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Biofield Energy Enriched Vitamin D_3: A New Horizons for Development of Bone Health Using MG-63 Cells

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Abstract: The current study investigates the effect of Consciousness Energy Healing based vitamin D_3 and DMEM medium on bone health parameters *in vitro* using MG-63 cells such as alkaline phosphatase enzyme (ALP) activity, collagen levels and bone mineralization. The test items (TI) *i.e.* vitamin D_3 and DMEM medium were divided into two parts. The test samples received Consciousness Energy Healing Treatment by Maire Anne Mayne and samples were defined as the Biofield Energy Treated (BT) samples, while the other parts of each sample were denoted as the untreated test items (UT). Cell viability using MTT assay showed that cell viability was more than 78% with safe and nontoxic profile on MG-63 cell line. The level of ALP was significantly increased by 141.9% and 25% at 50 and 100 µg/mL, respectively in the UT-DMEM+BT-TI group as compared with the untreated group. In addition, 144.6% (100 µg/mL) and 120.6% (1 µg/mL) increase in ALP activity in BT-DMEM+UT-TI and BT-DMEM+BT-TI group, respectively as compared with the untreated group. The level of collagen was significantly increased by 58.7% and 72.6% at 1 and 10 µg/mL, respectively in the UT-DMEM+BT-TI group, while 39.7% and 101.6% at 1 and 10 µg/mL, respectively in the BT-DMEM+UT-TI group as compared with the untreated group. In addition, BT-DMEM+BT-TI group showed a significant increased collagen level by 117.5%, 163.5%, and 169.4% at 0.1, 1, and 10 µg/mL, respectively as compared with the untreated test item and DMEM group. The percent of bone mineralization was significantly increased by 110.2%, 116.8%, and 81% at 10, 50, and 100 µg/mL, respectively in UT-DMEM+BT-TI group, while 148.8%, 180.1%, and 129.4% at 10, 50, and 100 µg/mL, respectively in BT-DMEM+UT-TI group as compared with the untreated group. In addition, BT-DMEM+BT-TI group showed a significant increased bone mineralization by 142%, 181.1%, and 113% at 10, 50, and 100 µg/mL, respectively as compared with the untreated group. The experimental data suggested that the Biofield Energy Treated vitamin D_3 and DMEM would play an important role in the promotion and maintenance of strong and healthy bones, which improve quality of life. Biofield Energy Treatment might be vital in maintaining the various clinical safety and quality of life by assisting them in maintaining optimal vitamin D levels. It regulates the osteoblast function, improves bone mineralization, and calcium absorption in wide range of bone disorders along with wide range of adverse health conditions, comprising cancer and certain autoimmune diseases.

Keywords: Biofield Energy, Bone Mass, Bone Strength, Osteosarcoma Cells, Vitamin D, 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 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, 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.

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. This Biofield Energy Healing Treatment was provided by Maire Anne Mayne remotely for ~5 minutes. Biofield Energy Healer was remotely located in the UK, 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. Maire Anne Mayne 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):

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

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

The cell viability of all the test samples were studied in MG-63 cells using MTT assay. The acquired results were compared with respect to rutin at various concentrations for the estimation of percentage cell viability. The cell viability among different groups results are graphically presented in Figure 1. The results of percentage cell viability in all the tested concentrations showed the cell viability range of 78% to 143% in different test item groups with DMEM, while for rutin group showed more than 86.6% cell viability (Figure 1). Overall, the 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, which were used for the estimation of other bone health parameters.

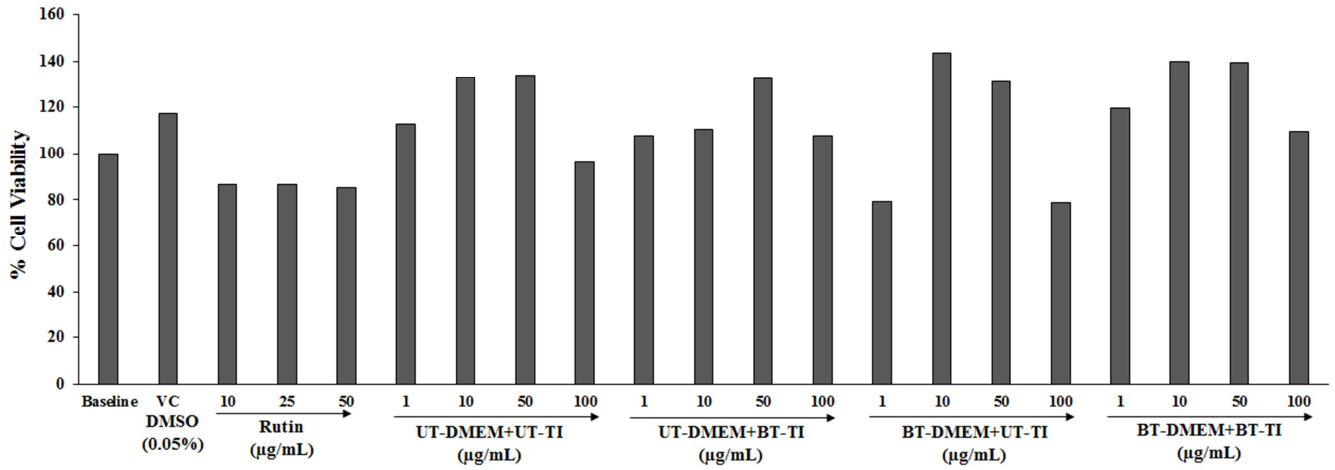

Figure 1. *Cell viability using MTT assays of the test formulations on MG-63 cell line after 72 hours. VC: Vehicle control (DMSO-0.05%), UT: Untreated; BT: Biofield Treated; TI: Test Item.*

3.2. Alkaline Phosphatase (ALP) Enzyme Activity

Bone alkaline phosphatase (BAP) is defined as the bone-specific isoform of ALP, which is a glycoprotein present on the surface of osteoblasts. It directly signifies the biosynthetic activity of the bone-forming cells. It is the bone health indicator and defines reliable indicator of bone metabolism. ALP level and its synthesis decreases with age, which can be overcome using health supplements such as calcium, vitamin D_3, and other natural sources [35-37]. Reduced level of ALP may lead to serious bone health diseases such as post-menopausal women, osteoporosis, bone cancers, Paget's disease of bone, healing fracture, bone growth, acromegaly, myelofibrosis, osteogenic sarcoma, or bone metastases, leukemia, and rarely myeloma [36]. The results of the Biofield Energy Healing Treatment on the level of ALP in MG-63 cells 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 4.3% increased level of ALP as compared with the untreated cells group. The positive control, rutin showed a significant increased value by 33.97%, 45.69%, and 79.66% 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 6.7%, 14.9%, 141.9%, and 25% at 1, 10, 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 1.8% and 144.6% at 50 and 100 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 120.6% and 5% at 1 and 10 µg/mL, respectively as compared with the untreated test item and DMEM group. Overall, the experimental data concluded that the Biofield Energy Healing Treatment in the test samples showed a significant improved level of the ALP, which could be the best supplementation to treat various bone and age related diseases. The experimental data well described that The Trivedi Effect®-Energy of Consciousness Healing based vit D_3 and DMEM could be used to improve the ALP concentration in many bone disorders.

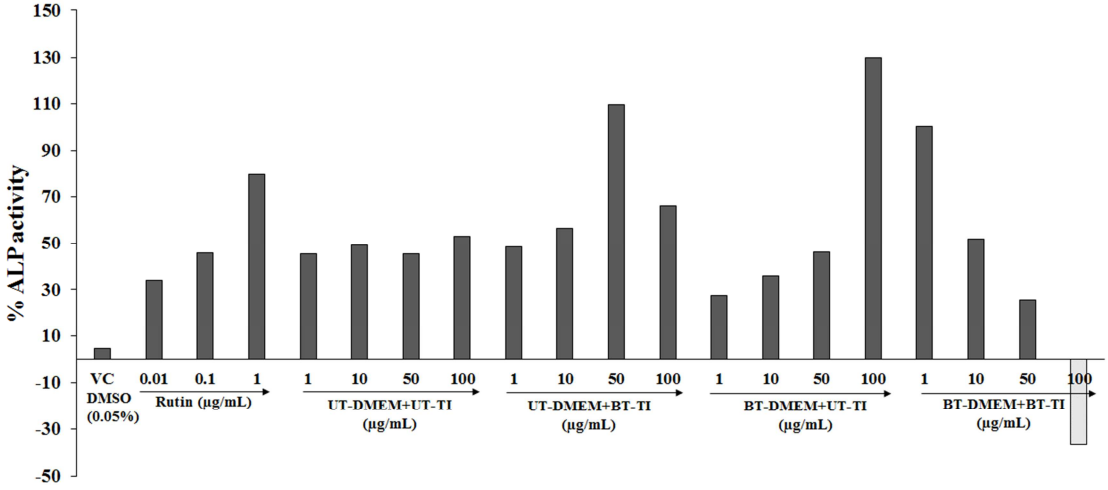

Figure 2. *Study of Alkaline Phosphatase (ALP) enzyme activity of the Biofield Energy Treated test items on MG-63 cell line. VC: Vehicle control (DMSO-0.05%), UT: Untreated; BT: Biofield Treated; TI: Test Item.*

3.3. Assessment of Collagen Synthesis

Collagen is an extremely important fibrous protein present in the connective tissue. Besides, present in skin as major component, collagen play important role in bones and joints. Fibrils are formed by collagen protein, which are arranged in regular pattern in long tubes of fibrous tissue. The synthesis of collagen hampered with age, which results in weakening of the joints, tendons, and ligaments that are more prone to injury. Bone mineralization has significant role of collagen type I, which is the most abundant matrix protein [38]. Overall, the reduced collagen synthesis results in serious bone diseases such as the type of bone loss experienced in osteoporosis, which can be overcome using various supplementation [39]. The collagen level among Biofield Energy Treated vit D_3 and DMEM was estimated at various safe concentrations and the data suggested significant increased collagen level. The results are presented as% values with respect to the untreated cells in Figure 3. The rutin hydrate showed a significant increased value of collagen by 25.81%, 51.61%, and 51.91% 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 5.3%, 58.7%, and 72.6% at 0.1, 1, and 10 µg/mL, respectively while BT-DMEM+UT-TI group showed a significant increased collagen level by 29.8%, 39.7%, and 101.6% 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 117.5%, 163.5%, and 169.4% at 0.1, 1, and 10 µg/mL, respectively as compared with the untreated test item and DMEM group. Thus, collagen supplementation in any form after certain age along with other nutritional factors is considered as the therapeutic agent to fight against bone diseases in case of osteoarthritis and osteoporosis. The Biofield Energy Treated vit D_3 and DMEM groups showed a significant improved level of collagen compared with the untreated group. Biofield Energy Treated vit D_3 (The Trivedi Effect®) that would improve the collagen level for bone health, which can be used to decrease aging process and bone inflammation.

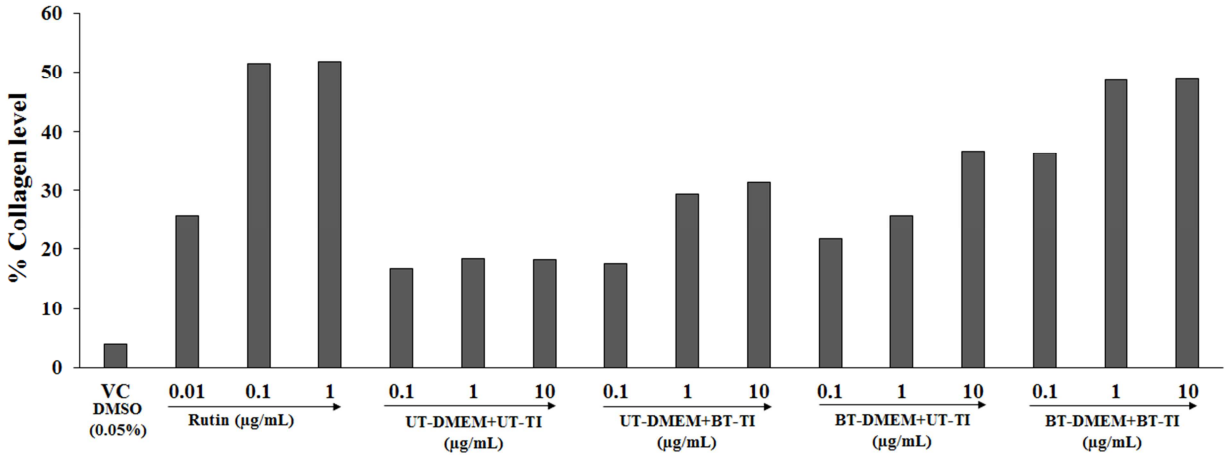

Figure 3. *Action of the test item on MG-63 cell line for collagen level. VC: Vehicle control (DMSO-0.05%), UT: Untreated; BT: Biofield Treated; TI: Test Item.*

3.4. Bone Mineralization

Bone mineralization play an important role in the management of various bone disorders such as osteoporosis or other bone diseases. Loss in bone mass results in decreased quality of bone and poor calcium absorption that leads to reduced bone mineral density (BMD) and various structural abnormalities [40, 41]. Bone health and its quality vary due to the bone architecture, bone remodeling rate, microdamage, apoptosis of bone cellular populations, and properties of the bone matrix *i.e.* crystals size, mineralization, collagen structure, and cross-linking [40]. The present study was conducted to check the alteration in percentage of bone mineralization in Biofield Energy Treated test samples with respect to the untreated test samples. Biofield Energy Treated vit D_3 and DMEM groups showed a significant improved bone mineralization on MG-63 cell line. The results are presented in term of percentage change of bone mineralization among different experimental groups in Figure 4. The positive control, rutin group showed a significant increased value of bone mineralization by 60.89%, 83.68%, and 137.12% 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 110.2%, 116.8%, and 81% at 10, 50, and 100 µg/mL, respectively while BT-DMEM+UT-TI group showed a significantly increased bone mineralization by 148.8%, 180.1%, and 129.4% 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 bone mineralization by 142%, 181.1%, and 113% at 10, 50, and 100 µg/mL, respectively as compared with the untreated test item and DMEM group. The experimental test groups showed that Biofield Energy Healing Treatment significantly improved the rate of bone mineralization compared with the untreated groups.

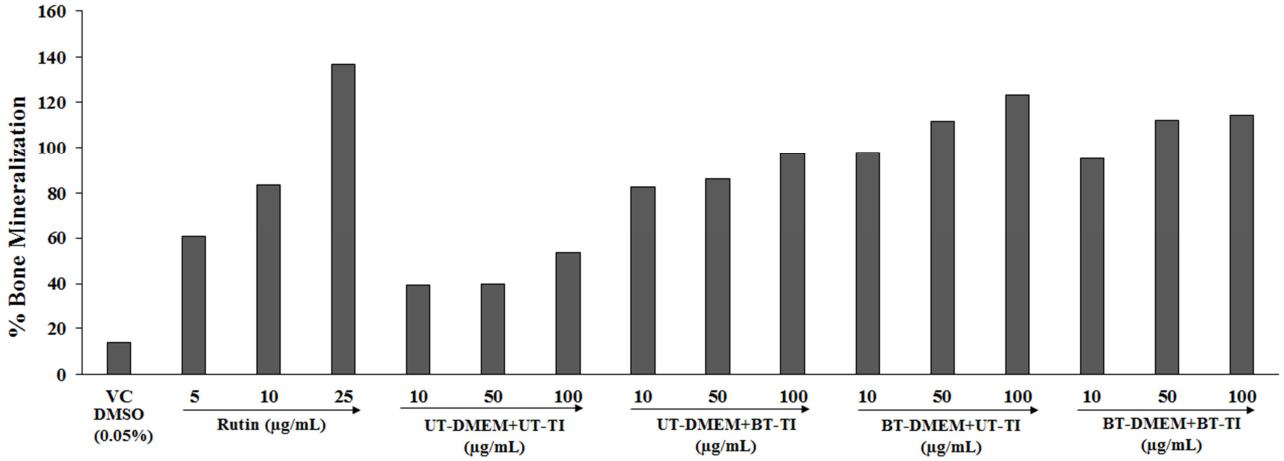

Figure 4. *Consequence of the test item on MG-63 cell line for bone mineralization. VC: Vehicle control (DMSO-0.05%), UT: Untreated; BT: Biofield Treated; TI: Test Item.*

4. Conclusions

The study includes the effect of Biofield Energy Treatment on various bone health parameters. MTT assay data showed that a significant improved viability with more than 78% among the tested groups. Bone health parameters such as the level of ALP was increased by 141.9%, and 25% at 50 and 100 µg/mL, respectively in the UT-DMEM+BT-TI, while 144.6% at 100 µg/mL in the BT-DMEM+UT-TI group as compared with the untreated test item and DMEM group. BT-DMEM+BT-TI group showed an increased ALP level by 120.6% at 1 µg/mL. The level of collagen was significantly increased by 58.7% and 72.6% at 1 and 10 µg/mL, respectively in the UT-DMEM+BT-TI, while 29.8%, 39.7%, and 101.6% at 0.1, 1, and 10 µg/mL, respectively in the BT-DMEM+UT-TI group. In addition, collagen level was increased by 117.5%, 163.5%, and 169.4% at 0.1, 1, and 10 µg/mL, respectively in BT-DMEM+BT-TI group as compared with the untreated test item and DMEM group. Similarly, the bone mineralization percent was significantly increased by 110.2%, 116.8%, and 81% at 10, 50, and 100 µg/mL, respectively in the UT-DMEM+BT-TI group, while 148.8%, 180.1%, and 129.4% at 10, 50, and 100 µg/mL, respectively in the BT-DMEM+UT-TI group as compared with the untreated group. In addition, BT-DMEM+BT-TI group showed a significant increased bone mineralization by 142%, 181.1%, and 113% at 10, 50, and 100 µg/mL, respectively as compared with the untreated 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.* 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, 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, Alzheimer's Disease, Dermatitis, Irritable Bowel Syndrome, Asthma, Hashimoto Thyroiditis, Pernicious Anemia, Sjogren Syndrome, Multiple Sclerosis, Aplastic Anemia, Hepatitis, Diverticulitis, Graves' Disease, Dermatomyositis, Diabetes, Myasthenia Gravis, Parkinson's Disease, Atherosclerosis, Systemic Lupus Erythematosus, stress, etc. with a safe therapeutic index to improve overall health, and quality of life.

Abbreviations

CAM: Complementary and Alternative Medicine, NCCAM: National Center for Complementary and Alternative Medicine; MG-63: Human Bone Osteosarcoma Cells, ALP: Alkaline phosphatase, DMEM: Dulbecco's Modified Eagle's Medium, FBS: Fetal Bovine Serum, FBS: Fetal bovine serum; EDTA: Ethylene Diamine Tetra Acetic Acid, UT: Untreated, BT: Biofield Energy Treated, TI: Test Item.

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