

Influence of the Biofield Energy Treated Vitamin D₃ on Human Osteoblast-Like Cells

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Abstract: The quality of life after rapid bone loss among individuals is one of the key health issues today, which might be due to hormonal factors and lack of proper nutrition that lead to an increased risk of fractures. The experimental data suggested a significant potential of Consciousness Energy Healing based vitamin D₃ and DMEM medium on various bone health parameters. Vitamin D₃ and DMEM medium as test items (TI), which were divided into two parts. Each sample received the Consciousness Energy Healing Treatment by Shirley Theresa Holmlund 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 measured. The cell viability (>92%) using MTT assay showed that the test items were found to be safe. ALP level was significantly increased by 137.8%, 126.2%, and 102.5% in the UT-DMEM+BT-TI, BT-DMEM+UT-TI, and BT-DMEM+BT-TI groups, respectively at 100µg/mL. Collagen content was significantly increased by 141.5% and 307.3% in the UT-DMEM+BT-TI and BT-DMEM+BT-TI group, respectively at 1µg/mL as compared with the untreated group. Moreover, the percent of bone mineralization was significantly increased by 293.7% and 76.3% at 0.1 and 1µg/mL, respectively in UT-DMEM+BT-TI, while 122.9% (at 1µg/mL) and 165.1% (at 0.1µg/mL) in BT-DMEM+UT-TI and BT-DMEM+BT-TI groups, respectively as compared with the untreated group. In conclusion, the Biofield Energy Treated vitamin D₃ and DMEM would play an important role in the promotion and maintenance of strong and healthy bones, which improve quality of life. Biofield Energy Treatment might also control the osteoblast function, improves bone mineralization, and calcium absorption in many bone disorders (such as 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), along with wide range of adverse health conditions, comprising cancer and certain autoimmune diseases.

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-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, 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, Rolfing 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 TCI, Japan, while vitamin D₃ (denoted as test item) and L-ascorbic acid were obtained from Sigma-Aldrich, USA. Fetal bovine serum

(FBS) and Dulbecco's Modified Eagle's Medium (DMEM) were purchased from Life Technology, USA. 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 and was defined as the untreated samples. This Biofield Energy Healing Treatment was provided by Shirley Theresa Holmlund 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. Shirley Theresa Holmlund 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):

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

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*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):

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

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):

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

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):

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

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

The test samples viz. Biofield Energy Treated vitamin D₃ and DMEM were tested for cell viability using MTT assay in MG-63 cells. It is one of the primary method to evaluate and screen any test items for its cell growth, proliferation, and morphological effects. The experimental results showed that both the test samples in combination at various concentrations were found to have significant cell viability with more than 92% among the experimental test groups. The cell viability due to various tested samples in terms of percentage are presented graphically 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 100µg/mL against the tested MG-63 cells. These concentrations are used to study the bone health parameters such as on the levels of alkaline phosphatase (ALP) activity, collagen synthesis, and bone mineralization in MG-63 cells.

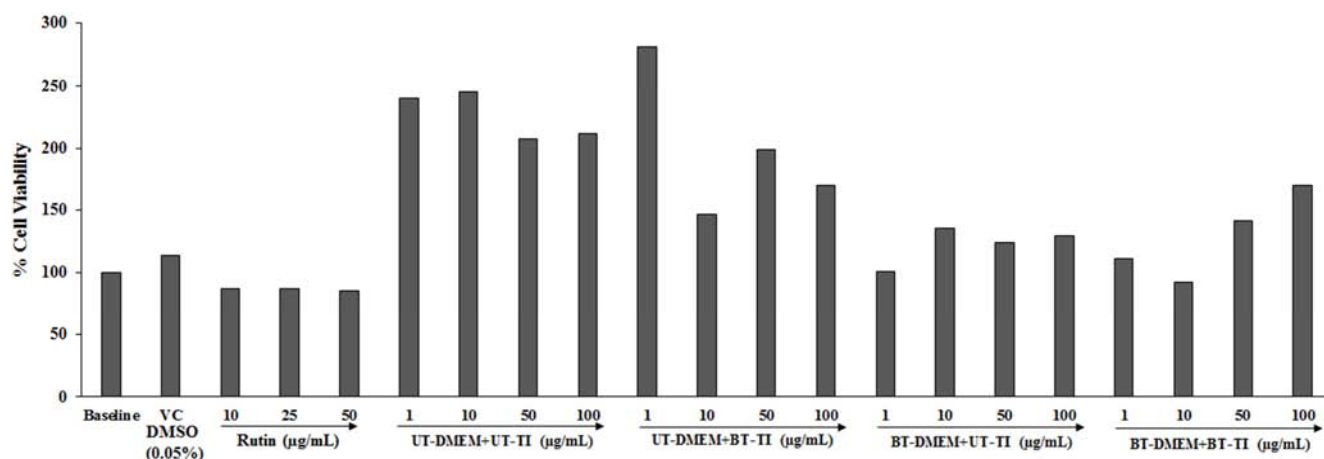


Figure 1. Cell viability study using MTT assays on MG-63 cell line after 72 hours. VC: Vehicle control (DMSO-0.05%), UT: Untreated; BT: Biofield Treated; TI: Test Item.

3.2. Study of Alkaline Phosphatase (ALP) Enzyme Activity

Biofield Energy Treated test item and DMEM were tested for ALP concentrations at various concentrations on MG-63 cell line (Figure 2). Data showed that the vehicle control group showed 5.7% increased level of ALP as compared with the baseline untreated cells group. The positive control, rutin showed a significant increased value increased values of ALP 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 21.7%, 65.9%, and 137.8% at 10, 50, and 100 µg/mL, respectively while Biofield Treated medium and untreated Test item (BT-DMEM+UT-TI) showed a significant increased ALP level by 70.4% and 126.2% at 50 and 100 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 56.9% and 102.5% at 50 and 100 µg/mL, respectively as compared with the untreated test item and DMEM group.

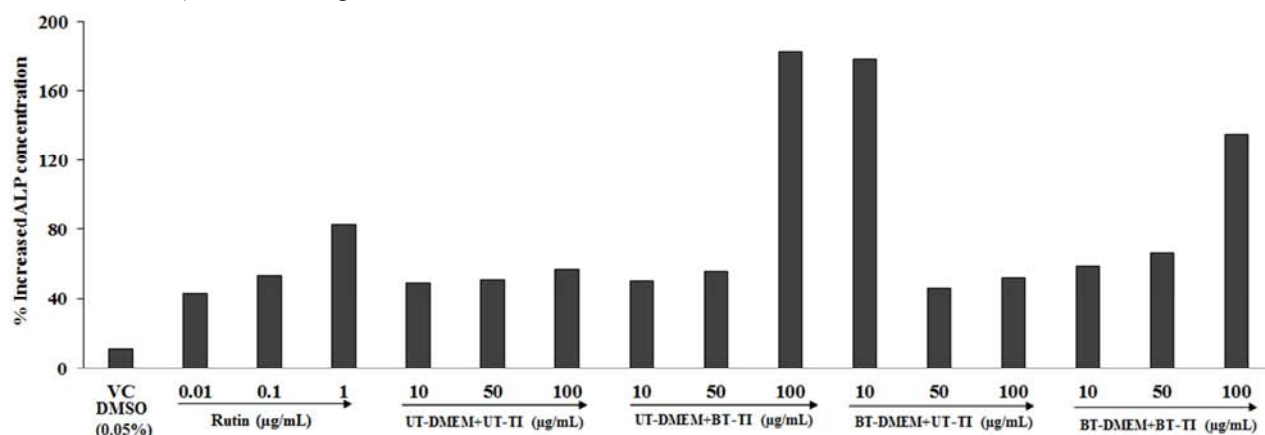


Figure 2. Effect of the Biofield Energy Treated test items on MG-63 cell line for the level of Alkaline Phosphatase (ALP) enzyme activity. VC: Vehicle control (DMSO-0.05%), UT: Untreated; BT: Biofield Treated; TI: Test Item.

ALP is a bone health biomarker present in the liver, bone, intestine, spleen, kidney, and placenta. It help in bone formation and mineralization, which play an important role in bone disorders such as post-menopausal women, osteoporosis, bone cancers, Paget's disease of bone, healing fracture, bone growth, acromegaly, myelofibrosis, osteogenic sarcoma, or bone metastases, leukemia, and rarely myeloma [35, 36]. In order to replenish the ALP level, various natural and synthetic supplements has been used to improve the quality of life. [37]. Overall, all the experimental test group's data showed a significant improved level of ALP at all the tested concentrations. This suggest that Biofield Energy

Treated test samples can be used against various bone related disorders such as osteoporosis, in middle and old-aged, post-and menopausal women [36]. Hence, The Trivedi Effect®-Energy of Consciousness Healing based vit D₃ and DMEM could be used to improve the ALP concentration against many bone disorders.

3.3. Estimation of Collagen Synthesis

Biofield Energy Treated vit D₃ and DMEM was tested for the level of collagen at various concentrations, and the data suggested significant increased collagen level. The experimental results in term of% increase in collagen

synthesis with respect to the untreated cells are presented in Figure 3. The rutin hydrate showed a significant increased value of collagen by 33.8%, 45.5%, and 54.5% at 0.01, 0.1, and 1 μ g/mL, respectively. Besides, the experimental test groups such as UT-DMEM+BT-TI showed a significant increased collagen level by 141.5%, 56.5%, and 38.9% at 1, 10, and 50 μ g/mL, respectively while BT-DMEM+UT-TI

group showed a significant increased collagen level by 22% and 8.6% at 10 and 50 μ g/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 307.3%, 80.1%, and 76.7% at 1, 10, and 50 μ g/mL, respectively as compared with the untreated test item and DMEM group.

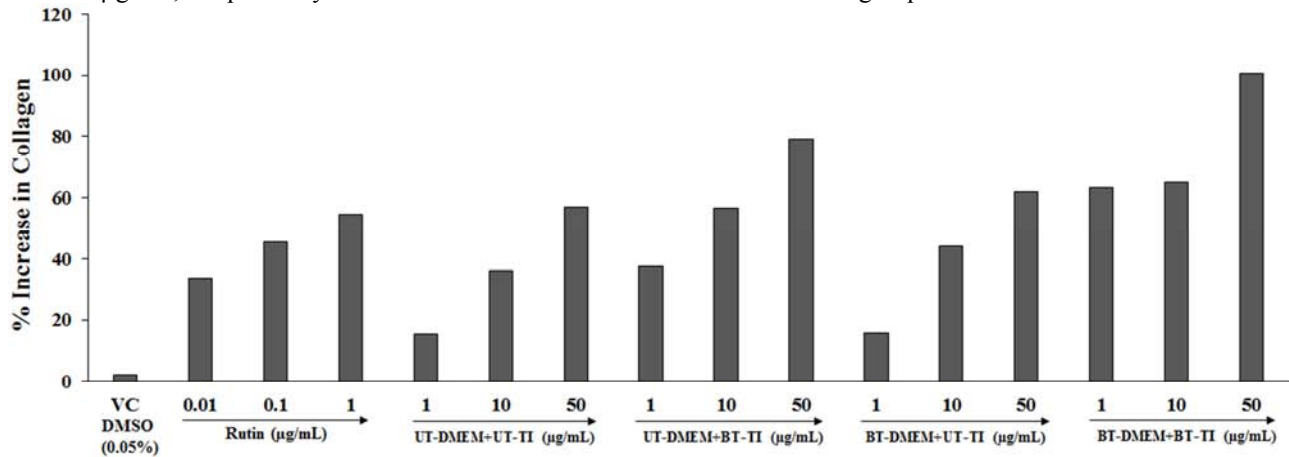


Figure 3. Effect of the test item on MG-63 cell line for collagen level. VC: Vehicle control (DMSO-0.05%), UT: Untreated; BT: Biofield Treated; TI: Test Item.

Collagen is found in muscles, bones, skin, blood vessels, digestive system and tendons. It is one of the important factor for the development of bone mass and muscle tissues. Collagen acts as glue to our joints and tendons that helps to hold the body together. Collagen production would be degenerated with aging process and results in sagging skin and joint pains due to weaker or decreased cartilage. Lack of proper nutritional factors such as high sugar, smoking and high amounts of sun exposure can contribute to depleting collagen levels. Thus, it would lead to collagen-related diseases that might be due to the combination of either genetic defects, poor intake of collagen-rich foods, nutritional deficiencies and digestive problems affecting production or collagen synthesis. However, rich diet, supplements, vitamin supplements, nutritional factors along with physical activity and exercises would improve the bone mass during growth period with improved collagen level [38, 39]. The present experiment suggested that the Biofield Energy Treated vit D₃ and DMEM groups showed a significant improved level of collagen compared with the untreated group. Biofield Energy Treated vit D₃ (The Trivedi Effect[®]) would be used to improve the collagen level and other macromolecules for bone health. Biofield Energy Treated vit D₃ would be very useful to reduce inflammation, decrease aging process, repair of cartilage, control bone loss, and elasticity of joints against different orthopedic diseases.

3.4. Study of Bone Mineralization

The data of Biofield Energy Treated vit D₃ and DMEM results suggest that the significant improved effect on bone mineralization and calcium synthesis with respect to the percentage of bone mineralization on MG-63 cell line. The

results in term of percentage change with respect to untreated cells of bone mineralization among different experimental groups are presented in Figure 4. The positive control, rutin group showed a significant increased value of bone mineralization by 49.5%, 66%, and 126.4% at 5, 10, and 25 μ g/mL, respectively. The experimental data among test group's viz. UT-DMEM+BT-TI showed a significant increased bone mineralization by 293.7%, 76.3%, and 36.5% at 0.1, 1, 10 μ g/mL, while BT-DMEM+UT-TI group showed a significantly increased bone mineralization by 4.8%, 122.9%, and 20.8% at 0.1, 1, and 10 μ g/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 165.1% at 0.1 μ g/mL as compared with the untreated test item and DMEM group.

Vitamin D, a secosteroid hormone reported to be deficient in most of the population worldwide. Lack of sufficient vitamin D₃ and calcium results in rickets in children and osteomalacia in adults, loss of bone mass, decreased bone mineralization capacity, and poor calcium absorption, which leads to decreased bone mineral density (BMD) and various structural abnormalities. Vitamin D₃ and calcium supplements are the preferred choice of treatment in osteoporosis or other bone diseases [40, 41]. Thus, vitamin D and its metabolites have direct effect on bone health and increased bone mineralization. The experimental data of the test groups such as 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. Biofield Energy Treated vit D₃ and DEMEM would play a vital role in various bone related disorders and have distinct roles during the bone recovery process and initiation of mineral formation.

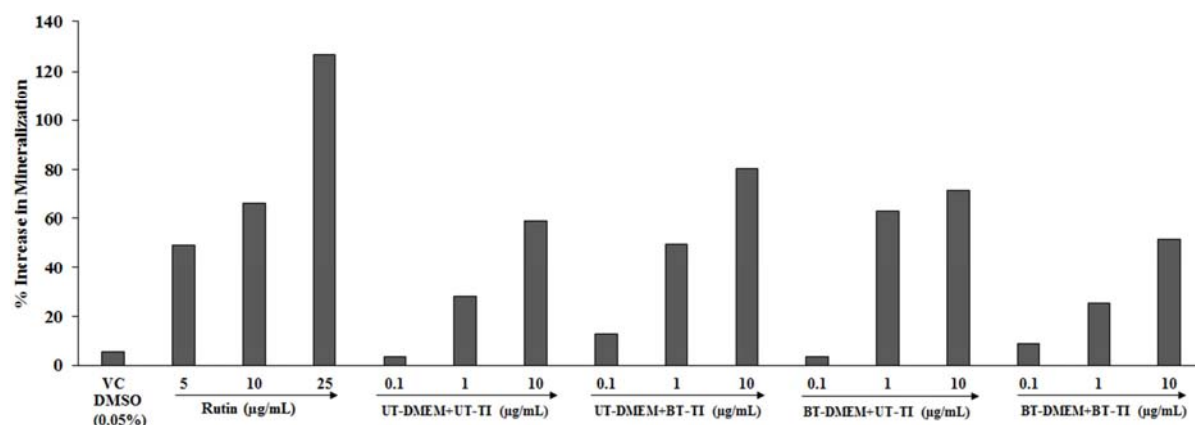


Figure 4. Effect of the test item on MG-63 cell line for bone mineralization. VC: Vehicle control (DMSO-0.05%), UT: Untreated; BT: Biofield Treated; TI: Test Item.

4. Conclusions

Based on the study outcomes, test samples using MTT assay was assessed for cell viability and data showed more than 92% among the tested groups, which suggest significant cell growth, proliferation, and morphological effects. ALP data showed that the Biofield Energy Treated test samples by 65.9%, and 137.8% at 50 and 100µg/mL, respectively in UT-DMEM+BT-TI, while BT-DMEM+UT-TI group showed a significant increased ALP level by 70.4% and 126.2% at 50 and 100µg/mL, respectively. In addition, BT-DMEM+BT-TI group showed a significant increased ALP level by 56.9% and 102.5% at 50 and 100µg/mL, respectively as compared with the untreated group. Collagen level was significantly increased by 141.5%, 56.5%, and 38.9% at 1, 10, and 50µg/mL, respectively in UT-DMEM+BT-TI, while 307.3%, 80.1%, and 76.7% at 1, 10, and 50µg/mL, respectively in BT-DMEM+BT-TI group as compared with the untreated group. Similarly, the bone mineralization percent was significantly increased by 293.7% and 76.3% at 0.1 and 1µg/mL, respectively in UT-DMEM+BT-TI group, while 122.9% (at 1µg/mL) and 165.1% (at 0.1µg/mL) in BT-DMEM+UT-TI and BT-DMEM+BT-TI groups, respectively 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, 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.* osteoma, rickets, low bone density and osteoporosis, osteomalacia, Paget's disease of bone, bone and/or joint pain, osteogenesis imperfecta, increased frequency of fractures, deformed bones, 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-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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