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Antioxidative Potential of Biofield Energy Treated DMEM in Pulmonary Disease Using Lung Adenocarcinoma Cell Line (A549)

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Abstract

The present *in vitro* investigation was aimed to explore the effect of Consciousness Energy Healing Treated DMEM medium for oxidative potential using oxidative biomarkers *viz*. Superoxide Dismutase (SOD) enzyme activity and protection against oxidative stress measured in A549 cells. The test item, DMEM was divided into two parts. First part of the test item 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 second part did not receive any sort of Treatment and defined as the Untreated DMEM (control) group. The cell viability of the test sample using MTT assay showed an increase cell viability by 136.4% in the Biofield Energy Treated DMEM group, suggesting a safe and nontoxic nature of the test items. Protection against oxidative stress was significantly increased by 23.80% in the Biofield Energy Treated DMEM group compared with the untreated DMEM group. Thus, the overall data suggest that the Biofield Energy Healing Treatment showed a significant improvement of SOD enzyme level along with an improved protection against oxidative damage, which can be used in various human lungs disorders such as asthma, Chronic Obstructive Pulmonary Disease (COPD), lung malignancies and parenchymal lung diseases like idiopathic pulmonary fibrosis and lung granulomatous diseases.

Keywords: Antioxidant; A549 cell; Biofield Energy Healing; DMEM; Lung disorder; Oxidative stress; SOD

Abbreviations: ANOVA: One-Way Analysis of Variance; CAM: Complementary and Alternative Medicine; CD-FBS: Charcoal Dextran Stripped FBS; COPD: Chronic Obstructive Pulmonary Disease; DMEM: Dulbecco's Modified Eagle's Medium; FBS: Fetal Bovine Serum; NCCAM: National Center for Complementary and Alternative Medicine; NIH: National Institute of Health, RNS: Reactive Nitrogen Species; ROS: Reactive Oxygen Species; SOD: Superoxide Dismutase

Introduction

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Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) are the endogenous products of cellular metabolism, act as a secondary messenger. Redox-regulatory mechanisms are involved by endogenous antioxidants under physiological conditions to protect cells against oxidative stress. Overproduction of ROS and RNS leads to excessive stimulation of reduced NADP (Nicotinamide Adenine Dinucleotide Phosphate) by cytokines or mitochondrial Electron Transport Chain (ETC) and Xanthine Oxidase (XO) that ultimately leads to a significant oxidative stress. The imbalance and an increased level of ROS and RNS can directly affect the lipids, DNA, carbohydrates, and proteins [1]. The lung damage and its related disorders have the serious clinical implications due to oxidative stress, leads to an injury and inflammation of the lungs. The lung is one of the vital organ that exposed to various endogenous and exogenous oxidants (such as cigarette smoke, mineral dust, ozone, and radiation). Several degenerative disorders and cancer have a significant correlation with the oxidative damage [2]. Oxidants produced in the respiratory system significantly enhanced the production of mediators of pulmonary inflammation and initiate or promote the mechanism of carcinogenesis [3]. These oxidants produce the high level of Citation: Trivedi D and Jana S (2019) Antioxidative Potential of Biofield Energy Treated DMEM in Pulmonary Disease Using Lung Adenocarcinoma Cell Line (A549). Appl Clin Pharmacol Toxicol: ACPT-119. DOI : 10.29011/ACPT-119.100019

free radicals, while ROS and RNS are produced by phagocytes as well as by alveolar, polymorphonuclear, bronchial, and endothelial cells [4]. However, the role of oxidative stress in the pathogenesis of lung diseases has been widely reported such as asthma, Chronic Obstructive Pulmonary Disease (COPD), lung malignancies and parenchymal lung diseases like idiopathic pulmonary fibrosis and lung granulomatous diseases [5]. Superoxide Dismutase (SOD) antioxidant enzyme is one of the common defense mechanism in lung cells and it worked against ROS by converting superoxide radicals to the hydrogen peroxide. According to their specific role and distribution, ROS can be categorized such as cytosolic copper-zinc, mitochondrial manganese, and extracellular SODs. To combat the oxidants induced damage against oxidative stress and improved SOD enzyme in lung tissues would be the best approach towards lung health [6,7]. The importance of SOD in protecting the lung tissues and other body tissues has well established. However, very few studies have been reported the SOD level in different cell lines. Hence, in this experiment, A549 (lung adenocarcinoma) cells were used to study the effect of the Biofield Energy Treated DMEM media as a test item on lung health using SOD and oxidative damage protection as biomarkers.

National Center for Complementary and Alternative Medicine (NCCAM), has been recognized the importance and clinical use of Biofield Energy Therapies as one of the complementary medicine domain. Human Biofield, a low electromagnetic field that permeates and surrounds the living organisms [8]. The scientific data suggested that most of the U.S. population has been focused towards the use of natural products as a Complementary And Alternative Medicine (CAM) [8]. Biofield Energy Healing Therapies or putative energy fields are characterized under CAM that have significant impact on living organisms and non-living materials. These therapies are based on the concept that human beings are pervaded with a subtle form of energy, which have the capacity to transform the living organisms and non-living materials. Besides, an increasing demand of CAM therapies, Biofield Energy Treatment proofed to have significant benefits in various scientific 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 [9]. The Biofield Energy Healing Treatment (The Trivedi Effect®) was practiced by renowned Biofield Energy Healer, which is channeled by renowned practitioners from a distance. Biofield Energy Healing showed a significant outcome in biological studies [10]. However, NCCAM also mentioned with well-known clinical benefits of Biofield Energy in the subcategory of Energy

Therapies [11]. The Trivedi Effect[®]- Consciousness Energy Healing Treatment has been reported with significant revolution in the physicochemical properties of metals, chemicals, ceramics and polymers [12-14], an improved agricultural crop yield, productivity, and quality [15, 16], transformed antimicrobial characteristics [17-19], biotechnology [20,21], improved bioavailability [22-24], skin health [25,26], nutraceuticals [27,28], cancer research [29,30], bone health [31-33], human health and wellness.

In this study, authors evaluated the impact of the Biofield Energy Treatment (The Trivedi Effect[®]) on DMEM as the test sample for lung health with respect to SOD and oxidative stress as biomarkers using standard *in vitro* assay in A549 cells (lung adenocarcinoma).

Material and Methods

Chemicals and Reagents

Antibiotics 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. Quercetin was purchased from Alfa Aesar, India. Fetal bovine serum (FBS) and Dulbecco's Modified Eagle's Medium (DMEM) were purchased from Life Technology, USA. *t*-BHP (tert-butyl hydroperoxide) was purchased from Sigma, India. All the other chemicals used in this experiment were analytical grade procured from India.

Cell Culture

A549 (lung adenocarcinoma) from muscle tissue of *Mus musculus* was used as the test system in the present study. Ishikawa cell line was maintained in DMEM growth medium for routine culture supplemented with 10% FBS. Growth conditions were maintained at 37° C, 5%CO₂, and 95% humidity and sub-cultured by trypsinisation followed by splitting the cell suspension into fresh flasks and supplementing with fresh cell growth medium. Before the start of the experiment, the growth medium of near-confluent cells was replaced with fresh phenol-free DMEM, supplemented with 10% Charcoal-Dextran Stripped FBS (CD-FBS) and 1% penicillin-streptomycin for 3 days [34].

Experimental Design

The experimental groups consisted of group 1 (G-I) with cells in baseline control with DMEM. Group 2 (G-II) consisted of positive control at non-cytotoxic concentrations. Further, group 3 (G-III) included the Biofield Energy Treated DMEM.

Consciousness Energy Healing Treatment Strategies

The test item, DMEM was divided into two parts, first part was treated with the Biofield Energy by a renowned Biofield Energy

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Healer (The Trivedi Effect[®]), Dahryn Trivedi remotely for ~5 minutes and coded as the Biofield Energy Treated DMEM, and the second part did not receive any sort of treatment and denoted as the untreated DMEM group (control). The Biofield Energy Healer was located in the USA, while the test item was located in the research laboratory of Dabur Research Foundation, New Delhi, India. This Biofield Energy Treatment was provided through the Healer's unique Energy Transmission process remotely to the test sample under laboratory conditions. Biofield Energy healer in this study never visited the laboratory in person, nor had any contact with the test item. Further, the untreated DMEM (control) group was treated with "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.

Identification of Non-Cytotoxic Concentration

The cell viability was performed by MTT assay in lung adenocarcinoma cell line (A549). The cells were counted and plated in 96-well plates at the density corresponding to 10 X 10³ cells/well/180 µL of cell growth medium. 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 test item (DMEM) and positive control. The cells in the above plate(s) were incubated for 24 hours in a 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 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 [35]. The percentage cytotoxicity at each tested concentrations of the test substance were calculated using the following equation (1):

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

Where, X = Absorbance of 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 \geq 70% cell viability was considered as non-cytotoxic.

Assessment of Cellular Protection against Oxidative Damage

The A549 cells were counted using an hemocytometer and plated in 96-well plates at the density corresponding to 1 X 10^4

cells/well followed by overnight incubation in a CO₂ incubator at 37°C, 5%CO₂, and 95% humidity. Following overnight incubation, the cells were treated with the positive control and test item. To induce oxidative damage, co-treatment of with *t*-BHP (150 μ M) was added. The cells corresponding to the positive control group were treated with quercetin. The untreated cells served as negative control [36]. After incubation, the plates were taken out and the percentage cell viability corresponding to each treatment group was calculated using the following equation (3):

% Protection = $[(Absorbance_{sample} - Absorbance_{t-BHP})]*100/$ [Absorbance_untreated - Absorbance_t_BHP]--(3)

Assessment of Intracellular Superoxide Dismutase (SOD) Enzyme Activity

The A549 cells were counted using an hemocytometer and plated in 24-well plates at the density corresponding to 2 X 104 cells/ well followed by overnight incubation in a CO₂ incubator at 37°C, 5% CO₂, and 95% humidity. Following overnight incubation, the cells were treated with the positive control and test item. To induce oxidative damage, co-treatment with t-BHP (150 μ M) was added. The cells corresponding to positive control group were treated with quercetin. The untreated cells served as negative control. After 24 hours of treatment, cell lysates were prepared by freezethaw lysis. SOD activity of the cells was assessed using cayman superoxide dismutase assay kit as per manufacturer's protocol. Further, 10μ L of the standard or the sample was added to 200 μ L of radical detector in a designated well on the plate. Reaction was initiated using xanthine oxidase (20 µL) in all the wells. After 30 minutes of incubation on shaker, absorbance was read at 450 nm using Synergy HT microplate reader. SOD activity of the samples was calculated using linear regression equation of the standard curve [37]. However, the percentage increase in SOD activity with respect to the t-BHP was calculated as per equation (4)-

Percentage increase= $[(SOD_{sample} - SOD_{tBHP})/ (SOD_{untreated} - SOD_{tBHP})]*100-----(4)$

Statistical Analysis

All the values were represented as Mean \pm SEM (standard error of mean) of three independent experiments. 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 $p \le 0.05$.

Results and Discussion

Cell Viability Study Using MTT

The data of cell viability using MTT assay expressed as percentage is shown in Figure 1. The test items were found to have significant cell viability with 136.4% in the Biofield Energy Treated DMEM group and more than 72% in the quercetin (positive

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control) group at various concentrations. Thus, the data suggested that the Biofield Energy Treated DMEM was found to be safe in A549 cells as compared with the untreated DMEM group.

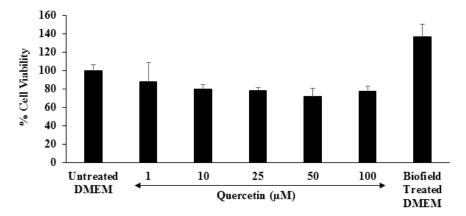


Figure 1: Cell viability using MTT assay of quercetin and DMEM in A549 cells. Data were expressed as Mean \pm SEM of three independent experiments.

Effect of the Test Items on Protection against Oxidative Damage

The effect of test items for the cellular protection against oxidative damage in A549 cells is shown in Figure 2. The level of protection against oxidative stress in terms of percentage values was presented in comparison with the untreated DMEM (control) group. The quercetin (positive control) group showed a significantly increase cellular protection by 20.12%, 35.97%, and 57.95% at 1, 10, and 50 μ M, respectively with respect to the untreated DMEM group. The Biofield Energy Treated DMEM group showed a significant increase cellular protection against oxidative damage by 23.80% compared with the untreated DMEM group. This suggest that Biofield Energy Treatment has the significant capacity to reduce the oxidative stress and can produce a balance between the oxidants and antioxidants. Oxidative stress plays a vital role in therapeutic development of various lung diseases, which results in the loss of cell function by damaging the lipids, DNA, carbohydrates and proteins by the interaction of ROS [38]. Scientific report suggested that the imbalance in systemic oxidant/antioxidant along with the downstream systemic leads to COPD and other respiratory diseases [39]. Oxidative stress leads to loss of cell by altering the cell function and oxidative damage in pathology of respiratory diseases [40]. However, Biofield Energy Healing Treatment might significantly improve cellular protection against oxidative damage which can significantly improve the clinical symptoms of lung diseases.

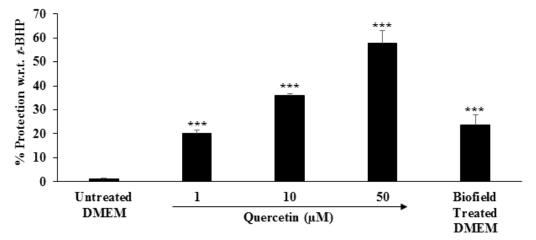


Figure 2: Protection against oxidative damage of the Biofield Energy Treated DMEM on A549 cells. Data were expressed as Mean \pm SEM of three independent experiments. ***p \leq 0.001 compared to the untreated DMEM group.

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Effect of the Test Items on Intracellular Superoxide Dismutase (SOD) Enzyme Activity

The SOD enzyme activity for the test items and positive control was assessed in A549 cells and the data are presented in Figure 3. The positive control, quercetin showed a significantly increase cellular protection by 35.76%, 66.79%, and 121.13% at 0.25, 0.5, and 1 μ M, respectively with respect to the untreated DMEM group. The Biofield Energy Treated DMEM group showed a significant increase level of SOD enzyme by 40.4% compared with the untreated DMEM group.

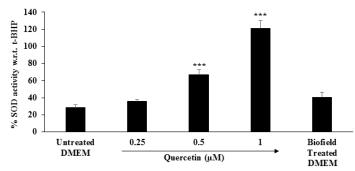


Figure 3: Super Oxide Dismutase (SOD) enzyme activity of the Biofield Energy Treated DMEM on A549 cells. Data were expressed as Mean \pm SEM of three independent experiments. ***p \leq 0.001 compared to the untreated DMEM group.

The SOD enzyme is regarded as one of the first-line defense antioxidants (like Superoxide Dismutase (SOD), catalase (CAT) and Glutathione Peroxidase (GPx)). These enzymes collectively act against free radicals and fight against various oxidative diseases. The reduced level of SOD and other enzymes would result in inflammation and cause lung diseases such as COPD, asthmatic airways, and parenchymal lung diseases. In order to improve the level of SOD enzyme, different types of antioxidants have been used to reduce the level of free radicals [41,42]. However, *in vitro* cell line experimental study advocates that the Biofield Energy Treatment has the significant capacity to work as an antioxidant and to remove the free radicals that play a vital role for the management of lung health and human lung diseases.

Conclusions

The cell viability using MTT assay showed 136.40% viable cells in the Biofield Energy Treated DMEM group indicating a safe and non-toxic profile of the test item. Moreover, the cellular protection against oxidative stress induced by *t*-BHP was significantly increased by 23.80% in the Biofield Energy Treated DMEM group compared with the untreated DMEM group. Besides, SOD enzyme activity was significantly increased by 40.40% in the Biofield Energy Treated DMEM group compared with the untreated DMEM group. Thus, the experimental result

suggests that the Biofield Energy Treated (The Trivedi Effect®) DMEM were found to have a significant impact to protect from oxidative damage in adenocarcinoma cells (A549). It can also be useful for the management of various human lung disorders such as Bronchiectasis, Asbestosis, Asthma, Bronchitis, Chronic Cough, Chronic Obstructive Pulmonary Disease (COPD), Common Cold, Cystic Fibrosis, Hantavirus, Idiopathic Pulmonary Fibrosis, Influenza, Lung Cancer, Pertussis, Pandemic Flu, Pleurisy, Pneumonia, Pulmonary Embolism, Respiratory Syncytial Virus (RSV), Sarcoidosis, Sleep Apnea, Sudden Infant Death Syndrome (SIDS), Tuberculosis, and Work-Related Asthma. Besides, it may also control the immune related disease conditions such as Hashimoto Thyroiditis, Aplastic Anemia, Hepatitis, Diverticulitis, Pernicious Anemia, Sjogren Syndrome, Myasthenia Gravis, Parkinson's Disease, Graves' Disease, Dermatomyositis, Multiple Sclerosis, Ulcerative Colitis, Alzheimer's Disease, Dermatitis, Irritable Bowel Syndrome, Diabetes, Atherosclerosis, Systemic Lupus Erythematosus, stress, etc. with a safe therapeutic index to improve overall health, and the Quality of Life.

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