

Impact of the Trivedi Effect®-Energy of Consciousness Healing on the Proliferation of Plant, Mouse and Human Stem Cells

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Abstract

The objective of the present study was to evaluate the effect of Biofield Energy Healing on the plant callus, mouse and human derived Mesenchymal Stem Cells (MSCs) for their cell count and cellular proliferation. All the three type stem cells and DMEM media for the stem cell culture were divided into two parts, one received Biofield Energy Treatment (known as The Trivedi Effect®-Energy of Consciousness Healing Treatment) by a renowned Biofield Energy Healer, Mahendra Kumar Trivedi through the Healer's unique Energy Transmission process remotely and defined as Biofield Energy Treated (BT) group. On day 9 after treatment, the plant callus flasks of mandukparni, amla, and *Centella* were observed and visualized which showed an increased percentage in weight of the callus by 11.4%, 24.9%, and 51.7%, respectively as compared with the untreated groups. In addition, stem cell assay in murine bone marrow derived Mesenchymal Stem Cells (MSCs) showed an increased cellular proliferation by 115.2%, 127.6%, 161.9%, and 123.6%, in Biofield Energy Treated DMEM in the passage 1, 2, 3, and 4, respectively. Similarly, human derived Mesenchymal Stem Cells, Adipose-Derived (AD-MSC) cells showed a significant increased cell count by 139%, 119%, and 182% in the Biofield Energy Treated DMEM group with the passage 1, 2, and 3, respectively. Similarly, bone marrow (BM-MSC) cells showed a significant increased cell count by 102%, 170%, and 141% in the Biofield Energy Treated DMEM group with the passage 1, 2, and 3, respectively. However, BrdU assay for % cellular proliferation in the AD-MSC cells showed a significant increased cellular proliferation by 122%, 120%, and 184% in the passage 1, 2, and 3, respectively. On the other hand, BM-MSC cells showed a significant increased cellular proliferation by 125%, 146%, and 157% in the passage 1, 2, and 3, respectively. Hence, the results suggest that there was a significant growth in plant callus weight, mouse and human stem cell growth and proliferation after The Trivedi Effect®-Energy of Consciousness Treatment. It can be concluded that The Trivedi Effect® can be used as a complementary and alternate therapy for the stem cells regeneration, improvement of tissue injury that might be every effective against various autoimmune human diseases such as arthritis, osteoarthritis, and different neurodegenerative disorders.

Keywords: Energy of Consciousness Healing; The Trivedi Effect®; Yield Improvement; Plant Tissue Culture; Mesenchymal Stem Cells (Mscs); Human Stem Cells

Introduction

Stem cells are significantly used for repair and regeneration therapies to repair the injured tissues of body and are one of the best approaches for associated experimental studies. Besides, human, mouse, and plant stem cells are emerged as the promising therapeutic system where tissues generated from the stem cells, which are grafted on the damaged tissue for repair [1]. In addition, stem cells have the significant capability to differentiate into a range of cell types, like osteoblasts, adipocytes, myocytes, chondrocytes, myocytes, tendon cells, and adipocytes. However, stem cells approach has been reported to treat various incurable diseases such as diabetes mellitus [2], Alzheimer's disease [3], Parkinson's disease [4], osteoporosis, neurodegenerative disorders, and other tissue degenerative disorders.

Human mesenchymal cells (MSCs) can be derived from adipose tissue, bone marrow, and medical waste material such as umbilical cord and placenta, while these are the multipotent cells, which are effortlessly isolated and worked in many aspects [5]. Because of its advantageous properties of cellular proliferation, this is significantly used in stem cell therapy. In addition, murine MSCs are generated from the bone marrow cells due to its adherence property. Bone marrow cells isolated from mice can be plated into the culture petridishes. Upon incubation, stem cells have the property of adherence with the petriplates due to their property of adherence to plastic surface. The medium containing non-adherent cells is discarded and the adherent cells (MSCs) are cultured and propagated by trypsinization [6]. Similarly, the study was performed with the well-established method of plant stem cells, as plant callus is one of the best sources of natural products synthesis in plant cells with wide range of applications. The plant callus culture is one of the best experimental approaches from experimental to the commercial platform in form of high-value phytoconstituents [7]. However, the plants are regarded as best source of wide variety of chemical molecules and scientific communities have found more than 200,000 high-value natural products that are currently available in some form of significant clinical applications such as codeine, morphine, and paclitaxel [8]. Plant callus culture along with the cellular regeneration have been well defined platform in various experimental methods [9], thus the present study also included the growth and yield of Mandukparni (*Centella asiatica*), Katsarika (*Barleria cristata*) and Amla (*Phyllanthus emblica*) plant callus.

Biofield Energy as a Complementary and Alternative Medicine (CAM) that is defined as energy and information field both putative and subtle, which has the ability to regulate the living organisms homeodynamic function's along with a substantial role in perceptive and guiding various health processes [10,11]. However, many researchers have been interested in the potential mechanisms of certain Energy-Healing Practices in different biological fields [12,13]. Various pre-clinical, *in vitro* cell lines, analytical, agricultural, and many more reports of The Trivedi Effect® have been published so far. The Trivedi Effect®-Consciousness Energy Healing Treatment has been reported with significant transformation in the physicochemical properties of metals, chemicals, ceramics and polymers [14-17], improved overall agricultural crop yield [18-20], altered antimicrobial characteristics of various pathogenic microbes [21-23], improved biological activity of nutraceutical compounds in skin health [24,25], livestock [26], and many more.

Materials and Methods

Chemicals and Reagents

Plant callus culture required liquid MS medium (Murashige Skoog's), which was purchased from Sigma-Aldrich. Serological pipettes and T75 culture flask were procured from Thermo Scientific, while culture petridish and 200 μ L microtips were purchased from Tarson, US. For mouse and human stem cell cultures, RPMI-1640 and antibiotics solution (Penicillin-Streptomycin) were purchased from HiMedia, India. EDTA, sodium bicarbonate, and trypsin were purchased from Sigma, India, while BrdU kit was procured from Roche, India. All the other chemicals used in this experiment were analytical grade procured from local vendors.

Experimental Design

The experimental groups were consisted of major two group's *viz.* stem cells of plant, mouse and human stem cells in Biofield Energy Treated and Untreated DMEM medium. All the groups were studied for their cell proliferation and BrdU uptake in case of mouse and human stem cells, while the plant callus cells were visualized for growth and yield of the callus on day 0 and day 7.

Energy of Consciousness Treatment Strategies

The plant callus (mandukparni, katsarika, and amla) containing flasks and MS media, while DMEM media for

mouse and human stem cells were divided into two parts. One part of the each sample (plant callus, MS media, and DMEM) was treated with the Biofield Energy (The Trivedi Effect®) and coded as the Biofield Energy Treated test samples, while the second part of the each sample did not receive any sort of treatment and was defined as the untreated test samples. This Biofield Energy Healing Treatment was provided by a renowned Biofield Energy Healer, Mahendra Kumar Trivedi, who performed the Biofield Energy Treatment remotely for ~3 minutes. Biofield Energy Healer was remotely located in the USA, while the test samples were located in the research laboratory of Dabur Research Foundation near New Delhi, India. This Biofield Energy Treatment was administered for ~3 minutes through the Healer's unique Energy Transmission process remotely to all the test samples. The Biofield Energy Healing Treatment was performed without any contact with the test samples. Biofield Energy Healer in this study did not visit the experimental laboratory nor had any contact with the samples. Further, the control groups of all plant callus, mouse and human stem cells were treated with a sham healer for comparative purposes. The sham healer did not have any knowledge about the Biofield Energy Treatment. After that, the Biofield Energy Treated and untreated samples were kept in similar sealed conditions for experimental study.

Assessment of The Trivedi Effect® Energy Healing on Yield of Plant Stem Cells

Healthy leaflet of mandukparni, katsarika, and amla explants were collected, which were thoroughly washed with running tap water for 20 min in order to remove the traces of dust, etc. followed by washing with fungicide solution. Further, the explants were surface sterilized using 70% ethanol for about 40 seconds and again washed with 4-5 times with sterile double distilled water. Further, fine cuts were made in the leaf (wound induction) and were cultured on MS (Murashige and Skoog) basal medium, gelled with Agar. The cultures were incubated in a culture room at 25±2°C with a photoperiod of 16 hour at 3000 lux light intensity provided by cool white fluorescent tubes. After some days, callus induction was observed. The suspension culture was made from the friable creamish calli and was transferred to T-75 culture flasks in liquid MS media supplemented with different phytohormones like BAP, KIN, NAA and 2,4D. The flasks received Biofield Energy Treatment (known as The Trivedi Effect®) at this stage without touching the cells. Following the treatment, the above T-75 flasks were incubated till day 9 in a shaking incubator (120 rpm)

under specified photoperiod. The incubation conditions were similar to that of untreated cells. On day 9, the flasks were observed and visualized under phase contrast microscope for monitoring cell growth and photomicrographs were captured at 200X magnification. The wet weight of all the callus was taken after day 7 [27].

Assessment of the Trivedi Effect® Energy Healing on yield of Mouse Mesenchymal Stem Cells (MSCs)

Bone marrow was isolated from C57BL/6 mice and the cell suspension was washed twice (250g x8 min) in growth medium (RPMI+15%FBS), which were counted using hemocytometer. Further, 25 x 10⁶ cells were plated in T-25 culture flasks and incubated for 3 days in a CO₂ incubator. After 3 days, non-adherent cells in the medium were discarded and rest cells were replenished with fresh growth medium (RPMI+15% FBS) and incubated in a CO₂ incubator. The cells were trypsinized when confluent (70-80%) and the cells at passage 1 to passage-4 were plated at density of 5000 cells/well in 96-well culture plates, which were treated with untreated test medium and Biofield Energy Healing based medium for 2 days to 6 days. After incubation, BrdU assay was performed using BrdU ELISA as per manufacturer's instructions using FixDenat solution fixation method. The percentage increase in the cellular proliferation was calculated as-

$$\% \text{ increase} = \left[\frac{\text{Absorbance of cells treated with Test Item} - \text{Absorbance of control cells}}{\text{Absorbance of control cells}} \right] * 100 \text{-----} (1)$$

Assessment of the Trivedi Effect® Energy Healing on yield of Human Adipose/ Bone Marrow/ Umbilical cord tissue - Derived Mesenchymal Stem cells (MSCs)

Three types of human stem cells, adipose-Derived Mesenchymal Stem cells (AD-MSC), Bone Marrow-Derived Mesenchymal Stem cells (BM-MSC), and Umbilical cord tissue- Derived Mesenchymal Stem cells (UC-MSC). The effect on cellular proliferation was assessed using Trypan Blue Exclusion Method (Cell Count) and BrdU uptake (DNA synthesis). The cells were counted using hemocytometer and plated in 60-mm petridish at the density corresponding to 30,000 cells/plate. The above cells were then incubated overnight under growth conditions as described in section 9 to allow cell recovery and exponential growth. Following overnight incubation, the above cells were incubated with test medium. After 3 days of incubation, cells were trypsinized and counted by

Trypan Blue Exclusion Method. This was counted as Passage 1. Trypsinized cells were re-seeded in 60-mm petridish at the density corresponding to 30,000 cells/plate. Further, the cells were trypsinized after 3 days and counted as mentioned above. The above step was repeated till passage 6. The percentage increase in cell count was calculated as-

$$\% \text{ increase} = \frac{[(\text{Cell Count of cells treated with Test Item} - \text{Cell Count of control cells}) / \text{Cell Count of control cells}] * 100}{\text{-----}} \quad (2)$$

Statistical Analysis

Data analysis was performed with Sigma Plot Statistical Software (Version 11.0). Differences between means (in triplicates) were assessed for the statistical differences using Student's *t*-test (between two groups) and for multiple comparison one-way analyses of variance (ANOVA) and *post-hoc* analysis were done by Dunnett's test. $p \leq 0.05$ was considered as statistically significant. The results are shown as mean \pm standard error of mean (SEM).

Results and Discussion

Effect of Biofield Energy Healing Treatment on Plant Callus Growth and Yield

The results of growth and yield of plant callus used in the study was examined after Biofield Energy Healing Treatment by Mahindra Kumar Trivedi, a renowned Biofield Energy Healer. The results are presented in (Figure 1). After day 9 of receiving the treatment, the effect on the growth of Mandukparni (*Centella asiatica*), Amla (*Phyllanthus emblica*) and Katsarika (*Baleria cristata*) calli were observed microscopically and compared with day 0, as demonstrated in Figure 1 (A-C) respectively. All the three plant callus flasks were observed and visualized under phase contrast microscope for cell growth and photomicrographs were captured at 200X magnification on day 0 and day 9. The plant callus at day 9 as presented showed significant growth after Biofield Energy Treatment, while the callus growth was high on day 9 as compared with the untreated plant callus and MS media.

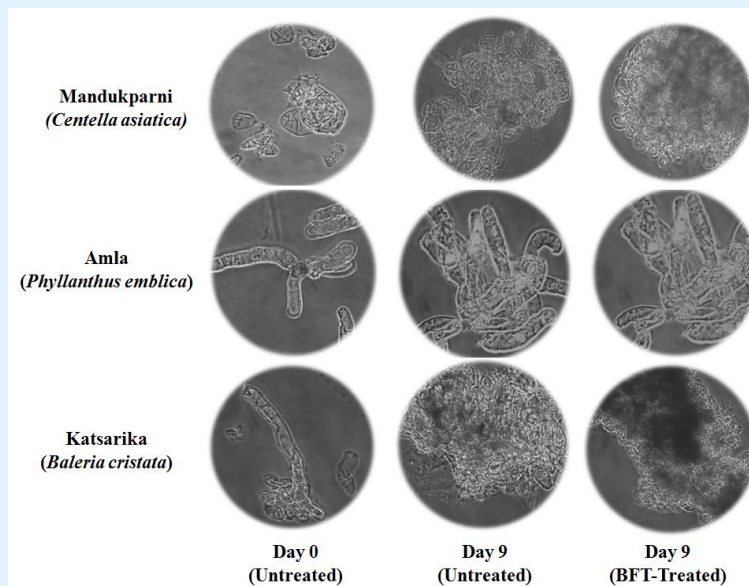
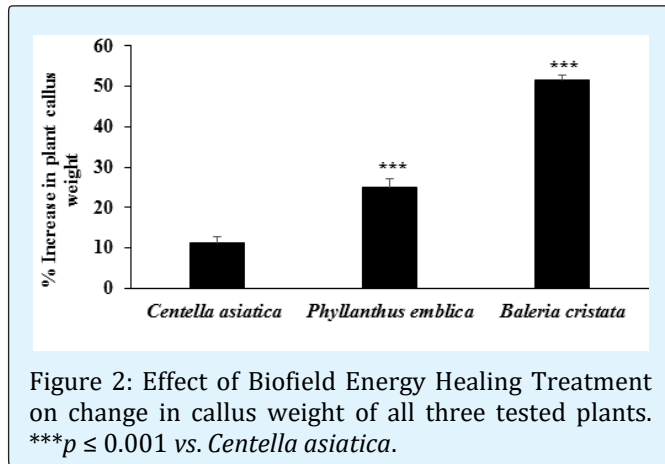


Figure 1: Representative pictures of studied plant callus on day 0 and 9 in control and Biofield Energy Treated groups.

However, experimental data suggested that the Mandukparni weight of callus in the untreated group was 260 mg, while it was 289.6 mg in the Biofield Energy Treated group. Overall, the data showed 11.4% increase in plant callus growth as compared with the control group. In addition, data of amla plant callus weight in the

untreated group was 250 mg, while it was 312.2 mg in the Biofield Energy Treated group. Overall, the data showed 24.9% increase in amla plant callus growth as compared with the untreated control. Similarly, the experimental data suggested that the katsarika weight of callus in the

untreated group was 321 mg, while it was 487 mg in the Biofield Energy Treated group (Figure 2).

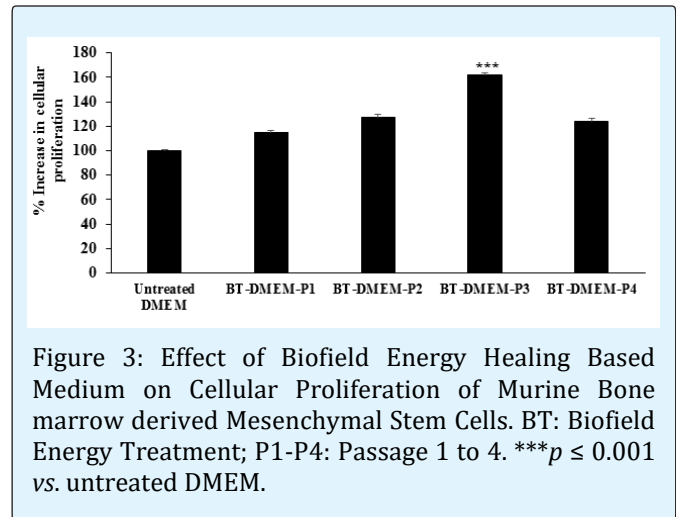


Thus, the data showed 51.7% increase in plant callus growth as compared with the untreated control. The tested plant callus, *Centella asiatica* contains many phytochemicals, which can be used against wide range of biological activities such as antileprotic, antibacterial, antioxidant, antifeedant, and antituberculosis properties due to the presence of asiaticosides and other antioxidants [28,29]. Amla is the best source of vitamin C along with other phytoconstituents such as emblicol, phyllembelic acid, phyllembelin, rutin, phenolic compounds, and tannins, which could be useful for antioxidation activity, antidiarrheal, analgesic, wound healing, hepatoprotective, and nephroprotective [30]. Katsarika test results assumed that Biofield Energy Healing Treatment has significant capacity to improve the phytoconstituents of *Barleria cristata* including iridoids, flavonoids, and phenylethanoid derivatives. These phytochemicals has been reported to have significant action against skin diseases, bronchitis, blood diseases, and asthma [31]. Overall, it was concluded that The Trivedi Effect® has significant capacity to improve the plant callus growth in all the plants, which suggests its action against many biological activities.

Effect of Biofield Energy Healing Treatment on Murine Bone marrow derived Mesenchymal Stem Cells (MSCs)

Murine bone marrow derived MSCs were cultured and treated with Biofield Energy Healing Based Medium at different passages during subculture. (Figure 3) demonstrated the effect of Biofield Energy Healing Based Medium on cellular proliferation of BM-MSCs by increase

in DNA synthesis (BrdU uptake). The experimental data suggested that untreated group was defined as 100%, while Biofield Energy Treated passage, BT-DMEM-P1 showed 115.2%, while BT-DMEM-P2, BT-DMEM-P3, and BT-DMEM-P4 were reported as increased cellular proliferation by 127.6%, 161.9%, and 123.6%, respectively.



In the present study, murine bone marrow derived MSCs were cultured and treated with Biofield Energy Based Medium and untreated medium till passage 4. Cellular proliferation was estimated by increase in DNA synthesis by BrdU uptake. The test medium showed an increased cellular proliferation of BM-MSCs by maximum of 61.9% as compared to untreated test Medium at passage-3. The results obtained suggest that Biofield Energy Based Medium induces a positive effect on the activity of stem cells, thus maintaining the growth and the proliferative activity. This translates into enhanced ability to regenerate and rejuvenate cells and tissues.

Effect of Biofield Energy Healing Treatment on Human derived Mesenchymal Stem Cells (MSCs)

The cells were cultured and received Biofield Energy Treated Medium till passage 6. (Figure 4) demonstrated the effect of Biofield Energy Based Medium on cellular proliferation by cell count and BrdU uptake in AD-MSC, BM-MSC, and UC-MSC's. However, cell count and BrdU uptake data showed no significant change in the UC-MSC's. However, AD-MSC cells showed a significant increased cell count by 139%, 119%, and 182% in the BT-DMEM-P1, BT-DMEM-P2, and BT-DMEM-P3, respectively. Similarly,

BM-MSC cells showed a significant increased cell count by 102%, 170%, and 141% in the BT-DMEM-P1, BT-DMEM-P2, and BT-DMEM-P3, respectively [Figure 4A]. In addition, % cellular proliferation using BrdU assay showed that in AD-MSC cells showed a significant increased proliferation by 122%, 120%, and 184% in the BT-DMEM-P1, BT-DMEM-P2, and BT-DMEM-P3, respectively. Similarly, BM-MSC cells showed a significant increase % cellular proliferation by 125%, 146%, and 157% in the BT-DMEM-P1, BT-DMEM-P2, and BT-DMEM-P3, respectively [Figure 4B].

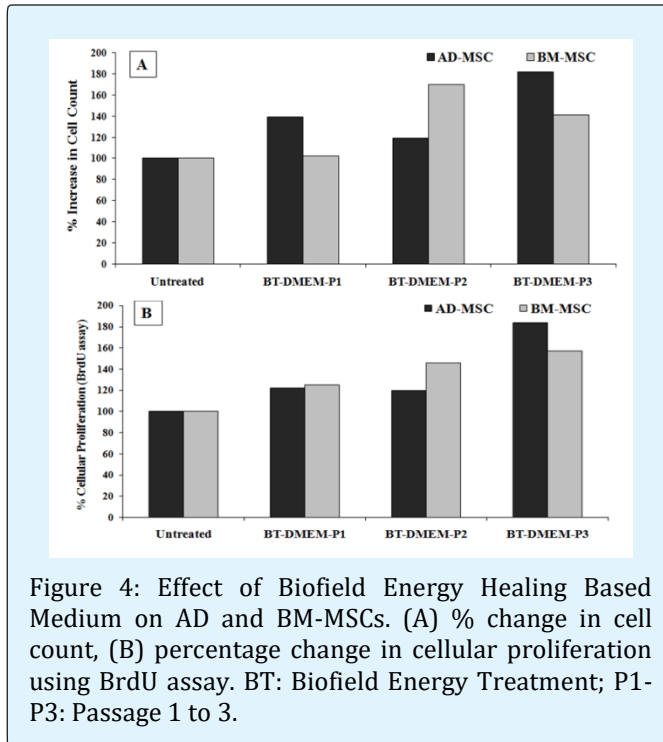


Figure 4: Effect of Biofield Energy Healing Based Medium on AD and BM-MSCs. (A) % change in cell count, (B) percentage change in cellular proliferation using BrdU assay. BT: Biofield Energy Treatment; P1-P3: Passage 1 to 3.

Biofield Energy Healing Treatment as a complementary and alternative medicine approach, which can be used to induce significant cell growth and improved cell number with high proliferation rate. Although, human stem cells plays a significant role in the tissue engineering and in various regenerative medicines. Thus, the improved cell growth using Biofield Energy Treatment can be one of the best approaches for cell survival and growth, which is one of the major limitations in front of stem cell technologies during translation against wide range of clinical applications [32,33]. Thus, it might be expecting that Biofield Energy Healing Treatment (The Trivedi Effect®) can be used in order to overcome the limitations of stem cell culture and their proliferation rate. Biofield Energy Treatment would be

the alternative approach that have long plethora of applications in case of different medicines, regenerative medicine, an emerging branch of medical science, which can be used against restoration of tissues or organs in various injuries or chronic diseases.

Conclusions

Biofield Energy Healing Treatment has been shown significant improvement of cell growth and proliferation of plant, mouse, and human stem cells. The Trivedi Effect®-Energy of Consciousness Healing Treatment on plant, mouse, and human stem cells showed significant growth (yield) of all the plant callus cultures as observed by phase contrast microscope. Overall, the plant yield (growth) of mandukparni, amla, and *Centella* callus increased by 11.4%, 24.9%, and 51.7%, respectively on day 9 as compared with the control. Similarly, the murine bone marrow derived Mesenchymal Stem Cells (MSCs) showed an increased cellular proliferation by 115.2%, 127.6%, 161.9%, and 123.6%, in BT-DMEM-P2, BT-DMEM-P3, and BT-DMEM-P4, respectively. In addition, among human derived MSCs, AD-MSC cells showed a significant increased cell count by 139%, 119%, and 182% in the BT-DMEM-P1, BT-DMEM-P2, and BT-DMEM-P3, respectively as compared to the control group. Similarly, BM-MSC cells also showed a significant increased cell count by 102%, 170%, and 141% in the BT-DMEM-P1, BT-DMEM-P2, and BT-DMEM-P3, respectively. On other hand, BrdU assay in AD-MSC cells showed a significant increase % proliferation by 122%, 120%, and 184% in the BT-DMEM-P1, BT-DMEM-P2, and BT-DMEM-P3, respectively. BM-MSC cells showed a significant increase % cellular proliferation by 125%, 146%, and 157% in the BT-DMEM-P1, BT-DMEM-P2, and BT-DMEM-P3, respectively as compared to the control group. Overall, data suggested that Biofield Energy Healing Treatment by a renowned Biofield Energy Healer, Mahendra Kumar Trivedi has shown the significant improvement of the stem cell growth and cell count in plant callus, mouse, and human stem cells. Therefore, The Trivedi Effect® can be used as a complementary and alternate therapy for the stem cells regeneration, and improvement of tissue injury that might be every effective against various autoimmune human diseases such as arthritis, osteoarthritis, and different neurodegenerative disorders.

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