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Full Length Research.

Improved Overall Bone Health Activity in MG-63 Cell Line after Biofield Energy Healing Treatment on Vitamin D_3.

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The aim of the study is to evaluate the role of The Trivedi Effect®-Consciousness Energy Healing based vitamin D_3 and DMEM medium on bone health parameters *in vitro* using MG-63 cells. The parameters of bone health tested are 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 Kathleen Starr Vagt 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 exhibited increased cell viability more than 72% with safe and nontoxic profile among test samples on MG-63 cell line. ALP was significantly increased by 140.8% (50 µg/mL), 167.3% (100 µg/mL),and 223.5% (50 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. The level of collagen was significantly increased by 120.7% and 93% at 1 and 10 µg/mL, respectively in the UT-DMEM+BT-TI group, while 46.7%, 100.9%, and 29.2% at 1, 10, and 50 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 collagen level by 60.9%, 93%, and 14.4% at 1, 10, and 50 µg/mL, respectively as compared with the untreated test item and DMEM group. The percent of bone mineralization was significantly increased by 95.9% at 100 µg/mL in the UT-DMEM+BT-TI group, while 162% and 35.5% at 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 57% and 107.4% at 50 and 100 µg/mL, respectively as compared with the untreated group.T hus, the study results concluded that The Trivedi Effect Treatment would be the best alternative treatment for the maintenance of strong and healthy bones and quality of life. Further, it regulates the osteoblast function and improved the level of collagen, ALP, and calcium absorption in wide range of bone disorders along with wide range of adverse bone health conditions.

Keywords: The Trivedi Effect®, Biofield Healing, Bone Health, Osteosarcoma Cells, Vitamin D, Bone Mineralization.

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.

INTRODUCTION

Vitamin D has multiple effects which regulate the functions indifferent 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.(Holick, 1996). 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 (Holick, 1996).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) (van Leeuwen et al. 2001). 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 (Bikle, 2012). Vitamin D insufficiency and deficiency is the major health problem, which causes metabolic bone disease in the young and elderly populations (Lips, 2001). 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 (Hossein-nezhad and Holick, 2013).

Various *in vitro* studies have readily established 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 (Czekanska et al. 2012). Hence, MG-63 cell line is widely used for studying the potential of any test compounds to improve the bone health (Luo and Liao, 2003). 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 (Iba et al. 2004). 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 arrays of an organic matrix known as Osteoid (Viguet-Carrin et al. 2006). Likewise, calcium phosphate is deposited in the Osteoid and gets mineralized (combination of calcium phosphate and hydroxyapatite) and provides rigidity to the bone (Bhattarai, 2014). 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* (Rubik, 2002). Biofield Energy Healing Treatment (The Trivedi Effect) contains a putative bioenergy, which is channeled by a renowned practitioner from a distance. Biofield Energy Healing as a CAM showed a significant results in biological studies (Barneset al. 2008). However, the National Center for Complementary and Alternative Medicine (NCCAM), well-defined Biofield therapies in the subcategory of Energy Therapies (Frasset al. 2012). The Trivedi Effect

Consciousness Energy Healing Treatment has been reported with significant revolution in the physicochemical properties of metals, chemicals, ceramics and polymers (Trivedi and Tallapragada, 2008;Trivedi et al. 2015a, b), improved agricultural crop yield, productivity, and quality (Trivedi et al. 2015c, d), transformed antimicrobial characteristics (Trivedi et al. 2015e, f), bone health (Ansari et al. 2018; Koster et al. 2018), biotechnology (Nayak and Altekar, 2015), improved bioavailability (Branton and Jana, 2017a, b, c), skin health (Kinney et al. 2017; Singh et al. 2017), nutraceuticals (Trivedi et al. 2017a, b), cancer research (Trivedi et al. 2015g, h), 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.

MATERIAL AND METHODS

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-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium) (MTT), Direct Red 80, and ethylene ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma, USA. All the other chemicals used in this experiment were analytical grade procured from India.

Cell Culture

Human bone osteosarcoma cell line -MG-63 was used as 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, 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 (Czekanska et al. 2012).

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.

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 Kathleen Starr Vagt 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. Kathleen Starr Vagt in this study never visited the laboratory in person, nor had any contact with the test item and medium. Further, the control group was treated with a sham healer for comparative purposes. The sham healer did not have any knowledge about the Biofield Energy Treatment. After that, the Biofield Energy Treated and untreated samples were kept in similar sealed conditions for experimental study.

Determination of Non-cytotoxic Concentration

The cell viability was performed by MTT assay in 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 microplate reader, BioTek, USA (Riss et al. 2013). The percentage cytotoxicity at each tested concentrations of the test substance was calculated using the following equation (1):

% Cytotoxicity = (1-X/R)*100……(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):

% Cell Viability = 100 - % Cytotoxicity…… (2)

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

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 microplate reader (Biotek, USA). The absorbance values obtained were normalized with substrate blank (*p*NPP solution alone) absorbance values(Czekanska, 2012). The percentage increase in ALP enzyme activity with respect to the untreated cells (baseline group) was calculated using equation (3):

% Increase = [(X-R)/R)]*100……(3)

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

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

Assessment of Collagen Synthesis

The MG-63 cells were counted using 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 microplate reader. The level of collagen was extrapolated using standard curve obtained from purified Calf Collagen Bornstein and Traub Type I (Sigma Type III) (Czekanska, 2012). The percentage increase in collagen level with respect to the untreated cells (baseline group) was calculated using equation (4):

% Increase = [(X-R)/R]*100……...(4)

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

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

Assessment of Bone Mineralization by Alizarin Red S Staining

The MG-63 cells were counted using an hemocytometer and plated in 24-well plate at the density corresponding to 10 x10^3 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 microplate reader (Czekanska, 2012). The percentage increase in bone mineralization with respect to the untreated cells (baseline group) was calculated using the following equation (5):

% Increase = [(X-R)/R]*100……(5)

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

Statistical Analysis

All the values were represented as percentage of 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 $p \leq 0.05$.

RESULTS AND DISCUSSION

Cell Viability using MTT Assay

MTT assay of cell viability showed a significant improved percentage among the Biofield Energy Treated vitamin D_3 and DMEM in MG-63 cells are shown in Figure 1. All the cell viability data was compared with the untreated group and was presented in terms of percentage cell viability. The percentage cell viability in all the tested cell lines showed the cell viability range of 72% to 127% in different test item groups with DMEM, while for rutin group showed more than 85% cell viability. Overall, the data suggest that all the test samples were found safe with maximum concentration upto 100 µg/mL against the tested MG-63 cells, which were used for the estimation of other bone health parameters such as ALP, collagen and bone mineralization.

Alkaline Phosphatase (ALP) Enzyme Activity

The Trivedi Effect®-Energy of Consciousness Healing based vit D_3 and DMEM showed a significant increased level of ALP in different test groups as compared with the untreated group. The percentage change in ALP data at various concentrations in different groups were presented in Figure 2 in terms of percentage values. The positive control, rutin showed a significant increased value by 30.02%, 34.31%, and 51.47% at 0.001, 0.01, and 0.1 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 140.8% and 15.2% 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 167.3% at 100 µg/mLas 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 223.5% at 50 µg/mL as compared with the untreated test item and DMEM group. Thus, the overall data suggested a significant improved ALP level after Biofield Energy Treatment. Biofield Energy Treated test samples could be useful against patients suffering from bone disorders to improve the skeletal structure and overall health. ALP is the bone specific glycoprotein (zinc metalloprotein enzymes). Obstructive liver disease, osteoblastic activity, and metabolic bone disease would results in reduced level of bone ALP, which affect bone skeleton. The altered level of ALP would lead to Paget disease or rickets/osteomalacia. Altered bone ALP level results in reduced bone growth, acromegaly, osteogenic sarcoma, or bone metastases, healing fracture, myelofibrosis, leukemia, and rarely myeloma. Thus, bone ALP is also used as a bone health and tumor biomarker (Saraç and Saygılı, 2014; Kubo et al. 2012; Deftos et al. 1991). Hence, it was concluded that The Trivedi Effect®-Energy of Consciousness Healing based vit D_3 can be used bone health supplements against many bone disorders. The Trivedi Effect®-Biofield Energy Treated vit D_3 as bone health supplements could be beneficial to maintain a healthy skeletal structure for the patients suffering from various bone-related disorders.

Estimation of Collagen Synthesis

The Trivedi Effect® Biofield Energy Treated vit D_3 and DMEM showed as significant improved level of collagen synthesis as compared with the untreated test samples. Theresults are presented in percentage collagen values in Figure 3. The rutin hydrate (positive control group) showed a significant increased value of collagen by 39.60%, 42.81%, and 90.13% 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 120.7%, 93%, and

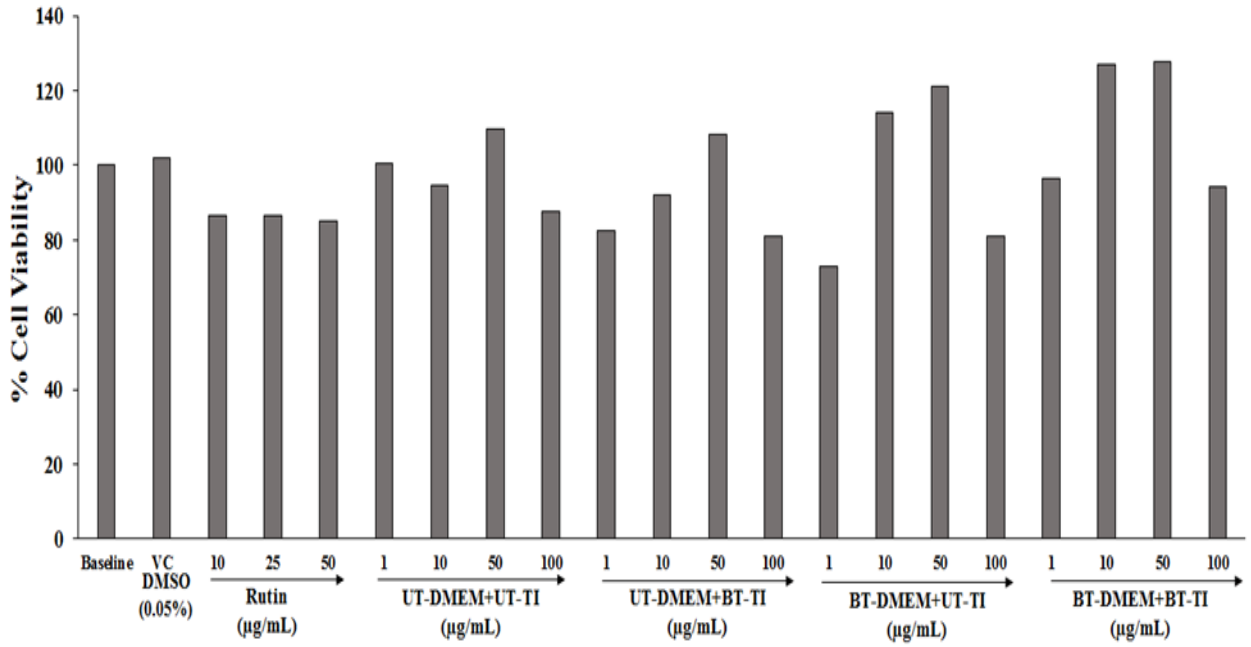

Figure 1: Cell viability using MTT assays of the test items on MG-63 cell line after 72 hours. VC: Vehicle control (DMSO-0.05%), UT: Untreated; BT: Biofield Treated; TI: Test Item.

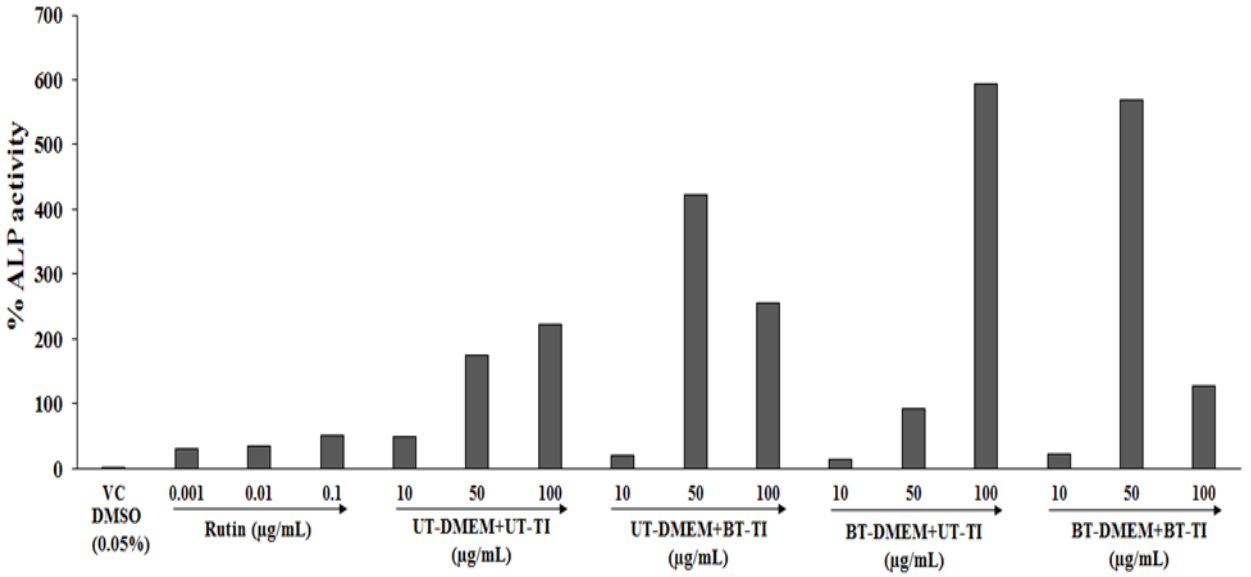

Figure 2: Study of Alkaline Phosphatase (ALP) enzyme activity of the Biofield Energy Treated test items on MG-63 cell line. VC: Vehicle control (DMSO-0.05%), UT: Untreated; BT: Biofield Treated; TI: Test Item.

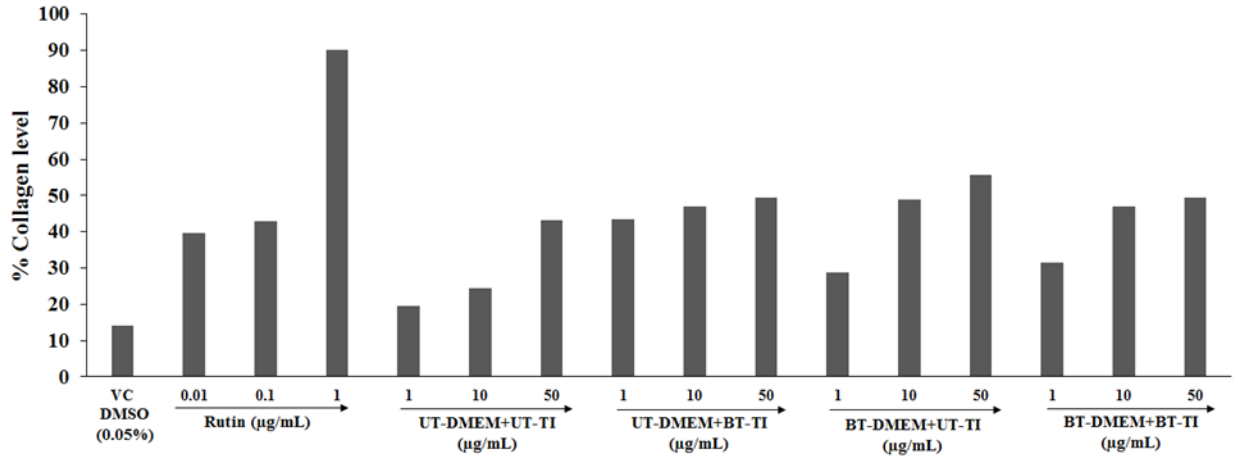

Figure 3: Action of the test item on MG-63 cell line for collagen level. VC: Vehicle control (DMSO-0.05%), UT: Untreated; BT: Biofield Treated; TI: Test Item.

14.4% at 1, 10, and 50 µg/mL, respectively while BT-DMEM+UT-TI group showed a significant increased collagen level by 46.7%, 100.9%, and 29.2% 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 collagen level by 60.9%, 93%, and 14.4% at 1, 10, and 50 µg/mL, respectively as compared with the untreated test item and DMEM group. The experimental data showed a significant increased level of collagen, which is a complex tissue that helped to resist mechanical forces and fractures. Collagen type I have significant role in bone health, which is the most abundant matrix protein (Barnes et al. 1973; Bring hurst and Potts, 1982). The Trivedi Effect-Biofield Energy Treated vit D_3 as compared with the untreated vitamin D could be beneficial to maintain a healthy skeletal structure against various bone-related disorders. Overall, The Trivedi Effect-Biofield Energy Treated vit D_3 and DMEM can be used as bone health supplements to improve collagen level, which play an important role against weaken joints, tendons, and ligaments.

Bone Mineralization

The Trivedi Effect®-Biofield Energy Treated vit D_3 and DMEM showed a significant increase in bone mineralization in the test samples. Vitamin D is the hormone of bone and Biofield Energy Treatment significantly increased the level of mineralization of various vital constituents, which would improve the bone health. The results in term of percentage bone mineralization was presented in Figure 4. The positive control, rut in group showed a significant increased value of bone mineralization by 61.72%, 82.15%, and 141.72% 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 95.9% at 100µg/mL,while BT-DMEM+UT-TI group showed a significantly increased bone mineralization by 19.7%, 162%, and 35.5% at 10, 50, and 100µg/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 57% and 107.4%at 50 and 100µg/mL, respectively as compared with the untreated test item and DMEM group. The improved bone mineralization might significantly increase the bone mineral density, improve precipitation of bone minerals, organic matrix, water, and other nutrients such as calcium and phosphorus, which can improve the various patients of bone disorders (Ruppel et al. 2008; van Driel and van Leeuwen, 2017). The Trivedi Effect®-Biofield Energy Treated vit D_3 as compared with the untreated vitamin D could be beneficial to maintain a healthy skeletal structure for the patients suffering from various bone-related disorders.

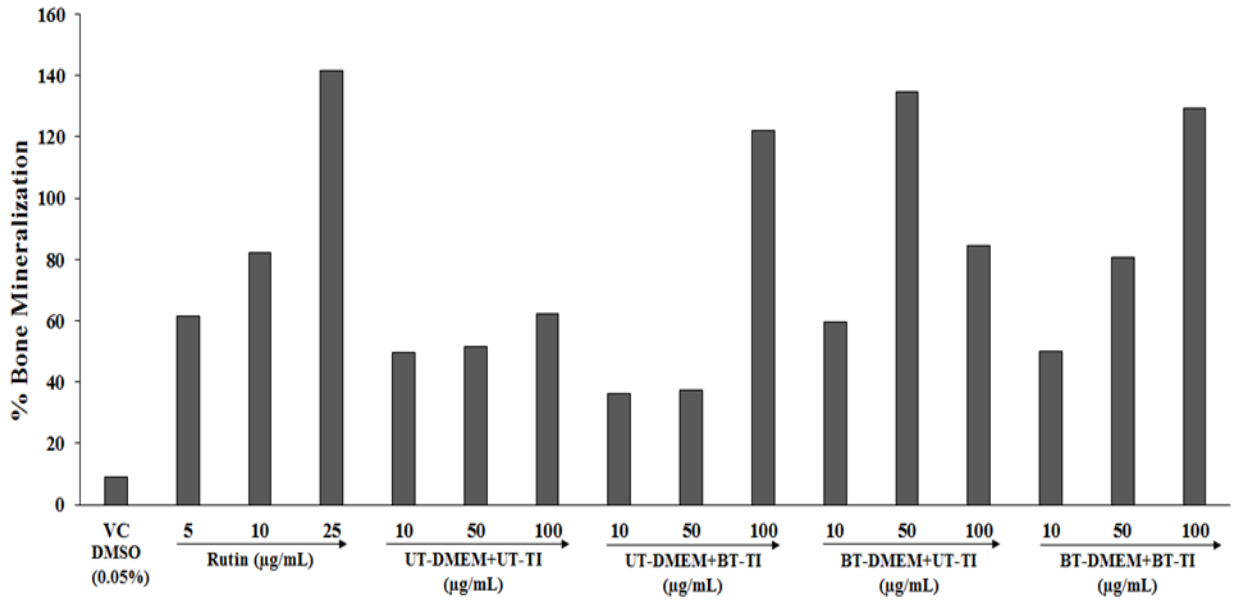

Figure 4: Consequence of the test item on MG-63 cell line for bone mineralization. VC: Vehicle control (DMSO-0.05%), UT: Untreated; BT: Biofield Treated; TI: Test Item.

CONCLUSIONS

The Consciousness Energy Healing based vitamin D_3 showed a significant improved overall bone health. MTT assay data showed significant improved cell viability with more than 72% among different experimental groups. Bone health parameters such as ALP was increased by 140.8% and 15.2% at 50 and 100 µg/mL, respectively in the UT-DMEM+BT-TI, while 167.3% at 100 µg/mL in the BT-DMEM+UT-TI group as compared with the untreated test item and DMEM group. In addition, BT-DMEM+BT-TI group showed an increased ALP level by 223.5% at 50 µg/mL. The level of collagen was significantly increased by 120.7%, 93%, and 14.4% at 1, 10, and 50 µg/mL, respectively in the UT-DMEM+BT-TI, while46.7%, 100.9%, and 29.2% at 1, 10, and 50 µg/mL, respectively in the BT-DMEM+UT-TI group. The level of collagen was increased by 60.9%, 93%, and 14.4% at 1, 10, and 50 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 by95.9% at 100 µg/mL in the UT-DMEM+BT-TI group, while 19.7%, 162%, and 35.5% 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 57% and 107.4% at 50 and 100 µg/mL, respectively as compared with the untreated group. The Bone health parameters were significantly improved among the Biofield Energy Treated vitamin D_3 test samples in MG-63 cells. 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 hormonal imbalance, aging, and various immune related disease conditions such as Multiple Sclerosis, Alzheimer's Disease, Dermatitis, Atherosclerosis, stress, Irritable Bowel Syndrome, Systemic Lupus Erythematosus, Pernicious Anemia, Aplastic Anemia, Hepatitis, Diverticulitis, Graves' Disease, Dermatomyositis, Asthma, Hashimoto Thyroiditis,Diabetes, Myasthenia Gravis, Ulcerative Colitis, Sjogren Syndrome, Parkinson's Disease, etc. with a safe therapeutic index to improve overall health, and quality of life.

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