



Exploring the Effects of Biofield Energy Treated Vitamin D₃ on Bone Health Parameters in MG-63 Cell Line

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Abstract: The aim of the present experiment is to define the potential of Consciousness Energy Healing based vitamin D₃ and DMEM medium on various bone health parameters such as alkaline phosphatase enzyme (ALP) activity, collagen level, and bone mineralization. Both the test items (TI) *i.e.* vitamin D₃ and DMEM medium were divided into two parts. The test samples were received Consciousness Energy Healing Treatment by Rolando Baptista Piedad 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). Cell viability of test samples using MTT assay showed that cell viability was more than 87% with safe and nontoxic profile on MG-63 cell line. ALP level was significantly increased by 196.1% (at 10 µg/mL) and 131.6% (at 0.1 µg/mL) in the UT-DMEM+BT-TI and BT-DMEM+UT-TI groups, respectively. In addition, ALP level was significantly increased by 342.1%, 205.2%, and 289.5% at 0.1, 1, and 10 µg/mL, respectively in the BT-DMEM+BT-TI groups as compared with the untreated test item and DMEM group. The percentage of collagen level was significantly increased by 109.5%, 67.0%, and 100.9% at 0.1, 1, and 10 µg/mL, respectively in UT-DMEM+BT-TI group, while 228.4%, 157.8%, and 154.1% at 0.1, 1, and 10 µg/mL, respectively in BT-DMEM+UT-TI as compared with the untreated group. The percent of bone mineralization was significantly increased by 40.8% at 0.1 µg/mL in UT-DMEM+ BT-TI group, while 59.2% at 0.1 µg/mL in BT-DMEM+UT-TI group as compared with the untreated group. In addition, BT-DMEM+BT-TI group showed a significant increased bone mineralization by 128% and 174.1% at 50 and 100 µg/mL, respectively as compared with the untreated group. Biofield Energy Treated vitamin D₃ and DMEM would enhance the promotion and maintenance of strong and healthy bones. Biofield Energy Treatment might also regulates the osteoblast function, improves bone mineralization, and calcium absorption in wide range of bone disorders along with wide range of autoimmune diseases, adverse bone health conditions, comprising cancer, and bone disorders such as Rickets, Osteogenesis Imperfecta, Osteomalacia, Perthes' Disease, Fibrous Dysplasia, Osteomyelitis, Paget's Disease, and Osteoporosis.

Keywords: Biofield Energy, Osteosarcoma Cells, Vitamin D, Osteoporosis, Bone Disorders, Bone Mineralization

1. Introduction

Vitamin D has multiple effects which regulate the functions in different organs such as brain, lungs, liver, kidneys, and heart, immune, skeletal, and reproductive systems. Moreover, it has significant anti-inflammatory, anti-arthritic, anti-osteoporosis, anti-stress, anti-aging, anti-apoptotic, wound healing, anti-cancer, anti-psychotic, and

anti-fibrotic roles. Vitamin D receptors (VDRs) are widely present in most of the body organs like brain, heart, lungs, kidney, liver, pancreas, large and small intestines, muscles, reproductive, nervous system, etc. [1]. VDRs influence cell-to-cell communication, normal cell growth, cell differentiation, cell cycling and proliferation, hormonal balance, neurotransmission, skin health, immune and cardiovascular functions. Bone-related health issues become

a major problem among the population from village to the cities. Vitamin D plays a vital role in preserving a healthy mineralized skeleton of most of the vertebrates including humans. Cod liver oil, irradiation of other foods including plants, sunlight, etc. are found to be effective against bone related disorders, which lead to discovering the active principle- vitamin D [1]. The role of vitamin D has been well defined not only for improving the bone mineralization but also with increased bone resorption, aging, inflammation and overall quality of life. Vitamin D₃ is synthesized in the skin by sunlight and once formed it sequentially metabolized in the liver and kidney to 1,25-dihydroxyvitamin D (calcitriol, the vitamin D hormone) [2]. Calcitriol play an important role in maintaining the normal level of calcium and phosphorus, promotes bone mineralization, induce or repress the genes responsible for conserving the mineral homeostasis and skeletal integrity, and inhibit hypertension, kidney damage, cardiovascular and immune disorders (such as Lupus, Addison Disease, Graves' Disease, Hashimoto Thyroiditis, Multiple Sclerosis, Myasthenia Gravis, Anemia, Sjogren Syndrome, Systemic Lupus Erythematosus, Diabetes, Alopecia Areata, Fibromyalgia, Vitiligo, Psoriasis, Scleroderma, Chronic Fatigue Syndrome and Vasculitis), and the secondary hyperparathyroidism [3]. Vitamin D insufficiency and deficiency is the major health problem, which causes metabolic bone disease in the young and elderly populations [4]. Fortified foods have a variable amount of vitamin D and most of the foods do not contain vitamin D, which can be fulfilled using some supplements. In order to avoid the bone related disorders such as osteomalacia, exacerbate osteoporosis, hyperparathyroidism, immune disorders, etc. calcium 1000-1500 mg/day along with vitamin D supplement around 400 IU/day is very important for maintaining the good bone health [5].

Various *in vitro* studies have readily demonstrated the role of bone health using cell lines and its resorbing effects using three important key biomarkers, such as alkaline phosphatase (ALP), collagen and calcium. MG-63 cell line derived from juxtacortical osteosarcoma, which represents an immature osteoblast phenotype and undergoes temporal development in long term culture. The response of MG-63 cells to 1,25-dihydroxyvitamin D₃ (1,25 (OH)₂D₃) administration has been studied to be similar to normal human osteoblast cells [6]. Hence, MG-63 cell line is widely used for studying the potential of any test compounds to improve the bone health [7]. The formation of new bone involves a complex series of events including the proliferation and differentiation of osteoblasts, and eventually the formation of a mineralized extracellular matrix. ALP is a phenotypic marker for the early differentiation and maturation of osteoblasts. ALP increases the local concentration of inorganic phosphate for bone mineralization and hence is an important marker for osteogenic activity [8]. Similarly, active osteoblasts synthesize and extrude collagen, which plays an important role in the formation of bone extracellular matrix by providing strength and flexibility. Collagen fibrils formed an arrays of an organic matrix known as Osteoid [9]. Likewise,

calcium phosphate is deposited in the Osteoid and gets mineralized (combination of calcium phosphate and hydroxyapatite) and provides rigidity to the bone [10]. Thus, these parameters are very essential in order to study the bone health in cell lines. Authors evaluated the *in vitro* effect of the Biofield Energy Treated vitamin D₃ as a test item, a Complementary and Alternative Medicine (CAM) on bone health using MG-63 cell line for major biomarkers.

Within the burgeoning ground of CAM therapies, Biofield Energy Treatment or energy medicine, is emerging with significant benefits in various scientific fields. The effects of the CAM therapies have great potential, which include external qigong, Johrei, Reiki, therapeutic touch, yoga, Qi Gong, polarity therapy, Tai Chi, pranic healing, deep breathing, chiropractic/osteopathic manipulation, guided imagery, meditation, massage, homeopathy, hypnotherapy, progressive relaxation, acupressure, acupuncture, special diets, relaxation techniques, Roling structural integration, healing touch, movement therapy, pilates, mindfulness, Ayurvedic medicine, traditional Chinese herbs and medicines in biological systems both *in vitro* and *in vivo* [11]. Biofield Energy Healing Treatment (The Trivedi Effect[®]) contain a putative bioenergy, which is channeled by a renowned practitioners from a distance. Biofield Energy Healing as a CAM showed a significant results in biological studies [12]. However, the National Center for Complementary and Alternative Medicine (NCCAM), well-defined Biofield therapies in the subcategory of Energy Therapies [13]. The Trivedi Effect[®]- Consciousness Energy Healing Treatment has been reported with significant revolution in the physicochemical properties of metals, chemicals, ceramics and polymers [14-16], improved agricultural crop yield, productivity, and quality [17, 18], transformed antimicrobial characteristics [19-21], biotechnology [22-23], improved bioavailability [24-26], skin health [27, 28], nutraceuticals [29, 30], cancer research [31, 32], and human health and wellness.

Based on the significant outcomes of Biofield Energy Treatment and vital role of vitamin D₃ on bone health, authors sought to evaluate the impact of the Biofield Energy Treatment (The Trivedi Effect[®]) on vitamin D₃ as test sample for bone health activity with respect to the assessment of different bone health parameters like ALP, collagen content, and bone mineralization using standard *in vitro* assays in MG-63 cells.

2. Material and Methods

2.1. Chemicals and Reagents

Rutin hydrate was purchased from Tokyo Chemical Industry, India, 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. Antibiotics 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

Human bone osteosarcoma cell line -MG-63 was used as test system in the present study. The MG-63 cell line was maintained in DMEM growth medium for routine culture 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 [33].

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), 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 Untreated-DMEM + Untreated-Test item (UT-TI), UT-DMEM + Biofield Energy Treated test item (BT-TI), BT-DMEM + UT-TI, and BT-DMEM + BT-TI.

2.4. Consciousness Energy Healing Treatment Strategies

The test item and DMEM were divided into two parts. One part each of the test item and DMEM was treated with the Biofield Energy by a renowned Biofield Energy Healer (also known as The Trivedi Effect[®]) and coded as the Biofield Energy Treated item, while the second part did not receive any sort of treatment. This Biofield Energy Healing Treatment was provided by Rolando Baptista Piedad 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. This Biofield Energy Treatment was administered for 5 minutes through the Healer's unique Energy Transmission process remotely to the test samples under laboratory conditions. Rolando Baptista Piedad 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 was performed by MTT assay in 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 the cell recovery and exponential growth, which were subjected to serum stripping or starvation. The cells were treated with the test item, DMEM, and positive control. The untreated cells were 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 were added to all the wells followed by 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 Synergy HT micro plate reader, BioTek, USA [34]. The percentage cytotoxicity at each tested concentrations of the test substance were 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 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.

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 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 [33]. The percentage increase in ALP enzyme activity with respect to the untreated cells (baseline group) was calculated using equation (3):

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

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 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 hours 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) [33]. The percentage increase in collagen level with respect to the untreated cells (baseline group) was calculated using equation (4):

$$\% \text{ Increase} = [(X-R)/R]*100 \quad (4)$$

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 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 will be subjected to serum stripping for 24 hours. The cells will be then be treated with non-cytotoxic concentrations of the test samples and positive control. After 3-7 days of incubation with the test samples and positive control, the plates were taken out cell layers and processed further for 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 [33]. 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 respective parameters. For multiple group comparison, one-way analysis of variance (ANOVA) was used followed by post-hoc analysis by Dunnett's test. Statistically significant values were set at the level of $p \leq 0.05$.

3. Results and Discussion

3.1. Cell Viability Study Using MTT

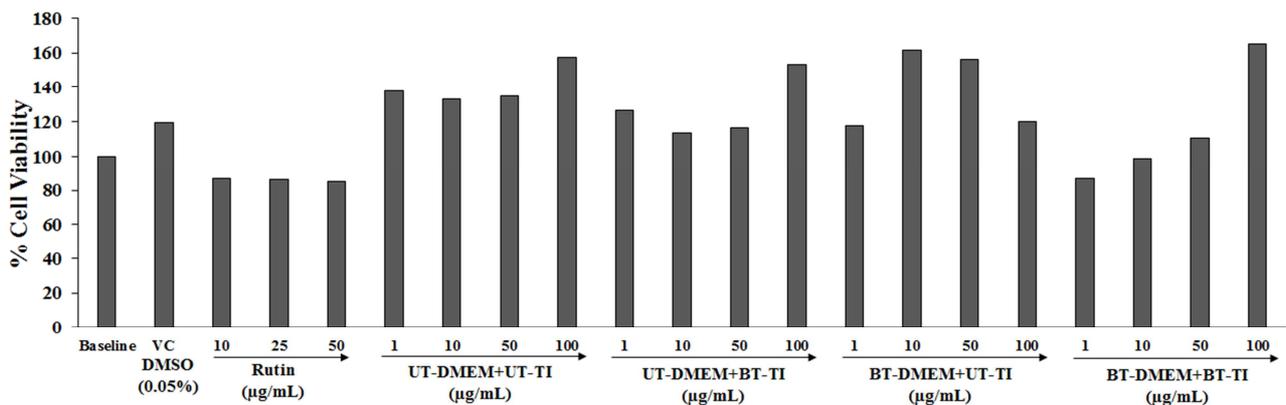


Figure 1. MTT assays of the test formulations on MG-63 cell line after 72 hours. VC: Vehicle control (DMSO-0.05%), UT: Untreated; BT: Biofield Treated; TI: Test Item.

MTT assay was done as the cytotoxicity test of the test samples, it is one of the preliminary method of biological

evaluation and screening tests of any test sample using cells in order to check the growth, proliferation, its reproduction

and morphological effects on cells. All the test samples were initially screened for cell viability assay in MG-63 cells. The percent cell viability results are graphically presented in Figure 1. MTT assay data showed that all the tested samples at various concentrations along with positive control, rutin were found to have significant cell viability with more than 85%. In addition, the Biofield Energy Treated test samples were found to have cell viability with more than 87% at all the concentrations. In addition, Biofield Energy Healing Treatment on test samples showed a significant improved cell viability in different tested groups. Overall, the test data suggests that the test item along with DMEM groups were found safe at all the tested concentrations range up to maximum of 100 µg/mL against the tested MG-63 cells and was tested for various bone health parameters such as on the levels of alkaline phosphatase (ALP) activity, collagen synthesis, and bone mineralization.

3.2. Alkaline Phosphatase (ALP) Enzyme Activity

Alkaline Phosphatase (ALP) has been categorized as ALP-1 and 2 depends upon the location in liver and bone, respectively. Increased level of ALP is reported during the growth phase of bones. However, abnormal level of ALP bone isoenzyme is reported in various pathological conditions, such as certain bone cancers, Paget's disease of bone, osteoporosis, healing fracture, bone growth, acromegaly, myelofibrosis, osteogenic sarcoma, or bone metastases, leukemia, and rarely myeloma. The level can be

improved using supplementation of vitamin D, calcium and ALP enzymes [35-37]. The test samples were screened for the level of ALP in different groups at various concentrations on MG-63 cell line (Figure 2). The results in terms of percentage ALP were described and compared with respect to the untreated group. The positive control, rutin showed a significant increased values of ALP by 30.02%, 34.31%, and 51.47% at 0.001, 0.01, and 0.1 µg/mL, respectively. The experimental test group's viz. untreated medium and Biofield Treated Test item (UT-DMEM+BT-TI) showed a significant increase in the ALP level by 37.9% and 196.1% at 1 and 10 µg/mL, respectively while Biofield Treated medium and untreated Test item (BT-DMEM+UT-TI) showed a significant increased ALP level by 131.6%, 46.6%, and 78.9% at 0.1, 1, and 10 µg/mL, respectively as compared with the untreated test item and DMEM group. However, the Biofield Energy Treated medium and Biofield Energy Treated Test item (BT-DMEM+BT-TI) showed a significant increased ALP level by 342.1%, 205.2%, and 289.5% at 0.1, 1, and 10 µg/mL, respectively as compared with the untreated test item and DMEM group. Thus, overall data concluded that The Trivedi Effect[®]-Energy of Consciousness Healing based vit D₃ and DMEM could be used to improve the ALP concentration in many bone disorders [36]. In conclusion, the experimental data suggest that Biofield Energy Healing Treatment in test samples showed a significant growth of bone health that can be used as supplementation against many bone related diseases.

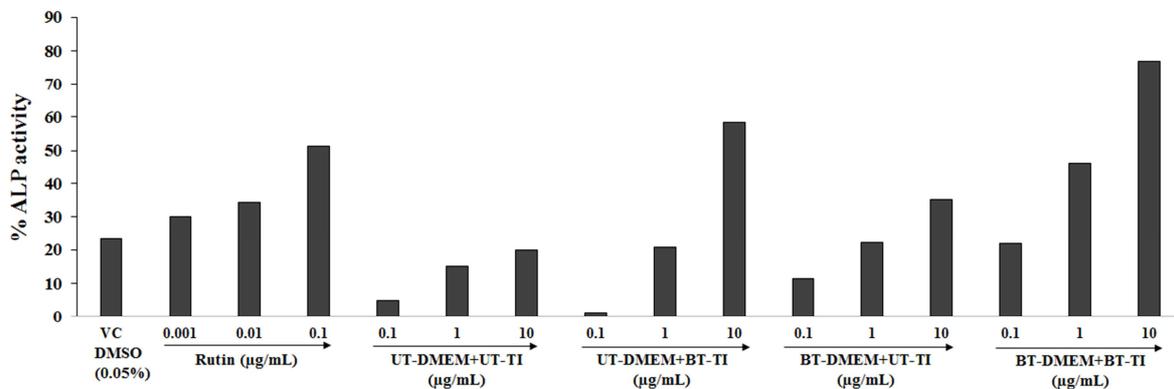


Figure 2. Estimation of Alkaline Phosphatase (ALP) enzyme activity of the Biofield Energy Treated test items on MG-63 cell line. VC: Vehicle control (DMSO-0.05%), UT: Untreated; BT: Biofield Treated; TI: Test Item.

3.3. Collagen Synthesis

Collagen, one of the most abundant protein in body worked as a beneficial therapeutic agent of potential utility in the treatment of major bone diseases such as osteoarthritis and osteoporosis. The test samples such as Biofield Energy Treated vit D₃ and DMEM showed a significant improved level of collagen among various tested concentrations. The results of collagen are presented as % values with respect to the untreated cells in Figure 3. The rutin hydrate showed a significant increased value of collagen by 17.76%, 28.27%, and 46.50% at 0.001, 0.01, and 0.1 µg/mL, respectively. Besides, the experimental test groups such as UT-DMEM+BT-TI showed a significant increased collagen level

by 109.5%, 67.0%, and 100.9% at 0.1, 1, and 10 µg/mL, respectively while BT-DMEM+UT-TI group showed a significant increased collagen level by 228.4%, 157.8%, and 154.1% at 0.1, 1, and 10 µg/mL, respectively as compared with the untreated test item and DMEM group. However, BT-DMEM+BT-TI group showed a significant increased collagen level by 39.2%, 3.7%, and 82.9% at 0.1, 1, and 10 µg/mL, respectively as compared with the untreated test item and DMEM group. Collagen imbalance would result in decreased bone mass and leads to various joint diseases. The level of collagen can be maintained using various collagen supplements in case of many chronic bone disorders. It was reported that the level of collagen was decreased with age,

which can be maintained using collagen supplementation and with other nutritional factors [38, 39]. Overall, the present experimentation suggested that the Biofield Energy (The

Trivedi Effect[®]) Treated vit D₃ showed a significant improved level of collagen that can be used to improve the bone health.

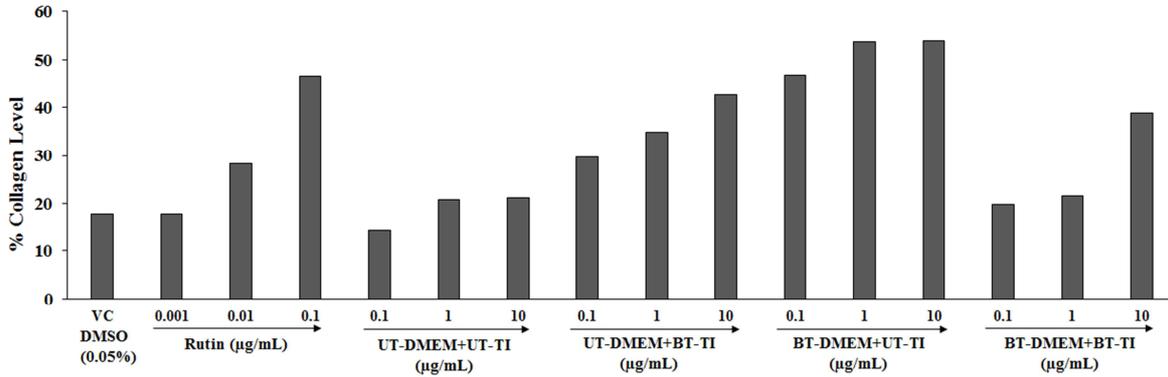


Figure 3. Effect of the test item on MG-63 cell line for collagen level. VC: Vehicle control (DMSO-0.05%), UT: Untreated; BT: Biofield Treated; TI: Test Item.

3.4. Bone Mineralization

Bone mineralization activity was done in order to check the bone calcification, which play an important role in the treatment of osteoporosis or other bone diseases. Bone mineralization activity was done in test samples such as Biofield Energy Treated vit D₃ and DMEM groups, which showed a significant improved bone mineralization on MG-63 cell line. The results of bone mineralization activity are presented in term of percentage change among different experimental groups in Figure 4. The positive control, rutin group showed a significant increased value of bone mineralization by 61.72%, 82.15%, and 141.72% at 5, 10, and 25 µg/mL, respectively. The experimental data among test group's viz. UT-DMEM+BT-TI showed a significant increased bone mineralization by 40.8%, 8.4%, 28%, and 28.1% at 0.1, 10, 50, and 100 µg/mL, respectively while BT-

DMEM+UT-TI group showed a significantly increased bone mineralization by 59.2%, 12.6%, and 10.5% at 0.1, 10, and 100 µg/mL, respectively as compared with the untreated test item and DMEM group. However, BT-DMEM+BT-TI group showed a significant increased bone mineralization by 128% and 174.1% at 50 and 100 µg/mL, respectively as compared with the untreated test item and DMEM group. The experimental test groups showed that Biofield Energy Healing Treatment significantly improved the rate of bone mineralization compared with the untreated groups, which can be used in various bone related disorders and recovery process. Improved bone mass after Biofield Energy Treatment results due to the improved bone mineralization capacity, and increased calcium absorption in bones, which leads to improved bone mineral density (BMD) and various structural abnormalities [40, 41].

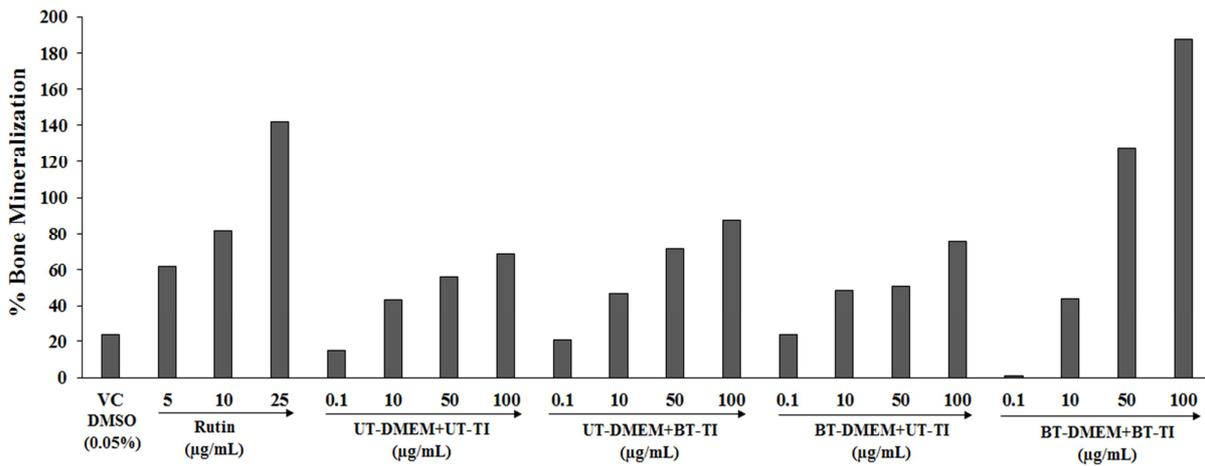


Figure 4. Effect of the test item on MG-63 cell line for bone mineralization. VC: Vehicle control (DMSO-0.05%), UT: Untreated; BT: Biofield Treated; TI: Test Item.

4. Conclusions

Cell viability using MTT assay with more than 87% among the tested groups, which suggest that test samples are

found as safe and nontoxic. ALP level was increased by 196.1% at 10 µg/mL in UT-DMEM+BT-TI, while 131.6% and 78.9% at 0.1 and 10 µg/mL, respectively, in the BT-DMEM+UT-TI group as compared with the untreated test item and DMEM group. In addition, BT-DMEM+BT-TI

group showed a significant increased ALP level by 342.1%, 205.2%, and 289.5% at 0.1, 1, and 10 µg/mL, respectively as compared with the untreated group. Collagen level was significantly increased by 109.5%, 67.0%, and 100.9% at 0.1, 1, and 10 µg/mL, respectively in the UT-DMEM+BT-TI, while 228.4%, 157.8%, and 154.1% at 0.1, 1, and 10 µg/mL, respectively in the BT-DMEM+UT-TI group. In addition, 39.2% and 82.9% increased collagen was reported at 0.1 and 10 µg/mL, respectively as compared with the untreated test item and DMEM group. Similarly, the bone mineralization percent was significantly increased by 40.8%, 28%, and 28.1% at 0.1, 50, and 100 µg/mL, respectively in the UT-DMEM+BT-TI group, while 59.2%, 12.6%, and 10.5% at 0.1, 10, and 100 µg/mL, respectively, in the BT-DMEM+UT-TI group as compared with the untreated group. In addition, BT-DMEM+BT-TI group showed a significant increased bone mineralization by 128% and 174.1% at 50 and 100 µg/mL, respectively as compared with the untreated group. Overall, the Biofield Energy Treated (The Trivedi Effect[®]) test samples were found to have a significant impact on tested bone health parameters *viz.* collagen, bone mineralization, and ALP, which are very vital to combat the bone disorders. Therefore, the Consciousness Energy Healing based vitamin D₃ might be a suitable alternative nutritional supplement, which could be useful for the management of various bone related disorders *viz.* osteoporosis, Paget's disease of bone, rickets, deformed bones, osteomalacia, bone and/or joint pain, increased frequency of fractures, osteoma, hormonal imbalance, stress, aging, bone loss and fractures, and other bone diseases that are caused by poor nutrition, genetics, or problems with the rate of bone growth or rebuilding. Biofield Energy Treated Vitamin D₃ can be useful as anti-inflammatory, anti-aging, anti-stress, anti-arthritis, anti-osteoporosis, anti-cancer, anti-apoptotic, wound healing, anti-psychotic and anti-fibrotic roles. It also influence cell-to-cell communication, normal cell growth, cell differentiation, neurotransmission, cell cycling and proliferation, hormonal balance, skin health, immune and cardiovascular functions. Besides, it can also be utilized in organ transplants (for example kidney transplants, liver transplants and heart transplants), hormonal imbalance, aging, and various immune related disease conditions such as Ulcerative Colitis, Alzheimer's Disease, Dermatitis, Irritable Bowel Syndrome, Asthma, Hashimoto Thyroiditis, Pernicious Anemia, Sjogren Syndrome, Multiple Sclerosis, Aplastic Anemia, Hepatitis, Diverticulitis, Graves' Disease, Dermatomyositis, Diabetes, Myasthenia Gravis, Parkinson's Disease, Atherosclerosis, Systemic Lupus Erythematosus, stress, etc. with a safe therapeutic index to improve overall health, and quality of life.

Abbreviations

CAM: Complementary and Alternative Medicine, NCCAM: National Center for Complementary and Alternative Medicine; MG-63: Human Bone Osteosarcoma Cells, ALP: Alkaline phosphatase, DMEM: Dulbecco's

Modified Eagle's Medium, FBS: Fetal Bovine Serum, FBS: Fetal bovine serum; EDTA: Ethylene Diamine Tetra Acetic Acid, UT: Untreated, BT: Biofield Energy Treated, TI: Test Item.

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