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Biofield Energy Enriched Vitamin D_3 versus Vitamin D_3 in Preventing Fractures and Bone Loss Using MG-63 Cells

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Abstract

Bone is a dynamic tissue, which continually adapting its structure during the development process. The aim of the study was to investigate the effect of Consciousness Energy Healing based vitamin D_3 and DMEM medium on bone health parameters such as alkaline phosphatase enzyme (ALP) activity, collagen levels and bone mineralization using MG-63 cells. The test items (TI) i.e. vitamin D_3 and DMEM medium were divided into two parts. The test samples received Consciousness Energy Healing Treatment by Lorraine Marie Hachfeld 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 showed that cell viability was more than 79% with a safe and nontoxic profile on MG-63 cell line. The level of ALP was increased by 400%, 84%, and 179.4% at 0.1, 1, and 10µg/mL, respectively in the UT-DMEM+BT-TI, while 314.3% at 10µg/mL in the BT-DMEM+UT-TI group as compared with the untreated test item and DMEM group. BT-DMEM+BT-TI group showed an increased ALP level by 128% and 60.3% at 1 and 10µg/mL, respectively. Collagen was significantly increased by 180% and 128.1% at 0.1 and 1µg/mL, respectively in the UT-DMEM+BT-TI group, while 84.4% and 69.7% at 1 and 10µg/mL, respectively in BT-DMEM+UT-TI group as compared with the untreated group. Furthermore, BT-DMEM+BT-TI group showed a significant increased collagen level by 123.4% and 184.8% at 1 and 10 µg/mL, respectively as compared with the untreated test item and DMEM group. The percent of bone mineralization was significantly increased by 385.1% at 0.1µg/mL in the UT-DMEM+BT-TI group, while 53.2%, 30.2%, and 163.9% at 0.1, 1, and 10µg/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 bone mineralization by 283%, 65.1%, and 197.3% at 0.1, 1, and 10µg/mL, respectively as compared with the untreated group. The experimental data suggested that the Biofield Energy Treated vitamin D_3 and DMEM would play an important role in the promotion and maintenance of strong and healthy bones, which improve quality of life. It regulates the osteoblast function, improves the 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 healing; Bone mass; Osteosarcoma cells; Calcium absorption; Vitamin D; Bone mineralization

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

Introduction

In order to maintain normal blood levels of calcium and phosphate, nerve conduction, muscle contraction, and general cellular function, vitamin D plays a vital role by maintaining the bone mineralization, collagen, and other hormones regulations for normal cell functioning [1]. The active form of vitamin D is 1,25-dihydroxyvitamin D [1,25-$(OH)_2$D], or calcitriol, which is formed by metabolism in liver and kidney. Calcitriol regulates the calcium and phosphorus dependent genes and its regulation for calcium-transporting proteins and bone matrix proteins [2]. It also regulates the cell cycle proteins transcription, which improves the cell differentiation and reduced the cell proliferation of specialized number of cells such as enterocytes, osteoclastic precursors, keratinocytes, etc. Due to this unique action of vitamin D, it plays a vital role in bone resorption, intestinal calcium transport, and skin. Significant immuno-modulatory actions have been reported in different *in vivo* infection models. Vitamin D regulates vital body functions and acts significantly as anti-osteoporosis, anti-cancer, anti-inflammatory, anti-aging, anti-arthritic, anti-stress, anti-apoptotic, wound healing, anti-psychotic, and anti-fibrotic roles. Vitamin D receptors (VDRs) present in most of the body organs

and these receptors helps to modulate and transmit cell-to-cell communication, improve cell proliferation, cell differentiation, cell cycling and hormonal balance, skin health, immune, and cardiovascular functions [3]. Thus, it is believed that vitamin D is supposed to be one of the oldest hormones, which can be used against most of the bone-related disorders. It can be supplied through some fortified foods or with food supplements, which could be useful to improve the process of bone mineralization, reduced the bone resorption, aging, and inflammation. Deficiency of vitamin D is the major health problem leading to bone metabolic diseases [4], and it can be overcome using the recommended daily dose of vitamin D using some fortified foods or supplements. In order to avoid, bone disorders, calcium along with vitamin D with 1000-1500mg/day and 400IU/day, respectively is recommended to improve bone health [5]. MG-63 cell line has been used to study the bone health biomarkers such as alkaline phosphatase (ALP), collagen and calcium. Scientific reports suggested that 1,25-dihydroxyvitamin D3 ($1,25(OH)_2D_3$) was reported with significant response in MG-63 cells [6]. Thus, MG-63 cells are considered as the best for in vitro study to find the potential of any test compounds [7]. ALP, collagen, and calcium are the phenotypic marker, which play important role in differentiation and maturation of bone cells, formation of bone extracellular matrix, and improved bone mineralized [8-10].

Biofield Energy Therapies, as Complementary and Alternative Medicine (CAM) are the noninvasive therapies in which the renowned Biofield Energy practitioner explicitly interacts with the energy fields that surround the living systems, which would significantly, stimulates the healing capacity. Due to the significant outcomes, Biofield Energy Treatment or energy medicine emerged as one of the best alternative approach of treatment. CAM therapies are practiced worldwide, some are commonly known as Reiki, therapeutic touch, pranic healing, external qigong, Tai Chi, Johrei, Qi Gong, polarity therapy, yoga, deep breathing, chiropractic/osteopathic manipulation, massage, meditation, mindfulness, Rolfing structural integration, homeopathy, movement therapy, progressive relaxation, hypnotherapy, acupressure, acupuncture, relaxation techniques, pilates, and some medicinal approaches to Ayurvedic medicine or traditional Chinese herbs [11]. Among CAM, The Trivedi Effect®- Biofield Energy Healing Treatment has been reported to have significant scientific results and the outcomes have been published in peer-reviewed journals [12]. National Center for Complementary and Alternative Medicine (NCCAM), classified the Biofield therapies under the subcategory of Energy Therapies [13]. Consciousness Energy Healing Treatment has been reported with significant revolution in the material science [14-16], agricultural science [17,18], microbiology [19,20], bone health [21,22], biotechnology [23], increased bioavailability [24-26], enhanced skin health [27,28], used as a nutraceutical [29,30], cancer science[31,32], and human health and wellness.Thus, authors evaluated the *in vitro* effect of the Biofield Energy Treated vitamin D_3 as a test item for bone health using MG-63 cell line for major biomarkers.

Material and Methods

Chemicals and reagents

Fetal bovine serum (FBS) and Dulbecco's Modified Eagle's Medium (DMEM) were procured from Life Technology, USA. Similarly, the rutin hydrate was purchased from TCI, Japan. Vitamin D_3 and L-ascorbic acid were obtained from Sigma-Aldrich, USA. Antibiotics solution (penicillin-streptomycin) was procured from HiMedia, India. 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium) (MTT), Direct Red 80, and ethylenediaminetetraacetic acid (EDTA) were obtained from Sigma, USA.

Cell culture

Human bone osteosarcoma (MG-63) cell line was used in this experiment and was maintained with DMEM growth medium for routine culture, which was supplemented with 10% FBS. Growth conditions were maintained at 37°C, 5% CO_2 and 95% humidity and sub-cultured by trypsinisation followed by splitting of the cell suspension into fresh flasks and further supplementing with fresh cell growth medium. 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 [6].

Experimental design

The following groups were defined in the study, such as baseline control, vehicle control groups (0.05% DMSO with Biofield Energy Treated and untreated DMEM media), positive control group included rutin hydrate and the experimental test groups. The experimental test sample groups have combination of the Biofield Energy Treated and untreated vitamin D_3/DMEM. 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 sample and the DMEM were treated with the Biofield Energy (also known as The Trivedi Effect®) by a renowned Biofield Energy Healer, Lorraine Marie Hachfeld remotely for ~5 minutes. These test groups were divided as treated and untreated test samples. The renowned Biofield Energy Healer was remotely located in the Canada and on the other side the test samples were located in the Dabur Research Foundation, New Delhi, India, which were treated through the Healer's unique Energy Transmission process remotely. Lorraine Marie Hachfeld in this study never visited the laboratory in person, nor had any contact with the test item and medium. In addition, the experimental control group was treated with a sham healer, and sham healer did not have any knowledge about the Biofield Energy Treatment. The samples were stored under laboratory condition for experimental test setup.

Determination of non-cytotoxic concentration

MTT assay was used in order to test the cell viability in MG-63 cells for treated and untreated test samples. The details procedure

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of cell viability assay was followed by Ansari et al. [21]with slight modification. The cytotoxicity of each tested concentration of the test items was calculated with the help of Equation (1):

$$\%Cytotoxicity = \left\{\frac{1-X}{R}\right\} * 100 \quad (1)$$

Where, X=Absorbance of treated cells; R=Absorbance of untreated cells

The percentage of cell viability corresponding to each treatment group was calculated by Equation (2):

$$\%Cell\,Viability = (100 - \%Cytotoxicity) \quad (2)$$

The concentration exhibiting ≥70% cell viability was defined as non-cytotoxic [33].

Assessment of alkaline phosphatase (ALP) activity

For the estimation of ALP activity of the Biofield Energy Treatment on the test items in MG-63 cells. The procedure of cell counting, plating, and treatment was followed as per Koster et al. [6,22]. The percent increase in ALP activity with respect to the untreated cells was calculated using Equation (3):

$$\%\,Increase\,in\,ALP = \left\{\frac{X-R}{R}\right\} * 100 \quad (3)$$

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

Assessment of collagen synthesis

For the estimation of collagen level in MG-63 cells, standard methods were used for the evaluation of the potential of Biofield Treated test items and the procedure in details was as per Koster et al.[6,22]with few modifications.The increase collagen level with respect to the untreated cells was calculated using Equation (4):

$$\%\,Increase\,in\,collagen\,levels = \left\{\frac{X-R}{R}\right\} * 100 \quad (4)$$

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

Assessment of bone mineralization by alizarin red s staining

For the evaluation of the percent alteration in bone mineralization after treatment of the Biofield Treated test items in MG-63 cells, and the details steps were followed according to Ansari et al. [29,30]. The percentage increase in bone mineralization compared to the untreated cells was calculated using Equation (5):

$$\%\,Increase = \left\{\frac{X-R}{R}\right\} * 100 \quad (5)$$

Where, X=Absorbance in cells corresponding to positive control or test groups; R=Absorbance in cells corresponding to 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.

Results and Discussion

Cell viability study using MTT

The results of the cell viability using MTT assay of the Biofield Energy Treated vitamin D_3 and DMEM in MG-63 cells are shown in Figure 1. All the results were compared with respect to rutin and untreated test samples at various concentrations for the estimation of percentage cell viability. The data showed that the test samples in combination found as nontoxic and safe (as evidence of cell viability approximately greater than 79%) across all the tested concentrations with range up to maximum of 100µg/mL. These safe concentrations are used for estimation of different bone health parameters in MG-63 cells.

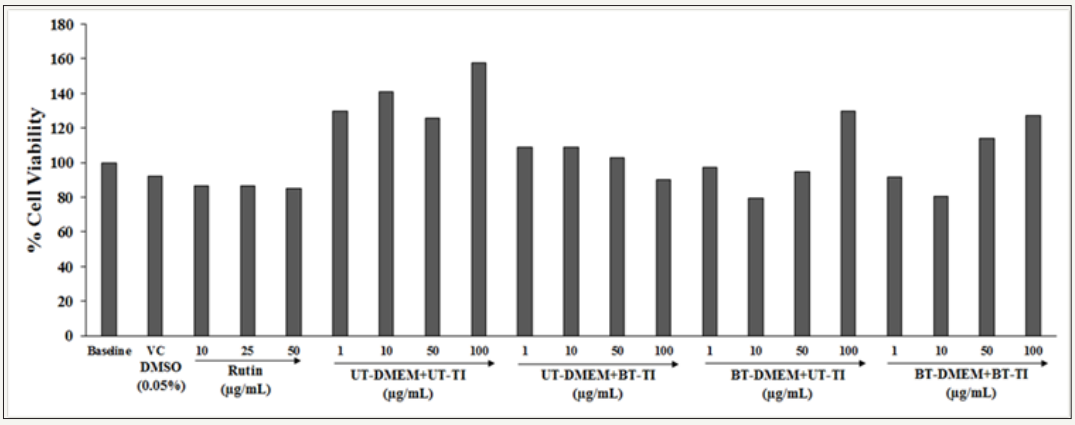

Figure 1: Cell viability using MTT assays of the test items on MG-63 cell line after 72 hours.
VC: Vehicle Control (DMSO-0.05%); UT: Untreated; BT: Biofield Treated; TI: Test Item.

Alkaline phosphatase (ALP) enzyme activity

The ALP activity results of the Biofield Energy Treated vitamin D_3 and DMEM on the level of ALP in MG-63 cells is shown in the Figure 2. The ALP concentrations after Biofield Energy Treatment with the test samples *viz.* Biofield Energy Treated test item and DMEM were studies at various concentrations and compared with

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the untreated test samples. The vehicle control group showed 17.4% increased level of ALP as compared with the untreated cells group. The positive control, rutin showed a significant increased value by 40.89%, 59.53%, and 60.38% at 0.001, 0.01, and 0.1µg/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 400%, 84%, and 179.4% at 0.1, 1, and 10µg/mL, respectively while Biofield Treated medium and untreated Test item (BT-DMEM+UT-TI) showed a significant increased ALP level by 314.3% at 10µg/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 128% and 60.3% at 1 and 10µg/mL, respectively as compared with the untreated test item and DMEM group. ALP is one of the biomarker used to access the growth of bone and bone formation and resorption along with other biomarkers such as bone-specific alkaline phosphatase (BALP), osteocalcin (OC), procollagen type 1 N-terminal propeptide (P1NP) and procollagen type 1 C-terminal propeptide (P1CP). BALP, a bone-specific isoform of ALP present on the surface of osteoblasts defines the biosynthetic activity of the bone-forming cells. In addition, ALP level continuously decreased with age, that might results in diseases 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. These bone health diseases can be overcome using some health supplements rich in calcium and vitamin D_3 [34-36]. Overall, the experimental data concluded that the Biofield Energy Healing Treatment in the test samples showed a significant improved level of the ALP used against various age related bone diseases. The experimental data well described that The Trivedi Effect®-Energy of Consciousness Healing based vit D_3 and DMEM could be used to improve the ALP concentration in many bone disorders.

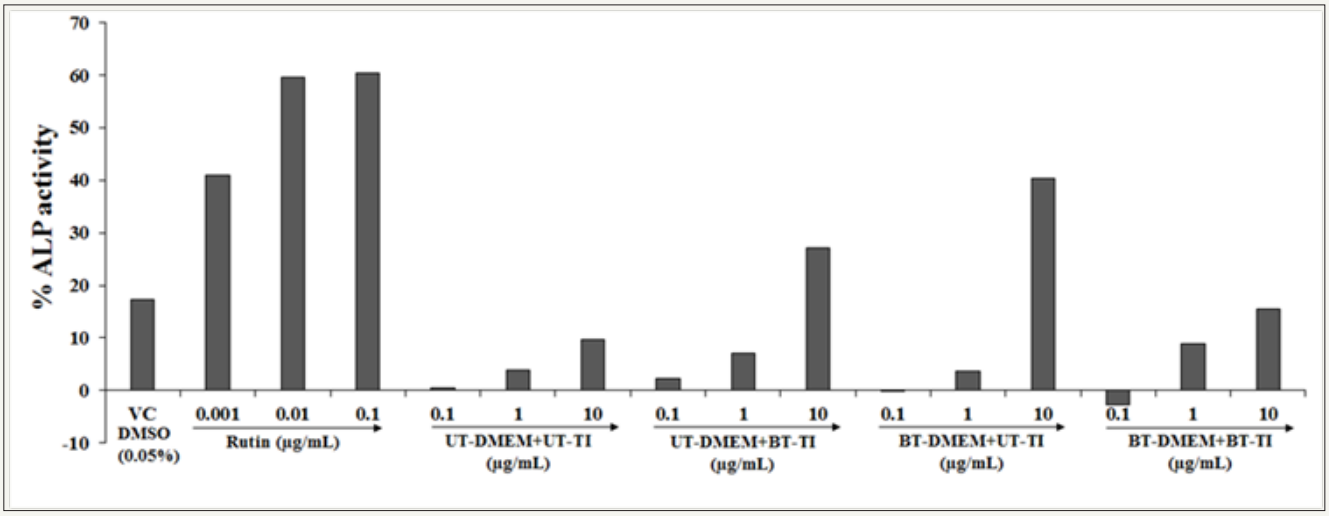

Figure 2: Alkaline Phosphatase (ALP) enzyme activity of the Biofield Energy Treated test items on MG-63 cell line. VC: Vehicle Control (DMSO-0.05%); UT: Untreated; BT: Biofield Treated; TI: Test Item.

Assessment of collagen synthesis

The collagen level among Biofield Energy Treated vit D_3 and DMEM was estimated at various safe concentrations and the data suggested significant increased collagen level with. The results are presented as % values with respect to the untreated cells in Figure 3. The rutin hydrate showed a significant increased value of collagen by 40.55%, 45.70%, and 58.59% at 0.01, 0.1, and 1µg/mL, respectively. Besides, the experimental test groups such as UT-DMEM+BT-TI showed a significant increased collagen level by 180%, 128.1%, and 38.6% at 0.1, 1, and 10µg/mL, respectively while BT-DMEM+UT-TI group showed a significant increased collagen level by 84.4% and 69.7% at 1 and 10µg/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 44.4%, 123.4%, and 184.8% at 0.1, 1, and 10µg/mL, respectively as compared with the untreated test item and DMEM group. The data suggested that the overall the collagen level was increased after Biofield Energy Treatment as compared with the untreated test samples. Collagen play important role in bones and joints, it is extremely important fibrous protein present in the connective tissue. Further, it was reported that collagen synthesis decreases with age and leads to high chance of bone, joints, muscle injuries. Collagen type I is the most abundant protein, which form the strength in bone health [37]. Thus, some reduced collagen synthesis results in serious bone diseases such as the type of bone loss experienced in osteoporosis, which can be overcome using various supplementations [38]. The experimental data suggest that Biofield Energy Treated vit D_3would be the best form of supplement in order to retain the bone health irrespective to age. The data showed a significant improved level of collagen compared with the untreated group. Biofield Energy Treated vit D_3 (The Trivedi Effect®) demonstrated a significant improved level of collagen for bone health, which can be used to decrease the aging process and bone inflammation.

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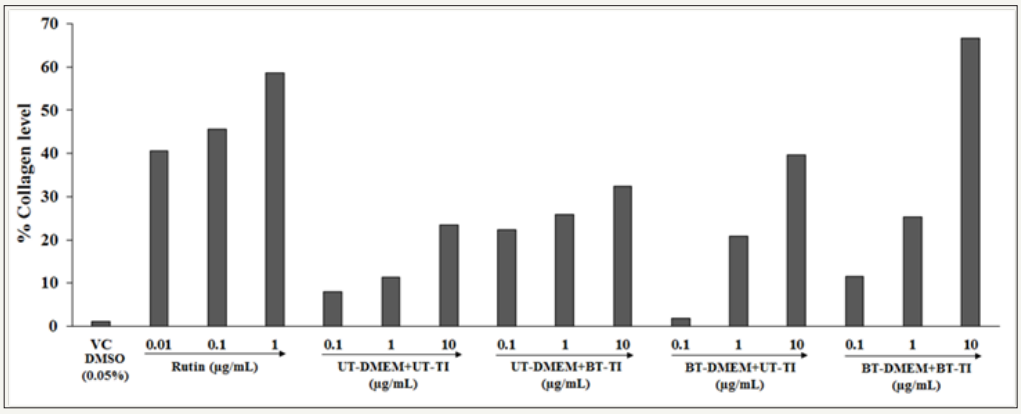

Figure 3: Evaluation of collagen activity on MG-63 cell line.
VC: Vehicle Control (DMSO-0.05%); UT: Untreated; BT: Biofield Treated; TI: Test Item.

Bone mineralization

Vitamin D and calcium has been well-recognized with its significant role in bone health by normal bone formation and normal mineralization. Poor bone mineralization results in various bone disorders such as osteoporosis or other bone diseases. Bone mineralization and remodeling would renew the skeleton along with sequential involvement of the bone resorption and formation at the same spatial location. In addition, bone remodeling affects bone material assets such as microdamage, mineralization, and collagen cross-linking. Both these processes affect the bone mineral density (BMD), quality of bone, and structural abnormalities [39,40]. The present study was conducted to check the alteration in percentage of bone mineralization in Biofield Energy Treated test samples with respect to the untreated test samples. Biofield Energy Treated vit D_3 and DMEM groups showed a significant improved bone mineralization on MG-63 cell line. The results 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 47.98%, 59.73%, and 139.02% at 5, 10, and 25µg/mL, respectively. The experimental data among test group's *viz*. UT-DMEM+BT-TI showed a significant increased bone mineralization by 385.1%, 28.7%, and 19.7% at 0.1, 1, and 10µg/mL, respectively while BT-DMEM+UT-TI group showed a significantly increased bone mineralization by 53.2%, 30.2%, and 163.9% 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 283%, 65.1%, and 197.3% at 0.1, 1, and 10µg/mL, respectively as compared with the untreated test item and DMEM group. The experimental test groups showed that Biofield Energy Healing Treatment significantly improved the rate of bone mineralization compared with the untreated groups.

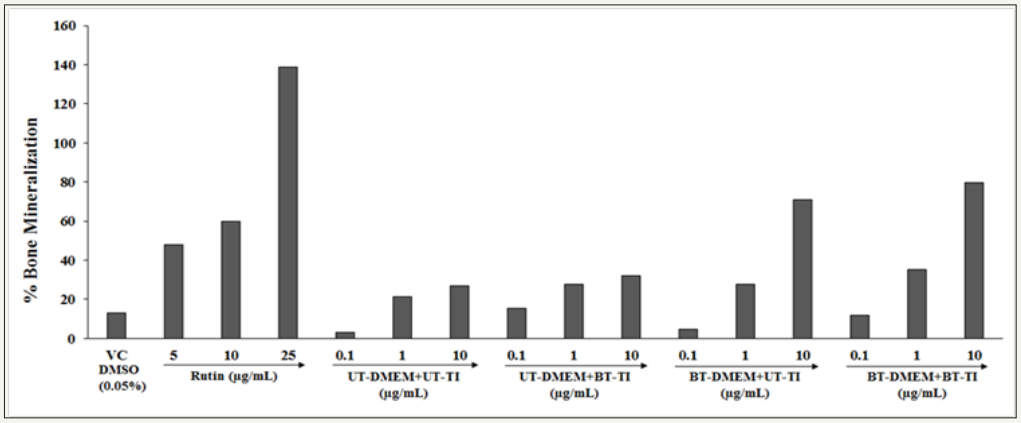

Figure 4: Effect of the test item on MG-63 cell line for bone mineralization.
VC: Vehicle Control (DMSO-0.05%); UT: Untreated; BT: Biofield Treated; TI: Test Item.

Conclusion

Biofield Energy Treated vitamin D_3 and DMEM was studied for bone health parameters. MTT study for cell viability data showed that a significant improved cell viability with more than 79% among all the tested groups. The level of ALP was increased by 400%, 84%, and 179.4% at 0.1, 1, and 10µg/mL, respectively in the UT-DMEM+BT-TI, while 314.3% at 10µg/mL in the BT-DMEM+BT-TI group as compared with the untreated test item and DMEM group. BT-DMEM+BT-TI group showed an increased ALP level by 128% and 60.3% at 1 and 10µg/mL, respectively. The

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level of collagen was significantly increased by 180%, 128.1%, and 38.6% at 0.1, 1, and 10µg/mL, respectively in the UT-DMEM+BT-TI, while 84.4% and 69.7% at 1 and 10µg/mL, respectively in the BT-DMEM+UT-TI group. In addition, collagen level was increased by 44.4%, 123.4%, and 184.8% at 0.1, 1, and 10µg/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 385.1%, 28.7%, and 19.7% at 0.1, 1, and 10µg/mL, respectively in the UT-DMEM+BT-TI group, while 53.2%, 30.2%, and 163.9% at 0.1, 1, and 10µg/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 283%, 65.1%, and 197.3% at 0.1, 1, and 10µg/mL, respectively as compared with the untreated group. Thus, experimental results demonstrated that Biofield Energy Healing Treatment can be utilized in managing various bone health parameters *viz*. collagen, bone mineralization, and ALP to combat the bone disorders. Thus, The Trivedi Effect®-Consciousness Energy Healing based vitamin D_3 showed a significant improved bone health, which supports its use as the best nutritional supplement to treat bone related disorders such as rickets, deformed bones, osteomalacia, osteoma, osteoporosis, Paget's disease, aging, bone and/or joint pain, increased tendency of fractures, hormonal imbalance, stress, and other associated bone diseases. Besides, this approach can also supports its use against many various immune related disease conditions and worked as anti-arthritic, anti-aging, anti-apoptotic, anti-osteoporosis, anti-inflammatory, anti-stress, anti-cancer, wound healing, and anti-fibrotic roles. It would improve the cell-to-cell communication, cell differentiation, cell cycling and proliferation, normal cell growth, neurotransmission, skin health, and also in vital cardiovascular functions. Besides, it can also be utilized against Dermatitis, Multiple Sclerosis, Pernicious Anemia, Asthma, Irritable Bowel Syndrome, Diabetes, Diverticulitis, Hashimoto Thyroiditis, Myasthenia Gravis, Sjogren Syndrome, Aplastic Anemia, Hepatitis, Parkinson's disease, Atherosclerosis, and many more.

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