



***In vitro* Study on Human Bone Osteosarcoma Cells (MG-63): Role of Biofield Energy Treated Vitamin D₃**

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Abstract: The study was aimed to evaluate the potential of Consciousness Energy Healing based 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 Gary Richard Gerber 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). ALP, collagen, and bone mineralization activities were performed to assess bone health in human bone osteosarcoma cells (MG-63). The cell viability assay (MTT) data showed that the test samples were found as safe in all the tested concentrations. The level of ALP was significantly increased by 74.54% and 98.26% in the UT-DMEM + BT-Test item and BT-DMEM + UT-Test item groups, respectively at 10 µg/mL compared to the UT-DMEM + UT-Test item group. Further, the ALP level was significantly elevated by 111.17% in the BT-DMEM + BT-Test item group at 100 µg/mL compared to the UT-DMEM + UT-Test item group. Collagen was significantly increased by 147.46%, 59.67%, and 35.36% in the UT-DMEM + BT-Test item group at 1, 10, and 50 µg/mL, respectively compared to the untreated group. Moreover, the level of collagen was significantly increased by 89.88%, 86.42%, and 82.08% 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 untreated group. Further, the collagen level was significantly increased by 365.76%, 80.10%, and 76.09% in the BT-DMEM + BT-Test item group at 0.1, 10, and 50 µg/mL compared to the untreated group. Besides, the percent of bone mineralization was distinctly increased by 59.30%, 30.69%, and 177.7% 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. The percent of bone mineralization was distinctly increased by 29.44%, and 45.51% in the BT-DMEM + UT-Test item and BT-DMEM + BT-Test item groups, respectively at 100 µg/mL compared to the untreated group. Overall, the Biofield Energy Treated vitamin D₃ was remarkably improved the bone health parameters and help to combat vitamin D₃ deficiency and fight against various bone related problems including low bone density, osteoporosis, osteogenesis imperfecta, Paget's disease of bone, rickets, osteomalacia, bone and joint pain, bone fractures, deformed bones, osteoma, chondrodystrophia fetalis, autoimmune and inflammatory diseases, stress management and prevention, and anti-aging by improving overall health.

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

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-arthritic, 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, large and small 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]. 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]. Thus, this is the one of the most critical health issues in today's world is the quality of life for menopausal women. 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

Fetal bovine serum (FBS) and Dulbecco's Modified Eagle's Medium (DMEM) were purchased from Life Technology, USA. Rutin hydrate was purchased from TCI, Japan, while vitamin D₃ (denoted as test item) and L-ascorbic acid were obtained from Sigma-Aldrich, 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 cell line, MG-63, was used as the test system in the present study. The MG-63 cell line was maintained under the DMEM growth medium for routine culture and supplemented with 10% FBS. 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 before 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 untreated cells group (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. 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 Gary Richard Gerber, who participated in this study and performed the Biofield Energy Treatment remotely for ~5 minutes. Gary Richard Gerber was remotely located in the Canada, 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. Gary Richard Gerber 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 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 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

The cell viability data of the Biofield Energy Treated vitamin D₃ and DMEM by MTT assay in MG-63 cells are depicted in Figure 1. The data showed that the test samples in combination did not exhibit any cytotoxicity (as evidence of cell viability approximately greater than 89%) across all the tested concentrations up to 100 µg/mL. Hence, the same concentrations were assessed further 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.

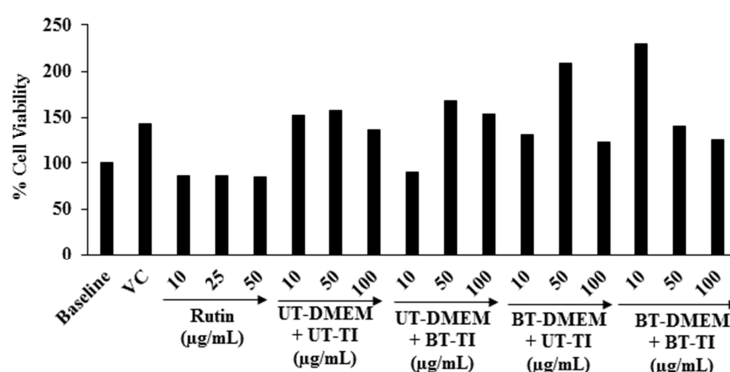


Figure 1. The cell viability of the test samples (vitamin D₃ and DMEM medium) in different concentrations in MG-63 cells after 72 hours of treatment. VC: Vehicle control (0.05% DMSO); UT: Untreated; BT: Biofield Energy Treated; TI: Test item.

3.2. Alkaline Phosphatase (ALP) Activity

The effect of the Biofield Energy Treated test items on the

level of alkaline phosphatase (ALP) in human bone osteosarcoma cells is presented in Figure 2. The level of ALP

was found as 29% in the vehicle control (VC) group compared to the untreated cells (baseline control) group. The ALP activity was significantly increased in a dose dependent manner by 39.07%, 46.45%, and 80.87% in the positive control group at the concentration of 0.01, 0.1, and 1 $\mu\text{g/mL}$, respectively compared to the untreated cells group. The level of ALP was increased by 74.54% and 98.26% in the UT-DMEM + BT-Test item and BT-DMEM + UT-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 significant increased by 4.48% and 111.17% in the UT-DMEM + BT-Test item and BT-DMEM + BT-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. Vitamin D is essential for the maintenance of health, due to the presence of its highly specific vitamin D receptors (VDRs) in all body tissues which regulates more than 200 genes [40]. Therefore, the deficiency of vitamin D affects on various tissues. ALP is a group of zinc metalloenzymes present in the cell membrane that catalyze to split off a terminal phosphate group from an organic phosphate ester and generate an organic radical and inorganic phosphate. It is expressed mainly in bone and liver and released in the blood [41, 42]. Apart from bone cells development, increased level of ALP also responsible for initiating calcification [43], extended to cardiovascular calcification [44]. In this experiment, it was also evident that the Biofield Energy Treated vitamin D₃ significantly increased the level of ALP expression, which might be very advantageous to maintain a healthy skeletal structure for the patients suffering from various bone related disorders.

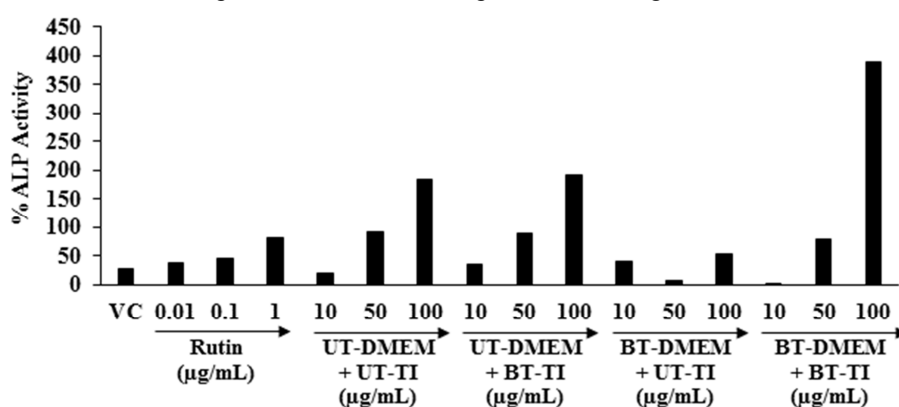


Figure 2. The effect of the Biofield Energy Treated test samples on alkaline phosphatase enzyme activity in human bone osteosarcoma cell. VC: Vehicle control (0.05% DMSO), UT: Untreated; BT: Biofield Energy Treated; TI: Test item.

3.3. Assessment of Collagen Activity

The effect of the test samples on the collagen content in human bone osteosarcoma cells is shown in Figure 3. Collagen level in the VC group was found as 3.8% as compared to the normal control group. The level of collagen synthesis was significantly increased by 29.56%, 46.80%, and 51.48% at 0.01, 0.1, and 1 $\mu\text{g/mL}$, respectively in the positive control group compared to the untreated cells group. The collagen synthesis was significantly increased by 365.76% in the BT-DMEM + BT-Test item group at 0.1 $\mu\text{g/mL}$ compared to the UT-DMEM + UT-Test item group. Moreover, the collagen level was significantly increased by 147.46%, 2.57%, and 3.15% in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item groups, respectively at 1 $\mu\text{g/mL}$ compared to the UT-DMEM + UT-Test item group. Additionally, at 10 $\mu\text{g/mL}$ the level of collagen was also significantly increased by 59.67%, 20.41%, and 80.10% 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. Further, at 50 $\mu\text{g/mL}$ the level of collagen was also significantly increased by 35.36%, 8.41%, and

76.09% 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. The level of collagen was significantly increased by 89.88%, 86.42%, and 82.08% in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item groups, respectively at 100 $\mu\text{g/mL}$ with respect to the UT-DMEM + UT-Test item group (Figure 3). The content of bone mineral provides a mechanical rigidity and load-bearing strength to the bone, while the organic matrix provides elasticity and flexibility [45]. Type I collagen is the major structural protein responsible for bone calcification and also promotes osteoblast differentiation [46]. From literature, it was reported that an abnormality of collagen synthesis and stability leads to age-related bone loss like osteogenesis imperfecta and osteoporosis [47-51]. 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.

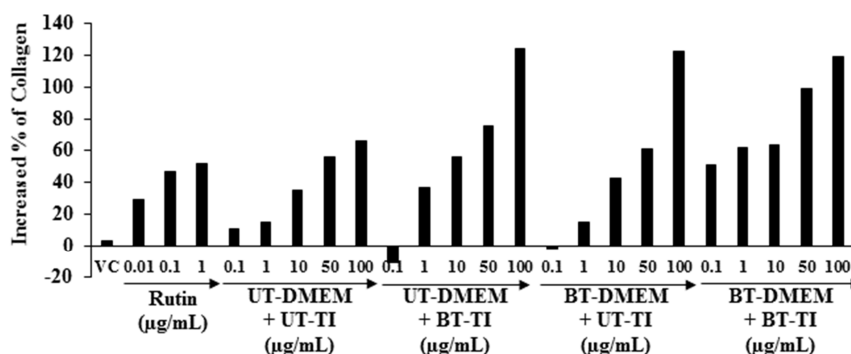


Figure 3. The effect of the test samples on collagen activity in human bone osteosarcoma cells. VC: Vehicle control (0.05% DMSO), UT: Untreated; BT: Biofield Energy Treated; TI: Test item.

3.4. Assessment of Bone Mineralization by Alizarin Red S (ARS) Staining

The Alizarin red S (ARS) staining is widely utilized for the assessment of calcium-rich deposits in the cell culture study. Formation of bone involves the mineralization of the extracellular matrix formed by osteoblasts. In this process vitamin D stimulates the bone mineralization of human osteoblasts but is often found inhibitory for mineralization of murine osteoblasts [52, 53]. According to Delleng *et al.*, reported that the lowering the threshold value of pathologic lack of bone mineralization to 1.2%, simultaneously there was a chance of the fraction of osteomalacia increases up to 43.41% [54]. The 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 49.45%, 66.01%, and 126.45% 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

20.96% in the BT-DMEM + UT-Test item group at 10 µg/mL compared to the UT-DMEM + UT-Test item group. Further, a noticeably increased percentage of bone mineralization by 59.30%, 30.69%, and 177.7% 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. In addition, the data showed a significant increased the bone mineralization by 15.97%, 29.44%, and 45.51% in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item groups, respectively than the UT-DMEM + UT-Test item group (Figure 4) at 100 µg/mL. Thus, based on the above findings it showed 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) with respect to the all others treatment groups.

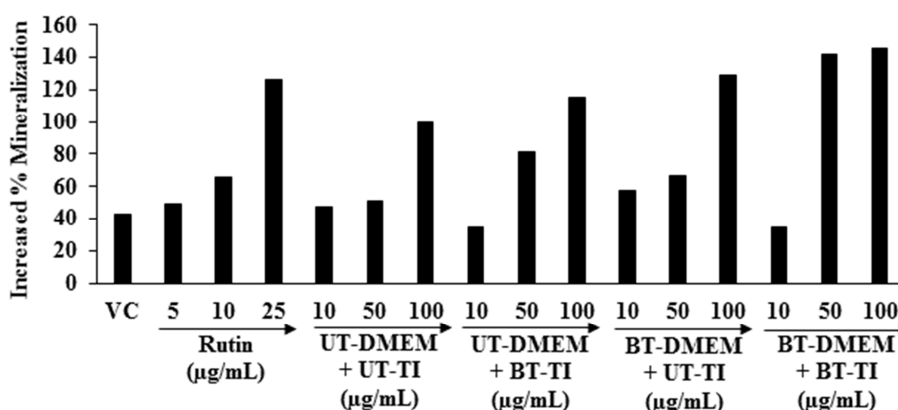


Figure 4. The effect of the Biofield Energy Treated test samples on human bone osteosarcoma cells for the assessment of bone mineralization activity. VC: Vehicle control (0.05% DMSO), UT: Untreated; BT: Biofield Energy Treated; TI: Test item.

4. Conclusions

The MTT cell viability assay data showed more than 89% cells were viable, which indicated that the test samples were safe and nontoxic in all the tested concentrations. ALP was

significantly increased by 74.54% and 98.26% in the UT-DMEM + BT-Test item and BT-DMEM + UT-Test item groups, respectively at 50 µg/mL; while increased by 111.17% in the BT-DMEM + BT-Test item group at 100 µg/mL compared to the UT-DMEM + UT-Test item group. Collagen was significantly increased by 147.46%, 59.67%,

and 35.36% in the UT-DMEM + BT-Test item group at 1, 10, and 50 µg/mL, respectively compared to the untreated group. Additionally, the level of collagen was significantly increased by 89.88%, 86.42%, and 82.08% 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 untreated group. Further, the collagen level was significantly increased by 365.76%, 80.10%, and 76.09% in the BT-DMEM + BT-Test item group at 0.1, 10, and 50 µg/mL compared to the untreated group. Besides, the percent of bone mineralization was distinctly increased by 59.30%, 30.69%, and 177.7% 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. The percent of bone mineralization was distinctly increased by 29.44% and 45.51% in the BT-DMEM + UT-Test item and BT-DMEM + BT-Test item groups, respectively at 100 µg/mL compared to the untreated 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, TI: Test item

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References

- [1] Holick MF (2004) Sunlight and vitamin D for bone health and prevention of autoimmune diseases cancers, and cardiovascular disease. *Am J Clin Nutr* 80: 1678S-1688S.
- [2] Holick MF (1996) Vitamin D and bone health. *J Nutr* 126: 1159S-1164S.
- [3] Matsuoka LY, Ide L, Wortsman J, MacLaughlin JA, Holick MF (1987) Sunscreens suppress vitamin D₃ synthesis. *J Clin Endocrinol Metab* 64: 1165-1168.
- [4] Barnes MS, Robson JP, Bonham MP, Strain J, Wallace J (2006) Vitamin D: Status, supplementation and immunodulation. *Cur Nut Food Sci* 2: 315-336.
- [5] Laird E, Ward M, McSorley E, Strain JJ, Wallace J (2010) Vitamin D and bone health; Potential mechanisms. *Nutrients* 2: 693-724.
- [6] Bhattarai T, Bhattacharya K, Chaudhuri P, Sengupta P (2014) Correlation of common biochemical markers for bone turnover, serum calcium, and alkaline phosphatase in post-menopausal women. *The Malaysian Journal of Medical Sciences : MJMS* 21: 58-61.
- [7] Iba K, Takada J, Yamashita T (2004) The serum level of bone-specific alkaline phosphatase activity is associated with aortic calcification in osteoporosis patients. *J Bone Miner Metab* 22: 594-596.
- [8] Holick MF, Garabedian M (2006) Vitamin D: Photobiology, metabolism, mechanism of action, and clinical applications. *Primer on the metabolic bone diseases and disorders of mineral metabolism*. Edited by: Favus MJ, Washington, DC.
- [9] DeLuca HF (2004) Overview of general physiologic features and functions of vitamin D. *Am J Clin Nutr* 80: 1689S-1696S.
- [10] Viguet-Carrin S, Garnero P, Delmas PD (2006) The role of collagen in bone strength. *Osteoporos Int* 17: 319-336.
- [11] Sroga GE, Vashishth D (2012) Effects of bone matrix proteins on fracture and fragility in osteoporosis. *Curr Osteoporos Rep* 10: 141-150.
- [12] Lutgendorf SK, Mullen-Houser E, Russell D, Degeest K, Jacobson G, Hart L, Bender D, Anderson B, Buekers TE, Goodheart MJ, Antoni MH, Sood AK, Lubaroff DM (2010) Preservation of immune function in cervical cancer patients during chemoradiation using a novel integrative approach. *Brain Behav and Immun* 24: 1231-1240.
- [13] Ironson G, Field T, Scafidi F, Hashimoto M, Kumar M, Kumar A, Price A, Goncalves A, Burman I, Tetenman C, Patarca R, Fletcher MA (1996) Massage therapy is associated with enhancement of the immune system's cytotoxic capacity. *Int J Neurosci* 84: 205-217.

- [14] Jain S, Hammerschlag R, Mills P, Cohen L, Krieger R, Vieten C, Lutgendorf S (2015) Clinical studies of biofield therapies: Summary, methodological challenges, and recommendations. *Glob Adv Health Med* 4: 58-66.
- [15] Rubik B (2002) The biofield hypothesis: Its biophysical basis and role in medicine. *J Altern Complement Med* 8: 703-717.
- [16] Trivedi MK, Patil S, Shettigar H, Mondal SC, Jana S (2015) The potential impact of biofield treatment on human brain tumor cells: A time-lapse video microscopy. *J Integr Oncol* 4: 141.
- [17] Trivedi MK, Patil S, Shettigar H, Gangwar M, Jana S (2015) *In vitro* evaluation of biofield treatment on cancer biomarkers involved in endometrial and prostate cancer cell lines. *J Cancer Sci Ther* 7: 253-257.
- [18] Trivedi MK, Patil S, Shettigar H, Mondal SC, Jana S (2015) *In vitro* evaluation of biofield treatment on *Enterobacter cloacae*: Impact on antimicrobial susceptibility and biotype. *J Bacteriol Parasitol* 6: 241.
- [19] Trivedi MK, Patil S, Shettigar H, Mondal SC, Jana S (2015) Evaluation of biofield modality on viral load of hepatitis B and C Viruses. *J Antivir Antiretrovir* 7: 083-088.
- [20] Trivedi MK, Patil S, Shettigar H, Mondal SC, Jana S (2015) An impact of biofield treatment: Antimycobacterial susceptibility potential using BACTEC 460/MGIT-TB System. *Mycobact Dis* 5: 189.
- [21] Trivedi MK, Branton A, Trivedi D, Nayak G, Mondal SC, Jana S (2015) Antimicrobial sensitivity, biochemical characteristics and biotyping of *Staphylococcus saprophyticus*: An impact of biofield energy treatment. *J Women's Health Care* 4: 271.
- [22] Trivedi MK, Branton A, Trivedi D, Nayak G, Mondal SC, Jana S (2015) Evaluation of antibiogram, genotype and phylogenetic analysis of biofield treated *Nocardia otitidis*. *Biol Syst Open Access* 4: 143.
- [23] Trivedi MK, Branton A, Trivedi D, Nayak G, Charan S, Jana S (2015) Phenotyping and 16S rDNA analysis after biofield treatment on *Citrobacter braakii*: A urinary pathogen. *J Clin Med Genom* 3: 129.
- [24] Trivedi MK, Patil S, Shettigar H, Bairwa K, Jana S (2015) Spectroscopic characterization of chloramphenicol and tetracycline: An impact of biofield. *Pharm Anal Acta* 6:395.
- [25] Trivedi MK, Patil S, Shettigar H, Bairwa K, Jana S (2015) Spectroscopic characterization of biofield treated metronidazole and tinidazole. *Med Chem* 5: 340-344.
- [26] Trivedi MK, Patil S, Shettigar H, Bairwa K, Jana S (2015) Effect of biofield treatment on spectral properties of paracetamol and piroxicam. *Chem Sci J* 6: 98.
- [27] Trivedi MK, Branton A, Trivedi D, Shettigar H, Bairwa K, Jana S (2015) Fourier transform infrared and ultraviolet-visible spectroscopic characterization of biofield treated salicylic acid and sparfloxacin. *Nat Prod Chem Res* 3: 186.
- [28] Trivedi MK, Branton A, Trivedi D, Nayak G, Gangwar M, Jana S (2016) Molecular analysis of biofield treated eggplant and watermelon crops. *Adv Crop Sci Tech* 4: 208.
- [29] Trivedi MK, Branton A, Trivedi D, Nayak G, Mondal SC, Jana S (2015) Morphological characterization, quality, yield and DNA fingerprinting of biofield energy treated alphonso mango (*Mangifera indica* L.). *Journal of Food and Nutrition Sciences* 3: 245-250.
- [30] Trivedi MK, Branton A, Trivedi D, Nayak G, Mondal SC, Jana S (2015) Evaluation of plant growth, yield and yield attributes of biofield energy treated mustard (*Brassica juncea*) and chick pea (*Cicer arietinum*) seeds. *Agriculture, Forestry and Fisheries* 4: 291-295.
- [31] Trivedi MK, Branton A, Trivedi D, Nayak G, Mondal SC, Jana S (2015) Evaluation of plant growth regulator, immunity and DNA fingerprinting of biofield energy treated mustard seeds (*Brassica juncea*). *Agriculture, Forestry and Fisheries* 4: 269-274.
- [32] Trivedi MK, Tallapragada RM, Branton A, Trivedi D, Nayak G, Jana S (2015) Characterization of physical and structural properties of aluminum carbide powder: Impact of biofield treatment. *J Aeronaut Aerospace Eng* 4: 142.
- [33] Trivedi MK, Nayak G, Patil S, Tallapragada RM, Latiyal O, Jana S (2015) Impact of biofield treatment on atomic and structural characteristics of barium titanate powder. *Ind Eng Manage* 4: 166.
- [34] Trivedi MK, Patil S, Nayak G, Jana S, Latiyal O (2015) Influence of biofield treatment on physical, structural and spectral properties of boron nitride. *J Material Sci Eng* 4: 181.
- [35] Trivedi MK, Nayak G, Patil S, Tallapragada RM, Latiyal O, Jana S (2015) Characterization of physical and structural properties of brass powder after biofield treatment. *J Powder Metall Min* 4: 134.
- [36] Trivedi MK, Nayak G, Patil S, Tallapragada RM, Jana S, Mishra RK (2015) Bio-field treatment: An effective strategy to improve the quality of beef extract and meat infusion powder. *J Nutr Food Sci* 5: 389.
- [37] Trivedi MK, Tallapragada RM, Branton A, Trivedi D, Nayak G, Mishra RK, Jana S (2015) Biofield treatment: A potential strategy for modification of physical and thermal properties of gluten hydrolysate and ipomoea macroelements. *J Nutr Food Sci* 5: 414.
- [38] Czekanska EM, Stoddart MJ, Richards RG, Hayes JS (2012) In search of an osteoblast cell model for *in vitro* research. *Eur Cells Mater* 24: 1-17.
- [39] Biological evaluation of medical devices - Part 5: Tests for *in vitro* cytotoxicity (ISO 10993-5:2009), I. S. EN ISO, 10993-5:20093.
- [40] Holick MF (2007) Vitamin D deficiency. *N Engl J Med* 357: 266-281.
- [41] Warnes TW (1972) Alkaline phosphatase. *Gut* 13: 926-937.
- [42] Saraç F, Saygılı F (2007) Causes of high bone alkaline phosphatase. *Biotechnol Biotechnol Equip* 21: 194-197.
- [43] Whyte MP (1994) Hypophosphatasia and the role of alkaline phosphatase in skeletal mineralization. *Endocr Rev* 15: 439-461.
- [44] Jaffe IZ, Tintut Y, Newfell BG, Demer LL, Mendelsohn ME (2007) Mineralocorticoid receptor activation promotes vascular cell calcification. *Arterioscler Thromb Vasc Biol* 27: 799-805.

- [45] Landis WJ (1995) The strength of a calcified tissue depends in part on the molecular structure and organization of its constituent mineral crystals in their organic matrix. *Bone* 16: 533-544.
- [46] Oishi Y, Fu ZW, Ohnuki Y, Kato H, Noguchi T (2002) Molecular basis of the alteration in skin collagen metabolism in response to *in vivo* dexamethasone treatment: effects on the synthesis of collagen type I and III, collagenase, and tissue inhibitors of metalloproteinases. *Br J Dermatol* 147: 859-868.
- [47] Takeuchi Y, Nakayama K, Matsumoto T (1996) Differentiation and cell surface expression of transforming growth factor-beta receptors are regulated by interaction with matrix collagen in murine osteoblastic cells. *J Biol Chem* 271: 3938-3944.
- [48] Adam M, Spacek P, Hulejová H, Galiánová A, Blahos J (1996) Postmenopausal osteoporosis. Treatment with calcitonin and a diet rich in collagen proteins. *Cas Lek Cesk* 135: 74-78.
- [49] Viguet-Carrin S, Garnero P, Delmas PD (2005) The role of collagen in bone strength. *Osteoporos Int* 17: 319-336.
- [50] Viguet-Carrin S, Roux JP, Arlot ME, Merabet Z, Leeming DJ, Byrjalsen I, Delmas PD, Buxsein ML (2006) Contribution of the advanced glycation end product pentosidine and of maturation of type I collagen to compressive biomechanical properties of human lumbar vertebrae. *Bone* 39: 1073-1079.
- [51] Mechanic GL, Toverud SU, Ramp WK, Gonnerman WA (1975) The effect of vitamin D on the structural crosslinks and maturation of chick bone collagen. *Biochim Biophys Acta* 393: 419-425.
- [52] van Driel M, van Leeuwen JPTM (2017) Vitamin D endocrinology of bone mineralization. *Mol Cell Endocrinol* 453: 46-51.
- [53] van Leeuwen JP, van Driel M, van den Bemd GJ, Pols HA (2001) Vitamin D control of osteoblast function and bone extracellular matrix mineralization. *Crit Rev Eukaryot Gene Expr* 11: 199-226.
- [54] Delling G (1987) Bone morphology in primary hyperparathyroidism: A qualitative and quantitative study of 391 cases. *Appl Pathol* 5: 147-159.