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In vitro Assessment of Biofield Energy Treated DMEM on Thermogenesis Using Myoblasts Cell Line (C2C12)

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Abstract

Mitochondrial dysfunction lead to various serious disorders, which are considered as one of the important components related with the aging, such as type-2 diabetes and Alzheimer's disease. 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 medium) was divided into two parts, one part received Consciousness Energy Healing Treatment by a renowned Biofield Energy Healer, Dahryn Trivedi and was labeled as the Biofield Energy Treated DMEM group, while the other part did not receive any kind of Treatment and denoted as the untreated DMEM group. The level of mitochondrial mass content was assessed using 10-N-nonyl acridine orange (NAO) dye method. Cell viability of the test items using MTT assay showed 72.32% and 125.32% viable cells in the untreated DMEM and Biofield Energy Treated DMEM groups, respectively suggested a safe and nontoxic profile of the test items. Besides, the mitochondrial mass content in terms of Fluorescence Unit (FU) was significantly ($p \leq 0.05$) increased by 81.78% in the Biofield Energy Treated DMEM group compared to the untreated DMEM group. Overall, the experimental data suggested that the Consciousness Energy Healing Based DMEM showed a significant improvement of mitochondrial mass content and results in better thermogenesis with respect to naive DMEM. Thus, an increased level of NAO dye accumulation in muscle cells indicated increased mitochondrial mass content and hence, 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 test sample has the potential to improve thermogenesis, which can be used against various metabolic diseases, such as insulin resistance, type-2 diabetes, and cardiovascular diseases, etc.

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

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

Introduction

Mitochondria (also known as power generator of the cell) produce most of the vital energy required for the cellular function through oxidative phosphorylation involved in electron transport and ATP synthesis. They produce ATP through the process of cellular respiration mainly aerobic respiration, which requires oxygen. Number and amount of mitochondria in a cell be governed by the energy requirement of the cell [1]. For example, the muscle cells have found comparatively more number of mitochondria since, they need to produce energy to move the body. On the other hand, red blood cells carry oxygen to other cells, do not need to produce energy as compared with the muscle cells. Mitochondria are the powerhouse in the cell, which produce energy from basic components. They undergo fusion, fission, transport, and degradation, all of the process is vital to maintain a healthy mitochondrial population [1]. However, the mitochondrial biogenesis process is 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]. Physiologic, metabolic, and pathologic changes along with morphological and functional adaptability are the vital factor to regulate the process of mitochondrial biogenesis. In addition, 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 wide number of diseases such as metabolic syndrome, neurodegenerative disorders, sarcopenia, cardiac pathophysiology and physiological processes like aging [4]. Nonyl-acridine orange (NAO) is a non-fluorescent dye that converts into fluorescent dye in the presence of oxidative species

[5]. NAO assay is one of the gold standard assays to detect the mitochondrial mass alteration, which is a metachromatic dye that binds to cardiolipin, an inner mitochondrial membrane lipid, regardless of the energetic state of the cell. Therefore, mitochondrial mass of the cells could be estimated by studying accumulation of the fluorescent dye in the mitochondria. Furthermore, an alternative therapies such as nuclear gene was reported to regulate total mitochondrial mass in response to mitochondrial dysfunction [6]. In order to improve the mitochondrial mass content *via* thermogenesis process, some alternative treatment approach without any associated side-effect is needed.

Biofield Energy Healing is a categorized as one of the Complementary and Alternative Medicine (CAM) accepted worldwide for the various treatment. Biofield Energy Therapy was accepted by National Center for Complementary and Alternative Medicine (NCCAM). Biofield Energy Healing is one of the emerging frontier in medicine, which has been increased in order to promote wellness by uncovering the root cause of diseases with universal solutions. CAM therapies have shown various significant clinical benefits. Over the past few decades, many energy healing practices have been reported a 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®) contain a putative bioenergy, which is channeled by a renowned practitioner from a distance. Biofield Energy Healing as a CAM showed a significant results in biological studies [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 staining using standard *in vitro* assay in murine myoblasts (C2C12) cells.

Material and Methods

Chemicals and reagents

Fetal bovine serum (FBS) and Dulbecco's Modified Eagle's Medium (DMEM) were purchased from Life Technology, USA. Antibiotics solution (penicillin-streptomycin) were procured from HiMedia, India, and ethylenediaminetetraacetic acid (EDTA) was purchased from Sigma, 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 and group 2 (G-II) included the Biofield Energy Treated DMEM.

Consciousness energy healing treatment strategies

The test item, DMEM was divided into two parts. One part of the test item was treated with the Biofield Energy by a renowned Biofield Energy Healer, Dahryn Trivedi remotely for ~5 minutes and coded as the Biofield Energy Treated DMEM, while the second part did not receive any sort of treatment and denoted as the untreated DMEM group. Biofield Energy Healer was located in the USA, while the test items were located in the research laboratory of Dabur Research Foundation, New Delhi, India. This Biofield Energy Treatment was administered through Healer's unique Energy Transmission process to the test sample under laboratory conditions. Dahryn Trivedi in this study never visited the laboratory in person, nor had any contact with the test item (DMEM). 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 samples 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/180µL of cell

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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 item. 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 20µL 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 150µL of DMSO was added to each well to dissolve formazan crystals. The absorbance of each well was read at 540nm using Synergy HT microplate reader, Bio Tek, USA [29]. The percentage cytotoxicity at each tested concentrations of the test items were calculated using the following equation (1):

$$\%cytotoxicity = (1 - X / R) * 100 \text{.....(1)}$$

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):

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

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

$$\% \text{ increase} = [\text{Average FU}_{Treated} - Average\ FU_{Untreated}] * 100 / Average\ \text{FU}_{Untreated} \text{.....(3)}$$

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 Sigma Plot statistical software (v11.0). For two groups comparison student's t-test was used. Statistically significant values were set at the level of $p \leq 0.05$.

Results and Discussion

Cell viability using MTT assay

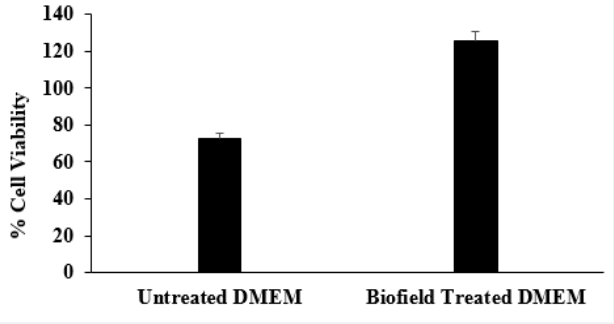

Figure 1: Cell viability using MTT assay of the test items on murine myoblasts (C2C12) cell-line.

Cell viability data of the untreated and Biofield Energy Treated DMEM groups in C2C12 cells using MTT assay is shown in Figure 1. The percentage of cell viability in the untreated DMEM group was 72.32%, while it was 125.32% in the Biofield Energy Treated DMEM group (Figure 1). Overall, data suggest that all the test samples were found safe against the tested C2C12 cells, which were used for the estimation of mitochondrial mass content, which indicate extend of thermogenesis.

Extend of mitochondrial mass content in C2C12 cells

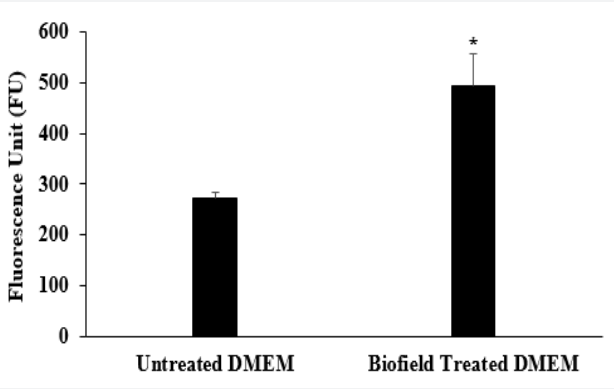

Figure 2: Effect of the test items on mitochondrial mass in terms of increase average Fluorescence Unit (FU) on C2C12 cell line. Values are represented as mean ± SEM of three independent experiments. *$p \leq 0.05$ vs. Untreated DMEM group.

Mitochondrial mass content or biogenesis showed a significant effects against various metabolic diseases. The increased cell capacity to control and maintain the cell metabolism, signal transduction, and regulation of mitochondrial ROS production [2]. Alteration or decrease in the mitochondrial biogenesis are related with the mitochondrial dysfunction and mitochondrial oxidative stress, which leads to various diseases [32]. Mitochondrial mass content results in the improved production of ATP as a response to greater energy expenditure [33]. Various factors such as physical exercise, nutritional factors, etc. reported to have an improved mitochondrial mass content, which results in greater glucose

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uptake by muscles, along with an increased metabolic enzymes level for glycolysis, oxidative phosphorylation and ultimately a greater mitochondrial metabolic capacity [34]. Aging process leads to decrease level of mitochondrial mass content and results in various diseases such as enhanced aging, insulin resistance, type-2 diabetes, cardiovascular diseases, obesity, etc. [35]. The experiment was conducted to study the influence of Biofield Energy Healing Treatment on mitochondrial content in C2C12 cells using NAO dye assay. The results of mitochondrial mass content in terms of increase number of fluorescence unit (FU) among different groups in C2C12 cells using NAO dye assay are presented in Figure 2. The untreated DMEM group showed 272.3 ± 12.14 FU. Besides, the Biofield Energy Treated DMEM group showed 81.78% increase the level of mitochondrial mass in terms of FU, compared to the untreated DMEM group (Figure 2).

Thus, the data suggested that the Consciousness Energy Blessed DMEM showed a significant improvement of thermogenesis, which results in mitochondrial mass content. This phenomenon can be significantly used against various metabolic diseases, such as insulin resistance, type 2 diabetes, and cardiovascular diseases. Overall, the 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.

Conclusion

MTT data showed a significant cell viability with ranges from 72.32% to 125.32% in different test item groups, which indicated that the test items were safe and non-toxic in nature. Correspondingly, The Trivedi Effect® showed a significant change in mitochondrial mass among different groups. The Biofield Energy Treated DMEM group demonstrated significant increase in the mitochondrial mass by 81.78% as 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, 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 Treatment 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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Conflict of Interest

Authors declare no conflict of action.

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