

Evaluation of Biofield Energy Treated Vitamin D₃ in Human Osteoblasts Cells

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Abstract: Bone formation, remodeling and healing processes involves a coordinated action of different cell types. Vitamin D and calcium significantly improves the patients' quality of life with respect to various bone disorders and comorbidities most prevalent in the elderly. The current study aimed to evaluate the potential of Consciousness Energy Healing based vitamin D₃ and DMEM medium on bone health. Vitamin D₃ and DMEM medium as test items (TI), were divided into two parts. One part of each sample received the Consciousness Energy Healing Treatment by Vicki Lynn Kindy 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). Bone health biomarkers such as alkaline phosphatase enzyme (ALP) activity, collagen levels and bone mineralization were evaluated. Cell viability using 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium) (MTT) data showed that the test items were found to be safe. ALP level was significantly increased by 621.2%, 638.9%, and 102.2% at 1, 10, and 50 µg/mL, respectively in UT-DMEM+BT-TI, as compared to the untreated group. Collagen content was significantly increased up to 284.6%, 134%, and 183.4% at 1, 10, and 50 µg/mL, respectively in the UT-DMEM+BT-TI group, while 109.6% and 159.6% increased collagen in BT-DMEM+UT-TI and BT-DMEM+BT-TI groups, respectively at 1 µg/mL as compared with the untreated group. Moreover, the percent of bone mineralization was significantly increased by 120% and 229.4% at 50 and 100 µg/mL, respectively in UT-DMEM+BT-TI group, while 254.7% and 78.1% in BT-DMEM+UT-TI and BT-DMEM+BT-TI groups, respectively at 10 µg/mL as compared with the untreated group. Thus, Biofield Energy Treated vitamin D₃ and DMEM would play an important role to control the osteoblast function, improves bone mineralization, and calcium absorption in many bone disorders. Moreover, the bone health parameters such as collagen, calcium and ALP were significantly improved and can be used as supplement to improve bone health. Based on the outstanding results, it is assumed that the Biofield Energy Treated vitamin D₃ could be a powerful alternative dietary sources and supplements to fight against various bone related diseases including low bone density and osteoporosis, osteogenesis imperfecta, Paget's disease of bone, rickets, osteomalacia, bone and/or joint pain, increased frequency of fractures, deformed bones, osteoma, chondrodystrophia fetalis, hormonal imbalance, stress, aging, bone loss and fractures.

Keywords: The Trivedi Effect[®], Bone Disorders, Osteosarcoma Cells (MG-63), Alizarin Red S Staining, ALP, Collagen, 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-arthritis, 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, polarity therapy, pranic healing, 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, Roling structural integration, 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 [14-17], improved agricultural crop yield, productivity, and quality [18-20], transformed antimicrobial characteristics at genetic level [21-23], biotechnology [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 Tokoyo Chemical

Industry Co., Ltd. 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. Antibiotics solution (penicillin-streptomycin) was procured from HiMedia, India, while 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 and was defined as the untreated samples. This Biofield Energy Healing Treatment was provided by Vicki Lynn Kindy 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. The Biofield Energy Healer, Vicki Lynn Kindy 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 5×10^3 to 10×10^3 cells/well/180 μ L 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 μ L 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 μ L 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):

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

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 $\geq 70\%$ 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 1×10^4 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 μ L 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*NP 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. MTT Assay-Non-Cytotoxic Effect of the Test Item

The cytotoxicity test is one of the preliminary method of biological evaluation and screening tests of any test sample using cells *in vitro* to observe the cell growth, proliferation, its reproduction and morphological effects after treatment. The test samples were screened for cell viability in MG-63 cells. The results showed that all the tested samples at different concentrations along with positive control, rutin were found to have significant cell viability with more than 70%. In addition, the Biofield Energy Treated test samples were found to have cell viability with more than 80% at all the concentrations. The cell viability results are graphically presented in Figure 1. These 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.

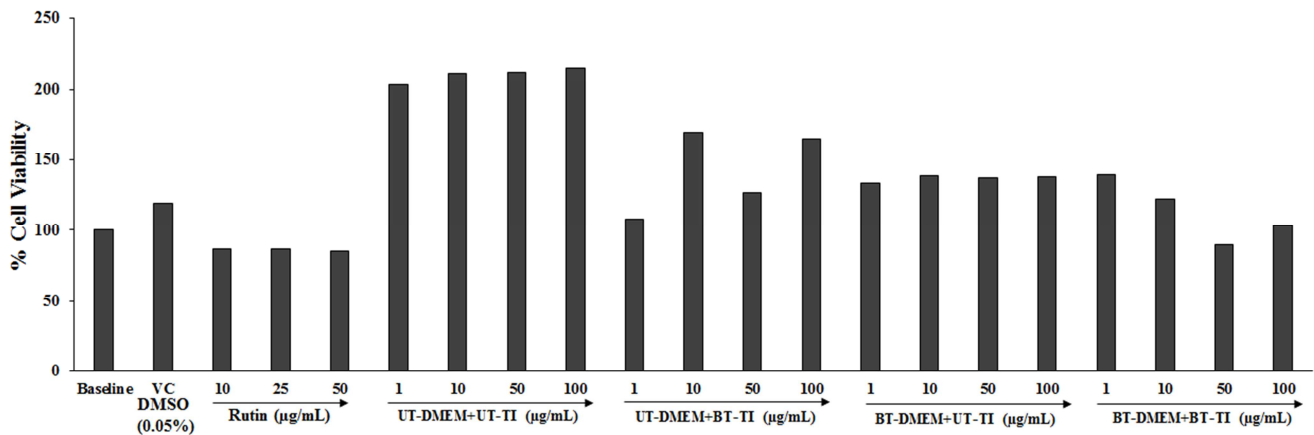


Figure 1. MTT assays of the test item on MG-63 cell line for cell viability after 72 hours. VC: Vehicle control (DMSO-0.05%), UT: Untreated; BT: Biofield Treated; TI: Test Item.

3.2. Evaluation of Alkaline Phosphatase (ALP) Enzyme Activity

The effect of the Biofield Energy Treated test item and DMEM on the ALP level showed a significantly increased at various experimental test item concentrations on MG-63 cell line (Figure 2). The positive control, rutin showed a significant increased value by 34.3%, 51.5%, and 189.4% at 0.01, 0.1, and 1 µg/mL, respectively with respect to the untreated cells. The experimental test group's viz. untreated medium and Biofield Treated Test item (UT-DMEM+BT-TI) showed a significant increase in ALP level by 621.2%, 638.9%, and 102.2% at 1, 10, and 50 µg/mL, respectively

while Biofield Treated medium and untreated Test item (BT-DMEM+UT-TI) showed a significant increased ALP level by 151.9% and 202.1% at 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 215.4% and 127.4% at 1 and 10 µg/mL, respectively as compared with the untreated test item and DMEM group. Overall, all the experimental test groups showed a significant improved level of ALP at all the tested concentrations.

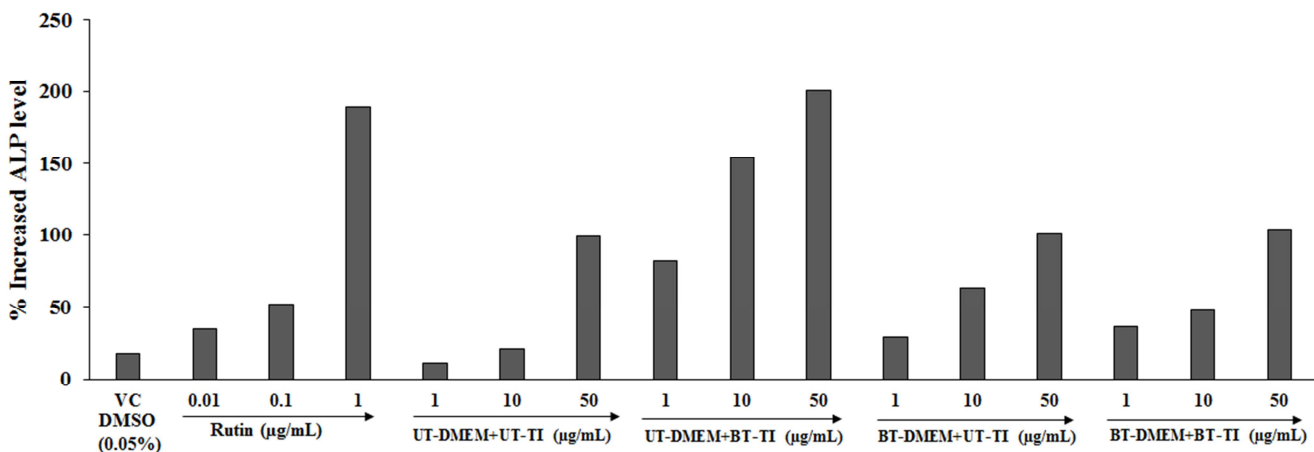


Figure 2. Effect of the Biofield Energy Treated test items on MG-63 cell line for the level of Alkaline Phosphatase (ALP) enzyme activity. VC: Vehicle control (DMSO-0.05%), UT: Untreated; BT: Biofield Treated; TI: Test Item.

ALP enzymes present throughout the body, it is the zinc metalloprotein enzymes that functions to split off a terminal phosphate group from an organic phosphate ester. Variations of ALP depends on where it is located in the body known as isoenzymes. Bone ALP isoenzyme is known as ALP-2, while liver ALP isoenzyme known as ALP-1. During growth phase of bones, or when bone cells are active, increased level of ALP is reported. 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 [35]. In addition, the abnormal level of bone ALP might also be associated with chronic bone and joint pain, bones that are brittle or break easily, and the deformed bones [36]. Vitamin D might improve the ALP level, which is a good predictor of neotissue mineralization that could provide beneficial therapeutic prospects for the treatment of various bone diseases [37]. Hence, The Trivedi

Effect[®]-Energy of Consciousness Healing based vit D₃ and DMEM can be used to improve the ALP concentration in many bone disorders.

3.3. Effect of Test Items on Collagen Synthesis

Biofield Energy Treated vit D₃ and DMEM were studied for change in the collagen level and data showed a significant increase in the collagen level at various experimental tested concentrations. The results in term of % increase in collagen synthesis are presented in Figure 3. The positive control, rutin showed a significant increased value of collagen by 39.6%, 42.8%, and 90.1% at 0.01, 0.1, and 1 µg/mL, respectively. The experimental test group's *viz.* UT-DMEM+BT-TI showed a significant increased collagen

level by 284.6%, 134%, and 183.4% at 1, 10, and 50 µg/mL, respectively while BT-DMEM+UT-TI group showed a significant increased collagen level by 109.6%, 55.7%, and 40.8% at 1, 10, and 50 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 159.6%, 86.6%, and 26.1% at 1, 10, and 50 µg/mL, respectively as compared with the untreated test item and DMEM group. Overall, all the experimental Biofield Energy Treated test item and DMEM groups showed a significant improved level of collagen at all the tested concentrations compared with the untreated group.

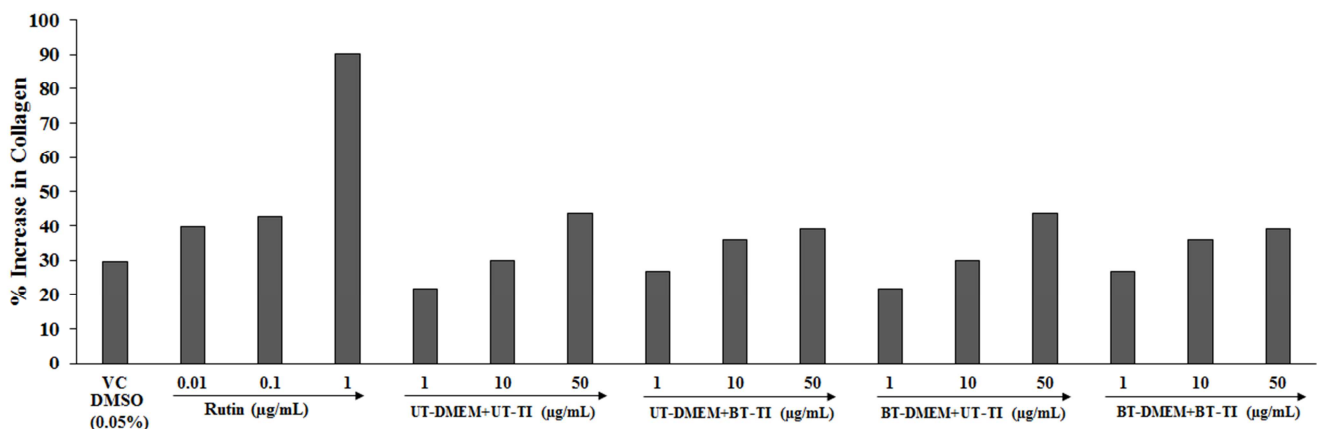


Figure 3. Effect of the test item on MG-63 cell line for collagen level. VC: Vehicle control (DMSO-0.05%), UT: Untreated; BT: Biofield Treated; TI: Test Item.

Collagen is the building block of the body, stimulates cartilage growth, comforts and supports weak joints. However, it was reported that collagen is highly effective as compared with the glucosamine and chondroitin [38]. It directly signifies the bone strength, joints support on the top of beautiful skin. Besides, it gives support and elasticity to the connective tissue, such as cartilage, bones, tendons and ligaments. It also provides the significant support for the growth and repair of most of the cartilage tissue, as well as relieve joint inflammation and pain. In addition, type II collagen, mainly present in the joints, which are areas of the body that provide more prone to wear and tear, microarchitecture of the trabecular bones, and its turnover [39]. Thus, it can be concluded that Biofield Energy (The Trivedi Effect[®]) Treated vit D₃ and DMEM would be an important source to improve the level of collagen and its mineralization process against different orthopedic diseases.

3.4. Effect of Test Items on Bone Mineralization

The effect of the Biofield Energy Treated vit D₃ and DMEM were studied on bone mineralization and data

showed a significant increase in the percentage bone mineralization process at various experimental tested concentrations on MG-63 cell line. The results of bone mineralization among different experimental groups have been presented in Figure 4. The positive control, rutin showed a significant increased value of bone mineralization by 61.7%, 82.2%, and 141.7% 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 120% and 229.4% at 50 and 100 µg/mL, respectively while BT-DMEM+UT-TI group showed a significant increased bone mineralization by 254.7%, 18.7%, and 39.1% at 10, 50, 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 78.1%, 39.6%, and 52.0% at 10, 50, and 100 µg/mL, respectively as compared with the untreated test item and DMEM group. In conclusion, all the experimental Biofield Energy Treated test item and DMEM groups showed a significant improved level of bone mineralization at all the tested concentrations compared with the untreated groups.

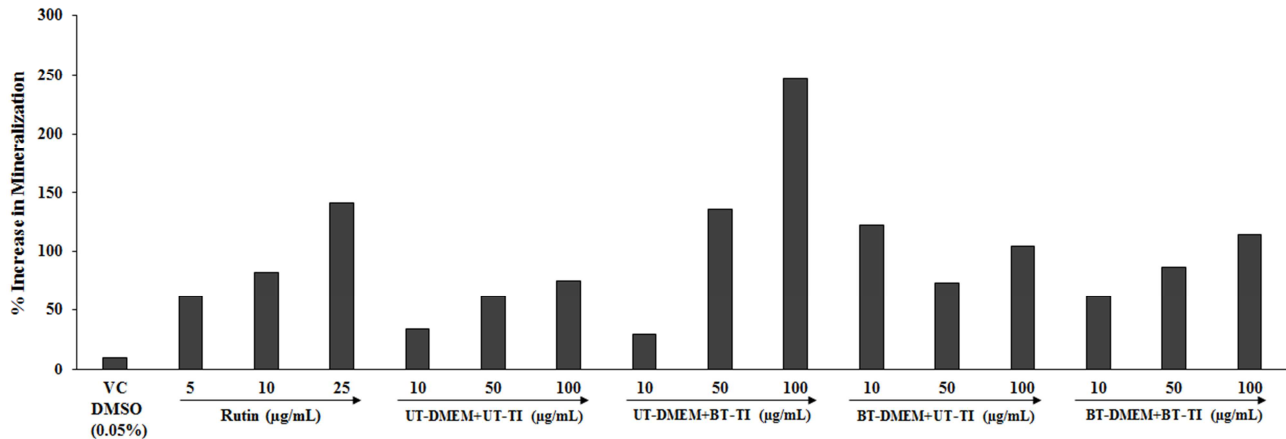


Figure 4. Effect of the test item on MG-63 cell line for bone mineralization. VC: Vehicle control (DMSO-0.05%), UT: Untreated; BT: Biofield Treated; TI: Test Item.

Bone is a complex and dynamic vascular mineralized tissue composed of mineral phase of hydroxyapatite and amorphous calcium phosphate crystals deposited in an organic matrix used for various functions. Calcium and vitamin-D deficiency is a major risk factor for osteoporosis. Moreover, calcium is very essential for fracture-callus mineralization. In osteoporosis patients, there were a progressive decline in bone health with increased fracture risk. Hence, vitamin D3 supplementation would improve the pathological condition of the patients with deficiency in calcium and vitamin D3. Thus calcium and vitamin supplements improve the bone health [40, 41]. Experimentation results showed that the Biofield Energy Treated vit D3 and DEMEM would play a major role in various bone related disorders and have distinct roles during the bone recovery process.

4. Conclusions

In conclusion, overall data suggested that Biofield Energy Healing based vitamin D₃ and DMEM have significant effect on bone health in MG-63 cell line. MTT assay data showed a significant improved cell viability with more than 80% in all the test samples along with improved cell viability as compared with the untreated group. In addition, Biofield Energy Treated test samples showed a significant improved level of ALP by 621.2%, 638.9%, and 102.2% in UT-DMEM+BT-TI at 1, 10, and 50 µg/mL, respectively, while 151.9% and 202.1% at 1 and 10 µg/mL in BT-DMEM+UT-TI group. In addition, the ALP level was increased 215.4% and 127.4% at 1 and 10 µg/mL, respectively in BT-DMEM+BT-TI group as compared with the untreated test item and DMEM group. The level of collagen was significantly increased by 284.6%, 134%, and 183.4% at 1, 10, and 50 µg/mL, respectively in UT-DMEM+BT-TI while 109.6%, 55.7%, and 40.8% increased collagen was reported at 1, 10, and 50, respectively in BT-DMEM+UT-TI group as compared with the untreated test item and DMEM group. In addition, the level of collagen was increased by 159.6%, 86.6%, and 26.1% at 1, 10, and 50 µg/mL, respectively in

BT-DMEM+BT-TI group as compared with the untreated test group. Similarly, the bone mineralization percent was significantly increased by 120% and 229.4% at 50 and 100 µg/mL, respectively in UT-DMEM+BT-TI group, 254.7% and 78.1% in BT-DMEM+UT-TI and BT-DMEM+BT-TI groups, respectively at 10 µg/mL as compared with the untreated test 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, calcium 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.* low bone density and osteoporosis, osteogenesis imperfecta, Paget's disease of bone, rickets, osteomalacia, bone and/or joint pain, increased frequency of fractures, deformed bones, osteoma, chondrodystrophia fetalis, 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-arthritis, anti-osteoporosis, anti-stress, anti-aging, anti-apoptotic, wound healing, anti-cancer, anti-psychotic and anti-fibrotic roles. It also influence cell-to-cell communication, normal cell growth, cell differentiation, cell cycling and proliferation, hormonal balance, neurotransmission, 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 Asthma, Ulcerative Colitis, Alzheimer's Disease, Atherosclerosis, Dermatitis, Diverticulitis, Dermatomyositis, Graves' Disease, Hashimoto Thyroiditis, Multiple Sclerosis, Myasthenia Gravis, Pernicious Anemia, Aplastic Anemia, Sjogren Syndrome, Systemic Lupus Erythematosus, Diabetes, Hepatitis, Irritable Bowel Syndrome, Parkinson's Disease, stress etc. with a safe therapeutic index to improve overall health and quality of life.

Abbreviations

MG-63: Human Bone Osteosarcoma Cells, ALP: Alkaline phosphatase, CAM: Complementary and Alternative Medicine, NCCAM: National Center for Complementary and Alternative Medicine, DMEM: Dulbecco's modified eagle's medium, FBS: Fetal bovine serum, UT: Untreated, BT: Biofield Energy Treated, TI: Test Item; FBS: Fetal bovine serum; EDTA: Ethylene diamine tetra acetic acid.

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