

Role of Vitamin D₃ on Alkaline Phosphatase, Collagen, and Bone Mineralization: Impact of Biofield Energy Healing Treatment

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Abstract: The objective of this study was to assess the potential of Biofield Energy Treated vitamin D₃ and DMEM medium on bone health. The test items, were divided into two parts. One part of each sample received the Consciousness Energy Healing Treatment by Bonnie Patrice Hegarty-Diaz and those samples were labeled as the Biofield Energy Treated (BT) samples, while the other parts of each sample were denoted as the untreated test items (UT). Various parameters were performed to evaluate bone strength and integrity such as ALP, collagen, and bone mineralization in human bone osteosarcoma cells (MG-63). The test samples were found as safe in the tested concentrations by MTT assay. ALP was significantly increased by 50.77% and 50.27% in the BT-DMEM + UT-Test item and BT-DMEM + BT-Test item, respectively at 10 µg/mL compared to the UT-DMEM + UT-Test item group. Further, the ALP level was significantly elevated by 137.29% and 88.08% and 107.93% 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. Further, the collagen level was significantly increased by 66.15%, 88.18%, and 55.87% in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item and BT-DMEM + BT-Test item groups, respectively at 0.1 µg/mL compared to the untreated group. Collagen was significantly increased by 39.44%, 39.44%, and 49.66% 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 untreated group. Additionally, the percent of bone mineralization was distinctly increased by 13.51% and 47.21% in the UT-DMEM + BT-Test item and BT-DMEM + UT-Test item groups, respectively at 10 µg/mL compared to the untreated group. The percent of bone mineralization was distinctly increased by 11.51%, 109.53%, and 83.73% 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. Overall, the Biofield Energy Treated vitamin D₃ was significantly improved the bone health parameters and it could be a powerful alternative nutraceutical supplement to combat vitamin D₃ deficiency and fight against various bone related problems including rickets, osteomalacia, osteoporosis, osteogenesis imperfecta, Paget's disease of bone, bone and joint pain, low bone density, bone fractures, deformed bones, osteoma, chondrodystrophia fetalis, stress management and prevention, autoimmune and inflammatory diseases, and anti-aging by improving overall health.

Keywords: The Trivedi Effect[®], Biofield Energy Healing Treatment, Osteosarcoma Cells (MG-63), Alizarin Red S Staining, Bone Mineralization, Vitamin D₃ Deficiency

1. Introduction

Vitamin D has multiple effects, which regulate the functions in different organs *viz.* brain, liver, lungs, heart, kidneys, skeletal, immune and reproductive systems. Moreover, it has 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 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. Vitamin D receptors 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 the presence of sunlight in the skin [2]. Most foods do not contain any vitamin D, additionally now-a-days due to aging, use of sunscreen, and change of zenith angle of sun the production of vitamin D₃ has reduced [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 for menopausal women is one of the most critical health problem in the today 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 in 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, several scientific reports and clinical trials have revealed the useful effects of Biofield Energy Treatments, which have shown to enhance immune function in cases 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 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 (17.7%) of the Americans used dietary supplements as a complementary health approach as compared with other practices in 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, 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 purchased from TCI, Japan, while vitamin D₃ (denoted as test item) 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, while 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium) (MTT), Direct Red 80, and ethylene diamine tetra acetic acid (EDTA) were purchased from Sigma, USA. 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 [38].

2.3. Experimental Design

The experimental groups consisted of cells in baseline control, 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 Bonnie Patrice Hegarty-Diaz, 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. Bonnie Patrice Hegarty-Diaz 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. 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 [39].

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 10×10^3 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 10×10^3 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

The percent of viable cells after exposure with the Biofield Energy Healing Treated vitamin D₃ and DMEM by MTT assay in MG-63 cells are shown in Figure 1. The percentage of cell viability was found in the ranges of 75% to 131% in the tested concentrations of all the groups and did not exhibit any cytotoxicity (as evidence of cell viability approximately greater than 75%) across all the tested concentrations from 1 µg/mL to 50 µg/mL. Therefore, 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.

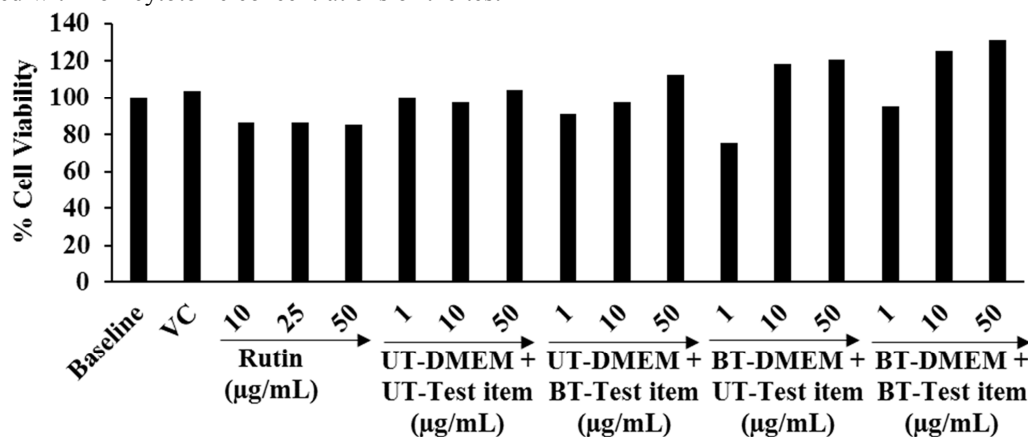


Figure 1. Assessment of the cell viability of the test items (vitamin D₃ and DMEM medium) after Biofield Energy Treatment in different tested concentrations in MG-63 cells. VC: Vehicle control (0.05% DMSO); UT: Untreated; BT: Biofield Energy Treated.

3.2. Alkaline Phosphatase (ALP) Assay

Various biomarkers are available for the assessment of bone formation and resorption *viz.* alkaline phosphatase (ALP), bone-specific alkaline phosphatase (BALP), osteocalcin (OC), procollagen type 1 N-terminal propeptide (P1NP) and procollagen type 1 C-terminal propeptide (P1CP) [40, 6]. ALPs are the group of enzymes that facilitate the calcification of osteoblasts and to protect them from enzymatic degradation. Primarily, ALP have three isotypes found in the liver (ALP-1), bone (ALP-2), and intestines (ALP-3). Apart from these organs this enzyme also present in small amount in the placenta and the proximal part of the kidney [41]. This enzyme can be available in the serum either from liver or bone [42-44]. Numerous studies reported that besides ALP, calcium, vitamin D, and anaerobic exercise also equal role for the development and remodeling of bone [45-47]. The effect of the Biofield Energy Treated test items on ALP in human bone osteosarcoma cells is shown in Figure 2. The level of ALP was found as 39.1% in the vehicle control (VC) group compared to the baseline control group. The ALP activity was significantly

increased by 31.64% and 384.37% in the positive control group at the concentration of 0.1 and 1 $\mu\text{g/mL}$, respectively compared to the VC group. The level of ALP was increased by 50.77% and 50.27% in the BT-DMEM + UT-Test item and BT-DMEM + BT-Test item group, at the concentration of 10 $\mu\text{g/mL}$ compared to the UT-DMEM + UT-Test item group. Further, the level of ALP was significantly increased by 137.29% and 88.08% and 107.93% in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item groups, respectively at 50 $\mu\text{g/mL}$ compared to the UT-DMEM + UT-Test item group. Further, the ALP level was significantly elevated by 1.41% and 53.31% in the UT-DMEM + BT-Test item and BT-DMEM + UT-Test item groups, respectively at 100 $\mu\text{g/mL}$ compared to the UT-DMEM + UT-Test item group. Overall, the Consciousness Energy Healing Treated (The Trivedi Effect[®]) test item group (*i.e.*, vitamin D₃) showed an improved synthesis of ALP level in the human osteosarcoma cells with respect to the untreated item items group, which might be very helpful to the patients suffering from various bone-related disorders.

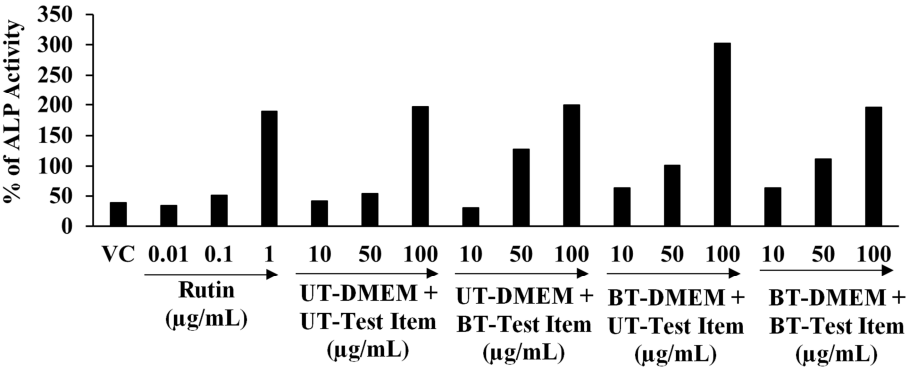


Figure 2. The effect of the Biofield Energy Treated test items on alkaline phosphatase (ALP) enzyme activity in human bone osteosarcoma cell. VC: Vehicle control (0.05% DMSO), UT: Untreated; BT: Biofield Energy Treated.

3.3. Assessment of Collagen Activity

The effect of the test items on collagen level in human bone osteosarcoma cells is shown in Figure 3. Collagen level in the VC group was found as 25.1% as compared to the baseline control group. The positive control rutin hydrate exhibited 63.44%, 63.83%, and 79.45% level of collagen content at 0.01, 0.1, and 1 $\mu\text{g/mL}$, respectively compared to the untreated cells group. The collagen synthesis was significantly increased by 66.15%, 88.18%, and 55.87% in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item groups, respectively at 0.1 $\mu\text{g/mL}$ compared to the UT-DMEM + UT-Test item group. Moreover, the collagen synthesis was significantly increased by 17.31% and 6.79% in the UT-DMEM + BT-Test item and BT-DMEM + UT-Test item groups, respectively at 1 $\mu\text{g/mL}$ compared to the UT-DMEM + UT-Test item group. The collagen synthesis was significantly increased by 44.34%, 23.95%, and 5.63% 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. Moreover,

the collagen level was significantly increased by 39.44%, 39.44%, and 49.66% in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item groups, respectively at 50 $\mu\text{g/mL}$ compared to the UT-DMEM + UT-Test item group. Additionally, at 100 $\mu\text{g/mL}$ the level of collagen was also significantly increased by 11.35%, 29.91%, and 46.41% 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). Altogether, the Consciousness Energy Healing based test item group (*i.e.*, vitamin D₃) showed an improved synthesis of collagen content in the human osteosarcoma cells with respect to all the treatment groups. Collagen comprises about 25% to 35% of the total body proteins content, mainly distributed in the extracellular matrix compartment. Naturally, after the age of 25 years body start to loses collagen at the rate of 1% per year [48]. It is well established that collagen is responsible for maintaining the structural integrity of various organs such as skin, tendons, ligaments, cartilages etc. in the vertebrates and multicellular organisms. In the Chinese drugstore, the collagen peptides are

extensively utilized as health supplements and are very expensive [10]. A lots of literatures reported that supplementation of collagen peptides enhanced skin elasticity, bone density, and reduced the severity of bone-related disorders [49-52]. In this experiment, 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 potential to improve the bone health in various skeletal disorders.

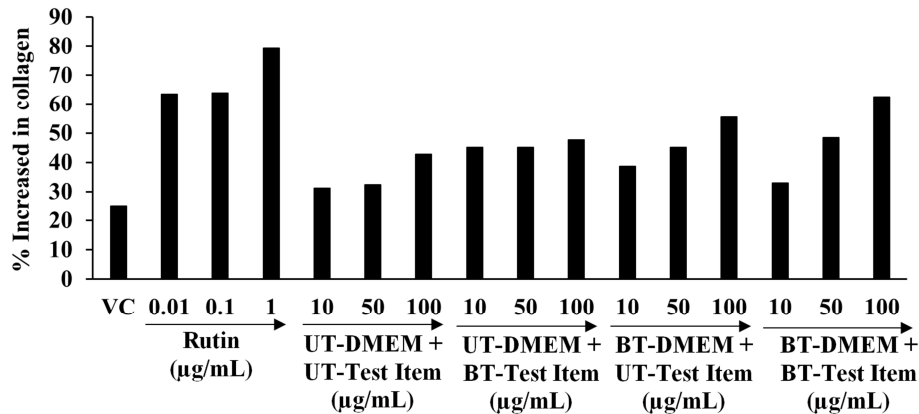


Figure 3. The effect of the test samples on collagen level in human bone osteosarcoma cells. VC: Vehicle control (0.05% DMSO), UT: Untreated; BT: Biofield Energy Treated.

3.4. Bone Mineralization

The effect of Biofield Energy Treatment on bone mineralization in human bone osteosarcoma cells is shown in Figure 4. The percentage of bone mineralization was significantly increased in a concentration dependent manner by 746.8%, 986.7%, and 1278.6% at 5, 10, and 25 µg/mL, respectively in the positive control group compared to the VC group. The percent of bone mineralization was distinctly increased by 13.51% and 47.21% in the UT-DMEM + BT-Test item and BT-DMEM + UT-Test item group, respectively at 10 µg/mL compared to the UT-DMEM + UT-Test item group. Further, a significant increased percentage of bone mineralization was observed by 11.51%, 109.53%, and 83.73% in the UT-DMEM + BT-Test item and BT-DMEM + UT-Test item group, respectively at 100 µg/mL with respect to the UT-DMEM + UT-Test item group (Figure 4). Bone-related disorders like osteoporosis is a silent and potentially disabling skeletal disease. Deficiency of calcium and vitamin-D is a major risk factor for osteoporosis. Most of

postmenopausal women are high risk for developing this disease. After menopause there was a massive reduction of bone mass that leads to an imbalance of bone resorption and formation [53]. There are numerous factors for the etiology of postmenopausal osteoporosis such as aging, genetic, environmental, hormonal, nutritional, and life style factors [54]. To combat these factors some medications are available such as direct supplementation with estrogen, calcitonin, bisphosphonate, calcium, vitamin D₃ [55, 56]. Thus, in this experiment, authors have found that the Biofield Energy Treated vitamin D3 significantly enhanced the levels 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).

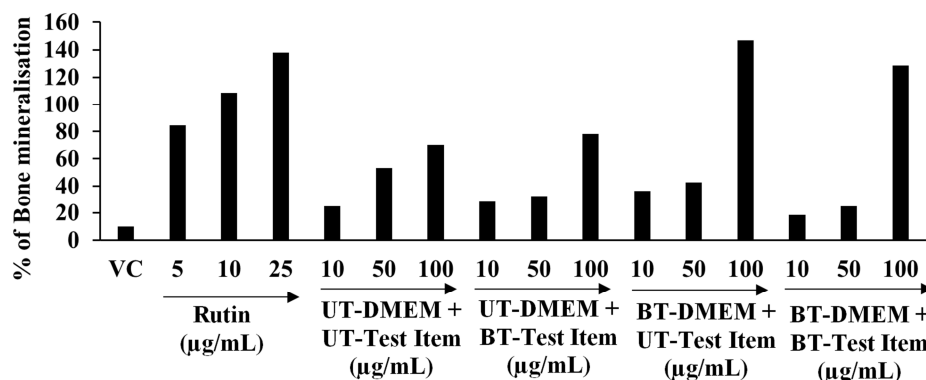


Figure 4. The effect of the test samples on bone mineralization in human bone osteosarcoma cells. VC: Vehicle control (0.05% DMSO), UT: Untreated; BT: Biofield Energy Treated.

4. Conclusions

The results of cell viability by MTT assay data showed more than 75% cells were viable, which indicated that the test samples were safe and nontoxic in all the tested concentrations. ALP was significantly increased by 50.77% and 50.27% in the BT-DMEM + UT-Test item and BT-DMEM + BT-Test item, respectively at 10 µg/mL compared to the UT-DMEM + UT-Test item group. Moreover, ALP level was significantly increased by 137.29% and 88.08% and 107.93% 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. Further, the collagen level was significantly increased by 66.15%, 88.18%, and 55.87% in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item and BT-DMEM + BT-Test item, respectively at 0.1 µg/mL compared to the untreated group. Collagen was significantly increased by 39.44%, 39.44%, and 49.66% in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item group at 50 µg/mL, respectively compared to the untreated group. Further, the collagen level was significantly increased by 29.91% and 46.41% in the BT-DMEM + UT-Test item and BT-DMEM + BT-Test item, respectively at 100 µg/mL compared to the untreated group. Further, the percent of bone mineralization was distinctly increased by 13.51% and 47.21% in the UT-DMEM + BT-Test item and BT-DMEM + UT-Test item groups, respectively at 10 µg/mL compared to the untreated group. Moreover, the percent of bone mineralization was distinctly increased by 11.51%, 109.53%, and 83.73% 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. low bone density and osteoporosis, osteogenesis imperfecta, Paget's disease of bone, rickets, osteomalacia, bone and joint pain, bone fractures, deformed bones, osteoma, 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, Multiple Sclerosis, Myasthenia Gravis, Pernicious Anemia, Aplastic Anemia, Reactive Arthritis, Rheumatoid Arthritis, Sjogren Syndrome, Systemic Lupus Erythematosus, Type 1 Diabetes, Alopecia Areata, Crohn's Disease, Fibromyalgia, Vitiligo, Psoriasis, Scleroderma, Chronic Fatigue Syndrome and Vasculitis, as well as inflammatory disorders such as Asthma, Ulcerative Colitis,

Alzheimer's Disease, Atherosclerosis, Dermatitis, Diverticulitis, Hepatitis, Irritable Bowel Syndrome, inflammatory diseases, anti-inflammatory, anti-stress, anti-arthritis, anti-osteoporosis, anti-apoptotic, wound healing, anti-cancer, anti-psychotic and anti-fibrotic actions stress management and prevention, and anti-aging by improving overall health, Parkinson's Disease and stress etc. to modulate the immune system by improving overall health.

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, AGE: Advanced glycation end products.

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