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A Comparative Analysis of Vitamin D₃ and Biofield Energy Treated Vitamin D₃ in Bone Health Using MG-63 Cell Line



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Abstract

Poor bone health is related with various bone disease that predisposes to fractures, while sufficient supplementation with calcium and vitamin D suggested prevention of bone diseases. The present study aimed to find the effect of Consciousness Energy Healing based vitamin D₃ and DMEM medium on *in vitro* bone health parameters using MG-63 cells such as alkaline phosphates enzyme (ALP) activity, collagen levels, and bone mineralization. The test items (TI) i.e. vitamin D₃ and DMEM medium were divided into two parts. The test samples received Consciousness Energy Healing Treatment by Lauree Ann Duprey-Reed 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 range 76% to 139% with safe and nontoxic profile of test samples on MG-63 cell line. ALP was significantly increased by 171.8% and 189.7% at 10µg/mL in the UT-DMEM+BT-TI and BT-DMEM+BT-TI groups, respectively as compared with the untreated group.

In addition, 100% and 33.3% increased ALP at 0.1 and 10µg/mL, respectively in BT-DMEM+UT-TI group as compared with the untreated group. The level of collagen was significantly increased at 10µg/mL by 145.8% in the UT-DMEM+BT-TI group, while 65.8% and 145.8% at 1 and 10µg/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 86.3% and 136.1% at 1 and 10µg/mL, respectively as compared with the untreated test item and DMEM group.

The percent of bone mineralization was significantly increased by 150%, 21%, and 73.2% at 0.1, 10, and 100µg/mL, respectively in the UT-DMEM+BT-TI group, while 258.3% and 126.8% at 0.1 and 100µg/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 33.3%, 32.7%, and 187.4% at 0.1, 10, and 100µg/mL, respectively as compared with the untreated group. Biofield Energy Treatment might be vital in promotion and maintenance of strong and healthy bones 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 many autoimmune disorders.

Keywords: Biofield energy; Bone strength; 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; EDTA: Ethylenediaminetetraacetic Acid; UT: Untreated, BT: Biofield Energy Treated, TI: Test Item

Introduction

Our good health depends on various factors such as adequate input of essential vitamins and minerals. One of the extreme important vitamin, is vitamin D not only to strengthen bone and teeth and bones, but also provide protection against serious diseases. Some primary function of vitamin D included the calcium absorption, which helps to build bones strong and healthy [1]. Besides, it also blocks parathyroid hormone release in the body, which protects the body from reabsorbing the bone tissue and lead to bones thin and

brittle. Vitamin D receptors (VDRs) are present in everybody or gans and significantly regulate the cell-to-cell communication, cell differentiation, hormonal balance, cell cycling and proliferation [2].

The synthesis of vitamin D is complex process and the energy of ultraviolet B rays in sunlight would convert the cholesterol present in skin into vitamin D₃ (inactive form), which travels through blood to liver to form 25-hydroxy-vitamin D. Further, in kidney it produces 1,25-dihydroxy vitamin D, also known as calcitriol (bio-

logical active form) [2]. Calcitriol maintains the normal level of calcium and phosphorus, promote the bone mineralization, induce or repress the genes responsible for conserving the mineral homeostasis and skeletal integrity, and inhibit the health issues such as hypertension, kidney damage, cardiovascular and immune disorders [3] in the young and elderly populations [4]. Vitamin D supplement (approx. 400IU/day) along with the calcium 1000-1500mg/day is very essential for maintaining the excellent bone health [5]. *In vitro* cell studies of bone health using key some vital biomarkers, such as alkaline phosphates (ALP), collagen and calcium found to be significantly reported using MG-63 cell line. MG-63 cells and the effect of 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃) has been studied and reported [6]. Thus, MG-63 cell line is widely used to test any formulation or sample for bone health [7]. ALP is a phenotypic marker and it improves the concentration of inorganic phosphate for bone mineralization [8], while collagen fibrils formed arrays of an organic matrix known as Osteoid [9]. Similarly, collagen and calcium are important biomarkers along with ALP that provides rigidity to the bone [10].

Putative Energy Fields (also known as Biofield) have been reported to date significant treatment reproducible methods in many fields. Biofield Energy Healing Therapies are based on the concept that human has the capacity to harness a subtle form of energy and transmit it into living and non-living objects [11]. This Consciousness Energy Healing Treatment included a life force, which has been known under different names. However, some vital energy therapies or Complementary and Alternative Medicine (CAM) therapies are commonly known as Qi Gong, polarity therapy, Tai Chi, pranic healing, deep breathing, relaxation techniques, Roling structural integration, healing touch, movement therapy, pilates, mindfulness, and traditional Chinese herbs treatment. Biofield Energy Healing Treatment (The Trivedi Effect®), one of the best Biofield Energy Healing Treatment worldwide has been proofed with significant scientific results [12]. National Center for Complementary and Alternative Medicine (NCCAM) also mentioned and included the Biofield therapies in the subcategory of the Energy Therapies [13]. The Trivedi Effect® has been reported with significant revolution in the physicochemical properties of metals [14-16], agricultural crops [17,18], antimicrobial action [19,20], improved bone health [21,22], advanced biotechnology [23], increased bioavailability [24-26], enhanced skin health [27,28], used as a nutraceutical [29,30], cancer science [31,32], and human health and wellness. Thus, authors in this study evaluated the impact of the Biofield Energy Treatment on vitamin D₃ (test sample) for bone health activity using biomarkers such as ALP, collagen content, and bone mineralization in the MG-63 cells.

Material and Methods

Chemicals and reagents

Fetal bovine serum (FBS) and Dulbecco's Modified Eagle's Medium (DMEM) were procured from Life Technology, USA. Similarly, the rutin hydrate was purchased from TCI, Japan. Vitamin D₃ and L-ascorbic acid were obtained from Sigma-Aldrich, USA. Antibiot-

ics solution (penicillin-streptomycin) was procured from HiMedia, India. 3-(4, 5-dimethyl -2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium (MTT), Direct Red 80, and Ethylenediaminetetraacetic Acid (EDTA) were obtained from Sigma, USA.

Cell culture

Human bone osteosarcoma (MG-63) cell line was used and maintained with DMEM growth medium for routine culture, which was supplemented with 10% FBS. Growth conditions were maintained at 37 °C, 5% CO₂ and 95% humidity and sub-cultured by trypsinisation followed by splitting of the cell suspension into fresh flasks and further supplementing with fresh cell growth medium. Before 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 [33].

Experimental design

The following groups were defined in the study, such as baseline control, vehicle control groups (0.05% DMSO with Biofield Energy Treated and untreated DMEM media), positive control group included rutin hydrate, and the experimental test groups. The experimental test sample groups have combination of the Biofield Energy Treated and untreated vitamin D₃/DMEM. Four major treatment groups on specified cells were selected 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 sample and the DMEM were treated with the Biofield Energy (also known as The Trivedi Effect®) by a renowned Biofield Energy Healer, Lauree Ann Duprey-Reed remotely for ~5 minutes. These test groups were divided as treated and untreated test samples. The renowned Biofield Energy Healer was remotely located in the Canada and on the other side the test samples were in the Dabur Research Foundation, New Delhi, India, which were treated through the Healer's unique Energy Transmission process remotely. Lauree Ann Duprey-Reed in this study never visited the laboratory in person, nor had any contact with the test item and medium. In addition, the experimental control group was treated with a sham healer, and sham healer did not have any knowledge about the Biofield Energy Treatment. The samples were stored under laboratory condition for experimental test setup.

Determination of non-cytotoxic concentration

MTT assay was used to test the cell viability in MG-63 cells for treated and untreated test samples. The details procedure of cell viability assay was followed by Ansari et al. (2018) with slight modification [21]. The cytotoxicity of each tested concentration of the test items was calculated with the help of Equation (1):

$$\% \text{Cytotoxicity} = \left\{ \frac{1-X}{R} \right\} * 100 \dots \dots \dots (1)$$

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

The percentage of cell viability corresponding to each treatment group was calculated by Equation (2):

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

The concentration exhibiting ≥70% cell viability was defined as non-cytotoxic [34].

Assessment of alkaline phosphatase (ALP) activity

For the estimation of ALP activity of the Biofield Energy Treatment on the test items in MG-63 cells. The procedure of cell counting, plating, and treatment was followed as per Koster et al. [22,33]. The percent increase in ALP activity with respect to the untreated cells was calculated using Equation (3):

$$\% \text{Increase in ALP} = \left\{ \frac{X-R}{R} \right\} * 100 \dots \dots \dots (3)$$

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

R =Absorbance of cells corresponding to untreated cells

Assessment of collagen synthesis

For the estimation of collagen level in MG-63 cells, standard methods were used for the evaluation of the potential of Biofield Treated test items and the procedure in details was as per Koster et al. with few modifications [22,33]. The increase collagen level with respect to the untreated cells was calculated using Equation (4):

$$\% \text{Increase in collagen levels} = \left\{ \frac{X-R}{R} \right\} * 100 \dots \dots \dots (4)$$

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

R =Collagen levels in cells corresponding to untreated cells

Assessment of bone mineralization by alizarin red s staining

For the evaluation of the percent alteration in bone mineralization after treatment with the Biofield Energy Treated test items in MG-63 cells, and the details steps were followed according to Ansari et al. [21,33]. The percentage increase in bone mineralization compared to the untreated cells was calculated using Equation (5):

$$\% \text{Increase} = \left\{ \frac{X-R}{R} \right\} * 100 \dots \dots \dots (5)$$

Where, X =Absorbance in cells corresponding to positive control or test groups; R =Absorbance in cells corresponding to 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.

Results and Discussion

Cell viability study using MTT

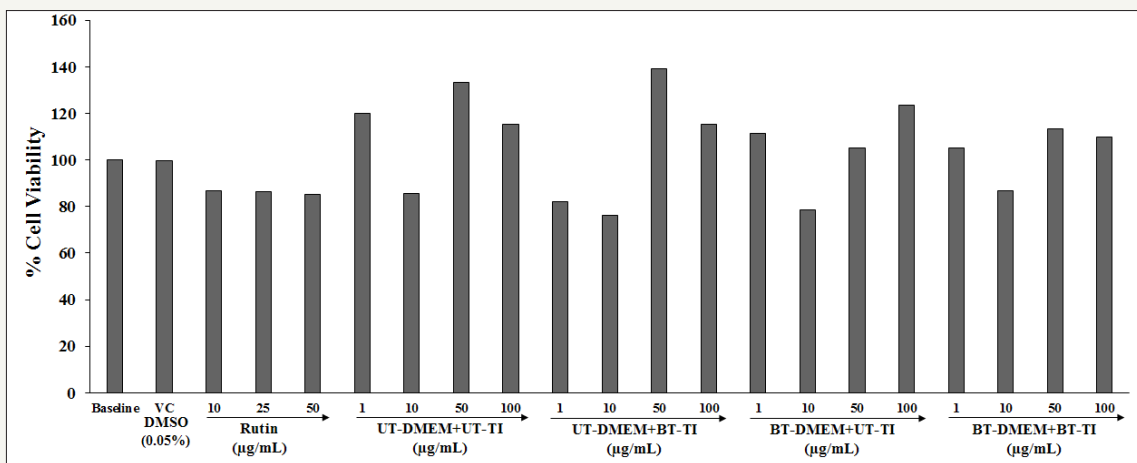


Figure 1: Cell viability using MTT assays of the test items on MG-63 cell line after 72 hours.

VC: Vehicle Control (DMSO-0.05%), UT: Untreated; BT: Biofield Treated; TI: Test Item

MTT assay for cell viability against all the test samples were studied in MG-63 cells. All the viability 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 cell lines showed the cell viability range from 76% to 139% in different test item groups with DMEM,

while for rutin group showed more than 86.6% cell viability (Figure 1). MTT data suggested that the test items along with DMEM groups were found safe at all the tested concentrations range up to maximum of 100µg/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 results of ALP level in MG-63 cells in Biofield Energy Healing Treated vitamin D₃ and DMEM at various concentrations is shown in the Figure 2. The vehicle control group showed 1.2% increased level of ALP as compared with the untreated cells group. 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 concentrations, respectively with respect to the untreated cells. The experimental test group's viz. untreated medium and Biofield Energy Treated

Test item (UT-DMEM+BT-TI) showed a significant increased level of ALP by 171.8% at 10 µg/mL, while Biofield Energy Treated medium and untreated Test item (BT-DMEM+UT-TI) showed a significant increased ALP level by 100% and 33.3% at 0.1 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 50% and 189.7% at 0.1 and 10 µg/mL, respectively as compared with the untreated test item and DMEM group.

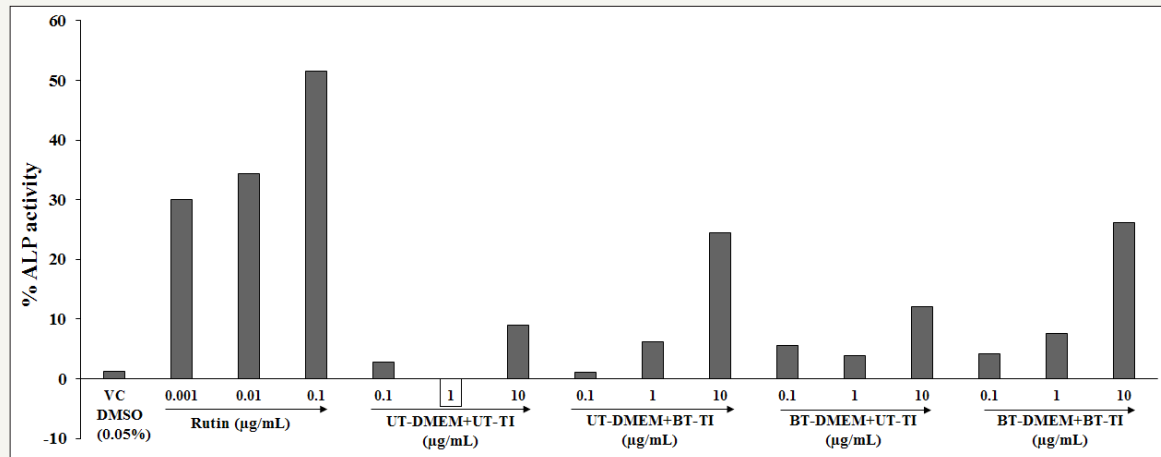


Figure 2: Study of Alkaline Phosphatase (ALP) enzyme activity of the Biofield Energy Treated test items on MG-63 cell line.

VC: Vehicle Control (DMSO-0.05%), UT: Untreated; BT: Biofield Treated; TI: Test Item

Skeletal or bone-specific alkaline phosphatase (BAP) is a glycoprotein found on osteoblasts cell surfaces. However, bone is constantly undergoing a metabolic process called remodeling, which mainly includes a degradation process, bone resorption, facilitated by osteoclasts action and a building process, bone formation and that is controlled by the osteoblasts. ALP, vitamin D, calcium, etc. are required for bone remodeling in order for maintenance and overall bone health [35-37]. It was reported by many reports that decreased level of ALP due to age factor or other reasons might lead to 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]. With respect to ALP level, 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 D₃ and DMEM could be used to improve the ALP concentration in many bone disorders.

Assessment of collagen synthesis

The collagen level among the tested samples i.e. Biofield Energy Treated vit D₃ and DMEM was estimated at various safe concentra-

tions and the data suggested significant increased collagen level. All the collagen results are presented as % values and were compared with the untreated cells in Figure 3. The rutin hydrate 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 35.6% and 145.8% at 1 and 10 µg/mL, respectively while BT-DMEM+UT-TI group showed a significant increased collagen level by 65.8% and 145.8% at 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 86.3%, 136.1%, and 15.1% at 1, 10, and 50 µg/mL, respectively as compared with the untreated test item and DMEM group. Collagen is one of the major insoluble fibrous proteins in the extra cellular matrix and in the connective tissue. Bone and joints strength largely depends upon the percentage of collagen and its other nutritional factors such as vitamin D and calcium. Various factors such as age, pathological condition, genetic makeup, etc. would affect the collagen synthesis that would weaken the joints, tendons, and ligaments. Bone mineralization has significant role of collagen type I, which is the most abundant matrix protein [38]. In addition, experimental results showed that collagen synthesis was significantly improved after Biofield Energy Treatment. This might be a significant approach to combat the reduced collagen synthesis, which could

lead to serious bone diseases such as the type of bone loss experienced in osteoporosis [39]. The Biofield Energy Treated vitamin D₃ and DMEM groups showed a significant improved level of collagen

compared with the untreated group. Biofield Energy Treated vitamin D₃ (The Trivedi Effect®) showed improved collagen level, which can be used to decrease aging process and bone inflammation.

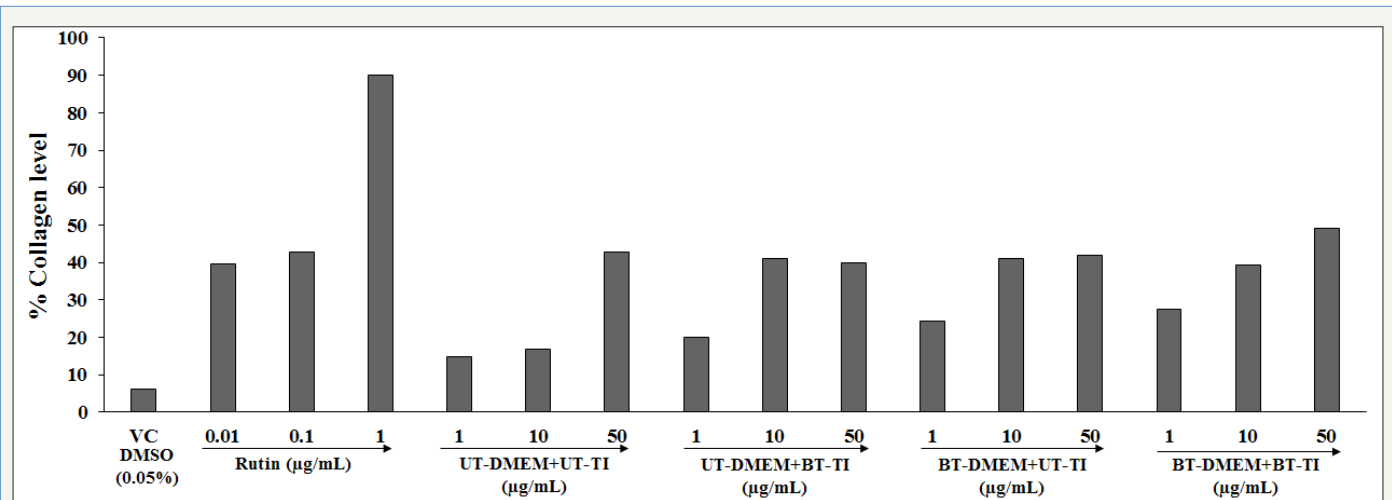


Figure 3: Effect of the test item on MG-63 cell line for collagen level.

VC: Vehicle Control (DMSO-0.05%), UT: Untreated; BT: Biofield Treated; TI: Test Item

Bone mineralization

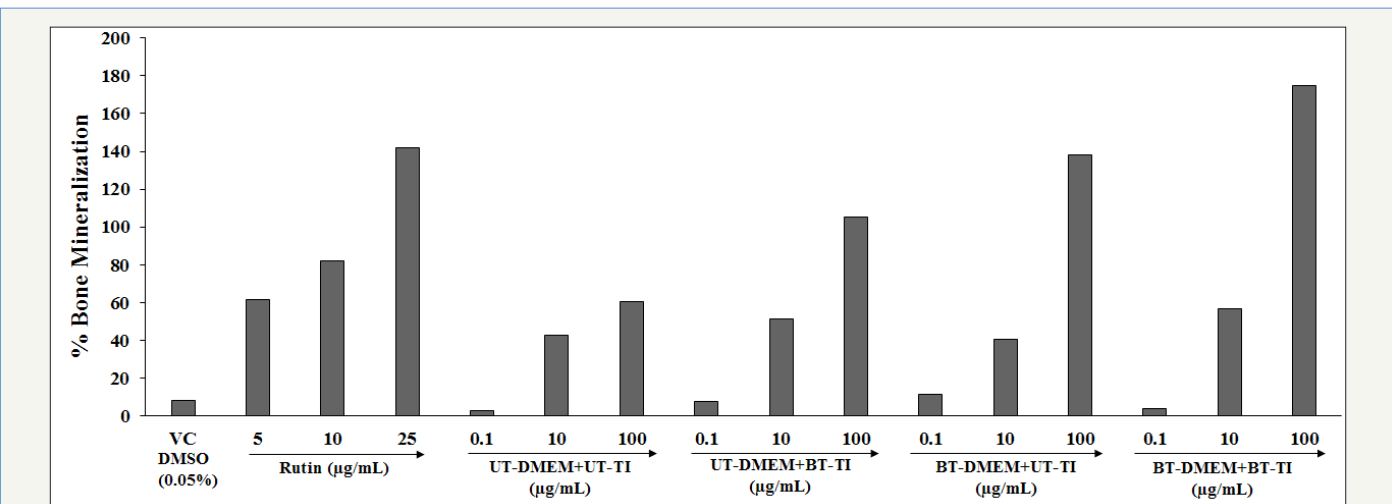


Figure 4: Evaluation of the test item for bone mineralization using MG-63 cell line.

VC: Vehicle Control (DMSO-0.05%), UT: Untreated; BT: Biofield Treated; TI: Test Item

The experimental analysis of bone mineralization on MG-63 cell line data suggested that the Biofield Energy Treated vitamin D₃ and DMEM groups showed a significant improved bone mineralization. All 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 61.72%, 82.15%, and 141.72% at 5, 10, and 25 µg/mL, respectively. The experimental data among test groups viz. UT-DMEM+BT-TI showed a significant increased bone mineralization by 150%, 21%, and 73.2% at 0.1, 10, and 100 µg/mL, respectively while BT-DMEM+UT-TI group showed a significantly increased bone mineralization by 258.3% and 126.8% at 0.1

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 33.3%, 32.7%, and 187.4% at 0.1, 10, and 100 µg/mL, respectively as compared with the untreated test item and DMEM group. The present study was conducted to check the alteration in percentage of bone mineralization in the Biofield Energy Treated test samples with respect to the untreated test samples. Bone mineralization process includes the precipitation of the inorganic substance in an organic matrix. This process starts during normal biological process with respect to the bone formation, teeth, and other body exoskeletons. This process controls various bone disorders such as osteoporosis or

other bone diseases. Bone mass and bone mineral density (BMD) depends upon the process of mineralization and various structural abnormalities [40,41]. The results after Biofield Energy Treatment suggested significant improved bone mineralization, which would improve the bone mass and resolve many bone related disorders.

Conclusion

Biofield Energy Treated vitamin D₃ demonstrated significant improved bone health parameters in MG-63 cells. MTT assay data showed that a significant improved cell viability with more than 76% among various test groups. Bone health parameters such as the level of ALP were increased by 171.8% at 10 µg/mL in the UT-DMEM+BT-TI, while 100% and 33.3% at 0.1 and 10 µg/mL, respectively 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 50% and 189.7% at 0.1 and 10 µg/mL, respectively. The level of collagen was significantly increased by 35.6% and 145.8% at 1 and 10 µg/mL, respectively in the UT-DMEM+BT-TI, while 65.8% and 145.8% at 1 and 10 µg/mL, respectively in the BT-DMEM+UT-TI group. The level of collagen was increased by 86.3%, 136.1%, and 15.1% 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 by 150%, 21%, and 73.2% at 0.1, 10, and 100 µg/mL, respectively in the UT-DMEM+BT-TI group, while 258.3% and 126.8% at 0.1 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 33.3%, 32.7%, and 187.4% at 0.1, 10, and 100 µg/mL, respectively as compared with the untreated group. Thus, experimental results demonstrated that The Trivedi Effect[®] treated test samples can be utilized in managing various bone health parameters viz. collagen, bone mineralization, and ALP to combat the bone disorders. Thus, The Trivedi Effect[®] based vitamin D₃ showed a significant improved bone health, which supports its use as the best nutritional supplement to treat bone related disorders such as rickets, deformed bones, osteomalacia, osteoma, osteoporosis, Paget's disease, aging, bone and/or joint pain, increased tendency of fractures, hormonal imbalance, stress, and other associated many more bone diseases.

Acknowledgement

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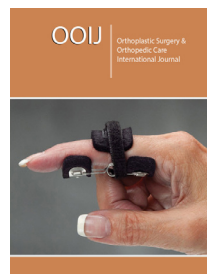
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