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The Impact of Biofield Energy Treated Vitamin D_3 on the Structural Crosslinks, Maturation, and Bone Mineralization in Human Bone Osteosarcoma Cells (MG-63)

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Abstract

The objective was to investigate the potential of Biofield Treated vitamin D_3 and DMEM on bone markers. The test items (TIs) were separated into two parts. One part of each sample was received Consciousness Energy Treatment by Alice Branton and termed as Biofield Treated (BT) samples, while other parts of each sample denoted as untreated TI (UT). MTT data found TIs were safe and nontoxic at tested concentrations. ALP was significantly elevated by 390% and 200% in UT-DMEM + BT-TI at 0.1 and 1µg/mL, respectively; while, increased by 135.29% in BT-DMEM + BT-TI at 0.1µg/mL than UT-DMEM + UT-TI. ALP was significantly increased by 106.34%, 138.47%, and 212.68% in UT-DMEM + BT-TI, BT-DMEM + UT-TI, and BT-DMEM + BT-TI, respectively at 10µg/mL than untreated. Collagen was significantly increased by 288.68% and 106.96% in UT-DMEM + BT-TI and BT-DMEM + BT-TI, respectively at 10µg/mL than untreated. Further, collagen was significantly increased by 435.78% and 139.72% in UT-DMEM + BT-TI and BT-DMEM + UT-TI, respectively at 50µg/mL than untreated. Percent of bone mineralization was significantly increased by 299.13%, 239.91%, and 218.77% in BT-DMEM + BT-TI at 0.1, 1, and 10µg/mL, respectively than untreated. Besides, percent of bone mineralization was distinctly increased by 192.51% in BT-DMEM + UT-TI at 10µg/mL than untreated. Overall, Biofield Treated vitamin D_3 remarkably improved bone parameters in MG-63 cells and could be an alternative to vitamin D_3 deficiency and used various bone disorders *viz.* low bone density, osteoporosis, autoimmune and inflammatory diseases, stress management and prevention, and anti-aging by improving overall health.

Keywords: Consciousness Energy Healing; The Trivedi Effect®; Biofield Energy Healing Treatment; Osteosarcoma cells (MG-63); ALP; Collagen; Bone mineralization

Abbreviations: CAM: Complementary and alternative medicine, MG-63: Human Bone Osteosarcoma Cells, ALP: Alkaline phosphatase, AGE: Advanced glycation end products, NHIS: National Health Interview Survey, DMEM: Dulbecco's modified eagle's medium, NCCIH: National Center of Complementary and Integrative Health, FBS: Fetal bovine serum, UT: Untreated, BT: Biofield Energy Treated

Introduction

Vitamin D has multiple effects in different organs *viz.* liver, heart, brain, kidneys, lungs, etc. It also possess a significant anti-aging, anti-inflammatory, anti-osteoporosis, anti-stress, anti-arthritic, anti-apoptotic, anti-cancer, wound healing, anti-fibrotic actions and anti-psychotic [1]. Vitamin D receptors (VDRs) are present in different organs *viz.* liver, brain, kidneys, heart, pancreas, lungs, muscles, large and small intestines, nervous system, reproductive, etc. VDRs can influence communication from one cell to another cell, neurotransmission, cell growth and differentiation, cell cycling and proliferation, immune and cardiovascular functions, hormonal balance, skin health, etc. Most of the living vertebrates, vitamin D is essential for maintaining a good skeletal structure and bone. Naturally, it is synthesized in presence of sunlight in the skin [2]. Most of foods vitamin D is almost absent. However, in current scenario due to several factors like aging, over use of sun protectives, and also due to alteration of zenith angle of sun, etc. the production of vitamin D_3 has drastically reduced [3]. It was well established that aging causes bone marrow depression and also reduced muscle strength, that ultimately great impact on the immune and inflammatory responses [4]. Deficiency of vitamin D_3 causes metabolic bone diseases like osteomalacia and exacerbate osteoporosis, etc. [5]. The quality of life for menopause women is one of the most critical health problem in the today world. The metabolic bone disorder like osteoporosis is mainly prevalent in post-menopausal women. Due to hormonal factors and a rapid bone loss in post-menopausal women that lead to an increased risk of fracture of bones [6]. Therefore, the serum calcium and alkaline phosphatase (ALP) levels are the two most suitable markers in post-menopausal women for the assessment of bone metabolism. Besides, bone-specific ALP

is also an important marker for osteoblast differentiation [7]. Further, increased calcium intake along with adequate supply of vitamin D is important for maintaining a good bone structure. Vitamin D also further responsible for maintaining an adequate level of serum calcium and phosphorus [8-9]. The strength of bone depends on the microarchitecture, quality, mineral content, and collagen content. Collagen is the primary structural protein mainly responsible for calcification of bone. In aged peoples, the mechanical properties of bone became reduced and the bone get became fragile, that causes various bone-related disorders associated with bone-collagen-abnormalities and bone fragility, such as osteogenesis imperfecta and osteoporosis [10-11].

In recent years, several scientific reports and clinical trials have revealed that the useful effects of the Biofield Energy Treatment, which has shown enhanced immune function in cases of cervical cancer patients with therapeutic touch [12], massage therapy [13], etc. Complementary and Alternative Medicine (CAM) therapies are now rising as preferred models of treatment, among which Biofield Therapy (or Healing Modalities) is one approach that has been reported to have several benefits to enhance physical, mental and emotional human wellness. However, the National Health Interview Survey (NHIS), 2012, reported approximately 20% Americans used dietary supplement as complementary health approached compared with conventional therapeutics. The National Center of Complementary and Integrative Health (NCCIH) has supported Biofield Energy Healing is a types of CAM health care approach in addition to other therapies, medicines and practices such as natural products, Qi Gong, Reiki, deep breathing, special diets, yoga, Tai Chi, homeopathy, chiropractic/osteopathic manipulation, acupuncture, meditation, massage, guided imagery, progressive relaxation, relaxation techniques, acupressure, healing touch, hypnotherapy, movement therapy, rolfing structural integration, pilates, Ayurvedic medicine, naturopathy, mindfulness, traditional Chinese herbs and medicines, aromatherapy, essential oils, and cranial sacral therapy. Human Biofield Energy has subtle form of energy that has the ability to work in an effective manner [14]. This energy can be harnessed from the environment and transmitted by the experts into both living and non-living things *via* the process of Biofield Energy Healing Treatment. CAM therapies have been practiced worldwide with reported many clinical benefits in different disease conditions [15]. Biofield Energy Treatment (The Trivedi Effect®) has been published in numerous peer-reviewed science journals and contributes the significant outcomes in many scientific fields such as cancer research [16-17], microbiology [18-19], biotechnology [20-21], pharmaceutical science [22-25], agricultural science [26-27], materials science [28-31], nutraceuticals [32,33], skin health [34,35], bone health [36-37], human health and wellness.

Based on the literature information and importance of vitamin D_3 on bone health, authors designed a study to evaluate the impact of Biofield Energy Treatment (The Trivedi Effect®) on the test samples (vitamin D_3 and DMEM) for bone health activity with respect to ALP, collagen content, and bone mineralization using standard assay in MG-63 cells.

Materials and Methods

Chemicals and reagents

Rutin hydrate was purchased from TCI, Japan, while vitamin D_3 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. 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. 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 a test system in this experiment. The MG-63 cells was maintained with DMEM for routine culture and supplemented with 10% FBS. At 37 °C, 5% CO_2, and 95% humidity were maintained as growth conditions and sub-cultured by trypsinisation followed by splitting the cell suspension into fresh flasks and supplementing with fresh cell growth medium. Three days prior start of the study (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 (untreated cells group), vehicle control groups (0.05% DMSO with Biofield Energy Treated and untreated DMEM), positive control group (rutin hydrate) and experimental test groups. Experimental groups included the combination of the Biofield Energy Treated and untreated vitamin D_3/DMEM. It consisted of four major treatment groups on specified cells with UT-DMEM + UT-Test item, UT-DMEM + Biofield Energy Treated test item (BT-TI), BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item.

Biofield energy healing strategies

The test items (vitamin D_3 and DMEM) were divided into two parts. One part each test item was treated with Biofield Energy by a renowned Biofield Energy Healer (The Trivedi Effect®) and referred as Biofield Treated test items and the second part did not receive any treatment and was defined as untreated test items. This Biofield Energy Healing Treatment was provided by the renowned Biofield Energy Healer, Alice Branton, who participated in this study and performed the Biofield Energy Treatment remotely for ~5 minutes through the Healer's unique Energy Transmission process under laboratory conditions. Healer remotely located in USA, while the test samples were located in research laboratory of Dabur Research Foundation, New Delhi, India. Healer, 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 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.

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MTT assay

The cell viability was performed using MTT assay in MG-63 cells. The details procedure of cell viability assay was followed by Karen BA et al.[39] with slight modification. The cytotoxicity of each tested concentration of the test items was calculated with the help of Equation (1):

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

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):

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

The concentration exhibiting ≥70% cell viability was appraise as non-cytotoxic [40].

Alkaline phosphatase (ALP) activity

The effect of the Biofield Energy Treatment on the test items for the evaluation of ALP activity in MG-63 cells. The procedure of cell counting, plating, and treatment was followed as per Liu SC, et al. [41]. The percent increase in ALP activity with respect to the untreated cells was calculated using Equation (3):

$$\% \text{ Increase in ALP} = \left\{\frac{1-X}{R}\right\} * 100 \quad (3)$$

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

R = Absorbance of cells corresponding to untreated cells

Collagen activity

The MG-63 cells were used for the evaluation of the potential of Biofield Energy Treated test items and the procedure in details was as per Parulkar VR et al. [42] with few modifications. The increase collagen level with respect to the untreated cells was calculated using Equation (4):

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

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

R = Collagen levels in cells corresponding to untreated cells

Bone mineralization activity

Evaluation of the percent increased of mineralization after treatment of the Biofield Treated test items in MG-63 cells, and the details steps were followed according to Slade TC et al. [43]. The percentage increase in bone mineralization compared to the untreated cells was calculated using Equation (5):

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

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

Statistical analysis

The obtained data were expressed as percentage (%) of the respective study parameters. Sigma-Plot (version 11.0) was used as a statistical tool for data interpretation. 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 Biofield Energy Treated vitamin D_3 and DMEM medium using MTT assay in MG-63 cells are presented 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 81%) across all the tested concentrations upto 100µg/mL. Hence, the non-cytotoxic concentrations were used for the evaluation of alkaline phosphatase (ALP) activity, collagen synthesis, and bone mineralization in MG-63 cells.

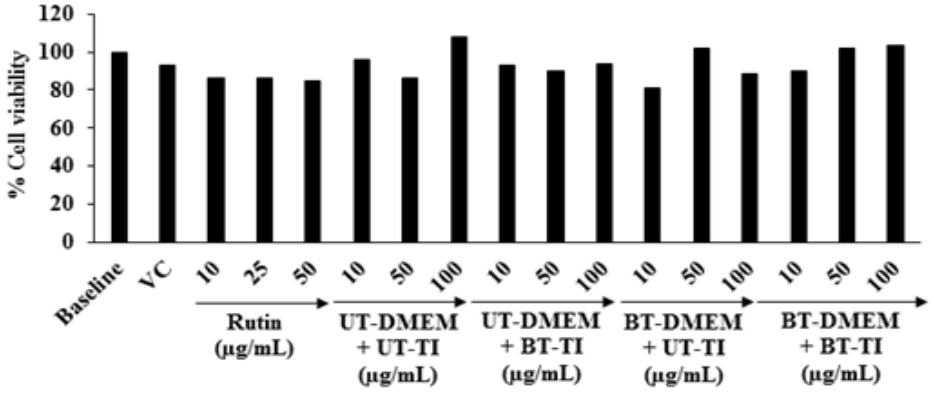

Figure 1: Percentage cell viability of the test samples (vitamin D3 and DMEM) in different concentrations in MG-63 cells after 72 hours of treatment. VC: Vehicle control; UT: Untreated; BT: Biofield Energy Treated, TI: Test item.

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Alkaline phosphatase (ALP) activity

The effect of the Biofield Energy Treatment on alkaline phosphatase (ALP) enzyme activity in MG-63 cells is shown in Figure 2. The level of ALP was found as 15% in the vehicle control (VC) group compared to the untreated cells group. The ALP activity was significantly increased in a dose dependent manner by 30.02%, 34.31%, and 51.47% in the positive control group at the concentration of 0.001, 0.01, and 0.1µg/mL, respectively compared to the untreated cells group. The level of ALP was significantly increased by 390%, 90%, and 10% in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item groups, respectively at the concentration of 0.1µg/mL compared to the UT-DMEM + UT-Test item group. Additionally, at 1µg/mL the level of ALP was significantly increased by 200% and 135.29% in the UT-DMEM + BT-Test item and BT-DMEM + BT-Test item groups, respectively compared to the UT-DMEM + UT-Test item group. Moreover, the level of ALP was significantly increased by 106.45%, 138.71%, and 212.9% in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item groups, respectively at the concentration of 10µg/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 D_3) showed an improved synthesis of ALP level in the human osteosarcoma cells with respect to the UT-DMEM + UT-Test item group. The ALP are membrane-bound ectoenzymes. Based on the numerous literature, reported that ALP plays an important role for bone differentiation, maturation, osteogenesis, and calcification process [44,45]. The enzymes split-up organic phosphate to formed inorganic phosphate (p_i), which thus combined with soluble calcium ion (Ca^{2+}) present in the tissue fluids and formed calcium phosphate. After reaching sufficient concentration of calcium phosphate it chemically precipitated into osteoid [46,47]. Here, the results support that the Biofield Treated vitamin D_3 significantly increased the level of ALP expression, which might be very advantageous to maintain a healthy skeletal structure to the patients suffering from various bone-related disorders.

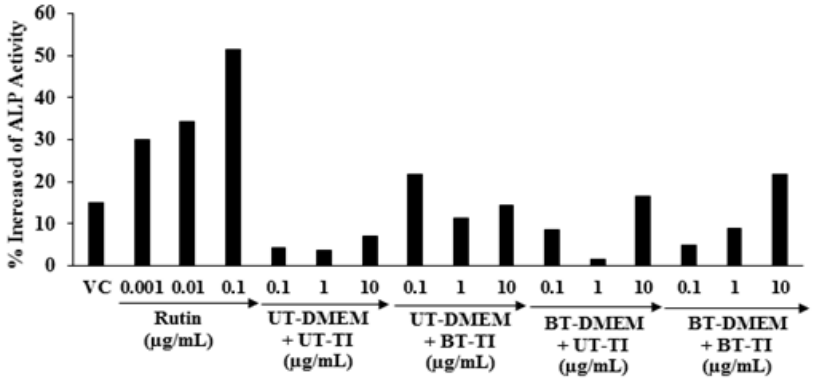

Figure 2: The effect of the Biofield Energy Treatment on test samples in human bone osteosarcoma cell for the assessment of alkaline phosphatase (ALP) enzyme activity. VC: Vehicle control, UT: Untreated; BT: Biofield Energy Treated, TI: Test item.

Collagen activity

The effect of the test samples on collagen in human bone osteosarcoma cell is demonstrated in Figure 3. The level of collagen in the VC group was observed as 24.5% as compared to the untreated cells group. Besides, 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 as compared to the untreated cells group. The collagen synthesis was significantly increased by 288.68%, 58.70%, and 106.96% in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item groups, respectively at 10µg/mL compared to the UT-DMEM + UT-Test item group. Moreover, the collagen level was significantly increased by 435.78%, 139.72%, and 70.63% in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item groups, respectively at 50µg/mL compared to the UT-DMEM + UT-Test item group. Additionally, at 100µg/mL the level of collagen was also significantly increased by 34.18%, 8.51%, and 9.27% in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item groups, respectively than untreated group (Figure 3). Thus, the Biofield Treated vitamin D_3 showed an improved synthesis of collagen fibers in the human osteosarcoma cells. The bone health depends on both the quantity as well as quality of bone tissue components. Apart from minerals, the collagen that has an important role in both health. The stability and maturation of collagen depends on the nature of cross-links of collagen microstructure. This cross-linking occurs through post translational modification of collagen fibers through either in enzymatic (i.e., activation of lysyl oxidase) and non-enzymatic process through the formation of advanced glycation end products (AGE) [48,49]. In disease state like osteogenesis imperfecta and osteoporosis there was an abnormality of collagen synthesis, stability and maturation [50,51]. From literature, it was reported that the collagen crosslink provides one of the earliest and most sensitive indications of a bone disturbance due to vitamin D deficiency and it also suggest that vitamin D specifically acts to increase the rate of maturation of bone collagen [52]. Henceforth, to maintain a healthy bone adequate concentration of vitamin D is required in human. In this experiment, the Biofield Energy Treated vitamin D_3 was significantly enhanced the synthesis of collagen in MG-63 cells. These improvements of collagen content

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might be due to The Trivedi Effect® - Treated vitamin D_3, 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. Another literature described that the osteoblast differentiation and maturation are crucial events in the formation of new bone tissue and for the determination of bone quality due to the synthesis of collagen [53]. Overall, The Trivedi Effect® - Consciousness Energy Healing Treatment modality showed a significant improvement of collagen level in human osteosarcoma cells. Thus, results suggested that The Trivedi Effect® has the potential to improve the bone health in various skeletal disorders.

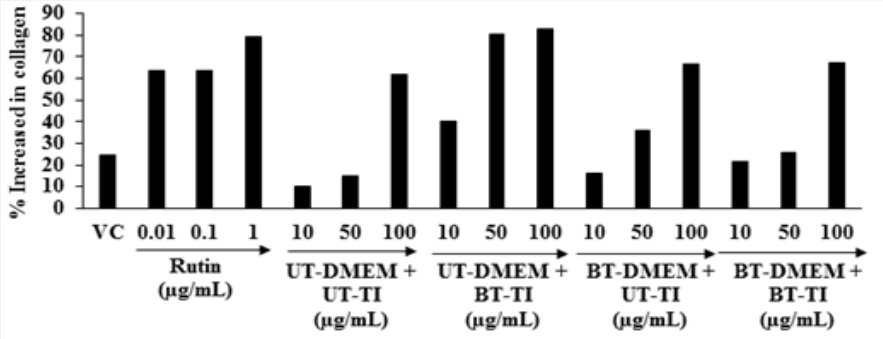

Figure 3: The effect of the test samples on collagen activity in human bone osteosarcoma cells. VC: Vehicle control, UT: Untreated; BT: Biofield Energy Treated, TI: Test item.

Assessment of bone mineralization by alizarin red s (ars) staining

The Alizarin red S (ARS) staining is widely utilized for the assessment of calcium-rich deposits or calcium crystals in the cell culture study. This is a versatile, sensitive, and semi-quantitative method in which the dye can be extracted from the stained monolayer and assayed [54,55]. ARS has the greatest sensitivity for the detection of calcium pyrophosphate crystals based on the sensitivity of crystals are stained weakly or strongly birefringent [56]. The effect of the test samples on the percentage increase of bone mineralization in the different treatment groups are shown in Figure 4. The bone mineralization in human bone osteosarcoma cell is shown in Figure 4. The level of collagen in the VC group was observed as 2% as compared to the untreated cells group. 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 significantly enhanced by 16.33%, 125.66%, and 299.13% in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item groups, respectively at 0.1µg/mL compared to the UT-DMEM + UT-Test item group. Additionally, the level of bone mineralization was remarkably elevated by 0.67%, 144.17%, and 239.91% in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item groups, respectively at 1µg/mL compared to the UT-DMEM + UT-Test item group. The percent of bone mineralization was distinctly increased by 17.51%, 192.51%, and 218.77% in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item groups, respectively at 10µg/mL compared to the UT-DMEM + UT-Test item group. Further, a noticeably increased percent of bone mineralization by 2.09%, 163.97%, and 183.39% in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item groups, respectively was observed at 50µg/mL with respect to the UT-DMEM + UT-Test item group. In addition to, the percent of bone mineralization data showed a significantly increased by 140.66%, 112.96%, and 150.86% in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item groups, respectively than the UT-DMEM + UT-Test item group (Figure 4) at 100µg/mL. Thus, based on the above outcomes suggested that the Consciousness Energy Healing Treatment (The Trivedi Effect®) based test item groups (i.e., vitamin D_3) showed a remarkably improvement of bone mineralization content assessed by *in vitro* in the human osteosarcoma cells (MG-63) with respect to the all others treatment groups.

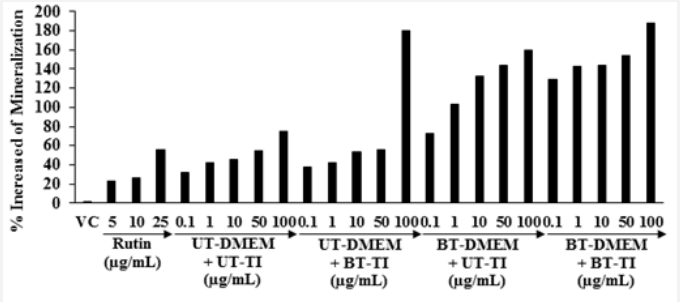

Figure 4: The effect of the Biofield Energy Treated test samples on human bone osteosarcoma cell for the assessment of bone mineralization activity. VC: Vehicle control, UT: Untreated; BT: Biofield Energy Treated, TI: Test item.

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Conclusion

The MTT cell viability assay data showed more than 81% cells were viable, which indicated that the test samples were safe and nontoxic in all the tested concentrations. ALP level was significantly elevated by 200% and 135.29% in the UT-DMEM + BT-Test item and BT-DMEM + BT-Test item groups, respectively at 1µg/mL while, it was also increased by 390% in the UT-DMEM + BT-Test item at 0.1µg/mL compared to the UT-DMEM + UT-Test item group. Further, ALP was significantly increased by 106.34%, 138.47%, and 212.68% in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item, respectively at 10µg/mL compared to the UT-DMEM + UT-Test item group. Collagen was significantly increased by 288.68% and 106.96% in the UT-DMEM + BT-Test item and BT-DMEM + B T-Test item groups, respectively at 10µg/mL compared to the untreated group. Further, the collagen level was significantly increased by 435.78%, 139.72%, and 70.63% in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item, respectively at 50µg/mL compared to the untreated group. The percent of bone mineralization was significantly enhanced by 125.66% and 299.13% in the BT-DMEM + UT-Test item and BT-DMEM + BT-Test item groups, respectively at 0.1µg/mL however, it was also increased by 144.17% and 239.91% in the BT-DMEM + UT-Test item and BT-DMEM + BT-Test item groups, respectively at 1µg/mL compared to the UT-DMEM + UT-Test item group. Besides, the percent of bone mineralization was distinctly increased by 192.51% and 218.77% in the BT-DMEM + UT-Test item and BT-DMEM + BT-Test item, respectively at 10µg/mL compared to the untreated group. A noticeably increased the percent of bone mineralization by 163.97% and 183.39% in the BT-DMEM + UT-Test item and BT-DMEM + BT-Test item, respectively at 50µg/mL with respect to the untreated group. Altogether, the Biofield Energy Treated test samples (The Trivedi Effect®) showed a significant impact on bone health parameters.

Therefore, the Consciousness Energy Healing-based vitamin D_3 might be suitable as supplement for vitamin D_3 deficiency, which could be useful for the management of various bone-related disorders *viz.* low bone density, osteomalacia, osteoporosis, rickets, osteogenesis imperfecta, osteoma, Paget's disease, deformed bones, chondrodystrophia fetalis, etc. Besides, it can also be used in organ transplants (kidney, liver, and heart transplants), autoimmune disorders (Addison Disease, Celiac Disease, Graves' Disease, Hashimoto Thyroiditis, Multiple Sclerosis, Systemic Lupus Erythematosus, Myasthenia Gravis, Type 1 Diabetes, Pernicious Anemia, Aplastic Anemia, Rheumatoid Arthritis, Alopecia Areata, Vitiligo, Psoriasis, Scleroderma, Chronic Fatigue Syndrome and Vasculitis, as well as inflammatory disorders (Ulcerative Colitis, Crohn's Disease, Irritable Bowel Syndrome). Further, it could be beneficial for the management of Alzheimer's Disease, Atherosclerosis, Dermatitis, Diverticulitis, and Hepatitis, anti-stress, anti-apoptotic, wound healing, anti-cancer, anti-psychotic and anti-fibrotic actions and anti-aging by improving overall health.

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