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Antioxidative Potential of Consciousness Energy Healing Treatment on HepG2 Cells and DMEM after Oxidative Stress Induced by Hydrogen Peroxide

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Abstract

Antioxidants can reduce oxidative stress in cells is used for the treatment of several disorders such as cancer, cardiovascular, and inflammatory diseases. The present study was evaluated the antioxidant potential of the Consciousness Energy Healing (The Trivedi Effect®) Treated human hepatoma cell line (HepG2) and Dulbecco's Modified Eagle Medium (DMEM) for the assessment of cell viability under hydrogen peroxide-induced oxidative stress. The Biofield Energy Treated HepG2 cells group was maintained for 23 days under standard conditions. On the next day, the cells were challenged with 1 mM of H_2O_2 for the generation of oxidative stress. The ability of the Biofield Energy Healing Treatment to protect from the oxidative stress was determined by MTT cell viability assay and compared with the negative control group. The percentage of cell viability was significantly ($p \leq 0.001$) increased by 13.6% in the Biofield Energy Treated DMEM group; while altered by 3.2% in the Biofield Energy Treated HepG2 cells group compared to the negative control group. Overall, the Biofield Energy Treated DMEM showed a better antioxidative protection against oxidative stress than HepG2 cells group, which was induced by H_2O_2. Therefore, the results envisaged that The Trivedi Effect®-Biofield Energy Healing Treatment has an impact on the protection of various vital organs from oxidative stress; which might be helpful in the development of powerful/energized growth medium for the accelerated study with a cost-effective manner.

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Introduction

Oxidative stress, is defined as the imbalance between the generation of reactive oxygen species (ROS) and antioxidant defense system. It has been well known that ischemia (an inadequate blood supply to an organ) initiates the noxious generation of ROS, however the re-oxygenation process is responsible for the production of ROS, activation of complement system, and inflammatory response [1]. Antioxidant enzymes, such as catalase (CAT) and superoxide dismutase (SOD) that detoxify H_2O_2 and superoxide, are highly potent and specific agents to the ROS-induced injury [2]. Oxidative stress also causes the pathogenesis of various acute or chronic neurodegenerative processes [3]. Among several free radicals such as superoxide anions and hydroxyl radicals; hydrogen peroxide is one of the main ROS that causes cytotoxicity. It has been suggested that overproduction of superoxide anions is involved in N-methyl-D-aspartate (NMDA)-induced neurotoxicity [4]. The more production of hydrogen peroxide leads to aggregation of amyloid precursor proteins (APP) [5], oxidation of dopamine (DA), and ischemia/reperfusion in the brain [6]. Further, cellular conversion of hydrogen peroxide into hydroxyl radicals damages the cellular macromolecules such as lipids, proteins, and DNA [7]. At lower concentrations, ROS and reactive nitrogen species (RNS) are important signaling molecules. ROS, including H_2O_2, also participate in pathway signaling related to cellular proliferation, migration, and apoptosis. At higher concentrations, ROS and RNS participate in the alteration of cellular phenotype from a basal state resulting in increased inflammatory signaling and more ROS and RNS formation [8, 9]. Antioxidants play an important role in inhibiting and scavenging the free radicals. Recent concern emphasized that the detrimental side-effects of synthetic additives or antioxidants. For example, the most commonly used synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are toxic and damages to DNA [10]. Besides, most of the allopathic drugs also having more or less side-effects to the several organs [11]. In current global scenario, Energy Therapy like Biofield Energy Healing has been widely used and recommended as an alternative method that has an impact on various properties of living organisms in a cost-effective manner [12]. The Trivedi Effect® - Biofield Energy Healing has been known to improve the potential beneficial effects in a broad spectrum field around the Globe. Thus, a healing practitioner has the ability to harness the energy from environment/Universe and can transmit into any object (living organism or non -living material) around the globe. The object(s) always receive the energy and respond it into a useful way that is called Biofield Energy. The Trivedi Effect® has improved the overall productivity of crops in agriculture and livestock [13-16], positive impact on cancer [17, 18], and altered characteristics features of microbes in the field of microbiology [19-21]. It also altered the structural, physical, and thermal properties of several metals and ceramics [22-24], cause chromosomal changes in microbes [25, 26], and improved various nutraceutical compounds in the areas of nutraceuticals [27, 28] and biotechnology [29-31]. Oxidative stress caused by ROS that damages the cellular DNA, proteins, and lipids and is widely recognized as one of the causes for the development of chronic diseases such as cancer, diabetes, neurodegenerative, and cardiovascular diseases. Since, the liver is the main detoxifying organ of the human body, human hepatocyte (HepG2 cell line and human hepatoma) was used as the test system in the present study to assess the protective effect of Biofield Energy Treatment (The Trivedi effect®) against hydrogen peroxide-induced oxidative damage.

Materials and Methods

Chemicals and Reagents

Antibiotics solution (penicillin-streptomycin) was procured from HiMedia, India. DMEM and FBS were procured from GIBCO, USA. 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium) (MTT), quercetin, trypsin, and ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma Chemical Co. St. Louis, MO, USA. H_2O_2 and DMSO were obtained from Fisher Scientific, India. All the other chemicals used in this experiment were analytical grade procured from India.

Cell Culture and Maintenance

Human hepatoma cell line (HepG2) was procured from National Centre for Cell Science (NCCS), Pune, India, used as a test system in the present study. HepG2 cell line was maintained under DMEM medium supplemented with 10% fetal bovine serum (FBS) for

routine culture. Growth conditions were maintained at 37°C, 5% CO_2, and 95% humidity and sub-cultured by tapping the flask and splitting the cell suspension into fresh flasks and supplementing with fresh cell growth medium.

Biofield Energy Treatment Modalities

The DMEM growth medium and HepG2 cells were subjected to the Biofield Energy Treatment (The Trivedi Effect®) by Mahendra Kumar Trivedi under standard laboratory conditions for ~3 minutes from a distance of ~25 cm. The energy transmission was done without touching the medium and HepG2 cells. Following the Biofield Energy Treatment, the DMEM and HepG2 cells were used for this experiment.

Experimental Design

The experiment was performed with four different groups. Group (G) 1 contained untreated HepG2 cells and untreated DMEM supplemented with 1% FBS and served as a baseline control. G2 served as negative control includes untreated HepG2 cells, untreated DMEM supplemented with 1% FBS, and 1 mM H_2O_2. G3 assigned as positive control consisted of untreated HepG2 cells, untreated DMEM supplemented with 1% FBS, H_2O_2 (1 mM), and quercetin at two different concentrations *viz.* 10 µM and 50 µM. G4 defined as the Biofield Energy Treated DMEM along with the untreated HepG2 cells supplemented with 1% FBS and 1 mM H_2O_2. Moreover, G5 referred as the Biofield Energy Treated HepG2 cells in addition to the untreated DMEM supplemented with 1% FBS and H_2O_2 (1 mM).

Assessment of Cell Proliferation

Human hepatoma cell line (HepG2) was used in this experiment for the assessment of cell proliferation. The DMEM and HepG2 cells were trypsinized, counted, and plated in wells of flat bottom 96-well plates at the density corresponding to 10 X 10^3 cells/well/180 µL of growth medium. Following respective treatments, the cells in the 96-well plates were incubated for 16 hours in a CO_2 incubator at 37°C, 5% CO_2, and 95% humidity. After 16 hours of incubation, the plates were taken out and 20 µL of 5 mg/mL of MTT 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide solution was 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 then read at 540 nm using Synergy HT microplate reader. Concentrations were determined and the experiment was done in triplicates. The percentage cell viability was calculated using formula (1):

% Cell viability = (X/R)*100.....(1)

Where, X = Absorbance of cells corresponding to positive control and test group

R = Absorbance of cells corresponding to baseline control

Statistical Analysis

The data were expressed as the mean ± standard deviation (SD) of three independent experiments. The analysis was performed with SigmaPlot Statistical Software (Version 11.0). Differences between means were assessed for the statistical differences using Student's *t*-test (between two groups) and for multiple comparison one-way analysis of variance (ANOVA) and *post-hoc* analysis was done by Dunnett's test. $p \leq 0.05$ was considered as statistically significant.

Results and Discussion

Assessment of Protective Effect of the Biofield Energy Treated DMEM against H_2O_2 Induced Loss of Cell Viability

The effect of the Biofield Energy Treated DMEM on cell viability (as absorbance) after challenged with hydrogen peroxide in HepG2 cells is represented in Table 1. The cell viability was evaluated with the help of MTT assay. The effect of the Biofield Energy Treatment on the percent cell viability in HepG2 cells and DMEM after challenged with H_2O_2 is shown in Figure 1. The percent cell viability in the baseline control group was defined as 100%. After challenged with H_2O_2 the percent cell viability was significantly ($p \leq 0.001$) decreased by 81.3% in the negative control group due to generation of oxidative stress compared to the baseline control group. The reference item, quercetin showed a significant ($p \leq 0.001$) increment of cell viability by 14.2% and 52.7% at the concentration of 10 and 50 µM, respectively compared to the negative control group. Zhang *et al.* 2011, demonstrated that the antioxidant

Table 1. Effect of the Biofield Energy Healing Treatment on cell viability of H_2O_2 challenged HepG2 cells and DMEM after 16 hours of incubation.

Treatment	**Description**	**Absorbance at 540 nm (Mean ± SD)**
G1: Baseline control (equivalent to no stress)	Untreated cells + DMEM + 1% FBS	0.53 ± 0.06
G2: Negative control (equivalent to stress)	Untreated cells + DMEM + 1% FBS + 1 mM H_2O_2	0.10 ± 0.01
G3 (Positive control)	Untreated cells + DMEM + 1% FBS + 1 mM H_2O_2 + Quercetin (10 µM)	0.17 ± 0.03
	Untreated cells + DMEM + 1 % FBS + 1 mM H_2O_2 + Quercetin (50 µM)	0.38 ± 0.04
G4 (Biofield Energy Treated DMEM)	Biofield Energy Treated DMEM + Untreated Cells + 1% FBS + 1 mM H_2O_2	0.17 ± 0.02
G5 (Biofield Energy Treated HepG2)	Biofield Energy Treated cells + Untreated DMEM + 1% FBS + 1 mM H_2O_2	0.08 ± 0.01

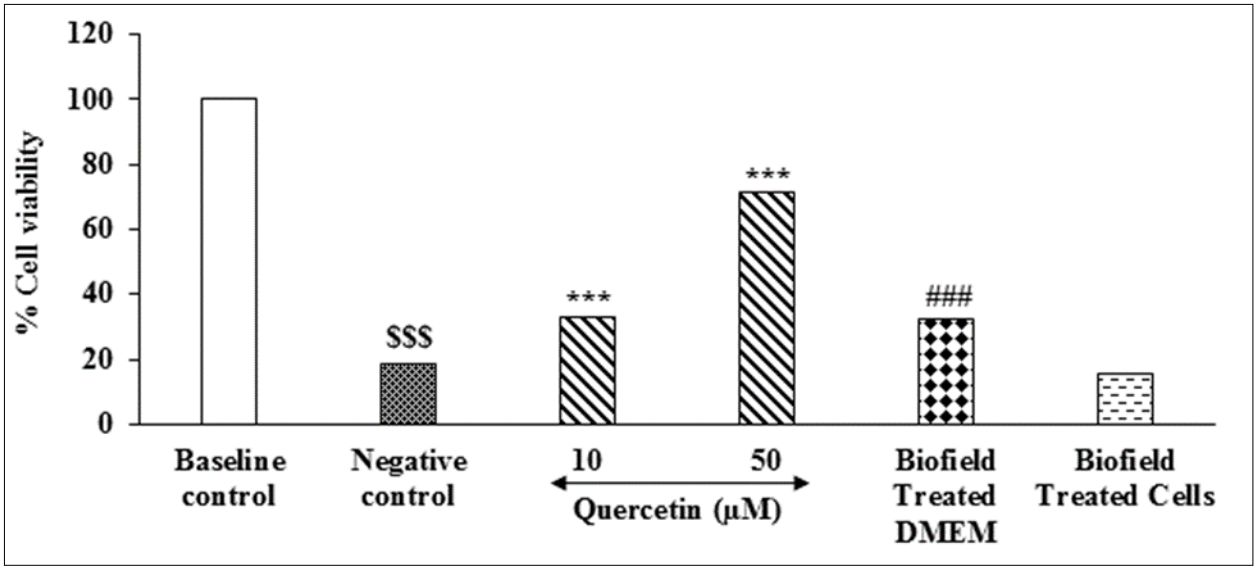

Figure 1. The effects of the Biofield Energy Treatment on HepG2 cells and DMEM for the assessment of cell viability after challenged with H_2O_2. All the groups were challenged with H_2O_2 except baseline control. $^{***}p \leq 0.001$ vs negative control and $^{\$\$\$}p \leq 0.001$ vs baseline control group (using one-way ANOVA). $^{\#\#\#}p \leq 0.001$ vs negative control group (using Student's *t*-test).

properties of quercetin, a plant-derived aglycone has been used as a nutritional supplement and may be beneficial against a variety of diseases, including cancer [32]. Besides, the percent cell viability was significantly ($p \leq 0.001$) increased by 13.6% in the Biofield Energy Treated DMEM group; while decreased by 3.2% in the Biofield Energy Treated HepG2 cells group compared to the negative control group.

Overall, the Consciousness Energy Treated DMEM possess better protection against oxidative stress, which was induced by H_2O_2. On the other hand, the Biofield Energy Treated HepG2 cells altered the protection against oxidative stress as compared to the negative control group. Hence, the study results demonstrated that The Trivedi Effect® - Consciousness Energy Healing Treatment on DMEM showed a significant antioxidant activity rather than Biofield Energy Treated HepG2 cells. Bioenergy Therapy is considered as an important supplement to conventional cancer treatment. A lot of evidence exists on the benefits of Bioenergy Therapies for stress management and boosting the immune system. From literature, it has been stated that the Biofield Energy Treatment was free from side-effects and it helps cancer patients both physically and emotionally [33]. Numerous literatures reported the beneficial effects of Bioenergy Therapies on stress management, reducing fatigue and anxiety, and increasing the quality of life and immune system among cancer patients [34-36]. In this experiment, authors found a significant impact of Consciousness Energy Healing Treatment (The Trivedi Effect®) against oxidative stress. It is assumed that The Trivedi Effect® - Consciousness Energy Healing could be use as potential antioxidant activity to protect various vital organs disorders.

Conclusions

Overall, the percent cell viability was significantly ($p \leq 0.001$) increased by 13.6% in the Biofield Energy Treated DMEM group; while altered by 3.2% in the Biofield Energy Treated HepG2 cells group compared to the negative control group. Based on the study outcomes, it is assumed that the Biofield Energy Treatment (The Trivedi Effect®) could be beneficial as a suitable antioxidant and thus simultaneously could protect various vital organs from oxidative stress such as *viz.* Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), lou gehrig's disease, atherosclerosis, cancer, charcot–marie–tooth disease (CMT), chronic obstructive pulmonary disease (COPD), chronic traumatic encephalopathy, creutzfeldt–jakob disease, *etc.* It is also presumed that in comparison to synthetic additives, antioxidants or even allopathic drugs the antioxidant/organ protection activity of The Trivedi Effect® - Consciousness Energy Healing Treatment would be better to some extent for the development of powerful/energized and cost-effective growth medium in a near future.

Abbreviations:

DMEM: Dulbecco's Modified Eagle's Medium

FBS: Fetal bovine serum

ROS: Reactive oxygen species

MTT:3-(4,5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide

SOD: Super oxide dismutase

RNS: Reactive nitrogen species

BHA: Butylated hydroxyanisole

BHT: Butylated hydroxytoluene

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