

Assessment of Biofield Energy Healing Based Vitamin D₃ Effects on Bone Health Parameters Using Human Osteoblast Cell Line (MG-63)

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Abstract

Poor bone health is the primary health issue, which leads to significant health problems, stress and worsening the patients' quality of life. The potential of The Trivedi Effect[®]- Biofield Energy Healing on vitamin D₃ as a test item (TI) and DMEM on MG-63 cells was investigated. The test items were treated with The Trivedi Effect[®] by Mahendra Kumar Trivedi and divided as Biofield Energy Treated (BT) and untreated (UT) test items. An increase in ALP activity, collagen levels, and bone mineralization was considered as the biomarker for bone health. MTT data showed that the test samples observed nontoxic in the tested concentrations. The level of ALP was significantly increased by 832.9% and 209.4% in the UT-DMEM+BT-TI and BT-DMEM+UT-TI groups, respectively at 10 µg/mL, while 222.9% increase in the BT-DMEM+BT-TI at 1 µg/mL compared to the untreated group. Collagen was significantly increased by 487.7% and 544.5% in the BT-DMEM+UT-TI and BT-DMEM+BT-TI groups, respectively at 100 µg/mL, while 116.2% at 1 µg/mL in UT-DMEM+BT-TI compared to the untreated group. Moreover, the percent of bone mineralization was significantly increased in the UT-DMEM+BT-TI and BT-DMEM+UT-TI groups by 344.9% and 149.7%, respectively at 50 µg/mL, while 183.6% in the BT-DMEM+BT-TI group at 100 µg/mL compared to the untreated group. Thus, the role of Biofield Energy Treated vitamin D₃ and DMEM in order to control osteoblast function and its direct effects on bone mineralization can be used to improve bone disorders.

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Introduction

Bones play an important role for skeletal structure, organ protection, muscles anchor, and storing excess calcium for future utilization. Bones are always changing *i.e.*, new bones are forming and old bones are decaying. In certain condition, when bone decaying is higher than bone formation leads to various bone-related disorders like osteoporosis, osteomalacia, etc. [1]. Osteoporosis is a progressive metabolic bone disorder characterized by reduced bone mass and increased chances of bone fractures [2]. Postmenopausal osteoporosis is a major health issue for women. A numbers of factors are responsible for the increased incidence of osteoporosis such as lifestyle, diets, ethnicity, smoking, alcoholism, and diet have been linked with an increased incidence of the osteoporosis [3]. For prevention of bone-related disorders and to maintain a good bone health usually required optimum ingestion of calcium (Ca). Although, increased level of bone density after adequate supplementation of Ca is not always impact on bone strength and low risks from fracture [4-6]. Vitamin D is a hormone acts on vitamin D receptors (VDRs), present in various vital organs like heart, brain, lungs, kidney, liver, etc. and essential for absorption of calcium and bone mineralization which is directly linked with the bone mineral density (BMD) [7]. Deficiency of vitamin D₃ causes rickets in children and osteomalacia in adult's primarily. Literature reported that vitamin D prevent fractures through muscle function and inflammation [8]. A number of experiment reported the role of bone health using cell lines and analyzing the bone -related parameters like alkaline phosphatase (ALP), collagen and bone mineralization in terms of calcium deposition [9]. MG-63 cell line (an immature osteoblast phenotype) is derived from juxtacortical osteosarcoma. The response of MG-63 cells to the active metabolites of vitamin D₃ *i.e.*, 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃) administration has been studied to be similar to normal human osteoblast cells [10]. Hence, MG-63 cell line is used extensively for the evaluation of bone health parameters [11]. For early differentiation and maturation of osteoblasts cells ALP acts as a phenotypic marker. It increases the local concentration of inorganic phosphate (pi) for bone mineralization and hence, it is used an important biomarker for osteogenic activity [12].

Similarly, collagen plays an important role in the formation of bone extracellular matrix (ECM) by providing strength and flexibility [13]. Similarly, calcium phosphate is deposited and gets mineralized (combination of calcium phosphate and hydroxyapatite) and gives rigidity to the bone [14]. Thus, these parameters are very essential to study the bone health in cell lines. Hence, authors evaluated the *in vitro* effect of the Biofield Energy Treated vitamin D₃ on bone health using MG-63 cell line for major biomarkers. Energy medicine is emerging with significant benefits in various scientific fields.

The effects of the complementary and alternative medicine (CAM) therapies have great importance, which include Qi Gong, Reiki, Tai Chi, meditation, acupressure, acupuncture, Johrei, yoga, polarity therapy, pranic healing, therapeutic touch, chiropractic/osteopathic manipulation, deep breathing, massage, homeopathy, progressive relaxation, special diets, guided imagery, relaxation techniques, hypnotherapy, pilates, traditional Chinese herbs and medicines, healing touch, Roling structural integration, movement therapy, mindfulness, Ayurvedic medicine, etc. [15]. Consciousness Energy Healing Treatment (The Trivedi Effect[®]) contain a putative bioenergy, which is channeled by a renowned practitioner from a distance to livings and non-livings objects. Biofield Energy Healing as a CAM showed a significant results in biological studies [16]. However, the National Center for Complementary and Alternative Medicine (NCCAM), well-defined Biofield therapies in the subcategory of Energy Therapies [17]. The Trivedi Effect[®]-Consciousness Energy Healing Treatment has been reported with significant revolution in multiple fields [18-28] and human health and wellness. Based on the outstanding outcomes of Biofield Energy Treatment and vital role of vitamin D₃ on bone health, authors performed this experiment to investigate the effects of Biofield Treated vitamin D₃ on bone health parameters like ALP, collagen content, and bone mineralization using standard *in vitro* assays in MG-63 cells.

Methods

Chemicals and Reagents

Dulbecco's Modified Eagle's Medium (DMEM) and fetal bovine serum (FBS) were obtained from Life

Technology, 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, vitamin D₃ and L-ascorbic acid, and ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma, USA. Rutin hydrate was purchased from TCI, Japan. Other chemicals used in this study were analytical grade procured from India.

Cell Culture

The human bone osteosarcoma cell line (MG-63) was used as a test system here. The MG-63 cells were cultured in DMEM growth medium for routine culture and supplemented with 10% FBS. At 37°C, 5% CO₂ and 95% humidity Growth conditions were maintained and sub-cultured by trypsinization followed by splitting the cell suspension into fresh flasks and supplementing with fresh cell growth medium. Before three days 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 [29].

Experimental Design

The study groups contain cells in baseline control, vehicle control groups (0.05% DMSO with Biofield Energy Treated and untreated DMEM), positive control group (rutin hydrate) and test groups. The test 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 (vitamin D₃ and DMEM) was divided into two parts. First part each of the test items was treated with the Biofield Energy (The Trivedi Effect®) by a renowned Biofield Energy Healer, Mahendra Kumar Trivedi remotely through his unique Energy Transmission process for ~3 minutes under laboratory conditions and defined as the Biofield Treated test items. While the second part did not receive any sort of treatment and referred as the untreated test items. The Healer was remotely located in the USA, while the test items were located at Dabur

Research Foundation, New Delhi, India. Healer, in this study did not visit the laboratory in person, nor had any contact with the test items. Besides, the control group was treated with a sham healer for better comparative purposes. The sham healer did not have any knowledge about the Biofield Treatment. After Energy Treatment, the Biofield Energy treated and untreated samples were kept in similar sealed conditions till the end of the experiment.

Determination of Non-cytotoxic Concentration

MTT assay was used for the evaluation of viable cells in MG-63 cells after treatment with Biofield Energy Treated and untreated test items. The details procedure of cell viability assay was followed by Lauree *et al.* (2018) with slight modification [30]. The cytotoxicity of each tested concentration of the test items was calculated with the help of Equation (1):

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

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):

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

The concentration exhibiting ≥70% cell viability was defined as non-cytotoxic [31].

Assessment of Alkaline Phosphatase (ALP) Activity

The ALP enzyme activity was performed in the Biofield Energy Treated test items in MG-63 cells. The procedure of cell counting, plating, and treatment was followed as per Krista *et al.* with slight modification [32]. The percent increase in ALP activity with respect to the untreated cells was calculated using Equation (3):

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

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

R = Absorbance of cells corresponding to untreated cells

Assessment of Collagen Synthesis

The collagen level in MG-63 cells, standard

methods were used for the evaluation of the potential of Biofield Energy Treated test items and the procedure in details was as per Lorraine et al. with few modifications [33]. The increase collagen level with respect to the untreated cells was calculated using Equation (4):

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

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 with the Biofield Energy Treated test items in MG-63 cells, and the details steps were followed according to Balmer et al. with slight changes [34]. The percentage increase in bone mineralization compared to the untreated cells was calculated using Equation (5):

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

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

Statistical Analysis

Data were represented as the percentage of respective parameters. One-way analysis of variance (ANOVA) was used for multiple group comparison followed by *post-hoc* analysis by Dunnett's test. Statistically significant was set at the level of $p \leq 0.05$.

Results

MTT Assay

The cytotoxic effects of test items were tested on MG-63 cells, and the percentage of cell viability is shown in Figure 1. The results of percentage cell viability in all the tested cell lines showed the cell viability ranges from 80% to 121% in different test item groups with DMEM, while for rutin group showed more than 85% cell viability (Figure 1). These data suggest that the test item along with DMEM groups were found as safe at all the tested concentrations range up to a maximum of

100 µg/mL against the tested MG-63 cells.

Alkaline Phosphatase (ALP) Activity

The effect of the Biofield Energy Treated test item and DMEM on the ALP level showed a significant increased at various experimental test item concentrations on MG-63 cell line (Figure 2). The positive control (rutin) showed a significant increased ALP by 80.90%, 58.70%, and 35.80% at 1, 5 and 10 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 ALP level by 654.2%, 832.9%, and 106.1% at 1, 10, and 50 µg/mL, respectively while Biofield Treated medium and untreated Test item (BT-DMEM+UT-TI) showed a significant increased ALP level by 133.3% and 209.4% at 1 and 10 µg/mL, respectively as compared with the untreated group. However, the Biofield Energy Treated medium and Biofield Energy Treated Test item (BT-DMEM+BT-TI) showed a significant increased ALP level by 222.9% and 130.6% at 1 and 10 µg/mL, respectively than untreated. Overall, result showed a significantly improved the level of ALP at the tested concentrations. The ALP is a phenotypic marker for the early differentiation and maturation of osteoblasts. ALP is reported to improve the local concentration of inorganic phosphate, a mineralization promoter along with inhibition of extracellular pyrophosphate concentrations, an inhibitor of mineral formation. Thus, it might be expected that Biofield Energy Treated vitamin D₃ improved the enzyme expression, which is a good predictor of new bone mineralization. The Trivedi Effect[®]-Energy of Consciousness Healing based vitamin D₃ and DMEM could provide therapeutic prospects for the treatment of bone diseases, and boost the ability to create useful bone biomaterials [35].

Effect of Test Items on Collagen Synthesis

The result of collagen synthesis is presented in Figure 3. The positive control showed a significantly increased collagen by 51.9%, 47.5%, and 42.5% at 1, 5, and 10 µg/mL, respectively. The experimental test group's *viz.* UT-DMEM+BT-TI showed a significantly increased collagen level by 116.2%, 36.9%, 68%, and 60.3% at 1, 10, 50, and 100 µg/mL, respectively; while

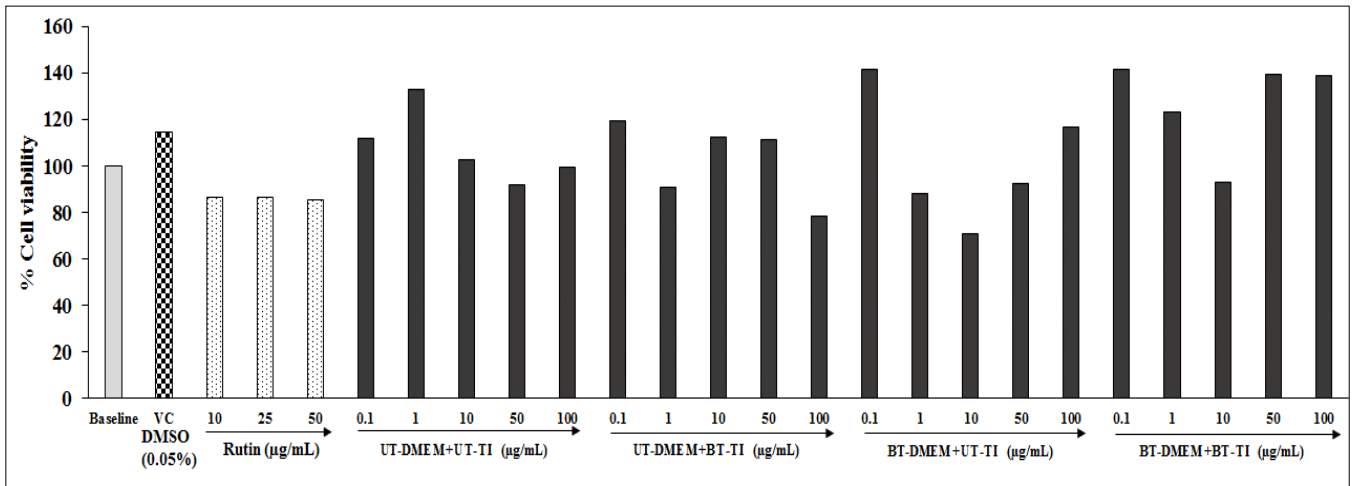


Figure 1. Effect of the test items on MG-63 cell line for cell viability after 72 hours using the MTT assays. VC: Vehicle control (DMSO-0.05%), UT: Untreated; BT: Biofield Treated; TI: Test Item.

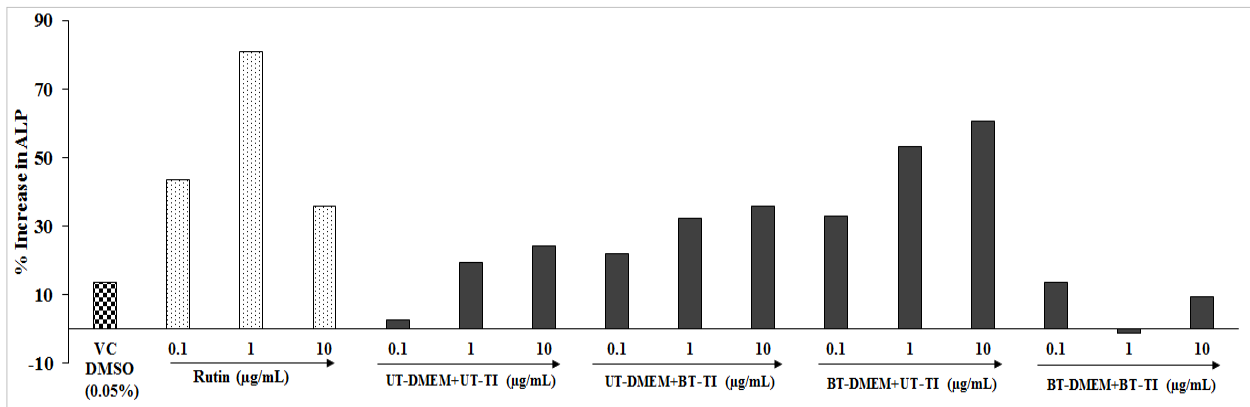


Figure 2. Effect of the test items on MG-63 cell lines for the level of alkaline phosphatase (ALP) enzyme activity. VC: Vehicle control (DMSO-0.05%), UT: Untreated; BT: Biofield Treated; TI: Test Item.

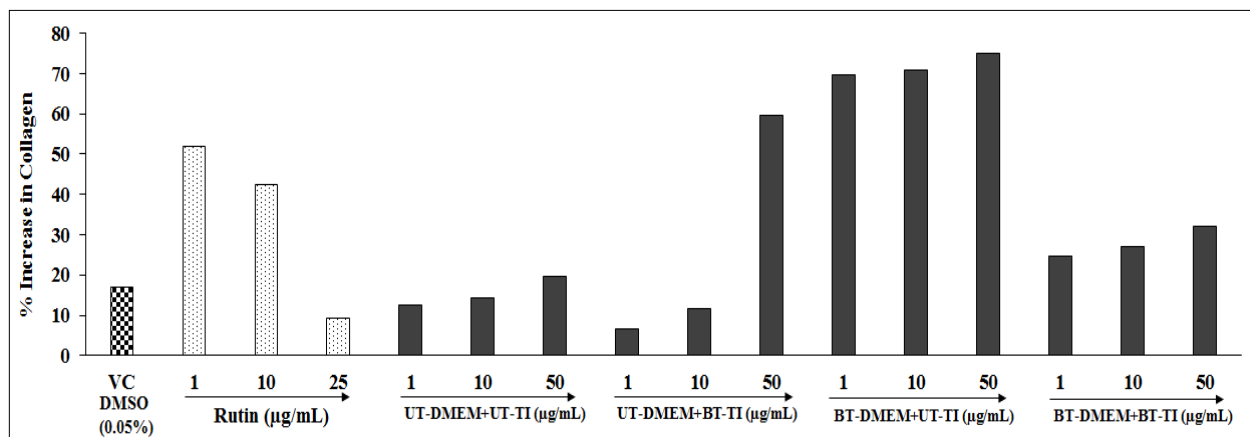


Figure 3. Effect of the test items on MG-63 cell line for collagen level. VC: Vehicle control (DMSO-0.05%), UT: Untreated; BT: Biofield Treated; TI: Test Item.

BT-DMEM+UT-TI group showed a significantly increased collagen level by 191.2%, 172.1%, 196.1%, and 487.7% at 1, 10, 50, and 100 µg/mL, respectively as compared with the untreated test item and DMEM group. However, BT-DMEM+BT-TI group showed a significantly increased collagen level by 182.4%, 82.9%, 132.8%, and 544.5% at 1, 10, 50, and 100 µg/mL, respectively as compared with the untreated group. Overall, results showed a significantly improved the level of collagen at the tested concentrations. The complexity of bone is composed of collagen fibrils, which form a scaffold for a highly organized arrangement of uniaxially oriented apatite crystals. The molecule of collagen enhances the infiltration of the fibrils with amorphous calcium phosphate (ACP), which results in enhanced bone apatite formation [36, 37]. Thus, the results envisaged that the Biofield Energy (The Trivedi Effect®) Treated vitamin D₃ could be an important source to improve the level of collagen against different orthopedic diseases.

Effect of the Test Items on Bone Mineralization

The result of bone mineralization among different groups is shown in Figure 4. The positive control showed a significantly increased the bone mineralization by 55.5%, 84.7%, and 108.7% at 1, 5, and 10 µg/mL, respectively. The UT-DMEM+BT-TI group showed a significantly increased in bone mineralization by 155.7%, 344.9%, and 284.4% at 10, 50, and 100 µg/mL, respectively while BT-DMEM+UT-TI group showed a significantly increased bone mineralization by 104.9%, 149.7%, and 125.3% at 10, 50, and 100 µg/

mL, respectively as compared with the untreated group. However, BT-DMEM+BT-TI group showed a significantly increased bone mineralization by 95.1%, 129.3%, and 183.6% at 10, 50, and 100 µg/mL, respectively than the untreated group. Overall, the Biofield Energy Treated test items and DMEM groups showed a significantly improved level of bone mineralization at the tested concentrations. Calcium deficiency in an organism can be protected by vitamin D, which is the major regulator of calcium homeostasis. Mineralization of bone might be disturbed through the vitamin D endocrine system, while vitamin D receptors also play a major role in the bone mineralization. However, various literature suggest that the important relationship between the intracellular calcium phosphate in osteoblasts and their role in mineralizing the extracellular matrix (ECM), on which apatite crystals subsequently form. Different bone disorders have reported that calcium supplementation restores the bone mineralization [38].

Conclusions

The result suggests that the cell viability using MTT assay showed a significantly improved the cell viability with more than 80% among different test groups, while Biofield Energy Treated test items also improved the cell viability as compared with the untreated group. MTT data indicated that the test items such as vitamin D and DMEM were safe and nontoxic in all the tested concentrations. After Biofield Treatment, the ALP level was significantly increased by 832.9% and 209.4% in the UT-DMEM+BT-TI and BT-DMEM+UT-TI groups, respectively at 10 µg/mL along with 222.9%

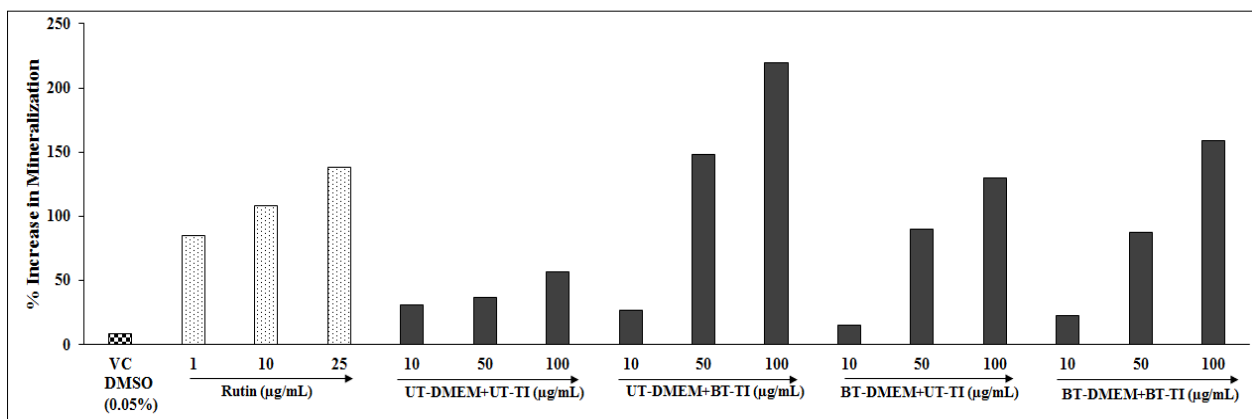


Figure 4. Effect of the test items on MG-63 cell line for bone mineralization. VC: Vehicle control (DMSO-0.05%), UT: Untreated; BT: Biofield Treated; TI: Test Item.

increased ALP in the BT-DMEM+BT-TI group at 1 µg/mL compared to the untreated group. In addition to, the level of collagen was significantly increased by 487.7% and 544.5% in the BT-DMEM+UT-TI and BT-DMEM+BT-TI groups, respectively at 100 µg/mL, along with 116.2% increased in collagen at 1 µg/mL in the UT-DMEM+BT-TI compared to the untreated group. Likewise, the percent of bone mineralization was significantly increased in the UT-DMEM+BT-TI and BT-DMEM+UT-TI groups by 344.9% and 149.7% at 50 µg/mL, respectively and 183.6% increased in bone mineralization in the BT-DMEM+BT-TI group at 100 µg/mL as compared to the untreated group. Overall, the Biofield Energy Treated (The Trivedi Effect®) test samples showed a significant impact on bone health parameters *viz.* collagen, calcium, and ALP, which play a vital role in maintaining bone disorders. Therefore, the Consciousness Energy Healing-based vitamin D₃ might be suitable alternative for nutritional supplement, which could use various bone disorders *viz.* low bone density and osteoporosis, osteogenesis imperfecta, Paget's disease of bone, rickets, osteomalacia, bone and/or joint pain, increased frequency of fractures, osteoma, deformed bones, chondrodystrophia fetalis, etc. Biofield Energy Treated Vitamin D₃ can also be used as anti-inflammatory, anti-arthritis, anti-osteoporosis, anti-stress, anti-aging, anti-apoptotic, wound healing, anti-cancer, anti-psychotic and anti-fibrotic roles. It also influences cell-to-cell communication, cell growth, differentiation, cycling and proliferation, hormonal balance, skin health, neurotransmission, and immune and cardiovascular functions. Besides, it can also use in organ transplants like kidney, liver, and heart transplants, aging, hormonal imbalance, and various immune-related disease conditions such as Asthma, Ulcerative Colitis, Alzheimer's Disease, Atherosclerosis, Dermatitis, Diverticulitis, Dermatomyositis, Graves' Disease, Hashimoto Thyroiditis, Multiple Sclerosis, Myasthenia Gravis, Pernicious Anemia, Aplastic Anemia, Sjogren Syndrome, Systemic Lupus Erythematosus, Diabetes, Hepatitis, Irritable Bowel Syndrome, Parkinson's Disease, stress etc.

Abbreviations

MG-63: Human Bone Osteosarcoma Cells, ALP: Alkaline phosphatase, CAM: Complementary and alternative medicine, NHIS: National Health Interview

Survey, NCCIH: National Center of Complementary and Integrative Health, DMEM: Dulbecco's modified eagle's medium, FBS: Fetal bovine serum, UT: Untreated, BT: Biofield Energy Treated, TI: Test Item; FBS: Fetal bovine serum; EDTA: Ethylenediaminetetraacetic acid.

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Conflict of interest

The authors declare that they have no competing interests.

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