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Assessment of Biofield Energy Treatment on Lung Health Using Lung Adenocarcinoma Cell Line (A549)

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Abstract

Oxidative stress is one of the major molecular mechanism responsible for human lung diseases. The objective of the study was to examine the effect of a Consciousness Energy Healing (The Trivedi Effect®) Treated DMEM medium for its anti-oxidative potential using various parameters such as the protection against oxidative stress and Superoxide Dismutase (SOD), antioxidant enzyme activity in A549 cells. The test item, DMEM was divided into two parts. One part received the Consciousness Energy Healing Treatment by a renowned Biofield Energy Healer, Mahendra Kumar Trivedi and was labeled as the Biofield Energy Treated test item, while the other part did not receive any sort of treatment and is defined as the untreated DMEM group. The cell viability of the test sample using MTT assay showed 122.67% viable cells, indicating a safe and nontoxic profile of the test item. Protection against oxidative stress was significantly increased by 64.70% in the Biofield Energy Treated DMEM group compared to the untreated DMEM group. In addition, Antioxidant Enzyme (SOD) activity was significantly increased by 45.1% in the Biofield Energy Treated DMEM group compared to the untreated DMEM group. Thus, these data suggest that Biofield Energy Healing Treatment showed a significant improvement of the Antioxidant Enzyme Activity (SOD level) along with an improved protection against oxidative damage, which can be used in various human lung disorders such as asthma, Chronic Obstructive Pulmonary Disease (COPD), lung malignancies and parenchymal lung diseases like idiopathic pulmonary fibrosis and lung granulomatous diseases.

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

Abbrevations: CAM: Complementary and Alternative Medicine; NCCAM: National Center for Complementary and Alternative Medicine; NIH: National Institute of Health; ANOVA: One-way analysis of variance; DMEM: Dulbecco's Modified Eagle's Medium; FBS: Fetal Bovine Serum; CD-FBS: Charcoal dextran stripped FBS; SOD: Superoxide dismutase; COPD: Chronic obstructive pulmonary disease; ROS: Reactive oxygen species; RNS: Reactive nitrogen species

Introduction

Increased level of Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) leads to oxidative stress, which is an imbalance between the oxidants and antioxidants. This imbalance and increased ROS can directly affect the lipids, DNA, carbohydrates, and proteins [1] in the human cells. Oxidative damage is considered to play a pivotal role in ageing, chronic inflammation, metabolic disorders, several degenerative diseases, and cancer [2]. Oxidative stress in the respiratory system increases the production of mediators of pulmonary inflammation and initiate or promote mechanisms of carcinogenesis [3]. Lung is one of the major organs, which is highly exposed by various oxidants i.e. endogenous and exogenous oxidants (cigarette smoke, mineral dust, ozone, and radiation). These oxidants produced 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]. The reduced level of the antioxidants and increased level of oxidants results in idiopathic pulmonary fibrosis and its associated lung diseases.

Lung tissue has significant capacity to fight against these oxidants through various antioxidant mechanisms. The most common and effective defense mechanism in lung cells is the Superoxide Dismutase (SOD), which is an enzyme that worked against ROS and convert superoxide radicals to the hydrogen peroxide. SODs are of three categories, which have their specific role and distribution, *viz.* cytosolic copper-zinc, mitochondrial manganese,

and extracellular SODs. SOD importance in protecting the lung tissues and other body tissues has been reported widely. However, very few studies have been accompanied on SOD level in the normal human lung for protecting lung health. To combat the oxidants damage against oxidative stress and improved SOD enzyme in lung tissues would be the best approach towards lung health [6].

Oxidative species can react with cellular membrane phospholipids generating Lipoperoxides Radicals ($LPO^{\cdot}$) and other toxic aldehydes (malondialdehyde, MDA) that can modify the lung membrane permeability and microcirculation [7]. Lipid peroxidation and inflammation is produced by the MDA, which reacts with nucleobases to form multiple adducts with mutagenic potential. Hence, in the present study, A549 (lung adenocarcinoma) cells was used to evaluate the effect of the Biofield Energy Treated DMEM as a test item on lung health using SOD and oxidative damage protection parameters.

Biofield Energy Healing Therapies or putative energy fields (also called Biofield) are characterized under Complementary and Alternative Medicine (CAM), which has been reported a significant impact on living organisms and non-living materials. National Centre for Complementary and Alternative Medicine (NCCAM)/ National Institute of Health (NIH) approved CAM as an alternative treatment in health care sector [8]. 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 Complementary and Alternative Medicine (CAM) therapies, Biofield Energy Treatment proofed to have a significant benefit 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 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 result in biological studies [10]. However, the National Center for Complementary and Alternative Medicine (NCCAM), well-defined Biofield Therapies 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. On the basis of Biofield Energy Treatment outcome, authors evaluated the impact of the Biofield Energy Treatment (The Trivedi Effect®) on DMEM as the test sample for lung health in A549 cells (lung adenocarcinoma).

Material and Methods

Chemicals and reagents

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. 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. 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% CO_2, and 95% humidity and sub-cultured by trypsinization 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 regimen

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, one part was treated with the Biofield Energy by a renowned Biofield Energy Healer (The Trivedi Effect®), Mahendra Kumar Trivedi remotely for ~3 minutes and coded as the Biofield Energy Treated DMEM, and the other part did not receive any sort of treatment and denoted as the untreated DMEM. The Biofield Energy Healer was 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 administered 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.

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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 10^3 cells/well/180µL 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 CO_2 incubator at 37°C, 5% CO_2, and 95% humidity. Following incubation, the plates were taken out and 20µL of 5mg/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, BioTek, USA [35]. The percentage cytotoxicity at each tested concentrations of the test substance were calculated using the following equation (1):

$$\%\ Cytotoxicity\ =\ \left(1-X\ /\ R\right)*100 \text{ ---------- (1)}$$

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):

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

The concentrations exhibiting ≥70% cell viability was considered as non-cytotoxic.

Assessment of test item protection against oxidative damage

The A549 cells were counted using a hemocytometer and plated in 96-well plates at the density corresponding to 1 X 10^4 cells/well followed by overnight incubation in a CO_2 incubator at 37°C, 5%CO_2, 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 (150µM) 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):

$$\%Protection\ =\ \left[\left(Absorbance_{sample}-Absorbance_{t-BHP}\right)\right]*100\ /\left[Absorbance_{untreated}-Absorbance_{t-BHP}\right] \text{- (3)}$$

Assessment of intracellular superoxide dismutase (sod) enzyme activity

The A549 cells were counted using a hemocytometer and plated in 24-well plates at the density corresponding to 2 X 10^4 cells/well followed by overnight incubation in a CO_2 incubator at 37°C, 5% CO_2, 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 (150µM) 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 freeze-thaw lysis. SOD activity of the cells was assessed using cayman superoxide dismutase assay kit as per manufacturer's protocol. Further, 10µL of the standard or the sample was added to 200µL of radical detector in a designated well on the plate. Reaction was initiated using xanthine oxidase (20µL) in all the wells. After 30 minutes of incubation on shaker, absorbance was read at 450nm 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)-

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

Statistical analysis

All the values were represented as Mean ± SEM 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 $p \leq 0.05$.

Results and Discussion

Cell viability study using MTT

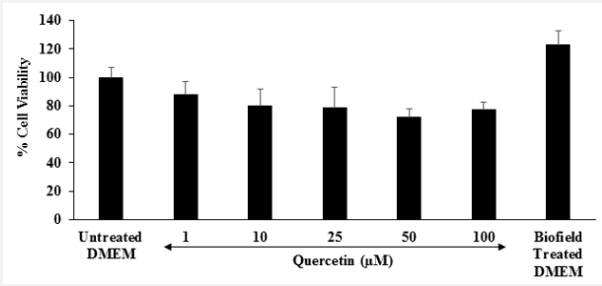

Figure 1: Cell viability using MTT assay of the quercetin and DMEM in A549 cells. Data were expressed as Mean ± SEM of three independent assay.

Cell viability data using MTT assay in A549 cells are represented in Figure 1. The data of MTT assay for cell viability are presented in term of percentage values. The MTT data showed that the test samples were found to have significant cell viability with 122.7% in the Biofield Energy Treated test sample group, while more than 72% viable cells were observed in the quercetin (positive control) group. Thus, the MTT data suggested that the Biofield Energy Treated DMEM was found to be safe in the A549 cells.

Effect of the test items on protection against oxidative damage

Protection against oxidative damage for the Biofield Energy Treated DMEM and positive control data in A549 cells are presented in Figure 2. The positive control, quercetin showed cel-

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lular protection by 20.12%, 35.97%, and 57.95% at 1, 10, and 50 µM, respectively with respect to the untreated DMEM group. The Biofield Energy Treated DMEM group showed a significantly ($p \leq 0.001$) increased the cellular protection against oxidative damage by 64.70% as compared with the untreated DMEM group. This suggests that Biofield Energy Treatment has the significant capacity to reduce the oxidative stress and can maintain a balance between the oxidants and antioxidants. Oxidative stress results in loss of cell function, which might be due to damage in lipids, DNA, carbohydrates, and proteins that lead to new reductive damaging mechanism in cellular processes [38]. The viability of the A549 cells was significantly reduced following exposure to *t*-BHP in a dose-dependent manner along with high cytotoxicity due to ROS production. ROS interact with cellular proteins, lipids or DNA, cell dysfunction, and cause apoptosis. This implicating severe oxidative damage that leads to pathogenesis of various diseases [39]. However, Biofield Energy Healing Treatment might significantly improve the protection level against oxidative damage that leads to a better lung health against many lung diseases.

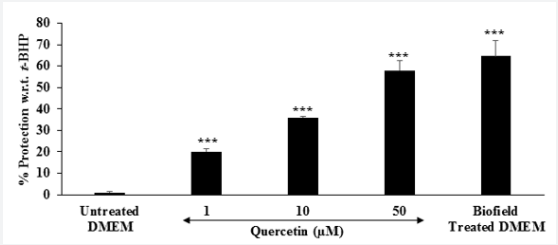

Figure 2: Protection against oxidative damage of the Biofield Energy Treated DMEM on A549 cells. Data were expressed as Mean ± SEM of three independent experiments. ***$p \leq 0.001$ compared to the untreated DMEM group.

Effect of test items on intracellular superoxide dismutase (sod) enzyme activity

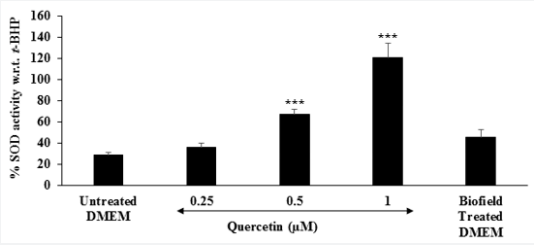

Figure 3: Superoxide dismutase (SOD) enzyme activity of the Biofield Energy Treated DMEM on A549 cells. Data were expressed as Mean ± SEM of three independent experiments. ***$p \leq 0.001$ compared to the untreated DMEM group.

The effect of the test items for antioxidant enzyme SOD activity in A549 cells is shown in Figure 3. The positive control, quercetin showed a concentration-dependent increased cellular protection by 35.76%, 66.79%, and 121.13% at 0.25, 0.5, and 1µM, respectively with respect to the untreated DMEM group. The Biofield Energy Treated DMEM group showed a significantly increased the level of SOD enzyme by 45.1% compared with the untreated DMEM group.

It is well established that the level of SOD in lung tissue indicate the lung health status. The level of SOD is downregulated during inflammation, asthmatic airways, and in parenchymal lung diseases in human. In order to replenish the level of SOD, various types of antioxidants have been used to improve the level of antioxidant enzymes and to reduce the level of free radicals [40,41]. However, these results suggest that the Biofield Energy Treatment has shown the significant protection against oxidative stress and also increased the level of SOD (antioxidant enzyme) to combat the free radicals that play a vital role in the human lung diseases.

Conclusion

The Biofield Energy Healing Treated (The Trivedi Effect®) DMEM showed a significant improvement of cellular protection against oxidative stress and increased the antioxidant enzyme capacity with respect to SOD level in human lung cells. The cell viability data using MTT assay showed 122.7% viable cells in the Biofield Energy Treated DMEM group, while more than 72% viable cells was observed in the positive control group suggested that the test samples were found as safe and nontoxic. Further, the antioxidative protection level against oxidative stress (induced by *t*-BHP) was significantly increased by 64.70% in the Biofield Energy Treated DMEM group compared with the untreated DMEM group. Moreover, the SOD level was significantly increased by 45.1% in the Biofield Energy Treated DMEM group compared with the untreated DMEM group. Thus, these data suggest that the Consciousness Energy Treated (The Trivedi Effect®) DMEM was found to have a significant impact on oxidative protection and antioxidant activity (SOD level) in human lung adenocarcinoma cells (A549), which results in improved lung health against human lung diseases. It can also be useful for the management of various human lung disorders such as Asbestosis, Asthma, Bronchiectasis, Bronchitis, Chronic Cough, Chronic Obstructive Pulmonary Disease (COPD), Common Cold, Cystic Fibrosis, Hantavirus, Idiopathic Pulmonary Fibrosis, Influenza, Lung Cancer, Pandemic Flu, Pertussis, 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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