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Increase in Bone Mass Density and Overall Bone Health Following High-Impact of Biofield Energy Treated Vitamin D3 in MG-63 Cell Line

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Increase in Bone Mass Density and Overall Bone Health Following High-Impact of Biofield Energy Treated Vitamin D₃ in MG-63 Cell Line

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Abstract

Vitamin D₃ and calcium are the main bone health supplements required for quality of life. The present study aimed to find out the role of Consciousness Energy Healing based vitamin D₃ and DMEM medium on bone health parameters *in vitro* using MG-63 cells. The parameters of bone health tested are 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 Krista Joanne Callas 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). Cell viability using MTT assay exhibited increased cell viability range from 72% to 122% with safe and nontoxic profile among test samples on MG-63 cell line. ALP was significantly increased by 129.6%, 392% and 255.7% in the UT-DMEM+BT-TI, BT-DMEM+UT-TI and BT-DMEM+BT-TI groups, respectively at 100µg/mL as compared with the untreated group.

The level of collagen was significantly increased by 35.9% and 235.3% at 10 and 50µg/mL, respectively in the UT-DMEM+BT-TI group, while 81.7% and 197.6% at 50 and 100µg/mL, respectively in 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 184.2% and 82.3% at 50 and 100µg/mL, respectively as compared with the untreated test item and DMEM group. The percent of bone mineralization was significantly increased by 198.6% and 53.5% at 1 and 10µg/mL, respectively in the UT-DMEM+BT-TI group, while 267.1% at 1µg/mL 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 146.2%, 91.4%, and 66.8% at 0.1, 1 and 10µg/mL, respectively as compared with the untreated group. Thus, the study results concluded that Biofield Energy Treatment would be the best alternative treatment for the maintenance of strong and healthy bones and quality of life. Further, it regulates the osteoblast function and improved the level of collagen, ALP, and calcium absorption in wide range of bone disorders along with wide range of adverse bone health conditions.

Keywords: Biofield healing; Bone health; Osteosarcoma cells; Vitamin D; Bone mineralization

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; EDTA: Ethylenediaminetetraacetic Acid; UT: Untreated, BT: Biofield Energy Treated; TI: Test Item

Introduction

Vitamin D has multiple effects which regulate the function of 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 plays 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, Jorgen 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 1000-1500mg/day along with vitamin D supplement around 400IU/day is very important for maintaining the good bone health [5].

In vitro studies have readily established the role of bone health using cell lines and its resorbing effects using three important key biomarkers, such 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 array of an organic matrix known as Osteoid [9]. Likewise, calcium phosphate is deposited in the Osteoid and gets mineralized (combination of calcium phosphate and hydroxyapatite) and provides rigidity to the bone [10]. Thus, these parameters are very essential in order to study the bone health in cell lines. Authors evaluated the *in vitro* effect of the Biofield Energy Treated vitamin D₃ as a test item, a Complementary and Alternative Medicine (CAM) on bone health using MG-63 cell line for major biomarkers.

Within the burgeoning ground of CAM therapies, Biofield Energy Treatment or energy medicine, is emerging with significant benefits in various scientific fields. The effects of the CAM therapies have great potential, which include external qigong, Johrei, Reiki, therapeutic touch, yoga, Qi Gong, polarity therapy, Tai Chi, pranic healing, deep breathing, chiropractic/osteopathic manipulation, guided imagery, meditation, massage, homeopathy, hypnotherapy, progressive relaxation, acupressure, acupuncture, special diets, relaxation techniques, Roling structural integration, healing touch,

movement therapy, pilates, mindfulness, Ayurvedic medicine, traditional Chinese herbs and medicines in biological systems both *in vitro* and *in vivo* [11]. Biofield Energy Healing Treatment (The Trivedi Effect®) contains a putative bio-energy, which is channeled by 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 [19,20], bone health [21,22], biotechnology [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.

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 Ethylenediaminetetraacetic 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 [33].

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 Krista Joanne Callas 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. Krista Joanne Callas 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 5X10³ to 10X10³ cells/well/180µL 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 20µL of 5mg/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 150µL 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 [34]. The percentage cytotoxicity at each tested concentrations of the test substance was calculated using the following equation (1):

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

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):

$$\% \text{Cell Viability} = 100 - \% \text{Cytotoxicity} \text{-----} (2)$$

The concentrations exhibiting ≥70% cell viability was considered as non-cytotoxic.

Assessment of alkaline phosphatase (ALP) activity

The cells were counted using ahemocytometer and plated in a 24-well plate at the density corresponding 1x10⁴ 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 1XPBS 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, 50µL of substrate solution i.e., 5mM of *p*-nitrophenyl phosphate (*p*NPP) in 1M di-ethanolamine and 0.24mM magnesium chloride (MgCl₂) solution (pH10.4) was added to all the wells followed by incubation for 1 hour at 37°C. The absorbance of the above solution was read at 405nm using Synergy HT microplate reader (Biotek, USA). The absorbance values obtained were normalized with substrate blank (*p*NPP solution alone) absorbance values [33]. The percentage increase in ALP enzyme activity with respect to the untreated cells (baseline group) was calculated using equation (3):

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

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 a hemocytometer and plated in 24-well plate at the density corresponding to 10x10³ 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 hour at Room Temperature (RT). After 1 hour of incubation, the above wells were washed with milliQ water and air dried. The cells were then stained with Sirius red dye solution for 1 hour at RT followed by washing in 0.01N HCl to remove unbound dye. The collagen dye complex obtained in the above step was dissolved in 0.1N NaOH and absorbance was read at 540nm 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) [33]. The percentage increase in collagen level with respect to the untreated cells (baseline group) was calculated using equation (4):

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

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

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

Assessment of bone mineralization by alizarin red S staining

The MG-63 cells were counted using a 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 (40µm; 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 solubilised with 10% cetylpyridinium chloride for 15 minutes with shaking. Absorbance was measured at 562nm using Biotek Synergy HT microplate reader [33]. The percentage increase in bone mineralization with respect to the untreated cells (baseline group) was calculated using the following equation (5):

$$\% \text{ Increase} = \frac{X-R}{R} \times 100 \text{----- (5)}$$

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 using MTT assay

The MTT results for cell viability data showed significant improved percentage among the Biofield Energy Treated vitamin D₃ and DMEM in MG-63 cells are shown in Figure 1. The data was compared with the untreated group and effect of the Biofield Energy Treated groups was presented in terms of percentage cell viability. The percentage cell viability in all the tested cell lines showed the cell viability range of 72% to 122% in different test item groups with DMEM, while for rutingroup showed more than 85% cell viability. The cell viability using MTT data suggested that the test samples were found safe with maximum concentration upto 100µg/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.

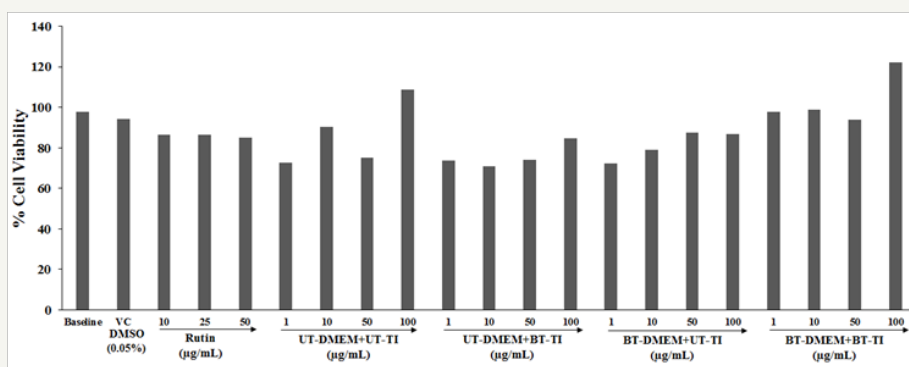


Figure 1: Cell viability using MTT assays of the test items on MG-63 cell line after 72 hours.

VC: Vehicle Control (DMSO-0.05%); UT: Untreated; BT: Biofield Treated; TI: Test Item

Alkaline phosphatase (ALP) enzyme activity

The ALP data in different test groups showed a significant improved level as compared with the untreated group in terms of percentage values. The percentage change in ALP data at various concentrations in different groups were presented in Figure 2. The vehicle control group showed 5.3% 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 1µg/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 21.8% and 129.6% at 50 and 100µg/mL, respectively while Biofield Treated medium and untreated Test item (BT-DMEM+UT-TI) showed a significant increased ALP level by 17.7% and 392% at 10 and 100µg/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 13% and 255.7% at 50 and 100µg/mL, respectively as compared with the untreated test item and DMEM group.

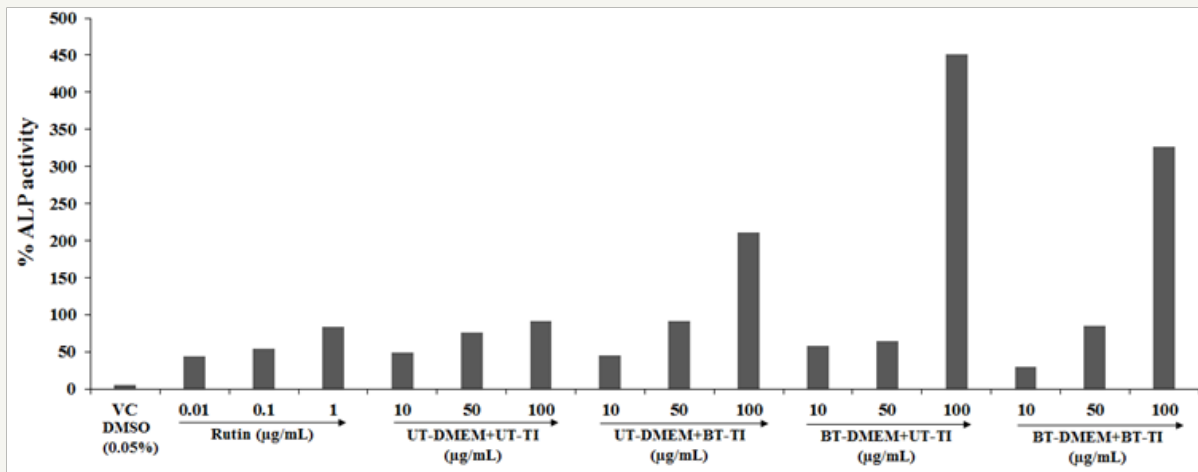


Figure 2: Study of Alkaline Phosphatase (ALP) enzyme activity of the Biofield Energy Treated test items on MG-63 cell line.

VC: Vehicle Control (DMSO-0.05%), UT: Untreated; BT: Biofield Treated; TI: Test Item

Thus, data suggest significant improved ALP, which is a bone specific glycoprotein and have wide applicability in bone health. Bone ALP control various metabolic activities such as bone remodelling, degradation process, bone resorption, bone building process, etc. using osteoclast and osteoblast cells. Different nutritional factors such as vitamins, minerals, etc. are required to maintain bone health [35-37]. Hence, The Trivedi Effect®-Energy of Consciousness Healing based vit D₃ could be used as bone health supplements which would maintain a healthy skeletal structure against many bone disorders such as Paget's disease of bone, healing fracture, bone growth, cessation of bone growth, achondroplasia, acromegaly, myelofibrosis, osteogenic sarcoma, or bone metastases, leukemia, and rarely myeloma [36].

Estimation of Collagen Synthesis: Collagen synthesis data showed a significant effect after Biofield Energy Treatment *in vitro* D₃. The results are presented in percentage 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, respectively. Besides, the experimental test groups such as UT-DMEM+BT-TI showed a significant increased collagen level by 35.9%, 235.3%, and 7% at 10, 50, and 100 µg/mL, respectively while BT-DMEM+UT-TI group showed a significant increased collagen level by 81.7% and 197.6% at 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 collagen level by 184.2% and 82.3% at 50 and 100 µg/mL, respectively as compared with the untreated test item and DMEM group. Thus, experimental data showed significant improved collagen level, which is a complex tissue that helped to resist mechanical forces and fractures. Collagen type I have significant role in bone health, which is the most abundant matrix protein [38,39]. Overall, The Trivedi Effect® Biofield Energy Treated vit D₃ and DMEM can be used as bone health supplements which would maintain a healthy skeletal structure in many patients against various bone disorders to improve collagen level, which play an important role against weaken joints, tendons, and ligaments.

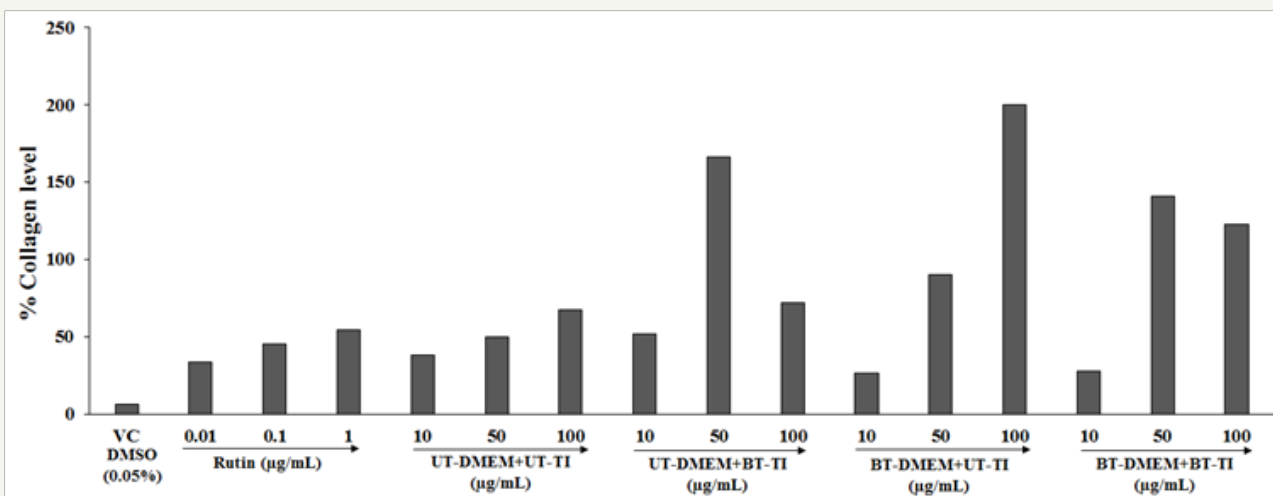


Figure 3: Action of the test item on MG-63 cell line for collagen level.

VC: Vehicle control (DMSO-0.05%), UT: Untreated; BT: Biofield Treated; TI: Test Item

Bone mineralization

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 49.5%, 66%, and 126.46% 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 198.6% and 53.5% at 1 and 10 µg/mL, respectively while BT-DMEM+UT-TI group showed a significantly increased bone mineralization by 11.5% and 267.1% at 0.1, 1 and 10 µg/mL, respectively as compared with the untreated test item and DMEM group. However, BT-DMEM+BT-TI group showed a significant increased bone mineralization by 146.2%, 91.4%, and 66.8% at 0.1, 1, and 10 µg/mL, respectively as compared with the untreated test item and DMEM group. Thus, overall data of bone mineralization suggest a significant improved level that could progress the bone mass, matrix, and bone mass density. The Trivedi Effect®-Biofield Energy Treated vit D₃ and DMEM would increase precipitation of bone minerals, organic matrix, water, and other nutrients such as calcium and phosphorus. This would maintain a healthy skeletal structure in many patients against various bone disorders. Bone mineralization would control various bone diseases as it controls bone mass and improve bone mineral density [40,41].

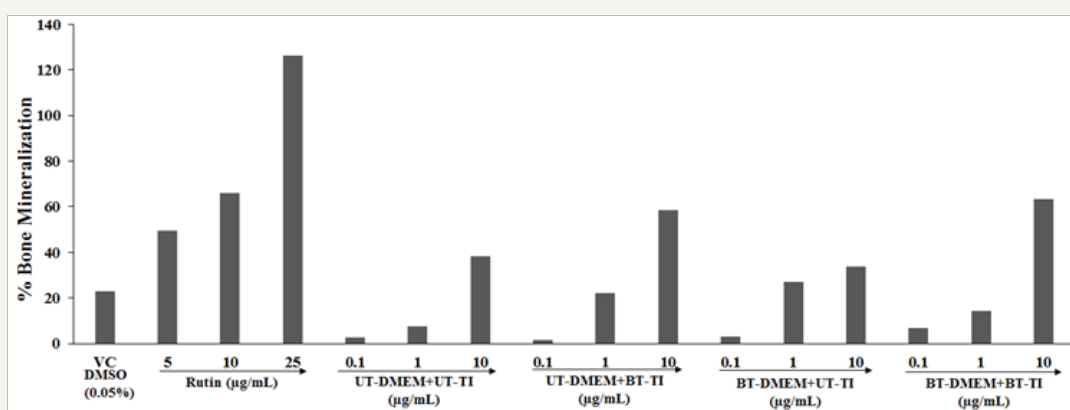


Figure 4: Consequence of the test item on MG-63 cell line for bone mineralization.

VC: Vehicle Control (DMSO-0.05%), UT: Untreated; BT: Biofield Treated; TI: Test Item

Conclusion

The cell viability using MTT assay data showed significant improved cell viability with more than 72% among various experimental groups. Various bone health parameters like level of ALP was increased by 21.8% and 129.6% at 50 and 100 µg/mL, respectively in the UT-DMEM+BT-TI, while 17.7% and 392% at 10 and 100 µg/mL, respectively in the BT-DMEM+UT-TI group as compared with the untreated test item and DMEM group. In addition, BT-DMEM+BT-TI group showed an increased ALP level by 13% and 255.7% at 50 and 100 µg/mL, respectively. The level of collagen was significantly increased by 35.9% and 235.3% at 10 and 50 µg/mL, respectively in the UT-DMEM+BT-TI, while 81.7% and 197.6% at 50 and 100 µg/mL, respectively in the BT-DMEM+UT-TI group. The level of collagen was increased by 184.2% and 82.3% at 50 and 100 µg/mL, respectively 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 198.6% and 53.5% at 1 and 10 µg/mL, respectively in the UT-DMEM+BT-TI group, while 11.5% and 267.1% at 0.1 and 1 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 146.2%, 91.4%, and 66.8% at 0.1, 1 and 10 µg/mL, respectively as compared with the untreated

group. The Bone health parameters were significantly improved among the Biofield Energy Treated vitamin D₃ test samples in MG-63 cells. Overall, the Biofield Energy Treated (The Trivedi Effect®) test samples were found to have a significant impact on tested bone health parameters viz. collagen, bone mineralization, and ALP, which are very vital to combat the bone disorders.

Therefore, the Consciousness Energy Healing based vitamin D₃ might be a suitable alternative nutritional supplement, which could be useful for the management of various bone related disorders viz. osteoporosis, Paget's disease of bone, rickets, deformed bones, osteomalacia, bone and/or joint pain, increased frequency of fractures, osteoma, hormonal imbalance, stress, aging, bone loss and fractures, and other bone diseases that are caused by poor nutrition, genetics, or problems with the rate of bone growth or rebuilding. Biofield Energy Treated Vitamin D₃ can be useful as anti-inflammatory, anti-aging, anti-stress, anti-arthritis, anti-osteoporosis, anti-cancer, anti-apoptotic, wound healing, anti-psychotic and anti-fibrotic roles. It also influences 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 hormonal imbalance, aging, and various immune related disease conditions such as Multiple

Sclerosis, Alzheimer's Disease, Dermatitis, Atherosclerosis, stress, Irritable Bowel Syndrome, Systemic Lupus Erythematosus, Pernicious Anemia, Aplastic Anemia, Hepatitis, Diverticulitis, Graves' Disease, Dermatomyositis, Asthma, Hashimoto Thyroiditis, Diabetes, Myasthenia Gravis, Ulcerative Colitis, Sjogren Syndrome, Parkinson's Disease, etc. with a safe therapeutic index to improve overall health, healthy skeletal structure against various bone disorders, and overall quality of life.

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