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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'<br>R: 5'-AGCTTCTTGGTGGACGCATC-3' | 101 bp | 65 |
| *Bcl-2* | F: 5'-TGTGGATGACTGAGTACCTGAACC-3'<br>R: 5'-CAGGCAGGAGAAATCAAACAGAG-3' | 122 bp | 66 |
| *GAPDH* | F: 5'-CGTCTGCCCTATCAACTTTCG-3'<br>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 $2.0\times10^5$ cells/mL, $1.5\times10^5$ cells/mL, and $1.4\times10^5$ 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.

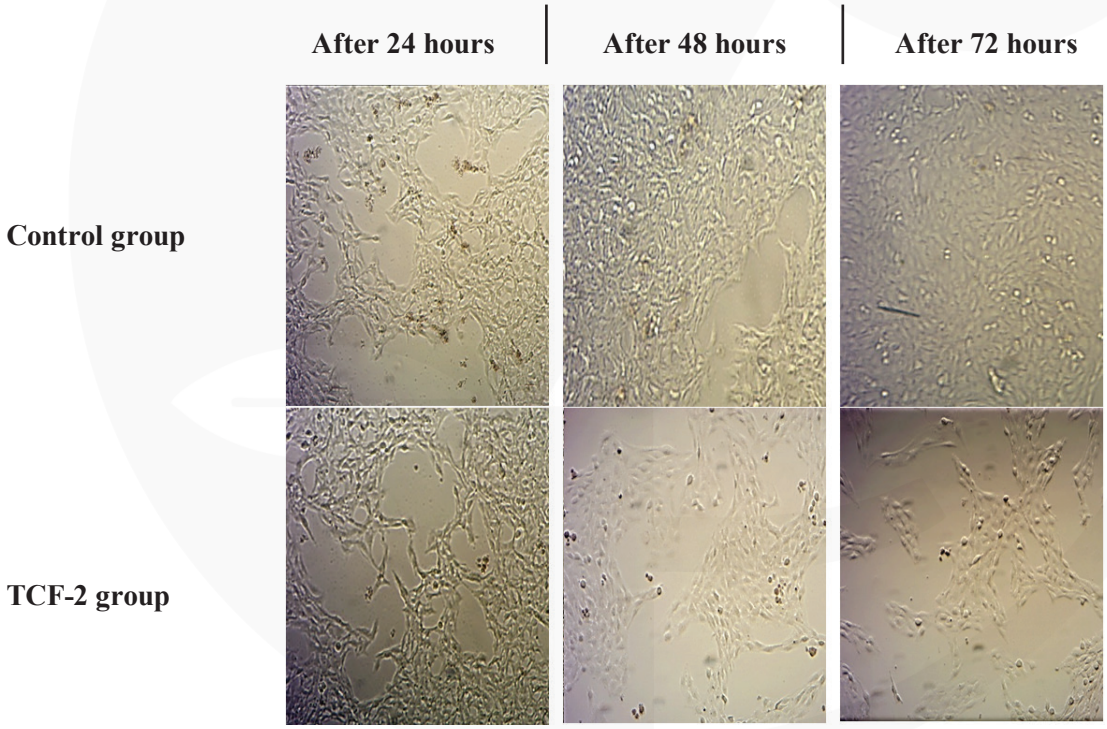



Figure 1. The microscopic evaluation of control and TCF-2 affected cells at 24, 48, and 72 hours after culture. In the TCF-2 group, cells did not fully occupy the culture surface compared to the control group, and intercellular gaps remained visible.

**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 ($p < 0.05$).

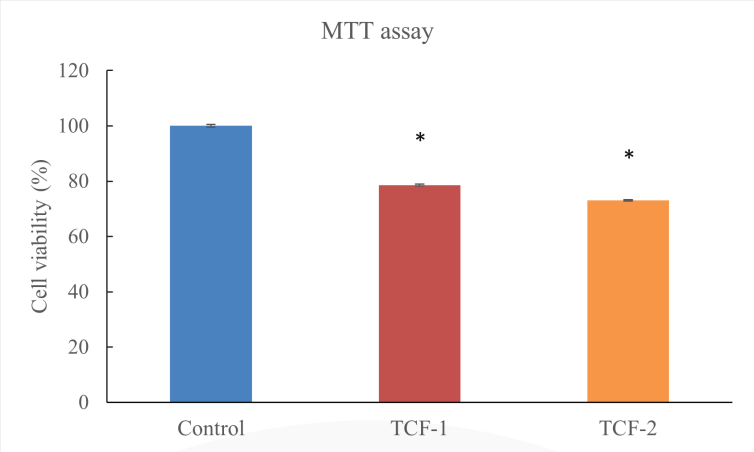
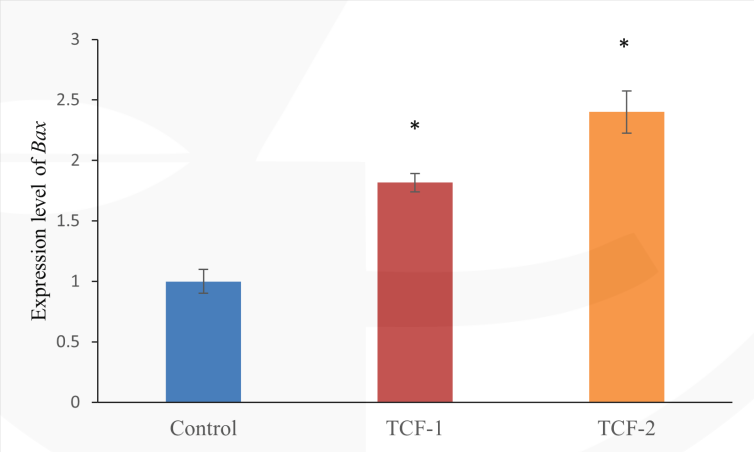
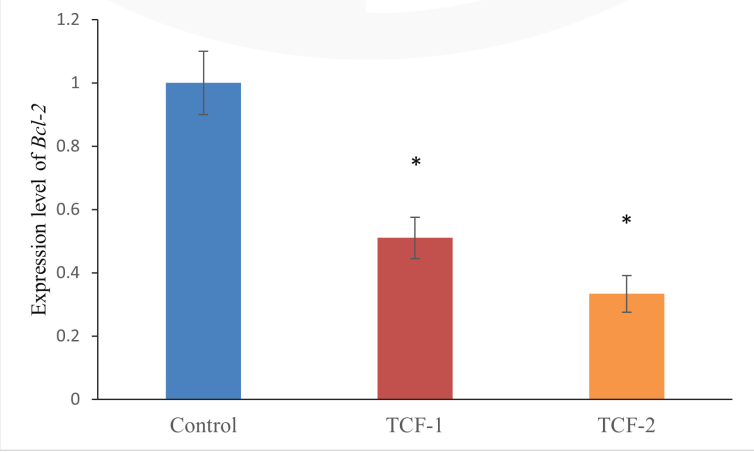



Figure 2. Viability of TCF-1 and TCF-2 treated cells compared with the control group. The asterisk (*) indicates a statistically significant difference ($p < 0.05$) between the experimental and control groups.

## *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).



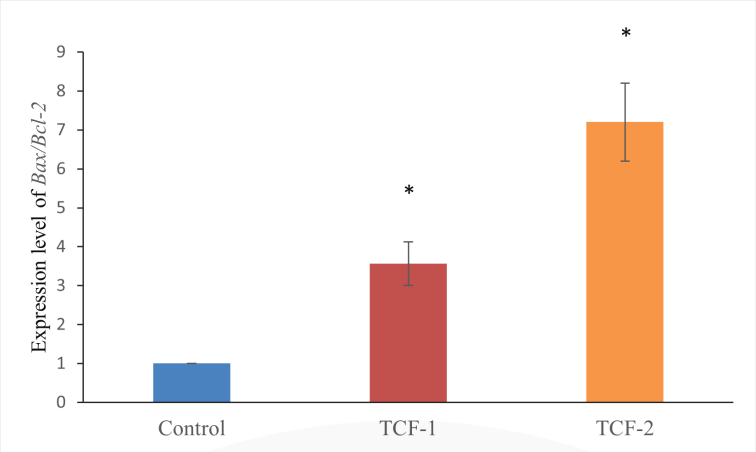



Figure 3. Expression of *Bax* (a) and *Bcl-2* (b) and the ratio of *Bax/Bcl-2* (c) in TCF-1 and TCF-2 treated cells compared with control. The asterisk (*) indicates a significant difference between the TCFs and control groups ($p < 0.05$).

**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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