

Role of Vitamin D₃ on Bone Health in Human Bone Osteosarcoma Cells (MG-63): Influence of Biofield Energy Healing Treatment



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Abstract

The aim was to evaluate the impact of Biofield Treated vitamin D₃ and DMEM on bone health. The test items (TI), were divided into two parts. One part of each sample received Consciousness Energy Treatment by Gopal Nayak and those samples were labeled as Biofield Treated (BT), while other parts of each sample were denoted as untreated test items (UT). MTT data showed test samples were found safe and nontoxic in tested concentrations. ALP was significantly increased by 213.46% and 218.66% in BT-DMEM + UT-TI and BT-DMEM + BT-TI, respectively at 1 µg/mL compared to UT-DMEM + UT-TI. Moreover, ALP was significantly increased by 200.79% and 187.09% in BT-DMEM + UT-TI and BT-DMEM + BT-TI, respectively at 10 µg/mL than untreated. Collagen synthesis was significantly increased by 551.25% and 130.31% in BT-DMEM + UT-TI and BT-DMEM + BT-TI, respectively at 1 µg/mL than untreated. Collagen was significantly increased by 401.97% in BT-DMEM + UT-TI at 10 µg/mL, while increased by 197.57% and 276.82% in UT-DMEM + BT-TI and BT-DMEM + UT-TI, respectively at 50 µg/mL than untreated. Percent bone mineralization was significantly increased by 186.86% and 188.23% in BT-DMEM + UT-TI and BT-DMEM + BT-TI, respectively at 10 µg/mL than untreated. It was further increased significantly by 224.04% and 156.72% in BT-DMEM + UT-TI and BT-DMEM + BT-TI, respectively at 50 µg/mL than untreated. Overall, data suggests that Biofield Treated vitamin D₃ could be a potential alternative nutraceutical supplement to combat vitamin D₃ deficiency and fight against various bone disorders.

Keywords: Consciousness energy healing; The Trivedi Effect®; Biofield energy healing treatment; Osteosarcoma cells (MG-63); Osteoporosis

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; ATCC: American Type Culture Collection; UT: Untreated; BT: Biofield Energy Treated

Introduction

Vitamin D plays an important role and maintains a healthy skeletal structure and it is very essential for bone cells growth and development. Naturally, it is synthesized presence of sunlight in the skin and has multidisciplinary functions, which regulate the functions in different organs viz. brain, liver, lungs, heart, skeletal, kidneys, immune and reproductive systems. Moreover, it has significant anti-inflammatory, anti-aging, anti-stress, anti-arthritis, anti-osteoporosis, anti-apoptotic, wound healing, anti-cancer, anti-psychotic and anti-fibrotic actions [1]. Vitamin D receptors (VDRs) are widely distributed in most of the body organs viz. brain, liver, heart, lungs, kidney, pancreas, large and small intestines, muscles, reproductive, nervous system, etc. Vitamin D receptors influence cell-to-cell communication, normal cell growth, cell differentiation, cell cycling and proliferation, hormonal balance, neurotransmission process, skin health, immune and cardiovascular functions [2]. Nowadays due to aging, indiscriminate use of sunscreen, and change of zenith angle of sun

the production of vitamin D₃ has reduced [3]. Increasing age is not only decrease in bone marrow and muscle strength but also marked changes in the immune and inflammatory responses [4].

Vitamin D₃ deficiency causes metabolic bone diseases like osteomalacia and exacerbate osteoporosis, etc. [5]. In the today world, the metabolic bone disorders are very prone to post-menopausal women leads to an increased risk of fractures [6]. Hence, calcium and alkaline phosphatase (ALP) levels in post-menopausal women are the main two vital biochemical markers. While, bone-specific ALP is the most important marker for osteoblast differentiation [7]. Vitamin D has a great impact in forming and maintaining strong bones [8,9]. Collagen is a major structural protein responsible for bone calcification. In the aging condition, the mechanical properties of the bones become hampered and the bones get fragile, that causes various clinical disorders associated with bone collagen abnormalities and

bone fragility, such as osteogenesis imperfecta and osteoporosis [10,11].

A huge scientific reports and clinical trials have supported the impact of Biofield Therapy, which have shown to enhance immune function [12], massage therapy [13], etc. Complementary and Alternative Medicine (CAM) therapies are now increasing trend as a preferred model of treatment, among which Energy Therapy (or Healing Modalities) is one of them to accelerate the physical, mental and emotional human wellness. Based on data from the National Health Interview Survey (NHIS) reported that highest percentage (approximately 18%) of the Americans used dietary supplements as a complementary health approach as compared with other practices.

The National Center of Complementary and Integrative Health (NCCIH) has recognized and accepted Biofield Energy Healing as a CAM health care approach in addition to other therapies, medicines and practices such as deep breathing, yoga, hypnotherapy, natural products, progressive relaxation, Qi Gong, chiropractic/osteopathic manipulation, meditation, pilates, massage, special diets, homeopathy, guided imagery, acupressure, aromatherapy, Tai Chi, acupuncture, essential oils, relaxation techniques, traditional Chinese herbs and medicines, healing touch, movement therapy, rolfing structural integration, mindfulness, Ayurvedic medicine, naturopathy, Reiki, and cranial sacral therapy. Human Biofield Energy has subtle energy that has the capacity to work in an effective manner [14]. CAM therapies have been extensively practiced worldwide with reported clinical benefits in different health disease profiles [15]. This energy can be harnessed and transmitted by the experts into living and non-living things via the process of Biofield Energy Transmission.

The impact of The Trivedi Effect® has been contributed in various peer-reviewed scientific journals with excellent findings in many scientific fields viz. cancer research [16,17], microbiology [18-21], biotechnology [22,23], pharmaceutical science [24-27], agricultural science [28-31], materials science [32-35], nutraceuticals [36,37], skin health, human health and wellness. Depending on the literature and importance of vitamin D₃ on bone health, authors designed this experiment for bone health activity with respect to ALP, collagen content, and bone mineralization using standard assays in MG-63 cells.

Materials and Methods

Chemicals and Reagents

Fetal bovine serum (FBS) and Dulbecco's Modified Eagle's Medium (DMEM) were purchased from Life Technology, USA. Rutin hydrate was purchased from TCI, Japan, while vitamin D₃ and L-ascorbic acid were obtained from Sigma-Aldrich, USA. Antibiotic 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. Other chemicals used in this experiment were analytical grade procured from India.

Cell Culture

The human bone osteosarcoma cell line (MG-63) was used as the test system in the present study. The MG-63 cell line was maintained under the DMEM growth medium for routine culture and supplemented with 10% FBS. Growth conditions were maintained as 37°C, 5% CO₂ and 95% humidity and subcultured by trypsinisation followed by splitting the cell suspension into fresh flasks and supplementing with fresh cell growth medium. Three days before the start of the experiment (i.e., day -3), the growth medium of near-confluent cells was replaced with fresh phenol-free DMEM, supplemented with 10% charcoal dextran stripped FBS (CD-FBS) and 1% penicillin-streptomycin [38].

Experimental Design

The experimental groups consisted of cells in baseline control, vehicle control groups (0.05% DMSO with Biofield Energy Treated and untreated DMEM), a 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-DMEM + 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 items (vitamin D₃ and DMEM) were divided into two parts. One part each of the test item were treated with the Biofield Energy (The Trivedi Effect®) and coded as the Biofield Energy Treated items, while the second part did not receive any sort of treatment and was defined as the untreated samples. This Biofield Energy Healing Treatment was provided by Gopal Nayak, who participated in this study and performed the Biofield Energy Treatment remotely for ~5 minutes. Gopal Nayak was remotely located, while the test samples were in the research laboratory of Dabur Research Foundation, New Delhi, India. The Biofield Energy Treatment was administered for 5 minutes through the healer's unique Energy Transmission process to the test samples under laboratory conditions. Healer in this study, never visited the laboratory in person, nor had any contact with the test items. Further, the control group was treated with a sham healer for better 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

MTT assay was used for the evaluation of viable cells in MG-63 cells after treatment with Biofield Energy Treated and untreated test samples. The details procedure of cell viability assay was followed by Lauree et al. (2018) with slight modification [39]. The cytotoxicity of each tested concentration of the test items was calculated with the help of Equation (1):

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

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 $\geq 70\%$ cell viability was considered as non-cytotoxic [40].

Assessment of Alkaline Phosphatase (ALP) Activity

The level of ALP enzyme 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 Krista et al. [41]. The percent increase in ALP activity with respect to the untreated cells was calculated using Equation (3):

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

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 level of collagen 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 Lorraine et al. with few modifications [42]. The increase collagen level with respect to the untreated cells was calculated using Equation (4):

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

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. [43]. The percentage increase in bone mineralization compared to the untreated cells was calculated using 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 untreated group.

Statistical Analysis

All the values were represented as percentage of the respective parameters. For statistical analysis Sigma-Plot (version 11.0) was used as a statistical tool. Statistically significant values were set at the level of $p \leq 0.05$.

Results and Discussion

Measurement of Non-cytotoxic Concentrations by MTT Assay

The cell viability results of the Biofield Energy Treated vitamin D₃ and DMEM medium using MTT assay in MG-63 cells are shown in (Figure 1). The data indicated that the test samples in combination did not exhibit any cytotoxicity (as evidence of cell viability approximately greater than 74%) across all the tested concentrations upto 100µg/mL. Hence, the same concentrations were assessed further to see the effect of the test samples on the levels of alkaline phosphatase (ALP) activity, collagen synthesis, and bone mineralization in MG-63 cells.

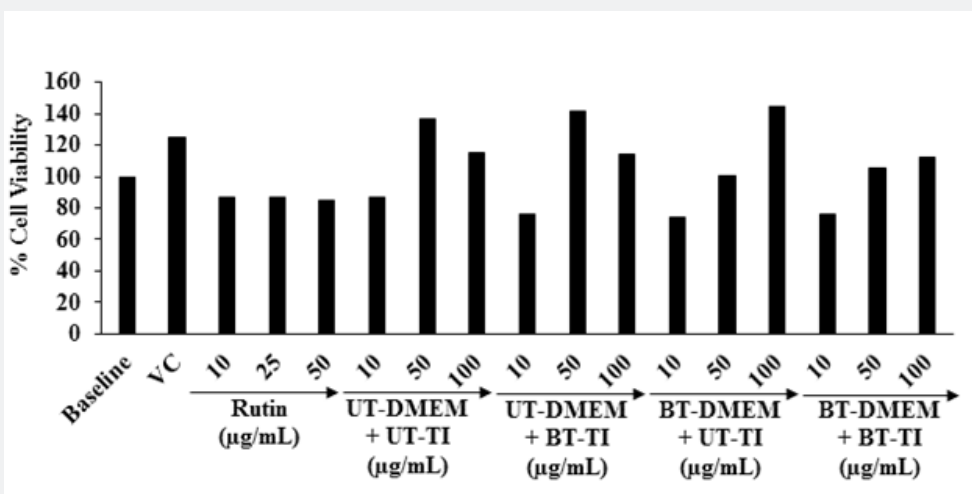


Figure 1: The effect of the Biofield Energy Treatment on the cell viability of the test samples (vitamin D₃ and DMEM medium) in different tested concentrations in MG-63 cells after 72 hours of treatment. VC: Vehicle control (0.05% DMSO); UT: Untreated; BT: Biofield Energy Treated; TI: Test item.

Assessment of Alkaline Phosphatase (ALP) Activity

The effect of the Biofield Energy Healing Treatment on the level of alkaline phosphatase (ALP) in human bone osteosarcoma

cells (MG-63) is shown in the (Figure 2). The level of ALP was 8.8% in the vehicle control (VC) group as compared to the baseline control (untreated cells) group. The ALP activity was significantly

increased in a dose dependent manner by 38.78%, 43.61%, and 80.92% in the positive control group at the concentration of 0.01, 0.1, and 1µg/mL, respectively compared to the untreated cells group. The level of ALP was significantly increased by 100.06%, 213.46%, and 218.66% in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item groups, respectively at the concentration of 1 µg/mL compared to the UT-DMEM + UT-Test item group. Moreover, the level of ALP was significantly

increased by 95.98%, 200.79%, and 187.09% in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item groups, respectively at the concentration of 10 µg/mL compared to the UT-DMEM + UT-Test item group. Additionally, ALP data showed a significant increased level by 24.46%, 101.75%, and 83.55% at 50 µg/mL in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item groups, respectively compared to the UT-DMEM + UT-Test item group.

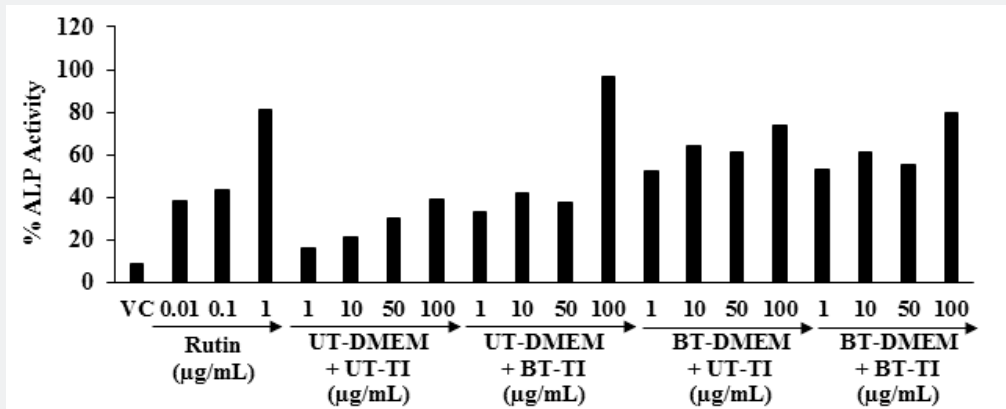


Figure 2: The effect of the Biofield Energy Treated test samples on alkaline phosphatase (ALP) enzyme activity in human bone osteosarcoma cell. VC: Vehicle control (0.05% DMSO), UT: Untreated; BT: Biofield Energy Treated, TI: Test item.

Further, the ALP level was significantly elevated by 147.75%, 90.25%, and 105.29% in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item groups, respectively at 100µg/mL compared to the UT-DMEM + UT-Test item group. Overall, the Consciousness Energy Healing Treated (The Trivedi Effect®) test item group (i.e., vitamin D₃) showed an improved synthesis of ALP level in the human osteosarcoma cells with respect to the UT-DMEM + UT-Test item group. Osteoporosis is characterized by reduced bone mass, quality and strength, changes in skeletal micro-architecture, and increased fracture risk. Osteoblast cells play an important role in bone formation through

the process of proliferation and differentiation. Osteoblastic differentiation is a complex process of sequential expression of marker proteins such as alkaline phosphatase (ALP), osteocalcin and osteopontin [44-46]. In this experiment, authors want to find out the impact of Biofield Energy Healing Treatment approach an anabolic agent on the stimulation of ALP, a potential marker of bone cell differentiation. In this experiment, it was also evident that the Biofield Energy Treated vitamin D₃ group significantly increased the level of ALP expression, which might be very advantageous to maintain a healthy skeletal structure for the patients suffering from various bone related disorders.

Assessment of Collagen Activity

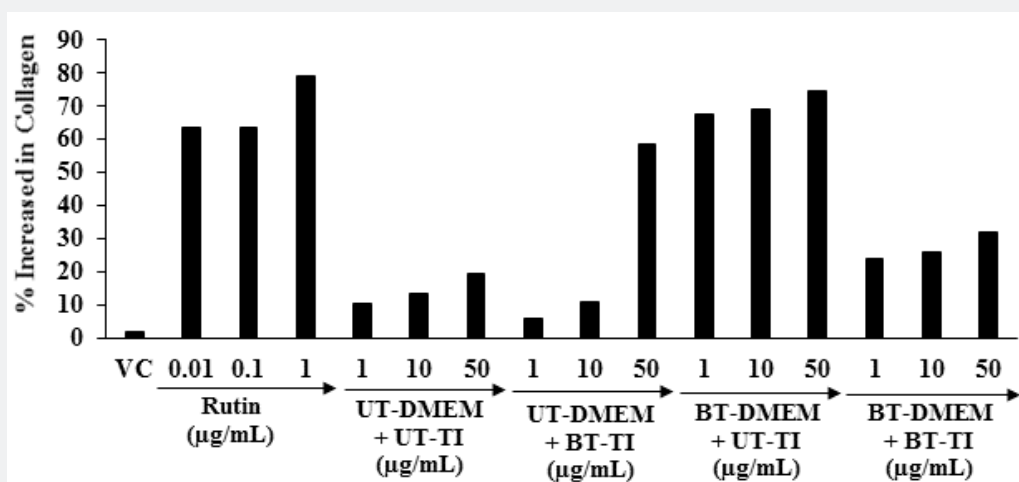


Figure 3: The effect of the test samples on collagen activity in human bone osteosarcoma cells. VC: Vehicle control (0.05% DMSO), UT: Untreated; BT: Biofield Energy Treated, TI: Test item.

The effect of the test samples on the collagen content in human bone osteosarcoma cells is shown in (Figure 3). The level of collagen in the VC group was observed as 2.2% as compared to the untreated cells group. Additionally, the level of collagen synthesis was significantly increased by 63.4%, 63.8%, and 79.4% at 0.01, 0.1, and 1µg/mL, respectively in the positive control group compared to the untreated cells group. The collagen synthesis was significantly increased by 551.25% and 130.31% in the BT-DMEM + UT-Test item and BT-DMEM + BT-Test item groups, respectively at 1 µg/mL compared to the UT-DMEM + UT-Test item group. The collagen synthesis was significantly increased by 401.97% and 89.51% in the BT-DMEM + UT-Test item and BT-DMEM + BT-Test item groups, respectively at 10 µg/mL compared to the UT-DMEM + UT-Test item group. Moreover, the collagen level was significantly increased by 197.57%, 276.82%, and 63.41% in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item groups, respectively at 50 µg/mL compared to the UT-DMEM + UT-Test item group (Figure 3). Overall, the Consciousness Energy Healing based test item group (i.e., vitamin D₃) showed an improved synthesis of collagen content in the human osteosarcoma cells with respect to all the treatment groups.

Assessment of Collagen Activity

The effect of the test samples on the collagen content in human bone osteosarcoma cells is shown in (Figure 3). The level of collagen in the VC group was observed as 2.2% as compared to the untreated cells group. Additionally, the level of collagen synthesis was significantly increased by 63.4%, 63.8%, and 79.4% at 0.01, 0.1, and 1µg/mL, respectively in the positive control group compared to the untreated cells group. The collagen synthesis was significantly increased by 551.25% and 130.31% in the BT-DMEM + UT-Test item and BT-DMEM + BT-Test item groups, respectively at 1 µg/mL compared to the UT-DMEM + UT-Test item group. The collagen synthesis was significantly increased by 401.97% and 89.51% in the BT-DMEM + UT-Test item and BT-DMEM + BT-Test item groups, respectively at 10 µg/mL compared to the UT-DMEM + UT-Test item group. Moreover, the collagen level was significantly increased by 197.57%, 276.82%, and 63.41% in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item groups, respectively at 50 µg/mL compared to the UT-DMEM + UT-Test item group (Figure 3). Overall, the Consciousness Energy Healing based test item group (i.e., vitamin D₃) showed an improved synthesis of collagen content in the human osteosarcoma cells with respect to all the treatment groups.

BT-Test item groups, respectively at 10 µg/mL compared to the UT-DMEM + UT-Test item group. Moreover, the collagen level was significantly increased by 197.57%, 276.82%, and 63.41% in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item groups, respectively at 50 µg/mL compared to the UT-DMEM + UT-Test item group (Figure 3). Overall, the Consciousness Energy Healing based test item group (i.e., vitamin D₃) showed an improved synthesis of collagen content in the human osteosarcoma cells with respect to all the treatment groups.

The bone health depends on both the quantity as well as quality of bone tissue components. Apart from minerals, collagen has an important role in both health. Bone loss is due to an imbalance of bone formation and resorption and excessive activity of osteoclasts on osteoblasts that leads to increased bone remodeling especially in women after menopause [47]. Bone cells replication and differentiation is occurred due to change in bone collagen synthesis [48]. Based on the literature, it is to speculate that a collagen-enriched diet can provides benefits for the skeleton system specially to collagen-derived products such as gelatin or hydrolyzed collagen (HC) [49]. Henceforth, to maintain a healthy bone an adequate concentration of vitamin D is required in humans. In this experiment, the Biofield Energy Treated vitamin D₃ significantly enhanced the synthesis of collagen in the MG-63 cells. This improvement of collagen content might be due to The Trivedi Effect® - Consciousness Energy Healing Treated vitamin D₃, which could be through various pathways viz. increased formation of AGE, activation of lysyl oxidase or due to post-translational modifications of type I collagen [50]. Overall, The Trivedi Effect® - Consciousness Energy Healing Treatment modality showed a significant improvement of the collagen level in human osteosarcoma cells. Thus, it is assumed that The Trivedi Effect® has the potential to improve the bone health in various skeletal disorders.

Assessment of Bone Mineralization by Alizarin Red S (ARS) Staining

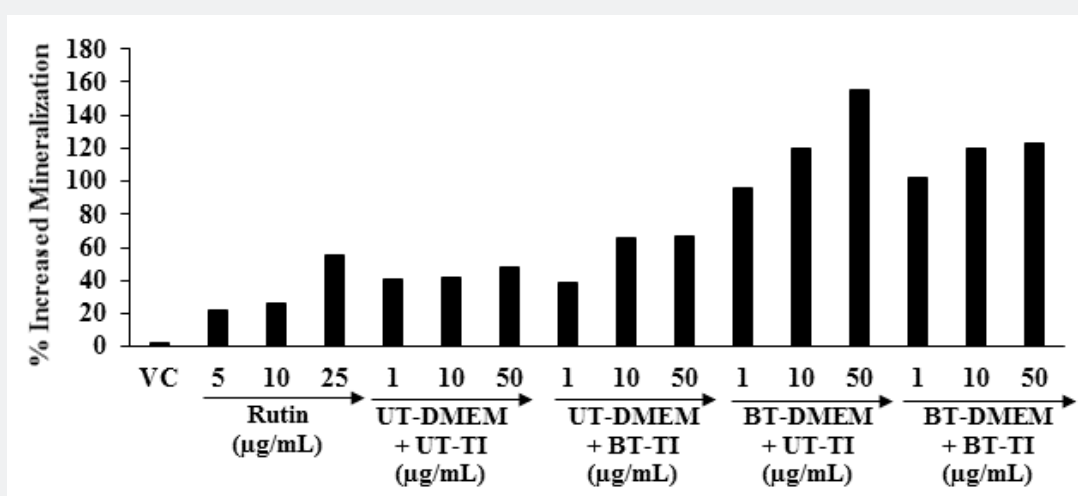


Figure 4: The effect of the Biofield Energy Treated test samples on human bone osteosarcoma cells for the assessment of bone mineralization activity. VC: Vehicle control (0.05% DMSO), UT: Untreated; BT: Biofield Energy Treated, TI: Test item.

The Alizarin red S (ARS) staining is mostly used for the assessment of calcium-rich deposits or crystals in the cell culture study. This is a versatile, sensitive, and semi-quantitative method has the greatest sensitivity for the detection of calcium pyrophosphate crystals based on the sensitivity of crystals that are stained weakly or strongly birefringent [51-53]. Vitamin D has an important role in skeletal system and required for normal bone formation and bone mineralization. Another study underpins a critical role of vitamin D in normal calcium and bone/tooth/growth plate homeostasis and improved bone mineralization [54]. The effect of the test samples on the percentage increase of bone mineralization in the different treatment groups are shown in (Figure 4). The percentage of bone mineralization was significantly increased in a concentration dependent manner by 22.5%, 26.6%, and 55.5% at 5, 10, and 25µg/mL, respectively in the positive control group compared to the untreated cells group. The percent of bone mineralization was distinctly increased by 135.52% and 150.71% in the BT-DMEM + UT-Test item and BT-DMEM + BT-Test item groups, respectively at 1 µg/mL compared to the UT-DMEM + UT-Test item group.

The percent of bone mineralization was distinctly increased by 56.55%, 186.86%, and 188.23% in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item groups, respectively at 10 µg/mL compared to the UT-DMEM + UT-Test item group. Further, a noticeably increased percentage of bone mineralization by 39.79%, 224.04%, and 156.72% in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item groups, respectively was observed at 50 µg/mL with respect to the UT-DMEM + UT-Test item group (Figure 4). Thus, based on the above outcomes it is postulated that the Consciousness Energy Healing Treatment (The Trivedi Effect®) based test item groups (i.e., vitamin D₃) showed a remarkable improvement of bone mineralization content assessed by *in vitro* in the human osteosarcoma cells (MG-63) with respect to the all others treatment groups.

Conclusion

The MTT cell viability assay data showed more than 74% cells were viable, which indicated that the test samples were safe and nontoxic in all the tested concentrations. ALP was significantly increased by 100.06%, 213.46%, and 218.66% in UT-DMEM + BT-Test item, BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item, respectively at 1 µg/mL compared to the UT-DMEM + UT-Test item group. ALP was significantly increased by 95.98%, 200.79%, and 187.09% in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item, respectively at 10 µg/mL compared to the UT-DMEM + UT-Test item group. Further, the ALP level was significantly elevated by 147.75%, 90.25%, and 105.29% in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item groups, respectively at 100 µg/mL compared to the untreated group.

The collagen synthesis was significantly increased by 551.25% and 130.31% in the BT-DMEM + UT-Test item and BT-

DMEM + BT-Test item groups, respectively at 1 µg/mL compared to the UT-DMEM + UT-Test item group. Collagen was significantly increased by 401.97% and 89.51% in the BT-DMEM + UT-Test item and BT-DMEM + BT-Test item groups, respectively at 10 µg/mL compared to the untreated group. Further, the collagen level was significantly increased by 197.57% and 276.82% in UT-DMEM + BT-Test item and BT-DMEM + UT-Test item, respectively at 50 µg/mL compared to untreated. Further, the percent of bone mineralization was distinctly increased by 186.86% and 188.23% in the BT-DMEM + UT-Test item and BT-DMEM + BT-Test item, respectively at 10 µg/mL compared to the untreated group. The percentage of bone mineralization notably increased by 224.04% and 156.72% in the BT-DMEM + UT-Test item and BT-DMEM + BT-Test item, respectively at 50 µg/mL with respect to the untreated group.

Altogether, the Biofield Energy Treated test samples (The Trivedi Effect®) demonstrated a significant impact on bone health parameters. Therefore, the Consciousness Energy Healing based vitamin D₃ might be suitable for the development of an alternative and more effective supplement for vitamin D₃ deficiency, which could be useful for the management of various bone related disorders *viz.* low bone density and osteoporosis, osteogenesis imperfecta, Paget's disease of bone, rickets, osteomalacia, bone and joint pain, bone fractures, deformed bones, osteoma, chondrodystrophia fetalis, etc. Besides, it can also be utilized in organ transplants (for example kidney transplants, liver transplants and heart transplants), various autoimmune disorders such as Addison Disease, Graves' Disease, Hashimoto Thyroiditis, Multiple Sclerosis, Myasthenia Gravis, Pernicious Anemia, Aplastic Anemia, Reactive Arthritis, Rheumatoid Arthritis, Systemic Lupus Erythematosus, Type 1 Diabetes, Alopecia Areata, Crohn's Disease, Psoriasis, Vasculitis, as well as inflammatory disorders such as Asthma, Ulcerative Colitis, Irritable Bowel Syndrome, anti-inflammatory, anti-stress, anti-arthritic, anti-osteoporosis, anti-apoptotic, wound healing, anti-cancer, Besides, it can also be utilized in stress management and prevention, and anti-aging by improving overall health.

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Conflict of Interest

Authors declare no conflict of interest.

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