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Implication of Biofield Energy Healing Based Vitamin D_3 on Osteoblastic Differentiation

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Abstract: Bone is one of the dynamic organ of the endoskeleton, which play a vital role in maintain the structural integrity, blood production, coagulation, mineral reservoirs, and body immunity. The present study investigates the potential of Consciousness Energy Healing based vitamin D_3 and DMEM medium on bone health parameters in MG-63 cell line. Vitamin D_3 and DMEM medium as test items (TI), were divided into two parts, and each sample received the Consciousness Energy Healing Treatment by Su-Mei Chen Liu and labeled as the Biofield Energy Treated (BT) samples, while the other parts of each sample were denoted as the untreated test items (UT). Bone health parameters such as alkaline phosphatase enzyme (ALP) activity, collagen levels and bone mineralization as bone biomarkers were studied. The cell viability using MTT assay showed more than 84% in test items groups were found to be safe. ALP level was significantly increased by 217% (100 µg/mL), 272.8% (10 µg/mL), and 233.9% (100 µg/mL) in the UT-DMEM+BT-TI, BT-DMEM+UT-TI, and BT-DMEM+BT-TI groups, respectively. Collagen content was significantly increased by 121.6%, 134.3%, and 86% in the UT-DMEM+BT-TI, BT-DMEM+UT-TI, and BT-DMEM+BT-TI groups, respectively at 100 µg/mL as compared with the untreated group. Additionally, the percent of bone mineralization was significantly increased by 213.5% (at 10 µg/mL), 109.5% (at 100 µg/mL), and 283.7% (at 100 µg/mL) in the UT-DMEM+BT-TI, BT-DMEM+UT-TI, and BT-DMEM+BT-TI groups, respectively as compared with the untreated group. In conclusion, the Biofield Energy Treated vitamin D_3 and DMEM would play an important role to improve the bone mineralization and calcium absorption. The bone health parameters such as collagen, calcium and ALP were significantly improved and can be used as supplement to improve bone health. Further, it is assumed that the Biofield Energy Treated vitamin D_3 could be a powerful alternative dietary sources and supplements to fight against various bone related diseases including low bone density and osteoporosis, Paget's disease of bone, rickets, deformed bones, osteomalacia, bone and/or joint pain, increased frequency of fractures, osteoma, hormonal imbalance, stress, aging, bone loss, and fractures.

Keywords: Biofield Energy Healing, Osteosarcoma Cells (MG-63), Vitamin D, Osteoporosis, Bone Biomarkers, Bone Mineralization

1. Introduction

Vitamin D has multiple effects which regulate the functions in different organs such as brain, lungs, liver, kidneys, and heart, immune, skeletal, and reproductive systems. Moreover, it has significant anti-inflammatory, anti-arthritic, anti-osteoporosis, anti-stress, anti-aging, anti-apoptotic, wound healing, anti-cancer, anti-psychotic, and anti-fibrotic roles. Vitamin D receptors (VDRs) are widely present in most of the body organs like brain, heart, lungs, kidney, liver, pancreas, large and small intestines, muscles, reproductive, nervous system, etc. [1]. VDRs influence cell-

to-cell communication, normal cell growth, cell differentiation, cell cycling and proliferation, hormonal balance, neurotransmission, skin health, immune and cardiovascular functions. Bone-related health issues become a major problem among the population from village to the cities. Vitamin D plays a vital role in preserving a healthy mineralized skeleton of most of the vertebrates including humans. Cod liver oil, irradiation of other foods including plants, sunlight, etc. are found to be effective against bone related disorders, which lead to discovering the active principle- vitamin D [1]. The role of vitamin D has been well defined for improving the bone mineralization along with the increased bone resorption, aging, inflammation and overall quality of life. Vitamin D_3 is synthesized in the skin by sunlight and once formed it sequentially metabolized in the liver and kidney to 1,25-dihydroxyvitamin D (calcitriol, the vitamin D hormone) [2]. Calcitriol play an important role in maintaining the normal level of calcium and phosphorus, promotes bone mineralization, induce or repress the genes responsible for conserving the mineral homeostasis and skeletal integrity, and inhibit hypertension, kidney damage, cardiovascular and immune disorders (such as Lupus, Addison Disease, Graves' Disease, Hashimoto Thyroiditis, Multiple Sclerosis, Myasthenia Gravis, Anemia, Alopecia Areata, Fibromyalgia, Sjogren Syndrome, Scleroderma, Systemic Lupus Erythematosus, Diabetes, Vitiligo, Psoriasis, Chronic Fatigue Syndrome and Vasculitis), and the secondary hyperparathyroidism [3]. Vitamin D insufficiency and deficiency is the major health problem, which causes metabolic bone disease in the young and elderly populations [4]. Fortified foods have a variable amount of vitamin D and most of the foods do not contain vitamin D, which can be fulfilled using some supplements. In order to avoid the bone related disorders such as osteomalacia, exacerbate osteoporosis, hyperparathyroidism, immune disorders, etc. calcium 1000-1500 mg/day along with vitamin D supplement around 400 IU/day is very important for maintaining the good bone health [5].

Various *in vitro* studies have readily demonstrated the role of bone health using cell lines and its resorbing effects using three important key biomarkers, such as alkaline phosphatase (ALP), collagen and calcium. MG-63 cell line derived from juxtacortical osteosarcoma, which represents an immature osteoblast phenotype and undergoes temporal development in long term culture. The response of MG-63 cells to 1,25-dihydroxyvitamin D_3 (1,25 $(OH)2D_3$) administration has been studied to be similar to normal human osteoblast cells [6]. Hence, MG-63 cell line is widely used for studying the potential of any test compounds to improve the bone health [7]. The formation of new bone involves a complex series of events including the proliferation and differentiation of osteoblasts, and eventually the formation of a mineralized extracellular matrix. ALP is a phenotypic marker for the early differentiation and maturation of osteoblasts. ALP increases the local concentration of inorganic phosphate for bone mineralization and hence is an important marker for osteogenic activity [8]. Similarly, active osteoblasts synthesize and extrude collagen, which plays an important role in the formation of bone extracellular matrix by providing strength and flexibility. Collagen fibrils formed an arrays of an organic matrix known as Osteoid [9]. Likewise, calcium phosphate is deposited in the Osteoid and gets mineralized (combination of calcium phosphate and hydroxyapatite) and provides rigidity to the bone [10]. Thus, these parameters are very essential in order to study the bone health in cell lines. Authors evaluated the *in vitro* effect of the Biofield Energy Treated vitamin D_3 as a test item, a Complementary and Alternative Medicine (CAM) on bone health using MG-63 cell line for major biomarkers.

Within the burgeoning ground of CAM therapies, Biofield Energy Treatment or energy medicine, is emerging with significant benefits in various scientific fields. The effects of the CAM therapies have great potential, which include external qigong, Johrei, Reiki, therapeutic touch, yoga, Qi Gong, polarity therapy, Tai Chi, pranic healing, deep breathing, chiropractic/osteopathic manipulation, guided imagery, meditation, massage, homeopathy, hypnotherapy, progressive relaxation, acupressure, acupuncture, special diets, relaxation techniques, Rolfing structural integration, healing touch, movement therapy, pilates, mindfulness, Ayurvedic medicine, traditional Chinese herbs and medicines in biological systems both *in vitro* and *in vivo* [11]. Biofield Energy Healing Treatment (The Trivedi Effect®) contain a putative bioenergy, which is channeled by a renowned practitioners from a distance. Biofield Energy Healing as a CAM showed a significant results in biological studies [12]. However, the National Center for Complementary and Alternative Medicine (NCCAM), well-defined Biofield therapies in the subcategory of Energy Therapies [13]. The Trivedi Effect®- Consciousness Energy Healing Treatment has been reported with significant revolution in the physicochemical properties of metals, chemicals, ceramics and polymers [14-16], improved agricultural crop yield, productivity, and quality [17, 18], transformed antimicrobial characteristics [19-21], biotechnology [22-23], improved bioavailability [24-26], skin health [27, 28], nutraceuticals [29, 30], cancer research [31, 32], and human health and wellness.

On the basis of significant results of the Biofield Energy Treatment and vital role of vitamin D_3 on bone health, authors sought to evaluate the impact of the Biofield Energy Treatment (The Trivedi Effect®) on vitamin D_3 as test sample for bone health activity with respect to the assessment of different bone health parameters like ALP, collagen content, and bone mineralization using standard *in vitro* assays in MG-63 cells.

2. Material and Methods

2.1. Chemicals and Reagents

Rutin hydrate was purchased from TCI, Japan, while vitamin D_3 (denoted as test item) 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. Antibiotics solution (penicillin-streptomycin) was procured from Hi Media, India, while 3-(4,5-diamethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium) (MTT), Direct Red 80, and ethylene diamine tetra acetic acid (EDTA) were purchased from Sigma, USA. All the other chemicals used in this experiment were analytical grade procured from India.

2.2. Cell Culture

Human bone osteosarcoma cell line -MG-63 was used as test system in the present study. The MG-63 cell line was maintained in DMEM growth medium for routine culture supplemented with 10% FBS. Growth conditions were maintained as 37°C, 5% CO_2 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 [33].

2.3. Experimental Design

The experimental groups consisted of cells in baseline control, vehicle control groups (0.05% DMSO with Biofield Energy Treated and untreated DMEM), positive control group (rutin hydrate) and experimental test groups. The 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 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.

2.4. Consciousness Energy Healing Treatment Strategies

The test item and DMEM were divided into two parts. One part each of the test item and DMEM was treated with the Biofield Energy by a renowned Biofield Energy Healer (also known as The Trivedi Effect®) and coded as the Biofield Energy Treated item, 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 Su-Mei Chen Liu remotely for ~5 minutes. Biofield Energy Healer was remotely located in the USA, while the test samples were located in the research laboratory of Dabur Research Foundation, New Delhi, India. This Biofield Energy Treatment was administered for 5 minutes through the Healer's unique Energy Transmission process remotely to the test samples under laboratory conditions. Su-Mei Chen Liu 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.

2.5. Determination of Non-cytotoxic Concentration

The cell viability was performed by MTT assay in human bone osteosarcoma cell line (MG-63). The cells were counted and plated in 96 well plates at the density corresponding to 5 X 10^3 to 10 X 10^3 cells/well/180 µL of cell growth medium. The above cells were incubated overnight under growth conditions and allowed the cell recovery and exponential growth, which were subjected to serum stripping or starvation. The cells were treated with the test item, DMEM, and 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 CO_2 incubator at 37°C, 5% CO_2, and 95% humidity. Following incubation, the plates were taken out and 20 µL of 5 mg/mL of MTT solution were added to all the wells followed by 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 Synergy HT micro plate reader, BioTek, USA [34]. The percentage cytotoxicity at each tested concentrations of the test substance were calculated using the following equation (1):

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

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

The percentage cell viability corresponding to each treatment was obtained using the following equation (2):

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

The concentrations exhibiting ≥70% Cell viability was considered as non-cytotoxic.

2.6. Assessment of Alkaline Phosphatase (ALP) Activity

The cells were counted using an hemocytometer and plated in a 24-well plate at the density corresponding 1 x 10^4 cells/well in phenol free DMEM supplemented with 10% CD-FBS. Following respective treatments, the cells in the above plate were incubated for 48 hours in CO_2 incubator at 37°C, 5% CO_2, 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 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 (*p*NPP) in 1M diethanolamine and 0.24 mM magnesium chloride ($MgCl_2$) 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 micro plate reader (Biotek, USA). The absorbance values obtained were normalized with substrate blank (*p*NPP solution alone) absorbance values [33]. The percentage increase in ALP enzyme activity with respect to the untreated cells (baseline

group) was calculated using equation (3):

$$\% \text{ Increase} = [(X-R)/R)]*100 \quad (3)$$

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

R = Absorbance of cells corresponding to baseline group (untreated cells)

2.7. Assessment of Collagen Synthesis

The MG-63 cells were counted using an hemocytometer and plated in 24-well plate at the density corresponding to 10 x 10^3 cells/well in phenol free DMEM supplemented with 10% CD-FBS. Following respective treatments, the cells in the above plate were incubated for 48 hours in CO_2 incubator at 37°C, 5% CO_2, 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 hours 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 micro plate reader. The level of collagen was extrapolated using standard curve obtained from purified Calf Collagen Bornstein and Traub Type I (Sigma Type III) [33]. The percentage increase in collagen level with respect to the untreated cells (baseline group) was calculated using equation (4):

$$\% \text{ Increase} = [(X-R)/R]*100 \quad (4)$$

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

R = Collagen levels in cells corresponding to baseline group (untreated cells)

2.8. Assessment of Bone Mineralization by Alizarin Red S Staining

The MG-63 cells were counted using an hemocytometer and plated in 24-well plate at the density corresponding to 10 x 10^3 cells/well in phenol free DMEM supplemented with 10% CD-FBS. Following respective treatments, the cells in the above plate were incubated for 48 hours in CO_2 incubator at 37°C, 5% CO_2, and 95% humidity to allow cell recovery and exponential growth. Following overnight incubation, the above cells will be subjected to serum stripping for 24 hours. The cells will be then be treated with non-cytotoxic concentrations of the test samples and positive control. After 3-7 days of incubation with the test samples and positive control, the plates were taken out cell layers and processed further for 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 micro plate reader [33]. The percentage increase in bone mineralization with respect to the untreated cells (baseline group) was calculated using the following equation (5):

$$\% \text{ Increase} = [(X-R)/R]*100 \quad (5)$$

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

2.9. Statistical Analysis

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

3. Results and Discussion

3.1. Cell Viability Study Using MTT

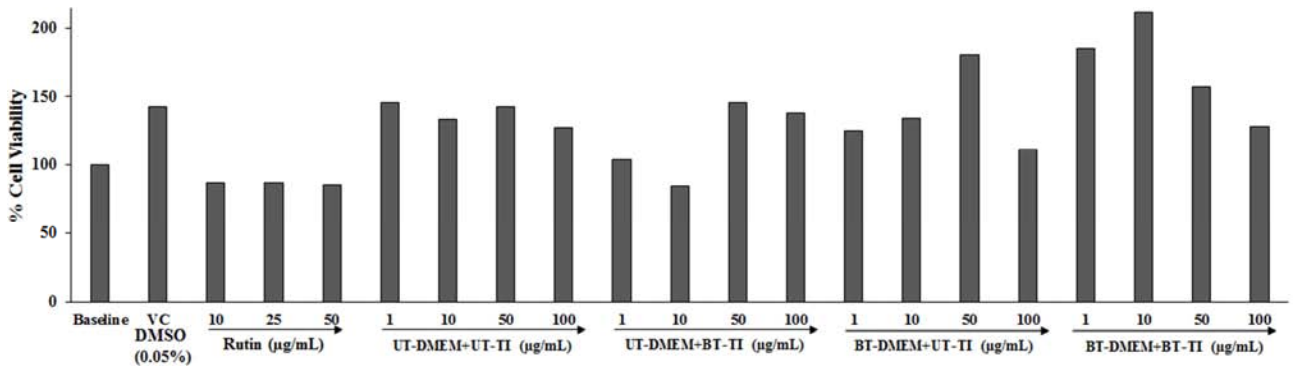

Figure 1. *Cell viability study using MTT assays on MG-63 cell line after 72 hours. VC: Vehicle control (DMSO-0.05%), UT: Untreated; BT: Biofield Treated; TI: Test Item.*

The cell viability results of the Biofield Energy Treated vitamin D_3 and DMEM medium using MTT assay in MG-63 cells are shown in Figure 1. All the test samples were screened for cell viability at different concentrations, and did not exhibit any cytotoxicity with greater than 84% across all the tested concentrations. Cell viability testing using MTT assay is one of the preliminary method of biological evaluation and screening tests of any test items for cell growth, proliferation, its reproduction and morphological effects. However, Biofield Energy Treatment showed a significant improved cell viability as compared with the untreated test samples. These data suggests that the test item along with DMEM groups were found safe at all the tested concentrations range up to maximum of 100 µg/mL against the tested MG-63 cells. These concentrations are used to study the bone health parameters such as on the levels of alkaline phosphatase (ALP) activity, collagen synthesis, and bone mineralization in MG-63 cells.

3.2. Study of Alkaline Phosphatase (ALP) Enzyme Activity

The results of the Biofield Energy Healing Treatment on the level of ALP in human bone osteosarcoma cells (MG-63) is shown in the Figure 2. ALP concentrations after treatment with the test samples *viz.* Biofield Energy Treated test item and DMEM were studies at various concentrations. The vehicle control group showed 11.5% increased level of ALP as compared with the untreated cells group. The positive control, rutin showed a significant increased value by 43.4%, 53.6%, and 83.3% at 0.01, 0.1, and 1 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 level of ALP by 9.5% and 217% at 50 and 100 µg/mL, respectively while Biofield Treated medium and untreated Test item (BT-DMEM+UT-TI) showed a significant increased ALP level by 272.8% at 10 µg/mL, respectively as compared with the untreated test item and DMEM group. However, the Biofield Energy Treated medium and Biofield Energy Treated Test item (BT-DMEM+BT-TI) showed a significant increased ALP level by 16.8%, 27.9%, and 233.9% at 10, 50, and 100 µg/mL, respectively as compared with the untreated test item and DMEM group.

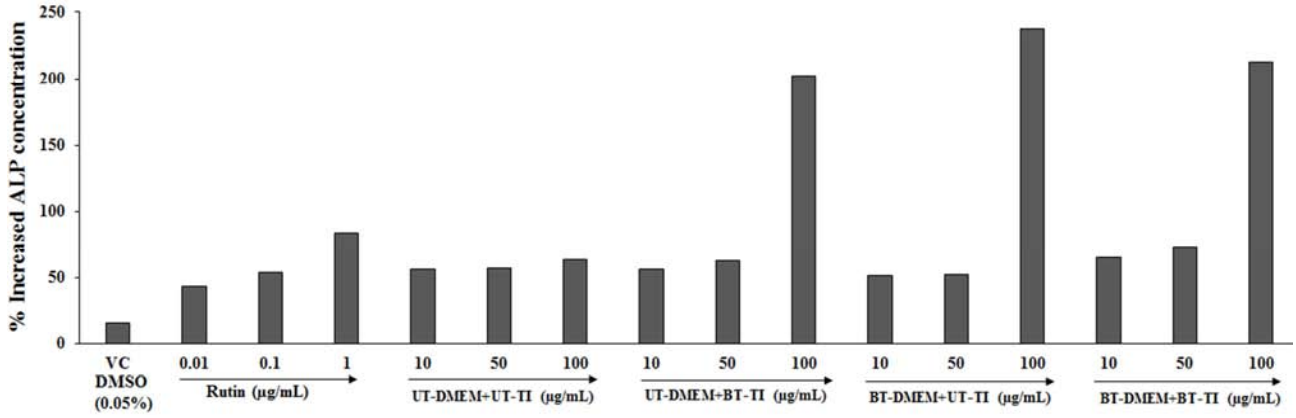

Figure 2. *Effect of the Biofield Energy Treated test items on MG-63 cell line for the level of Alkaline Phosphatase (ALP) enzyme activity. VC: Vehicle control (DMSO-0.05%), UT: Untreated; BT: Biofield Treated; TI: Test Item.*

ALP is the biomarker for bone health and its abnormal level might leads to various bone diseases such as bone cancers, Paget's disease of bone, osteoporosis, healing fracture, bone growth, acromegaly, myelofibrosis, osteogenic sarcoma, or bone metastases, leukemia, and rarely myeloma [35]. The experimental data showed that the Biofield Energy Treated test items were reported to have significant improved ALP level, which could be a better alternative supplement to combat the bone disorders in middle-aged, post-menopausal women, and older women [36]. In addition, these diseases leads to high morbidity affecting quality of life [37]. Overall, The Trivedi Effect®-Energy of Consciousness Healing based vit D_3 and DMEM can be used to improve the ALP concentration in many bone disorders.

3.3. Estimation of Collagen Synthesis

The level of collagen was estimated in all the test samples such as Biofield Energy Treated vit D_3 and DMEM at various concentrations, which suggested significant increase in the collagen level. The experimental results in term of% increase in collagen synthesis with respect to the untreated cells are presented in Figure 3. The positive control, rutin showed a significant increased value of collagen by 46.59%, 51.97%, and 65.41% at 0.01, 0.1, and 1 µg/mL, respectively. Besides, the experimental test groups such as UT-DMEM+BT-TI showed a significant increased collagen level by 38.2% and 121.6% at 50 and 100 µg/mL, respectively while BT-DMEM+UT-TI group showed a significant increased collagen level by 9.2%, 23.2%, and 134.3% at 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 significant increased collagen level by 70.4%, 73.7%, and 86% at 10, 50 and 100 µg/mL, respectively as compared with the untreated test item and DMEM group.

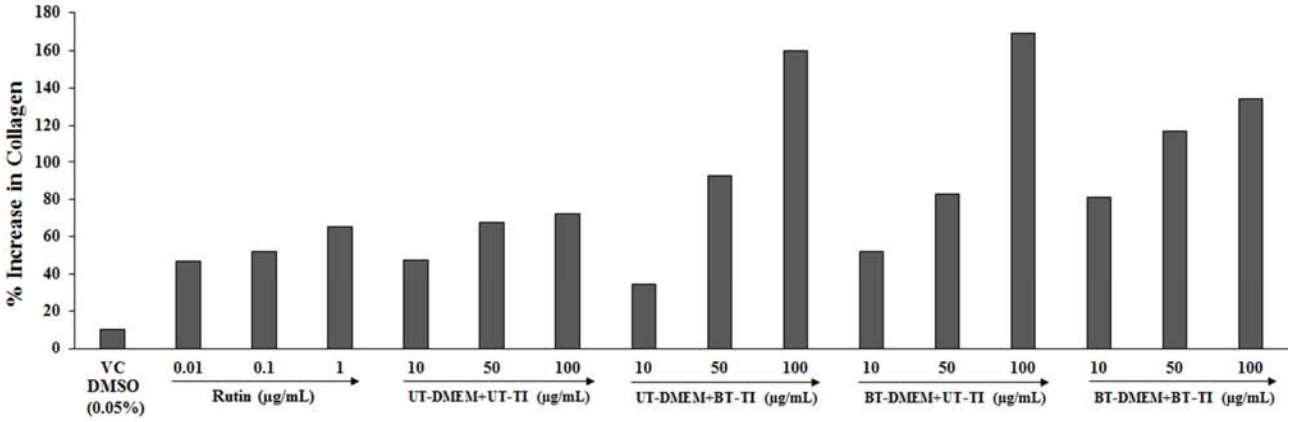

Figure 3. *Effect of the test item on MG-63 cell line for collagen level. VC: Vehicle control (DMSO-0.05%), UT: Untreated; BT: Biofield Treated; TI: Test Item.*

Vitamins, minerals, and collagen are the important role in both health. Collagen is the most abundant protein responsible for bone formation. Loss of bone or tissue is due to an imbalance of bone formation and resorption along with excessive activity of osteoclasts on osteoblasts, which results in increased bone remodeling especially in women after menopause. Collagen is present in muscles, bones, skin, blood vessels, digestive system and tendons, which can be improved using supplementation, dietary control, or physical exercise during growth period [39]. Collagen play vital role to control joints pain, degeneration, bone loss, weak joints, and many more. The experimental results showed that all the data among the Biofield Energy Treated vit D_3 and DMEM groups showed a significant improved collagen level at all the tested concentrations compared with the untreated group. Biofield Energy Treated vit D_3 (The Trivedi Effect®) would be used to replenish the level of collagen that helped to provide elasticity to the connective tissue, like cartilage, bones, tendons and ligaments, reduce inflammation, repair of cartilage, control bone loss and elasticity of joints against different orthopedic diseases.

3.4. Study of Bone Mineralization

The results of bone mineralization suggested significant effect of the Biofield Energy Treated vit D_3 and DMEM with improved percentage of bone mineralization on MG-63 cell line. The results in term of percentage change with respect to untreated cells of bone mineralization among different experimental groups are presented in Figure 4. The positive control, rutin group showed a significant increased value of bone mineralization by 47.98%, 59.73%, and 139.02% at 5, 10, and 25 µg/mL, respectively. The experimental data among test group's *viz.* UT-DMEM+BT-TI showed a significant increased bone mineralization by 213.5% and 11.5% at 10 and 100 µg/mL, while in the BT-DMEM+UT-TI group showed a significant increased bone mineralization by 47.2% and 109.5% at 10 and 100 µg/mL, respectively as compared with the untreated test item and DMEM group. However, BT-DMEM+BT-TI group showed a significant increased bone mineralization by 283.7% at 100 µg/mL as compared with the untreated test item and DMEM group.

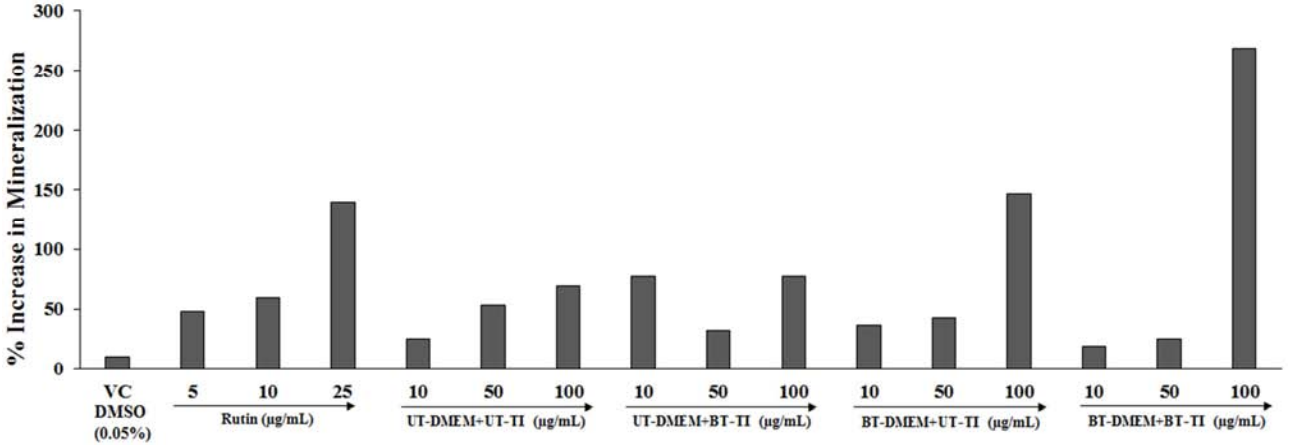

Figure 4. *Effect of the test item on MG-63 cell line for bone mineralization. VC: Vehicle control (DMSO-0.05%), UT: Untreated; BT: Biofield Treated; TI: Test Item.*

Vitamin D_3 and calcium deficiency might results in osteoporosis, which is a progressive decline in bone properties results in increased bone fractures. Vitamin D regulates calcium homeostasis by influencing intestinal calcium absorption, renal calcium reabsorption, and bone resorption by osteoclasts. Increased bone mineralization leads to improved Bone Mineral Density (BMD), which can be achieved using supplementations [40, 41]. In conclusion,

all the experimental Biofield Energy Treated test item and DMEM groups showed a significant improved level of bone mineralization at all the tested concentrations compared with the untreated groups. Biofield Energy Treated vit D3 and DEMEM would play a vital role in various bone related disorders and have distinct roles during the bone recovery process and initiation of mineral formation.

4. Conclusions

Biofield Energy Healing based vitamin D_3 and DMEM showed a significant improved bone health in various tested parameters in MG-63 cell line. MTT assay showed a significant improved cell viability with more than 84% in all the test samples at various tested concentrations. In addition, ALP data showed that the Biofield Energy Treated test samples in the UT-DMEM+BT-TI, BT-DMEM+UT-TI, and BT-DMEM+BT-TI groups were reported with improved ALP by 217% (100 µg/mL), 272.8% (10 µg/mL), and 233.9% (100 µg/mL) respectively, as compared with the untreated group. Collagen level was significantly increased by 38.2% and 121.6% at 50 and 100 µg/mL, respectively in the UT-DMEM+BT-TI group, while 23.2% and 134.3% at 50 and 100 µg/mL, respectively in the BT-DMEM+UT-TI group as compared with the untreated group. In addition, the level of collagen was increased by 70.4%, 73.7%, and 86% at 10, 50 and 100 µg/mL, respectively in BT-DMEM+BT-TI group as compared with the untreated test group. Similarly, the bone mineralization percent was significantly increased by 213.5% and 11.5% at 10 and 100 µg/mL, respectively in the UT-DMEM+BT-TI group, while 47.2% and 109.5% at 10 and 100 µg/mL, respectively in the BT-DMEM+UT-TI groups. In addition, bone mineralization was increased by 283.7% at 100 µg/mL respectively in the BT-DMEM+BT-TI group as compared with the untreated test group. Overall, the Biofield Energy Treated (The Trivedi Effect®) test samples were found to have a significant impact on tested bone health parameters *viz.* collagen, bone mineralization, and ALP, which are very vital to combat the bone disorders. Therefore, the Consciousness Energy Healing based vitamin D_3 might be a suitable alternative nutritional supplement, which could be useful for the management of various bone related disorders *viz.* osteoma, rickets, low bone density and osteoporosis, osteomalacia, Paget's disease of bone, bone and/or joint pain, osteogenesis imperfecta, increased frequency of fractures, deformed bones, chondrodystrophia fetalis, and other bone diseases that are caused by poor nutrition, genetics, or problems with the rate of bone growth or rebuilding. Biofield Energy Treated Vitamin D_3 can be useful as anti-inflammatory, anti-aging, anti-stress, anti-arthritic, anti-osteoporosis, anti-cancer, anti-apoptotic, wound healing, anti-psychotic and anti-fibrotic roles. It also influence cell-to-cell communication, normal cell growth, cell differentiation, neurotransmission, cell cycling and proliferation, hormonal balance, skin health, immune and cardiovascular functions. Besides, it can also be utilized in organ transplants (for example kidney transplants, liver transplants and heart transplants), hormonal imbalance, aging, and various immune related disease conditions such as Ulcerative Colitis, Aplastic Anemia, Hepatitis, Diabetes, Asthma, Alzheimer's Disease, Irritable Bowel Syndrome, Parkinson's Disease, Graves' Disease, Dermatitis, Dermatomyositis, Hashimoto Thyroiditis, Pernicious Anemia, Sjogren Syndrome, Multiple Sclerosis, Myasthenia Gravis, Atherosclerosis, Diverticulitis, Systemic Lupus Erythematosus, stress, etc. with a safe therapeutic index to improve overall health and quality of life.

Abbreviations

MG-63: Human Bone Osteosarcoma Cells, ALP: Alkaline phosphatase, CAM: Complementary and Alternative Medicine, NCCAM: National Center for Complementary and Alternative Medicine; 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: Ethylene Diamine Tetra Acetic Acid.

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References

[1] Holick MF (1996) Vitamin D and bone health. J Nutr 126: 1159S-64S.

[2] Van Leeuwen JP, van Driel M, van den Bemd GJ, Pols HA (2001) Vitamin D control of osteoblast function and bone extracellular matrix mineralization. Crit Rev Eukaryot Gene Expr 11: 199-226.

[3] Bikle DD (2012) Vitamin D and bone. Curr Osteoporos Rep 10: 151-159.

[4] Lips P (2001) Vitamin D deficiency and secondary hyperparathyroidism in the elderly: consequences for bone loss and fractures and therapeutic implications. Endocrine Rev 22: 477-501.

[5] Hossein-nezhad A, Holick MF (2013) Vitamin D for Health: A global perspective. Mayo Clinic proceedings Mayo Clinic 88: 720-755.

[6] Czekanska EM, Stoddart MJ, Richards RG, Hayes JS (2012) In search of an osteoblast cell model for *in vitro* research. Eur Cell Mater 24: 1-17.

[7] Luo XH, Liao EY (2003) Effects of estriol on the proliferation and differentiation of human osteoblastic MG-63 cells. Endocrine Res 29: 343-351.

[8] Iba K, Takada J, Yamashita T (2004) The serum level of bone-specific alkaline phosphatase activity is associated with aortic calcification in osteoporosis patients. J Bone Miner Metab 22: 594-596.

[9] Viguet-Carrin S, Garnero P, Delmas PD (2006) The role of collagen in bone strength. Osteoporos Int 17: 319-336.

[10] Bhattarai T, Bhattacharya K, Chaudhuri P, Sengupta P (2014) Correlation of common biochemical markers for bone turnover, serum calcium, and alkaline phosphatase in post-menopausal women. The Malaysian Journal of Medical Sciences : MJMS 21: 58-61.

[11] Rubik B (2002) The biofield hypothesis: Its biophysical basis and role in medicine. J Altern Complement Med 8: 703-717.

[12] Barnes PM, Bloom B, Nahin RL (2008) Complementary and alternative medicine use among adults and children: United States, 2007. Natl Health Stat Report 12: 1-23.

[13] Koithan M (2009) Introducing complementary and alternative therapies. J Nurse Pract 5: 18-20.

[14] Trivedi MK, Tallapragada RM (2008) A transcendental to changing metal powder characteristics. Met Powder Rep 63: 22-28, 31.

[15] Trivedi MK, Nayak G, Patil S, Tallapragada RM, Latiyal O (2015) Studies of the atomic and crystalline characteristics of ceramic oxide nano powders after bio field treatment. Ind Eng Manage 4: 161.

[16] Trivedi MK, Nayak G, Patil S, Tallapragada RM, Latiyal O, Jana S (2015) Effect of biofield energy treatment on physical and structural properties of calcium carbide and praseodymium oxide. International Journal of Materials Science and Applications 4: 390-395.

[17] Trivedi MK, Branton A, Trivedi D, Nayak G, Mondal SC, Jana S (2015) Morphological characterization, quality, yield and DNA fingerprinting of biofield energy treated alphonso mango (*Mangifera indica* L.). Journal of Food and Nutrition Sciences 3: 245-250.

[18] Trivedi MK, Branton A, Trivedi D, Nayak G, Mondal SC, Jana S (2015) Evaluation of biochemical marker – Glutathione and DNA fingerprinting of biofield energy treated *Oryza sativa*. American Journal of BioScience 3: 243-248.

[19] Trivedi MK, Branton A, Trivedi D, Nayak G, Charan S, Jana S (2015) Phenotyping and 16S rDNA analysis after biofield treatment on Citrobacter braakii: A urinary pathogen. J Clin Med Genom 3: 129.

[20] Trivedi MK, Patil S, Shettigar H, Mondal SC, Jana S (2015) Evaluation of biofield modality on viral load of Hepatitis B and C viruses. J Antivir Antiretrovir 7: 083-088.

[21] Trivedi MK, Patil S, Shettigar H, Mondal SC, Jana S (2015) An impact of biofield treatment: Antimycobacterial susceptibility potential using BACTEC 460/MGIT-TB System. Mycobact Dis 5: 189.

[22] Trivedi MK, Patil S, Shettigar H, Bairwa K, Jana S (2015) Phenotypic and biotypic characterization of *Klebsiella oxytoca*: An impact of biofield treatment. J Microb Biochem Technol 7: 203-206.

[23] Nayak G, Altekar N (2015) Effect of biofield treatment on plant growth and adaptation. J Environ Health Sci 1: 1-9.

[24] Branton A, Jana S (2017) The influence of energy of consciousness healing treatment on low bioavailable resveratrol in male Sprague Dawley rats. International Journal of Clinical and Developmental Anatomy 3: 9-15.

[25] Branton A, Jana S (2017) The use of novel and unique biofield energy healing treatment for the improvement of poorly bioavailable compound, berberine in male Sprague Dawley rats. American Journal of Clinical and Experimental Medicine 5: 138-144.

[26] Branton A, Jana S (2017) Effect of The biofield energy healing treatment on the pharmacokinetics of 25-hydroxyvitamin D3 [25 (OH)D3] in rats after a single oral dose of vitamin D3. American Journal of Pharmacology and Phytotherapy 2: 11-18.

[27] Kinney JP, Trivedi MK, Branton A, Trivedi D, Nayak G, Mondal SC, Jana S (2017) Overall skin health potential of the biofield energy healing based herbomineral formulation using various skin parameters. American Journal of Life Sciences 5: 65-74.

[28] Singh J, Trivedi MK, Branton A, Trivedi D, Nayak G, Gangwar M, Jana S (2017) Consciousness energy healing treatment based herbomineral formulation: A safe and effective approach for skin health. American Journal of Pharmacology and Phytotherapy 2: 1-10.

[29] Trivedi MK, Branton A, Trivedi D, Nayak G, Plikerd WD, Surguy PL, Kock RJ, Piedad RB, Callas RP, Ansari SA, Barrett SL, Friedman S, Christie SL, Liu SC, Starling SE, Jones S, Allen SM, Wasmus SK, Benczik TA, Slade TC, Orban T, Vannes VL, Schlosser VM, Albino YSY, Panda P, Sethi KK, Jana S (2017) A Systematic study of the biofield energy healing treatment on physicochemical, thermal, structural, and behavioral properties of magnesium gluconate. International Journal of Bioorganic Chemistry 2: 135-145.

[30] Trivedi MK, Branton A, Trivedi D, Nayak G, Plikerd WD, Surguy PL, Kock RJ, Piedad RB, Callas RP, Ansari SA, Barrett SL, Friedman S, Christie SL, Liu SC, Starling SE, Jones S, Allen SM, Wasmus SK, Benczik TA, Slade TC, Orban T, Vannes VL, Schlosser VM, Albino YSY, Panda P, Sethi KK, Jana S (2017) Chromatographic and spectroscopic characterization of the consciousness energy healing treated *Withania Somnifera* (ashwagandha) root extract. European Journal of Biophysics 5: 38-47.

[31] Trivedi MK, Patil S, Shettigar H, Mondal SC, Jana S (2015) The potential impact of biofield treatment on human brain tumor cells: A time-lapse video microscopy. J Integr Oncol 4: 141.

[32] Trivedi MK, Patil S, Shettigar H, Gangwar M, Jana S (2015) *In vitro* evaluation of biofield treatment on cancer biomarkers involved in endometrial and prostate cancer cell lines. J Cancer Sci Ther 7: 253-257.

[33] Czekanska EM, Stoddart MJ, Richards RG, Hayes JS (2012) In search of an osteoblast cell model for *in vitro* research. Eur Cells Mater 24: 1-17.

[34] Biological evaluation of medical devices - Part 5: Tests for *in vitro* cytotoxicity (ISO 10993-5: 2009), I. S. EN ISO, 10993-5: 20093.

[35] Sharma U, Pal D, Prasad R (2014) Alkaline phosphatase: An overview. Indian J Clin Biochem 29: 269-278.

[36] Orimo H (2010) The mechanism of mineralization and the role of alkaline phosphatase in health and disease. J Nippon Med Sch 77: 4-12.

[37] Golub EE, Boesze-Battaglia K (2007) The role of alkaline phosphatase in mineralization. Curr Opin Orthop 18: 444-448.

[38] Black C, Clar C, Henderson R, MacEachern C, McNamee P, Quayyum Z, Royle P, Thomas S (2009) The clinical effectiveness of glucosamine and chondroitin supplements in slowing or arresting progression of osteoarthritis of the knee: A systematic review and economic evaluation. Health Technol Assess 13: 1-148.

[39] Lodish H, Berk A, Zipursky SL, et al. Molecular Cell Biology. 4th edition. New York: W. H. Freeman; 2000. Section 22.3, Collagen: The Fibrous Proteins of the Matrix. Available from: https://www.ncbi.nlm.nih.gov/books/NBK21582/.

[40] Roschger P, Paschalis EP, Fratzl P, Klaushofer K (2008) Bone mineralization density distribution in health and disease. Bone 42: 456-466.

[41] Laird E, Ward M, McSorley E, Strain JJ, Wallace J (2010) Vitamin D and bone health; Potential mechanisms. Nutrients 2: 693-724.