

Full Length Research

A Prospective Study on Bone Health: Effect of Biofield Energy Treated Vitamin D₃ Using MG-63 Cell Line

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The aim of the present study was to evaluate the significant role of Consciousness Energy Healing based vitamin D₃ and DMEM medium on bone health parameters *in vitro* using MG-63 cells such as alkaline phosphatase enzyme (ALP) activity, collagen levels and bone mineralization. The test items (TI) i.e. vitamin D₃ and DMEM medium were divided into two parts. The test samples received Consciousness Energy Healing Treatment by Laura Nelson Streicher and samples were defined as the Biofield Energy Treated (BT) samples, while the other parts of each sample were denoted as the untreated test items (UT). MTT assay for cell viability showed that increased range 76% to 139% cell viability with safe and nontoxic profile of test samples on MG-63 cell line. ALP was significantly increased by 232.7% (1 µg/mL), 156.4% (100 µg/mL) and 262.5% (100 µg/mL) in the UT-DMEM+BT-TI, BT-DMEM+UT-TI, and BT-DMEM+BT-TI groups, respectively as compared with the untreated group. The level of collagen was significantly increased by 145.8% and 151.6% at 10 and 50 µg/mL, respectively in the UT-DMEM+BT-TI group, while 177.1%, 235.6%, and 165.3% at 1, 10, and 50 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 collagen level by 252.1% at 1 µg/mL as compared with the untreated test item and DMEM group. The percent of bone mineralization was significantly increased by 92.8%, 65.8%, and 32.2% at 0.1, 1, and 10 µg/mL, respectively in the UT-DMEM+BT-TI group, while 76.8%, 132.5%, and 7.6% at 0.1, 1, and 10 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 92.8%, 38.6%, and 12.3% at 0.1, 1, and 10 µg/mL, respectively as compared with the untreated group. Biofield Energy Treatment might be vital in promotion and maintenance of strong and healthy bones and quality of life by assisting them in maintaining optimal vitamin D levels. It regulates the osteoblast function, improves bone mineralization, and calcium absorption in wide range of bone disorders along with wide range of adverse health conditions, comprising cancer and certain autoimmune diseases.

Keywords: Biofield Energy, Bone strength, Osteosarcoma Cells, Vitamin D, Bone Mineralization

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. (Holick, 1996). 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 (Holick, 1996). 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) (van Leeuwen et al. 2001). 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 (Bikle, 2012). Vitamin D insufficiency and deficiency is the major health problem, which causes metabolic bone disease in the young and elderly populations (Lips, 2001). 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 (Hossein-nezhad and Holick, 2013).

Various *in vitro* studies have readily established 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 (Czekanska et al. 2012). Hence, MG-63 cell line is widely used for studying the potential of any test compounds to improve the bone health (Luo and Liao, 2003). 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 (Iba et al. 2004). 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 arrays of an organic matrix known as Osteoid (Viguet-Carrin et al. 2006). Likewise, calcium phosphate is deposited in the Osteoid and gets mineralized (combination of calcium phosphate and hydroxyapatite) and provides rigidity to the bone (Bhattarai, 2014). 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.

CAM therapies have been increased worldwide and 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* (Rubik, 2002). Biofield Energy Healing Treatment (The Trivedi Effect[®]) contains a putative bioenergy, which is channeled by a renowned practitioner from a distance. Biofield Energy Healing as a CAM showed a significant results in biological studies (Barnes et al. 2008). However, the National Center for Complementary and Alternative Medicine (NCCAM), well-defined Biofield therapies in the subcategory of Energy Therapies (Frass et al. 2012). The Trivedi Effect[®]- Consciousness Energy Healing Treatment has been reported with significant revolution in the physicochemical properties of metals, chemicals, ceramics and polymers (Trivedi and Tallapragada, 2008;

Trivedi et al. 2015a, b), improved agricultural crop yield, productivity, and quality (Trivedi et al. 2015c, d), transformed antimicrobial characteristics (Trivedi et al. 2015e, f), bone health (Ansari et al. 2018; Koster et al. 2018), biotechnology (Nayak and Altekar, 2015), improved bioavailability (Branton and Jana, 2017a, b, c), skin health (Kinney et al. 2017; Singh et al. 2017), nutraceuticals (Trivedi et al. 2017a, b), cancer research (Trivedi et al. 2015g, h), 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.

MATERIAL AND METHODS

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.

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, 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 (Czekanska et al. 2012).

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.

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 Laura Nelson Streicher 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. Laura Nelson Streicher 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.

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 microplate reader, BioTek, USA (Riss et al. 2013). The percentage cytotoxicity at each tested concentrations of the test substance were calculated using the following equation (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):

% Cell Viability = 100 - % Cytotoxicity.....(2)

The concentrations exhibiting $\geq 70\%$ cell viability was considered as non-cytotoxic.

Assessment of Alkaline Phosphatase (ALP) Activity

The cells were counted using 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 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 microplate reader (Biotek, USA). The absorbance values obtained were normalized with substrate blank (*p*NPP solution alone) absorbance values (Czekanska, 2012). The percentage increase in ALP enzyme activity with respect to the untreated cells (baseline group) was calculated using equation (3):

Where, X = Absorbance of cells corresponding to positive control and test groups

R = Absorbance of cells corresponding to baseline group (untreated cells)

Assessment of Collagen Synthesis

The MG-63 cells were counted using 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 microplate reader. The level of collagen was extrapolated using standard curve obtained from purified Calf Collagen Bornstein and Traub Type I (Sigma Type III) (Czekanska, 2012). The percentage increase in collagen level with respect to the untreated cells (baseline group) was calculated using equation (4):

Where, X = Collagen levels in positive control and test groups

R = Collagen levels in cells corresponding to baseline group (untreated cells)

Assessment of Bone Mineralization by Alizarin Red S Staining

The MG-63 cells were counted using 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 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 Bioteck Synergy HT microplate reader (Czekanska, 2012). The percentage increase in bone mineralization with respect to the untreated cells (baseline group) was calculated using the following equation (5):

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

Where, X = Absorbance in cells corresponding to positive control or test groups; R = Absorbance in cells corresponding to baseline (untreated) group.

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 $p \leq 0.05$.

RESULTS AND DISCUSSION

Cell Viability: MTT Assay

The graphical results of cell viability data in terms of percentage of the Biofield Energy Treated vitamin D₃ and DMEM using MTT assay in MG-63 cells are shown in Figure 1. The data was compared with the untreated group and effect of Biofield Energy Treated groups was presented in terms of percentage cell viability. The results of percentage cell viability in all the tested cell lines showed the cell viability range of 90% to 219% in different test item groups with DMEM, while for rutin group showed more than 85% cell viability. The cell viability using MTT data suggested that the test items were found safe with maximum concentration up to 100 µg/mL against the tested MG-63 cells, which were used for the estimation of other bone health parameters such as ALP, collagen and bone mineralization.

Alkaline Phosphatase (ALP) Enzyme Activity

Bone-specific alkaline phosphatase (BAP) is a glycoprotein found on osteoblasts cell surfaces. ALP controls the bone formation metabolic processes such as remodeling, degradation process, bone resorption, bone building process, etc. using osteoclast and osteoblast cells. BAP, vitamin D, calcium, and other nutritional factors are required for bone remodeling in

order for maintenance and overall bone health (Masrour and Roudsari, 2012; Mahjoub Kubo et al. 2012; Deftos et al. 1991). The experimental results of ALP in test samples suggest significant improved level of ALP in terms of percentage values. The data at various concentrations in different groups were presented in Figure 2. The vehicle control group showed 4.1% increased level of ALP as compared with the untreated cells group. The positive control, rutin showed a significant increased value by 43.44%, 53.55%, and 83.33% 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 increased level of ALP by 232.7%, 82.5%, and 34.1% at 1, 10, and 100 µg/mL, respectively while Biofield Treated medium and untreated Test item (BT-DMEM+UT-TI) showed a significant increased ALP level by 156.4% at 100 µg/mL 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 262.5% at 100 µg/mL as compared with the untreated test item and DMEM group. Overall, the experimental results showed significant increased level of the ALP, which could be highly useful in various bone related disorders such as Paget's disease of bone, healing fracture, bone growth, acromegaly, myelofibrosis, osteogenic sarcoma, or bone metastases, leukemia, and rarely myeloma (Kubo et al. 2012). Thus, The Trivedi Effect®-Energy of Consciousness Healing based vit D₃ and DMEM could be the best alternative to improve the ALP concentration.

Estimation of Collagen Synthesis

Bone is a very complex tissue and is very important to resist mechanical forces and fractures. Collagen, one of the major insoluble fibrous protein play a vital role in bone strength and growth. However, it was reported that the percentage of collagen and its other nutritional factors such as vitamin D and calcium reflects the strength of bone and joints. In addition, some other factors such as age, pathological condition, genetic makeup, etc. would affect the collagen synthesis that would weaken the joints, tendons, and ligaments. Collagen type I is shown to have significant effect on bone health, which is the most abundant matrix protein (Paschalis et al. 2003; Bringhurst and Potts, 1982). The test results showed that collagen has significant effect after Biofield Treatment in vit D₃. All the collagen results are presented as % values and was compared with the untreated cells in Figure 3. The rutin hydrate showed a significant increased value of collagen by 33.82%, 45.48%, and 54.52% at 0.01, 0.1, and 1 µg/mL,

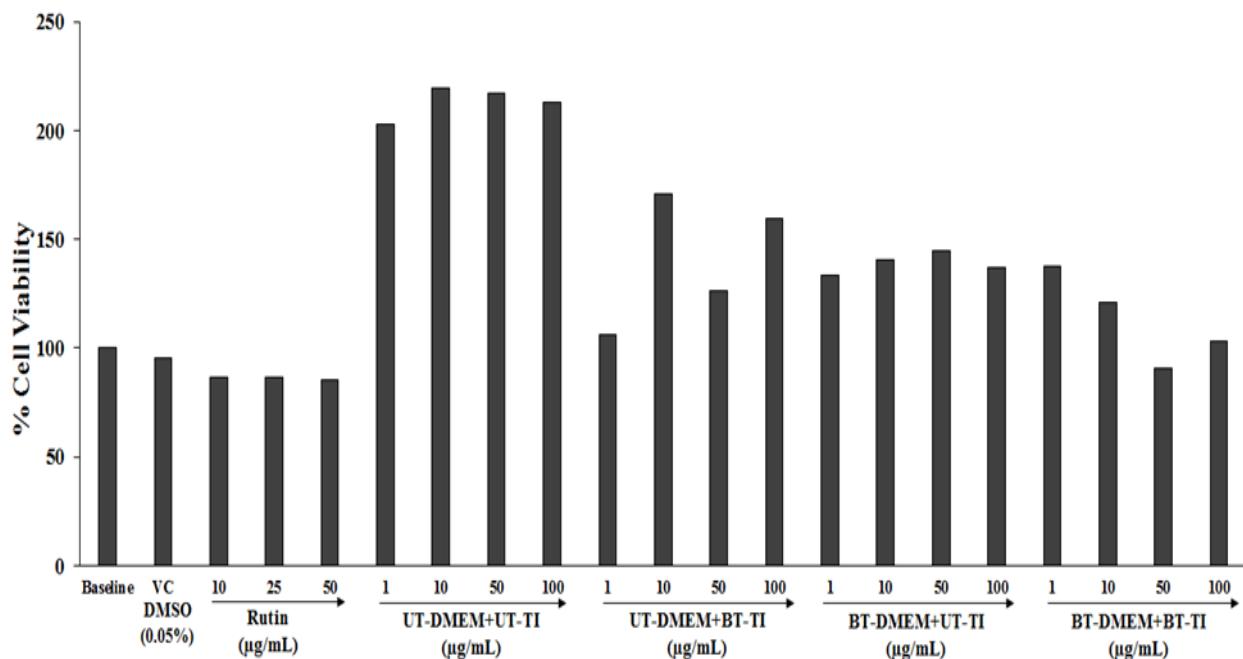


Figure 1: Cell viability using MTT assays of the test items on MG-63 cell line after 72 hours. VC: Vehicle control (DMSO-0.05%), UT: Untreated; BT: Biofield Treated; TI: Test Item.

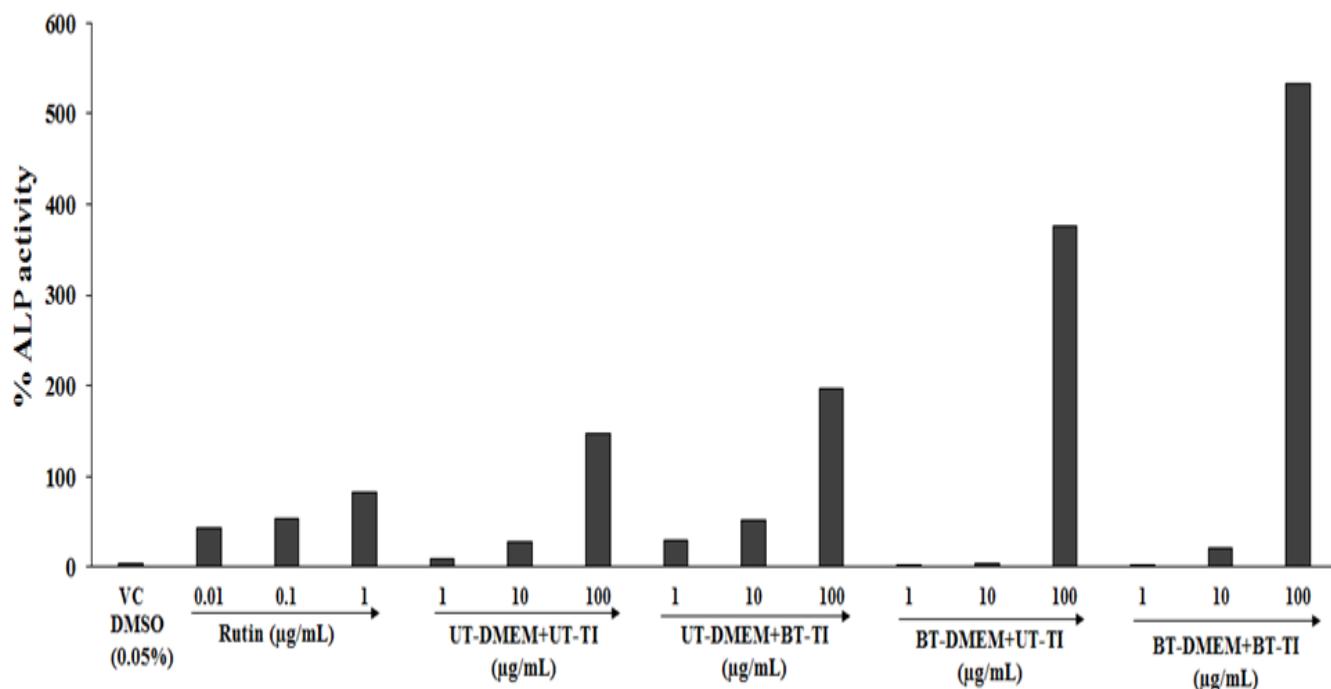


Figure 2. Study of Alkaline Phosphatase (ALP) enzyme activity of the Biofield Energy Treated test items on MG-63 cell line. VC: Vehicle control (DMSO-0.05%), UT: Untreated; BT: Biofield Treated; TI: Test Item.

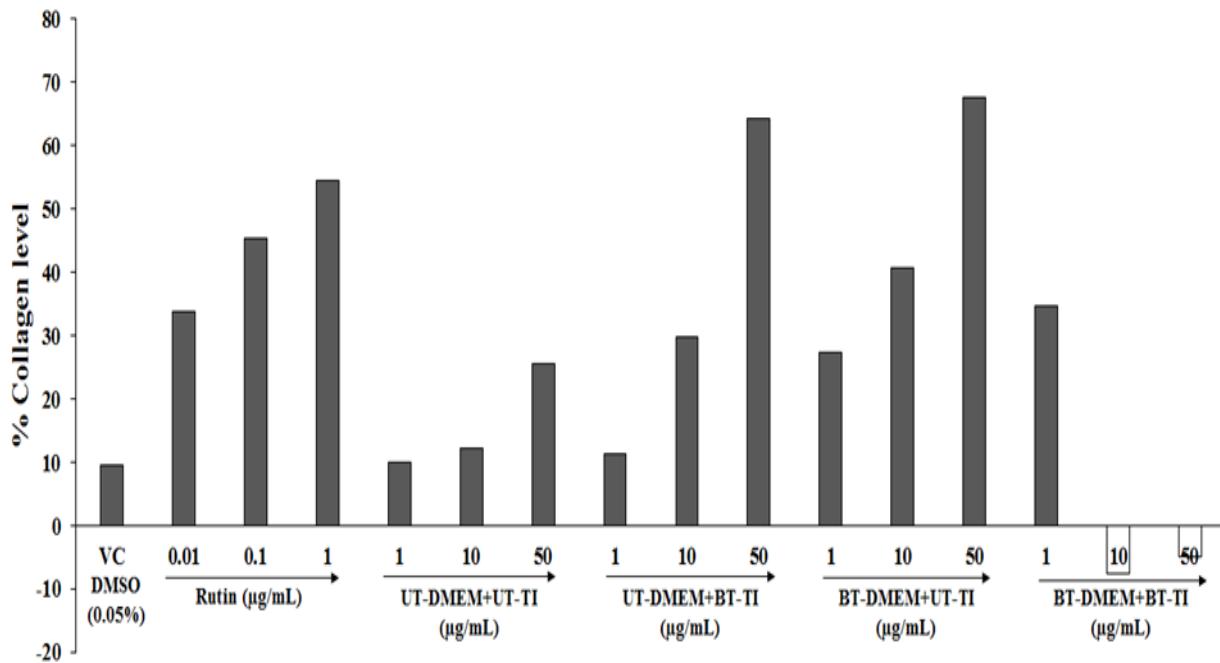


Figure 3: Action of the test item on MG-63 cell line for collagen level. VC: Vehicle control (DMSO-0.05%), UT: Untreated; BT: Biofield Treated; TI: Test Item.

respectively. Besides, the experimental test groups such as UT-DMEM+BT-TI showed a significant increased collagen level by 14.6%, 145.8%, and 151.6% at 1, 10, and 50 µg/mL, respectively while BT-DMEM+UT-TI group showed a significant increased collagen level by 177.1%, 235.6%, and 165.3% 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 252.1% at 1 µg/mL as compared with the untreated test item and DMEM group. Thus, collagen synthesis was significantly improved after Biofield Energy Treatment. This approach would fight against reduced collagen synthesis and work effectively against serious bone diseases such as the type of bone loss experienced in osteoporosis (Bringhurst and Potts, 1982). Thus, The Trivedi Effect® Biofield Energy Treated vit D₃ and DMEM groups showed a significant improved level of collagen compared with the untreated group.

Bone Mineralization

Bone mineralization process involve precipitation and laying down minerals in bone matrix, which were approximately 50 to 70% mineral, 20 to 40% organic matrix, 5 to 10% water, and less than 3% lipids. Besides, the chief minerals in bone matrix are calcium and phosphorus with small amount of carbonate and magnesium. Bone mineralization would control various bone diseases as it control bone mass and improve bone mineral density (BMD) (Ruppel et al. 2008; van Driel and van Leeuwen, 2017). The results of bone mineralization experiment on MG-63 cell line suggested that the Biofield Energy Treated vit D₃ and DMEM groups showed a significant improved bone mineralization. All the results in term of percentage are presented in term of percentage change of bone mineralization among different experimental groups in Figure 4. The positive control, rutin group showed a significant increased value of bone mineralization by 68.9%, 84.6%, and 134.5% 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 92.8%, 65.8%, and 32.2% at 0.1, 1, and 10 µg/mL, respectively while BT-DMEM+UT-TI group showed a significantly increased bone

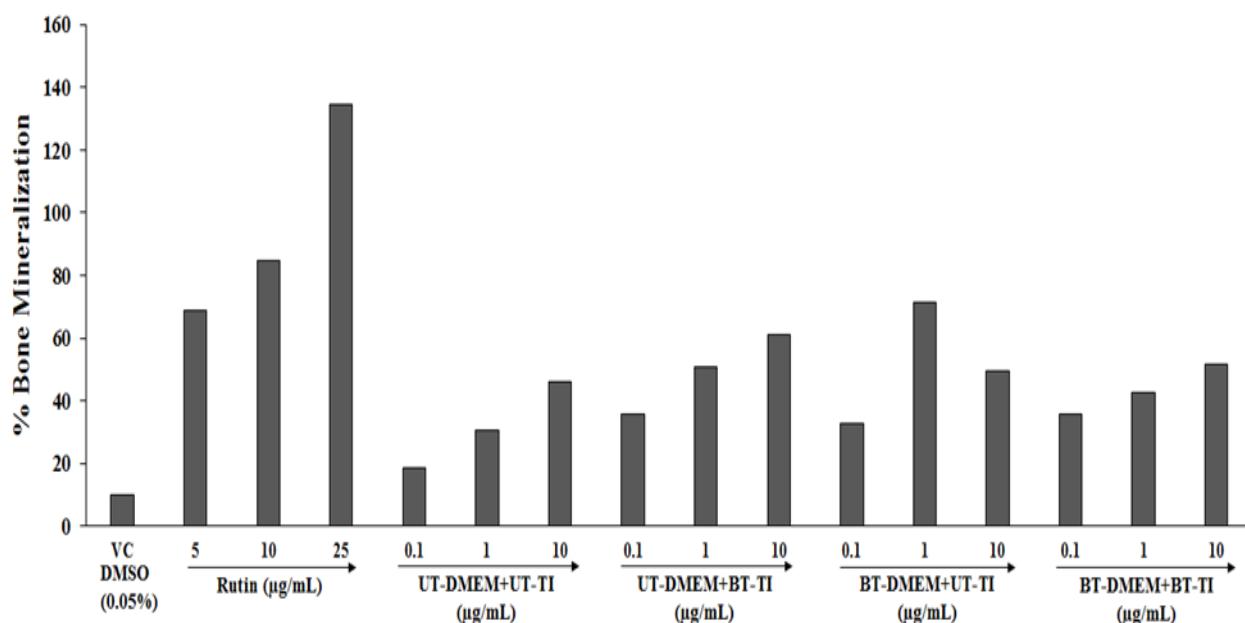


Figure 4: Consequence of the test item on MG-63 cell line for bone mineralization. VC: Vehicle control (DMSO-0.05%), UT: Untreated; BT: Biofield Treated; TI: Test Item.

mineralization by 76.8%, 132.5%, and 7.6% at 0.1, 1, and 10 $\mu\text{g/mL}$, respectively as compared with the untreated test item and DMEM group. However, BT-DMEM+BT-TI group showed a significant increased bone mineralization by 92.8%, 38.6%, and 12.3% at 0.1, 1, and 10 $\mu\text{g/mL}$, respectively as compared with the untreated test item and DMEM group. The present study results showed a significant improved bone mineralization, which would directly help to improve the bone matrix, bone mass and BMD. Thus, Biofield Energy Treated test samples results to improve various bone disorders with respect to the untreated test samples. The results after Biofield Energy Treatment suggested significant improved bone mineralization, which would improve the bone mass and resolve many bone related disorders.

CONCLUSIONS

Bone health parameters were significantly improved among the Biofield Energy Treated vitamin D₃ test samples in MG-63 cells. Cell viability testing using MTT assay data showed significant improved cell viability with more than 90% among various experimental groups. Other bone health parameters such as the level of ALP was increased by 232.7%, 82.5%, and 34.1% at 1, 10, and 100 $\mu\text{g/mL}$, respectively in the UT-DMEM+BT-TI, while 156.4% at 100 $\mu\text{g/mL}$ 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 an increased ALP level by 262.5% at 100 $\mu\text{g/mL}$. The level of collagen was significantly increased by 14.6%, 145.8%, and 151.6% at 1, 10, and 50 $\mu\text{g/mL}$, respectively in the UT-DMEM+BT-TI, while 177.1%, 235.6%, and 165.3% at 1, 10, and 50 $\mu\text{g/mL}$, respectively in the BT-DMEM+UT-TI group. The level of collagen was increased by 252.1% at 1 $\mu\text{g/mL}$ in BT-DMEM+BT-TI group as compared with the untreated test item and DMEM group. Similarly, the bone mineralization percent was significantly increased by 92.8%, 65.8%, and 32.2% at 0.1, 1, and 10 $\mu\text{g/mL}$, respectively in the UT-DMEM+BT-TI group, while 6.8%, 132.5%, and 7.6% at 0.1, 1, and 10 $\mu\text{g/mL}$, 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 92.8%, 38.6%, and 12.3% at 0.1, 1, and 10 $\mu\text{g/mL}$, 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 is 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 influences the 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 Multiple Sclerosis, Aplastic Anemia, Asthma, Hepatitis, Ulcerative Colitis, Alzheimer's Disease, Dermatomyositis, Dermatitis, Parkinson's Disease, Irritable Bowel Syndrome, Pernicious Anemia, Sjogren Syndrome, Graves' Disease, Diabetes, Myasthenia Gravis, and Atherosclerosis.

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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