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## Functional Modification of Bone Tissues After Treatment with the Biofield Energy Treated Vitamin D<sub>3</sub> in Human Bone Osteosarcoma Cells (MG-63)

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### Abstract

The potential of Consciousness Energy Treated vitamin D<sub>3</sub> and DMEM in human bone osteosarcoma cells (MG-63) was investigated. The Test Items (TI), were separated into two parts. One part of each sample was received Biofield Energy Treatment by Joy Angevin Balmer and defined as Biofield Treated (BT) samples, while other parts of each sample were denoted as Untreated Test Items (UT). The cell viability results revealed test samples were found as safe upto 100 µg/mL. ALP was significantly increased by 84.62% in BT-DMEM + BT-TI at 0.1 µg/mL, while increased by 118.24% and 241.62% in UT-DMEM + BT-TI and BT-DMEM + UT-TI, respectively at 1 µg/mL than UT-DMEM + UT-TI. Moreover, ALP was significantly elevated by 43.56% and 192.08% in UT-DMEM + BT-TI and BT-DMEM + UT-TI, respectively at 10 µg/mL than untreated. Collagen was significantly increased by 112.24%, 83.68%, and 142.88% in UT-DMEM + BT-TI, BT-DMEM + UT-TI, and BT-DMEM + BT-TI, respectively at 0.1 µg/mL than untreated. Further, collagen was significantly elevated by 75.39%, 106.15%, and 113.87% in UT-DMEM + BT-TI, BT-DMEM + UT-TI, and BT-DMEM + BT-TI at 1 µg/mL than UT-DMEM + UT-TI. Besides, bone mineralization was remarkably increased by 106.2% and 95.20% at 100 µg/mL in BT-DMEM + UT-TI and BT-DMEM + BT-TI, respectively than untreated. Overall, Biofield Energy Treated vitamin D<sub>3</sub> was significantly improved bone health parameters and could be able to fight against various bone-related disorders (osteoporosis, rickets, low bone density, osteogenesis imperfecta), autoimmune and inflammatory diseases, stress, and anti-aging improving overall health.

**Keywords:** Biofield Energy Treatment; Bone health; Osteosarcoma cells; Osteoporosis; The Trivedi Effect<sup>®</sup>; Vitamin D

### Abbreviations

ALP : Alkaline Phosphatase

ATCC : American Type Culture Collection

BT : Biofield Energy Treated

CAM : Complementary and Alternative Medicine

ECM : Extracellular Matrix

DMEM : Dulbecco's Modified Eagle's Medium

FBS : Fetal Bovine Serum

NHIS : National Health Interview Survey

NCCIH : National Center of Complementary and Integrative Health

MG-63 : Human Bone Osteosarcoma Cells

TI : Test Item

UT : Untreated

### Introduction

Vitamin D has multiple effects, which regulate the functions

in different organs *viz.* brain, liver, lungs, heart, kidneys, skeletal, 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 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. In any living vertebrates, vitamin D plays an important role in maintaining a healthy skeletal structure and is essential for bone health. Naturally, it is synthesized in the presence of sunlight in the skin [2]. Most foods do not contain any vitamin D, additionally now-a-days due to aging, use of sunscreen, and change of zenith angle of sun the production of vitamin D<sub>3</sub> has reduced [3]. Increasing age is not only related to a decrease in bone marrow depression and muscle strength but is also associated with marked changes in the immune and inflammatory responses [4]. Deficiency of vitamin D<sub>3</sub> causes metabolic bone diseases like osteomalacia and exacerbate osteoporosis, etc. [5].

The quality of life for menopausal women is one of the most critical health problem in the today world. Metabolic bone disorders like osteoporosis are mainly prevalent in post-menopausal women. Hormonal factors and rapid bone loss in post-menopausal women leads to an increased risk of fractures [6]. Hence, the serum calcium and Alkaline Phosphatase (ALP) levels in post-menopausal women are the main two vital biochemical markers of bone metabolism. However, bone-specific ALP is the most important marker for osteoblast differentiation [7]. Further, it is generally accepted that an increased calcium intake along with an adequate source of vitamin D is important for maintaining good bone health. Vitamin D also plays an important role in maintaining an adequate level of serum calcium and phosphorus. Therefore, vitamin D has a great impact in forming and maintaining strong bones [8,9]. Bone strength depends on the quality, geometry, shape, microarchitecture, turnover, mineral content, and the collagen content. Collagen is the major structural protein responsible for bone calcification. In the aging state, the mechanical properties of the bones become impaired 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].

In recent years, several scientific reports and clinical trials have revealed the useful effects of Biofield Energy Treatments, which have shown to enhance immune function in cases of cervical cancer patients *via* 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, as per the data of 2012 from the National Health Interview Survey (NHIS), which indicated that the highest percentage (17.7%) of the Americans used dietary supplements as a complementary health approach as compared with other practices in past years. 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 natural products, deep breathing, yoga, Tai Chi, Qi Gong, chiropractic/osteopathic manipulation, meditation, massage, special diets, homeopathy, progressive relaxation, guided imagery, acupressure, acupuncture, relaxation techniques, hypnotherapy, healing touch, movement therapy, pilates, rolfing structural integration, mindfulness, Ayurvedic medicine, traditional Chinese herbs and medicines, naturopathy, essential oils, aromatherapy, 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 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 Healing. Biofield Energy Treatment (The Trivedi Effect<sup>®</sup>) has been published in numerous peer-reviewed science journals with significant outcomes in many scientific fields such as cancer research [16,17], microbiology [18-20], biotechnology [21, 22], pharmaceutical science [23-26], agricultural science [27-29], materials science [30-32], nutraceuticals [33,34], skin health [35,36], human health and wellness.

Based on the literature information and importance of vitamin D<sub>3</sub> on bone health, the authors sought to evaluate the impact of the Biofield Energy Treatment (The Trivedi Effect<sup>®</sup>) on the test samples (vitamin D<sub>3</sub> and DMEM medium) for bone health activity with respect to the assessment of different bone health parameters like 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 D<sub>3</sub> (denoted as test item) and L-ascorbic acid were obtained from Sigma-Aldrich, USA. Antibiotic solution (penicillin-streptomycin) was procured from HI Media, 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

The human bone osteosarcoma (MG-63) cell line was used as test system, maintained under the DMEM growth medium for routine culture and supplemented with 10% FBS. Growth conditions were maintained as 37°C, 5% CO<sub>2</sub> 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 [37].

## Experimental Design

The experimental groups consisted of cells in baseline control (untreated cells), vehicle control groups (0.05% DMSO with Biofield Energy Treated and untreated DMEM), a positive control group (rutin hydrate) and experimental test groups. Experimental test groups included the combination of the Biofield Energy Treated and untreated vitamin D<sub>3</sub>/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-Test item), BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item.

## Consciousness Energy Healing Treatment Strategies

The test item (vitamin D<sub>3</sub>) and DMEM were divided into two parts. One part each of the test item and DMEM were treated with the Biofield Energy (also known as 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 Joy Angevin Balmer, who participated in this study and performed the Biofield Energy Treatment remotely for ~5 minutes. Joy Angevin Balmer remotely located in the USA, while the test samples were located 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 remotely to the test samples under laboratory conditions. Biofield Energy Healer's in this study, never visited the laboratory in person, nor had any contact with the test item and medium. Further, the control group was treated with a sham healer for comparative purposes. The sham healer did not have any knowledge about the Biofield Energy Treatment. After that, the Biofield Energy Treated and untreated samples were kept in similar sealed conditions for experimental study.

## Determination of Non-Cytotoxic Concentration

The cell viability was performed by MTT assay in MG-63 cell line. The cells were counted and plated in a 96-well plate at

the density corresponding to 5 X 10<sup>3</sup> to 10 X 10<sup>3</sup> cells/well/180 µL of cell growth medium. The above cells were incubated overnight under growth conditions and allowed for cell recovery and exponential growth, then they were subjected to serum stripping or starvation. The cells were treated with the test item, DMEM, and the positive control. The untreated cells were served as baseline control. The cells in the above plate(s) were incubated for a time point ranging from 24 to 72 hours in a CO<sub>2</sub> incubator at 37°C, 5% CO<sub>2</sub> and 95% humidity. After incubation, the plates were taken out and 20 µL of 5 mg/mL of MTT solution was added to all the wells followed by an additional incubation for 3 hours at 37°C. The supernatant was aspirated and 150 µL of DMSO was added to each well to dissolve formazan crystals. The absorbance of each well was read at 540 nm using a Synergy HT microplate reader, BioTek, USA. The percentage cytotoxicity at each tested concentration of the test substance was calculated using 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 cell viability corresponding to each treatment was then be obtained using Equation (2):

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

The concentration exhibiting ≥70% cell viability was considered as non-cytotoxic [38].

## Assessment of Alkaline Phosphatase (ALP) Activity

The cells were counted using a hemocytometer and plated in a 24-well plate at the density corresponding to 1 X 10<sup>4</sup> cells/well in phenol-free DMEM supplemented with 10% CD-FBS. After the respective treatments, the cells in the above plate were incubated for 48 hours in a CO<sub>2</sub> incubator at 37°C, 5% CO<sub>2</sub> and 95% humidity. After 48 hours of incubation, the plate was taken out and processed for the measurement of ALP enzyme activity. The cells were washed with 1X phosphate buffer saline (PBS) and lysed by freeze-thaw method *i.e.*, incubation at -80°C for 20 minutes followed by incubation at 37°C for 10 minutes. To the lysed cells, 50 µL of substrate solution *i.e.*, 5 mM of p-nitrophenyl phosphate (pNPP) in 1M diethanolamine and 0.24 mM magnesium chloride (MgCl<sub>2</sub>) solution (pH 10.4) was added to all the wells followed by incubation for 1 hour at 37°C. The absorbance of the above solution was read at 405 nm using Synergy HT microplate reader (Biotek, USA). The absorbance values obtained were normalized with substrate blank (pNPP solution alone) absorbance values. The percentage increase in ALP enzyme activity with respect to the untreated cells (baseline group) 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 baseline group (untreated cells)

**Assessment of Collagen Synthesis**

The MG-63 cells were counted using a hemocytometer and plated in a 24-well plate at the density corresponding to 10 X 10<sup>3</sup> cells/well in phenol-free DMEM supplemented with 10% CD-FBS. Following the respective treatments, the cells in the above plate were incubated for 48 hours in a CO<sub>2</sub> incubator at 37°C, 5% CO<sub>2</sub> and 95% humidity. After 48 hours of incubation, the plate was taken out and the amount of collagen accumulated in MG-63 cells corresponding to each treatment was measured by Direct Sirius red dye binding assay. In brief, the cell layers were washed with PBS and fixed in Bouin’s solution (5% acetic acid, 9% formaldehyde, and 0.9% picric acid) for 1 hour at room temperature (RT). After 1 hour of incubation, the above wells were washed with milliQ water and air dried. The cells were then stained with Sirius red dye solution for 1 hour at RT followed by washing in 0.01 N HCl to remove unbound dye. The collagen dye complex obtained in the above step was dissolved in 0.1 N NaOH and absorbance was read at 540 nm using Biotek Synergy HT microplate reader. The level of collagen was extrapolated using standard curve obtained from purified Calf Collagen Bornstein and Traub Type I (Sigma Type III). The percentage increase in collagen level with respect to the untreated cells (baseline group) 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 baseline group (untreated cells)

**Assessment of Bone Mineralization by Alizarin Red S Staining**

The MG-63 cells were counted using a hemocytometer and plated in 24-well plate at the density corresponding to 10 X 10<sup>3</sup> cells/well in phenol-free DMEM supplemented with 10% CD-FBS. Following the respective treatments, the cells in the above plate were incubated for 48 hours in a CO<sub>2</sub> incubator at 37°C, 5% CO<sub>2</sub> and 95% humidity to allow cell recovery and exponential growth. After overnight incubation, the above cells were subjected to serum stripping for 24 hours. The cells were then treated with non-cytotoxic concentrations of the test samples and positive

control. Following 3-7 days of incubation with the test samples and positive control, the plates were taken out, cell layers processed further by staining with Alizarin Red S dye. The cells were fixed in 70% ethanol for 1 hour, after which Alizarin Red solution (40 µm; pH 4.2) was added to the samples for 20 minutes with shaking. The cells were washed with distilled water to remove unbound dye. For quantitative analysis by absorbance evaluation, nodules were solubilized with 10% cetylpyridinium chloride for 15 minutes with shaking. Absorbance was measured at 562 nm using Biotek Synergy HT microplate reader. The percentage increase in bone mineralization with respect to the untreated cells (baseline group) 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 baseline (untreated) group.

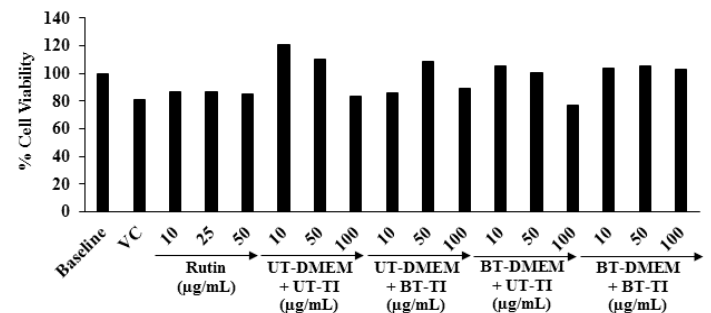
**Statistical Analysis**

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≤0.05.

**Results and Discussion**

**MTT Assay**

The data of MTT cell viability assay of the Biofield Energy Treated vitamin D<sub>3</sub> and DMEM in MG-63 cells are shown in (Figure 1). The data showed that the test samples was found as safe and nontoxic (as evidence of cell viability approximately greater than 77%) across all the tested concentrations upto 100 µg/mL. Hence, these concentrations were used for the estimation of Alkaline Phosphatase (ALP) activity, collagen level, and bone mineralization in MG-63 cells.

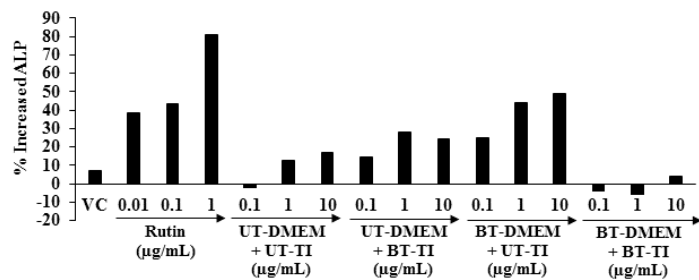


**Figure 1:** The effect of the test items (vitamin D<sub>3</sub> and DMEM medium) on cell viability in MG-63 cells after 72 hours of treatment. VC: Vehicle control (0.05% DMSO); UT: Untreated; BT: Biofield Energy Treated; TI: Test item.

## Alkaline Phosphatase (ALP) Activity

The response of the test items on ALP in MG-63 cells is shown in Figure 2. The Vehicle Control (VC) group showed 7.5% increased the level of ALP compared to the untreated cells (Normal Control) group. The ALP activity was significantly raised by 38.78%, 43.61%, and 80.92% in the positive control (rutin) group at the concentration of 0.01, 0.1, and 1 µg/mL, respectively compared to the untreated cells group. The level of ALP was significantly increased by 84.62% in the BT-DMEM + BT-Test item group at 0.1 µg/mL, while increased by 118.24% and 241.62% in the UT-DMEM + BT-Test item and BT-DMEM + UT-Test item groups, respectively at 1 µg/mL, respectively compared to the UT-DMEM + UT-Test item group. Moreover, the level of ALP was significantly increased by 43.56% and 192.08% in the UT-DMEM + BT-Test item and BT-DMEM + UT-Test item groups, respectively at 10 µg/mL, respectively compared to the UT-DMEM + UT-Test item group (Figure 2).

An increased the level of bone specific ALP in serum can give a valuable diagnostic information. The enzyme is elevated due to a result of increased osteoblastic activity [39]. Literature evident that thyroid hormone (T3) can stimulate the bone specific ALP activity through an osteoblast nuclear receptor-mediated process. Bone ALP contributes about 50% of the total circulating ALP in normal cases [40,41]. Overall, the Consciousness Energy Treated (The Trivedi Effect<sup>®</sup>) vitamin D<sub>3</sub> showed an improved synthesis of ALP in the human osteosarcoma cells with respect to the untreated item items group, which might be advantageous to maintain a healthy skeletal structure for the patients suffering from various bone-related disorders.

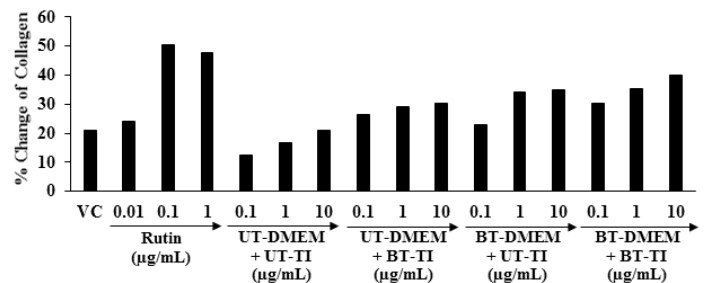


**Figure 2:** The effect of the test items (vitamin D<sub>3</sub> and DMEM medium) on alkaline phosphatase enzyme activity was assessed in human bone osteosarcoma cell after treatment with the Biofield Energy Treated test samples. VC: Vehicle control (0.05% DMSO), UT: Untreated; BT: Biofield Energy Treated; TI: Test item.

## Assessment of Collagen Activity

The response of the test items on collagen activity in MG-63 cells is shown in (Figure 3). Vehicle Control (VC) group showed 20.9% increase of collagen with respect to the untreated cells (normal control) group. Collagen synthesis was significantly

enhanced by 24%, 50.29%, and 47.71% at 0.01, 0.1, and 1 µg/mL, respectively in the positive control (rutin) group compared to the untreated cells group. Collagen synthesis was significantly increased by 112.24%, 83.68%, and 142.88% in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item groups, respectively at 0.1 µg/mL compared to the UT-DMEM + UT-Test item group. Moreover, the collagen level was significantly increased by 75.39%, 106.15%, and 113.87% in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item groups at 1 µg/mL compared to the UT-DMEM + UT-Test item group. Additionally, at 10 µg/mL the level of collagen was also significantly increased by 43.41%, 65.09%, and 89.18% in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item groups, respectively with respect to the UT-DMEM + UT-Test item group (Figure 3). Bone is a mineralized connective tissue that exhibits four types of cells: osteoblasts, bone lining cells, osteocytes, and osteoclasts [42]. In the process of deposition of organic matrix by osteoblasts after secretion of collagen proteins (mainly type-1) and others peptidoglycans which formed the organic matrix [43]. Type I collagen is one of the main structural protein in hard tissues, responsible for various functions on osteoblast such as initial attachment, proliferation, and differentiation etc. [44, 45]. Overall, the Consciousness Energy treated vitamin D<sub>3</sub> had significantly improved the synthesis of collagen fibers in the human osteosarcoma cells with respect to all the treatment groups. Hence, it is assumed that The Trivedi Effect<sup>®</sup> has the significant potential to improve the bone health in various skeletal disorders.



**Figure 3:** The effect of the test items (vitamin D<sub>3</sub> and DMEM medium) on collagen activity in MG-63 cells. VC: Vehicle control (0.05% DMSO), UT: Untreated; BT: Biofield Energy Treated, TI: Test item.

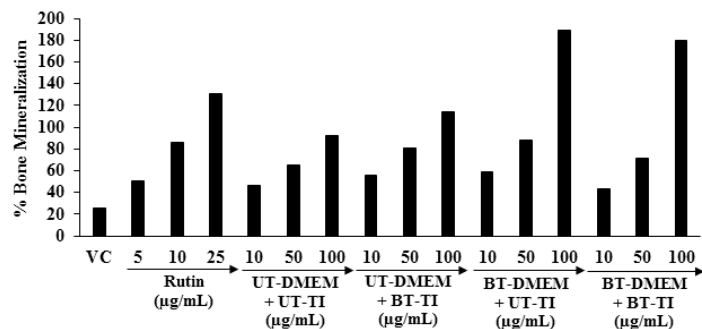
## Assessment of Bone Mineralization by Alizarin Red S (ARS) Staining

In the process of bone mineralization after its subsequent deposition of organic matrix by osteoblasts a secretion of enzymes that degrade the proteoglycans. After that, the calcium ions are released from the proteoglycans and cross the calcium channels and formed a rigid cellular matrix [46]. Staining with alizarin red S is a suitable technique for the screening of calcium-rich deposits by cells in culture [47]. The bone mineralization response of the

test items in MG-63 cells is shown in Figure 4.

The vehicle control (VC) group showed 25.4% increase in bone mineralization as compared to the untreated cells (normal control) group. The percentage of bone mineralization was significantly increased in a concentration-dependent manner by 50.46%, 86.16%, and 130.60% at 5, 10, and 25 µg/mL, respectively in the positive control group compared to the untreated cells group. The percent of bone mineralization was significantly raised by 19.34% and 25.53% in the UT-DMEM + BT-Test item and BT-DMEM + UT-Test item group at 10 µg/mL compared to the UT-DMEM + UT-Test item group. Moreover, a significant increased the percentage of bone mineralization by 24.71%, 36.24%, and 10.35% in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item groups, respectively at 50 µg/mL with respect to the UT-DMEM + UT-Test item group. In addition to, the data showed a significant increased of percent bone mineralization by 23.94%, 106.2%, and 95.20% 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 100 µg/mL.

The bone mineralization is essential for the growth and development of overall health. Alteration of bone mineralization process can lead to a variety of medical difficulties [45]. Thus, based on the outcomes it is assumed that the Biofield Energy Treated vitamin D<sub>3</sub> showed a remarkable improvement of bone mineralization content in the MG-63 with respect to the all others treatment groups.



**Figure 4:** The effect of the test items (vitamin D<sub>3</sub> and DMEM medium) on bone mineralization activity in human bone osteosarcoma cells. VC: Vehicle control (0.05% DMSO), UT: Untreated; BT: Biofield Energy Treated, TI: Test item

## Conclusions

The MTT cell viability assay results exhibited more than 77% cells were viable, which supported that the test samples were found as safe and nontoxic in all the tested concentrations. The UT-DMEM + BT-Test item group exhibited 118.24% and 43.56% increased the level of ALP at 1 and 10 µg/mL, respectively as

compared to the untreated group. Moreover, the BT-DMEM + UT-Test item group increased of ALP by 241.62% and 192.08% at 1 and 10 µg/mL, respectively while BT-DMEM + BT-TI showed 84.62% at 0.1 µg/mL compared to the untreated group. Collagen was significantly increased by 112.24%, 83.68%, and 142.88% in the UT-DMEM + BT-TI, BT-DMEM + UT-TI, and BT-DMEM + BT-TI groups, respectively at 0.1 µg/mL, while increased by 75.39%, 106.15%, and 113.87% in the UT-DMEM + BT-TI, BT-DMEM + UT-TI, and BT-DMEM + BT-TI groups, respectively at 1 µg/mL compared to the untreated group. Besides, the percent of bone mineralization was remarkably increased by 23.94%, 106.2%, and 95.20% at 100 µg/mL in the UT-DMEM + BT-TI, BT-DMEM + UT-TI, and BT-DMEM + BT-TI groups, respectively compared to the untreated group.

Thus, the Biofield Energy Treated test samples (The Trivedi Effect®) demonstrated a significant impact on bone health parameters. Therefore, the Consciousness Energy Healing based vitamin D<sub>3</sub> might be suitable for the development of an alternative and more effective supplement for vitamin D<sub>3</sub> 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 (kidney transplants, liver transplants and heart transplants), various autoimmune disorders such as Lupus, Addison Disease, Celiac Disease (gluten-sensitive enteropathy), Dermatomyositis, Graves' Disease, Hashimoto Thyroiditis, Multiple Sclerosis, Myasthenia Gravis, Pernicious Anemia, Aplastic Anemia, Reactive Arthritis, Rheumatoid Arthritis, Sjogren Syndrome, Systemic Lupus Erythematosus, Type 1 Diabetes, Alopecia Areata, Crohn's Disease, Fibromyalgia, Vitiligo, Psoriasis, Scleroderma, Chronic Fatigue Syndrome and Vasculitis, as well as inflammatory disorders such as Asthma, Ulcerative Colitis, Alzheimer's Disease, Atherosclerosis, Dermatitis, Diverticulitis, Hepatitis, Irritable Bowel Syndrome, inflammatory diseases, anti-inflammatory, anti-stress, anti-arthritis, anti-osteoporosis, anti-apoptotic, wound healing, anti-cancer, anti-psychotic and anti-fibrotic actions stress management and prevention, and anti-aging by improving overall health, Parkinson's Disease and stress etc. to modulate the immune system by improving overall health.

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## Conflict of Interest

Authors declare no conflict of action.

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