

Assessment of the Biofield Energy Healing-Based Proprietary Test Formulation on Modulation of Immune Response in Human Monocytic Cell Line (THP-1)



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Abstract

The present study was aimed to evaluate the effect of the Trivedi Effect®- Biofield Energy Treated/Blessed Test formulation/Item (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 cytokine (IL-6 and TNF- α) levels in Human monocytic cell line (THP-1) cell line in RPMI. Each constituents of 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/Blessing Treatment (BT) by a renowned Biofield Energy Healer, Mr. Mahendra Kumar Trivedi. The test items were treated with Biofield Energy Healing Treatment/Blessing and divided as Biofield Energy Treated (BT) and untreated (UT) test items. MTT data showed that the test formulation in various concentrations was found as safe and nontoxic in the tested concentrations with viability range 77% to 134%. IL-6 level was significantly inhibited by 48.7% and 21.1% at 5 and 10 μ g/mL, respectively in the BT-RPMI+UT-TI group, while 11.8%, 20.8%, and 44% inhibition of IL-6 was reported at 0.01, 5, and 10 μ g/mL respectively, in the UT-RPMI+BT-TI group, and 422.9%, 84.2%, and 14.7% inhibition of IL-6 at 0.01, 0.1, and 10 μ g/mL, respectively in the BT-RPMI+BT-TI group as compared with the untreated test formulation and RPMI group. Similarly, TNF- α inhibition by 26.1%, 29.2%, and 155.2% in the UT-RPMI+BT-TI group at 0.01, 0.1, and 5 μ g/mL respectively, as compared with the untreated group. However, BT-RPMI+UT-TI group showed a significant inhibition of TNF- α by 50% and 14.9% at 0.01 and 10 μ g/mL, respectively, while BT-RPMI+BT-TI group showed a significant inhibition of TNF- α by 46.4%, 17.5%, 21.2%, and 228.4% at 0.01, 0.1, 1, and 10 μ g/mL, respectively as compared with the untreated test formulation and RPMI group. Overall, the *in vitro* cell line data suggested significance inhibition of pro-inflammatory cytokines (TNF- α and IL-6) at all the testes concentration of Biofield Energy Treated test formulation. Overall, the experimental results showed the significant improved immune profile by regulation of cytokines profile, which can be used against immunity disorders, mental illness, stress, lethargic conditions, alertness, migraine, minimizing toxic effects of free radicals, and many other immune related disorders.

Keywords: Biofield treatment; IL-6; TNF- α ; The Trivedi Effect®; TMP-1

Introduction

Immune system plays a vital role in regulation of health of humans and animals. Immunity and immune functions can be varied by various factors including diets known as immune modulation [1]. To study the immunity profile and to study the effect of any test compound, cell line activity using estimation of cytokines would be the meaningful approach because it minimizes the effects of culture period and genetic variation [2]. In addition, it can also provide mechanistic approach and detailed information of any test compound or plant based novel formulations [3]. *In*

vitro-ex vivo systems are the initial step forward to mimic the *in vivo* situation and to study the immunity parameters; THP-1 (human leukemia monocytic cell line) has been extensively used to evaluate the monocyte/macrophage functions, signaling pathway, mechanism, and drug transport. Cytokines are the small, secreted proteins derived from immune cells, which play a major role in the differentiation, maturation, and activation of different immune function. IL-6 and TNF- α was used as the major cytokines estimation in lipopolysaccharide (LPS) induced THP-1 cell line. Anti-inflammatory cytokines are involved in most

of the immune-related disorders and defined as the standard parameter for evaluation the effect test compound. Cytokines are divided based on the specific local microenvironment i.e., pro-inflammatory or anti-inflammatory effects, or both [4,5]. They are responsible to create the bridge between innate and adaptive immune systems [6,7]. The present study focused on the detection of pro-inflammatory cytokines as a major target in THP-1 cell line. Thus, 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). Vitamins are the major source that builds immunity using different pathways to boost energy level. Vitamin C, D are the major immunity-based vitamins along with minerals used in the test formulations. All the minerals and vitamins used in the test formulation have significant functional role to provide vital physiological role [8-10]. Besides, cannabidiol itself has wide range of pharmacological profile and was reported to role in different disorders [11,12], while ginseng extract is regarded as the one of the best immune boosters for overall immunity [13]. Thus, using this novel test formulation, present study was designed to study the effect of test formulation on the level of cytokines in presence of LPS. The test formulation was treated with Biofield Energy Treatment (a Complementary and Alternative Medicine, CAM) by a renowned Biofield Energy Healer, on THP-1 cell line. 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, relaxation techniques, pranic healing, meditation, homeopathy, 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/Blessing 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 as materials science [20,21], agriculture science [22], antiaging [23], gut health [24], nutraceuticals [25], pharmaceuticals [26], 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 the level of cytokines (IL-6 and TNF- α) levels in THP-1 cell line.

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. Human monocytic cell line (THP-1) cell line was procured from NCCS, Pune.

Cell culture

THP-1 was used as test system in the present study. The cell line was maintained in RPMI growth medium for routine culture supplemented with 10% FBS. Growth conditions were maintained as 37°C, 5%CO₂, and 95% humidity and subculture 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 RPMI, supplemented with 10% charcoal dextran stripped FBS (CD-FBS) and 1% penicillin-streptomycin [27].

Experimental design

The experimental groups consisted of cells in baseline control, vehicle control groups (0.05% DMSO with Biofield Energy Treated/Blessed and untreated RPMI-1640), positive control group (calcitriol) and five different experimental test groups. The experimental groups included the combination of the Biofield Energy Treated/Blessed and untreated test formulation/Medium (RPMI-1640). It consisted of five major treatment groups on specified cells with Untreated-RPMI + Untreated-Test item (UT-TI), UT-RPMI + Biofield Energy Treated/Blessed test item (BT-TI), BT-RPMI + UT-TI, and BT-RPMI + 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/Blessing (Biofield Energy Treatment) by a renowned Biofield Energy Healer, Mr. Mahendra Kumar Trivedi under laboratory conditions for ~3 minutes 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 does 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 THP-1 cells was prepared in RPMI with 10% FBS. The cells were counted on a hemocytometer, while the cells were seeded with specific cell density (30,000 cells/well/180µL in RPMI-1640 + 10% FBS in 96-well plates). The cells were incubated in a CO₂ 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 24 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. A 20µL of 5mg/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 150µL of DMSO was added to all wells to dissolve formazan crystals. Further, all the wells were reported using optical density (OD) values at 540nm using Biotek Reader. The effect of the test formulation on viability of cells was determined using equation 1.

$$\% \text{ Cell viability} = 100 - \% \text{ Cytotoxicity} \text{ -----}$$

$$\text{-----(1)}$$

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

Estimation of an immunomodulation activity

The single cell suspension of THP-1 cells was prepared in RPMI and 10% FBS using a hemocytometer. The cells were seeded density of 0.25 X 10⁶ cells/well/0.5mL in 48-well plates and incubated in a CO₂ incubator for 24 hours and 95% humidity. The cells were centrifuged to obtain the pellet. Supernatants were removed and cells were resuspended in medium RPMI-1640 + 0% FBS. After treatment in all the experimental test groups, the cells were incubated in a 5% CO₂ incubator for 24 hours. After 24 hours of incubation, culture supernatants were collected from each well and stored at -20°C until analysis. Further, the level of cytokines (IL-6 and TNF-α) in culture supernatants of THP-1 cells was determined using ELISA as per manufacturer’s instructions. In general, ELISA assay was performed using assay diluents, which was added to each well followed by sample addition (supernatants of cells) were directly pipetted into the wells and incubated for 2 hours at room temperature (RT). After washing away any unbound substances for a total of 5 times, 200µL of IL-6/TNF-α conjugate was added to each well and incubated for 2 hours at RT. Following a wash (5 times) to remove any unbound conjugate, 200µL of substrate solution was added to the wells and incubated for 30 minutes at RT in dark. The reaction was stopped

by adding 50µL stop solution to each well. The optical density of the sample was measured at 450nm. Further, the concentration of IL-6 & TNF-α (pg/ml) was determined using the standard curve. Inhibitory effect of the Test Items on secretion of IL-6 & TNF-α against LPS stimulated level was determined as follows:

$$\% \text{ Inhibition of IL-6 \& TNF-}\alpha = [(A-B)/A]*100$$

Where A = Concentration of cytokines (pg/mL) in the Control cells (stimulated with LPS alone) and B = Concentration of cytokines (pg/mL) in cells treated with test formulation/positive control + LPS

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* ≤ 0.05 was considered as statistically significant.

Results and Discussion

MTT assay- non-cytotoxic effect of the test formulation

The cytotoxic effects of test item were tested on TMP-1 cell. The results were compared with respect defined positive controls such as calcitriols. The TMP-1 cell was treated with the test formulation for 24 hours. The effect on viability of cells was determined after 24 hours of treatment by MTT assay (Figure 1). The THP-1 cells were treated with the test formulation and in various experimental test group for 24 hours. Calcitriol (PC) demonstrated 121.2%, 128.1%, and 110.2% cell viability in the concentration 100 nM, 500 nM, and 1 µM respectively. Following test formulation resulted in more than 70% cell viability in the concentration range of 0.1µg/mL to 50µg/mL. The results of percentage cell viability range in all the tested cell lines showed the cell viability range of 77% to 134% in different test item groups with RPMI. These data suggests that the test formulation along with RPMI groups were found safe at all the tested concentrations range up to maximum of 10µg/mL against the tested TMP-1 cells.

Assessment of immunity assay on cytokines assay

Immunomodulatory effect of the test formulation was determined in THP-1 cells stimulated with LPS. The cells were co-treated with the test formulation and stimulated with 10µg/mL of LPS for 24 hours. The levels of IL-6 and TNF-α were determined by ELISA.

Determination of IL-6 against LPS stimulation

The result of IL-6 was determined among all the experimental test groups, and the results are demonstrated in (Figure 2). The data showed that the effect of the test formulation on secretion of IL-6 against LPS stimulation was significantly inhibited by the test formulation at various concentrations. Stimulation of THP-

1 cells with inflammatory stimulus; LPS (10µg/mL) resulted in enhanced secretion of IL-6 by 210% as compared to the untreated cells. Calcitriol in the concentration range 100 nM, 500 nM, and 1 µM showed inhibition of IL-6 from 42.1%, 49.2%, and 70.5% as compared to the control cells (LPS stimulated). However, the experimental test group's viz. untreated medium and Biofield Treated Test item (UT-RPMI+BT-TI) showed a significant inhibition of IL-6 by 11.8%, 20.8%, and 44% in the 0.01, 5 and 10µg/mL respectively as compared with the vehicle control, while 14.4% inhibition at 10µg/mL as compared with the untreated test formulation group. Biofield Treated medium and untreated Test item (BT-RPMI+UT-TI) showed a significant inhibition of IL-6 level 48.7% and 21.1% at 5 and 10µg/mL, respectively as compared with the untreated test formulation and RPMI group.

However, the Biofield Energy Treated medium and Biofield Energy Treated Test item (BT-RPMI+BT-TI) showed a significant inhibition of IL-6 level by 422.9%, 84.2%, and 14.7% at 0.01, 0.1, and 10µg/mL, respectively as compared with the untreated test formulation and RPMI group. High level of IL-6 results in various level of stress, which leads to various immunological disorders. Thus, alteration and management in the level of cytokines has wide range of implicated in major depressive and immunological illness. This is also regarded as the innate inflammatory response to many physical stressors (e.g., depression, traumatic stress, infection, inflammation) [27,28]. Overall, all the experimental test groups showed a significant benefit with improved immunity by inhibition of IL-6 level at all the tested concentrations compared with the untreated test medium.

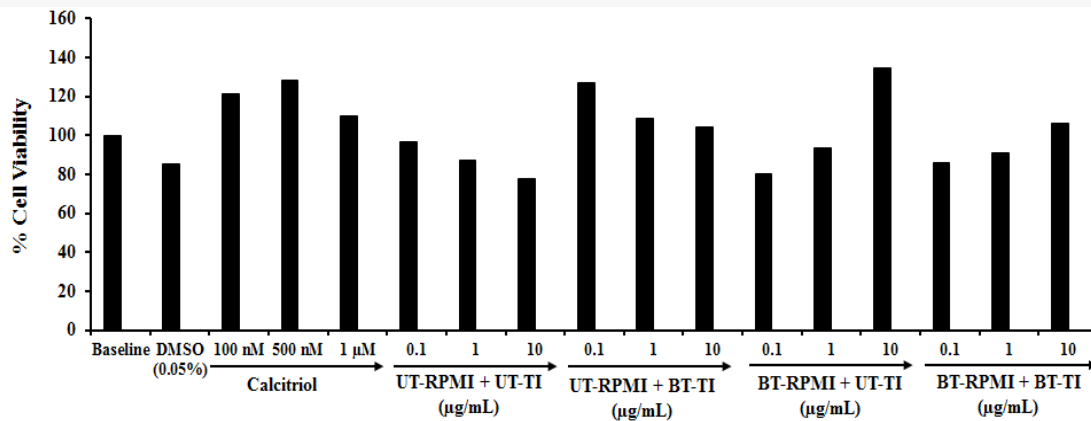


Figure 1: Effect of the test item on TMP-1 cell line for cell viability after 24 hours using the MTT assays. UT: Untreated; BT: Biofield Treated; TI: Test Item.

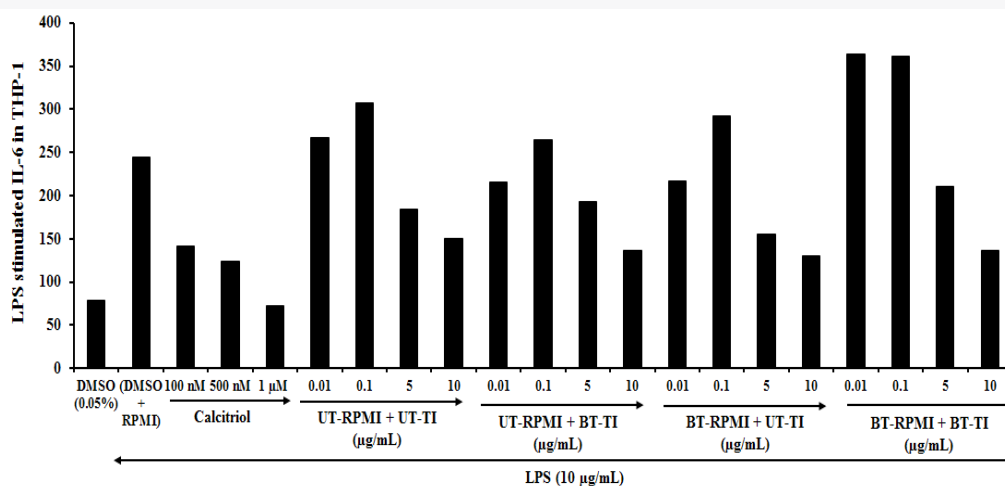


Figure 2: Effect of the test item on the level of IL-6 using TMP-1 cell line after 24 hours. Vehicle control (DMSO-0.05%), UT: Untreated; BT: Biofield Treated; TI: Test Item.

Determination of TNF- α against LPS stimulation

Similarly, TMP-1 cells also showed a significant inhibition of TNF- α as compared with the untreated test medium. The data showed a significant inhibition of TNF- α at various experimental concentrations on TMP-1 cell line (Figure 3). The positive control, calcitriol in TMP-1 cells showed a significant inhibition of TNF- α by 57.90%, 49%, and 28.40% at 100nM, 500nM, and 1 μ M, respectively. However, the experimental test group's *viz.* untreated medium and Biofield Treated Test item (UT-RPMI+BT-TI) showed a significant inhibition of TNF- α by 26.1%, 29.2%, and 155.2% at 0.01, 0.1, and 5 μ g/mL respectively as compared with the untreated group, while Biofield Treated medium and untreated Test item (BT-RPMI+UT-TI) showed a significant inhibition of TNF- α by 50% and 14.9% at 0.01 and 10 μ g/mL, respectively as compared with the untreated test formulation and RPMI group. However, the Biofield Energy Treated medium and Biofield Energy Treated Test item (BT-RPMI+BT-TI) showed a significant inhibition of TNF- α by 46.4%, 17.5%, 21.2%, and 228.4% at 0.01, 0.1, 1, and 10 μ g/mL, respectively as compared with the untreated test formulation and RPMI group. Overall, all the experimental

test groups showed a significant inhibition of TNF- α in the TMP-1 cells under LPS induced condition at all the tested concentrations compared with the untreated test medium. TNF- α , a pro-inflammatory cytokine is responsible for various inflammatory reactions in the body. These cells signaling protein are highly engaged in systemic inflammation along with its important role in acute phase reaction. TNF- α also mediates and regulates immune responses, inflammation, and immune activation, that may involve in depressive symptoms in many cases. Thus, its alteration may result in various immunological disorders [29,30]. In this research plan, the results showed the significant inhibition of IL-6 and TNF- α level in TMP-1 cell line, which helps in slowdown of the disease progression, disease-related all other symptoms/ complications and reduced the chances of disease susceptibility. Based on the overall data, it suggests that the Biofield Energy Healing Therapy was found to be most effective and benefited 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.

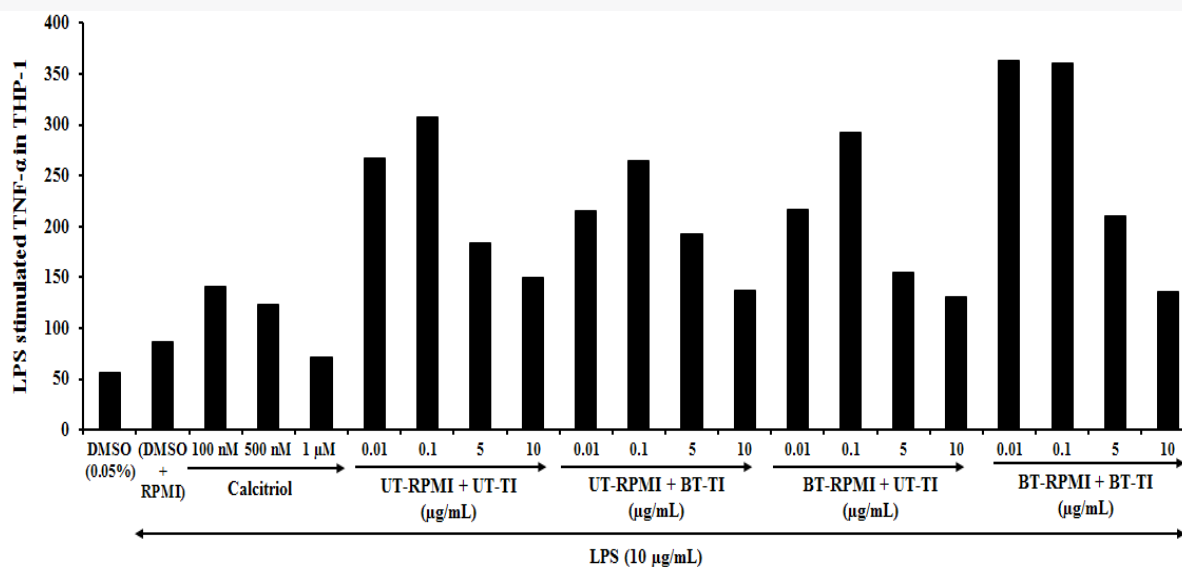


Figure 3: Effect of the test item on the level of TNF- α using TMP-1 cell line after 24 hours. Vehicle control (DMSO-0.05%), UT: Untreated; BT: Biofield Treated; TI: Test Item.

Conclusion

Immunological activity was tested for cytokines assay on TMP-1 cells. The experimental results with respect to IL-6 and TNF- α activity using Biofield Energy Treated/Blessed test formulation showed a significant inhibition as compared with the untreated test formulation. However, the cell viability MTT test of TMP-1 cell line assay showed a significant improved the cell viability with more than 77% cell viability among the different test groups, while Biofield Energy Treated/Blessed test item also showed

significantly improved the cell viability up to 134% as compared with the untreated test group. The percentage cell viability in all the tested cell lines showed the cell viability range of 77% to 134% in different test item groups with RPMI-1640. Thus, based on MTT data indicated that the test formulation and the medium, RPMI was found safe and nontoxic in all the tested concentrations. Further, the level of IL-6 was significantly inhibited by 48.7% and 21.1% at 5 and 10 μ g/mL, respectively in the BT-RPMI+UT-TI, while 11.8%, 20.8%, and 44% in the 0.01, 5 and 10 μ g/mL respectively in the UT-

RPMI+BT-TI group, and 422.9%, 84.2%, and 14.7% inhibition of IL-6 at 0.01, 0.1, and 10µg/mL, respectively in the BT-RPMI+BT-TI as compared with the untreated test formulation and RPMI group. On the hand, TNF-α inhibition was reported in the UT-RPMI+BT-TI group by 26.1%, 29.2%, and 155.2% at 0.01, 0.1, and 5µg/mL respectively, as compared with the untreated group. BT-RPMI+UT-TI group showed a significant inhibition of TNF-α by 50% and 14.9% at 0.01 and 10µg/mL, respectively, while BT-RPMI+BT-TI group showed a significant inhibition of TNF-α by 46.4%, 17.5%, 21.2%, and 228.4% at 0.01, 0.1, 1, and 10µg/mL, respectively as compared with the untreated test formulation and RPMI group. Overall, the Biofield Energy Treated/Blessed (the Trivedi Effect®) test formulation showed a significant impact on cytokine level in THP-1 cell line, which play a vital role in maintaining various immune related disorders, increase alertness, energy, attention, allergy, Alzheimer, cardiovascular, cancer, diabetes, eye, immune, inflammatory, or Parkinson. Therefore, the Consciousness Energy Healing based test formulation might be suitable alternative nutritional supplement, which could be useful for the management of various immune-related disorders that may be caused by poor nutrition, genetics, or problems with the rate of bone growth or rebuilding. Thus, in conclusion this therapy might also reduce the severity of any type of acute/chronic disease (auto-immune related and inflammatory disorders) progression rate and can be used in both before and after the manifestation of any disease symptoms in healthy, unhealthy, and ill peoples.

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