The Scientific Journal of CosmoIntel
The First Scientific Journal in T-Consciousness Research

The Impact of Faradarmani Consciousness Field on Controlling the Toxicity of Copper Nanoparticles on Lysosomal Membrane Rupture in Human Blood Lymphocytes

Mohammad Ali Taheri[1], Evelyn Assadian[2*], Neda Asghari Kollahi[3]

* Corresponding author: Evelyn Assadian
Email: evelyn.assadian@queenevelyn.com

1. Sciencefact R&D Department, Cosmointel Inc. Research Center, Ontario, Canada
2. Department of Pharmacology and Toxicology, Faculty of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3. Shahid Akbarabadi Clinical Research Development Unit (shACRDU), Iran University of Medical Sciences (IUMS), Tehran, Iran

DOI: https://doi.org/10.61450/joci.v4i18.220

Abstract

This study examines the effect of one type of T-Consciousness Field, named Faradarmani Consciousness Field (FCF), on mitigating the toxicity of copper oxide nanoparticles (CuO-NPs) in human blood lymphocytes. Nanoparticle toxicity, particularly its adverse effects on cellular systems, is a critical concern in nanotechnology. FCF, introduced by Mohammad Ali Taheri, is described as a novel field, neither matter nor energy, and is non-quantifiable. However, its effects can be assessed through standard scientific experiments. In this research, the toxicological effects of CuO-NPs with and without FCF treatment were analysed by evaluating lysosomal membrane integrity and cell viability. Lymphocytes were isolated from healthy individuals' blood and exposed to CuO-NPs at various concentrations for six hours. Results demonstrated that CuO-NPs reduced cell viability in a dose-dependent manner, with an IC50 of 420 µM. The toxicity was linked to oxidative stress, leading to lysosomal membrane rupture and decreased cell survival. Under the influence of FCF, lymphocytes showed improved survival rates despite CuO-NP exposure. FCF significantly (p-value< 0.05) reduced lysosomal membrane damage and increased cell viability. Interestingly, higher nanoparticle concentrations in the presence of FCF corresponded to enhanced cell survival compared to untreated controls. This study highlights the potential protective effect of FCF against the toxicity of nanoparticles, paving the way for further research into their mechanisms and applications in biomedical science.

Keywords: Faradarmani Consciousness Field, CuO nanoparticles, Lysosomes, Toxicology

Introduction

Nanotechnology has revolutionized numerous fields, from medicine to environmental science, by offering innovative solutions at the nanoscale. Among these advancements, copper oxide nanoparticles (CuO-NPs) have garnered significant attention due to their unique properties and wide applications. The pharmaceutical industry has increasingly embraced nanotechnology to enhance drug delivery (especially for cancer therapy), and therapeutic efficacy (Gaetke and Chow, 2003).

Copper oxide nanoparticles (CuO-NPs) have gained significant interest due to their diverse applications across various fields. In pharmaceuticals, they enhance drug solubility, stability, and controlled release, and their ability to generate reactive oxygen species (ROS) has been utilized in cancer treatments for selectively targeting tumor cells (Assadian et al., 2018). They are also being explored as contrast agents in medical imaging techniques such as MRI and fluorescence imaging. Additionally, CuO-NPs are used in biosensors for detecting biomolecules and pathogens, as well as in batteries, fuel cells, and photocatalysis due to their excellent electrical and thermal conductivity. In environmental applications, they aid in wastewater treatment by degrading organic pollutants through photocatalysis, while in agriculture, they serve as antimicrobial agents in pesticides and fertilizers, protecting crops from infections and facilitating the controlled release of nutrients to improve soil fertility (Patra et al., 2018).

Despite their advantages, the use of CuO-NPs in medicine raises concerns about toxicity, as they can induce oxidative stress, damage cellular structures, and cause lysosomal membrane rupture in human blood lymphocytes, leading to cell damage and reduced viability (Ahmed et al., 2010). To address these challenges, recent research has focused on mitigating nanoparticle-induced toxicity through approaches such as coating CuO-NPs with biocompatible materials to reduce surface reactivity while maintaining their functionality (Hussain et al., 2016).

Studies on exposure to CuO-NPs have shown that it leads to oxidative stress, DNA damage, growth inhibition in organisms, and cell death (Assadian et al., 2019). The toxicity of various nanomaterials has been investigated in mice. It was demonstrated that CuO-NPs, compared to titanium (Ti), iron (Fe), or silver (Ag) oxides, cause stronger inflammation in addition to increased total cell and neutrophil uptake into the lungs, elevated total protein levels, and lactate dehydrogenase activity in Broncho alveolar lavage (BAL) fluid. Moreover, along with evidence of inflammation induced in mice exposed sub-acutely to CuO-NPs, these nanoparticles are considered one of the key toxic nanomaterial's for mammals (Grassian et al., 2007).

Few studies have examined the adverse effects of CuO on the immune system, and the potential toxic impact of CuO-NPs on the human immune system has yet to be thoroughly investigated. This research aimed to study the effect of CuO-NPs on isolated human lymphocytes, as adverse effects on these crucial components of the immune system could disrupt its overall functioning, and investigated the possible role of Faradarmani Consciousness Field (FCF) in controlling CuO-NP toxicity by assessing their impact on lysosomal membrane integrity and lymphocyte survival. To determine the underlying effect of CuO-NP cytotoxicity, we studied the effects of half IC50, IC50, and 2IC50 concentrations of CuO-NPs on various cellular and intracellular factors. The IC50 of CuO nanoparticles refers to the concentration that kills 50% of lymphocytes during a 12-hour treatment. In the sample, all conditions are identical to the control, with the only difference being that all processes are influenced by FCF.

Given their extensive applications, it is crucial to explore strategies for minimizing nanoparticle-induced cellular damage to ensure safer use in advanced medicines. Since CuO-NPs can enter the body through the skin, eyes, or inhalation, their toxic effects are unavoidable (Fahmy and Cormier, 2009). Therefore, developing methods to control their toxicity and mitigate

their harmful effects on humans and biological systems, including animals, is essential (Siddiqui et al., 2013).

Materials and Methods

Faradarmani Consciousness Field Application

Faradarmani was applied to the samples according to protocols regulated by the COSMOintel research center (www.COSMOintel.com). More details are presented in general consideration of this issue. The samples without Faradarmani Consciousness Field application served as controls and for treated samples, this field was announced once at the beginning of the experiment.

Chemicals

Acridine orange, CuO nanoparticle, and trichloroacetic acid were purchased from Sigma-Aldrich Co. RPMI1640 and FBS (fetal bovine serum) were purchased from Gibco, Life Technologies, Grand Island, NY., bovine serum albumin, Ficoll-Paque PLUS was obtained from GE Healthcare Bio-Science Company.

Isolation and Treatment of Human Lymphocytes

Lymphocytes were obtained from the blood of 20 healthy donors aged 18 to 40 years who showed no signs of infectious disease at the time of sample collection. After dilution with an equal volume of phosphate-buffered saline (PBS), the blood was layered over 3 mL of Ficoll-Paque and centrifuged at 2000 × g for 20 minutes. The plasma layer was carefully removed, and the lymphocyte-containing layer (the buffy coat) was transferred to a new 15 mL tube. The transferred cells were diluted with 10 mL PBS and centrifuged at 1000 × g. The supernatant was discarded.

After the washing step, lymphocytes were suspended in red blood cell lysis buffer (150 mM NH4Cl, 10 mM NaHCO3, 1 mM EDTA, pH 7.4) and incubated at 37°C for 5 minutes. The cells were washed twice with PBS, and approximately 1–1.5 × 10^6 cells were resuspended in 1 mL of RPMI-1640 medium containing 10% FBS and 1% antibiotic (pen-strep) for further experiments. To assess cell viability, lymphocytes were treated with 0.1–5 mM CuO nanoparticles (CuO-NPs) for 12 hours. Additional experiments were performed by incubating lymphocytes with 0.2, 0.4, and 0.8 mM CuO-NPs for 2, 4, and 6 hours.

Cell Viability Assay

The trypan blue exclusion dye was used to evaluate cell viability, as it can penetrate the cell membrane and label dead cells. Approximately 1 × 10^4 human lymphocytes per well were cultured in 96-well plates and treated with different concentrations of CuO-NPs. An equal volume of the cell suspension was mixed with 0.4% trypan blue and loaded onto a hemocytometer. Dead and live cells were counted using a light microscope, and cell viability as well as EC50 values were calculated.

The $IC50_{12h}$ of a chemical/toxicant was defined as the concentration that kills 50% of the lymphocytes after 12 hours of exposure. To determine this value for the compound under investigation, dose-response curves were plotted for five different CuO-NP concentrations (0.1, 0.5, 1, 2, 5 mM) after 12 hours of exposure, and the EC50 was determined based on the regression plot. The $IC50_{12h}$ for CuO-NPs was determined to be 0.4 mM. Further experiments were conducted according to a standard protocol by incubating CuO-NPs at concentrations of IC50/2 (0.2 mM), IC50 (0.4 mM), and 2 × IC50 (0.8 mM). These concentrations covered all levels of CuO-NP toxicity in human blood lymphocytes, ranging from sub-toxic (0.2 mM), threshold toxic (0.4 mM), to highly toxic (0.8 mM).

To evaluate lysosomal membrane leakage and other mechanistic parameters, time intervals of 2, 4, and 6 hours were selected to avoid highly toxic conditions (i.e., excessive cell death).

These intervals were chosen based on the prior determination of the IC50 (50% cell death) for CuO-NPs in human lymphocytes after 12 hours of exposure (Pourahmad et al., 2011).

Assessment of Lysosomal Membrane Destabilization

The integrity of lysosomal membranes in human lymphocytes treated with CuO-NPs was assessed at 2, 4, and 6-hour intervals. For this purpose, a lipophilic dye was used, which accumulates in acidic organelles such as lysosomes. Upon lysosomal membrane damage, the dye leaks out of the organelle (Assadian et al., 2023).

100 µL of 5 µM acridine orange was added to 100 µL of the cell suspension and incubated at 37°C for 10 minutes. The lymphocytes were washed, and the fluorescence of released acridine orange was measured using a fluorescence spectrophotometer at excitation and emission wavelengths of 470 nm and 540 nm, respectively. Then, we subjected the sample to Faradarmani Consciousness Field to investigate its effects on the toxicity control of copper nanoparticles in human blood lymphocytes, considering the findings of our previous research on nanoparticle toxicity to analyze and interpret the results of this study (Taheri et al., 2022).

Statistical Analysis

GraphPad Prism 10 software (GraphPad, La Jolla, CA) was used for data analysis. Statistical analysis of the data was performed using one-way and two-way ANOVA, followed by Tukey's and Bonferroni post-hoc tests. A minimum of three independent experiments were conducted. A p-value of less than 0.05 was considered statistically significant. Results are presented as mean ± standard error of the mean (SEM).

Results

As shown in Figure 1, copper oxide nanoparticles (CuO-NPs) reduce the viability of human lymphocytes in a concentration-dependent manner over a 12-hour period, as assessed using the trypan blue exclusion dye method. A significant decrease in cell viability is observed at concentrations above 0.1 mM ($P < 0.05$), with highly significant effects at 0.5 mM and above ($P < 0.001$). The $IC50_{12h}$, defined as the concentration that reduces viability by 50% after 12 hours of exposure, is determined to be 420 µM (0.42 mM). These findings highlight the cytotoxic potential of CuO-NPs, emphasizing their dose-dependent impact on lymphocyte survival.

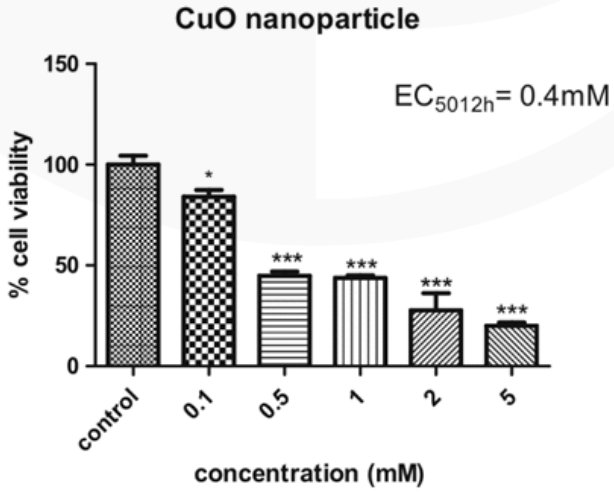

Figure. 1- Cell viability after treatment of human lymphocytes with CuO- NPs for 12 h. *P < 0.05, **P < 0.01, and ***P < 0.001

Figure 2 presents the significant effect of copper nanoparticles (CuO-NPs) on the survival of human lymphocytes and the effects of Faradarmani in reducing the cytotoxicity induced by these nanoparticles. CuO-NPs have been shown to exert dose- and time-dependent cytotoxic effects characterized by reduced cell survival, oxidative stress, and disruption of the lysosomal membrane. At higher concentrations and prolonged exposure times, these toxic effects become more pronounced, as observed in control samples, the highest concentration of CuO-NPs (0.8 mM) significantly induced lysosomal membrane leakage at all times. With the influence of FCF, the resistance of the lysosomal membrane increased at all concentrations, especially at 0.8 mM, resulting in a significant increase in viability (p-value< 0.05).

When comparing untreated control groups with FCF-exposed groups, a significant protective effect was observed in FCF-treated samples at all concentrations and time intervals. FCF exposure resulted in higher cell survival rates, indicating that FCF effectively counteracted the harmful effects of CuO-NPs.

Treatment with FCF significantly improved cell survival in the presence of CuO nanoparticles (CuO-NPs), with notable differences observed at specific concentrations and time points. At 0.2 mM, FCF led to a significant increase in survival after 2 hours compared to the corresponding CuO-treated control (Con-0.2) ($P < 0.05$). Similarly, at 0.4 mM, a statistically significant enhancement was observed at 2 hours relative to Con-0.4 ($P < 0.05$). The most prominent effect was observed at the concentration of 0.8 mM, where the Faradarmani Consciousness Field improved cell viability by 140% at 2 hours, 103% at 4 hours, and 89% at 6 hours. The highest increase was observed at 2 hours. ($P = 0.0008$). These results highlight the strong protective capacity of FCF, particularly at higher concentrations and early exposure times, against CuO-NP-induced cytotoxicity.

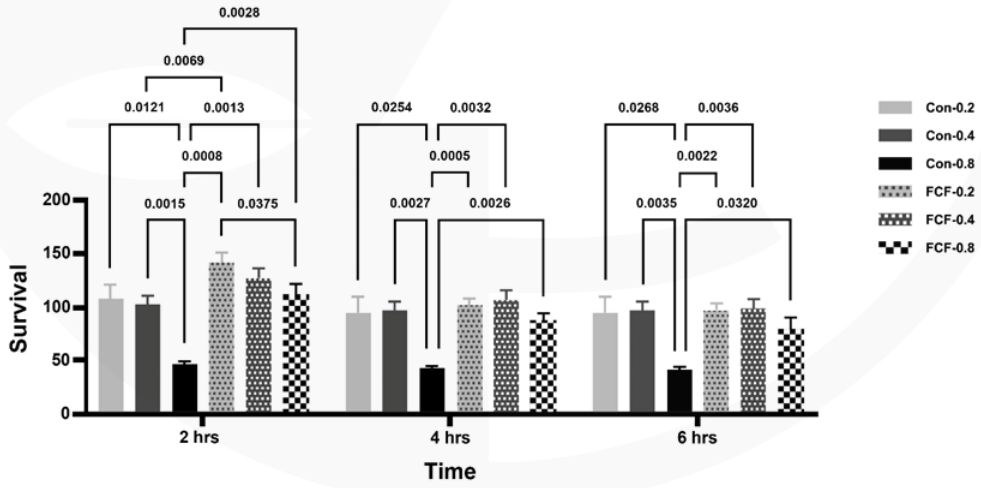

Figure 2. Lymphocyte survival rates after exposure to 0.2, 0.4, 0.8 concentrations of CuO nanoparticles (CuO-NPs) with and without Faradarmani Consciousness Field (FCF) over 2, 4, and 6 hours. Statistical significance (p-values) highlights the differences between conditions.

Discussion

Nanoparticles, particularly copper oxide nanoparticles (CuO-NPs), have demonstrated significant cytotoxic potential, raising concerns regarding their safe application in biomedical, industrial, and environmental fields. In this study, we examined the impact of CuO-NPs on human lymphocyte survival and investigated whether Taheri Consciousness Fields (FCF) could mitigate their toxic effects. The control group was treated with CuO-NPs alone, while the experimental group was exposed to both CuO-NPs and FCF. The results provided compelling evidence that FCF significantly improve cell survival, suggesting a potential protective mechanism against nanoparticle-induced toxicity.

The toxic effects of CuO-NPs have been widely reported, with multiple studies demonstrating a dose-dependent decrease in cell viability across various human cell lines. Previous research has shown CuO-NPs reduce cell viability in HepG2 cells (48% at 10 μg/mL), A549 lung epithelial cells (93% at 20 μg/cm² and 50% at 15 μg/mL) (Brandão et al.,2020), HEp-2 airway epithelial cells (60% at 80 μg/cm²) (Farshori et al.,2022), SH-SY5Y neuroblastoma cells (60–70% at 0.01–10 μM) (Brandão et al.,2020). Similarly, our findings revealed a 50% reduction in lymphocyte viability at 420 μM CuO-NPs, consistent with these studies (Assadian et al., 2023). The cytotoxicity of CuO-NPs is largely attributed to oxidative stress, lysosomal membrane disruption, and DNA damage, leading to cellular dysfunction and apoptosis (Kubo et al.,2020).

The results in Figure 2 further strengthen the hypothesis that FCF enhance cellular resistance to CuO-NP-induced cytotoxicity. Over a 2, 4, and 6-hour period, lymphocyte survival was consistently higher in FCF-exposed groups compared to CuO-NP-treated controls. The statistically significant p-values indicate a clear protective effect, particularly at higher CuO-NP concentrations. At 2 hours, cell viability in control groups significantly declined, especially at 0.8 mM CuO-NPs ($p < 0.05$), while FCF-treated samples exhibited notable resistance to cell death. This trend persisted at 4 and 6 hours, suggesting that FCF not only reduce immediate toxicity but may also facilitate long-term cellular adaptation and recovery.

A key observation was the stabilization of lysosomal membrane integrity in the presence of FCF. Given that CuO-NPs have been shown to cause lysosomal rupture, leading to the release of hydrolytic enzymes and subsequent cell death, our findings suggest that FCF may help maintain lysosomal integrity, thereby preventing premature cell death. These results align with previous research showing that Faradarmani and FCF mitigate environmental and biological stressors, including their ability to reduce oxidative stress in plants (Torabi et al., 2023) and decontaminate radioactive materials in aquatic environments (Taheri et al., 2022).

These findings offer new insights into the potential application of FCF as a protective treatment against nanoparticle-induced cytotoxicity. The observed increase in cell survival suggests that FCF may modulate key biological pathways involved in oxidative stress response, apoptosis regulation, and cellular repair mechanisms.

The exact molecular mechanisms underlying these effects remain unknown, warranting further exploration into mitochondrial function and its role in oxidative stress regulation, inflammatory responses triggered by CuO-NPs and the potential of FCF to modulate immune cell activity, and the long-term adaptation of cells to nanoparticle exposure in the presence of FCF.

Additionally, the integration of FCF with nanotechnology presents promising opportunities in biomedical applications. Given the increasing concerns surrounding nanoparticle safety, the use of FCF in drug delivery systems, regenerative medicine, and environmental decontamination could pave the way for safer and more efficient therapeutic strategies.

Conclusion

In conclusion, this study suggests that FCF treatment may reduce oxidative stress, stabilize lysosomal membranes, and enhance cellular repair mechanisms, thereby improving resilience against nanoparticle-induced damage. We suggest that this research be expanded using diverse cellular models and toxicity mechanisms to validate its applicability in nanomedicine and toxicology.

Acknowledgement

The authors would like to express their sincere appreciation to Shahid Akbarabadi Clinical Research Development Unit (shACRDU), Iran University of Medical Sciences (IUMS), Tehran, Iran, for providing data acquisition service for this research work.

References

Ahmed, M., Anjum, M., Riaz, S., Irfan, M., & Bhatti, H. N. (2010). Genotoxic potential of copper oxide nanoparticles in human lung epithelial cells. *Biochemical and Biophysical Research Communications, 396*(2), 578–583. https://doi.org/10.1016/j.bbrc.2010.04.156

Assadian, E., Jamali, Z., Salimi, A., & Pourahmad, J. (2023). Antioxidants and mitochondrial/lysosomal protective agents reverse toxicity induced by titanium dioxide nanoparticles on human lymphocytes. *Toxicology and Industrial Health, 39*(10), 594–602. https://doi.org/10.1177/07482337231196293

Assadian, E., Zarei, M. H., Gilani, A. G., & Pourahmad, J. (2018). Toxicity of copper oxide (CuO) nanoparticles on human blood lymphocytes. *Biological Trace Element Research, 184,* 350–357. https://doi.org/10.1007/s12011-017-1205-6

Assadian, E., Zarei, M. H., Gilani, A. G., & Pourahmad, J. (2019). Toxicity of Fe_2O_3 nanoparticles on human blood lymphocytes. *Biomedical and Molecular Toxicology, 33*(6). https://doi.org/10.1002/jbt.22303

Brandão, F., Fernández-Bertólez, N., Rosário, F., Bessa, M. J., Fraga, S., Pásaro, E., Teixeira, J. P., Laffon, B., Valdiglesias, V., & Costa, C. (2020). Genotoxicity of TiO_2 Nanoparticles in Four Different Human Cell Lines (A549, HEPG2, A172 and SH-SY5Y). *Nanomaterials*, *10*(3), 412. https://doi.org/10.3390/nano10030412

Fahmy, B., & Cormier, S. A. (2009). Copper oxide nanoparticles induce oxidative stress and cytotoxicity in airway epithelial cells. *Toxicology in Vitro, 23*(7), 1365–1371. https://doi.org/10.1016/j.tiv.2009.07.029

Farshori, N. N., Siddiqui, M. A., Al-Oqail, M. M., Al-Sheddi, E. S., Al-Massarani, S. M., Ahamed, M., Ahmad, J., & Al-Khedhairy, A. A. (2022). Copper Oxide Nanoparticles Exhibit Cell Death Through

Oxidative Stress Responses in Human Airway Epithelial Cells: a Mechanistic Study. *Biological trace element research*, *200*(12), 5042–5051. https://doi.org/10.1007/s12011-022-03107-8

Gaetke, L. M., & Chow, C. K. (2003). Copper toxicity, oxidative stress, and antioxidant nutrients. *Toxicology, 189*(1), 147–163. https://doi.org/10.1016/S0300-483X(03)00159-8

Grassian, V. H., Adamcakova-Dodd, A., Pettibone, J. M., & Thorne, P. S. (2007). Inflammatory response of mice to manufactured titanium dioxide nanoparticles: Comparison of size effects through different exposure routes. *Nanotoxicology, 1*(3), 211–226. https://doi.org/10.1080/17435390701763108

Hussain, S., Javorina, A. K., Schrand, A. M., Duhart, H. M., Ali, S. F., & Schlager, J. J. (2016). Size-dependent cytotoxicity of copper oxide nanoparticles in lung epithelial cells. *Environmental Science: Nano, 3*(3), 452–460. https://doi.org/10.1039/C5EN00271K

Kubo, A. L., Vasiliev, G., Vija, H., Krishtal, J., Tõugu, V., Visnapuu, M., Kisand, V., Kahru, A., & Bondarenko, O. M. (2020). Surface carboxylation or PEGylation decreases CuO nanoparticles' cytotoxicity to human cells in vitro without compromising their antibacterial properties. *Archives of toxicology, 94*(5), 1561–1573. https://doi.org/10.1007/s00204-020-02720-7

Patra, J. K., Das, G., Fraceto, L. F., Campos, E. V. R., Rodriguez-Torres, M. d. P., Acosta-Torres, L. S., ... & Shin, H.-S. (2018). Nano-based drug delivery systems: Recent developments and future prospects. *Journal of Nanobiotechnology, 16,* 71. https://doi.org/10.1186/s12951-018-0392-8

Pourahmad, J., Eskandari, M. R., & Daraei, B. (2011). Involvement of lysosomal destabilization and lysosomal/mitochondrial cross-talk in diclofenac-induced hepatotoxicity. *Iranian Journal of Pharmaceutical Research.*

Siddiqui, M. A., Kashyap, M. P., Kumar, V., Tripathi, S., & Pant, A. B. (2013). Copper oxide nanoparticles induced mitochondria-mediated apoptosis in human hepatocarcinoma cells. *PLoS ONE, 8*(8), e69534. https://doi.org/10.1371/journal.pone.0069534

Taheri, M. A. ., Amani, L., Khalili, A., Vaziri, A. Z., & Keyvani, H. (2022). Effect of Faradarmani Consciousness Field on Cell Culture, Bacterial Contamination of Cell Culture and SARS-CoV-2 Replication in vitro. *The Scientific Journal of Cosmointel*, *1*(1), 14–19. https://doi.org/10.61450/joci.v1i1.10

Taheri, M. A., Payervand, F., Ahmadkhanlou, F., Torabi, S., & Semsarha, F. (2022). Investigation of the properties of the Tc-99m radioisotope and water contaminated with Tc-99m under the influence of Taheri Consciousness Fields. *The Scientific Journal of Cosmointel*, *1*(4), 20-29. https://doi.org/10.61450/joci.v1i4.33

Torabi, S., Taheri, M. A., & Semsarha, F. (2023). Alleviative effects of Faradarmani Consciousness Field on *Triticum aestivum* L. under salinity stress. *F1000Research*, *9*, 1089. https://doi.org/10.12688/f1000research.25247.4