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

Mohammad Ali Taheri[1], Laleh Amani[2], Ali Zaman Vaziri[3], Ahmad Khalili[4*]

1. Sciencefact R&D Department, Cosmointel Inc. Research Center, Ontario, Canada

2. Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran

3.Cancer Biomedical Center (CBC) Research Institute, Tehran, Iran

4.Department of Virology, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran

* Corresponding author:

Ahmad Khalili, Cancer Biomedical Center (CBC) Research Institute, Tehran, Iran.

Email: Khalili.a.cbc@gmail.com

** Dr. Laleh Amani was an outstanding, compassionate, and enthusiastic researcher in the Cosmointel. Inc studies who passed away in 2021. We extend our sincere condolences and appreciation for her extraordinary efforts in this research and pray for her peace

ABSTRACT

The most common primary spinal cord and brain tumors are gliomas, representing 81% of malignant brain tumors. In neuro-oncology investigations, the rat C6 glioma cell line has mostly been utilized as an experimental model system. Taheri Consciousness Fields (TCFs) founded and 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 effect of TCFs (A and B) on the C6 glioma cell line in three and four treatments compared with the control (nono-treatment) group. To study the morphology and microscopic properties of the cells, cells were detached and stained with trypan blue then the dead cells were counted. An MTT assay was used in order to evaluate the effect of TCFs. The expression of *Bax* and *Bcl-2* genes was assessed by using the RT Real-time PCR. The results showed that the TCFs treatment not only reduced the number of the C6 glioma cells but also changed their morphology. Similarly, in the trypan blue dye count, more dead cells were counted in the TCFs groups in comparison to the control group. In the MTT assay, both TCFs displayed a cytotoxic effect in incubation times on the C6 glioma cells ($p< 0.05$). *Bax*/*Bcl2* ratio increased to 3.5 and 7-fold, compared to the control via TCF (A), and (B), respectively ($P<0.05$). The findings suggest that TCF (A) and (B) can induce apoptosis in the rat C6 glioma cells. The TCF (B) effect was greater than the effect of TCF (A) in all tests. The mechanism of action of TCFs is not still definable by researchers, and it can be useful to elucidate the effects of the TCFs treatment in vivo and in clinical research.

Keywords: Taheri Consciousness Fields, C6 glioma cells, Cell viability

INTRODUCTION

The most common tumors in 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 for progression of the tumor is only eight 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). Compared with the poor outcome and slowly developing technologies for radiotherapy and surgery, the innovation and application of new methods are crucial.

Despite the fact that several cancer treatments have been developed in recent decades, few drugs have been approved for the treatment of glioma by the Food and Drug Administration (FDA). One of the reasons for the lack of progress in the treatment of glioma is the blood-brain barrier, which limits the delivery of therapeutic agents to the brain. The unique CNS structure inhibits from entering the most anticancer drugs into the brain, poses challenges to the progress of anti-glioma 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.

The nature of consciousness and its place in science has received much attention in the current century. Many philosophical and scientific theories have been proposed in this area. In the1980s, Mohammad Ali Taheri introduced novel fields with a non-material/non-energetic nature named Taheri-Consciousness Fields (TCFs). In this perspective, T-Consciousness is one of the three existing elements of the universe apart from matter and energy. According to this theory, there are various TCFs with different functions, which are the subcategories of a networked universal internet called the Cosmic Consciousness Network (CCN). The major difference between the theory of TCFs and other theoretical concepts about consciousness is related to the practical application of the TCFs. TCFs can be applied to all living and non-living creatures, including plants, animals, microorganisms, materials, etc.

Mohammad Ali Taheri, the founder of Erfan Keyhani Halqeh, a school of thought, introduced a new science in 2020 as a branch of this school. He coined the term Sciencefact for this new science because it utilizes scientific investigations to prove the existence of T-Consciousness as an irrefutable phenomenon and a fact. Although science focuses solely on the study of matter and energy and Sciencefact, by contrast, explores the effects of the [non-material/non-energetic] TCFs, Sciencefact has provided a common ground between the two by conducting reproducible laboratory experiments in various scientific fields, and it has used the scientific approach in proving TCFs.

The influence of the TCFs begins with the Connection between CCN as the Whole Taheri Consciousness of the universe and the subjects of study as a part. This Connection called "Ettesal" is established by a Faradarmangar's mind (a certified and trained individual who has been entrusted with the TCFs). The human mind has an intermediary role (Announcer) which plays a part by fleeting attention to the subject of study and then the main achievement obtained as a result of the effects of the TCFs. These fields cannot be directly measured by science, but it is possible to investigate their effects on various subjects through reproducible laboratory experiments (Taheri 2013).

The research methodology in the study of T-Consciousness has been founded on the process

of Assumption, Argument, and Proof, in which the basic Assumption is: The Cosmos was formed by a third element called T-Consciousness that is different from matter and energy.

The Argument: The existence of TCFs can be demonstrated by their effects on matter and energy (e.g., humans, animals, plants, microorganisms, cells, materials, etc.)

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

Accordingly, to investigate and verify the existence, effects, and mechanisms of TCFs, the following five research phases (Phases 0 through 4), and the aims of each phase are outlined below.

Phase-0 studies aim to prove the existence of TCFs by observing their effects. The nature of T-Consciousness and what it is will not be addressed in this phase. Phase-1 explores the varied effects of different TCFs. Phase-2 examines the reason behind the varied effects of these fields. Phase-3 investigates the mechanism of TCFs effects on matter and energy. Finally, Phase-4 draws significant conclusions, particularly with regard to the *mind and memory of matter* and their relation to the T-Consciousness, etc.

In previous research, we observed the effects of the TCFs on MCF7 cancer cell line (Taheri et al., 2020a), Alzheimer's disease rat models (Taheri et al., 2021b), spatial memory and avoidance behavior of a rat model of Alzheimer's disease (Taheri et al., 2021c), wheat plant (Torabi et al., 2020), bacterial population growth (Taheri et al., 2021d), viral replication (Taheri et al., 2021a), 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 (A and B) on three and four announcements in the C6 glioma cell line compared with the non-treatment group (control) by counting and evaluating the number of dead cells, morphology, and microscopic characteristics in cell culture and then MTT test for cell viability and in another step quantitative evaluation of *Bax* apoptotic and *Bcl-2* anti-apoptotic genes expression by using RT Real-time PCR.

Methods and Materials

Application of the TCFs

TCFs were applied to the samples according to the protocols regulated by the COSMOintel research center (www.COSMOintel.com). A request for Connection to the CCN to utilize TCFs can be placed through the COSMOintel website in the "Assign Announcement" section. This access is available for everyone at no cost. In order to study and experience this Connection, the researchers can register on the website at any time and in order to report the experiment to the COSMOintel research center. Certain details of the experiment must be provided to the center; for example, the characteristics or number and name of samples and controls must be specified. This entire experiment was carried out as a double-blind method where lab technicians were completely unaware of TCFs theory, and the Faradarmangar at the COSMOintel research center who established the Connection was unaware of the details of the study. Double-blind is a gold standard that is common in science experiments in the field of medicine and psychology, involving theoretical and practical testing. Announcement programs are shown in Table 1.

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

Groups	Number of Announcements	Announcement intervals after seeding of cells			
TCF(A) group	3	20 min	24 hours	48 hours	
TCF(B) group	4	20 min	24 hours	48 hours	72 hours
Control	0	-	-	-	-

Cell culture and trypan blue staining assay

C6 glioma was prepared by the National Cell Bank of Iran. The cells were cultured in the DMEM F12 media, supplemented with 10% fetal bovine serum (FBS), and 1% penicillin/streptomycin, and incubated at 37 ℃, 90% humidity, and 5% CO_2.

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

In order to study the morphology and microscopic properties of the cells, cells of the 6-well plates were detached by trypsin and mixed with one part of cell suspension and one part of 0.4% trypan blue and were incubated for 3 min at room temperature. Then dead cells were counted by hemocytometer slide and were determined the percentage of living/dead cells. Three replications were considered for all groups.

The formula for calculation of the percentage (%) of the dead cells stained with trypan blue (Strober 2015):

$$\text{Dead cell (\%)} = (A_{\text{Dead cell}}/A_{\text{all counted cell}}) \times 100$$

MTT assay

In order to accurately evaluate the effect (cytotoxicity) of TCFs in the two groups, TCF(A) and TCF(B), in comparison with the non-treated group (control), the MTT assay was used. This is a colorimetric method based on the reduction of yellow tetrazolium by the enzyme succinate dehydrogenase. First, an appropriate number of the cells were seeded in each of the plate wells (10,000 cells per well). After 48 hours and 72 hours for the TCF(A) group and TCF(B) groups, respectively, following the microscopic examination of cells, each plate wells medium was replaced with the MTT solution (0.5 mg/ml MTT in fresh medium), and then they were incubated for 4 hours under 5% CO_2, and 37℃. 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 applied (Chueh et al., 2014), and also 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 Real-time PCR method

Expression of *Bax* and *Bcl-2* was assessed in C6 glioma cancer cells by using RT Real-time PCR. 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 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 instructions of the kit manufacturer.

The Real-time PCR reaction was performed in a MIC real-time PCR system (BioMolecular systems, London, UK). The final volume of each reaction was 20µl , which contained 10 picomoles of reciprocating primers of *Bax* and *Bcl-2* genes for each reaction, 40 ng cDNA, 10 µg SYBR green PCR master mix (Ampliqon, Denmark). In this study, the *GAPDH* gene was considered the reference gene. The sequences of primers utilized are listed in Table 2.

Table 2 . Name and sequence of the primers used in the real-time PCR.

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. For verification of the specificity amplicons, the analysis of the melting curve was performed. The comparative expression of genes was considered by the standard $2^{-\Delta\Delta ct}$ (Livak et al., 2001, Arocho et al., 2006).

Statistical analysis

The one-way ANOVA was applied to assess the TCFs effect on cell viability, genes expression level examination tests, and $p < 0.05$ was considered the significant level.

Results

Evaluation of morphology and microscopic properties

Microscopic examination of the C6 glioma cells showed that these cells, for the control group, averaged 20×10^4 cell/ml, for TCF(A) group, was 15×10^4 cell/ml, and for the TCF(B) group was 14×10^4 cell/ml.

In addition to reducing the number of cells, notable points about the effect of TCFs on cancer cells were changes in the morphology and specific appearance of the nucleus and cell widening. The cells of the TCFs groups were not significantly filled compared to the control group, and there were still empty spaces in the cell culture plate after 24, 48, and 72 h (Figure 1).

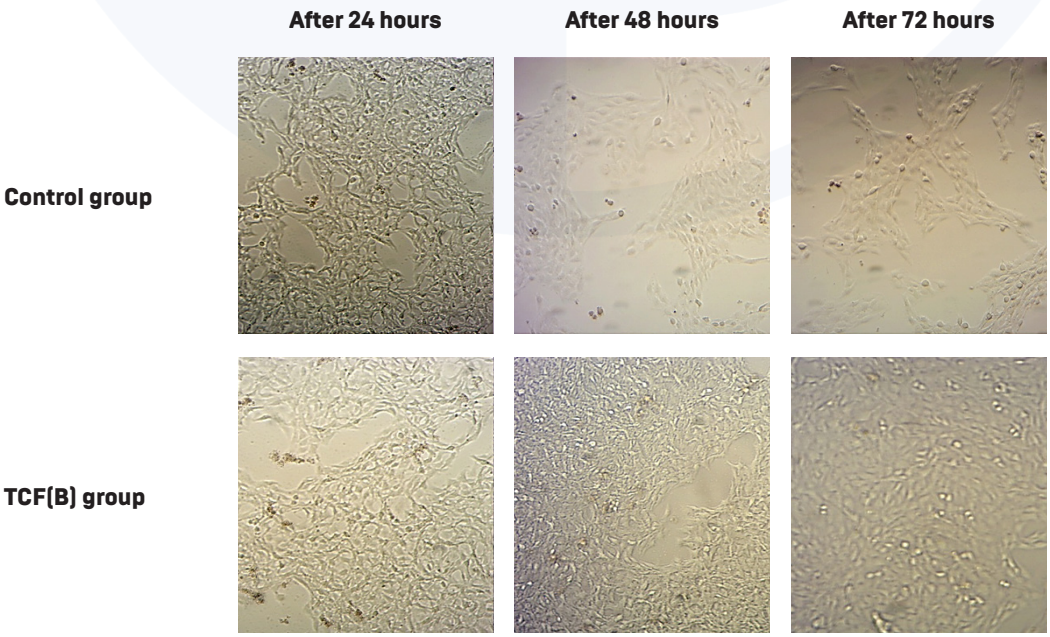


Figure 1. The microscopic evaluation of control and Taheri Consciousness Field (TCF) (B) affected cells at 24, 48, and 72 hours after culture. The TCF(B) group cells were not filled completely compared to the control group and there are still spaces among cells.

Trypan blue staining assay

On average, in trypan blue dye count, about 12%, more dead cancer cells were counted in the TCF (B) group, and 9% in the TCF(A) group compared with the control. This offers a significant, inducing effect of TCFs on the death of the C6 glioma cells ($p < 0.05$).

MTT test results of C6 glioma cell

In order to evaluate the viability of TCF (A) and TCF (B) treated cells, it was used from MTT assay. Results showed that both TCFs were significantly decreased the cells' viability compared with the control group ($p < 0.05$).

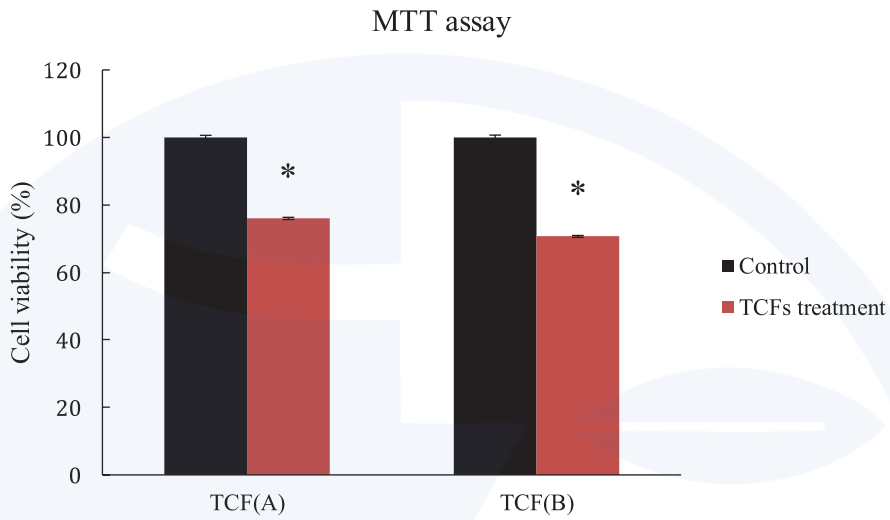
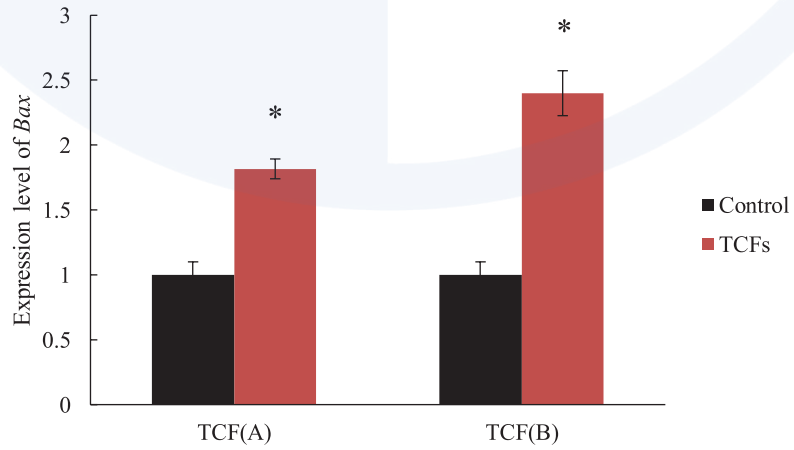


Figure 2. Viability of Taheri Consciousness Field (TCF) (A) and the TCF(B) treated cells compared with the control group. The asterisk (*) indicates a statistically significant difference ($p < 0.05$) between the experimental and control groups.

The RT Real-time PCR

The present study showed the alterations in the expression of *Bcl-2* (anti-apoptotic), Bax (pro-ap-optotic) genes, and the ratio of *Bax/Bcl-2* after stimulating the apoptotic state through TCF (A) and TCF (B) in the C6 glioma cell line. The expression of the *Bax* gene level was increased in both TCF (A), and TCF (B) treated cells compared with control (Figure3, a). The Bcl-2 expression was decreased in both TCFs groups significantly ($p<0.05$; Figure3, b). 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 ($p<0.05$; Figure3, c).

a



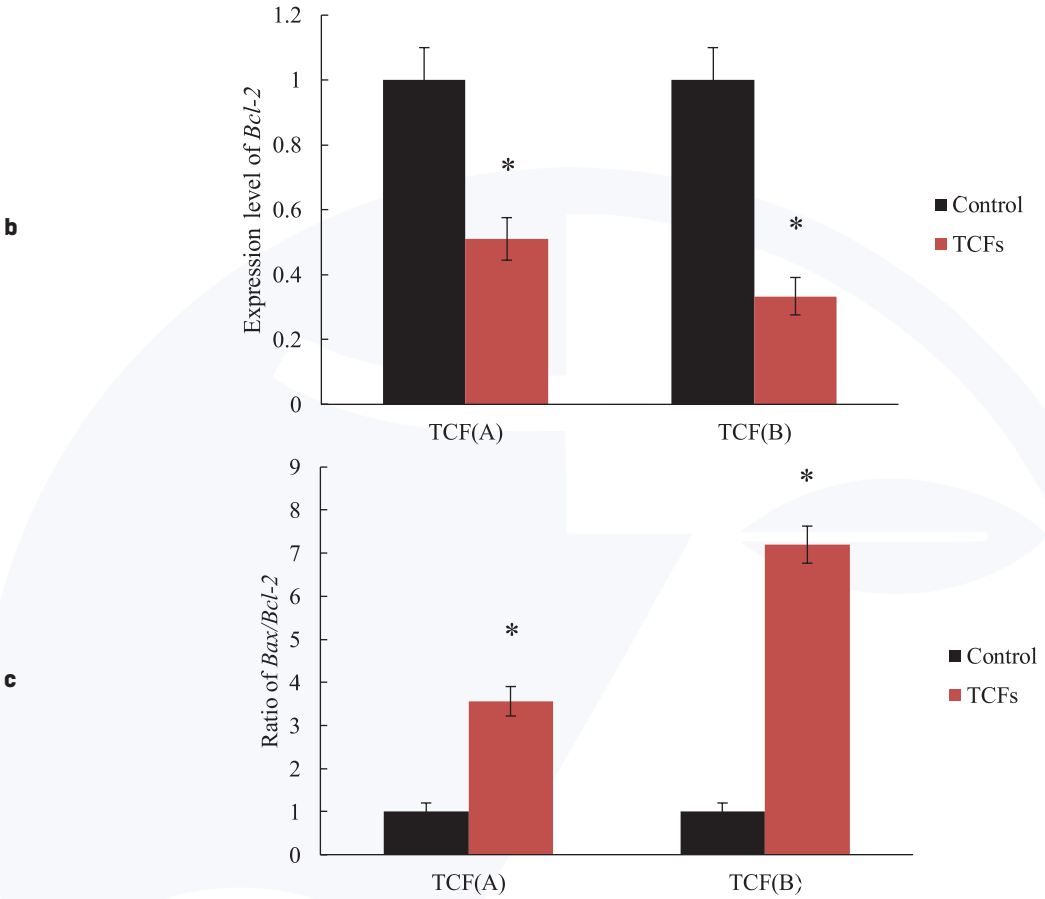


Figure 3. Expression of *Bax* (a) and *Bcl-2* (b) and the ratio of *Bax*/*Bcl-2* (c) in Taheri Consciousness Field (TCF) (A) and (B) treated cells compared with control. The asterisk (*) indicates a significant difference between the TCFs and control groups ($p < 0.05$).

Discussion

The effects of two types of TCFs (A and B) on the number of dead cells, morphology, and microscopic characteristics in cell culture were evaluated. In addition, alteration of the *Bax* apoptotic, and *Bcl-2* anti-apoptotic genes expression under the influence of TCFs was investigated. Using MTT assay for evaluation of cytotoxicity induced by TCF (A) and TCF (B), it was found that both TCFs displayed cytotoxic effect in mentioned incubation times on C6 glioma cells ($p < 0.05$). In trypan blue dye count, more dead cells were counted in TCFs groups compared with control. This observation may be related to the inducing effect of the TCFs on the death of the C6 glioma cells.

The Bcl-2 family members possess a critical role in the regulation of death or survival of the cells. Chemotherapeutic drugs control the expression of various members of the Bcl-2 family in cancerous cells partially. The members of this family contribute significantly to apoptosis via activation (Bax) or inhibition (Bcl-2) (Green et al., 1998, Rao et al., 1997).

Investigation of Bax and Bcl-2 genes expression, along with *Bax*/*Bcl-2* ratio exhibited that the expression of the *Bax* gene was increased and expression of the *Bcl-2* gene was decreased in both TCF (A) and TCF (B) groups compared with control. The *Bax*/*Bcl-2* ratio helps the cell destiny determination (Gross, 2001). *Bax*/*Bcl-2* ratio increased to 3.5, and 7-fold compared to control via TCF (A) and TCF (B), respectively ($P<0.05$), which show significantly the apoptosis induced by TCF (A) and TCF (B).

In the previous study, the effect of Faradarmani CF on the MCF7 cells was evaluated. The Faradarmani CF was applied every hour until the end of the experiment, which was at 6, 18, and 24 hours from the initial start time. Cell viability was assessed by the MTT assay, and the gene expression of *Bax* and *Bcl-2*

in MCF-7 cells was assessed via the RT Real-time PCR . technique. Faradarmani CF significantly increased the proliferation of the MCF-7 cells (18%) in comparison to the control in a time-dependent manner. Moreover, the RT Real-time PCR results showed that in cells treated with Faradarmani CF, the *Bax*/*Bcl-2* ratio was decreased (1-fold) compared with control, which suggested a higher MCF7 cells survival and resistance to death (Taheri et al., 2020a).

The effect of TCFs on the cancer cells seemingly shows different results depending on the type of the cancer cells, duration, and frequency of announcements. The effect of TCF (B) in all experiments was greater than the effect of TCF (A), which indicates the different effects of each TCFs. The findings indicate that it is necessary to investigate other TCFs further and perform laboratory tests in a variety of areas. The mechanism of action of TCFs is not still definable by researchers, and this study suggests that in vivo and clinical research can be useful to elucidate the effects of TCFs treatment.

Acknowledgments

This study was performed at the Cancer Biomedical Center (CBC) Research Institute in Tehran, Iran; we thank the members of this laboratory for their assistance in the experiments.

Conflicts of Inte est

The authors declare no conflict of interest.

References

A Ghotme, K., G. E Barreto, V. Echeverria, J. Gonzalez, R. H Bustos, M. Sanchez, J. Leszek, N. Sastry Yarla, R. Margarita Gomez & V. V Tarasov. (2017). Gliomas: new perspectives in diagnosis, treatment and prognosis. *Current topics in medicinal chemistry*, 17, 1438-1447.

Arocho, A., B. Chen, M. Ladanyi & Q. Pan. (2006). Validation of the 2-DeltaDeltaCt calculation as an alternate method of data analysis for quantitative PCR of BCR-ABL P210 transcripts. *Diagnostic Molecular Pathology*, 15, 56-61.

Ashby, L. S. & W. R. Shapiro. (2004). Low-grade glioma: supratentorial astrocytoma, oligodendroglioma, and oligoastrocytoma in adults. *Current neurology and neuroscience reports,* 4, 211-217.

Ballabh, P., A. Braun & M. Nedergaard. (2004). The blood–brain barrier: an overview: structure, regulation, and clinical implications. *Neurobiology of Dis-ease,* 16, 1-13.

Chueh, P. J., R.-Y. Liang, Y.-H. Lee, Z.-M. Zeng & S.-M. Chuang. (2014). Differential cytotoxic effects of gold nanoparticles in different mammalian cell lines. *Journal of hazardous materials*, 264, 303-312.

Green, D. R. & J. C. Reed (1998) Mitochondria and apoptosis. *Science*, 1309-1312.

Gross, A. (2001) BCL-2 proteins: regulators of the mitochondrial apoptotic program. *IUBMB life*, 52, 231-236.

Hanif, F., K. Muzaffar, K. Perveen, S. M. Malhi & S. U. Simjee. (2017). Glioblastoma multiforme: a review of its epidemiology and pathogenesis through clinical presentation and treatment. *Asian Pacifc journal of cancer prevention: APJCP,* 18, 3.

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* (San Diego, Calif.), 25(4), 402–408. https://doi.org/10.1006/meth.2001.1262.

Louis, D. N., A. Perry, G. Reifenberger, A. Von Deimling, D. Figarella-Branger, W. K. Cavenee, H. Ohgaki, O. D. Wiestler, P. Kleihues & D. W. Ellison. (2016). The 2016 World Health Organization classif cation of tumors of the central nervous system: a summary. *Acta neuropathologica,* 131, 803-820.

Oberoi, R. K., K. E. Parrish, T. T. Sio, R. K. Mittapalli, W. F. Elmquist & J. N. Sarkaria. (2015). Strategies to improve delivery of anticancer drugs across the blood-brain barrier to treat glioblastoma. *Neuro-oncology*, 18, 27-36.

Rao, L. & E. White. (1997). Bcl-2 and the ICE family of apoptotic regulators: making a connection. *Current opinion in genetics & development,* 7, 52-58.

Strober, W. (2015). Trypan blue exclusion test of cell viability. *Current protocols in immunology,* 111, A3. B. 1-A3. B. 3.

Taheri, M. A. 2013. Human from another outlook (2nd Edition). ISBN-13: 978-1939507006, ISBN- 10: 1939507006.

Taheri, M. A., M. R. Etemadi, S. Torabi, N. Nabavi & F. Semsarha. (2021a). Evaluation of the Inf uence of Faradarmani Consciousness Field on Viral Growth. Taheri, M. A., F. Semsarha, M. Mahdavi, Z. Afsartala & L. Amani. (2020a). The Influen e of the Faradarmani Consciousness Field on the Survival and Death of MCF-7 Breast Cancer Cells: An Optimization Perspective. Available at SSRN 3705537.

Taheri, M. A., F. Semsarha & F. Modarresi-Asem. (2020b). An Investigation on the Electrical Activity of the Brain during Fara-Darmani Connection in the Fara-Therapist Population.

Taheri, M. A., S. Torabi, N. Nabavi & F. Semsarha. (2021b). Faradarmani Consciousness Field Suppresses Alzheimer's Disease Development in Both in Vitro and in Vivo Models of The Disease.

Taheri, M. A., S. Torabi, N. Nabavi & F. Semsarha. (2021c). Influe ce of Faradarmani Consciousness Field (FCF) on Spatial Memory and Passive Avoidance Behavior of Scopolamine Model of Alzheimer Disease in Male Wistar Rats.

Taheri, M. A., G. Zarrini, S. Torabi, N. Nabavi & F. Semsarha. (2021d). Infl ence of Fara-darmani Consciousness Field on Bacterial Population Growth. BioRxiv. Torabi, S., M. A. Taheri & F. Semsarha. (2020). Alleviative effects of Fara-darmani Consciousness Field on Triticum aestivum L. under salinity stress. *F1000Research* , 9, 1089.