

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

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## ABSTRACT

Colorectal cancer accounts for 11% of all cancers diagnosed, and it is the second deadliest cancer worldwide. Surgery and chemotherapy or targeted therapy are generally used for the treatment of colorectal cancer. However, there are significant challenges, such as recurrence of tumor and drug resistance, so the application of novel methods is required for the treatment of this cancer. Taheri Consciousness Fields (TCFs) were founded and introduced by Mohammad Ali Taheri. These new fields are not energy or 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 TCFs (A and B) on the HT29 human colon cancer cells in two and three announcements compared with the control group. The morphology and microscopic properties of cells were investigated in TCF (A) and TCF (B) groups compared with the control, and the cells were detached and stained with trypan blue, then the dead cells were counted. To evaluate the inhibitory effect of the TCFs, the MTT assay was used. The expression of two apoptosis-related genes (Bax and Bcl-2) was assessed using RT Real-time PCR. The results demonstrated that TCFs decreased the cell number and changed the morphology of the HT29 cells. In trypan blue dye count, more dead cells were counted in the TCFs groups compared with the control. In the MTT assay, both TCFs decreased the viability of cells on HT29 cells during in-cubation times ( $p < 0.05$ ). *Bax/Bcl-2* ratio increased 4.9 and 7.6-fold in the TCF (A) and TCF (B) treated cells, respectively ( $P < 0.05$ ). Therefore, TCF (A) and TCF (B) induced apoptosis in HT29 cells. The TCF (B) effect was greater than the TCF (A) effect in all tests. There are still many ambiguities and questions about the nature and effects of TCFs. To clarify the issue, more research is needed in vitro, in vivo, and clinically.

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\*\* 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.

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

## The Theory of Taheri Consciousness Fields

The third most common type of cancer is colorectal cancer and 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). Modern investigations have shed light on the pathogenesis of colorectal cancer and offer advanced screening strategies. The prevalence of colorectal cancer is still increasing (Siegel et al., 2020). The current standard treatment of colorectal cancer removes the tumor with surgery and 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 and methods are essential for the treatment of metastatic disease.

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the tumor with surgery and 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 and methods are essential for the treatment of metastatic disease.

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 the 1980s, 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



Vol. 01  
No. 06  
April  
2022

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T-Consciousness Research

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 its 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, 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), tolerance of *Triticum aestivum* L. under salinity stress (Torabi et al., 2020), bacterial population growth (Taheri et al., 2021d), Vesicular Stomatitis Virus (VSV), Herpes Simplex Virus 1 (HSV1), Encephalomyocarditis Virus (EMCV), and Reovirus (Taheri et al., 2021a), and the electrical activity of the brain during Faradarmani in the Faradarmangars population (Taheri et al., 2020b) have been observed.

This study aimed to evaluate the effect of T-Consciousness Fields (A and B) with two and three announcements on HT29 human colon cancer cells compared with the non-treatment group (control). The comparison was made by counting and evaluating the number of dead cells, morphology, and microscopic characteristics in cell culture. MTT test to the comparison of cell viability in the treated and untreated group was done. Also, a quantitative evaluation of Bax apoptotic and Bcl-2 anti-apoptotic genes expression using the RT Real-time Polymerase Chain Reaction (PCR) was performed.

## 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](http://www.COSMOintel.com)). A re-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 2.** Announcement programs for the treatment of HT29 cells and control groups

Groups	Number of Announcements	Announcement intervals after seeding of cells		
TCF(A) group	2	min 20	hours 24	
TCF(B) group	3	min 20	hours 24	hours 48
Control	0	-	-	-

### *Cell culture and study of 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 it is receiving a special interest in studies of food bioavailability and digestion because of the 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% CO<sub>2</sub>. 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, which stains with a distinctive blue color the dead cells, while leaves living cells unstained are seen under a microscope. Living cells do not absorb stains because they have a healthy cell membrane (ref).

For the trypan blue staining assay, cells were detached from 6-well plates with trypsin, then an equal volume of cell suspension and trypan blue

(0.4%) were mixed and incubated for three minutes at room temperature. Then dead cells were counted by hemocytometer slide and were calculated the ratio of living/dead cells. Three replications were considered for all examinations.

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

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

### *MTT assay*

MTT assay was used to determine the effect of TCFs (A and B) on the viability of HT29 cells. First, the HT29 cells were seeded in 96 well microplates (10,000 cells per well). Then, after 24 hours for the TCF (A) group, and 48 hours for the TCF (B) group, the medium of wells was exchanged with MTT solution (0.5 mg/mL), and microplates were incubated at 37°C for four hours under 5% CO<sub>2</sub>. Then, the MTT solution was replaced with 100 $\mu$ l dimethyl sulfoxide (DMSO). A plate reader (BDSL Immunoskan MS, Finland) was used for the evaluation of the absorbance of wells at 570 nm. All tests were repeated three times. to evaluate the cell viability, the following formula was utilized (Chueh et al., 2014):

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



### Evaluation of gene expression by RT Real-time PCR method

The expression of *Bax* and *Bcl-2* in HT29 cells was assessed by RT Real-time PCR using 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 obtained RNA were evaluated through NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific).

The cDNA was synthesized using a cDNA synthesis kit (Biotechrabbit GmbH, Germany) following the manufacturer's instructions. 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 20  $\mu$ l, which contained 10 picomoles of reciprocating primers of *Bax* and *Bcl-2* genes for each reaction, 40 ng cDNA, 10  $\mu$ g SYBR green PCR master mix (Ampliqon, Denmark). In this study, the *GAPDH* gene was considered the reference gene. The sequences of primers utilized in the present study are listed in Table 2.

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

Target gene	Sequence of primers	Length	Annealing temperature
<i>Bax</i>	'F: 5'-TTGCTTCAGGGTTTCATCCAG-3 'R: 5'-AGCTTCTTGGTGAGCGCATC-3	101 bp	65
<i>Bcl-2</i>	'F: 5'-TGTGGATGACTGAGTACCTGAACC-3 'R: 5'-CAGGCAGGAGAAATCAAACAGAG-3	122 bp	66
<i>GAPDH</i>	'F: 5'-CGTCTGCCCTATCAACTTTCG-3 'R: 5'-CGTTTCTCAGGCTCCCTCT-3	74 bp	63

For the PCR reaction, the following conditions were provided: 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 genes expression was calculated with the standard  $2^{-\Delta\Delta ct}$  (Livak et al., 2001, Arocho et al. 2006).

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 intervention group (this could be due to cell weakness and mortality).

The cell confluency of the TCFs groups was significantly lower than the control group, and after incubation times, there were still empty spaces in the cell culture plate.

### Statistical analysis

One-way ANOVA was applied to evaluate the TCFs effects on cell viability, and genes expression level examinations, and  $p < 0.05$  was considered the significant level.

### Trypan blue staining assay

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

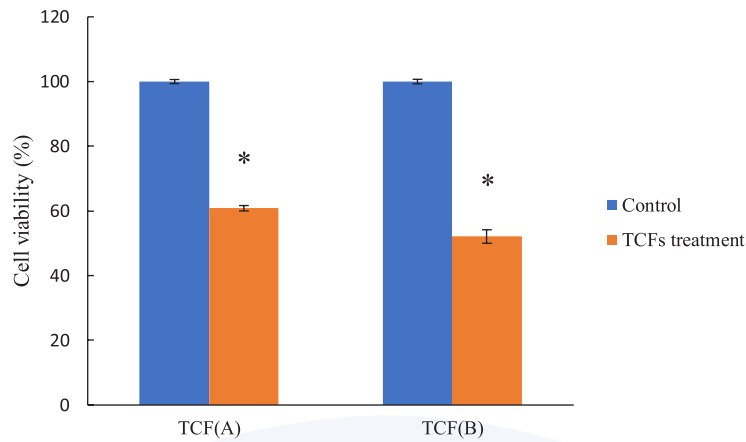
## Results

### Evaluation of morphology and microscopic properties

In a microscopic examination of HT29 cells in the TCFs groups, the colonies were smaller, the cells were

### MTT test results of HT29 human colon cancer cells

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



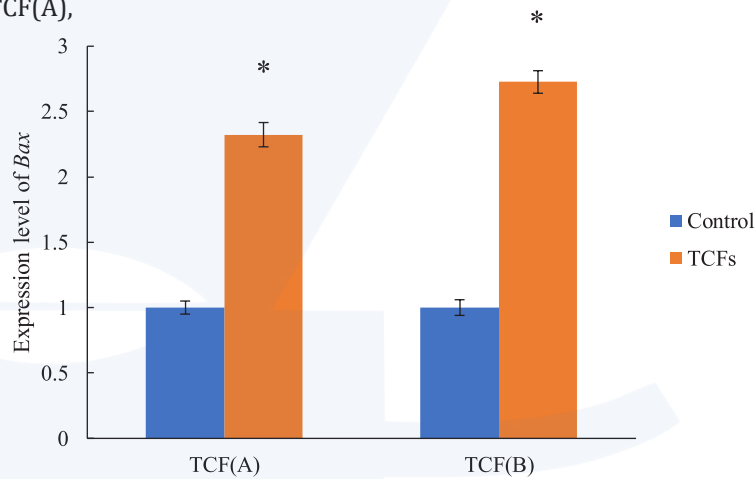
**Figure 1.** Cell viability of TCF (A) and TCF (B) treated groups compared with the control. The asterisk shows a statistically significant difference ( $p < 0.05$ ) between the experimental and control groups.

### RT Real-time PCR

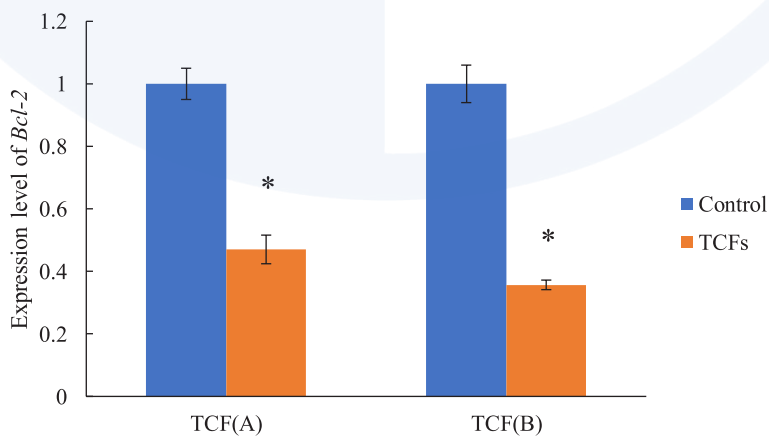
This study showed the change in the expression of *Bcl-2* and *Bax* genes and the ratio of *Bax/Bcl-2* in TCF (A) and TCF (B) treated HT29 cells. The expression of the *Bax* gene was increased in both TCF(A),

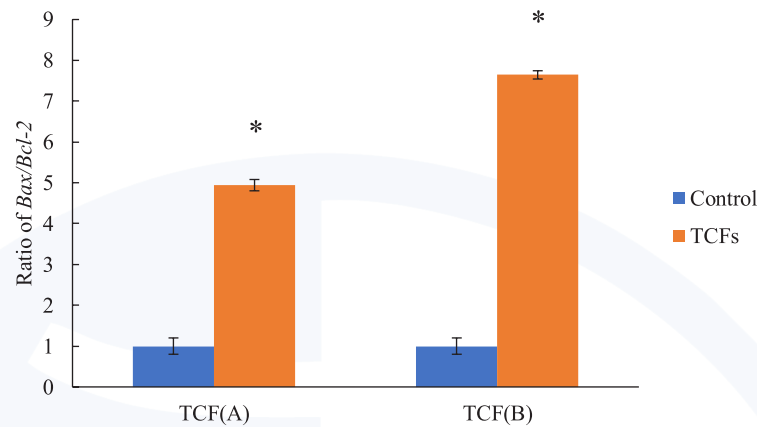
and TCF(B) treated cells compared with control (Figure2, a). The *Bcl-2* expression was significantly decreased in both TCFs groups ( $p < 0.05$ ; Figure2, b). *Bax/Bcl-2* ratio was significantly increased in the TCFs treated cells ( $p < 0.05$ ; Figure2, c).

**a**



**b**





**Figure 2 .** The expression of *Bax* [a] and *Bcl-2* [b] and the ratio of *Bax/Bcl-2* [c] in TCF [A] and TCF [B] 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 (A) and TCF (B) considerably influenced the number of cells and 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 the death of HT29 cells. In the MTT assay, both TCFs decreased the viability of cells at the mentioned incubation times ( $p < 0.05$ ).

The Bcl-2 family members have a critical role in the regulation of death or survival of the cells. Chemotherapeutic agents partially affect the expression of numerous members of the Bcl-2 family in cancerous cells. The members of the Bcl-2 family contribute to apoptosis through activation (*Bax*) or inhibition (*Bcl-2*) genes (Green et al., 1998, Rao et al., 1997).

The expression of *Bcl-2*, *Bax* genes, and the *Bax/Bcl-2* ratio was evaluated in the present study. The results showed that *Bax* gene expression increased and *Bcl-2* gene expression decreased in both TCF (A) and TCF (B) groups compared to the control. The ratio of *Bax/Bcl-2* is an indicator for determining cell susceptibility to apoptosis and helps the determination of cell destiny (Gross 2001). *Bax/Bcl-2* ratio increased 4.9 and 7.6-fold compared to control via TCF (A) and TCF (B), respectively ( $P < 0.05$ ) which shows apoptosis induced by both TCFs significantly.

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. The viability of cells was assessed by MTT assay. The expression of *Bax* and *Bcl-2* genes was assessed via RT Real-time PCR technique in the MCF-7 breast cancer cells. Faradarmani CF significantly increased the proliferation of the MCF-7 cells (18%) in comparison to the control in a time-dependent manner. Furthermore, in the cells treated with Faradarmani CF, the ratio of *Bax/Bcl-2* was decreased (1-fold), compared with the control suggesting a 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 TCFs showed a significant anticancer effect; however, the effects of TCF(B) were greater than TCF(A) in all experiments. It is necessary to further study of 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.

## 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 Interest

The authors declare no conflict of interest.

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