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In Vitro Effect of Biofield Energy Treated DMEM On Mitochondrial Biogenesis Using Myoblasts Cell Line, C2C12

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Abstract

Mitochondria, a signaling organelles are responsible for energy metabolism within the cell, while its dysfunction in any form could results in the cell death due to an increased oxidative stress and that leads to various metabolic diseases. The aim of the present study was to examine the effect of Consciousness Energy Healing based DMEM medium on murine myoblasts (C2C12) cells to evaluate the mitochondrial mass content using 10-N-nonyl acridine orange (NAO) dye assay. The test item, DMEM was divided into three parts. First part did not receive any sort of treatment and defined as the untreated DMEM group. The second and third parts were treated with the one-time and two-times Biofield Energy Treatment by a renowned Biofield Energy Healer, Alice Branton and coded as the one-time Biofield Energy Treated DMEM (BT-I) and two-times Biofield Energy Treated DMEM (BT-II) groups, respectively. Cell viability of the test items using MTT assay showed 72.32%, 97.89%, and 104.96% viable cells in the untreated DMEM, BT-I, and BT-II groups, respectively suggested that the test items were nontoxic and safe in murine myoblasts (C2C12) cells. Further, the mitochondrial mass content in terms of Fluorescence Unit (FU) was significantly ($p \leq 0.05$) increased by 43.11% and 64.52% in the BT-I and BT-II groups, respectively in C2C12 cells compared to the untreated DMEM group. Thus, overall experimental data suggested that two-times blessed DMEM showed a significant improvement of mitochondrial mass content and results in better thermogenesis. In the present study, results demonstrated that an increased mitochondrial mass content in the cells, when treated with The Trivedi Effect®. This indicates that the Biofield Energy Treated DMEM has the great potential to improve thermogenesis, which can be used against various metabolic diseases, such as insulin resistance, type-2 diabetes, and cardiovascular diseases.

Keywords: Biofield Energy; DMEM; Metabolic disorders; Mitochondrial biogenesis; Murine myoblast cell; The Trivedi Effect®; Thermogenesis

Abbreviations: CAM: Complementary and Alternative Medicine; DMEM: Dulbecco's Modified Eagle's Medium; FBS: Fetal Bovine Serum; NCCAM: National Center for Complementary and Alternative Medicine

Introduction

Mitochondria are well-known for their central bioenergetics role and they act as a power houses to produce Adenosine Triphosphate (ATP) from oxidation of nutrients. In addition, these vital cell organelles are highly dynamic and undergo fusion, fission, transport, and degradation, all of the process are vital to maintain a healthy mitochondrial population [1]. Mitochondria has central metabolic function, while mitochondrial dynamics and bioenergetics reciprocally influence to each. Mitochondrial biogenesis process involved an increased and controlled mitochondrial mass with number that helped to produce greater production of ATP as a response to greater energy expenditure [2]. Various factors are involved to regulate the process of mitochondrial biogenesis such as physiologic, metabolic, and pathologic changes along with morphological and functional adaptability. Besides, several proteins and transcription factors, upstream regulatory proteins and secondary mechanisms are also involved in the biogenesis process, which also stabilizes the

new mitochondrial DNA [3]. Mitochondrial biogenesis regulates and control various therapeutic interference in a wide number of diseases such as metabolic syndrome, neurodegenerative disorders, sarcopenia, cardiac pathophysiology and physiological processes like aging [4]. Mitochondrial mass alteration can be detected using various gold standard assay such as Nonyl-acridine orange (NAO) assay, which is a metachromatic dye that binds to cardiolipin, an inner mitochondrial membrane lipid, regardless of the energetic state of the cell. NAO is a non-fluorescent dye that converts into fluorescent dye in the presence of oxidative species [5]. Therefore, mitochondrial mass of the cells could be estimated by studying accumulation of the fluorescent dye in the mitochondria. Furthermore, an alternative therapies using a nuclear gene product (MIDAS/GPP34) was reported to regulate total mitochondrial mass in response to mitochondrial dysfunction [6]. However, an alternative treatment approach without any associated side-effect has not been reported so far to improve the mitochondrial mass content, which can be utilized against many metabolic disorders. Thus, some alternative or complementary therapeutic approach is required that might improve the thermogenesis process, which leads to regulate and improve the mitochondrial mas content without any side-effects.

Biofield Energy Healing is one of the emerging frontier in medicine, which has been increasing rapidly in order to promote wellness by uncovering the root cause of diseases with universal solutions. Complementary and Alternative Medicine (CAM) therapies have shown various significant clinical benefits. Over the past few decades, many energy healing practices have been reported with significant outcomes in various clinical and non-clinical 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* [7]. Every living organisms possess some kind of unique energy known as Biofield Energy, which is infinite, para-dimensional and electromagnetic field surrounding the human body. Biofield (Putative Energy Fields) based Energy Healing Therapies have been reported to have significant outcomes against various disease conditions. Biofield Energy Healing Treatment (The Trivedi Effect®) can be channeled by a renowned practitioner from a distance. Biofield Energy Healing as a CAM showed a significant result in biological studies [8]. However, the National Center for Complementary and Alternative Medicine (NCCAM), well-defined Biofield Therapies in the subcategory of Energy Therapies [9]. The Trivedi Effect®-Consciousness Energy Healing Treatment has been reported with significant revolution in the physicochemical properties of metals, chemicals, ceramics and polymers [10-12], improved agricultural crop yield, productivity, and quality [13,14], transformed antimicrobial characteristics [15-17], biotechnology [18,19], improved bioavailability [20-22], skin health [23,24], nutraceuticals [25,26], cancer research [27,28], bone health [29-31], human health and wellness.

On the basis of outstanding benefits of Biofield Energy Treatment, the present study was aimed to evaluate the impact of the Biofield Energy Treatment (The Trivedi Effect®) on DMEM as test sample to alter the mitochondrial mass content on thermogenesis using NAO dye stain assay in murine myoblasts (C2C12) cells.

Material and Methods

Chemicals and Reagents

Antibiotics solution (penicillin-streptomycin) were procured from HiMedia, India, and Ethylenediaminetetraacetic Acid (EDTA) was purchased from Sigma, USA. Fetal Bovine Serum (FBS) and Dulbecco's Modified Eagle's Medium (DMEM) were purchased from Life Technology, USA. All the other chemicals used in this experiment were analytical grade procured from India.

Cell Culture

C2C12 (murine myoblasts) was used as a test system in the present study. The C2C12 cell line was maintained in DMEM growth medium for routine culture supplemented with 10% FBS. Growth conditions were maintained at 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. Before initiation of the experiment, cells were incubated in DMEM + 2% horse serum (HS) for 3 days to allow the cells to differentiate into myotubes.

Experimental Design

The experimental groups consisted of group 1 (G-I) with untreated DMEM. Group 2 (G-II) included one-time blessed DMEM medium (BT-I), and group 3 (G-III) included two-times blessed DMEM and denoted as BT-II.

Consciousness Energy Healing Treatment Strategies

The test item, DMEM was divided into three parts. First part did not receive any sort of treatment and defined as the untreated DMEM group. The second and third parts were treated with the one-time and two-times Biofield Energy Treatment by a renowned Biofield Energy Healer (The Trivedi Effect®), Alice Branton remotely for ~5 minutes under laboratory conditions and coded as the one-time Biofield Energy Treated DMEM (BT-I) and two-times Biofield Energy Treated DMEM (BT-II) groups, respectively. Healer in this study never visited the laboratory in

person, nor had any contact with the test items (DMEM medium). Further, the untreated DMEM 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 test items were kept in similar sealed conditions for experimental study.

Assessment of Cell Viability Using MTT Assay

The cell viability was performed by MTT assay in C2C12 cell line. The cells were counted and plated in 96-well plates at the density corresponding to 10 X 10^3 cells/well/180 µL of cell growth medium (DMEM + 2% HS). 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 Untreated and Biofield Energy Treated test items. The cells in the above plate(s) were incubated for 24 to 72 hours in a 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 [29]. The percentage cytotoxicity at each tested concentrations of the test items were calculated using the following equation (1):

% Cytotoxicity = (1-X/R)*100 ---------- (1)

Where, X = Absorbance of the Biofield 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 Mitochondrial Content

For the assessment of mitochondrial mass, the cells were counted using an hemocytometer and plated at 4500 cells/well in dark walled 96-well plates in DMEM supplemented with 2% HS. The cells were incubated overnight under standard growth conditions to allow the cell recovery and exponential growth, which were treated by the test items in different groups followed by incubation with the test items for 72 hours. After incubation with the test items, mitochondrial content was determined by 10-N-nonyl acridine orange (NAO) dye. 50nM dye was added to each well and the cells were incubated for 30 minutes at 37˚C and 5%CO_2. After 30 minutes of incubation, media was discarded and cells were washed with phosphate buffer saline (PBS). 150µL of PBS was added to each well and fluorescence was read at 485/20 excitation, 528/20 emission filter using synergy HT microplate reader. The percentage increase in mitochondrial content was calculated using following equation-

% increase = [Average $FU_{Treated}$ - Average $FU_{Untreated}$]*100/Average $FU_{Untreated}$ ----------(3)

Where, FU denotes Fluorescence unit

Statistical Analysis

All the values were represented as Mean ± SEM (standard error of mean) of three independent experiments. The statistical analysis was performed using SigmaPlot statistical software (v11.0). For two groups comparison student's *t*-test was used. 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

The result of cell viability using MTT assay of the untreated and Biofield Energy Treated DMEM in C2C12 cells is shown in Figure 1. The percentage of cell viability in all the tested groups showed that the cell viability ranges from 72.32% to 104.96%. Overall, the data suggest that all the test samples were found safe against the tested C2C12 cells, which were used for the estimation of other parameter such as mitochondrial mass content, which indicate extend of thermogenesis.

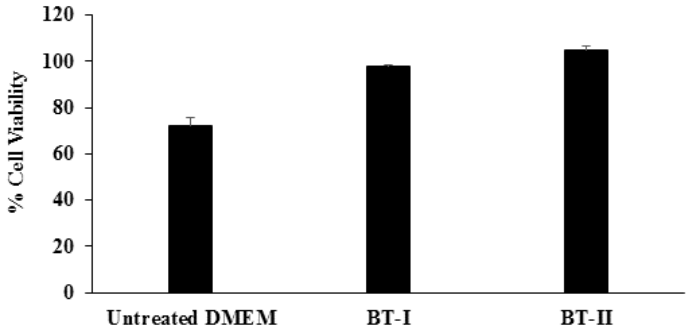

Figure 1: Cell viability using MTT assay of the test items on murine myoblasts (C2C12) cell-line. BT-I: One-time Biofield Energy Treated DMEM; BT-II: Two-times Biofield Energy Treated DMEM

Effect of the Test Samples on Mitochondrial Mass Content in C_2C_{12} Cells

The effect of the test samples on mitochondrial mass content was assessed in C2C12 cells using NAO dye assay. The results of mitochondrial mass content among different groups in terms of increase number of Fluorescence Unit (FU) is shown in Figure

2. Increase NAO dye accumulation in muscle cells indicated an increase FU i.e., the mitochondrial mass content. The untreated DMEM group showed 272.3 ± 12.14 FU. Moreover, the one-time Blessed DMEM group (BT-I) showed 43.11%, while the two-times Blessed DMEM group (BT-II) showed 64.52% increase the level of mitochondrial mass in terms of FU, respectively compared to the untreated DMEM group (Figure 2). Thus, the data suggested that two-times blessed DMEM group significantly improved thermogenesis, which results in mitochondrial mass content against various metabolic diseases, such as insulin resistance, type-2 diabetes, and cardiovascular diseases.

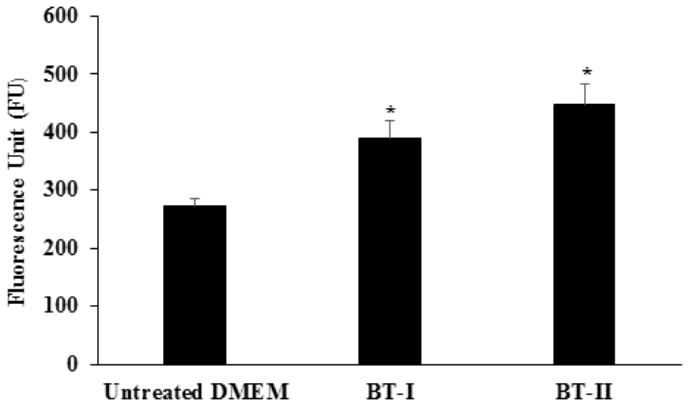

Figure 2: Effect of the test items for the change in mitochondrial mass on C2C12 cell line. BT-I: One-time Biofield Energy Treated DMEM; BT-II: Two-times Biofield Energy Treated DMEM. Values are represented as mean ± SEM of three independent experiments. *p≤0.05 vs. Untreated DMEM group.

According to the experimental results, it can be concluded that NAO dye accumulation in muscle cells was increased significantly which indicated an increased level of mitochondrial mass content and hence, Biofield Energy Healing Treatment showed a better thermogenesis. Thus, the mitochondrial biogenesis process was improved, which results in increased cell capacity and number that results in the improved production of ATP as a response to greater energy expenditure [32]. This results can also have assumed that it improved physical endurance training by improved mitochondrial content levels, leading to greater glucose uptake by muscles, along with an increased metabolic enzymes level for glycolysis, oxidative phosphorylation and ultimately a greater mitochondrial metabolic capacity [33]. From literature, it was reported that aging results in decreased level of mitochondrial mass content and leads to various diseases such as enhanced aging, insulin resistance, type-2 diabetes, cardiovascular diseases, obesity, etc. [34]. But, Biofield Energy Healing Treatment (The Trivedi Effect®) has the significant capacity to improve the overall Quality of life with an improved thermogenesis and mitochondrial content.

Conclusions

MTT assay of the test item (DMEM) in C2C12 cells showed a significant improved cell viability with the ranges from 97.89% to 104.96% in different test item groups. Besides, the mitochondrial mass content was significantly ($p \leq 0.05$) increased by 43.11% and 64.52% in the one-time Biofield Energy Treated DMEM (BT-I) and two-times Biofield Energy Treated DMEM (BT-II) groups, respectively in C2C12 cells compared to the untreated DMEM group. Thus, Biofield Energy Healing based DMEM can be significantly used to improve the energy level, endurance, body energy, which can be utilized against many diseases such aging, diabetes, cancer, depression, hypertension, cardiovascular disease, aging, and physical strength. The Biofield Energy Treated (The Trivedi Effect®) DMEM were found to have a significant impact on thermogenesis, which might significantly improve the mitochondrial content in muscle cells. Therefore, the Consciousness Energy Healing based DMEM might be a suitable alternative media for cell growth. It can be useful for the management of various disorders such as Lupus, Systemic Lupus Erythematosus, Fibromyalgia, Addison Disease, Hashimoto Thyroiditis, Celiac Disease (gluten-sensitive enteropathy), Multiple Sclerosis, Dermatomyositis, Graves' Disease, Myasthenia Gravis, Pernicious Anemia, Aplastic Anemia, Scleroderma, Psoriasis, Rheumatoid Arthritis, Reactive Arthritis, Type 1 Diabetes, Sjogren Syndrome, Crohn's Disease, Vasculitis, Vitiligo, Chronic Fatigue Syndrome and Alopecia Areata, as well as inflammatory disorders such as Irritable Bowel Syndrome (IBS), Asthma, Ulcerative Colitis, Alzheimer's Disease, Parkinson's Disease, Atherosclerosis, Dermatitis, Hepatitis, and Diverticulitis. Further, the Biofield Energy Healing Treated test formulation can also be used in the prevention of immune-mediated tissue damage in cases of organ transplants (for example heart transplants, kidney transplants and liver transplants), for anti-aging, stress prevention and management, and in the improvement of overall health and Quality of Life.

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