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Assessment of the Biofield Energy Healing-Based Proprietary Test Formulation for Energy Boosting Biomarker-ATP in MG63 and C2C12 Cell Lines

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Abstract

The objective of the present study was to evaluate the effect of the Trivedi Effect®- Biofield Energy Treated Test formulation (TI) containing 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 ATP levels in human osteoblast (MG-63, Osteosarcoma) and mouse myoblast (C2C12) cell lines. The 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 the test formulation received Biofield Energy Healing Treatment (BT) by a renowned Biofield Energy Healer, Mr. Mahendra Kumar Trivedi. The test items were treated with Biofield Energy Healing Treatment and known as Biofield Energy Treated (BT) test items. MTT results showed that the test formulation in various concentrations was found as safe and nontoxic, with the viability range from 82% to 159%. However, the level of adenosine tri phosphate (ATP) in MG63 cells was increased by 146.9% (at 0.001 µg/mL) in the UT-DMEM+BT-TI group as compared with the UT-DMEM+UT-TI group. However, 79.6%, 111.9%, and 180.7% increased the level of ATP was reported at 0.1, 1, and 10 µg/mL, respectively in the BT-DMEM+UT-TI group, while 456.9%, 91.5%, and 123.4% increased ATP level was reported at 0.001, 0.01, and 0.1 µg/mL, respectively in the BT-DMEM+BT-TI group as compared with the UT-DMEM+UT-TI group in MG63 cells. Similarly, in the C2C12 cell lines were also reported with significant increased the ATP level by 178.7% at 1 µg/mL in the UT-DMEM+BT-TI group compared to the UT-DMEM+UT-TI group. Further, the ATP level was increased by 637.5%, 240.1%, and 26.8% at 0.001, 0.01, and 0.1 µg/mL, respectively in the BT-DMEM+UT-TI group as compared to the UT-DMEM+UT-TI group. Moreover, the ATP level was increased by 776.2%, 22.9%, 234.2%, and 24.5% at 0.001, 0.01, 0.1, and 1 µg/mL, respectively in the BT-DMEM+BT-TI group as compared with the UT-DMEM+UT-TI group. Overall, data suggested that there was significant improvement of ATP level in the Biofield Energy Treated test formulation as well as Biofield Treated DMEM medium. Therefore, the results suggest that the Biofield Energy Treated test formulation and/or the Biofield Energy Treated DMEM medium can be used as energy booster and also it can improve the mental stamina, endurance, and physical strength of the muscles to show the better performance.

Keywords: Biofield Treatment; Energy Panel; ATP; The Trivedi Effect®; MG63; C2C12

Introduction

ATP is considered as a building block of genetic material that is a ubiquitous carrier of chemical energy in all living organisms. It also serves as an extracellular signaling molecule that plays important role in synaptic transmission, vascular tone, and cell death [1,2]. It has been noticed that the cellular ATP levels might give an idea about the cell viability, along with its survival, morphology, and growth [3,4]. Some scientific studies also reported that partial ATP depletion may induce apoptosis, while, complete ATP depletion might be a reason behind necrosis. Such studies are supported by the internucleosomal DNA cleavage, alterations in cellular morphology, and changes in the plasma membrane. Besides, mitochondria is responsible for the production of majority of ATP supply of the cell, and its biogenesis further depends on the coordinated expression of genes present in the nucleus and mitochondria [5]. ATP is also considered as the universal biological energy source due to the presence of its phosphoanhydride bond that helps in providing a driving force to various intracellular biosynthetic reactions [6].

Besides, the muscle cells also need large amounts of ATP for the proper movement of body and therefore, the phosphocreatine is used by them as a ready and available energy source for ATP production. In this process, the mitochondria also help by

replenishing the phosphocreatine store in those cells after the event of muscle contraction. In that scenario, measuring the time taken in replenishment of the phosphocreatine store is considered as the measure of mitochondrial efficiency, i.e., the shorter phosphocreatine recovery times, the better mitochondrial function. On the other hand, there is a balance between the amount of energy production and its expenditure, which further determines the energy charge of the cell and the ratio of ATP to the adenylate pool. The adenylate pool helps in determining the absolute ATP concentration, which, in turn, depends on the balance between the rates of AMP synthesis and their degradation [7,8]. Although, there is a natural balance and regulation in the ATP supply in the body to maintain the constant ATP levels in cells; however, in case of engineered cell, the intracellular ATP supply would change due to an imbalance between the ATP generation and its rate of consumption. In that case, there will be a need of improvements of the ATP supply that may further helps in the production of target molecules [9]. Therefore, the scientific fraternity has been doing various researches in search of the natural molecules that might help in increasing the ATP production in need.

Thus, in order to study the changes in ATP levels in cell lines, 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 vital extracts such as β-carotene, Ginseng, cannabidiol isolate (CBD). Vitamin D is considered as a vital source for efficient muscular work, good bone health, and boosting the energy levels. It is produced in the skin using the energy from sunlight, and can also be derived, to a lesser extent, from dietary sources. Nowadays, vitamin D deficiency is known as a significant public health problem due to various diagnosed cases of poor bone health and muscle fatigue, etc. that are its common deficiency symptoms. The recent studies indicated the reason behind such fatigue as a problem in the mitochondria, which is considered as the 'power stations' within each cell of the body [10]. Similarly, ginseng has also been since ancient time to enhance the energy, wellbeing, and for the recovery of physical strength; and various studies in this regard were done in animal models of severe fatigue due to its potential for recovery of strength [11,12]. The scientific studies reported that there was enhancement of physical work capacity in larger subjects' number, if the ginseng was taken for not less than 8 weeks with sufficient dosage [13].

Biofield Energy Healing Treatment has been considered as a Complementary and Alternative Medicine (CAM) treatment approach, which is also recommended by National Center for Complementary/Alternative Medicine (NCCAM) due to its significant effects against various disorders [14-16], when used along with the conventional treatment approach [17]. There are some other CAM therapies that have been accepted by the National Centre of Complementary and Integrative Health (NCCIH) along with Biofield Energy Healing, such as deep breathing, Tai Chi, yoga, therapeutic touch, Reiki, chiropractic/osteopathic manipulation, guided imagery, pranic healing, meditation, homeopathy, hypnotherapy, relaxation techniques, Ayurvedic medicine, movement therapy, mindfulness, traditional Chinese herbs and medicines in biological systems, etc. [18,19]. The impact of the Trivedi Effect®-Consciousness Energy Healing Treatment is similarly found to be useful due to its beneficial impact on various living and non-living things. Its effect has been reported by various scientific studies in different disciplines such agriculture science [20], material science [21,22], bioavailability studies [23,24], microbiology [25,26], skin health [27,28], bone health [29,30], biotechnology [31], herbomineral products [32], cancer research [33], and overall human health and wellness. In this study, the authors sought to study the impact of the Biofield Energy Treatment (the Trivedi Effect®) on the given novel test formulation on ATP Levels in cell lines. In the present study, the possible effect of the Test formulation, which was treated with Biofield Energy Treatment (a Complementary and Alternative Medicine, CAM) by a renowned Biofield Energy Healer, on ATP synthesis was determined in human osteoblasts (MG-63) and mouse myoblast cell lines (C2C12) by *in vitro* assays.

Material 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) sulfate, 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. Imipramine Hydrochloride was purchased from Sigma, USA. MG-63 (Human Osteosarcoma like cells) and C2C12 (Mouse Myoblast Cells) cell lines were procured from NCCS, Pune.

Cell Culture

The MG-63 and C2C12 cell lines were used as test system in the present study. Both the cell lines were 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. 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 DMEM, supplemented with 10% charcoal-dextran stripped FBS (CD-FBS) and 1% penicillin-streptomycin [34].

Experimental Design

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

Consciousness Energy Healing Strategies

The novel test formulation 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 and magnesium (II) gluconate. Each ingredient of the novel test formulation was divided into two parts. 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 Treatment (Biofield Energy Treatment) by a renowned Biofield Energy Healer, Mr. Mahendra Kumar Trivedi under laboratory conditions for ~3 minutes in the research laboratory, Dabur Research Foundation, New Delhi, India. The test formulation and cell line medium were kept in the research laboratory of Dabur Research Foundation, New Delhi, India. The energy transmission was done without touching the samples. After that, the Biofield Energy Treated 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 minutes energy treatment, under the same laboratory conditions. The "sham" healer did not have any knowledge about the Biofield Energy Treatment. The Biofield Energy Treated test medium was also taken back to experimental room for further culture methods.

Determination of Non-cytotoxic Concentration

The single cell suspension of MG-63 and C2C12 cells was prepared in DMEM with 10% FBS. The cells were counted on a hemocytometer, while the cells were seeded with specific cell density (i.e., MG-63 cell line – 3000 cells/well and C2C12 cell line – 8000 cells/well) in 180 µL in DMEM with 10 % FBS in 96-well plates. The cells were incubated in a CO_2 incubator for 24 hours. After 24 hours, medium was removed, and following treatments were given in medium along with the 10% FBS in various experimental groups. After incubation for 72 hours, 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 hours. 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 Biotek Reader. The effect of the test formulation on viability of cells was determined using equation 1.

$$\% \text{ Cell viability} = 100 - \% \text{ Cytotoxicity} \quad (1)$$

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

Estimation of Energy Level (ATP Values)

The single cell suspension of MG-63 and C2C12 cells was prepared in DMEM and 10% FBS using hemocytometer. The cells were seeded density of 0.3 X 10^6 cells/well/2 mL in 6-well plates and incubated in a CO_2 incubator for 24 hours. After 24 hours, medium was removed and replaced with differentiation inducing medium. The change in cell morphology was observed upon differentiation as they become elongated. Cells were incubated for differentiation such as MG-63 cells were added with differential medium of mineralization activation cocktail/MAC [β- glycerophosphate (5 mM), ascorbic acid (0.5 mM), Dexamethasone (10 nM)] with 0% FBS for 5 days inoculation. However, C2C12 cells were cultured with DMEM and 2% horse serum for 5 days, and the medium was changed for every 48 hours. After inducing differentiation, the cells were treated with the test formulation in respective differentiating medium in various experimental groups. After treatment, the cells were incubated in a 5% CO_2 incubator for 72 hours. After 72 hours of incubation, the cell lysates were prepared by gentle harvesting using trypsinization. The cells were pelleted at 500g and the supernatant was discarded. The cell pellets were resuspended in 100 µL of lysis buffer (Lysis buffer: 200 mM Tris HCl, pH 7.5, 2 M NaCl, 20 mM EDTA, 0.2 % Triton X-100). The cell suspension turned turbid, while the cell lysates were stored at -20 °C for 15 minutes. Frozen lysates were taken out, thawed at 4 °C and then centrifuged at 13000 rpm at 4 °C for 2 minutes. Supernatants were transferred to labelled tubes. Pellets were discarded and the supernatants were stored at -20 °C till analyzed for ATP level in the cell lysates using ATP kit as per manufacturer's instructions using equation 2.

$$\% \text{ Increase in ATP} = [(B-A)/A]*100 \quad (2)$$

Where, A is the concentration of ATP in test formulation treated cells, while B is the concentration of ATP in the control (untreated) cells.

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 effects of the test item were tested on MG-63 and C2C12 cells. The MG-63 and C2C12 cells were treated with the test formulation for 72 hours. The effect on viability of cells was determined after 72 hours of treatment by MTT assay (Figure 1). All the test formulation resulted with more than 70% cell viability upto the tested concentration at 10 µg/mL. However, the calcitriol and resveratrol also demonstrated more than 70% cell viability at the tested concentrations. The percentage cell viability in all the tested cell lines showed the cell viability ranges from 82% to 159% in different test item groups with DMEM, while for calcitriol and resveratrol groups showed more than 75% and 105% cell viability, respectively (Figure 1). These data suggests that the test formulation along with DMEM groups were found safe at all the tested concentrations range up to maximum of 10 µg/mL against the tested MG-63 and C2C12 cells.

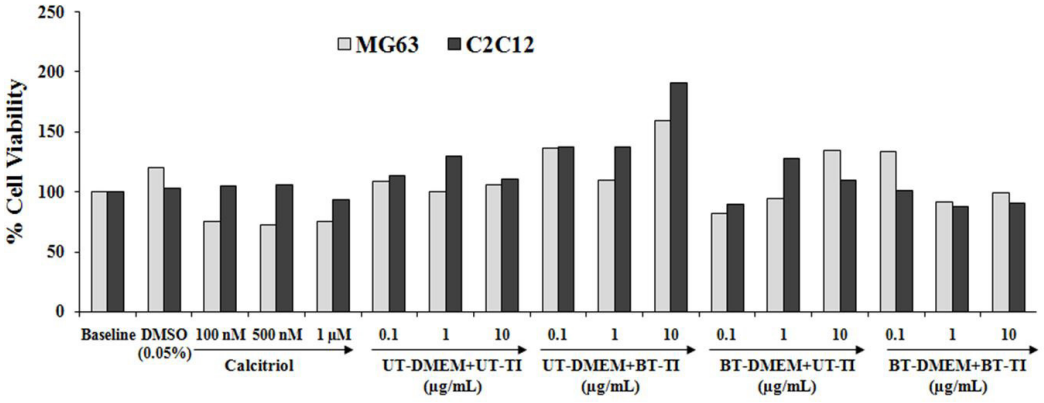

Figure 1: The effect of the test item on MG-63 and C2C12 cell lines for cell viability after 72 hours using the MTT assays. UT: Untreated; BT: Biofield Treated; TI: Test Item

Assessment of the Test Items on Adenosine Triphosphate (ATP) Activity

The ATP is a well-defined stress booster functions with respect to immune-related disorders and one of the golden energy booting biomarker [35]. ATP level was reported to be decreased in case of stressed or depressed patients [36]. ATP mediates various cellular reactions such as increased intracellular antioxidant synthesis, induction of DNA damage repair systems, cell-mediated immune responses, and cellular differentiation [37,38]. The effect of the Biofield Energy Treated test formulation and DMEM on the ATP level showed a significant increase the ATP level at various experimental concentrations on MG-63 cell line (Figure 2). The positive control, calcitriol in MG63 cells showed a significant increase by 82.4%, 108.1%, and 120.2% at 100 nM, 250 nM, and 500 nM, respectively. However, the experimental test group's *viz.* untreated medium and Biofield Treated Test item (UT-DMEM+BT-TI) showed a significant increase ATP level by 146.9% and 20.4% at 0.001 and 10 µg/mL, respectively while Biofield Treated medium and untreated Test item (BT-DMEM+UT-TI) showed a significant increase ATP level by 79.6%, 111.9%, and 180.7% at 0.1, 1, and 10 µg/mL, respectively as compared with the untreated test formulation and DMEM group. However, the Biofield Energy Treated medium and Biofield Energy Treated Test item (BT-DMEM+BT-TI) showed a significant increase ATP level by 456.9%, 91.5%, and 123.4% at 0.001, 0.01, and 0.1 µg/mL, respectively as compared with the untreated test formulation and DMEM group. Overall, all the experimental test groups showed a significant improved level of ATP at all the tested concentrations compared with the untreated test medium.

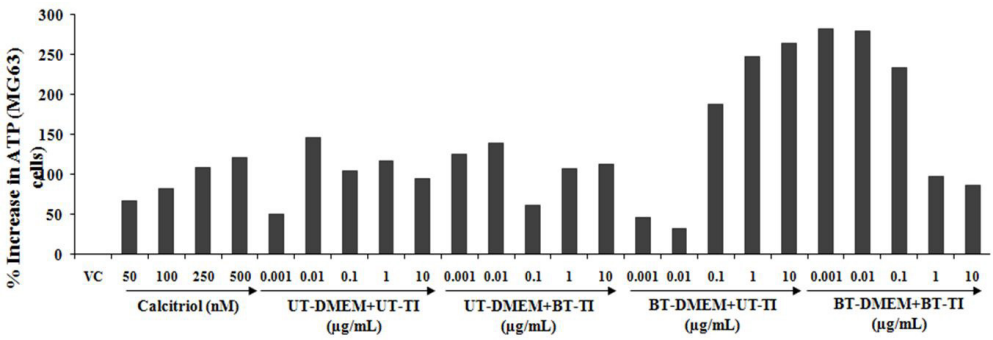

Figure 2: The effect of the test item on the level of ATP using MG-63 cell line after 72 hours. VC: Vehicle control (DMSO-0.05%), UT: Untreated; BT: Biofield Treated; TI: Test Item

Similarly, the C2C12 cells also showed a significant improved ATP level as compared with the untreated test medium. The data showed a significant increased ATP level at various experimental concentrations on C2C12 cell line (Figure 3). The positive control, resveratrol in C2C12 cells showed a significant increased by 60.1%, 53%, and 41.8% at 100 nM, 250 nM, and 500 nM, respectively. However, the experimental test group's *viz*. untreated medium and Biofield Treated Test item (UT-DMEM+BT-TI) showed a significant increased ATP level by 178.7% at 1 µg/mL, while Biofield Treated medium and untreated Test item (BT-DMEM+UT-TI) showed a significant increased ATP level by 637.5%, 240.1%, and 26.8% at 0.001, 0.01, and 0.1 µg/mL, respectively as compared with the untreated test formulation and DMEM group. However, the Biofield Energy Treated medium and Biofield Energy Treated Test item (BT-DMEM+BT-TI) showed a significant increased ATP level by 776.2%, 22.9%, 234.2%, and 24.5% at 0.001, 0.01, 0.1, and 1 µg/mL, respectively as compared with the untreated test formulation and DMEM group. Overall, all the experimental test groups showed a significant improved the level of ATP in C2C12 cells at all the tested concentrations compared with the untreated test medium.

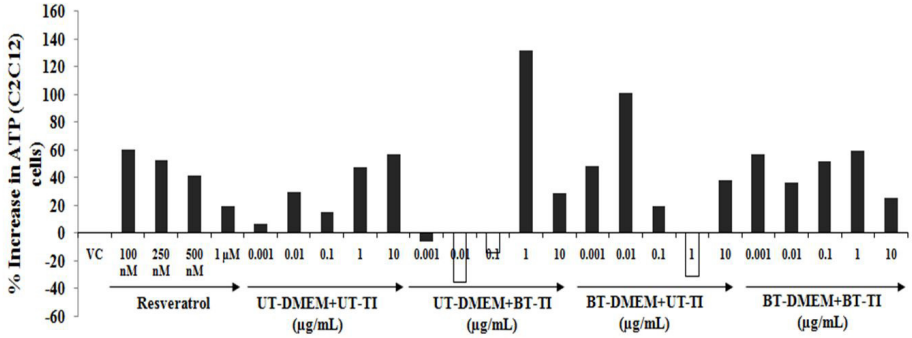

Figure 3: The effect of the test item on the level of ATP using C2C12 cell line after 72 hours. VC: Vehicle control (DMSO-0.05%), UT: Untreated; BT: Biofield Treated; TI: Test Item

Conclusions

The experimental results with respect to ATP activity using Biofield Energy Treated test formulation showed a significant effect in ATP synthesis. However, the cell viability test in both the cell lines MG63 and C2C12 using MTT assay showed a significant improved the cell viability with more than 82% among the different test groups, while Biofield Energy Treated test item also showed significantly improved the cell viability up to 159% as compared with the untreated test group. Thus, MTT data indicated that the test formulation and the medium, DMEM was found safe and nontoxic in all the tested concentrations. The MG63 cells were tested for ATP activity in cell line, and the results showed that the UT-DMEM+BT-TI group showed a significant increase ATP level by 146.9% and 20.4% at 0.001 and 10 µg/mL, respectively, while BT-DMEM+UT-TI group showed a significant increased ATP level by 79.6%, 111.9%, and 180.7% at 0.1, 1, and 10 µg/mL, respectively as compared with the untreated test formulation and DMEM group. Similarly, the BT-DMEM+BT-TI group showed a significant increase ATP level by 456.9%, 91.5%, and 123.4% at 0.001, 0.01, and 0.1 µg/mL, respectively as compared with the untreated test formulation and DMEM group in MG63 cells. The results showed that ATP level in C2C12 cells were improved by 178.7% at 1 µg/mL in the UT-DMEM+BT-TI, while 637.5%, 240.1%, and 26.8% at 0.001, 0.01, and 0.1 µg/mL, respectively in the BT-DMEM+UT-TI group as compared with the untreated test formulation and DMEM group. Besides, the ATP level was improved by 776.2%, 22.9%, 234.2%, and 24.5% at 0.001, 0.01, 0.1, and 1 µg/mL, respectively in the BT-DMEM+BT-TI group as compared with the untreated test formulation and DMEM group. Overall, the Biofield Energy Treated (the Trivedi Effect®) test formulation showed a significant impact on ATP levels in both the cell lines. Altogether, the data suggest that the Biofield Energy Treated test formulation and/ or the Biofield Energy Treated DMEM medium can be used as energy booster and also it can improve the mental stamina, endurance, and strength of the muscles to show the better performance.

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