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Regulation of Bone Health Parameters After Treatment with Biofield Energy Healing Based Vitamin D_3 on Human Osteoblast Cell Line (MG-63)

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Abstract: Bone disorders dramatically affecting the functional status of many individuals, which are suffering from bone diseases such as fractures, significant pain and height loss, disability to stand up, and walk. Vitamin D play an important role to improve the patients' quality of life with respect to bone disorders. The current study aimed to evaluate the potential of The Trivedi Effect®- Biofield Energy Healing on vitamin D_3 and DMEM as test item (TI) on bone cell differentiation using human osteoblast cell line (MG-63, Osteosarcoma). Bone health biomarkers such as alkaline phosphatase enzyme (ALP) activity, collagen levels and bone mineralization were evaluated. The test items were treated with The Trivedi Effect® by Victoria Lee Vannes and divided as Biofield Energy Treated (BT) and untreated (UT) test items. Cell viability using MTT data showed that the test items were found to be safe. ALP level was significantly increased by 114.3%, 304.8%, and 314.3% at 0.1 µg/mL in the UT-DMEM+BT-TI, BT-DMEM+UT-TI, and BT-DMEM+BT-TI groups, respectively as compared to the untreated group. Collagen content was significantly increased by 82.5%, 138.4%, and 100.8% at 0.1, 1, and 10 µg/mL, respectively in the UT-DMEM+BT-TI, while 120.6% and 64.6% increased collagen at 0.1 and 1 µg/mL in BT-DMEM+UT-TI group and 261.9%, 179.8%, and 116.0% increased collagen in BT-DMEM+BT-TI group at 0.1, 1, and 10 µg/mL, respectively as compared with the untreated group. Moreover, the percent of bone mineralization was significantly increased by 261.2% and 239.9% at 1 µg/mL UT-DMEM+BT-TI and BT-DMEM+UT-TI groups, respectively as compared with the untreated group. However, BT-DMEM+BT-TI group showed a significant increased bone mineralization by 324.5% at 50 µg/mL. Thus, Biofield Energy Treated vitamin D_3 and DMEM would play an important role to control the osteoblast function, improves bone mineralization, and calcium absorption in many bone disorders. Moreover, the bone health parameters such as collagen, calcium and ALP were significantly improved and can be used as supplement to improve bone health. Based on the outstanding results, 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, osteogenesis imperfecta, Paget's disease of bone, rickets, osteomalacia, bone and/or joint pain, increased frequency of fractures, deformed bones, osteoma, chondrodystrophia fetalis, hormonal imbalance, stress, aging, bone loss and fractures.

Keywords: The Trivedi Effect®, Bone Disorders, Osteosarcoma Cells (MG-63), Alizarin Red S Staining, ALP, Collagen, Bone Mineralization

1. Introduction

Vitamin D has multiple effects which regulate the functions in different organs such as brain, lungs, liver, kidneys, 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 not only for improving the bone mineralization but also with 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, Sjogren Syndrome, Systemic Lupus Erythematosus, Diabetes, Alopecia Areata, Fibromyalgia, Vitiligo, Psoriasis, Scleroderma, 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, polarity therapy, pranic healing, 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 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 [14-17], improved agricultural crop yield, productivity, and quality [18-20], transformed antimicrobial characteristics at genetic level [21-23], biotechnology [24-26], skin health [27, 28], nutraceuticals [29, 30], cancer research [31, 32], and human health and wellness.

Based on the significant outcomes of 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 HiMedia, 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 Victoria Lee Vannes 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. The Biofield Energy Healer, 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. MTT Assay- Non-cytotoxic Effect of the Test Item

The cell viability of the test samples were studied in MG-63 cells. The obtained results were compared with respect to rutin at defined concentrations for the estimation of percentage cell viability. The cell viability results are graphically presented in Figure 1. The results of percentage cell viability in all the tested cell lines showed the cell viability range of 70% to 126% in different test item groups with DMEM, while rutin showed more than 85% cell viability (Figure 1) at the tested concentrations. 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.

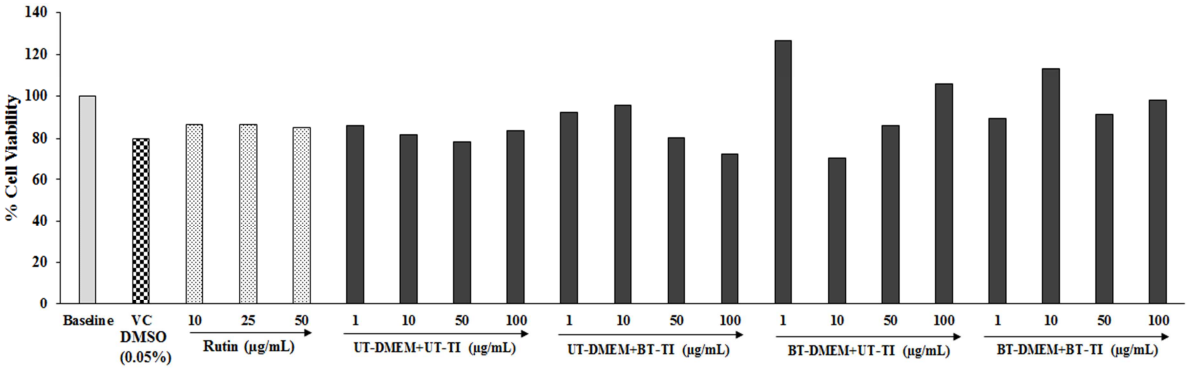

Figure 1. *Effect of the test item on MG-63 cell lines for cell viability after 72 hours using the MTT assays. VC: Vehicle control (DMSO-0.05%), UT: Untreated; BT: Biofield Treated; TI: Test Item.*

3.2. Assessment of Test Items Effects on Alkaline Phosphatase (ALP) Enzyme Activity

The effect of the Biofield Energy Treated test item and DMEM on the ALP level showed a significantly increased at various experimental test item concentrations on MG-63 cell line (Figure 2). The positive control, rutin showed a significant increased value by 38.78%, 43.61%, and 80.92% 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 increase in ALP level by 114.3% and 96.2% at 0.1 and 10 µg/mL, respectively while Biofield Treated medium and untreated Test item (BT-DMEM+UT-TI) showed a significant increased ALP level by 304.8%, 61.8%, and 64.2% at 0.1, 1, and 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 314.3% and 101.3% at 0.1 and 1 µg/mL, respectively as compared with the untreated test item and DMEM group. Overall, all the experimental test groups showed a significant improved level of ALP at all the tested concentrations.

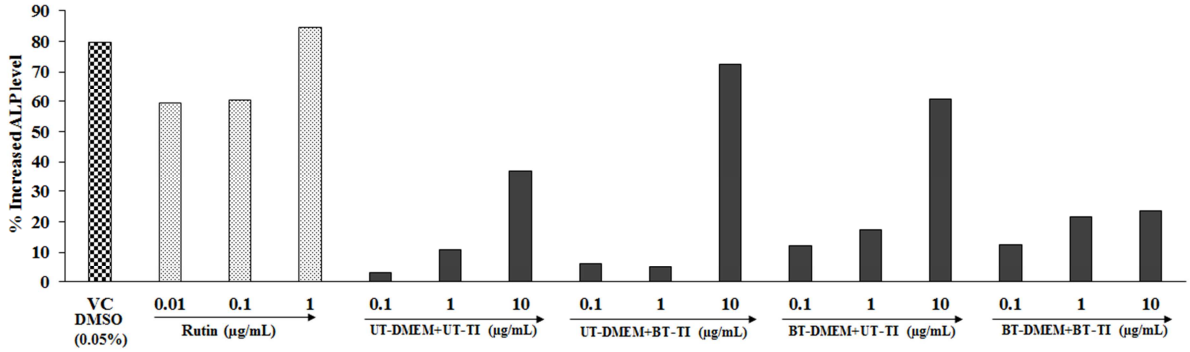

Figure 2. *Effect of the test items on MG-63 cell line for the level of Alkaline Phosphatase (ALP) enzyme activity. VC: Vehicle control (DMSO-0.05%), UT: Untreated; BT: Biofield Treated; TI: Test Item.*

Serum ALP belongs to the zinc metalloprotein enzymes family member, which functions to split off a terminal phosphate group from an organic phosphate ester. Scientific data suggested that liver diseases and metabolic done disorders would increase the ALP activity in serum. Bone specific ALP level is elevated due to high osteoblastic activity, which has been reported to be highest in some disease such as Paget disease or rickets/osteomalasia [35]. ALP is regarded and commonly used as a tumor marker and high ALP has been associated with the healing fracture, bone growth, acromegaly, myelofibrosis, osteogenic sarcoma, or bone metastases, leukemia, and rarely myeloma. It has been reported that isoenzymes has been reported to be high in children's and would decrease in old age over fifties. Hyperthyroidism has been reported to have increased ALP level [36].

Hence, 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. Thus, it might improve the ALP level, which is a good predictor of neotissue mineralization that could provide beneficial therapeutic prospects for the treatment of various bone diseases [37].

3.3. Effect of Test Items on Collagen Synthesis

Biofield Energy Treated vit D_3 and DMEM were studied for change in the collagen level and data showed a significant increase in the collagen level at various experimental tested concentrations. The results in term of % increase in collagen

synthesis are presented in Figure 3. The positive control, rutin showed a significant increased value of collagen by 40.5%, 45.7%, and 58.6% at 0.1, 1, and 10 µg/mL, respectively. The experimental test group's *viz.* UT-DMEM+BT-TI showed a significant increased collagen level by 182.5%, 138.4%, and 100.8% at 0.1, 1, and 10 µg/mL, respectively while BT-DMEM+UT-TI group showed a significant increased collagen level by 120.6%, 64.6%, and 35.1% at 0.1, 1, and 10 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 261.9%, 179.8%, and 116.0% at 0.1, 1, and 10 µg/mL, respectively as compared with the untreated test item and DMEM group. Overall, all the experimental Biofield Energy Treated test item and DMEM groups showed a significant improved level of collagen at all the tested concentrations compared with the untreated group.

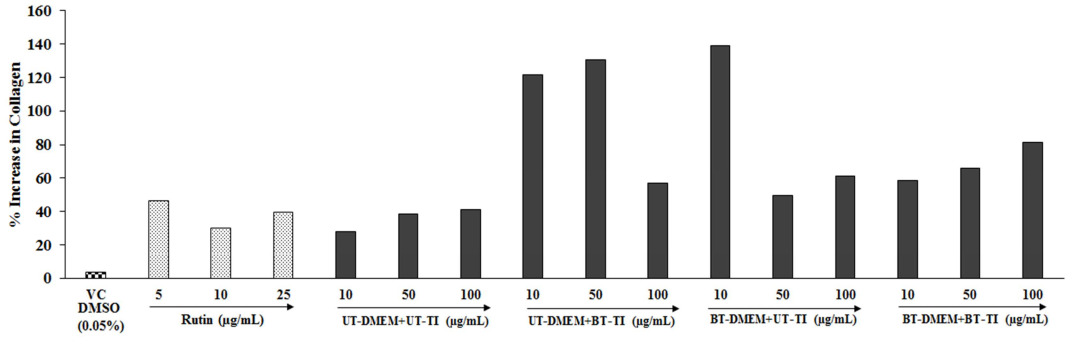

Figure 3. *Effect of the test item on MG-63 cell lines for collagen level. VC: Vehicle control (DMSO-0.05%), UT: Untreated; BT: Biofield Treated; TI: Test Item.*

The crystals of mineral bound to protein form a composite material in bone. Bone minerals are mainly bound in an orderly manner to a matrix, which is made up largely of a single protein known as collagen. Collagen signifies bone strength and is made up of bone cells, which is assembled as long thin rods containing three intertwined protein chains that forms the large fibers [38]. The collagen matrix is strengthen by other proteins present in the bone, which also regulates the ability to bind with other minerals. Collagen is most abundant protein used for bone tissue regeneration and enhanced bone apatite formation, which defines the geometry and the shape of bones, collagen content, minerals, microarchitecture of the trabecular bones, and its turnover [39]. Thus, it can be concluded that Biofield Energy (The Trivedi Effect®) Treated vit D_3 and DMEM would be an important source to improve the level of collagen and its mineralization process against different orthopedic diseases.

3.4. Effect of Test Items on Bone Mineralization

The effect of the Biofield Energy Treated vit D3 and DMEM were studied on bone mineralization and data showed a significant increase in the percentage bone mineralization process at various experimental tested concentrations on MG-63 cell line. The results of bone mineralization among different experimental groups have been presented in Figure 4. The positive control, rutin showed a significant increased value of bone mineralization by 48%, 59.7%, and 139.0% 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 261.2% at 1 µg/mL, while BT-DMEM+UT-TI group showed a significant increased bone mineralization by 239.9%, 21.5%, and 14.9% at 1, 10, and 50 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 24.5% and 324.5% at 10 and 50 µg/mL, respectively as compared with the untreated test item and DMEM group. 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.

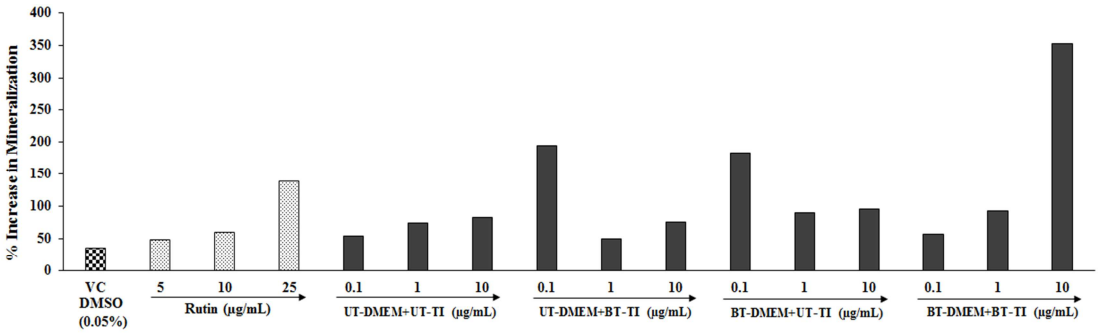

Figure 4. *Effect of the test item on MG-63 cell line for bone mineralization. VC: Vehicle control (DMSO-0.05%), UT: Untreated; BT: Biofield Treated; TI: Test Item.*

Vitamin D_3 and its metabolites have direct effect on bone health. Various studies suggested that with sufficient supply of calcium, vitamin D metabolites improve the calcium level and would facilitates the absorption of minerals along with osteoblasts maturation. Calcium and vitamin D through VDRs play vital role in bone mineralization and treating bone disorders [40]. Thus calcium and vitamin supplements improve the bone health [41, 42], but Biofield Energy Treated vit D3 and DEMEM would play a major role in various bone related disorders and have distinct roles during the bone recovery process.

4. Conclusions

Biofield Energy Healing based vitamin D_3 and DMEM has proved to be significant effect on bone health in MG-63 cell line. The test samples using MTT assay showed a significant improved cell viability with more than 80% cell viability and the test samples were also reported to be improved cell viability as compared with the untreated group. In addition, Biofield Energy Treated test samples showed a significant improved level of ALP by 114.3% and 96.2% in UT-DMEM+BT-TI at 0.1 and 10 µg/mL, respectively, while 304.8% (at 0.1 µg/mL) in BT-DMEM+UT-TI group. In addition, the ALP level was increased by 314.3% and 101.3% at 0.1 and 1 µg/mL, respectively as compared with the untreated test item and DMEM group. The level of collagen was significantly increased by 82.5%, 138.4%, and 100.8% at 0.1, 1, and 10 µg/mL, respectively, while 120.6% and 64.6% increased collagen was reported at 0.1 and 1 µg/mL, respectively in BT-DMEM+UT-TI group as compared with the untreated test item and DMEM group. In addition, the level of collagen was increased by 261.9%, 179.8%, and 116.0% at 0.1, 1, and 10 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 261.2% (at 1 µg/mL) in UT-DMEM+BT-TI group, 239.9% (at 1 µg/mL) in BT-DMEM+UT-TI group, and 324.5% (at 50 µg/mL) in 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, calcium 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.* low bone density and osteoporosis, osteogenesis imperfecta, Paget's disease of bone, rickets, osteomalacia, bone and/or joint pain, increased frequency of fractures, deformed bones, osteoma, 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-arthritic, anti-osteoporosis, anti-stress, anti-aging, anti-apoptotic, wound healing, anti-cancer, anti-psychotic and anti-fibrotic roles. It also influence cell-to-cell communication, normal cell growth, cell differentiation, cell cycling and proliferation, hormonal balance, neurotransmission, 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 Asthma, Ulcerative Colitis, Alzheimer's Disease, Atherosclerosis, Dermatitis, Diverticulitis, Dermatomyositis, Graves' Disease, Hashimoto Thyroiditis, Multiple Sclerosis, Myasthenia Gravis, Pernicious Anemia, Aplastic Anemia, Sjogren Syndrome, Systemic Lupus Erythematosus, Diabetes, Hepatitis, Irritable Bowel Syndrome, Parkinson's Disease, stress etc. 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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