Effect of Biofield Energy Treated Vitamin D$_3$ on the Proliferation of Osteoblast-Like MG-63 Human Osteosarcoma Cells in vitro

Abstract
The study objective was to evaluate the effect of Biofield Treated vitamin D$_3$ and DMEM on bone health. The Test Items (TI) were divided into two parts. One part of each sample was received Consciousness Energy Healing Treatment by Janice Patricia Kinney and those samples were labeled as Biofield Energy Treated (BT) samples, while other parts of each sample were denoted as Untreated Test items (UT). The cell viability showed test samples were found as safe in the tested concentrations. ALP was significantly increased by 212.57% and 134.55% in UT-DMEM + BT-TI and BT-DMEM + BT-TI groups, respectively at 50 µg/mL, while increased by 156.38% and 145.64% in UT-DMEM + BT-TI and BT-DMEM + BT-TI groups, respectively at 1 µg/mL compared to untreated group. Moreover, collagen was significantly increased by 198.62%, 111.72%, and 168.28% in UT-DMEM + BT-TI and BT-DMEM + BT-TI groups, respectively at 0.1 µg/mL compared to untreated group. Overall, Biofield Treated vitamin D$_3$ was remarkably improved the bone health parameters and could be beneficial against bone-related disorders (osteoporosis, osteomalacia etc.), stress, inflammatory and autoimmune disorders.

Keywords: The Trivedi effect; Biofield energy healing treatment; Osteosarcoma cells (MG-63); Vitamin D$_3$ deficiency; Osteoporosis; Low bone density.

Introduction
Vitamin D has multiple effects, which regulate the functions in different organs viz. brain, liver, lungs, heart, kidneys, skeletal, immune and reproductive systems. Moreover, it has significant anti-inflammatory, anti-aging, anti-stress, anti-arthritis, anti-osteoporosis, anti-apoptotic, wound healing, anti-cancer, anti-psychotic and anti-fibrotic actions [1]. Vitamin D receptors are widely distributed in most of the body organs viz. brain, liver, heart, lungs, kidney, pancreas, large and small intestines, muscles, reproductive, nervous system, etc. Vitamin D receptors influence cell-to-cell communication, normal cell growth, cell differentiation, cell cycling and proliferation, hormonal balance, neurotransmission process, skin health, immune and cardiovascular functions. In any living vertebrates, vitamin D plays an important role in maintaining a healthy skeletal structure and is essential for bone health. Naturally, it is synthesized in the presence of sunlight in the skin [2]. Most foods do not contain any vitamin D, additionally now-a-days due to aging, use of sunscreen, and change of zenith angle of sun the production of vitamin D$_3$ has reduced [3]. Increasing age is not only related to a decrease in bone marrow depression and muscle strength but is also associated with marked changes in the immune and inflammatory responses [4]. Deficiency of vitamin D$_3$ causes metabolic bone diseases like osteomalacia and exacerbate osteoporosis, etc. [5]. The quality of life for menopausal women is one of the most critical health problem in the today world. Metabolic bone disorders like osteoporosis are mainly prevalent in post-menopausal women. Hormonal factors and rapid bone loss in post-menopausal women leads to an increased risk of fractures [6]. Hence, the serum calcium and Alkaline Phosphatase (ALP) levels in post-menopausal women are the main two vital biochemical markers of bone metabolism. However, bone-specific ALP is the most important marker for osteoblast differentiation [7]. Further, it is generally accepted that an increased calcium intake along with an adequate source of vitamin D is important for maintaining good bone health. Vitamin D also plays an important role in maintaining an adequate level of serum calcium and phosphorus. Therefore, vitamin D has a great impact in forming and maintaining strong bones [8,9]. Bone strength depends on the quality, geometry, shape, micro architecture, turnover, mineral content, and the collagen content. Collagen is the major structural protein responsible for bone calcification. In the aging state, the mechanical properties of the bones become impaired and the bones get fragile, that causes various clinical disorders associated with bone collagen abnormalities and bone fragility, such as osteogenesis imperfecta and osteoporosis [10,11].

In recent years, several scientific reports and clinical trials have revealed the useful effects of Biofield Energy Treatments, which have

Abbreviations
MG-63: Human Bone Osteosarcoma Cells; ALP: Alkaline Phosphatase; CAM: Complementary and Integrative Medicine; NHIS: National Health Interview Survey; NCCIH: National Center of Complementary and Integrative Health; DMEM: Dulbecco’s Modified Eagle’s Medium; FBS: Fetal Bovine Serum; ATCC: American Type Culture Collection; UT: Untreated; BT: Biofield Energy Treated; TI: Test item

shown to enhance immune function in cases of cervical cancer patients via therapeutic touch [12], massage therapy [13], etc. Complementary and Alternative Medicine (CAM) therapies are now rising as preferred models of treatment, among which Biofield Therapy (or Healing Modalities) is one approach that has been reported to have several benefits to enhance physical, mental and emotional human wellness. However, as per the data of 2012 from the National Health Interview Survey (NHIS), which indicated that the highest percentage of the Americans used dietary supplements as a complementary health approach as compared with other practices in past years. The National Center of Complementary and Integrative Health (NCCIH) has recognized and accepted Biofield Energy Healing as a CAM health care approach in addition to other therapies, medicines and practices such as natural products, 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, rolffing structural integration, mindfulness, Ayurvedic medicine, traditional Chinese herbs and medicines, naturopathy, essential oils, aromatherapy, Reiki, and cranial sacral therapy.

The human biofield is the energetic matrix that surrounds the human body [14]. It directly links with the cellular function that allows the DNA to communicate faster than light and maintain intelligence in the organisms [15]. It can be measured with the help of Electromyography (EMG), Electrocardiography (ECG) and Electroencephalogram (EEG) [16]. Thus, a human has the capability to harness energy from environment/Universe and can transmit into any object (living or non-living) around the Globe. The object(s) always receive the energy and responded into useful way that is called ‘Biofield Energy’. This process is known as ‘Biofield Treatment’. Biofield Energy Treatment (The Trivedi Effect) has been published in numerous peer-reviewed science journals with significant outcomes in many scientific fields such as cancer research [17,18], microbiology [19-22], biotechnology [23,24], pharmaceutical science [25-28], agricultural science [29-32], materials science [33-36], nutraceuticals [37,38], skin health, human health and wellness. Based on the literature information and importance of vitamin D on bone health, the authors sought to evaluate the impact of the Biofield Energy Treatment (The Trivedi Effect) on the test samples (vitamin D3 and DMEM medium) for bone health activity with respect to the assessment of different bone health parameters like ALP, collagen content, and bone mineralization using standard assay in MG-63 cells.

Materials and Methods

Chemicals and reagents

Fetal Bovine Serum (FBS) and Dulbecco’s Modified Eagle’s Medium (DMEM) were purchased from Life Technology, USA. Rutin hydrate was purchased from TCI, Japan, while vitamin D3 and L-ascorbic acid were obtained from Sigma-Aldrich, USA. Antibiotic 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 Ethylenediaminetetraacetic Acid (EDTA) were purchased from Sigma, USA. All the other chemicals used in this experiment were analytical grade procured from India.

wells followed by an additional incubation for 3 hours at 37°C. The supernatant was aspirated and 150 μL of DMSO and was added to each well to dissolve formazan crystals. The absorbance of each well was read at 540 nm using a Synergy HT microplate reader, BioTek, USA. The percentage cytotoxicity at each tested concentration of the test substance was calculated using Equation (1):

\[
\% \text{ Cytotoxicity} = \frac{1}{1} \times \frac{X}{R} \times 100 \tag{1}
\]

Where, \( X \) = Absorbance of treated cells; \( R \) = Absorbance of untreated cells

The percentage cell viability corresponding to each treatment was then be obtained using Equation (2):

\[
\% \text{ Cell Viability} = 100 - \% \text{ Cytotoxicity} \tag{2}
\]

The concentration exhibiting ≥ 70% cell viability was considered as non-cytotoxic and safe [40].

**Assessment of alkaline phosphatase (ALP) activity**

The cells were counted using a 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 the respective treatments, the cells in the above plate were incubated for 48 hours in a 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 (pNPP) 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 (pNPP solution alone) absorbance values. The percentage increase in ALP enzyme activity with respect to the untreated cells (baseline group) was calculated using Equation (3):

\[
\% \text{ Increase in ALP} = \frac{(X-R)}{R} \times 100 \tag{3}
\]

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 a hemocytometer and plated in a 24-well plate at the density corresponding 10 X 10^4 cells/well in phenol-free DMEM supplemented with 10% CD-FBS. Following the respective treatments, the cells in the above plate were incubated for 48 hours in a 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 hour 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). The percentage increase in collagen level with respect to the untreated cells (baseline group) was calculated using Equation (4):

\[
\% \text{ Increase in collagen levels} = \frac{(X-R)}{R} \times 100 \tag{4}
\]

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 a hemocytometer and plated in a 24-well plate at the density corresponding to 10 X 10^4 cells/well in phenol-free DMEM supplemented with 10% CD-FBS. Following the respective treatments, the cells in the above plate were incubated for 48 hours in a 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 were subjected to serum stripping for 24 hours. The cells were then treated with non-cytotoxic concentrations of the test samples and positive control. Following 3-7 days of incubation with the test samples and positive control, the plates were taken out, cell layers processed further by 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. The percentage increase in bone mineralization with respect to the untreated cells (baseline group) was calculated using Equation (5):

\[
\% \text{ Increase} = \frac{(X-R)}{R} \times 100 \tag{5}
\]

Where,

\( X \) = Absorbance in cells corresponding to positive control or test groups

\( R \) = Absorbance in cells corresponding to baseline group (untreated cells)

**Statistical analysis**

All the values were represented as percentage of the respective parameters.

**Results and Discussion**

**MTT assay**

The cell viability data of the Biofield Energy Treated vitamin D_3,
and DMEM by MTT assay in MG-63 cells are depicted in Figure 1. The data showed that the test samples in combination did not exhibit any cytotoxicity (as evidence of cell viability approximately greater than 73%) across all the tested concentrations up to 100 µg/mL. Hence, the same concentrations were assessed further to see the effect of the test samples on the levels of Alkaline Phosphatase (ALP) activity, collagen synthesis, and bone mineralization in MG-63 cells.

**Alkaline phosphatase (ALP) activity**

The effect of the Biofield Energy Treated test items on the level of Alkaline Phosphatase (ALP) in human bone osteosarcoma cells is presented in Figure 2. The level of ALP was found as 7.4% in the Vehicle Control (VC) group compared to the untreated cells group. The ALP activity was significantly increased in a dose dependent manner by 39.07%, 46.45%, and 80.87% in the positive control group at the concentration of 0.01, 0.1, and 1 µg/mL, respectively compared to the untreated cells group. The level of ALP was significantly increased by 9.83%, 242.77%, and 32.95% in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item groups, respectively at the concentration of 10 µg/mL compared to the UT-DMEM + UT-Test item group. Further, the level of ALP was significantly increased by 212.57% and 134.55% in the UT-DMEM + BT-Test item group. Additionally, at 1 µg/mL the level of ALP was significantly increased by 39.07%, 46.45%, and 80.87% at 0.01, 0.1, and 1 µg/mL, respectively compared to the untreated cells group. The level of ALP was significantly increased by 9.83%, 242.77%, and 32.95% in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item groups, respectively at the concentration of 10 µg/mL compared to the UT-DMEM + UT-Test item group.

**Assessment of collagen activity**

The effect of the test samples on the collagen content in human bone osteosarcoma cells is shown in Figure 3. Collagen level in the VC group was found as 7.4%. The level of collagen synthesis was significantly increased by 39.07%, 46.45%, and 80.87% at 0.01, 0.1, and 1 µg/mL, respectively compared to the untreated cells group. The collagen synthesis was significantly increased by 172.04%, 293.55%, and 248.39% in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item groups, respectively at 0.1µg/mL compared to the UT-DMEM + UT-Test item group. Moreover, the collagen level was significantly increased by 99.25%, 41.35%, and 172.93% in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item groups, respectively with respect to the UT-DMEM + UT-Test item group. Additionally, 10 µg/mL the level of collagen was also significantly increased by 156.38% and 145.64% in the UT-DMEM + BT-Test item and BT-DMEM + BT-Test item groups, respectively with respect to the UT-DMEM + UT-Test item group Figure 3. Type I collagen is the major structural protein responsible for bone calcification and osteoblast differentiation [43]. From literature it was reported that vitamin D play an important role for the synthesis of collagen [44]. Apart from vitamin D, ascorbic acid also responsible for synthesis of collagen by enhancing the formation of hydroxyproline and hydroxylysine, the component of collagen fibers [45]. Overall, The Trivedi Effect - Consciousness Energy Healing Treatment modality showed a significant improvement of the collagen level in human osteosarcoma cells.

**Assessment of bone mineralization by alizarin red S (ARS) staining**

The Alizarin red S (ARS) staining is widely utilized for the evaluation of calcium-rich deposits in the cell culture study. Numerous research outcomes showed that inadequate vitamin D intakes for long time can lead to bone demineralization. Thus, deficiency of vitamin D leads to decreased calcium absorption and ultimately the release of calcium from the bones in order to maintain
circulating calcium concentrations and bone become fragile and spongy [46,47]. The percent change of bone mineralization results after treatment of the test items in human bone osteosarcoma cells is shown in Figure 4. The percentage of bone mineralization level in the Vehicle Control (VC) group was observed as 7.3% as compared to the untreated cells group. The percentage of bone mineralization was significantly increased in a concentration dependent manner by 49.45%, 66.01%, and 126.45% at 5, 10, and 25 µg/mL, respectively in the positive control group compared to the untreated cells group.

The percent of bone mineralization was significantly increased by 198.62%, 111.72%, and 168.28% in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item groups, respectively at 0.1 µg/mL compared to the UT-DMEM + UT-Test item group. Further, a noticeably increased percentage of bone mineralization by 83.25%, 99.52%, and 50.24% in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item groups, respectively at 1 µg/mL with respect to the UT-DMEM + UT-Test item group. In addition, the data showed a significant increase of bone mineralization by 212.57% and 134.55% in the UT-DMEM + BT-Test item group. In conclusion, the all others treatment groups.

Conclusion

Based on the study data, it was showed that more than 73% cells were viable, which indicated the test samples were found as safe and nontoxic up the tested concentrations. Bone-specific ALP was remarkably increased by 212.57% and 134.55% in the UT-DMEM + BT-Test item and BT-DMEM + BT-Test item groups, respectively at 50 µg/mL while increased by 242.77% in the BT-DMEM + UT-Test item group at 10 µg/mL compared to the UT-DMEM + UT-Test item group. The level of collagen was also significantly increased by 248.39%, 172.93%, and 145.64% in the BT-DMEM + BT-Test item group at 0.1, 1, and 10 µg/mL, respectively while increased by 293.55% in the BT-DMEM + UT-Test item at 0.1 µg/mL with respect to the untreated group. Besides, the percent of bone mineralization was distinctly increased by 198.62%, 111.72%, and 168.28% in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item groups, respectively at 0.1 µg/mL compared to the untreated group. Altogether, the Biofield Energy Treated test samples (The Trivedi Effect®) demonstrated a significant impact on bone health parameters. Therefore, the Consciousness Energy Healing based vitamin D₃ might be suitable for the development of an alternative and more effective supplement for vitamin D₃ deficiency, 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 joint pain, bone fractures, deformed bones, osteoma, chondrodystrophy fetalis, etc. Besides, it can also be utilized in organ transplants (for example kidney transplants, liver transplants and heart transplants), various autoimmune disorders such as Lupus, Addison Disease, Celiac Disease (gluten-sensitive enteropathy), Dermatomyositis, Graves’ Disease, Hashimoto Thyroiditis, Multiple Sclerosis, Myasthenia Gravis, Pernicious Anemia, Aplastic Anemia, Reactive Arthritis, Rheumatoid Arthritis, Sjogren Syndrome, Systemic Lupus Erythematosus, Type I Diabetes, Alopecia Areata, Cohn’s Disease, Fibromyalgia, Vitiligo, Psoriasis, Schleroderma, Chronic Fatigue Syndrome and Vasculitis, as well as inflammatory disorders such as Asthma, Ulcerative Colitis, Alzheimer’s Disease, Atherosclerosis, Dermatitis, Derciictilis, Hepatitis, Irritable Bowel Syndrome, inflammatory diseases, anti-inflammatory, anti-stress, anti-arthritis, anti-osteoporosis, anti-apoptotic, wound healing, anti-cancer, anti-psychotic and anti-fibrotic actions stress management and prevention, and anti-aging by improving overall health, Parkinson’s Disease and stress etc. to modulate the immune system by improving overall health.

References


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