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Effect of Biofield Energized Vitamin D$_3$ on Bone Health in MG-63 Cell-Line

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Abstract: The objective of current research work was to evaluate the potential of Consciousness Energy Healing-based vitamin D$_3$ and DMEM medium on bone health parameters such as alkaline phosphatase (ALP), collagen, and bone mineralization in human bone osteosarcoma cells (MG-63). The test items like vitamin D$_3$ and DMEM were divided into two parts. One part of each sample was received the Biofield Energy Treatment by Faith Ann Pyka and those samples were denoted as the Biofield Energy Treated (BT) samples, while the other parts of each sample were referred as the untreated test items (UT). The MTT cell viability assay revealed that the test samples were found as safe in the tested concentrations. The level of ALP was significantly increased by 191.45% in the BT-DMEM + UT-Test item (UT-TI) group at 1 µg/mL compared to the UT-DMEM + UT-TI group. Moreover, ALP level was significantly increased by 144.89% and 226.75% in the UT-DMEM + BT-TI and BT-DMEM + BT-TI groups, respectively at 50 µg/mL compared to the untreated group. Further, collagen level was significantly increased by 98.15%, 80.54%, and 49.98% in the UT-DMEM + BT-TI, BT-DMEM + UT-TI, and BT-DMEM + BT-TI groups, respectively at 10 µg/mL compared to the untreated group. Additionally, at 50 µg/mL level of collagen was significantly increased by 129.73%, 189.16%, and 94.60% in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item groups, respectively with respect to the untreated group. Apart from this, the percent of bone mineralization was distinctly enhanced by 283.74%, 27.91%, and 118.02% in the UT-DMEM + BT-TI, BT-DMEM + UT-TI, and BT-DMEM + BT-TI groups, respectively at 50 µg/mL compared to the untreated group. Moreover, the percentage of bone mineralization was significantly increased by 265.92%, 231.82%, and 158.2% in the UT-DMEM + BT-TI, BT-DMEM + UT-TI, and BT-DMEM + BT-TI groups, respectively at 100 µg/mL compared to the untreated group. Altogether, the Biofield Energy Treated vitamin D$_3$ was significantly improved the bone health parameters and it could be an alternative approach for nutraceutical supplement to combat vitamin D$_3$ deficiency and able to fight against various bone-related disorders including rickets, low bone density, osteomalacia, bone and joint pain, bone fractures, osteoporosis, osteoma, osteogenesis imperfecta, Paget’s disease, deformed bones, chondrodystrophia fetalis, stress management and prevention, autoimmune and inflammatory diseases, and anti-aging by improving overall health.

Keywords: The Trivedi Effect®, Vitamin D$_3$, Bone Health, Consciousness Healing Treatment, Osteosarcoma Cells, Osteoporosis

1. Introduction

Vitamin D has multiple effects, which regulate the functions in different target organs such as brain, liver, lungs, heart, kidneys, skeletal, immune, and reproductive systems. Additionally, it has a significant anti-inflammatory, anti-aging, anti-stress, anti-arthritic, anti-osteoporosis, anti-apoptotic, wound healing, anti-cancer, anti-psychotic and...
anti-fibrotic actions [1]. Vitamin D receptors (VDRs) are widely distributed in most of the body organs viz. brain, liver, heart, lungs, kidney, pancreas, small and large intestines, muscles, reproductive, nervous system, etc. VDRs can 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 presence of sunlight in the skin [2]. Most of the foods do not contain vitamin D, additionally now-a-days due to aging, use of sunscreen, and change of zenith angle of the sun, the production of vitamin D3 has been decreased [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 D3 causes metabolic bone diseases like osteomalacia and exacerbate osteoporosis, etc. [5]. The quality of life (QoL) for menopausal women is one of the most critical health problems in the modern 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 on forming and maintaining strong bones [8, 9]. Bone strength depends on the quality, geometry, shape, microarchitecture, 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, numerous scientific reports and clinical trials have revealed that the useful effects of Biofield Energy Treatment, which have shown to enhanced immune function in case 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 Energy 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 (~17%) of the Americans used dietary supplements as a complementary health approach as compared with other practices in the 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, rolfing structural integration, mindfulness, Ayurvedic medicine, traditional Chinese herbs and medicines, naturopathy, essential oils, aromatherapy, Reiki, and cranial sacral therapy. Human Biofield Energy has subtle energy that has the capacity to work in an effective manner [14]. CAM therapies have been practiced worldwide with reported clinical benefits in different health disease profiles [15]. This energy can be harnessed and transmitted by the experts into living and non-living things via the process of Biofield Energy Healing. 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 [16, 17], microbiology [18-21], biotechnology [22, 23], pharmaceutical science [24-27], agricultural science [28-31], materials science [32-35], nutraceuticals [36, 37], skin health [38, 39], human health and wellness.

Based on the literature information and importance of vitamin D3 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 assays in MG-63 cells.

2. Materials and Methods

2.1. Chemicals and Reagents

Rutin hydrate was obtained from TCI, Japan, while vitamin D3, 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium) (MTT), Direct Red 80, ethylene diamine tetra acetic acid (EDTA) 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. Antibiotic solution (penicillin-streptomycin) was procured from HiMedia, India. All the other chemicals used in this experiment were analytical grade procured from India.

2.2. Cell Culture

The human bone osteosarcoma cells (MG-63) were used as the test system in this experiment. The MG-63 cells supplemented with 10% FBS were maintained under the DMEM growth medium for routine culture. Growth conditions were maintained as 37°C, 5% CO2 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 prior 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 [40].

2.3. Experimental Design

The experimental groups consisted of cells in baseline control (untreated cells), vehicle control groups (0.05% DMSO with Biofield Energy Treated and untreated DMEM), a positive control group (rutin hydrate) and experimental test groups. The experimental groups included the combination of the Biofield Energy Treated and untreated vitamin D3/DMEM. It consisted of four major treatment groups on specified cells with UT-DMEM + UT-Test item, UT-DMEM + Biofield Energy Treated test item (BT-Test item), BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item.

2.4. Consciousness Energy Healing Treatment Strategies

The test item (vitamin D3) and DMEM were divided into two parts. One part each of the test item and DMEM were treated with the Biofield Energy (also known as The Trivedi Effect®) and coded as the Biofield Energy Treated items, while the second part did not receive any sort of treatment and was defined as the untreated samples. This Biofield Energy Healing Treatment was provided by Faith Ann Pyka, who participated in this study and performed the Biofield Energy Healing Treatment 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. The Biofield Energy Treatment was administered for 5 minutes through the healer’s unique Energy Transmission process remotely to the test samples under laboratory conditions. Faith Ann Pyka 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 test was performed by MTT assay in the 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 cell recovery and exponential growth, then they were subjected to serum stripping or starvation. The cells were treated with the test item, DMEM, and the positive control. The untreated cells served as baseline control (untreated cells). The cells in the above plate(s) were incubated for a time point ranging from 24 to 72 hours in CO2 incubator at 37°C, 5% CO2 and 95% humidity. Following incubation, the plates were taken out and 20 µL of 5 mg/mL of MTT solution was added to all the 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 micro plate reader, BioTek, USA. The percentage cytotoxicity at each tested concentration of the test substance was calculated using the following Equation 1:

$$\text{% Cytotoxicity} = (1 - \frac{X}{R}) \times 100$$  \hspace{1cm} (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 the following Equation 2:

$$\text{% Cell Viability} = 100 - \text{% Cytotoxicity}$$  \hspace{1cm} (2)

The concentrations exhibiting ≥70% Cell viability was considered as non-cytotoxic [41].

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 to 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 CO2 incubator at 37°C, 5% CO2 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 (MgCl2) 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 (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:

$$\text{% Increase in ALP} = \frac{(X - R)}{R} \times 100$$  \hspace{1cm} (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 10^4 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 CO\textsubscript{2} incubator at 37°C, 5% CO\textsubscript{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 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). The percentage increase in collagen level with respect to the untreated cells (baseline group) was calculated using Equation 4:

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

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 x10\textsuperscript{3} 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 CO\textsubscript{2} incubator at 37°C, 5% CO\textsubscript{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 micro plate reader. The percentage increase in bone mineralization with respect to the untreated cells (baseline group) was calculated using the following Equation 5:

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

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 the respective parameters. For statistical analysis Sigma-Plot (version 11.0) was used as a statistical tool. Statistically significant values were set at the level of \(p\leq0.05\).

3. Results and Discussion

3.1. MTT Assay

For the assessment of non-cytotoxic concentrations of the test samples \(i.e.,\) vitamin D\textsubscript{3} and DMEM medium the MTT cell viability assay was used in this experiment in MG-63 cells. The data are expressed as percentage and shown in Figure 1. The percentage of cell viability was observed in the ranges of 70% to 131% in the tested concentrations of all the groups and did not show any cytotoxicity (as evidence of cell viability approximately greater than 70%) across all the tested concentrations upto 50 µg/mL. Hence, the safe concentrations were used in this experiment 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.

![Figure 1. Study of the cell viability of the test items (vitamin D3 and DMEM medium) after Biofield Energy Treatment in different tested concentrations in MG-63 cells. VC: Vehicle control (0.05% DMSO); UT: Untreated; BT: Biofield Energy Treated; TI: Test item.](image-url)
3.2. Assessment of Alkaline Phosphatase (ALP) Enzyme Activity

The effect of the Biofield Energy Treated test samples on ALP activity in MG-63 cells is shown in Figure 2. The vehicle control (VC) group showed 5.5% level of ALP as compared to the untreated cells group. The reference standard was used in this experiment (rutin hydrate) showed 34.31%, 51.47%, and 189.39% increased the level of ALP at the concentration of 0.01, 0.1, and 1 µg/mL, respectively compared to the untreated cells group. The results of the test formulation showed that the level of ALP was significantly enhanced by 191.45% in the BT-DMEM + UT-TI groups at 1 µg/mL as compared to the UT-DMEM + UT-Test item group. Moreover, the level of ALP was significantly increased by 144.89% and 226.75% in the UT-DMEM + BT-TI and BT-DMEM + BT-TI, respectively at 50 µg/mL as compared to the UT-DMEM + UT-Test item group.

ALP is one of the biomarker for the evaluation of bone formation and resorption [42, 43]. Various in vitro study reported that ALP can be released in the soluble form by a phospholipase (an anchorless) and also in insoluble form by vesicle exocytosis (an anchor-intact). Regulation of skeletal activities by ALP is govern by these two putative process [44]. Overall, the Biofield Energy Treated (The Trivedi Effect®) test item group (i.e., vitamin D3) showed an improved synthesis of ALP enzyme in the MG-63 cells with respect to the untreated test item group, which might be advantageous to the patients those are suffering from various bone-related disorders.

![Figure 2. The effect of test samples on alkaline phosphatase (ALP) enzyme activity in human bone osteosarcoma cell (MG-63). VC: Vehicle control (0.05% DMSO); UT: Untreated; BT: Biofield Energy Treated; TI: Test item.](image)

3.3. Assessment of Collagen Activity

The collagen activity of the test items in human bone osteosarcoma cells is shown in Figure 3. Collagen level in the VC group was found as 28% increased as compared to the untreated cells group. The positive control (rutin hydrate) showed 28.27%, 46.5%, and 46.73% increased the level of collagen at 0.01, 0.1, and 1 µg/mL, respectively compared to the untreated cells group. The collagen synthesis was increased by 2.56% in the BT-DMEM + BT-Test item group at 1 µg/mL compared to the UT-DMEM + UT-Test item group. Moreover, the collagen level was significantly increased by 98.15%, 80.54%, and 49.98% in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item and BT-DMEM + BT-Test item groups, respectively at 10 µg/mL compared to the UT-DMEM + UT-Test item group. Additionally, at 50 µg/mL the level of collagen was also significantly increased by 129.73%, 189.16%, and 94.60% 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 (Figure 3). Collagen, the source of fibers is the most abundant constituent of all the connective tissue. Various evidence reported that collagen, plays a considerable role in both bone development and bone maintenance [45, 46]. ATF4, a transcription factor that stimulates amino-acid uptake and collagen synthesis in osteoblast. Thus, increased amino-acid uptake simultaneously increases the synthesis of type I collagen and osteocalcin expression that ultimately enhanced the differentiation of osteoblast [47]. Here, the Biofield Energy Treated vitamin D3 significantly improved the level of collagen which might be helpful to maintain a healthy bone in postmenopausal women selectively. Overall, The Trivedi Effect® - Consciousness Energy Healing Treatment modality showed a significant improvement of the collagen level in human osteosarcoma cells. Thus, it is assumed that The Trivedi effect® has the significant potential to improve the bone health in various skeletal disorders.
3.4. Bone Mineralization

The effect of Biofield Energy Treated test samples on bone mineralization in MG-63 cells is shown in Figure 4. The percentage of bone mineralization was significantly increased in a concentration-dependent manner by 75%, 105.94%, and 135.15% 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 distinctly increased by 69.19%, 62.44%, and 88.73% in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item groups, respectively at 10 µg/mL compared to the UT-DMEM + UT-Test item group. Further, an increased percentage of bone mineralization was observed by 283.74%, 27.91%, and 118.02% in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item groups, respectively at 50 µg/mL with respect to the UT-DMEM + UT-Test item group. Moreover, the percentage of bone mineralization was significantly increased by 265.92%, 231.82%, and 158.2% in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item groups, respectively at 100 µg/mL compared to the UT-DMEM + UT-Test item group (Figure 4).

Vitamin D is very essential for sufficient development of whole skeletal system. Unfortunately, most of foods naturally do not contain vitamin D, and only a few foods are fortified with vitamin D. That is why, vitamin D deficiency has become epidemic for all age groups in the both developed and under developing countries. Vitamin D deficiency not only causes metabolic bone disease among children and adults but also may increase the risk of many common chronic diseases [1]. Here, authors have found that the Biofield Energy Treated vitamin D₃ significantly enhanced the level of bone mineralization in the form of calcium, assessed by Alizarin Red S Staining technique. Therefore, based on the above findings it is hypothesized that the Consciousness Energy Healing Treatment (The Trivedi Effect®) based test item groups (i.e., vitamin D₃) showed a remarkable improvement of bone mineralization content assessed by in vitro in the human osteosarcoma cells (MG-63).

4. Conclusions

The cell viability assay data using MTT assay showed more than 70% cells were viable, which indicated that the test samples were safe and nontoxic in all the tested concentrations. The level of ALP was significantly increased by 144.89% and 226.75% in the UT-DMEM + BT-Test item groups, respectively.
and BT-DMEM + BT-TI groups, respectively at 10 µg/mL; while increased by 191.45% in the BT-DMEM + UT-Test item at 1 µg/mL compared to the UT-DMEM + UT-Test item group. The synthesis of collagen was significantly increased by 98.15%, 80.54%, and 49.98% in the UT-DMEM + BT-Test item, BT-DMEM + UT-TI, and BT-DMEM + BT-Test item groups, respectively at 10 µg/mL compared to the untreated group. Further, the collagen level was significantly increased by 129.73%, 189.16%, and 94.60% in the UT-DMEM + BT-TI, BT-DMEM + UT-TI, and BT-DMEM + UT-TI groups, at 50 µg/mL compared to the untreated group.

Besides, the percent of bone mineralization was distinctly increased 69.19%, 62.44%, and 88.73% in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item groups, respectively at 10 µg/mL compared to the untreated group. Further, the percentage of bone mineralization was significantly increased by 283.74%, 27.91%, and 118.02% in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item groups, respectively at 50 µg/mL compared to the UT-DMEM + UT-Test item group. Moreover, the percentage of bone mineralization was significantly increased by 265.92%, 231.82%, and 158.2% in the UT-DMEM + BT-Test item, BT-DMEM + UT-Test item, and BT-DMEM + BT-Test item groups, respectively at 100 µg/mL compared to the UT-DMEM + UT-Test item 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: Paget’s disease of bone, low bone density, osteoporosis, osteogenesis imperfecta, rickets, deformed bones, osteomalacia, osteoma, bone and joint pain, bone fractures, 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, Rheumatoid Arthritis, Multiple Sclerosis, Myasthenia Gravis, Pernicious Anemia, Scleroderma, Aplastic Anemia, Reactive Arthritis, Fibromyalgia, Sjogren Syndrome, Systemic Lupus Erythematous, Vitiligo, Type 1 Diabetes, Alopecia Areata, Crohn’s Disease, Psoriasis, Chronic Fatigue Syndrome and Vasculitis, as well as inflammatory disorders such as Asthma, Ulcerative Colitis, Irritable Bowel Syndrome, Atherosclerosis, Alzheimer’s Disease, Dermatitis, Diverticulitis, Hepatitis, inflammatory diseases, anti-inflammatory, anti-arthritis, anti-osteoporosis, anti-apoptotic, anti-stress, wound healing, anti-cancer, anti-aging, anti-psychotic and anti-fibrotic action, stress management and prevention, Parkinson’s Disease etc. to modulate the immune system by improving Quality of Life (QoL).

**Abbreviations**

MG-63: Human Bone Osteosarcoma Cells, ALP: Alkaline phosphatase, CAM: Complementary and alternative 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

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**References**

1. Holick MF (2004) Sunlight and vitamin D for bone health and prevention of autoimmune diseases cancers, and cardiovascular disease. Am J Clin Nut 80: 1678S-1688S.

2. Holick MF (1996) Vitamin D and bone health. J Nutr 126: 1159S-1164S.

3. Matsuoka LY, Ide L, Wortsman J, MacLaughlin JA, Holick MF (1987) Sunscreens suppress vitamin D₃ synthesis. J Clin Endocrinol Metab 64: 1165-1168.

4. Barnes MS, Robson JP, Bonham MP, Strain J, Wallace J (2006) Vitamin D: Status, supplementation and immunomodulation. Nutr Food Sci 2: 315-336.

5. Laird E, Ward M, McSorley E, Strain JJ, Wallace J (2010) Vitamin D and bone health; Potential mechanisms. Nutrients 2: 693-724.

6. Bhattacharai T, Bhattacharyya K, Chaudhuri P, Sengupta P (2014) Correlation of common biochemical markers for bone turnover, serum calcium, and alkaline phosphatase in post-menopausal women. Malays J Med Sci 21: 58-61.

7. Iba K, Takada J, Yamashita T (2004) The serum level of bone-specific alkaline phosphatase activity is associated with aortic calcification in osteoporosis patients. J Bone Miner Metab 22: 594-596.

8. Holick MF, Garabedian M (2006) Vitamin D: Photobiology, metabolism, mechanism of action, and clinical applications. Primer on the metabolic bone diseases and disorders of mineral metabolism. Edited by: Favus MJ, Washington, DC.

9. DeLuca HF (2004) Overview of general physiologic features and functions of vitamin D. Am J Clin Nutr 80: 1689S-1696S.

10. Vigué-Carrin S, Garnier P, Delmas PD (2006) The role of collagen in bone strength. Osteoporos Int 17: 319-336.

11. Sroga GE, Vashishth D (2012) Effects of bone matrix proteins on fracture and fragility in osteoporosis. Curr Osteoporos Rep 10: 141-150.

12. Lutgendorf SK, Mullen-Houser E, Russell D, Degeest K, Jacobson G, Hart L, Bender D, Anderson B, Buckers TE, Goodheart MJ, Antoni MH, Sood AK, Lubaroff DM (2010) Preservation of immune function in cervical cancer patients during chemoradiation using a novel integrative approach. Brain Behav and Immun 24: 1231-1240.
Trivedi MK, Patil S, Shettigar H, Mondal SC, Jana S (2015) The potential impact of biofield treatment on human brain tumor cells: A time-lapse video microscopy. J Integr Oncol 4: 141.

Trivedi MK, Patil S, Shettigar H, Gangwar M, Jana S (2015) In vitro evaluation of biofield treatment on cancer biomarkers involved in endometrial and prostate cancer cell lines. J Cancer Sci Ther 7: 253-257.

Trivedi MK, Branton A, Trivedi D, Nayak G, Mondal SC, Jana S (2015) Antibigram, biochemical reactions and biotyping of biofield treated Providencia rettgeri. American Journal of Health Research 3: 344-351.

Trivedi MK, Branton A, Trivedi D, Nayak G, Mondal SC, Jana S (2015) Antimicrobial sensitivity, biochemical characteristics and biotyping of Staphylococcus saprophyticus: An impact of biofield energy treatment. J Women’s Health Care 4: 271.

Trivedi MK, Branton A, Trivedi D, Nayak G, Shettigar H, Mondal SC, Jana S (2015) Antimicrobial susceptibility pattern, biochemical characteristics and biotyping of Salmonella paratyphi A: An impact of biofield treatment. Clin Microbiol 4: 215.

Trivedi MK, Branton A, Trivedi D, Nayak G, Mondal SC, Jana S (2015) Antibigram of biofield-treated Shigella boydii: Global burden of infections. Science Journal of Clinical Medicine 4: 121-126.

Trivedi MK, Branton A, Trivedi D, Nayak G, Mondal SC, Jana S (2015) Evaluation of antibiotic, genotype and phylogenetic analysis of biofield treated Nocardia otitidis. Biol Syst Open Access 4: 143.

Trivedi MK, Branton A, Trivedi D, Nayak G, Charan S, Jana S (2015) Phenotyping and 16S rDNA analysis after biofield treatment on Citrobacter braakii: A urinary pathogen. J Clin Med Genom 3: 129.

Trivedi MK, Patil S, Shettigar H, Bairwa K, Jana S (2015) Spectroscopic characterization of chloramphenicol and tetracycline: An impact of biofield. Pharm Anal Acta 6: 395.

Trivedi MK, Patil S, Shettigar H, Bairwa K, Jana S (2015) Spectroscopic characterization of biofield treated metronidazole and tinidazole. Med Chem 5: 340-344.

Trivedi MK, Patil S, Shettigar H, Bairwa K, Jana S (2015) Effect of biofield treatment on spectral properties of paracetamol and piroxicam. Chem Sci J 6: 98.

Trivedi MK, Branton A, Trivedi D, Shettigar H, Bairwa K, Jana S (2015) Fourier transform infrared and ultraviolet-visible spectroscopic characterization of biofield treated salicylic acid and spirularoxicin. Nat Prod Chem Res 3: 186.

Trivedi MK, Branton A, Trivedi D, Nayak G, Mondal SC, Jana S (2015) Morphological characterization, quality, yield and DNA fingerprinting of biofield energy treated alphonso mango (Mangifera indica L.). Journal of Food and Nutrition Sciences 3: 245-250.

Trivedi MK, Branton A, Trivedi D, Nayak G, Gangwar M, Jana S (2015) Agronomic characteristics, growth analysis, and yield response of biofield treated mustard, cowpea, horse gram, and groundnut. International Journal of Genetics and Genomics 3: 74-80.

Trivedi MK, Branton A, Trivedi D, Nayak G, Gangwar M, Jana S (2015) Analysis of genetic diversity using simple sequence repeat (SSR) markers and growth regulator response in biofield treated cotton (Gossypium hirsutum L.). American Journal of Agriculture and Forestry 3: 216-221.

Trivedi MK, Branton A, Trivedi D, Nayak G, Gangwar M, Jana S (2015) Evaluation of vegetative growth parameters in biofield treated bottle gourd (Lagenaria siceraria) and okra (Abelmoschus esculentus), International Journal of Nutrition and Food Sciences 4: 688-694.

Trivedi MK, Tallapragada RM, Branton A, Trivedi D, Nayak G, Latiyal O, Jana S (2015) Evaluation of atomic, physical, and thermal properties of bismuth oxide powder: An impact of biofield energy treatment. American Journal of Nano Research and Applications 3: 94-98.

Trivedi MK, Patil S, Nayak G, Jana S, Latiyal O (2015) Influence of biofield treatment on physical, structural and spectral properties of boron nitride. J Material Sci Eng 4: 181.

Trivedi MK, Nayak G, Patil S, Tallapragada RM, Latiyal O, Jana S (2015) Characterization of physical and structural properties of brass powder after biofield treatment. J Powder Metall Min 4: 134.

Trivedi MK, Nayak G, Patil S, Tallapragada RM, Latiyal O, Jana S (2015) Evaluation of biofield treatment on physical and structural properties of bronze powder. Adv Automob Eng 4: 119.

Trivedi MK, Nayak G, Patil S, Tallapragada RM, Jana S, Mishra RK (2015) Bio-field treatment: An effective strategy to improve the quality of beef extract and meat infusion powder. J Nutr Food Sci 5: 389.

Trivedi MK, Tallapragada RM, Branton A, Trivedi D, Nayak G, Mishra RK, Jana S (2015) Biofield treatment: A potential strategy for modification of physical and thermal properties of gluten hydrolysate and ipomoea macromolecules. J Nutr Food Sci 5: 414.

Kinney JP, Trived MK, Branton A, Trived D, Nayak G, Mondal SC, Jana S (2017) Overall skin health potential of the biofield energy healing based herbomineral formulation using various skin parameters. American Journal of Life Sciences 5: 65-74.

Singh J, Trivedi MK, Branton A, Trivedi D, Nayak G, Gangwar M, Jana S (2017) Consciousness energy healing treatment based herbomineral formulation: A safe and effective approach for skin health. American Journal of Pharmacology and Phytotherapy 2: 1-10.

Czekanska EM, Stoddart MJ, Richards RG, Hayes JS (2012) In search of an osteoblast cell model for in vitro research. Eur Cells Mater 24: 1-17.
[41] Biological evaluation of medical devices - Part 5: Tests for in vitro cytotoxicity (ISO 10993-5:2009), I.S.EN ISO, 10993-5:2009.

[42] Li BB, Yu SF, Meng XM (2003) The beneficial effect of genistein on mandible bone metabolism in ovariectomized rats. Zhonghua Kou Qiang Yi Xue Za Zhi 38: 336-338.

[43] Sultana S, Choudhury S, Choudhury SA (2002) Serum alkaline phosphatase and bone mineral density: To assess bone loss in oral contraceptive pill user. Mymensingh Med J 11: 107-109.

[44] Anh DJ, Dimai HP, Hall SL, Farley JR (1998) Skeletal alkaline phosphatase activity is primarily released from human osteoblasts in an insoluble form, and the net release is inhibited by calcium and skeletal growth factors. Calcif Tissue Int 62: 332-340.

[45] Daneault A, Prawitt J, Soulé VF, Coxm V, Wittrant Y (2017) Biological effect of hydrolyzed collagen on bone metabolism. Crit Rev Food Sci Nutr 57: 1922-1937.

[46] Zhang H, Zhao Y, Li Y, Sun X, Bai X, Zhao D (2010) Effects of deer tendons collagen on osteoporosis rats induced by retinoic acid. Journal of Chinese Medicinal Materials 33: 411-414.

[47] Elefteriou F, Benson MD, Sowa H (2006) ATF4 mediation of NF1 functions in osteoblast reveals a nutritional basis for congenital skeletal dysplasias. Cell Met 4: 441-451.