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Assessment of Biofield Energy Healing-Based Novel Proprietary Test Formulation Comprised of Minerals, Vitamins, Panax ginseng Extract, CBD Isolates, and β-carotene on Senescence of Fibroblasts (WI-38)

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ABSTRACT

The study was planned to evaluate the effect of the Trivedi Effect®- Biofield Energy Treated Test formulation (TI) composed of minerals (magnesium, zinc, copper, calcium, selenium, and iron), vitamins (ascorbic acid, pyridoxine HCl, alpha tocopherol, cyanocobalamin, and cholecalciferol), *Panax ginseng* extract, CBD isolates, and β-carotene on senescence of human lung fibroblasts (WI-38) cell line in EMEM medium. Growth rate (amount of doubling in one unit of time) was determined, while senescence was induced by H_2O_2 damage and then cells were allowed to recover for 48 h. The test formulation constituents of the test formulation were divided into two parts; one section was defined as the untreated test formulation (UT), while the other portion of test formulation received Biofield Energy Healing/Blessing Treatment (BT) by a renowned Biofield Energy Healer, Mr. Mahendra Kumar Trivedi. The test items were treated with Biofield Energy Healing Treatment and divided as Biofield Energy Treated (BT) and untreated (UT) test items. MTT data showed that the test formulation in various tested concentrations was found as safe and nontoxic with viability range 70% to 125%. The decreased reduction in doubling time was calculated on the basis of untreated test group of EMEM and test formulation. The UT-EMEM + BT-TI test group reported with decreased doubling time by 67% and 67.5% at 0.001 and 0.1 µg/mL, respectively, while 134.3%, 24.4%, and 50.5% decreased doubling time was found in the BT-EMEM + UT-TI group at 0.001, 0.01, and 0.1 µg/mL respectively, as compared with untreated test group. In addition, BT-EMEM + BT-TI test group showed a significant reduction in doubling time value by 191.6%, 33.2%, and 74.6% at 0.001, 0.01, and 0.1 µg/mL respectively, as compared with the UT-EMEM + UT-TI group. Overall, the results showed the significant decreased doubling time in WI-38 cells. Thus, the mechanical properties of cells such as cellular function, including shape, motility, differentiation, division, and adhesion to its surrounding extracellular matrix were improved. Overall, it can be useful in many disease progressions with significant anti-aging activity and its associated complications/symptoms.

Keywords: Biofield Treatment, Anti-aging, The Trivedi Effect®, Cellular senescence, WI-38

INTRODUCTION

The cellular senescence refers to the irreversible growth arrest which occurs when dividing cells encounter stress [1]. Stress in cells may be induced by an intracellular process associated with shortening and uncapping of telomeres or environmental factors and oxidative stress [2]. Replicative senescence is known as a process that can limit the population doubling of cells by reducing their proliferation rate [3]. The population doubling time is the period of time required for a cell to double in size or value [4]. Oxidative damage induces Stress Induced Premature Senescence (SIPS) in cells, slowing down the growth rate and increasing the doubling time. Antioxidants are capable of attenuating ROS-induced damage associated with oxidative stress by scavenging free radicals, including hydroxyl radicals and superoxide anions. This restores the population doubling time of near senescent cells and provides protection against SIPS [5]. Carnosine at a concentration range of 25 mM to 50

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mM retards the senescence in Human Diploid Fibroblasts [6]. It is also reported that ascorbic acid delays cellular senescence of normal human fibroblast cells [7]. Using $CuSO_4$-SIPS in WI-38 fibroblasts, resveratrol is shown to attenuate typical senescence alterations on cell morphology, senescence-associated beta-galactosidase activity, and cell proliferation [8]. Human diploid fibroblasts (WI-38) are a well-established experimental model for cellular aging-related studies [8]. This study was conducted to investigate whether the anti-aging effect of a novel test formulation could protect WI-38 human fibroblast cells against SIPS, by reversing the increase in doubling time upon H_2O_2 damage. Reduction of H_2O_2-induced increase in doubling time of WI-38 cell line has been employed as key end point to evaluate the anti-senescence properties of the test formulation. A novel test formulation was designed with the combination of vital minerals (Ca, Zn, Mg, Se, Fe, Cu), vitamins (B_{12}, E, D_3, C, B_6), and some biological active plant-based extracts such as β-carotene, ginseng, and cannabidiol isolate (CBD). Minerals and vitamins, which are used in the test formulation have significant functional role to provide vital physiological role [9-11]. Besides, cannabidiol itself has wide range of biological action [12,13], while ginseng extract is regarded as the one of the best immune boosters for overall immunity [14]. Vitamins are immunity builder and works through various pathways to boost energy level. Vitamin D improved strength, skin elasticity [15,16], improve arterial stiffness and neuronal plasticity [17]. Cannabidiol and *Panax ginseng* are also known to increase skin elasticity [18,19]. Hence, in the present study, effect of Biofield Energy Treatment/Blessing on media (EMEM) and a novel test formulation on senescence was determined in WI-38 cell line by calculation of doubling time.

The novel test formulation and cell line media (EMEM) was treated with Biofield Energy Healing/Blessing Treatment by a renowned Biofield Energy Healer to study senescence. Biofield Energy, as a Complementary and Alternative Medicine (CAM) one of the emerging CAM treatments that aimed in building a scientific network with respect to the complex homodynamic regulation of living systems. Biofield Energy therapy is supposed to be highly effective with respect to the physical, mental, and emotional human wellness [20] that improve the endogenous energy flows. CAM therapies have been accepted by the National Centre of Complementary and Integrative Health (NCCIH) along with the Biofield Energy Healing, such as deep breathing, Tai Chi, yoga, therapeutic touch, Reiki, chiropractic/osteopathic manipulation, relaxation techniques, pranic healing, meditation, homeopathy, Ayurvedic medicine, movement therapy, mindfulness, traditional Chinese herbs and medicines in biological systems, etc. [21]. However, impact of the Trivedi Effect®-Consciousness Energy Healing Treatment have significant clinical, preclinical, and scientific studies in different scientific disciplines such in materials science [22,23], agriculture science [24], antiaging [25], gut health [26], nutraceuticals [27], pharmaceuticals [28], and overall human health and wellness. In this study, the authors sought to study the impact of the Biofield Energy Healing/Blessing Treatment on the given novel test formulation for senescence in WI-38 cell line using doubling time.

MATERIALS AND METHODS

Chemicals and Reagents

Pyridoxine hydrochloride (vitamin B_6), calcitriol, zinc chloride, magnesium (II) gluconate, and β-carotene (retinol, provit A) were purchased from TCI, Japan. Copper chloride, cyanocobalamin (vitamin B_{12}), calcium chloride, vitamin E (alpha-tocopherol), cholecalciferol (vitamin D_3), iron (II) sulphate, and sodium carboxymethyl cellulose (Na-CMC) were procured from Sigma-Aldrich, USA. Ascorbic acid (vitamin C) and sodium selenate were obtained from Alfa Aesar, India. Cannabidiol isolate and panax ginseng extract were obtained from Panacea Phytoextracts, India and Standard Hemp Company, USA, respectively. Resveratrol was purchased from Across Organics, and EMEM was purchased from Lonza, USA. WI-38 (Human Lung Fibroblasts) cell line was procured from ATCC, USA. However, cell line medium such as DMSO, FBS, EDTA, and MTT were procured from Genexlife, Protaq Biomedical, Genexlife, and Parshuram and Parshuram traders, respectively.

Cell Culture

The WI-38 cell line was used as test system in the present study. The cell line was maintained in EMEM growth medium for routine culture supplemented with 10% FBS. Growth conditions were maintained at 37°C, 5%CO_2 and sub cultured by trypsinization followed by splitting the cell suspension into fresh flasks and supplementing with fresh cell growth medium. Three days before the start of the experiment (i.e., day -3), the growth medium of near-confluent cells was replaced with fresh phenol-free medium, supplemented with 10% charcoal dextran stripped FBS (CD-FBS) and 1% penicillin-streptomycin [29].

Experimental Design

The experimental groups consisted of cells in baseline control, vehicle control groups (0.05% DMSO with Biofield Energy Treated/Blessed and untreated EMEM), positive control group (resveratrol) and four different experimental test groups. The experimental groups included the combination of the Biofield Energy Treated and untreated test formulation/Medium (EMEM). It consisted of four major treatment groups on specified cells with Untreated-EMEM (UT-EMEM) + Untreated-Test item (UT-TI), UT-EMEM + Biofield Energy Treated test item (BT-TI), BT-EMEM + UT-TI, and BT- EMEM + BT-TI.

Consciousness Energy Healing Strategies

Consciousness Energy Healing (Blessing) Treatment was performed in the novel test formulation, which was consisted of zinc chloride, iron (II) sulfate, copper chloride, vitamin B_6, vitamin B_{12}, vitamin D_3, sodium selenate, calcium chloride, ascorbic acid, vitamin E, beta carotene, *Panax ginseng* extract, cannabidiol isolate, and magnesium (II) gluconate. Each ingredient of the test formulation was divided into two parts, one part of the test compound was not received any sort of treatment and were defined as the untreated or control sample. The second part of the test formulation was treated with the Trivedi Effect® - Energy of Consciousness Healing (Blessing) Treatment (Biofield Energy Treatment) by a renowned Biofield Energy Healer, Mr. Mahendra Kumar Trivedi under laboratory conditions for ~3 min in the research laboratory, Dabur Research Foundation, New Delhi, India without touching the samples. After that, the Biofield Energy Treated (Blessed) samples was kept in the similar sealed condition and used as per the study plan. In the same manner, the control test formulation group was subjected to "sham" healer for ~3 min, under the same laboratory conditions. The "sham" healer did not have any knowledge about the Biofield Energy Treatment. The Biofield Energy Treated/Blessed test medium was also taken back to experimental room for further culture methods.

Determination of Non-cytotoxic Concentration

The single cell suspension of WI38 cells were prepared in EMEM with 10% FBS. The cells were counted on a hemocytometer, while the cells were seeded with specific cell density (8000 cells/well in EMEM + 10% FBS in 96-well plates). The cells were incubated in a CO_2 incubator for 24 h. After 24 h, medium was removed, and following treatments were given in medium along with the 10% FBS in various experimental groups. After incubation for 24 h, the effect of the test formulation on cell viability was assessed by 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. 20 µL of 5 mg/mL of MTT was added to all the wells and incubated at 37°C for 3 h. The cells were centrifuged to obtain the pellet. The supernatant was removed and 150 µL of DMSO was added to all wells to dissolve formazan crystals. Further, all the wells were reported using optical density (OD) values at 540 nm using Synergy HT microplate reader. The effect of the test formulation on viability of cells was determined using equation 1.

$$\%Cell\ viablity = 100 - \%Cytotoxicity \quad (1)$$

Where; % Cytotoxicity = {(O.D. of Control cells – O.D. of cells treated with test formulation)/ OD of Control cells} *100

Estimation of Cellular Senescence

The single cell suspension of WI-38 cells was prepared in EMEM and 10% FBS using a hemocytometer. The initial density of cells was recorded (N (0)-0.2 million cells/well). The cells were incubated in a CO_2 incubator for 24 h and 95% humidity. The cells were centrifuged to obtain the pellet. Supernatants were removed and cells were resuspended in medium EMEM with 10% FBS. After respective treatments, the cells were incubated in a CO_2 incubator at 37°C, 5%CO_2, and 95% humidity for 24 h. After 24 h, the cells were treated with 300 µM H_2O_2 in serum-free medium for 30 min. After 30 min, the medium was replaced with EMEM containing 2% FBS and allowed to recover for 48 h. After 48 h, the cells were trypsinized and cell count was determined using trypan blue method. The cell number was harvested was recorded as N(t) for each sample. The population doubling time of each sample was calculated, which was based on the population doubling of treated cells *vs*. untreated, the beneficial effect of the test formulation against H_2O_2-induced cell damage was determined. The calculation of cell doubling time was calculated as follows using growth rate (amount of doubling in one unit of time):

Growth rate: ln{N(t)/N (0)}/t

N(t) are the number of cells at time of harvesting, N (0) ate the number of cells at time 0 at time of seeding, t defined as the time (in hours), and doubling time was calculated as ln (2)/growth rate.

Statistical Analysis

The data were represented as mean ± standard error of mean (SEM) and subjected to statistical analysis using Sigma-Plot statistical software (Version 11.0). For multiple comparison One-way analysis of variance (ANOVA) followed by post-hoc analysis by Dunnett's test and for between two groups comparison Student's *t*-test was performed. The $p \leq 0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

MTT Assay- Non-cytotoxic Effect of the Test Formulation

The cytotoxic effect of the test formulation was evaluated on WI-38 cells using MTT assay. The results were compared with respect to defined positive control, resveratrol. The cells were treated with the test formulation for 24 h. The effect on viability of cells was determined after 24 h of treatment by MTT assay **(Figure 1)**. The cells were treated with the test formulation and in various experimental test groups. Resveratrol (PC) demonstrated 120.2%, 103.5%, and 84.4% cell viability at 0.1, 1, and 20 µM concentration, respectively. In addition, the test formulation resulted in more than 73.11% cell viability in the concentration range of 0.001 µg/mL to 0.1 µg/mL. The results of percentage cell viability range in all the tested cell lines showed the cell viability range of 70% to 125% in different test formulation groups. Overall, the MTT data suggested that the test formulation along with EMEM groups were found safe at all the tested concentrations range up to maximum 0.1 µg/mL in the tested cell line.

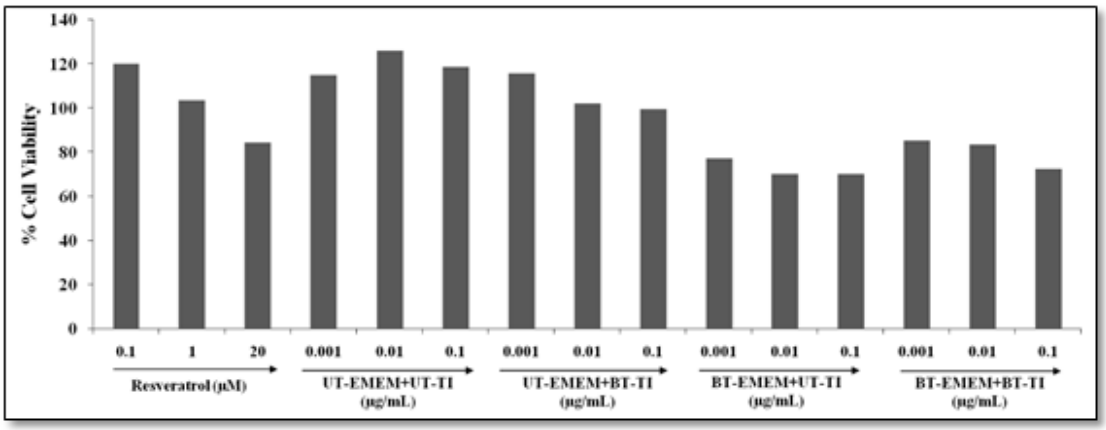

Figure 1. Effect of the test item on WI-38 cell line for cell viability using the MTT assays. UT: Untreated; BT: Biofield Energy Treated/Blessed; TI: Test Item.

Assessment of Cellular Senescence Assay in WI-38 Cells

Cellular senescence assay in WI-38 cells was determined using percentage change in cell viability and growth rate (amount of doubling in one unit of time). Senescence was induced by H_2O_2 damage and then cells were allowed to recover for 48 h. The protective effect of the test formulation on Stress Induced Premature Senescence (SIPS) was evaluated by calculating the doubling time using cell count. The cells were co-treated with the test formulation and stimulated with 0.001 µg/mL to 0.1 µg/mL for 24 h. The percentage reduction in doubling time was calculated in all the groups, which were compared and presented graphically (**Figure 2**). The decreased reduction in doubling time was calculated on the basis of the untreated test group of EMEM and test formulation. The doubling time of untreated WI-38 cells was 72.5 h, while in 300 µM H_2O_2 treatment, the growth rate of dividing WI-38 cells was reduced and doubling time was increased to 247.2 h. However, upon treatment with the BT-medium, the doubling time of cells was restored to 124 h as compared to the control (H_2O_2 alone). Resveratrol, positive control group data showed a significant reduction in doubling time by 82.8%, 77.5%, and 52.8% at 0.1, 1, and 20 µM concentrations, respectively as compared with the control cells. However, reduction in doubling time in all the treated experimental groups was significantly decreased as compared with the WI-38 cells in EMEM control group. Furthermore, the data of Biofield Energy Treated/untreated test formulation/EMEM combination was compared with respect to the untreated test formulation and EMEM group. Thus, the data suggested that UT-EMEM + BT-TI group showed a decreased doubling time by 67% and 67.5% at 0.001 and 0.1 µg/mL, respectively as compared with UT-EMEM + UT-TI group. However, 134.3%, 24.4%, and 50.5% decreased doubling time was also reported in the BT-EMEM + UT-TI group at 0.001, 0.01, and 0.1 µg/mL, respectively as compared with the UT-EMEM + UT-TI group. Besides, group BT-EMEM + BT-TI group showed a significant reduction in doubling time value by 191.6%, 33.2%, and 74.6% at 0.001, 0.01, and 0.1 µg/mL respectively, as compared with the UT-EMEM + UT-TI group.

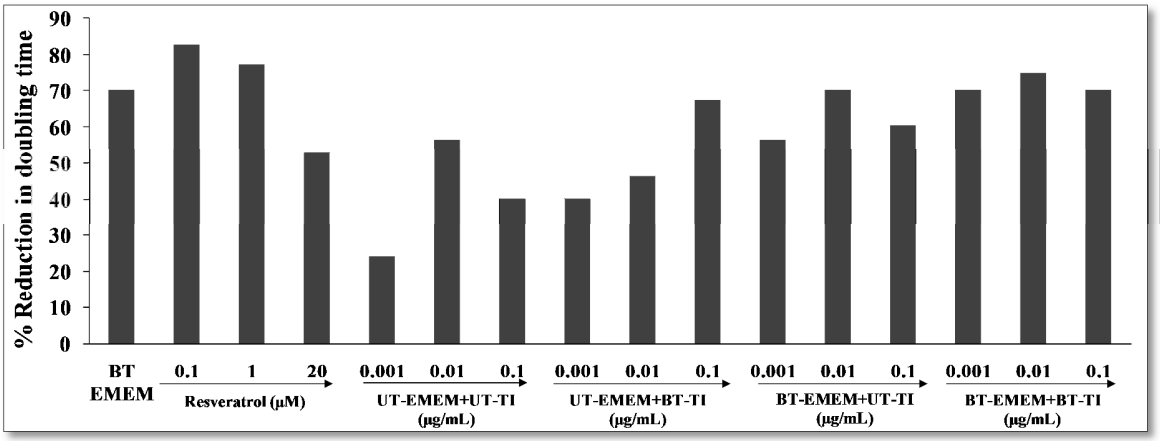

Figure 2. Effect of the test item on the level of percentage reduction in doubling time (with respect to H_2O_2 control) of WI-38 cell line after 24 h. UT: Untreated; BT: Biofield Treated/Blessed; TI: Test Item.

Overall, WI-38 cells in presence of Biofield Energy Treatment showed a significant decrease in doubling time values. The cell senescence is an inevitable process that is induced by external factors and oxidative stress [30]. Stress is the main factor which is responsible for senescence (an irreversible growth arrest). However, the senescence induced by H_2O_2 control was significantly reduced after treatment with the Biofield Energy Treated media EMEM and test formulation. Significant reduction of H_2O_2-induced increase in doubling time of WI-38 cell line has been employed as key end point, which showed a significant anti-senescence property of the test formulation.

Anti-senescence property of the cell line WI-35 was tested and the effect of the test formulation was evaluated at different concentrations. The study investigated the anti-aging effect of test formulation, which protects WI-38 human fibroblast cells against SIPS, by reversing the increase in doubling time upon H_2O_2 damage. In this research plan, the results showed the significant anti-senescence property in WI-38 cell-line, which helps in slowdown of the disease progression, disease-related all other symptoms/complications and also reduced the chances of disease susceptibility. This improved cellular differentiation, contractile functions, exonal extensions, and skin elasticity and firmness of the cell lines used in the study after treatment was very significant. Based on the overall data, it suggests that the Biofield Energy Healing Therapy was found to be most effective and benefited in order to prevent and protect from the occurrence of any type of diseases and can be used as significant way for energy boosting in various disease states that will ultimately improve the overall health and quality of life in human.

CONCLUSION

Cell viability data of the test formulation at various concentrations by MTT assay was found as 70% to 125%, which indicated that the test formulation was safe up to 0.1 µg/mL. Cellular senescence assay in WI-38 cells was calculated, which showed a significant decreased growth rate (amount of doubling in one unit of time) in the test formulation group. The doubling time of cells in absence of H_2O_2 was 72.5 h, while in presence of 300 µM H_2O_2 treatment, it was found to be significantly increased to 247.2 h. The UT-EMEM + BT-TI group showed a significant decreased doubling time by 67% and 67.5% at 0.001 and 0.1 µg/mL, respectively as compared with UT-EMEM + UT-TI test group. The BT-EMEM + UT-TI group showed 134.3%, 24.4%, and 50.5% decreased doubling time at 0.001, 0.01, and 0.1 µg/mL, respectively than untreated group. Moreover, the BT-EMEM + BT-TI group displayed a significant reduction in doubling time value by 191.6%, 33.2%, and 74.6% at 0.001, 0.01, and 0.1 µg/mL, respectively than untreated group. Overall, the Biofield Energy Treated (Blessed) test formulation showed a significant anti-aging in the tested cell line (WI-38), which play a vital role in maintaining various immune and life style-related disorders *viz.* diabetes, allergy, Alzheimer's, cardiovascular, cancer, etc. Therefore, the Consciousness Energy Healing (Blessing)-based test formulation might be suitable alternative nutritional supplement, which could be useful for the management of various immune-related disorders. Thus, in conclusion this therapy might also reduce the severity of many acute/chronic diseases and its progression rate near future.

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