

Effect of Biofield Energized Vitamin D₃ on Bone Health in MG-63 Cell-Line

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Abstract: The objective of current research work was to evaluate the potential of Consciousness Energy Healing-based vitamin D₃ and DMEM medium on bone health parameters such as alkaline phosphatase (ALP), collagen, and bone mineralization in human bone osteosarcoma cells (MG-63). The test items like vitamin D₃ and DMEM were divided into two parts. One part of each sample was received the Biofield Energy Treatment by Faith Ann Pyka and those samples were denoted as the Biofield Energy Treated (BT) samples, while the other parts of each sample were referred as the untreated test items (UT). The MTT cell viability assay revealed that the test samples were found as safe in the tested concentrations. The level of ALP was significantly increased by 191.45% in the BT-DMEM + UT-Test item (UT-TI) group at 1 µg/mL compared to the UT-DMEM + UT-TI group. Moreover, ALP level was significantly increased by 144.89% and 226.75% in the UT-DMEM + BT-TI and BT-DMEM + BT-TI groups, respectively at 50 µg/mL compared to the untreated group. Further, collagen level was significantly increased by 98.15%, 80.54%, and 49.98% in the UT-DMEM + BT-TI, BT-DMEM + UT-TI, and BT-DMEM + BT-TI groups, respectively at 10 µg/mL compared to the untreated group. Additionally, at 50 µg/mL level of collagen was significantly increased by 129.73%, 189.16%, and 94.60% in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item groups, respectively with respect to the untreated group. Apart from this, the percent of bone mineralization was distinctly enhanced by 283.74%, 27.91%, and 118.02% in the UT-DMEM + BT-TI, BT-DMEM + UT-TI, and BT-DMEM + BT-TI groups, respectively at 50 µg/mL compared to the untreated group. Moreover, the percentage of bone mineralization was significantly increased by 265.92%, 231.82%, and 158.2% in the UT-DMEM + BT-TI, BT-DMEM + UT-TI, and BT-DMEM + BT-TI groups, respectively at 100 µg/mL compared to the untreated group. Altogether, the Biofield Energy Treated vitamin D₃ was significantly improved the bone health parameters and it could be an alternative approach for nutraceutical supplement to combat vitamin D₃ deficiency and able to fight against various bone-related disorders including rickets, low bone density, osteomalacia, bone and joint pain, bone fractures, osteoporosis, osteoma, osteogenesis imperfecta, Paget's disease, deformed bones, chondrodystrophia fetalis, stress management and prevention, autoimmune and inflammatory diseases, and anti-aging by improving overall health.

Keywords: The Trivedi Effect[®], Vitamin D₃, Bone Health, Consciousness Healing Treatment, Osteosarcoma Cells, Osteoporosis

1. Introduction

Vitamin D has multiple effects, which regulate the functions in different target organs such as brain, liver, lungs,

heart, kidneys, skeletal, immune, and reproductive systems. Additionally, it has a significant anti-inflammatory, anti-aging, anti-stress, anti-arthritis, anti-osteoporosis, anti-apoptotic, wound healing, anti-cancer, anti-psychotic and

anti-fibrotic actions [1]. Vitamin D receptors (VDRs) are widely distributed in most of the body organs *viz.* brain, liver, heart, lungs, kidney, pancreas, small and large intestines, muscles, reproductive, nervous system, etc. VDRs can influence cell-to-cell communication, normal cell growth, cell differentiation, cell cycling and proliferation, hormonal balance, neurotransmission process, skin health, immune and cardiovascular functions. In any living vertebrates, vitamin D plays an important role in maintaining a healthy skeletal structure and is essential for bone health. Naturally, it is synthesized in presence of sunlight in the skin [2]. Most of the foods do not contain vitamin D, additionally now-a-days due to aging, use of sunscreen, and change of zenith angle of the sun, the production of vitamin D₃ has been decreased [3]. Increasing age is not only related to a decrease in bone marrow depression and muscle strength but is also associated with marked changes in the immune and inflammatory responses [4]. Deficiency of vitamin D₃ causes metabolic bone diseases like osteomalacia and exacerbate osteoporosis, etc. [5]. The quality of life (QoL) for menopausal women is one of the most critical health problems in the modern world. Metabolic bone disorders like osteoporosis are mainly prevalent in post-menopausal women. Hormonal factors and rapid bone loss in post-menopausal women leads to an increased risk of fractures [6]. Hence, the serum calcium and alkaline phosphatase (ALP) levels in post-menopausal women are the main two vital biochemical markers of bone metabolism. However, bone-specific ALP is the most important marker for osteoblast differentiation [7]. Further, it is generally accepted that an increased calcium intake along with an adequate source of vitamin D is important for maintaining good bone health. Vitamin D also plays an important role in maintaining an adequate level of serum calcium and phosphorus. Therefore, vitamin D has a great impact on forming and maintaining strong bones [8, 9]. Bone strength depends on the quality, geometry, shape, microarchitecture, turnover, mineral content, and the collagen content. Collagen is the major structural protein responsible for bone calcification. In the aging state, the mechanical properties of the bones become impaired, and the bones get fragile, that causes various clinical disorders associated with bone collagen abnormalities and bone fragility, such as osteogenesis imperfecta and osteoporosis [10, 11].

In recent years, numerous scientific reports and clinical trials have revealed that the useful effects of Biofield Energy Treatment, which have shown to enhanced immune function in case of cervical cancer patients *via* therapeutic touch [12], massage therapy [13], etc. Complementary and Alternative Medicine (CAM) therapies are now rising as preferred models of treatment, among which Biofield Energy Therapy (or Healing Modalities) is one approach that has been reported to have several benefits to enhance physical, mental and emotional human wellness. However, as per the data of 2012 from the National Health Interview Survey (NHIS), which indicated that the highest percentage (~17%) of the Americans used dietary supplements as a complementary health approach as compared with other practices in the past

years. The National Center of Complementary and Integrative Health (NCCIH) has recognized and accepted Biofield Energy Healing as a CAM health care approach in addition to other therapies, medicines and practices such as natural products, deep breathing, yoga, Tai Chi, Qi Gong, chiropractic/osteopathic manipulation, meditation, massage, special diets, homeopathy, progressive relaxation, guided imagery, acupressure, acupuncture, relaxation techniques, hypnotherapy, healing touch, movement therapy, pilates, rolfing structural integration, mindfulness, Ayurvedic medicine, traditional Chinese herbs and medicines, naturopathy, essential oils, aromatherapy, Reiki, and cranial sacral therapy. Human Biofield Energy has subtle energy that has the capacity to work in an effective manner [14]. CAM therapies have been practiced worldwide with reported clinical benefits in different health disease profiles [15]. This energy can be harnessed and transmitted by the experts into living and non-living things *via* the process of Biofield Energy Healing. Biofield Energy Treatment (The Trivedi Effect[®]) has been published in numerous peer-reviewed science journals with significant outcomes in many scientific fields such as cancer research [16, 17], microbiology [18-21], biotechnology [22, 23], pharmaceutical science [24-27], agricultural science [28-31], materials science [32-35], nutraceuticals [36, 37], skin health [38, 39], human health and wellness.

Based on the literature information and importance of vitamin D₃ on bone health, the authors sought to evaluate the impact of the Biofield Energy Treatment (The Trivedi Effect[®]) on the test samples (vitamin D₃ and DMEM medium) for bone health activity with respect to the assessment of different bone health parameters like ALP, collagen content, and bone mineralization using standard assays in MG-63 cells.

2. Materials and Methods

2.1. Chemicals and Reagents

Rutin hydrate was obtained from TCI, Japan, while vitamin D₃, 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium (MTT), Direct Red 80, ethylene diamine tetra acetic acid (EDTA) and L-ascorbic acid were obtained from Sigma-Aldrich, USA. Fetal bovine serum (FBS) and Dulbecco's Modified Eagle's Medium (DMEM) were purchased from Life Technology, USA. Antibiotic solution (penicillin-streptomycin) was procured from HiMedia, India. All the other chemicals used in this experiment were analytical grade procured from India.

2.2. Cell Culture

The human bone osteosarcoma cells (MG-63) were used as the test system in this experiment. The MG-63 cells supplemented with 10% FBS were maintained under the DMEM growth medium for routine culture. Growth conditions were maintained as 37°C, 5% CO₂ and 95% humidity and subcultured by trypsinisation followed by

splitting the cell suspension into fresh flasks and supplementing with fresh cell growth medium. Three days prior the start of the experiment (*i.e.*, day -3), the growth medium of near-confluent cells was replaced with fresh phenol-free DMEM, supplemented with 10% charcoal dextran stripped FBS (CD-FBS) and 1% penicillin-streptomycin [40].

2.3. Experimental Design

The experimental groups consisted of cells in baseline control (untreated cells), vehicle control groups (0.05% DMSO with Biofield Energy Treated and untreated DMEM), a positive control group (rutin hydrate) and experimental test groups. The experimental groups included the combination of the Biofield Energy Treated and untreated vitamin D₃/DMEM. It consisted of four major treatment groups on specified cells with UT-DMEM + UT-Test item, UT-DMEM + Biofield Energy Treated test item (BT-Test item), BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item.

2.4. Consciousness Energy Healing Treatment Strategies

The test item (vitamin D₃) and DMEM were divided into two parts. One part each of the test item and DMEM were treated with the Biofield Energy (also known as The Trivedi Effect[®]) and coded as the Biofield Energy Treated items, while the second part did not receive any sort of treatment and was defined as the untreated samples. This Biofield Energy Healing Treatment was provided by Faith Ann Pyka, who participated in this study and performed the Biofield Energy Healing Treatment remotely for ~5 minutes. Biofield Energy Healer was remotely located in the USA, while the test samples were located in the research laboratory of Dabur Research Foundation, New Delhi, India. The Biofield Energy Treatment was administered for 5 minutes through the healer's unique Energy Transmission process remotely to the test samples under laboratory conditions. Faith Ann Pyka in this study, never visited the laboratory in person, nor had any contact with the test item and medium. Further, the control group was 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.

2.5. Determination of Non-cytotoxic Concentration

The cell viability test was performed by MTT assay in the human bone osteosarcoma cell line (MG-63). The cells were counted and plated in 96 well plates at the density corresponding to 5 X 10³ to 10 X 10³ cells/well/180 μL of cell growth medium. The above cells were incubated overnight under growth conditions and allowed cell recovery and exponential growth, then they were subjected to serum stripping or starvation. The cells were treated with the test item, DMEM, and the positive control. The untreated cells served as baseline control (untreated cells). The cells in the above plate(s) were incubated for a time point ranging from

24 to 72 hours in CO₂ incubator at 37°C, 5% CO₂ and 95% humidity. Following incubation, the plates were taken out and 20 μL of 5 mg/mL of MTT solution was added to all the wells followed by an additional incubation for 3 hours at 37°C. The supernatant was aspirated and 150 μL of DMSO and was added to each well to dissolve formazan crystals. The absorbance of each well was read at 540 nm using a Synergy HT micro plate reader, BioTek, USA. The percentage cytotoxicity at each tested concentration of the test substance was calculated using the following Equation 1:

$$\% \text{ Cytotoxicity} = \{(1-X)/R\} * 100 \quad (1)$$

Where, X = Absorbance of treated cells; R = Absorbance of untreated cells

The percentage cell viability corresponding to each treatment was then be obtained using the following Equation 2:

$$\% \text{ Cell Viability} = 100 - \% \text{ Cytotoxicity} \quad (2)$$

The concentrations exhibiting ≥70% Cell viability was considered as non-cytotoxic [41].

2.6. Assessment of Alkaline Phosphatase (ALP) Activity

The cells were counted using an hemocytometer and plated in a 24-well plate at the density corresponding to 1 x 10⁴ cells/well in phenol free DMEM supplemented with 10% CD-FBS. Following the respective treatments, the cells in the above plate were incubated for 48 hours in CO₂ incubator at 37°C, 5% CO₂ and 95% humidity. After 48 hours of incubation, the plate was taken out and processed for the measurement of ALP enzyme activity. The cells were washed with 1X PBS and lysed by freeze thaw method *i.e.*, incubation at -80°C for 20 minutes followed by incubation at 37°C for 10 minutes. To the lysed cells, 50 μL of substrate solution *i.e.*, 5 mM of *p*-nitrophenyl phosphate (*p*NPP) in 1M diethanolamine and 0.24 mM magnesium chloride (MgCl₂) solution (pH 10.4) was added to all the wells followed by incubation for 1 hour at 37°C. The absorbance of the above solution was read at 405 nm using Synergy HT micro plate reader (Biotek, USA). The absorbance values obtained were normalized with substrate blank (*p*NPP solution alone) absorbance values. The percentage increase in ALP enzyme activity with respect to the untreated cells (baseline group) was calculated using Equation 3:

$$\% \text{ Increase in ALP} = \{(X-R)/R\} * 100 \quad (3)$$

Where, X = Absorbance of cells corresponding to positive control and test groups

R = Absorbance of cells corresponding to baseline group (untreated cells)

2.7. Assessment of Collagen Synthesis

The MG-63 cells were counted using an hemocytometer and plated in 24-well plate at the density corresponding to 10 x 10³ cells/well in phenol free DMEM supplemented with 10% CD-FBS. Following the respective treatments, the cells

in the above plate were incubated for 48 hours in CO₂ incubator at 37°C, 5% CO₂ and 95% humidity. After 48 hours of incubation, the plate was taken out and the amount of collagen accumulated in MG-63 cells corresponding to each treatment was measured by Direct Sirius red dye binding assay. In brief, the cell layers were washed with PBS and fixed in Bouin's solution (5% acetic acid, 9% formaldehyde and 0.9% picric acid) for 1 hour at room temperature (RT). After 1 hour of incubation, the above wells were washed with milliQ water and air dried. The cells were then stained with Sirius red dye solution for 1 hour at RT followed by washing in 0.01 N HCl to remove unbound dye. The collagen dye complex obtained in the above step was dissolved in 0.1 N NaOH and absorbance was read at 540 nm using Biotek Synergy HT micro plate reader. The level of collagen was extrapolated using standard curve obtained from purified Calf Collagen Bornstein and Traub Type I (Sigma Type III). The percentage increase in collagen level with respect to the untreated cells (baseline group) was calculated using Equation 4:

$$\% \text{ Increase in collagen levels} = \{(X-R)/R\} * 100 \quad (4)$$

Where, X = Collagen levels in cells corresponding to positive control and test groups

R = Collagen levels in cells corresponding to baseline group (untreated cells)

2.8. Assessment of Bone Mineralization by Alizarin Red S Staining

The MG-63 cells were counted using an hemocytometer and plated in 24-well plate at the density corresponding to 10 x 10³ cells/well in phenol free DMEM supplemented with 10% CD-FBS. Following the respective treatments, the cells in the above plate were incubated for 48 hours in CO₂ incubator at 37°C, 5% CO₂ and 95% humidity to allow cell recovery and exponential growth. Following overnight incubation, the above cells were subjected to serum stripping for 24 hours. The cells were then treated with non-cytotoxic concentrations of the test samples and positive control. Following 3-7 days of incubation with the test samples and positive control, the plates were taken out, cell layers

processed further by staining with Alizarin Red S dye. The cells were fixed in 70% ethanol for 1 hour, after which Alizarin Red solution (40 µm; pH 4.2) was added to the samples for 20 minutes with shaking. The cells were washed with distilled water to remove unbound dye. For quantitative analysis by absorbance evaluation, nodules were solubilized with 10% cetylpyridinium chloride for 15 minutes with shaking. Absorbance was measured at 562 nm using Biotek Synergy HT micro plate reader. The percentage increase in bone mineralization with respect to the untreated cells (baseline group) was calculated using the following Equation 5:

$$\% \text{ Increase} = \{(X-R)/R\} * 100 \quad (5)$$

Where, X = Absorbance in cells corresponding to positive control or test groups; R = Absorbance in cells corresponding to baseline (untreated) group.

2.9. Statistical Analysis

All the values were represented as percentage of the respective parameters. For statistical analysis Sigma-Plot (version 11.0) was used as a statistical tool. Statistically significant values were set at the level of $p \leq 0.05$.

3. Results and Discussion

3.1. MTT Assay

For the assessment of non-cytotoxic concentrations of the test samples *i.e.*, vitamin D₃ and DMEM medium the MTT cell viability assay was used in this experiment in MG-63 cells. The data are expressed as percentage and shown in Figure 1. The percentage of cell viability was observed in the ranges of 70% to 131% in the tested concentrations of all the groups and did not show any cytotoxicity (as evidence of cell viability approximately greater than 70%) across all the tested concentrations upto 50 µg/mL. Hence, the safe concentrations were used in this experiment to see the effect of the test samples on the levels of alkaline phosphatase (ALP) activity, collagen synthesis, and bone mineralization in MG-63 cells.

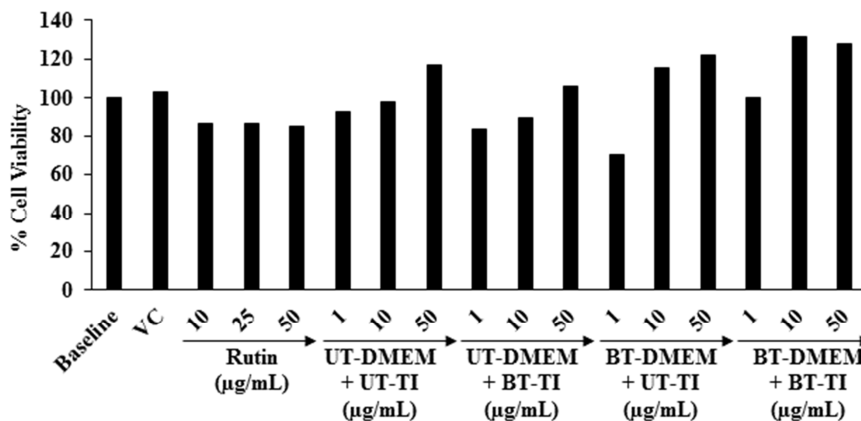


Figure 1. Study of the cell viability of the test items (vitamin D₃ and DMEM medium) after Biofield Energy Treatment in different tested concentrations in MG-63 cells. VC: Vehicle control (0.05% DMSO); UT: Untreated; BT: Biofield Energy Treated; TI: Test item.

3.2. Assessment of Alkaline Phosphatase (ALP) Enzyme Activity

The effect of the Biofield Energy Treated test samples on ALP activity in MG-63 cells is shown in Figure 2. The vehicle control (VC) group showed 5.5% level of ALP as compared to the untreated cells group. The reference standard was used in this experiment (rutin hydrate) showed 34.31%, 51.47%, and 189.39% increased the level of ALP at the concentration of 0.01, 0.1, and 1 $\mu\text{g/mL}$, respectively compared to the untreated cells group. The results of the test formulation showed that the level of ALP was significantly enhanced by 191.45% in the BT-DMEM + UT-TI groups at 1 $\mu\text{g/mL}$ as compared to the UT-DMEM + UT-Test item group. Moreover, the level of ALP was significantly increased by 144.89% and 226.75% in the UT-DMEM + BT-

TI and BT-DMEM + BT-TI, respectively at 50 $\mu\text{g/mL}$ as compared to the UT-DMEM + UT-Test item group.

ALP is one of the biomarker for the evaluation of bone formation and resorption [42, 43]. Various *in vitro* study reported that ALP can be released in the soluble form by a phospholipase (an anchorless) and also in insoluble form by vesicle exocytosis (an anchor-intact). Regulation of skeletal activities by ALP is govern by these two putative process [44]. Overall, the Biofield Energy Treated (The Trivedi Effect[®]) test item group (*i.e.*, vitamin D₃) showed an improved synthesis of ALP enzyme in the MG-63 cells with respect to the untreated test item group, which might be advantageous to the patients those are suffering from various bone-related disorders.

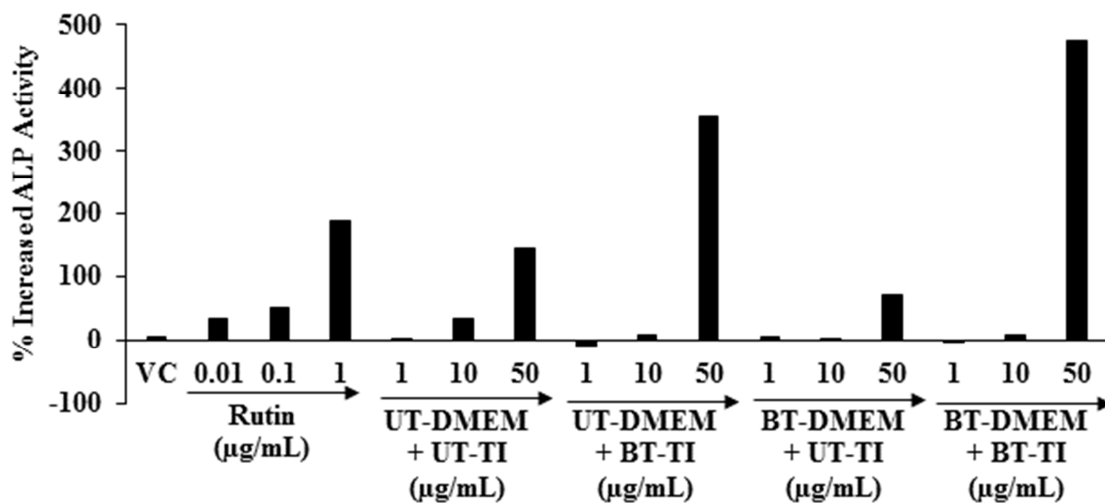


Figure 2. The effect of test samples on alkaline phosphatase (ALP) enzyme activity in human bone osteosarcoma cell (MG-63). VC: Vehicle control (0.05% DMSO), UT: Untreated; BT: Biofield Energy Treated; TI: Test item.

3.3. Assessment of Collagen Activity

The collagen activity of the test items in human bone osteosarcoma cells is shown in Figure 3. Collagen level in the VC group was found as 28% increased as compared to the untreated cells group. The positive control (rutin hydrate) showed 28.27%, 46.5%, and 46.73% increased the level of collagen at 0.01, 0.1, and 1 $\mu\text{g/mL}$, respectively compared to the untreated cells group. The collagen synthesis was increased by 2.56% in the BT-DMEM + BT-Test item group at 1 $\mu\text{g/mL}$ compared to the UT-DMEM + UT-Test item group. Moreover, the collagen level was significantly increased by 98.15%, 80.54%, and 49.98% in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item and BT-DMEM + BT-Test item groups, respectively at 10 $\mu\text{g/mL}$ compared to the UT-DMEM + UT-Test item group. Additionally, at 50 $\mu\text{g/mL}$ the level of collagen was also significantly increased by 129.73%, 189.16%, and 94.60% in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item groups, respectively with

respect to the UT-DMEM + UT-Test item group (Figure 3). Collagen, the source of fibers is the most abundant constituent of all the connective tissue. Various evidence reported that collagen, plays a considerable role in both bone development and bone maintenance [45, 46]. ATF4, a transcription factor that stimulates amino-acid uptake and collagen synthesis in osteoblast. Thus, increased amino-acid uptake simultaneously increases the synthesis of type I collagen and osteocalcin expression that ultimately enhanced the differentiation of osteoblast [47]. Here, the Biofield Energy Treated vitamin D₃ significantly improved the level of collagen which might be helpful to maintain a healthy bone in postmenopausal women selectively. Overall, The Trivedi Effect[®] - Consciousness Energy Healing Treatment modality showed a significant improvement of the collagen level in human osteosarcoma cells. Thus, it is assumed that The Trivedi effect[®] has the significant potential to improve the bone health in various skeletal disorders.

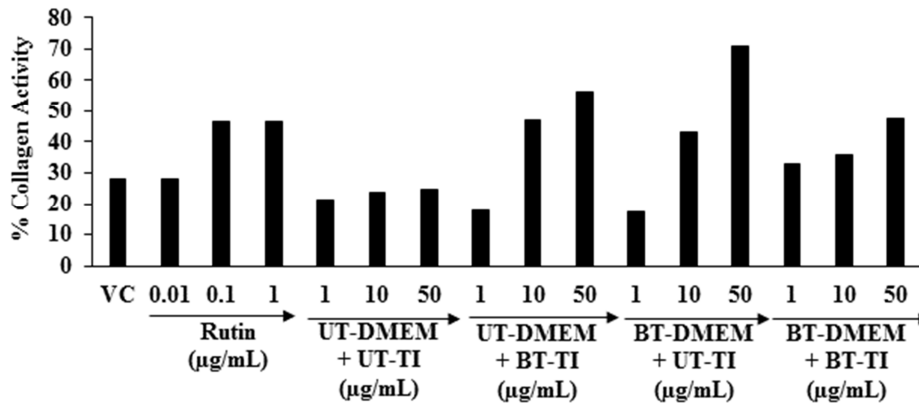


Figure 3. Analysis of the collagen level in human bone osteosarcoma cells after exposure with the Biofield Energy Treated test samples. VC: Vehicle control (0.05% DMSO), UT: Untreated; BT: Biofield Energy Treated; TI: Test item.

3.4. Bone Mineralization

The effect of Biofield Energy Treated test samples on bone mineralization in MG-63 cells is shown in Figure 4. The percentage of bone mineralization was significantly increased in a concentration-dependent manner by 75%, 105.94%, and 135.15% at 5, 10, and 25 µg/mL, respectively in the positive control group compared to the untreated cells group. The percent of bone mineralization was distinctly increased by 69.19%, 62.44%, and 88.73% in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item groups, respectively at 10 µg/mL compared to the UT-DMEM + UT-Test item group. Further, an increased percentage of bone mineralization was observed by 283.74%, 27.91%, and 118.02% in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item groups, respectively at 50 µg/mL with respect to the UT-DMEM + UT-Test item group. Moreover, the percentage of bone mineralization was significantly increased by 265.92%, 231.82%, and 158.2% in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item

groups, respectively at 100 µg/mL compared to the UT-DMEM + UT-Test item group (Figure 4). Vitamin D is very essential for sufficient development of whole skeletal system. Unfortunately, most of foods naturally do not contain vitamin D, and only a few foods are fortified with vitamin D. That is why, vitamin D deficiency has become epidemic for all age groups in the both developed and under developing countries. Vitamin D deficiency not only causes metabolic bone disease among children and adults but also may increase the risk of many common chronic diseases [1]. Here, authors have found that the Biofield Energy Treated vitamin D₃ significantly enhanced the level of bone mineralization in the form of calcium, assessed by Alizarin Red S Staining technique. Therefore, based on the above findings it is hypothesized that the Consciousness Energy Healing Treatment (The Trivedi Effect[®]) based test item groups (*i.e.*, vitamin D₃) showed a remarkable improvement of bone mineralization content assessed by *in vitro* in the human osteosarcoma cells (MG-63).

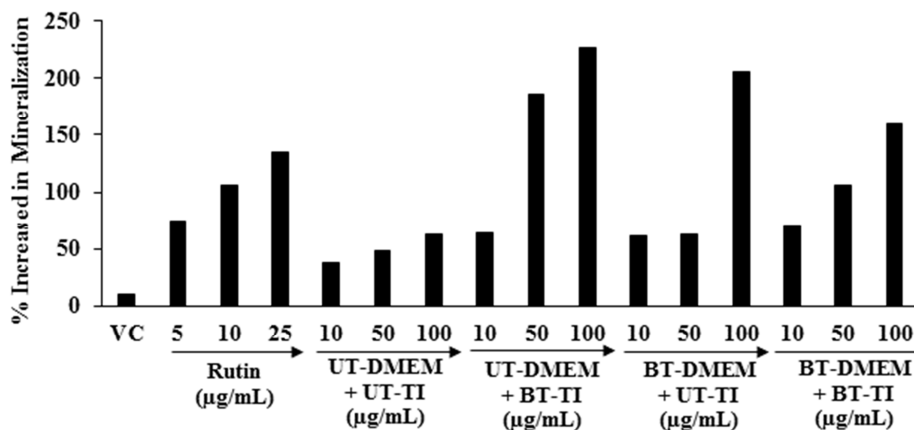


Figure 4. The bone mineralization activity of Biofield Energy Treated test samples in human bone osteosarcoma cells. VC: Vehicle control (0.05% DMSO), UT: Untreated; BT: Biofield Energy Treated.

4. Conclusions

The cell viability assay data using MTT assay showed more

than 70% cells were viable, which indicated that the test samples were safe and nontoxic in all the tested concentrations. The level of ALP was significantly increased by 144.89% and 226.75% in the UT-DMEM + BT-Test item

and BT-DMEM + BT-TI groups, respectively at 10 µg/mL; while increased by 191.45% in the BT-DMEM + UT-Test item at 1 µg/mL compared to the UT-DMEM + UT-Test item group. The synthesis of collagen was significantly increased by 98.15%, 80.54%, and 49.98% in the UT-DMEM + BT-Test item, BT-DMEM + UT-TI, and BT-DMEM + BT-Test item group, respectively at 10 µg/mL compared to the untreated group. Further, the collagen level was significantly increased by 129.73%, 189.16%, and 94.60% in the UT-DMEM + BT-TI, BT-DMEM + UT-TI, and BT-DMEM + UT-TI groups, at 50 µg/mL compared to the untreated group.

Besides, the percent of bone mineralization was distinctly increased 69.19%, 62.44%, and 88.73% in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item groups, respectively at 10 µg/mL compared to the untreated group. Further, the percentage of bone mineralization was significantly increased by 283.74%, 27.91%, and 118.02% in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item groups, respectively at 50 µg/mL compared to the UT-DMEM + UT-Test item group. Moreover, the percentage of bone mineralization was significantly increased by 265.92%, 231.82%, and 158.2% in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item groups, respectively at 100 µg/mL compared to the UT-DMEM + UT-Test item group. Altogether, the Biofield Energy Treated test samples (The Trivedi Effect[®]) demonstrated a significant impact on bone health parameters. Therefore, the Consciousness Energy Healing based vitamin D₃ might be suitable for the development of an alternative and more effective supplement for vitamin D₃ deficiency, which could be useful for the management of various bone related disorders *viz.* Paget's disease of bone, low bone density, osteoporosis, osteogenesis imperfecta, rickets, deformed bones, osteomalacia, osteoma, bone and joint pain, bone fractures, chondrodystrophia fetalis, etc. Besides, it can also be utilized in organ transplants (for example kidney transplants, liver transplants and heart transplants), various autoimmune disorders such as Lupus, Addison Disease, Celiac Disease (gluten-sensitive enteropathy), Dermatomyositis, Graves' Disease, Hashimoto Thyroiditis, Rheumatoid Arthritis, Multiple Sclerosis, Myasthenia Gravis, Pernicious Anemia, Scleroderma, Aplastic Anemia, Reactive Arthritis, Fibromyalgia, Sjogren Syndrome, Systemic Lupus Erythematosus, Vitiligo, Type 1 Diabetes, Alopecia Areata, Crohn's Disease, Psoriasis, Chronic Fatigue Syndrome and Vasculitis, as well as inflammatory disorders such as Asthma, Ulcerative Colitis, Irritable Bowel Syndrome, Atherosclerosis, Alzheimer's Disease, Dermatitis, Diverticulitis, Hepatitis, inflammatory diseases, anti-inflammatory, anti-arthritis, anti-osteoporosis, anti-apoptotic, anti-stress, wound healing, anti-cancer, anti-aging, anti-psychotic and anti-fibrotic action, stress management and prevention, Parkinson's Disease etc. to modulate the immune system by improving Quality of Life (QoL).

Abbreviations

MG-63: Human Bone Osteosarcoma Cells, ALP: Alkaline

phosphatase, CAM: Complementary and alternative medicine, NHIS: National Health Interview Survey, NCCIH: National Center of Complementary and Integrative Health, DMEM: Dulbecco's modified eagle's medium, FBS: Fetal bovine serum, ATCC: American type culture collection, UT: Untreated, BT: Biofield Energy Treated, TI: Test item

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