcripts.

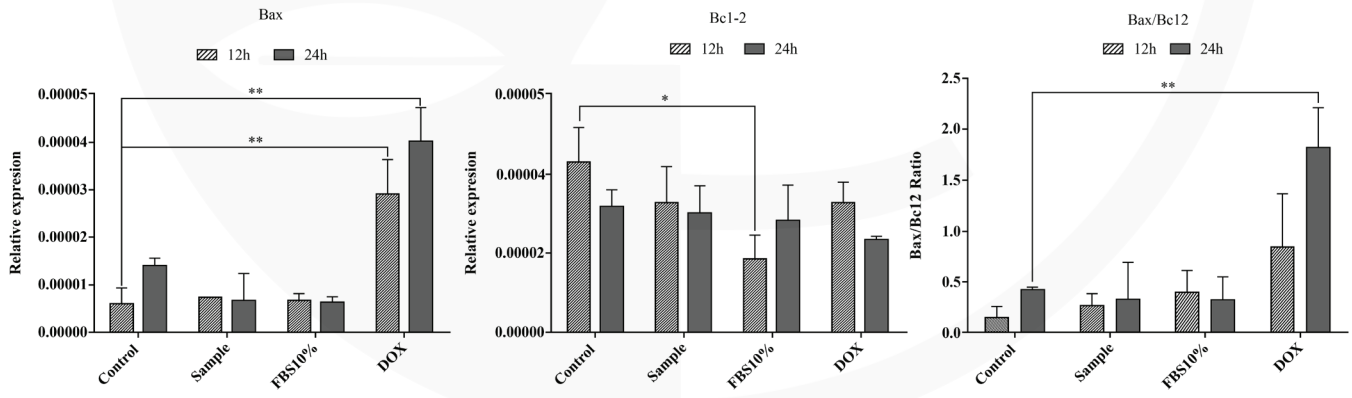


Figure 6. The expression changes of *Bax, Bcl2* genes in MCF-7 cells treated with Faradarmani CF (sample), Dox (positive control) and dietary factor (FBS 10%) compared to untreated or negative control cells. The cells were treated for 12-24 h and relative expression levels were measured by real-time RT-PCR. (*p <0.05, **p <0.001)

Discussion

In this study, using MCF-7 cell line, the hypothesize of the effectiveness of Faradarmani Consciousness Field was evaluated. According to Taheri, when a subject is affected by this field, it is possible that the behavior of the treated sample alters compared to the control (without field). The current study provides evidence of this effect.

In this experiment, three selective intervals between samples and controls were considered. Cells were monitored for proliferation, apoptosis, and cell cycle expression of genes related to cell death and survival. By examining the overall variations of cell populations compared to the own control state in each distance test, it is found that MCF-7 cancer cells gained more proliferation and survival at the middle distance (No. 1 condition) selected in this study because of Faradarmani CF treatment. On the other hand, by comparing changes in the cell populations of each group at three selective intervals between the sample and control groups, we find that the spatial condition has a significant additive effect on the growth conditions of the negative and positive control groups. Moreover, Faradarmani CF also increases resistance to toxicity, as shown in the MCF-7 cells treated with Dox. This significant incremental effect of the Faradarmani CF on cell populations was also demonstrated by cell cycle assays showing increased expression of survival genes. Three key points can be extrapolated from these results. First, Faradarmani CF affects cells at the cellular and molecular levels. Second, the MCF-7 cell line showed an enhancement in survival under the influence of Faradarmani. Lastly, it seems that the space between treated samples and controls can affect the obtained results. It is worth mentioning that according to Taheri, information transmitted by TCFs to the samples leads to cellular changes. In this experiment the cells were in the *in vitro* condition, to complete the evidence of the effects of TCFs, conducting more experiments in *in vivo* and *ex vivo* conditions is on the agenda of the authors of this study. It is expected that information transmitted from these fields alter based on different culture and experimental conditions. It is suggested that survival and death analyses of other cells, either cell lines or primary cells, be done to study the effect of Faradarmani CF on cancer cell behavior.

Data are available in Harvard Dataverse: https://doi.org/10.7910/DVN/UBC7FG.

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Effect of Faradarmani Consciousness Field on the Mice 4T1 Breast Cancer Model

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Abstract

The use of complementary and alternative medicine along with conventional methods of chemotherapy and radiation therapy, with the aim of cancer prevention and treatment, has been investigated and validated in various preclinical and clinical studies. Meanwhile, in contrast to the widespread use of medicinal plants and other complementary and alternative medicine methods in the preclinical trials with animal models of cancer, as an important step confirming the safe and effective use in humans, similar studies in the field of mind-body modalities are rarely examined. A new treatment method founded and introduced by Mohammad Ali Taheri provides a different type of consciousness that is neither matter nor energy. The effectiveness and capability of a new complementary and alternative medicine were examined in this study, and the effectiveness of one of T-Consciousness Fields (TCFs) named Faradarmani, was investigated in the 4T1 orthotopic breast cancer spontaneous metastasis Balb/c mouse model. According to the results, Faradarmani Consciousness Field treatment, during tumor progression, had a significant effect on inhibiting the growth of cancerous masses and preventing metastasis in the mice animal model under the study. Moreover, this treatment had a significant positive effect on survival behavior and natural vital functions of the treated mice in comparison with the untreated control group.

Keywords: Faradarmani; Taheri Consciousness Fields; Cosmic Consciousness Network; breast cancer; 4T1 model; complementary and alternative medicine

Introduction

Cancer is one of the leading causes of death in the world. According to the World Health Organization (WHO, 2020), there were 2.3 million women diagnosed with breast cancer and 685 000 deaths globally. Breast cancer is one of the world's most prevalent cancer diseases with high incidence rates in all countries (Ganz et al., 2015). After coronary heart disease and accidents, cancers are the third cause of death in Iran (Mousavi et al., 2009; Farhood et al, 2018). Metastasis represents the most destructive stage of cancer in which it is difficult to remove tumors as well as treat cancer. For solid tumors, about 66.7 % of cancer deaths were registered with metastases as a contributing cause (Dillekås et al., 2019). Thus, understanding the biology of metastases and accurate diagnosis in the early stage is a promising strategy to reduce cancer mortality.

Many patients with cancer use complementary and alternative medical (CAM) therapies and about 50% already use CAM alongside cancer therapy (Ciarlo et al., 2021). Given the high prevalence of CAM use in patients, more research is required to determine whether they are efficacious. Among CAMs, various mind-body therapies have also been used for disease prevention, immune system enhancement and symptom control by cancer survivors (Mayden, 2012).

According to the guidelines of WHO, in order to evaluate the effectiveness of any treatment for various human diseases, it is first necessary to conduct preclinical studies in cellular laboratory conditions (*In vitro*) and in animal models (*In vivo*). In the field of cancer research, the use of animal-based research can be a valuable tool for the preclinical investigation of anti-cancer therapeutics and cancer prevention. The mouse has been the traditional animal model for basic and preclinical studies of cancer (Yee et al., 2015). The breast carcinoma 4T1 cell line is an appropriate candidate for studying the processes of metastasis and understanding its molecular mechanisms (Aslakson et al., 1999). Mouse breast cancer has been used to investigate the effects of a variety of complementary and alternative medicine techniques, such as Therapeutic Touch (Gronowicz et al., 2015) and largely herbal medicine (Chen et al., 2007; Xu et al., 2011). The use of animal models for evaluating the effectiveness of conventional methods of mind-body therapy, such as meditation and mindfulness is impossible, due to the necessity for the active role of the recipient of the treatment.

The nature of consciousness and its place in science have received much attention in the current century. Many philosophical and scientific theories have been proposed in this area. In the 1980s, Mohammad Ali Taheri introduced novel fields with a non-material/ non-energy nature named T-Consciousness Fields (TCFs). In this perspective, T-Consciousness is one of the three existing elements of the universe apart from matter and energy. The influence of TCFs is initiated through a short attention to the subjects of the study. This feature has provided the opportunity of designing various experiments on living organisms and inanimate materials (Taheri, 2013). The present study was aimed to investigate the effects of Faradarmani, as a suggested complementary and alternative medicine approach on the 4T1 mice model of breast cancer.

Materials and Methods

Faradarmani CF (FCF) application

TCFs were applied to the samples according to the protocols regulated by the COSMOintel research center (www.cosmointel.com). More details are presented in the general considerations of this issue. In this research, two groups (mentioned in below) were under the influence of FCF for 4 weeks.

Cell Allograft Metastasis Mouse Model

The 4T1 Cell Culture and Harvest

4T1 mouse mammary tumor cells were cultured in RPMI 1640 medium containing 10% fetal bovine serum (FBS), 100 units/mL of penicillin and 100 µg/mL of streptomycin in a humidified atmosphere containing 5% CO2 at 37 ◦C. Then, the cells were collected, and after centrifugation; they were calculated by Neo-Bar lam (Yang et al., 2012).

Tumor cell injection to mice

Simultaneously with the previous step, twenty 6–8 weeks female Balb/c mice were obtained from the Faculty of Veterinary Medicine, Urmia University, and kept under standard laboratory conditions until the 4T1 cancer cells, triple-negative mouse breast cancer, (PR^-, ER^-, HER^-) were prepared for injection. 1×10^4 cells were inoculated into the back of each mouse subcutaneously. Fourteen days after injection, tumor nodules were observed in the back of the mice (Figure 1).

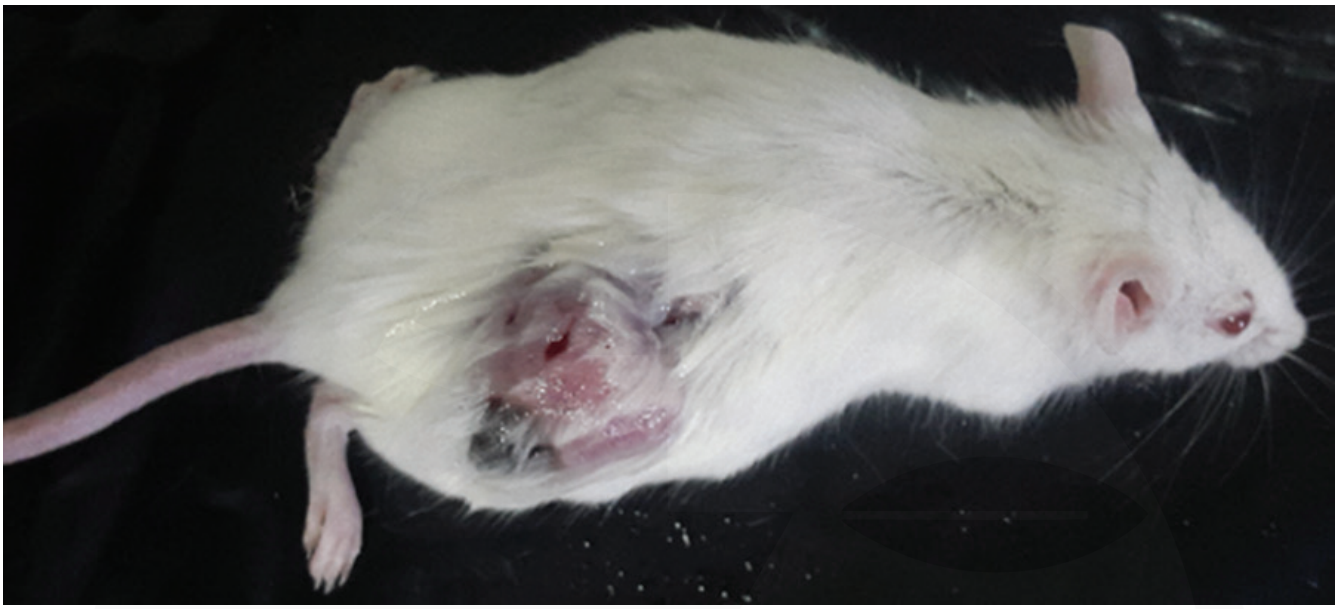


Figure 3. Figure1. The cancer mouse model, 14 days after the 4T1 cell injection.

There were four groups (n= 5 mice), including the negative control, the cancer FCF pretreatment group, the cancer FCF post-treatment group, and cancer control group. More explanation is mentioned in the first part of the result section.

Histology of tumor cells and selected organs

Tumor, liver and spleen samples were harvested and fixed in 10% neutral buffered formalin for 48 h, processed routinely, after which the tissues were embedded in paraffin, sectioned onto slides and their morphologies were observed using hematoxylin and eosin (H&E) staining (Hou et al., 2011).

Results

Tumor size in each group

Four groups are mentioned below, and an image of one member of each group is presented in Figure 2. Moreover, the tumors sizes of groups 2 and 3 are illustrated in Figures 3 and 4, respectively.

- Group 1- five mice without any injection (as the negative control group) (Figure 2a).
- Group 2- five mice received 1×10^4 4T1 cells subcutaneously and at the same time were influenced by FCF (as a pretreatment sample group which is getting cancer – the model of the preventive effect of FCF) (Figure 2b).
- Group 3- five mice received 1×10^4 4T1 cells subcutaneously, and after 14 days, were influenced by FCF (as a sample group that received cancer and then treated- the model of the corrective effect of FCF) (Figure 2c).
- Group 4- five mice received 1×10^4 4T1 cells subcutaneously without the FCF treatment (as the cancer control group) (Figure 2d)

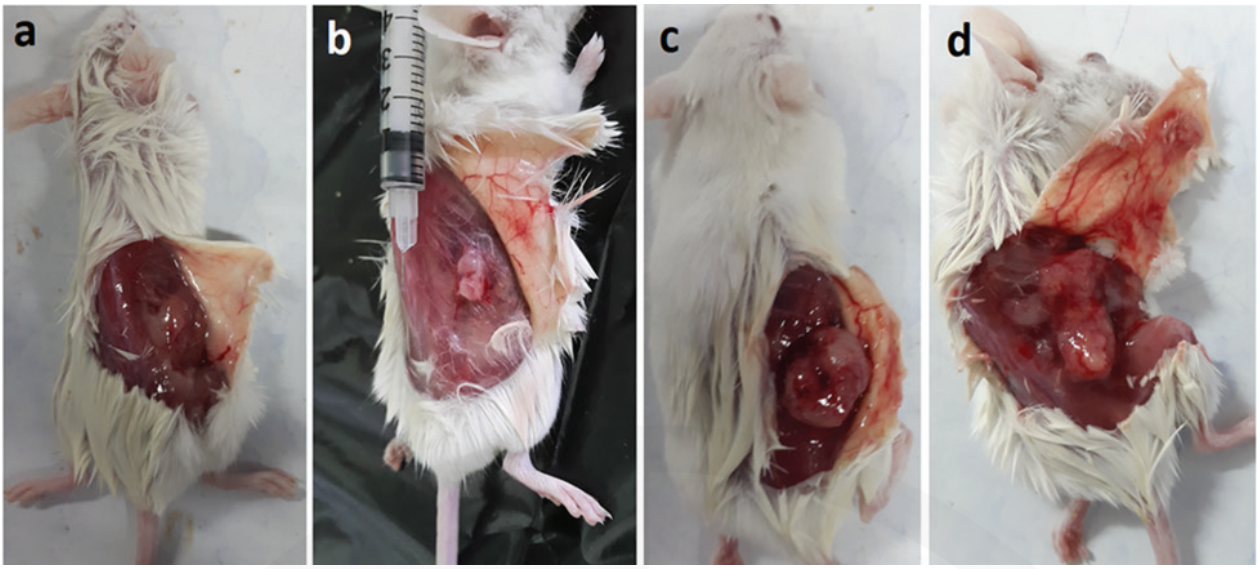


Figure 2. Dissection of one member of each group of the study. (a) The control mouse model. (b) The mouse model receiving cancer, and at the same time influenced by Faradarmani treatment (c) The mouse model received cancer, and then influenced by Faradarmani (d) The mouse model received cancer without Faradarmani treatment.

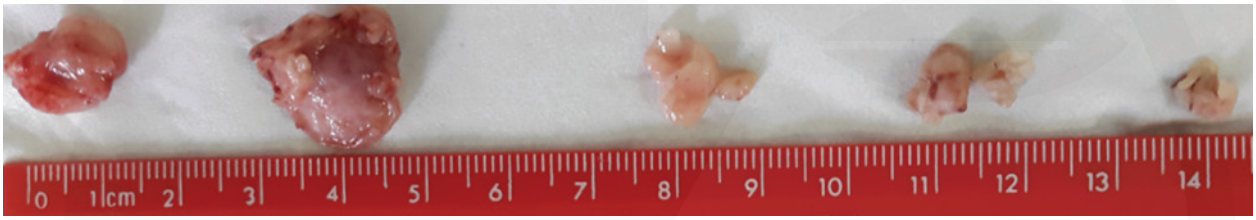


Figure 3. The size of tumors in group 2 (pretreatment model of the Faradarmani application).

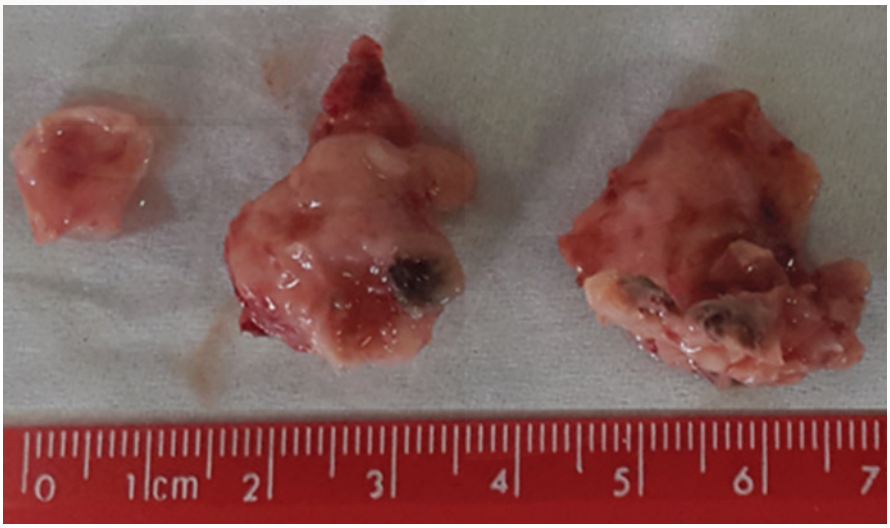


Figure 4. The size of tumors in group 3 (post-treatment model of the Faradarmani application).

After surgery, the weight, size and volume of tumors in the three groups (2, 3 and 4) were measured and compared with each other (Table 1). The data showed that the tumor volumes and weights of treated mice in the preventive approach (group 2) decreased significantly in comparison with the positive control mice (group 4) by about 95% and 75%, respectively. Moreover, according to the data, there is no significant difference in the size and weight of tumors in group 3 (the FCF post-cancer treatment), and group 4 (cancer without treatment).

Table 1. Average size, volume and weight of tumors in different mice groups of this study.

Groups	Average size/mm (length * width * diameter)	Average volume / mm^3	Volume range / mm^3	Average weight / gr	Weight range/ gr
2	10.2*8.2*0.76	63.56	2.4-459	0.38	0.04-0.7
3	18.1*16.3*3.6	1062	180-2622	1.42	0.21-2.1
4	19.2*17.1*3.5	1149	94.5-2990	1.51	0.46-2.4

Histology of tumor mass, liver and spleen in each group

Microscopic slides of tumor mass, liver and spleen cross-sections in each group of the present study can be seen in Figures 5, 6 and 7, respectively. In confirming and completing the results of the previous section, the histologic analysis showed the development of the tumor in the FCF pre-treated, and the FCF post-treated mice (Fig. 6) with remarkable differences in size.

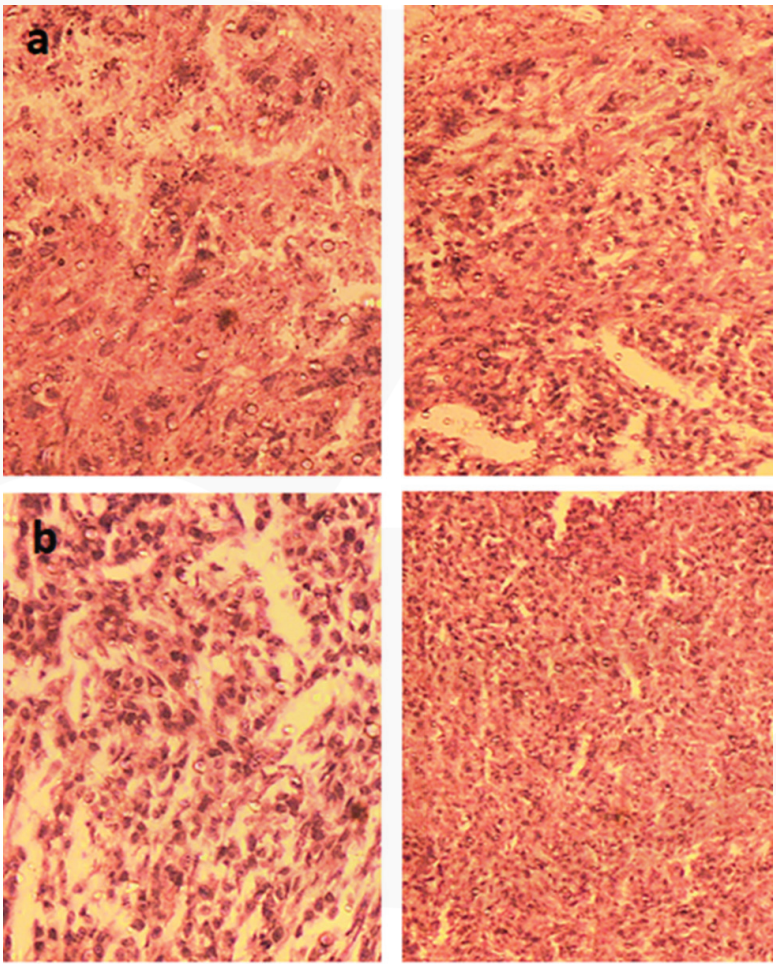


Figure 5. Microscopic slides of tumor mass in group 2 (a) and group 3 (b) in BALB/c mice. (The slides on the left show a scale bar of 200 µm, and the slides on the right show a scale bar of 100 µm.)

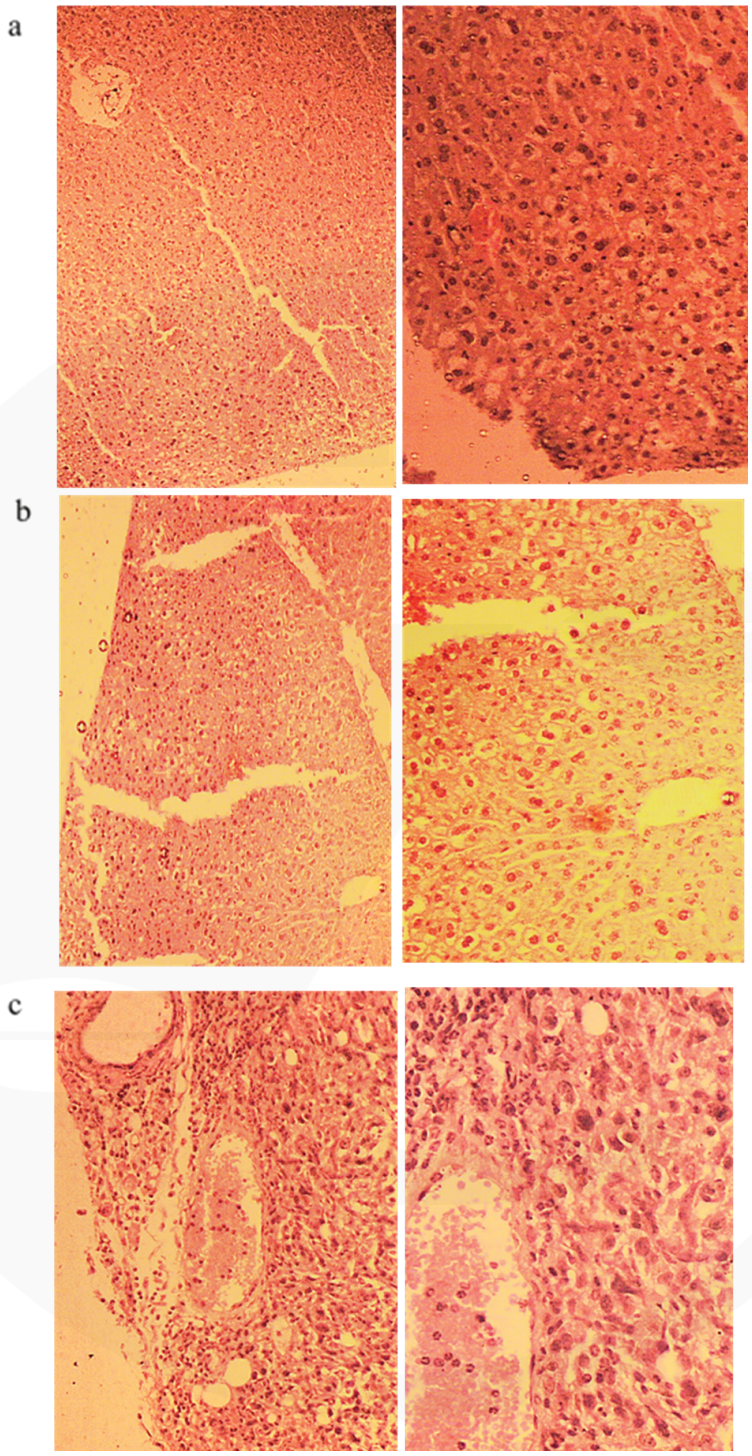


Figure 6. Microscopic slides of the liver in control (a), group 2 (b) and group 3 (c) in BALB/c mice. (The slides on the left show a scale bar of 100 μm and the slides on the right show a scale bar of 200 μm.)

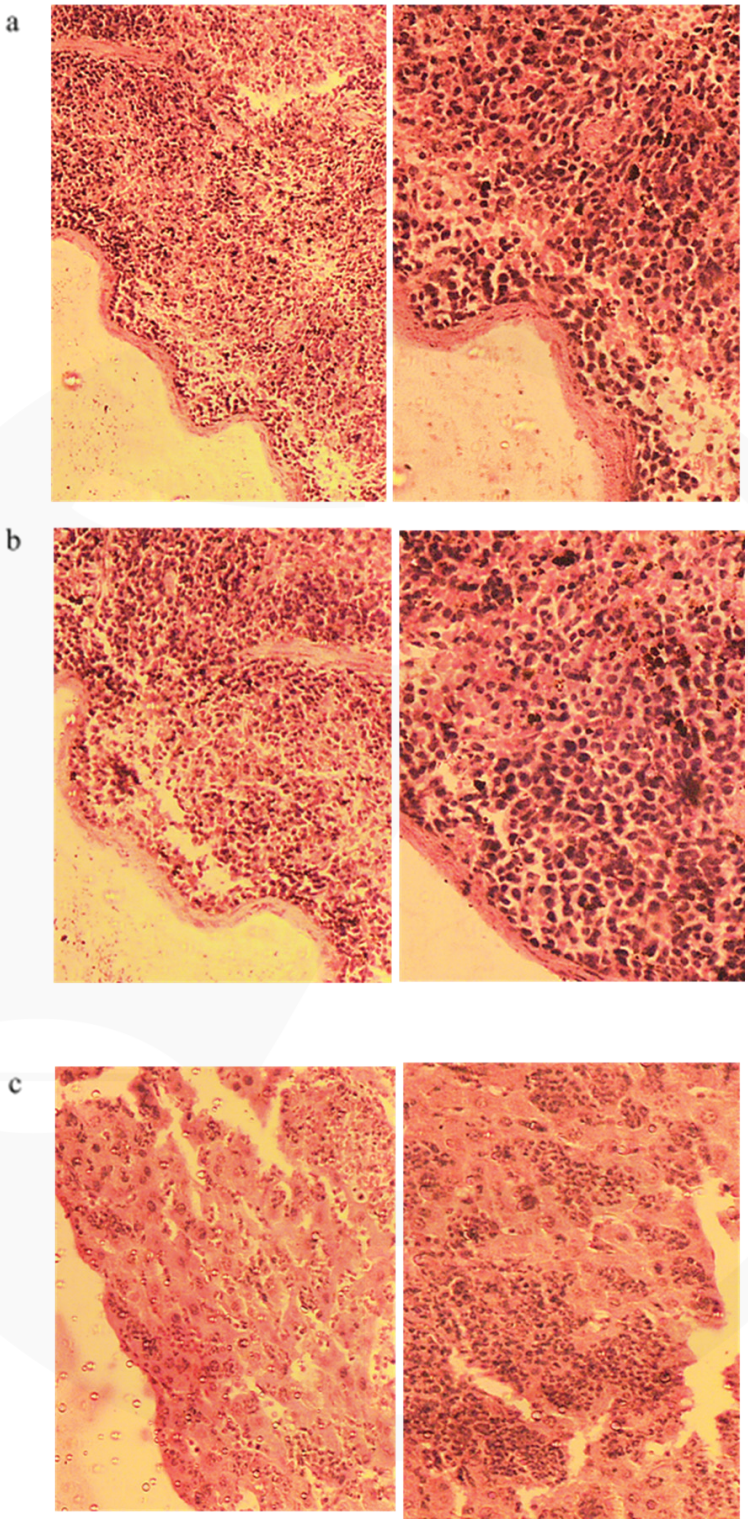


Figure 6. Microscopic slides of the spleen in control (a), group 2 (b) and group 3 (c) in BALB/c mice. (The slides on the left show a scale bar of 100 µm and the slides on the right show a scale bar of 200 µm.)

As can be seen in Figures 6 and 7, the FCF post-treated mice, in addition to tumor formation and enlargement, showed liver and spleen metastases, which was similar to group 4 (cancerous mice, untreated with FCF). However, FCF pre-treated mice had less tumor growth and their livers and spleens were similar to healthy mice (control group). In other words, the livers and spleens of the FCF pre-treated mice were not involved with the breast tumors.

Survival and behavior of mice

It is observed that two of the five mice in group 3 died before the end of the FCF treatment and the rest of them still had tumor burden, but with reduced tumor size. In contrast, the mice in group 2, had no evidence of disease and all five animals were alive. It was observed that one mouse in each control group (1 and 4) died. Interestingly, the only group without any death was group 2, in which pre-treatment with FCF resulted in significant improvement in the survival of treated animals. After surgery, it was found that not only the tumor sizes in groups 3 and 4 were remarkably larger than in group 2, but also a significant increase in liver and spleen size and tissue destruction occurred in group 2 (similar to negative control: group 1); these complications were not observed at all.

Discussion

Due to the prevalence of various types of cancer, using complementary and alternative medicine is one of the topics of interest and reference of researchers and experts globally. FCF as one of the TCFs has been introduced as a complementary therapy by Taheri. In this study, the effects of FCF on tumor growth and metastasis were examined. According to the results, the use of FCF in the model of induced cancer in mice, in the pretreatment model, completely led to the survival of mice, limitation of tumor size and no metastasis. On the other hand, its use after complete tumor formation in mice does not achieve these results. These data suggest that (simultaneous) pre-treatment of mice receiving cancer with FCF can lead to tumor control and inhibition. However, post-tumor therapy did not inhibit the tumor growth (group 3) which was similar to untreated tumors (group 4).

As it was explained briefly in the introduction section, this experiment provided evidence of effectiveness of FCF on an animal model. Although we cannot measure FCF directly and its mechanism is not clear yet, it is possible to investigate its effect through i*n vivo* and *in vitro* experiments (Phase 0 in studying TCFs). Since the effect of FCF is initiated without any kind of physical intervention, it can be considered a safe way to control and prevent diseases like cancer.

These observations were in accordance with previous studies (Taheri et al., 2020; Taheri et al., 2021b; Taheri et al., 2022). Due to the remarkable results of this study in reducing the size and weight of the tumor in the animal model of cancer, conducting a study at the level of primary cancer cells and clinical human studies of this and other types of cancer, is on the research agenda of the authors.

Acknowledgments

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The First Scientific Journal in T-Consciousness Research

Screening the Effect of Faradarmani Consciousness Field on *Ex vivo* Controlled Microenvironment on Solid 4T1 Tumors

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Abstract

Personalized cancer medicine is currently focused on knowledge of the cancer mutation repertoire and the tailored application of drugs that targets altered genes or pathways in individual patients. Thus, a critical need exists for more sophisticated *ex vivo* diagnostic methods that recapitulate human tumor biology and predict response to the targeted and immune-based therapies in real-time. T-Consciousness Fields are immaterial, and non-energy Fields, and their influence on the matter and energy has been observed in several biological and physical studies. In the present study, we developed a 3D Microfluidic controlled microenvironment device that consists of two media channels parallelly running. They are located on either side of an extended, central region, containing the tumor cell spheroids embedded within an extracellular matrix. In these conditions, we investigated the influence of Faradarmani Consciousness Field on this 4T1 cell line. According to the results, under the influence of the treatment of Faradarmani CF, the 4T1 cell line showed a significant increase in survival compared to the control groups.

Keywords: *ex vivo*, Microfluidic, Cell line, Cancer, T-Consciousness Fields, Faradarmani Consciousness Field

Introduction

Cancer is a prominent cause of death worldwide, accounting for nearly 10 million deaths in 2020 (Sung et al., 2021). The risk factors like genetic background, environmental stresses, and improper diet generally lead to molecular changes or mutations which cause the initiation of carcinogenesis (Padma, 2015). In malignant tumors, metastasis poses the biggest problem for cancer treatment. It has been hypothesized that the epithelial to mesenchymal transition (EMT) is an important event in the metastatic cascade. EMT is involved in the generation of tumor cells with stem cell properties and resistance to certain therapeutic interventions (Bullock et al., 2012; Nieto et al., 2016; Lambert et al., 2017). Therefore, it has been considered a viable therapeutic target to combat metastatic disease (Kalluri and Neilson, 2003). Spotting a primary tumor before metastasis is crucial. However, physical examination and traditional imaging methods like MRI, PET, CT, and X-ray have a detection limit for smaller metastasis (Van Dalum et al., 2012). In a patient with solid tumors, Circulating Tumor Cells (CTCs) can be found in the blood, so biomarkers may provide a way to monitor disease progression more directly than traditional imaging methods (Van Dalum et al., 2012, Yang et al., 2019). CTCs hold information about a tumor that could be a key to the cancer diagnosis or treatment (Williams, 2013).

Chemotherapy as an important treatment for cancer patients has some limitations, including a lack of selectivity for tumor cells over normal cells, systemic toxicity, and the appearance of drug-resistant tumor cells (Xu and McLeod, 2001). Numerous therapeutic strategies have been focused on drugs that target altered genes or pathways in individual patients. For example, *Kristen Rat Sarcoma viral oncogene* (KRAS) is one of the earliest oncogenic drivers, so inhibition of KRAS may be a viable therapeutic strategy for this disease (Muzumdar et al., 2017, Uras et al., 2020). A variety of *in vitro* approaches have been used in cancer biology and drug discovery. However, *in vitro* models do not entirely reproduce the cellular diversity and complexity of interactions in tumor niches (Bellido et al., 2020). Two-dimensional (2D) cell culture has many limitations, such as disturbance of interactions between the cellular and extracellular environments, changes in cell morphology, polarity, and method of division (Kapałczyńska et al., 2018). It has been reported that around 95% of new anticancer drugs eventually fail in clinical trials, despite robust indications of activity in existing *in vitro* pre-clinical models (Hickman et al., 2014).

Recent advances in the microfluidic cell or tissue culture technology can be used as a novel *in vitro* drug screening method with high-throughput applications (Tsui et al., 2013) and the development of "organs on chips", enable experimentalists to recapitulate the multicellular architecture, tissue-tissue interfaces and the physiologically relevant physical microenvironment of cancers growing within living human organs while sustaining vascular perfusion *in vitro* (Sontheimer-Phelps et al., 2019). Using 3D culture, the inhibition of KRAS-driven tumorigenicity by interruption of an autocrine cytokine circuit was investigated and identified concurrent inhibition of the TBK1/IKKε, Janus-activated kinase (JAK), and MEK signaling as an effective method to inhibit the actions of oncogenic KRAS (Zhu et al., 2014). A study, screening the therapeutic EMT blocking agents using 3D microfluidic systems, showed that there are significant differences in response to drugs between 2D and 3D and between monoculture and co-culture (Aref et al., 2013). In addition, Jenkins et al. and Aref et al. evaluated tumor-immune interaction in 3D culture using patient- or murine-derived organotypic tumor spheroids (MDOTS/PDOTS) (Jenkins et al., 2017; Aref et al., 2018). Their research showed that this platform can model response to the PD-1 blockade and facilitate precision immune-oncology efforts and ultimately personalized immunotherapy.

In this study, the effects of Faradarmani Consciousness Field on changes in 4T1 cell line was evaluated in *ex vivo* conditions. This field possesses a non-frequency nature. Therefore, it is not possible to measure it with quantitative instruments, but its effects can be recorded through designing experiments (Taheri, 2013). The influence of this field is initiated with a short attention to the subject under study. Previously, the effects of Faradarmani

Consciousness Field on MCF-7 cell line (Taheri et al., 2020), and the mice 4T1 breast cancer model were investigated (Taheri et al., 2022). The obtained results showed that although Faradarmani induced replication and survival of MCF-7 cell line, inhibited the growth of tumor cells and metastasis in 4T1 animal model. The current experiment with three-dimensional culture allows the reproducibility of previous experiment.

Methods and Materials

Application of Faradarmani CF

TCFs were applied to the samples according to the protocols regulated by the COSMOintel research center (www.cosmointel.com). More details are presented in the general considerations of this issue.

2D cell culture

The 4T1 cell line was cultured in a humidified atmosphere with 5% carbon dioxide at 37°C in Dulbecco modified Eagle medium/Nutrient Mixture F-12 Ham supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin solution.

Spheroid preparation

The adherent cells were grown to 80% confluence. Monolayers were rinsed twice with PBS. After draining well, 2 mL (for 100 mm plates) of 0.05% trypsin-1 mM EDTA was added and incubated at 37°C until cells detach. Next, the detached cells were transferred to a 15 mL conical tube and centrifuged at 1200 rpm for 5 mins. Then, the cell pellet was resuspended in a 2 mL complete culture medium and the cells were counted. Using a pipette, 20 µL of cells were deposited on an inverted lid from a 100 mm tissue culture dish. The lid containing 45 deposited cells was inverted in the PBS-filled chamber at 37°C under 5% CO_2 and 95% humidity and was monitored and incubated until the aggregates were formed. After 24h of incubation, drops were washed by the medium from the lid and centrifuged in a conical tube at 1200 rpm for 5 mins to obtain a spheroid pellet.

3D cell culture (day 0)

In order to achieve the proper fractions (40-100 µm), spheroids were filtered by 100 µm and 40 µm cell strainers respectively, and centrifuged at 1200 rpm for 5 min. Subsequently, cell pellets were resuspended in collagen hydrogels (type I rat tail collagen 2.5mg/ml, Corning Co.). Hydrogels containing spheroids were injected into the central channels of 3 (A, B, C) microfluidic devices and incubated for 30 min at 37 °C in humidified chambers. Microfluidic chips were designed by and manufactured at AIM BIOTECH (DAX-1, AIM BIOTECH, https://www.aimbiotech.com/). We used 3 devices for each sample as three replications for the experiments (3 for A, 3 for B, and, 3 for C). Hydrogels were hydrated with DMEM/F12 + 10% FBS + 1% pen/strep and incubated at 37°C for 24 hours.

Treatment of the spheroids (day 0 to day 3)

A (control: DMEM + FBS + pen/strep), B (treatment), and C (control: doxorubicin) were placed in different places in the incubator.

Live /dead assay

Spheroid viability was evaluated on day 3 after staining with AO/PI using the ViaStain™ AO/PI Staining Solution (Nexcelom, CS2-0106) according to the manufacturer's protocol.

Imaging of spheroids and data analysis

Spheroids were imaged on an inverted Nikon Eclipse Ti microscope equipped with a Nikon DS-Qi1Mc camera using NIS-Elements software. The total area of Acridine orange-stained live (green) cells versus Propidium iodide-stained dead (red) cells was quantified.

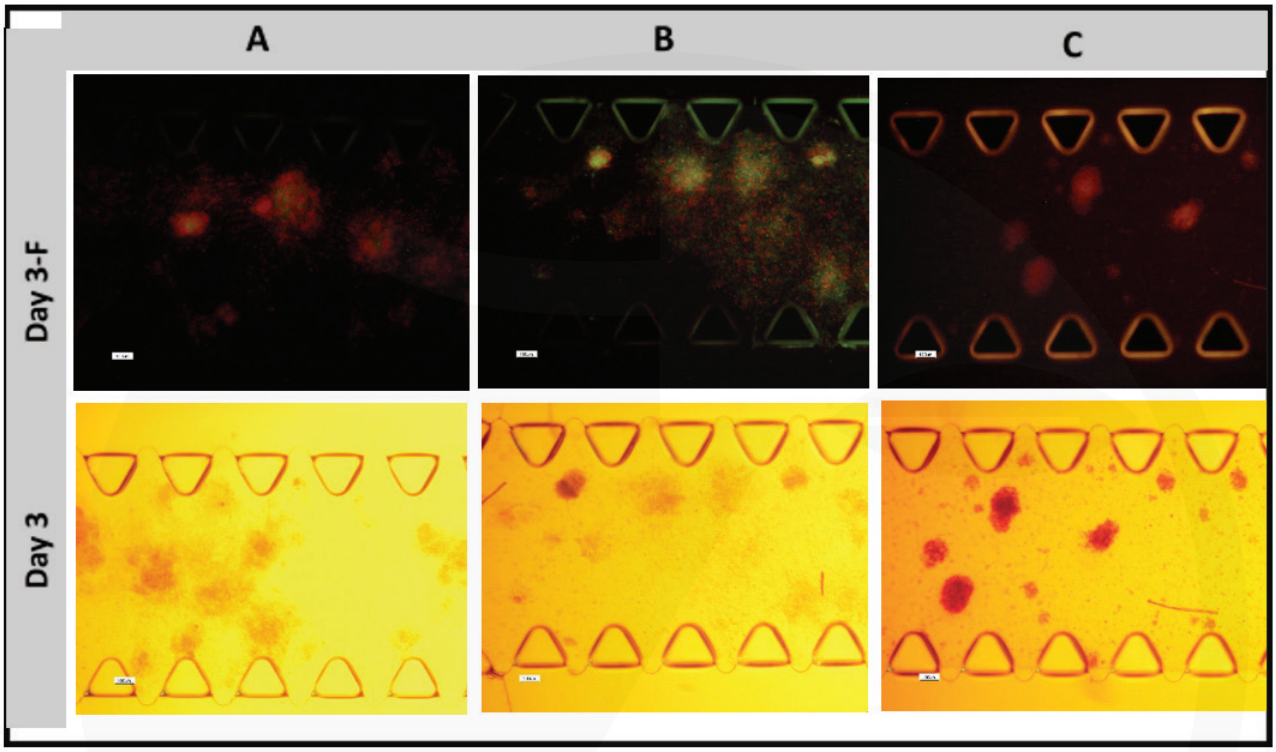
Statistical analysis

In this study there were three replicates in each group and no data were excluded. Data were statistically analyzed using analysis of variance one-way (ANOVA) with GraphPad software (version 9), and a confidence level of 95% considered as significant threshold of data.

Results and Conclusion

To evaluate the effects of the treatment on 4T1 spheroids in microfluidic devices, live and dead cells were counted in fluorescent images from cells in groups A (DMEM-treated cells), B (treated cells), and C (Doxorubicin-treated cells as positive control) on day 3 of the study. The results showed that the percentage of live cells in group B was significantly more than in groups A and C. In other words, treatment slightly improved the viability of the cells. Figure 1 presents the images and the graph.

A)



B)

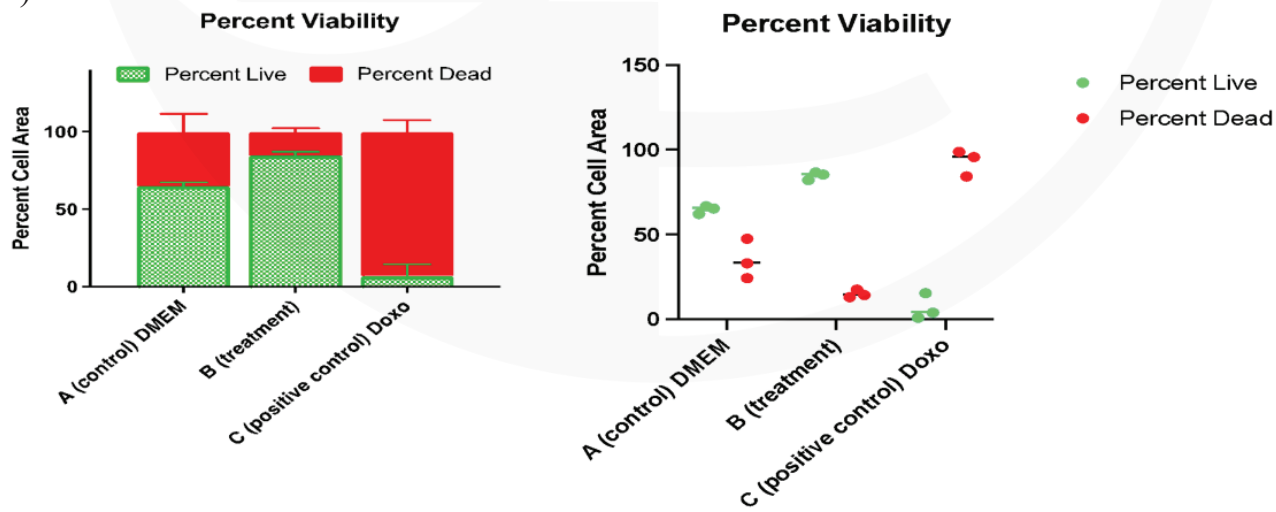


Figure 1. Percentage of cell viability on day 3 of the study. A) Optical and fluorescent Images from the spheroids on day 3. Green: live. Red: dead. Scale bars: 100µm. A: DMEM-treated cells (as control), B: Treated cells, C: Doxorubicin-treated cells (as positive control).

The obtained results in this study were in accordance with observations made on the *in vitro* MCF7 culture (Taheri et al., 2020). Similarly, in the current work, a significant enhancement in survival was observed in Faradarmani-treated 4T1 cell line compared to the control. According to Taheri, Faradarmani CF can transmit information to optimize the system under the study. Considering independent nature of cancerous cell lines in the body of living organisms, it seems that as observed with MCF7 cells, this cell line demonstrates increased proliferation outside the body when exposed to Faradarmani Consciousness Field. Further research on the primary cancer cells (patient), and also study the molecular and immunological changes in variety of cell lines under the influence of Faradarmani Consciousness Field are on the agenda of the authors of this study.

Acknowledgment

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Effect of Faradarmani Consciousness Field on Proliferation, Telomerase Activity, and Telomere Length of Human Mesenchymal Stem Cells

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Abstract

Taheri Consciousness (T-Consciousness) was introduced and defined by Mohammad Ali Taheri as one of the constituent components of the Cosmos, in addition to matter and energy, from which Taheri Consciousness Fields (TCFs) derive. TCFs are not matter or energy, but scientific experiments can prove them. The effect of Faradarmani CF, as one of the TCFs, was examined in this study. Telomerase is an important enzyme that adds DNA sequence repeats to the ends of chromosomes, thus preventing their shortening. Telomerase activity is associated with cell proliferation, organism growth, and aging. This study aimed to study proliferation, telomerase activity, and telomere length in the Faradarmani CF treated human mesenchymal stem cells (hMSCs). Human MSCs were isolated from bone marrow, and the morphology, cell proliferation, telomere length, and telomerase activity were evaluated in the Faradarmani CF treatment and control groups. The results showed that Faradarmani CF significantly increased proliferation, telomere length, and telomerase activity in hMSCs compared with the control group ($p < 0.05$). Given the effects of Faradarmani CF on telomere length and telomerase activity, which are essential factors in the treatment of several age-related diseases, it is recommended that additional experiments be conducted to clarify the effects of TCFs on aging.

Keywords: Taheri Consciousness Fields, Faradarmani Consciousness Field, telomerase activity, telomere length, mesenchymal stem cells

Introduction

The discovery of telomere structure and the accurate description of their functions were important achievements in molecular medicine, especially in gerontology (Blackburn, 2001; Kim et al., 2002). To date, several experimentations and data from long-term medical investigations have confirmed the validity of the telomere theory of aging that associates life expectancy and the progress of age-related diseases with telomere length (Calado et al., 2012; Hoen et al., 2011; Martin-Ruiz et al., 2005; Zglinicki et al., 2005). A reverse association has been found between telomere length and various forms of cancer (Broberg et al., 2005; Bagheri et al., 2006), renal failure (Boxall et al., 2006; Ramírez et al., 2005), cardiovascular disease (Demissie et al., 2006; Zhan et al., 2019), Parkinson's disease (Maeda et al., 2012), and Alzheimer's disease (Cai et al., 2013). In most cases of these diseases, a direct relationship has been found between telomere shortening and a persistent high level of oxidative stress (Wolkowitz et al., 2011; Spivak et al., 2016).

Stem cells (SCs) are undifferentiated cells that can extensively proliferate (self-renew) and differentiate into many cell types in the body. The high differentiation potential of mesenchymal stem cells (MSCs) suggests that any change in their proliferation and metabolism may have unexpected consequences (Brown et al., 2019). Therefore, it seems that hMSCs can be suitable models and reference cells for evaluating the results of Faradarmani CF in biological systems.

Mohammad Ali Taheri introduced novel fields with a non-material/non-energy nature named Taheri Consciousness Fields (TCFs). From this perspective, T-Consciousness is the main component of the universe, and matter, energy, and information are derived from it. According to this theory, there are various TCFs with different functions that are non-frequency-based. These fields cannot be directly measured by science, but their effects can be investigated across various subjects through reproducible laboratory experiments. The effect of TCFs begins with the brief and immediate attention of the Faradarmangar (trained individual) to the subject of study (Taheri, 2013). This study evaluated the effect of Faradarmani CF on stem cell proliferation, telomerase activity and telomere length.

Methods and Materials

Application of the T-Consciousness Fields

TCFs were applied to the samples according to the protocols regulated by the COSMOintel Research Center (www.COSMOintel.com). More details are provided in the Common Considerations section of this issue.

Isolation and cultivation of mesenchymal stem cells

Human MSCs were isolated from a bone marrow sample of a volunteer female donor (25 years old) after obtaining informed consent. To put it briefly, the sample was washed with sterile phosphate-buffered saline (PBS) to remove debris and red blood cells. The sample was treated with 0.075% type I collagenase in PBS for 30 min at 37° C under alternating shaking. Then, collagenase I was neutralized with an equal volume of Dulbecco's Modified Eagle's Medium (DMEM) and 10% fetal bovine serum and centrifuged at 800×rpm for 10 min. The cell pellet was dissolved and cultured in DMEM high-glucose medium with 10% FBS and 1% penicillin/streptomycin. After 24 h of cultivation, non-adherent cells were discarded, and adherent cells were washed twice with PBS. After 80% confluency, the cells were passaged. After two passages, hMSCs were cultured on 6-well plates and kept in an incubator at 37 ˚C and 5% CO_2.

Cell count and telomerase tests

The morphology and number of cells were evaluated 24 hours after cultivation in Faradarmani CF-treated and control cells. After 4 days, the cells were examined under a microscope to assess contamination and cell density. When no contamination was observed, and the cells reached the appropriate confluency, they were passaged. After trypsinisation, the number of cells was counted on the hemocytometer. One mL of suspension was used to evaluate telomerase activity and telomere length.

DNA and RNA extraction

Genomic DNA extraction was performed according to the manufacturer's protocol using a kit (SinaClon, Tehran, Iran). Briefly, after the fourth and fifth passages, hMSCs were lysed in lysis buffer containing 10 mM EDTA pH 8.0, 10 mM Tris-HCl pH 8.0, 100 mM NaCl, 0.5% SDS w/v, and 100 µg/mL proteinase K for 1.5 h at 37 °C. After inactivation of proteinase K by incubation at 75 °C for 15 min, the lysate was treated with 5 µL RNase A (10 mg/mL), then centrifuged, and the supernatant was transferred to a new microtube. An equal volume of phenol:chloroform (1:1) was added and mixed thoroughly. The aqueous phase was transferred to a new tube, and genomic DNA was isolated using the ethanol precipitation method.

RNA extraction was performed using the FavorPrep™ Blood/Cultured Cell Total RNA Mini Kit (Favorgen® Biotech Corporation, Taiwan) according to the manufacturer's protocol. The concentration of RNA was measured using a NanoDrop spectrophotometer (Thermo Fisher Scientific), and samples were stored at −70 °C.

Real-Time PCR

Real-time PCR was performed to measure the relative telomere length and telomerase activity in MSCs. The master mix kit used was the Takara kit from Japan. Real-time PCR was conducted using telomerespecific primers, with 36B4 serving as the single-copy reference gene encoding acidic ribosomal phosphoprotein P0 (located on chromosome 12). The reactions were performed for 30 cycles on an Applied Biosystems 7500 system (USA).

Tel-F (5′-GGTTTTTGAGGGTGAGGGT-GAGGGTGAGGGTGAGGGT-3′), Tel-R (5′-TCCCGTATCCCTATCCCTATCCCTATC-CCTATCCCTA-3′), 36B4-F (5′-CAGCAAGT-GGGAAGGTGTAATCC-3′), and 36B4-R (5′-CCCATTCTATCATCAACGGGTACAA-3′) primers were used. The T/S ratio was calculated from the cycle threshold (Ct) values obtained for the telomere and 36B4 reactions using the comparative Ct method and was expressed as the relative telomere repeat copy number normalized to the 36B4 single-copy reference gene. In addition, telomerase activity was measured using a real-time PCR-based TRAP assay.

Data were analyzed using statistical analysis software (SPSS version 21). Data are presented as mean and standard deviation. A Student's t-test was used to compare the differences between normally distributed variables. A *p-value* < 0.05 was considered statistically significant.

Results

Results of MSCs culture

The average number of mesenchymal stem cells was counted in six wells after 24 h. The number of MSCs in the Faradarmani CF-treated group was significantly higher than that in the control group, showing an approximately 20% increase ($p < 0.05$). Morphologically, cells in the control group appeared more spread. In addition, MSCs in the treatment group appeared to detach from the plate surface more rapidly during trypsinization compared with the control group.

Results of telomere length and telomerase activity

As shown in Table 1, the effect of the Faradarmani CF on the telomere length of MSCs and the telomerase activity was statistically significant compared to the control group. Faradarmani CF significantly increased telomere length and telomerase activity in MSCs ($p < 0.05$).

Table 1. Telomere length and telomerase activity in the control and treatment group under Faradarmani CF.

Variables	Treatment group	Control group	p-value
Length of telomere	1.84±0.03	1.64±0.07	< 0.05
Telomerase activity[a]	0.10±0.005	0.09±0.002	< 0.05

Data are presented as mean ± standard deviation. [a]Telomerase activity was assessed by real-time PCR-based TRAP assay and expressed as relative activity calculated from normalized Ct values using the 2^-ΔΔCt method.

Discussion and conclusion

The aging and age-related diseases are extensively associated not only with medication but also with psychological interventions. These include proper diet, self-control, and mental and physical training to increase the telomerase activity in non-tumor cells and thus counteract age-related telomere shortening and decreased telomerase activity. These are the critical health principles that form a good way to have a long and healthy life (Epel, 2012; Farzaneh-Far et al., 2010; Spivak et al., 2016).

The present study showed that Faradarmeni CF significantly increased proliferation, telomere length, and telomerase activity in hMSCs ($p < 0.05$). Telomere length and telomerase activity are considered important biomarkers of cellular aging and potential therapeutic targets in age-related diseases, which Faradarmani CF affects, as was mentioned in this study.

As mentioned in the introduction section, TCFs, as non-material and non-energy fields, can be applied to all living and non-living beings, including plants, animals, and microorganisms, etc. Although we cannot measure TCFs directly, we can investigate their effects indirectly through various experiments.

In previous studies, we observed the effects of the TCFs on the MCF7 cancer cell line (Taheri et al., 2020a), spatial memory and avoidance behaviour of a rat model of Alzheimer's disease (Taheri et al., 2021a), wheat plant (Torabi et al., 2020), bacterial population growth (Taheri et al., 2021b), and the electrical activity of the brain during Faradarmani in the Faradarmangars population (Taheri et al., 2020b).

The present research was a study on the effect of Faradarmani CF on telomerase activity and telomere length *in vitro*, so additional research on other cell types, and *in vivo* models are required to clarify the biological relevance of these findings.

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Conflicts of Interest

The authors declare no conflict of interest.

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Ms. Laleh Amani, a distinguished, compassionate, and enthusiastic Cosmonautics researcher, passed away in 2021. We express our sincere condolences and gratitude for her extraordinary efforts in this research and pray for her peace of mind.

T-Consciousness Fields and Their Application in Cancer Research

According to Taheri's theory, consciousness is the fundamental constituent of the universe, from which matter, energy, and information originate. This perspective introduces various T-Consciousness Fields that possess a non-frequency nature. It is possible to design different experiments to record their effects. The hypothesis suggests that information transmitted from these fields may lead to changes in the subject under study. In this issue, cancerous and primary cell lines, both in culture conditions and in animal models, were exposed to these fields, and the experimental results have been reported.

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