Current Trends in Pharmacology and Clinical Trials
ISSN: 2642-0848

Academic Strive
Researcher's perception is our reality

Research Article *Volume 2; Issue 1*

An Impact of the Biofield Energy Healing Treatment Based Novel Proprietary Formulation on Pro-inflammatory Cytokines for Immunomodulation in Mouse Splenocyte Cells

Trivedi MK[1] and Jana S[2]*

[1]Trivedi Global, Inc, Henderson, USA

[2]Trivedi Science Research Laboratory Pvt Ltd, India

***Corresponding author:** Dr. Snehasis Jana, Trivedi Science Research Laboratory Pvt. Ltd, Thane (W), India, Email: publication@trivedisrl.com

Received Date: April 22, 2019; **Published Date:** June 04, 2019

Abstract

Phytoconstituents, minerals, and vitamins have been widely evaluated for their significant biological properties. A new proprietary test formulation was prepared with a mixture of the nanocurcumin, zinc chloride, magnesium (II) gluconate, sodium selenate, ascorbic acid (vitamin C), cholecalciferol (vitamin D_3), iron (II) sulfate, and copper chloride. The study was aimed to evaluate the immunomodulatory potential of Biofield Energy Healing (The Trivedi Effect®) Treatment on the test formulation in splenocyte cells isolated from the Biofield Energy Treated mice. The test formulation was distributed in two different parts, one part was denoted as control and the other part was demarcated as the Biofield Energy Treated sample, which received Biofield Energy Healing Treatment by a renowned Biofield Energy Healer, Mahendra Kumar Trivedi. MTT assay data showed more than 70% cell viability in the test formulation group and was found to be safe and non-cytotoxic in splenocyte cells. Proinflammatory cytokine tumor necrosis factor-α (TNF-α) expression was significantly ($p \leq 0.01$) reduced by 25.73%, 19.05%, and 18.05% at 0.1, 0.5, and 5.2 µg/mL, respectively as compared to the vehicle control (VC) group. The level of macrophage inflammatory proteins-1α (MIP-1α) was significantly reduced by 20.32% in the Biofield Energy Treated test formulation at 3 µg/mL than VC group. Additionally, the level of interleukin-1β (IL-1β) secretion was significantly inhibited in the Biofield Energy Treated test formulation by 16.09% and 10.71% at 0.1 and 1 µg/mL, respectively with respect to the VC group. Overall, results suggest that The Trivedi Effect®-Consciousness Energy Healing Treatment has significantly improved and modulate the immunomodulatory effect of the test formulation in the Biofield Treated mouse splenocytes. Therefore, data anticipated that the Biofield Energy Treated test formulation can be utilized for the management of many inflammatory disorders *viz.* rheumatoid arthritis, inflammatory bowel diseases, psoriasis, scleroderma, multiple sclerosis, and type 1 diabetes mellitus, aging, stress, and immune-related disease conditions.

Keywords: Immunity, Biofield Energy Healing, The Trivedi Effect®, Splenocytes, Pro-inflammatory cytokines, TNF-α

Abbreviations: NCCAM: The National Center for Complementary/Alternative Medicine; LPS: Lipopolysaccharide; DMSO: Dimethyl Sulfoxide; FBS: Fetal Bovine Serum; MTT: 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide; PBS: Phosphate Buffer Saline; ELISA: Enzyme-linked Immunosorbent Assay;

Citation: Trivedi MK. An Impact of the Biofield Energy Healing Treatment Based Novel Proprietary Formulation on Pro-inflammatory Cytokines for Immunomodulation in Mouse Splenocyte Cells. Curr Trends Pharma Clinical Trials 2019, 2(1): 180019.

Copyright © 2019 Trivedi MK.

NCCAM: National Center for Complementary and Alternative Medicine; CAM: Complementary and Alternative Medicine.

Introduction

Plant based products are the herbal medicines, which modulate the immune function in human and considered as an accepted alternative therapeutic approach [1]. Minerals along with plant metabolites have been used and accepted worldwide for treating various ailment and diseases. Modulation of immune response has been recognized as an alternative and complementary approach to conventional chemotherapy against various disease states [2]. Some conditions such as in case of autoimmune disorders (like rheumatoid arthritis, inflammatory bowel diseases, multiple sclerosis, type 1 diabetes mellitus, etc.) and organ transplantation, where immunosuppressant must be included or host defense mechanism has been activated required safe immunomodulatory agents. Immunodeficiency might be due to stress and infectious diseases such as acute respiratory tract infections, diarrheal diseases, yellow fever, hepatitis A and E, tuberculosis, and HIV/AIDS [3]. Cytokines (small secreted proteins from immune cells) play a major role in differentiation, maturation, and activation of different immune function. Irregular discharge and malfunctioning of various cytokines are involved in most of the immune-related disorders, while they are considered as the standard parameter for evaluation the effect of any compound in *in vitro* and *in vivo* models. Cytokines are classified depending upon the specific local microenvironment as the pro-inflammatory or anti-inflammatory effects, or both [4,5]. Moreover, cytokines are considered as the critical mediators that constitute a rather complex immune response network, which creates the bridge between innate and adaptive immune systems [6,7]. Authors focused on pro-inflammatory cytokines as a major target and based on the literature, authors formulated a new proprietary formulation including nanocurcumin, zinc chloride, magnesium (II) gluconate hydrate, sodium selenate, ascorbic acid (vitamin C), cholecalciferol (vitamin D_3), iron (II) sulfate, and copper chloride.

All the ingredients selected for the present study in the test formulation reported to have significant biological activities such as immune modulating properties, antioxidant, anti-inflammatory, anti-infective, and anti-viral [8-10]. The novel formulation was treated with Biofield Energy Healing Treatment. Biofield Energy Healing Treatment as CAM approach was reported with significant therapeutic outcome. However, various scientific reports and data suggest the use of Biofield Energy against many disorders, like cervical cancer [11,12]. The use of CAM therapies has been recommended by The National Center for Complementary/Alternative Medicine (NCCAM). Human biofield energy has subtle form of energy has the potential to work in a positive manner [13], and its clinical benefits are reported worldwide [14]. This energy can be harness and transmit it into living and non-living things by the process of Biofield Energy Healing Treatment. Biofield Energy Treatment (The Trivedi Effect®- Consciousness Energy Healing Treatment) had been extensively studied with significant outcomes in the field of pharmaceuticals [15-17], nutraceuticals [18,19], metals and ceramics [20-22], microbiology [23-25], microbial genetics [26,27], cancer research [28,29], livestock, and agriculture science [30-32]. The current study aimed to evaluate the immunomodulatory potential of Biofield Energy Treated new proprietary test formulation, which was studied for the modulation of pro-inflammatory cytokines (tumor necrosis factor-alpha), chemokine (macrophage inflammatory protein-1α), and interleukin (interleukin-1β) in splenocyte cells isolated from Biofield Energy Healing Treated mice.

Materials and Methods

Requirement

3-(4, 5-dimethyl-2-thiazolyl) 2, 5 diphenyl-2 H-tetrazolium) (MTT), lipopolysaccharide (LPS), L-glutamine, Roswell Park Memorial Institute (RPMI-1640), streptomycin, penicillin4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 2-mercaptoethanol, and rapamycin were procured from Sigma Chemical Corp. (St. Louis, MO). Macrophage inflammatory protein-1α (MIP-1α), tumor necrosis factor alpha (TNF-α), and interleukin-1 beta (IL-1β) were purchased from R&D Systems, USA. Ascorbic acid and sodium selenate were obtained from Alfa Aesar, USA. Fetal bovine serum (FBS) was obtained from GIBCO, USA. Iron sulfate, copper chloride, and cholecalciferol (vitamin D_3) were obtained from Sigma Chemical Co. (St. Louis, MO). Magnesium gluconate and zinc chloride were purchased from TCI, Japan. Nanocurcumin was purchased from Sanat Products Ltd., India.

Test formulation and reference standard

The novel proprietary test formulation contained a combination of nanocurcumin along with minerals *viz.* copper chloride, iron sulfate, zinc chloride and vitamins *viz.* cholecalciferol (vitamin D_3) and magnesium (II) gluconate hydrate, ascorbic acid (vitamin C). Rapamycin was used as a positive control for immune response; while LPS was used as an inflammatory stimulant.

Experimental animal

The male C57BL/6 mice approximately 8 weeks old and ~25 gm body weight were obtained from Vivo Bio Tech Ltd., Hyderabad, India. The animals were acclimatized for one week before commencement of experiment. The animals were housed with specified controlled condition (temperature 22 ± 3°C, humidity 30% to 70%, and 12-hour light/12-hour dark cycle) with normal pellet diet (NPD) drinking water *ad libitum*. The animals used in this experiment were subjected to prior approval of the Institutional Animal Ethics Committee (IAEC) to carrying out the animal experiment.

Biofield energy healing approaches

One part of the test formulation did not receive any treatment and was defined as the control group, while other part received the Biofield Energy Treatment known as Biofield Energy Treated Test formulation. Besides, one group of animals also received the Biofield Energy Healing Treatment *per se* by Mr. Mahendra Kumar Trivedi for three minutes and were used to isolate the splenocyte cells (Figure 1). These isolated splenocyte cells were known as Biofield Energy Treated splenocyte cells. The Biofield Energy Treatment was administered by Mr. Mahendra Kumar Trivedi, a renowned Biofield Energy Healer (The Trivedi Effect®) under similar conditions for ~3 minutes through unique Energy Transmission process to the test formulation and animals. Further, the control group was treated with a "sham" healer, who did not have any knowledge about the Biofield Energy Treatment. After treatment, the Biofield Energy Treated and untreated samples were kept in similar sealed conditions for cytokines estimation.

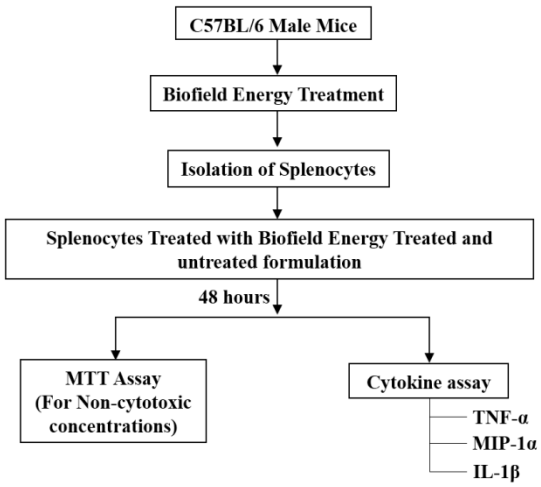

Figure 1*:* Schematic experimental design of Biofield Energy Healing Treatment.

Experimental design

Splenocytes isolated from Biofield Energy Treated animals were divided into various experimental groups *viz.* vehicle control, positive control, untreated test formulation, and Biofield Energy Treated test formulation at various concentrations. All the groups were treated with lipopolysaccharides (LPS) at the rate of 50 ng/mL as inflammatory stimulant. After 48 hours of incubation, supernatants were analyzed for the secreted levels of TNF-α, MIP-1α, and IL-1β using ELISA as per the manufacturer's instructions. Concentrations were determined in triplicate wells of each sample.

Isolation of splenocyte cells

The Biofield Energy Treated male mice (C57BL/6) were sacrificed and the spleens were aseptically removed, grounded by passing through a sterile plastic strainer under aseptic conditions. The cells were centrifuged twice at 1000 *g* for 5 minutes, erythrocytes were lysed by a lysis buffer (0.15 M NH_4Cl, 0.01 M $NaHCO_3$, and 0.1 mM EDTA, pH 7.4) and then the cell pellets were washed twice with the RPMI-1640 medium. Further, the cells were resuspended in the complete RPMI-1640 medium (RPMI 1640 medium plus 10% fetal bovine serum, 2 mM glutamine, 100 IU/mL of penicillin and streptomycin, 15 mM HEPES and 50 mM 2-mercaptoethanol). The cell counts were performed using a hemocytometer and cell viability was determined using the trypan-blue dye exclusion technique with ≥95% viable cells. The cells were cultured in 96-well tissue culture plates with 0.2 X 10^6 cells per well. They were incubated at 37°C in a humidified atmosphere of 5% CO_2 for the indicated period [33].

Cell culture and test formulation treatment

Splenocyte (0.2 X 10^6 cells per well) cells were isolated from Biofield Energy Treated animals and grown in 96-well culture plates using a RPMI-1640 medium supplemented with 10% FBS, 100 units/mL of penicillin, and 100 µg/mL of streptomycin. LPS (50 ng/mL) induced splenocyte cell cultures were grown in a humidified CO_2 incubator (5% CO_2) for 48 hours at 37°C. The effect of cytotoxicity of the test formulation was tested by treating cells with different concentrations of the test formulation in RPMI-1640 medium.

Cytotoxicity by MTT assay

The effect of the Biofield Energy Treated and untreated test formulations at the concentration range of 0.01 to 10.4 µg/mL were tested for cell viability using 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. The details procedure was followed as per Trivedi et al. 2016 [34]. The cell viability was determined

using equation 1 and results showed more than 75% cell viability were selected s for cytokine estimation.

% Cell viability = 100 − % cytotoxicity---------- (1)

Where; % cytotoxicity = [(O.D. of control cells – O.D. of cells treated with the test formulation)/O.D. of control cells]*100.

Estimation of TNF-α, IL-1β, and MIP-1α using ELISA assay

The enzyme-linked immunosorbent assay (ELISA) was used for the estimation of TNF-α, MIP-1α, and IL-1β. The ELISA plates were coated with an antibody in a coating buffer at the recommended concentration and kept overnight at 4ºC. After washing with PBS-T (PBS with 0.05% Tween 20), the plates were blocked with assay diluent for at least 2 hours at room temperature. A total of 100 µL cell culture supernatant from different experimental samples and standards were incubated overnight at 4ºC and, after three washes, biotinylated anti-mice cytokine (TNF-α, MIP-1α, and IL-1β) antibodies at the recommended concentrations were incubated for 1 hour at room temperature and the plate was incubated for 45 minutes at room temperature with gentle shaking. The plates were again washed 3 times and then 100 µL of horseradish per-oxidase (HRP)–streptavidin conjugate solution was added and the plate was incubated for 45 minutes at room temperature with gentle shaking. Further, the plate wells were washed 3 times as previous and 100 µL of 3,3,5,5'-tetramethylbenzidine (TMB) one-step substrate reagent was added, followed by a 30-minute incubation at room temperature in the dark. In addition, 50 µL of 0.2 mol/L sulphuric acid was added to each well to stop the reaction and the plates were read for absorbance at 450 nm using a BioTek Reader (SIAFRT/Synergy HT multimode reader). The standards were run in parallel to the samples, and the concentrations were determined in triplicate for each sample [35].

Statistical analysis

Data were expressed as mean of three replicates ± SEM and were subjected to one-way analysis of variance (ANOVA) for multiple groups followed by Dunnett's test and Student's *t*-test for two groups comparison. Statistical significance was considered at $p \leq 0.05$.

Results and Discussion

MTT Assay on splenocyte cells

Splenocyte cells isolated from the Biofield Treated mice were used for assessment of cell viability using MTT assay. The cell viability results are summarized in Figure 2. The Biofield Energy Healing Treated test formulation significantly improved the cell viability at the tested concentrations. The cell viability of the positive control (rapamycin) group showed percentage cell viability with more than 168% in the presence of LPS. The test formulation were tested at various concentrations ranges from 0.01 to 25 µg/mL, in which upto 10 µg/mL were found safe and nontoxic. These concentrations ranges were selected for the estimation of cytokines on splenocyte cells. However, all the tested concentrations of test formulation have shown more than 70% cell viability.

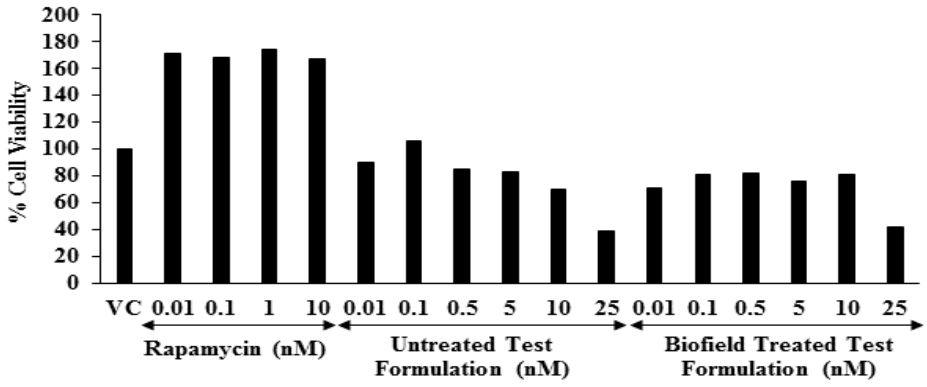

Figure 2: Effect of the test formulation on cell viability by MTT assay under the stimulation of lipopolysaccharide (LPS) in splenocyte cells measured at 540 nm.

MTT assay is extensively used for the cell toxicity against any test formulations. In addition, this assay was found as more rapid, less costly, less time consuming, and non-radioactive method as compared with the other assays. This assay display cell proliferation results on the basis of the cell growth and metabolic activity [36]. MTT assay

suggest that the concentrations of the test formulation were found safe up to 10 µg/mL with respect to the viability of the isolated splenocyte cells. However, the cell viability percentage was significantly increased after Biofield Energy Treatment on the test formulation. This cell viability assay defines the metabolic activity by evaluating the activity of succinate dehydrogenase, mitochondrial enzyme.

Effect of the test formulation on cytokines expression in biofield treated mouse splenocyte cells

The effect of novel test formulation was evaluated for pro-inflammatory cytokines and chemokines levels in the splenocyte cells isolated from Biofield Energy Treated animals. The pro-inflammatory cytokines and chemokines have important role in inflammation, immune modulation, and lymphocyte activation. Therefore, the safe concentrations were examined for the expression of pro-inflammatory cytokines such as TNF-α, MIP-1α and IL-1β in splenocyte cells isolated from the Biofield Treated animals. The effect of test formulation on pro-inflammatory cytokines was estimated after 48 hours of incubation with the test formulation using ELISA assay.

Estimation of TNF-α expression

The level of TNF-α expression in splenocyte cells is shown in Figure 3. The level of TNF-α was observed 553.9% in the vehicle control (VC) group. The untreated test formulation showed 15.04%, 16.22%, and 18.7% decrease the level of TNF-α at the concentration of 0.1, 0.5, and 5.2 µg/mL, respectively as compared to the VC group. Besides, TNF-α level was significantly ($p \leq 0.01$) reduced by 25.73%, 19.05% and 18.05% in the Biofield Energy Treated test formulation at 0.1, 0.5, and 5.2 µg/mL, respectively as compared to the VC group. Overall, the Biofield Energy Treated test formulation showed immunomodulatory activity by significantly reducing the concentration of TNF-α as compared with the vehicle control group. Therefore, the Biofield Treated test formulation has the potential to reduce the level of proinflammatory cytokine, TNF-α, which plays a major role in immune disorders and also defined as controlling factor for many diseases [37]. Thus, overall findings suggest that the Biofield Energy Treated test formulation can be used in many inflammatory disorders.

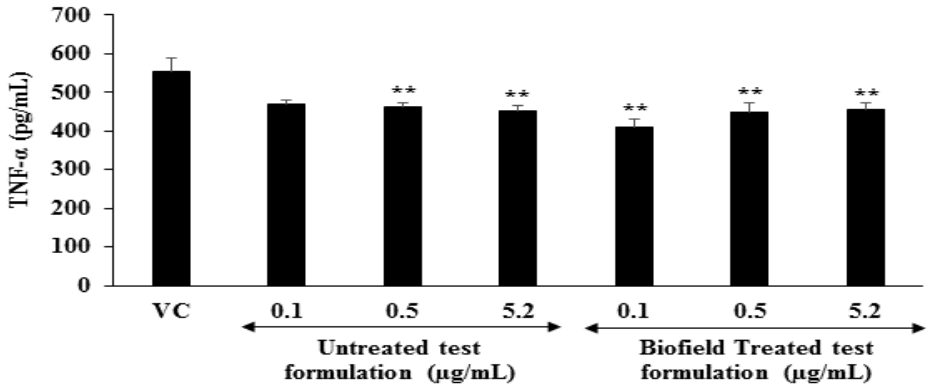

Figure 3: Effect of the test formulation on the level of TNF-α in splenocyte cells was measured after 48 hours of treatment under the influence of LPS (50 ng/mL). Data are assigned as pg/mL as mean ± SEM. **$p \leq 0.01$ *vs*. VC.

Estimation of MIP-1α expression

The experimental data suggested that the expression of MIP-1α in splenocyte cells isolated from the Biofield Energy Treated animals was changed significantly after exposure of the test formulation is shown in the Figure 4. The level of MIP-1α was found as 1534.7 ± 30.7 pg/mL in the vehicle control (VC) group. However, the positive control, rapamycin significantly ($p \leq 0.001$) reduced the level of MIP-1α by 36.8%, 42.26%, and 42.45% at the concentrations of 0.01, 0.1, and 1 nM, respectively compared to the vehicle control group. The untreated test formulation was reduced by 25.49% (at 1 µg/mL), while the Biofield Energy Treated test formulation was significantly reduced by 20.32% (at 3 µg/mL) the level of MIP-1α as compared to the VC group. As a result, the Biofield Energy Healing Treated test formulation enhanced the immune response property than the untreated test formulation group.

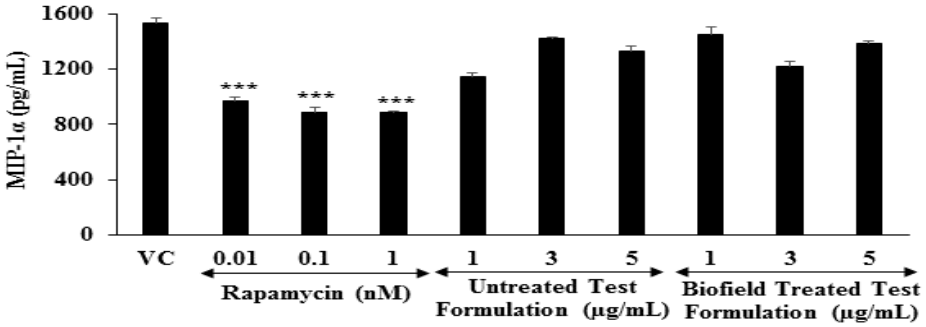

Figure 4: Effect of the test formulation on the level of MIP-1α under the stimulation of lipopolysaccharide (LPS) measured after 48-hours of treatment. Data are shown as pg/mL as mean ± SEM. $^{***}p \leq 0.001$ *vs.* VC.

Overall, the experimental data suggested that Biofield Energy Healing Treatment has the significant capacity to improve immune response with respect to vehicle control and untreated test formulation groups. Several scientific data suggested that MIP-1α would be beneficial in minimizing the inflammatory responses in several diseases [38].

Measurement of IL-1β

The level of IL-1β in the presence of novel proprietary test formulation is presented in Figure 5. The vehicle control group showed values of IL-1β as 40.90 ± 3.40 pg/mL. The level of IL-1β was significantly decreased by 16.09% and 10.71% at 0.1 and 1 µg/mL, respectively in the Biofield Treated test formulation group with respect to the vehicle control group. This suggests that Biofield Energy Treated test formulation has significant immunomodulatory activity. Scientific reports suggest that various immunological and inflammatory functions of IL-1β plays a significant role in controlling the immune response during infections and the result of specific modulation of transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (*NF-κB*) [39,40].

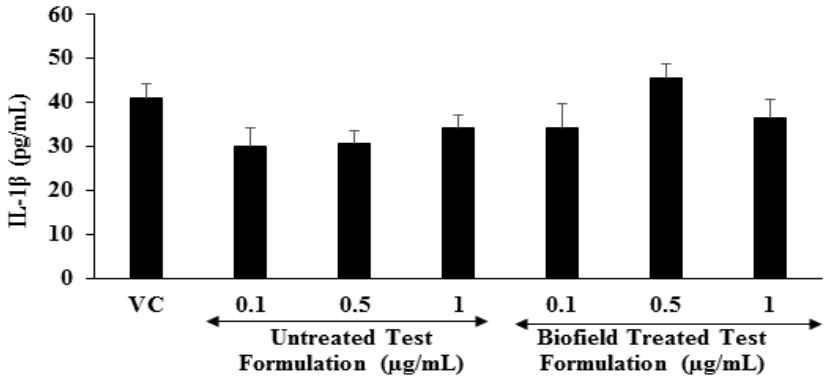

Figure 5: Effect of the test formulation on the level of IL-1β under the stimulation of lipopolysaccharide (LPS) measured after 48-hours of treatment. Data are shown as pg/mL as mean ± SEM.

Worldwide scope of alternative medicine and its outcomes have been increased significantly. However, an important phytoconstituents along with minerals and vitamins are reported to have beneficial role against many diseases such as diabetes, indigestion, inflammation of intestine, osteomalacia, blood disorders, infertility, potent revitalizer, etc. [41]. Due to high safety profile with the wide therapeutic action of alternative medicines, the scope has been increased worldwide [42]. Besides, the individual constituents of test formulation has been reported to have substantial immunomodulatory action, and the Biofield Energy Healing Treatment significantly reduced the expression of cytokines. Overall, the Biofield Energy Healing Treatment on the test formulation can be a novel approach in supports of the use of Biofield

Treated test formulation for various types of autoimmune disorders on Biofield Treated mice splenocyte cells.

Conclusions

MTT assay data suggest that the Biofield Energy Treated test formulation showed more than 70% cell viability and considered as safe and non-toxic. The level of TNF-α was significantly decreased by 25.73%, 19.05%, and 18.05% in Biofield Energy Treated test formulation at concentration 0.1, 0.5, and 5.2 µg/mL, respectively, with respect to the vehicle control group. In case of MIP-1α, the Biofield Energy Treated test formulation showed significant suppression by 20.32% at 3 µg/mL as compared with the untreated test formulation. The level of IL-1β secretion in Biofield Energy Treated test formulation group was decreased by 16.09% at 0.1 µg/mL as compared with the vehicle control group. On the basis of current study findings, it is concluded that the novel test formulation showed significant immunomodulatory action on the tested cytokines (TNF-α, MIP-1α, and IL-1β) in splenocyte cells isolated from the Biofield Energy Treated mice after administration of the Biofield Energy Treated formulation. On the basis of experimental results of various tested cytokines and their expression, significant immunosuppressive activity was reported in the new proprietary formulation after treated with The Trivedi Effect®- Biofield Energy Healing by a renowned Biofield Energy Healer, Mr. Mahendra Kumar Trivedi. Biofield Energy Treated test formulation can be used as a Complementary and Alternative Medicine (CAM) to prevent the immune-mediated diseases such as Irritable Bowel Syndrome, Rheumatoid arthritis, Ulcerative colitis and Crohn's disease, Stress, Asthma, and many more with safe therapeutic index. It can also be used for organ transplants, autoimmune disorders like Diabetes, Addison Disease, Multiple Sclerosis, Myasthenia Gravis, Pernicious Anemia, Aplastic Anemia, Systemic Lupus Erythematosus, Alopecia Areata, Vitiligo, Psoriasis, and Vasculitis, etc.

Acknowledgements

Authors are grateful to Dabur Research Foundation, Trivedi Global, Inc., Trivedi Science, Trivedi Testimonials and Trivedi Master Wellness for their support throughout the work.

Conflict of Interest

No conflict of interest was declared by the authors.

References

1. Duke JA (1985) CRC Handbook of Medicinal Herbs. CRC Press Inter Inc 272-276.
2. Singh N, Tailang M, Mehta SC (2016) A Review on Herbal Plants as Immunomodulators. Int J Pharm Sci Res 7: 3602-3610.
3. Cooper MD, Schroeder HW (2005) Primary immune deficiency diseases. In: Harrison TR, editor. Harrison's Principles of Internal Medicine. Mc Graw-Hill 1939-1941.
4. Zhang JM, An J (2007) Cytokines, Inflammation and Pain. Int Anesthesiol Clin 45(2): 27-37.
5. Coppack SW (2001) Pro-inflammatory cytokines and adipose tissue. Proc Nutr Soc 60(3): 349-356.
6. Su DL, Lu ZM, Shen MN, Li X, Sun LY (2012) Roles of pro- and anti-inflammatory cytokines in the pathogenesis of SLE. J Biomed Biotech 2012: 347.
7. Velasco-Ramirez SF, Rosales-Rivera LY, Ramirez-Anguiano AC, Bitzer-Quintero OK (2013) Cytokines and the nervous system: The relationship between seizures and epilepsy. Rev Neurol 57: 171-177.
8. Wintergerst ES, Maggini S, Hornig DH (2007) Contribution of selected vitamins and trace elements to immune function. Ann Nutr Metab 51: 301-323.
9. Sankar P, Telang AG, Suresh S, Kesavan M, Kannan K, et al. (2013) Immunomodulatory effects of nanocurcumin in arsenic-exposed rats. Int Immunopharmacol 17(1): 65-70.
10. Mora JR, Iwata M, von Andrian UH (2008) Vitamin effects on the immune system: Vitamins A and D take centre stage. Nat Rev Immunol 8: 685-698.
11. Peck SD (1998) The efficacy of therapeutic touch for improving functional ability in elders with degenerative arthritis. Nurs Sci Q 11(3): 123-132.
12. Turner JG, Clark AJ, Gauthier DK, Williams M (1998) The effect of therapeutic touch on pain and anxiety in burn patients. J Adv Nurs 28(1): 10-20.
13. Barnes PM, Bloom B, Nahin RL (2008) Complementary and alternative medicine use among adults and children: United States, 2007. Natl Health Stat Report 12: 1-23.
14. Rubik B (2002) The biofield hypothesis: Its biophysical basis and role in medicine. J Altern Complement Med 8(6): 703-717.
15. Trivedi MK, Patil S, Shettigar H, Bairwa K, Jana S et al. (2015) Spectroscopic characterization of

chloramphenicol and tetracycline: An impact of biofield Treatment. Pharm Anal Acta 6: 7.

16. Trivedi MK, Patil S, Shettigar H, Bairwa K, Jana S (2015) Spectroscopic characterization of biofield treated metronidazole and tinidazole. Med Chem 5: 340-344.

17. Trivedi MK, Branton A, Trivedi D, Shettigar H, Bairwa K, et al. (2015) Fourier transform infrared and ultraviolet-visible spectroscopic characterization of biofield treated salicylic acid and sparfloxacin. Nat Prod Chem Res 3: 186.

18. Trivedi MK, Branton A, Trivedi D, Nayak G, Plikerd WD, et al. (2017) A Systematic study of the biofield energy healing treatment on physicochemical, thermal, structural, and behavioral properties of magnesium gluconate. International Journal of Bioorganic Chemistry 2: 135-145.

19. Trivedi MK, Branton A, Trivedi D, Nayak G, Plikerd WD, et al. (2017) Chromatographic and spectroscopic characterization of the consciousness energy healing treated *Withania somnifera* (ashwagandha) root extract. European Journal of Biophysics 5: 38-47.

20. Trivedi MK, Tallapragada RM, Branton A, Trivedi D, Nayak G, et al. (2015) Characterization of physical and structural properties of aluminum carbide powder: Impact of biofield treatment. J Aeronaut Aerospace Eng 4: 142.

21. Trivedi MK, Nayak G, Patil S, Tallapragada RM, Latiyal O et al. (2015) Impact of biofield treatment on atomic and structural characteristics of barium titanate powder. Ind Eng Manage 4: 166.

22. Trivedi MK, Patil S, Nayak G, Jana S, Latiyal O (2015) Influence of biofield treatment on physical, structural and spectral properties of boron nitride. J Material Sci Eng 4: 181.

23. Trivedi MK, Patil S, Shettigar H, Bairwa K, Jana S (2015) Phenotypic and biotypic characterization of *Klebsiella oxytoca*: An impact of biofield treatment. J Microb Biochem Technol 7: 202-205.

24. Trivedi MK, Branton A, Trivedi D, Gangwar M, Jana S (2015) Antimicrobial susceptibility, biochemical characterization and molecular typing of biofield treated *Klebsiella pneumoniae*. J Health Med Inform 6: 206.

25. Trivedi MK, Branton A, Trivedi D, Nayak G, Gangwar M, et al. (2015) Antibiogram, biochemical reactions, and genotypic pattern of biofield treated *Pseudomonas aeruginosa*. J Trop Dis 4: 181.

26. Trivedi MK, Branton A, Trivedi D, Nayak G, Mondal SC, et al. (2015) Evaluation of antibiogram, genotype and phylogenetic analysis of biofield treated *Nocardia otitidis*. Biol Syst Open Access 4: 143.

27. Trivedi MK, Branton A, Trivedi D, Nayak G, Charan S, et al. (2015) Phenotyping and 16S rDNA analysis after biofield treatment on *Citrobacter braakii*: A urinary pathogen. J Clin Med Genom 3: 129.

28. Trivedi MK, Patil S, Shettigar H, Mondal SC, Jana S (2015) The potential impact of biofield treatment on human brain tumor cells: A time-lapse video microscopy. J Integr Oncol 4: 141.

29. Trivedi MK, Patil S, Shettigar H, Gangwar M, Jana S (2015) *In vitro* evaluation of biofield treatment on cancer biomarkers involved in endometrial and prostate cancer cell lines. J Cancer Sci Ther 7: 253-257.

30. Trivedi MK, Branton A, Trivedi D, Nayak G, Gangwar M, et al. (2015) Effect of biofield energy treatment on chlorophyll content, pathological study, and molecular analysis of cashew plant (*Anacardium occidentale* L.). Journal of Plant Sciences 3: 372-382.

31. Trivedi MK, Branton A, Trivedi D, Nayak G, Gangwar M, et al. (2016) Molecular analysis of biofield treated eggplant and watermelon crops. Adv Crop Sci Tech 4(1): 208.

32. Trivedi MK, Branton A, Trivedi D, Nayak G, Mondal SC, et al. (2015) Effect of biofield treated energized water on the growth and health status in chicken (*Gallus gallus domesticus*). Poult Fish Wildl Sci 3(2): 140.

33. Trivedi MK, Mondal SC, Gangwar M, Jana S (2017) Effect of a novel ashwagandha-based herbomineral formulation on pro-inflammatory cytokines expression in mouse splenocyte cells: A potential immunomodulator. Pharmacogn Mag 13: S90-S94.

34. Trivedi MK, Branton A, Trivedi D, Nayak G, Afaganis AE, et al. (2016) An impact of the Trivedi Effect® - Biofield Energy Healing based herbomineral formulation on pro-inflammatory cytokines expression in mouse splenocytes. American Journal of Life Sciences 4(6): 164-174.

35. Dinarello CA (2009) Immunological and inflammatory functions of the interleukin-1 family. Annu Rev Immunol 27: 519-550.

36. Seo Y, Lee HJ, Kim Y, Youn H, Lee BJ (2005) Effects of several salt marsh plants on mouse spleen and thymus cell proliferation using MTT assay. OSJ 40: 209-212.

37. Bemelmans MH, van Tits LJ, Buurman WA (1996) Tumor necrosis factor: Function, release and clearance. Crit Rev Immunol 16(1): 1-11.

38. Hsieh CH, Frink M, Hsieh YC, Kan WH, Hsu JT, et al. (2008) The role of MIP-1 alpha in the development of systemic inflammatory response and organ injury following trauma hemorrhage. J Immunol 181: 2806-2812.

39. Dinarello CA (2009) Immunological and inflammatory functions of the interleukin-1 family. Annu Rev Immunol 27: 519-550.

40. Schultz MJ, Rijneveld AW, Florquin S, Edwards CK, Dinarello CA, et al. (2002) Role of interleukin-1 in the pulmonary immune response during *Pseudomonas aeruginosa* pneumonia. Am J Physiol Lung Cell Mol Physiol 282(2): L285-L290.

41. Joshi D (1998) Varanasi, India: Chaukambha Sanskrit Bhawan; Rasa Amritam; pp. 127-129.

42. Bodeker G, Kronenberg F (2002) A public health agenda for traditional, complementary, and alternative medicine. Am J Public Health 92: 1582-1591.