Biochemistry and Molecular Biology
2017; 2(6): 120-130
http://www.sciencepublishinggroup.com/j/bmb
doi: 10.11648/j.bmb.20170206.18
ISSN: 2575-5064 (Print); ISSN: 2575-5048 (Online)

Differential Regulation of Immune Biomarkers by the Trivedi Effect®- Energy of Consciousness Healing Treatment Based Herbomineral Formulation in Male *Sprague Dawley* Rats

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To cite this article:

Mahendra Kumar Trivedi, Alice Branton, Dahryn Trivedi, Gopal Nayak, Ariadne Esmene Afaganis, Barbara Marie Bader, Brian A. Weekes, Daphne Luisa Dumas, Denise Marie Fiedler, Dennille Mellesia Smith, Desi Pano, Donna Felice Galla, Donna Maria Alija, Elaine Barbara Mullins, Elaine M. Scorza, Ellia O'Donnell, Fabio Massimo Paciucci, Frances Goodman Warlick, Haddon Norman Salt, Inthirani Arul, Jacqueline Y. Andrews, James Jay McLeran, James Stephen Burnett, Jean Caroline White, Mayank Gangwar, Snehasis Jana. Differential Regulation of Immune Biomarkers by the Trivedi Effect®- Energy of Consciousness Healing Treatment Based Herbomineral Formulation in Male *Sprague Dawley* Rats. *Biochemistry and Molecular Biology*. Vol. 2, No. 6, 2017, pp. 120-130. doi: 10.11648/j.bmb.20170206.18

Received: October 30, 2017; **Accepted**: November 11, 2017; **Published**: December 11, 2017

Abstract: A new proprietary herbomineral formulation was formulated, consisting of essential ingredients *viz.* herbal root extract of ashwagandha and minerals (zinc, magnesium, and selenium). The present study aimed to evaluate the impact of The Trivedi Effect®- Energy of Consciousness Healing Treatment (Biofield Energy Healing Treatment) based herbomineral formulation in male *Sprague Dawley* (SD) rats for immune biomarkers modulation. The test formulation was divided into two parts. One part was denoted as the control without any Biofield Energy Treatment, while the other part was defined as the Biofield Treated sample, which received the Biofield Energy Healing Treatment remotely from twenty renowned Biofield Energy Healers. The immunomodulatory potential was studied in male SD rats using various immune biomarkers such as IgG and IgM, $CD4^+$ and $CD8^+$, hematology, lipid profile, hepatic enzymes, sex hormone, and antioxidant profile. The humoral immune response showed an increase IgG level by 6.10% in the Biofield Energy Treated test formulation (G4) group compared with the disease control (G2) group. Cellular immune response *i.e.* $CD4^+$ and $CD8^+$ counts were significantly improved by 51.64% and 52.09%, respectively in G4 compared with the G2 group. The lymphocytes level were increased by 38.87%, while TLC, neutrophils, eosinophils, and monocytes count were decreased by 7.38%, 19.35%, 21.14%, and 3.43%, respectively in G4 compared with the G2 group. Lipid analysis showed that triglycerides and VLDL levels were decreased by 6.02% and 5.97%, respectively while HDL level was significantly increased by 28.44% in G4 compared with the G2 group. Hepatic biomarkers *viz.* SGOT, CK-MB, total protein, total bilirubin, and globulin by 6.27%, 14.97%, 2.85%, 16.66%, and 5.90%, respectively in G4 compared with the G2 group. However, the testosterone level was significantly increased in G4 and untreated test formulation (G5) groups by 135.74% and 28.09%, respectively compared with the G2 group. Additionally, the antioxidant assay showed that the lipid peroxidation (LPO) level was decreased, while superoxide dismutase (SOD) and catalase levels were increased by 59.72% and 20.07%, respectively in the G4 compared with the G2 group. Overall, the study

results suggested that the Biofield Energy Treated test formulation showed an improved cellular and humoral immune responses along with improved hematological and biochemical animal profile compared with the untreated test formulation. Consequently, it can be established that The Trivedi Effect®-Biofield Energy Healing has the significant capacity for immunomodulatory effect, which may also be useful in organ transplants, anti-aging, and stress management by improving overall health and quality of life.

Keywords: Biofield Energy Healers, Consciousness Energy Healing Treatment, The Trivedi Effect®, Herbomineral Formulation, Cardiac Biomarker, Testosterone, Inflammatory Diseases, Anti-aging

1. Introduction

Herbomineral formulations have always been a major target of scientific research due to their significant immunomodulatory potential. The plant products and their extracts are used in both allopathic health care as well as complementary and alternative health care in order to improve overall health and the immune system. Substantial progress in the field of natural medicine and molecular immunology, translational research has succeeded for rapid development of herbal based formulation with respect to the immune biomarkers modulation. In general, the biomarkers are the biological evaluation that can be used to analyze the risk associated with diseases, to recognize the disease direction, and to observe the consequences of some therapeutic mediations [1]. In this context, measurement of specific immune biomarkers are beneficial, while developing any novel herbomineral formulations. Herbomineral formulations plays an important role in immunomodulatory action due to the secondary metabolites of plants extract and minerals [2-4]. Therefore, minerals and herbal based medicines are the major products to modulate the immune system due to its low toxicity profile compared with the synthetic drugs against infections [5, 6]. Immunomodulatory therapies modulates the major immune biomarkers and has now been considered as primary treatment in many disease conditions. To evaluate the therapeutic immunomodulatory potential of any herbomineral formulations, biomarkers with respect to hepatic, cardiac, lipid, hematology, cellular and humoral response are considered as the standard method of analysis [7]. Herbomineral formulations have been reported and accepted worldwide against many health related disorders and as a supplement product to improve the quality of life (QoL). According to the scientific literatures, and as per the best medicinal activity of herbal extract, a new proprietary herbomineral formulation was formulated with a combination of the herb ashwagandha (*Withania somnifera)* root extract and three minerals *viz.* zinc, magnesium, and selenium. All the ingredients of the formulation in this present study possess important activities such as immune-modulatory, anti-inflammatory, antioxidant, anti-infective, and anti-viral properties [8-10]. Besides, several significant biological effects of herbal products and minerals, very low or no toxicity profile was also reported, when compared with the currently available synthetic drugs. Immunomodulatory activity is always considered as the primary therapeutic potential of any formulations against various infections, while estimation of immune biomarkers such as hepatic, cardiac, lipid, hematology, cellular and humoral response are considered as the gold standard for activity analysis. Ashwagandha due to the presence of withanolides are used as complementary medicine as antibacterial, immunomodulatory and antitumor effects, with many clinical and preclinical data [11, 12]. The importance of minerals such as selenium, zinc, and magnesium has been well defined to modulate the immune system. Zinc regulates most of the biochemical reaction in the living organism because of enzyme catalyzing activity, while selenium work as significant immunomodulatory effect by altering $CD8^+$ lymphocyte function. Similarly, magnesium also responsible for cytokine production through NF-κB pathways activation, a novel innate immunomodulatory mechanism [13-16].

Scientific research has been reported that the combination of minerals and herbal medicines have been found to exhibit significant immunomodulatory action [6]. Herbomineral formulations can be used for better therapeutic effect in immune compromised patients that are affected by cardiovascular diseases, age, stress related diseases, cancer, and autoimmune disorders. Along with the herbomineral formulations, the Biofield Energy Healers in this study have used Energy Medicine (Biofield Energy Healing Treatment) as a complementary and alternative approach to study the impact of Biofield Energy Healing Treatment on the herbomineral formulation for its immunomodulatory potential in male *Sprague Dawley* rats.

Amidst many Complementary and Alternative Medicine (CAM) therapies, there have been an extensive number of scientific reports that showed that the Biofield Therapy (or Healing Modalities) as preferred models of treatment with several benefits to enhance physical, mental and emotional human wellness. The National Center of Complementary and Integrative Health (NCCIH) has been recognized and accepted Biofield Energy Healing as a CAM health care approach in addition to other therapies, medicines and practices such as natural products, deep breathing, yoga, Tai Chi, Qi Gong, chiropractic/osteopathic manipulation, meditation, massage, special diets, homeopathy, progressive relaxation, guided imagery, acupressure, acupuncture, relaxation techniques, hypnotherapy, healing touch, movement therapy, pilates, Rolfing structural integration, mindfulness, Ayurvedic medicine, traditional Chinese herbs and medicines, naturopathy, essential oils, aromatherapy, Reiki, and cranial sacral therapy. Human Biofield Energy has subtle energy that has the capacity to work in an effective manner [17]. CAM therapies have been practiced worldwide

with reported clinical benefits in different health disease profiles [18]. Biofield Energy Healing Treatment has gained rapid rapport as a holistic alternative and complementary medicine therapy that has significant impact on living organisms and nonliving materials without any adverse effects and in a manner that is more cost-effective than more available conventional methods. Biofield Energy Heling Treatment (The Trivedi Effect®) significant outcomes has been published in numerous peer-reviewed science journals in many scientific fields such as cancer and biotechnology research [19], microbiology [20-22], genetics [23, 24], pharmaceutics [25, 26], nutraceuticals [27], organic compounds [28, 29], agricultural science [30, 31], and changing the structure of the atom in relation to various metals, ceramics, polymers, chemicals in materials science [32-34], human health and wellness.

In this study, the authors sought to explore the impact of The Trivedi Effect®- Energy of Consciousness Healing Treatment (Biofield Energy Healing Treatment) on the given herbomineral formulation for its immunomodulatory properties *viz.* humoral and cellular immune responses, hematology, lipid profile, hepatic enzymes, sex hormone, antioxidant study in male *Sprague Dawley* (SD) rats.

2. Materials and Methods

2.1. Chemicals and Reagents

Cyclophosphamide was used as an immunosuppressive agent, procured from Zydus Oncosciences, India. Levamisole hydrochloride was used as a reference standard (positive control) for immunostimulatory activity, which was obtained from Sigma-Aldrich, USA. Sodium carboxymethyl cellulose (Na-CMC) was used as a vehicle for formulation of the test herbomineral formulation and was procured from Sigma-Aldrich, USA. Ashwagandha root extract powder was procured from Sanat Products Ltd., India. Zinc chloride and magnesium (II) gluconate hydrate were procured from TCI, Japan. Sodium selenate was procured from Alfa Aesar, USA. However, other common laboratory reagents used in this experiment were of analytical grade available in India.

2.2. Laboratory Animals

All healthy male *Sprague Dawley* (SD) rats, weighing between 220 to 290 grams, were used for the study. The animals were purchased from M/s. Vivo Bio Tech Ltd., Hyderabad, India. Standard rodent diet was procured from M/s. Golden feeds, Mehrauli, New Delhi, India and provided *ad libitum* to all the groups of animals during the experiment under controlled conditions with a temperature of 22 ± 3°C, humidity of 30% to 70% and a 12-hour light/12-hour dark cycle. The animals were acclimatized for the period of 5 days prior to the experiment, and all were accessed once daily for clinical signs, behaviors, morbidity and mortality. All the procedures were in strict accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health. The approval of the Institutional Animal Ethics Committee was obtained prior to carrying out the animal experiment.

2.3. Energy of Consciousness Treatment Strategies

The test formulation was divided into two parts. One part of the test formulation was treated with Biofield Energy by renowned Biofield Energy Healers (also known as The Trivedi Effect®) and coded as the Biofield Energy Treated formulation, while the second part of the test formulation did not receive any sort of treatment and was defined as the untreated test formulation. The Trivedi Effect®- Energy of Consciousness Healing Treatment (Biofield Energy Healing Treatment) was provided through a group of twenty Biofield Energy Healers who participated in this study and performed the Biofield Energy Treatment remotely. The total of eighteen Biofield Energy Healers were remotely located in the U.S.A and two were located in Canada, while the test herbomineral formulation was located in the research laboratory of Dabur Research Foundation, New Delhi, India. This Biofield Energy Treatment was administered for 5 minutes through the Healer's unique Energy Transmission process remotely to the test formulation under laboratory conditions. None of the Biofield Energy Healers in this study visited the laboratory in person, nor had any contact with the herbomineral samples. Further, the control group was treated with a "sham" healer for comparative purposes. The sham healer did not have any knowledge about the Biofield Energy Treatment. After that, the Biofield Energy treated and untreated samples were kept in similar sealed conditions and used for identification of immunological parameters.

2.4. Antigen (Sheep RBC, sRBC)

The fresh sheep blood was collected aseptically from the jugular vein of a healthy sheep and transferred immediately to the heparinized tube. The collected erythrocytes were separated from plasma by centrifugation (400 *g*, 10°C, 10 minutes), washed twice with the normal saline and then further diluted in saline, which were analyzed using Hematology analyzer (Abbott Model-CD-3700). Based on the number of erythrocytes, the samples were further diluted (using saline) before injecting to the rat [35].

2.5. Experimental Procedure

The animals were randomized and grouped according to their body weight. A total of five groups (G) were included *i.e.* Group 1 (G1) was served as a normal control (*i.e.* vehicle control), and G2 was served as a disease control; both the groups were received 0.5% Na-CMC, while G3 group animals received levamisole (75 mg/kg; *p.o.*). G4 group animals were received Biofield Energy Treated test formulation at a dose of 1105.005 mg/kg. Similarly, G5 animals were received untreated test formulation at a same dose. However, during the experimental period, all the animals except normal control (G1) were received with cyclophosphamide (10 mg/kg, *p.o.*) daily to induce the immunosuppression action. Cyclophosphamide was given 1

hour prior to the oral administration of test formulation for initial period of 13 days. The treatment was continued to all the tested groups (G1 to G5) with 5 mL/kg body weight dose volume for 22 day experiment. Further, on day 7 and 13, all the groups (G1 to G5) received sRBC (0.5 X 10^9/100 gm body weight; *i.p*.) as the antigenic material to sensitize them for immunological studies. On the last day of experiment, the animals were kept under fasting over night and on next day, blood was collected again from retro-orbital plexus from each animal under isoflurane anaesthesia. At the end of the study; animals were euthanized by CO_2 asphyxiation as per in-house approved standard protocol. Whole blood was analysed for haematological parameters and serum was analysed for serum biochemistry. Further, the blood samples were analyzed for cellular immune biomarkers ($CD4^+$ and $CD8^+$), biochemical markers, testosterone level and humoral immune markers (IgG and IgM). A portion of liver samples were snap frozen and stored in -80°C for the estimation of anti-oxidant parameters such as superoxide dismutase (SOD), catalase (CAT), and lipid peroxidation (LPO).

2.6. Assessment of Cellular and Humoral Responses

Humoral immune response, IgG and IgM were estimated using a Mini Vidas, Biomeurix (French) from serum, using commercially available kits. Flow cytometry was used to evaluate the $CD4^+$ and $CD8^+$ cells in blood as a measure of the cellular immune response. The mean value was calculated for each group with SEM. The percent change in the Biofield Energy Treated group was calculated compared to the vehicle treatment group.

2.7. Assessment of Hematology Parameters

Hematological parameters such as total leukocyte count (TLC), and differential leukocyte counts (DLC), were analyzed using a Hematology analyzer (Abbott Model-CD-3700) in blood samples.

2.8. Assessment of Lipid Profile and Hepatic Enzymes

Glucose, total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL), high density lipoprotein (HDL), very low density lipoprotein (VLDL), alkaline phosphatase (ALP), serum glutamic oxaloacetic transaminase (SGOT), and serum glutamate-pyruvate transaminase (SGPT) were analyzed using serum [36, 37].

2.9. Assessment of Sex Hormone - Testosterone

The level of testosterone was analyzed in serum using commercial kits. The mean value was calculated for each group with SEM. The percent change in the treated group was calculated compared to the vehicle treatment group.

2.10. Assessment of Antioxidant Profile by ELISA Assay

Superoxide dismutase (SOD), catalase and lipid peroxidase (LPO) were analyzed by ELISA assay using liver homogenate sample [38-40].

2.11. Statistical Analysis

All the results were expressed as mean ± standard error of mean (SEM) and subjected to statistical analysis using a Sigma Plot (Version 11.0). Student's *t*-test was performed for comparison of the individual treatment group with control. The $p \leq 0.05$ was considered as statistically significant.

3. Results and Discussion

3.1. Measurement of Humoral Immune Response

The results immunoglobulin levels (IgG and IgM) after oral administration of the Biofield Energy Treated and untreated test formulation are presented in the Figure 1. The study data suggested that the level of IgG was increased by 6.10% in the Biofield Energy Treated test formulation (G4) compared with the disease control group (G2). The values of IgG in G2 and G4 were 2.62 ± 0.12 and 2.78 ± 0.09 mg/mL, respectively. However, the level of IgM remained unchanged with respect to the disease control group *i.e.* 0.23 ± 0.01 and 0.23 ± 0.02 mg/mL in G2 and G4 groups, respectively. Besides, the level of IgM was decreased in the untreated test formulation (G5) by 13.03% compared with the G2 group. Overall, it can be concluded that the Biofield Energy Healing Treatment based test formulation would improve the humoral immune response with respect to the untreated test formulation.

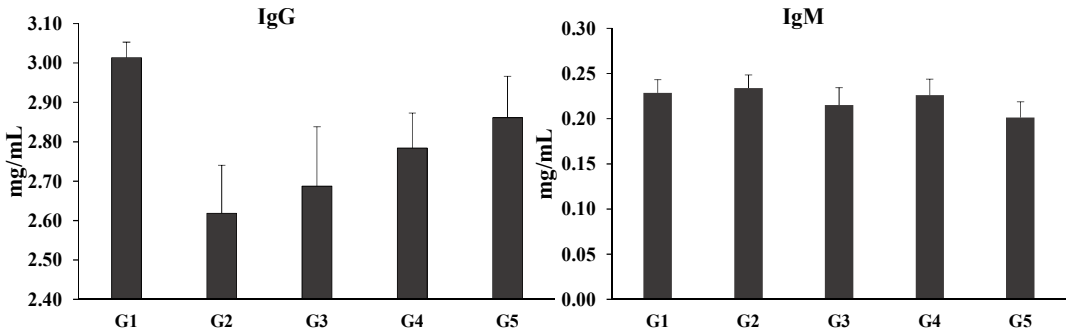

Figure 1. *The effect of the test formulation on immunoglobulins, IgM and IgG after treatment on various groups (G1 – G5) in male SD rats. G1: Normal control; G2: Disease control; G3: Levamisole; G4: Biofield Energy Treated test formulation; G5: Untreated test formulation. All the values are represented as mean ± SEM (n=8).*

IgG and IgM are the major immunoglobulins that are considered as an important role in complement activation, opsonization, neutralization of toxins, *etc*. The study results suggested that the level of IgG in the Biofield Energy Treated test formulation group was significantly altered, while the IgM response did not show any change compared with the disease control group. However, Biofield Energy Treated test formulation showed an increased level of IgG compared with the IgM. Literature data suggested that ashwagandha and minerals such as zinc, selenium, and magnesium have been reported with an improved immunoglobulin production [41, 42]. The test formulation is the combination of ashwagandha root extract and minerals, thus it can be suggested that the constituents are responsible for altered humoral immunity, while Biofield Energy Healing Treatment significantly altered the immunoglobulin production.

3.2. Measurement of Cellular Responses

The cellular immune response was estimated by calculating the percentage of $CD4^+$ and $CD8^+$ in male *Sprague Dawley* rats after oral administration of the Biofield Energy Treated and untreated test formulation. The results are presented in the Figure 2. The results showed the percentage of $CD4^+$ in the Biofield Energy Treated test formulation (G4) was significantly increased by 51.64% compared with the disease control group (G2). On the other hand, untreated test formulation only showed an increased level by 27.92% in the $CD4^+$ percentage with respect to the G2 group. Thus, the Biofield Energy Treated test formulation (G4) was observed with significantly improved cellular immune response compared with the untreated test formulation (G5). Similarly, the results of $CD8^+$ in the Biofield Energy Treated test formulation (G4) was significantly increased by 52.09% compared with the disease control (G2) group.

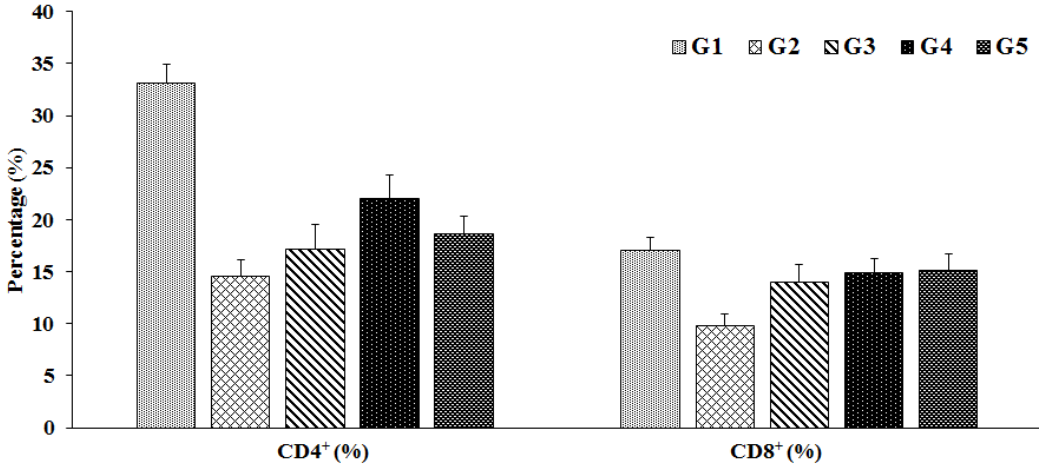

Figure 2. *The effect of the test formulation on the ratio of cellular biomarkers ($CD4^+$/$CD8^+$ ration) after treatment on various groups (G1 – G5) in blood sample of male SD rats. G1: Normal control; G2: Disease control; G3: Levamisole; G4: Biofield Energy Treated test formulation; G5: Untreated test formulation. All the values are represented as mean ± SEM (n=8).*

T-cells are mainly of two types, *i.e.* T4 and T8. $CD4^+$ or T4 cells are known as helper cells and they always lead against any infection. On the other hand, T8 cells or $CD8^+$ are defined as the suppressor or killer cells, they have the capacity to kill the cancerous cells and also those cells which are infected by virus. These are the subpopulations of lymphocytes and are the important white blood cells of the immune system. Their strength are directly related with the immunity and the power to fight against multiple infection [43, 44]. Thus, higher count of these cell and its role in T cell activation are always considered for strong immune system [45]. Overall, it can be concluded that the Biofield Energy Healing (The Trivedi Effect®) based herbomineral formulation showed a significant improved cellular immunity to fight against various autoimmune and anti-inflammatory disorders.

3.3. Assessment of Hematology Parameters

The results of hematology study such as total and differential leucocytes counts after oral administration of the Biofield Energy Treated and untreated test formulation in different groups (G1 to G5) are summarized in the Table 1. The study results suggested that the animal hematology profile was significantly improved compared with the disease control group. Hematology parameters such as TLC, neutrophils, lymphocytes, eosinophils, and monocytes exhibited alterations such as decreased TLC, neutrophils, eosinophils, and monocytes counts by 7.38%, 19.35%, 21.14%, and 3.43%, respectively in the Biofield Energy Treated test formulation group (G4) compared with the disease control group (G2). Besides, the level of lymphocytes was increased by 38.87% in G4 compared with the G2 group.

Table 1. *Effect of the test formulation on hematological parameters in Sprague Dawley rats.*

Group (G)	TLC (thousand/mm^3)	Neutrophils (%)	Lymphocytes (%)	Eosinophils (%)	Monocyte (%)
G1	12.15 ± 1.06	16.63 ± 1.41	78.75 ± 2.48	1.88 ± 0.35	2.75 ± 1.19
G2	11.11 ± 1.91	32.38 ± 3.77	62.38 ± 4.04	1.75 ± 0.16	3.50 ± 0.46
G3	10.71 ± 1.60	29.75 ± 2.79	65.50 ± 3.13	1.50 ± 0.19	3.25 ± 0.45
G4	10.29 ± 1.75	27.13 ± 1.84	68.13 ± 2.38	1.38 ± 0.18	3.38 ± 0.60
G5	8.43 ± 1.23	30.25 ± 2.37	64.63 ± 2.87	1.75 ± 0.25	3.38 ± 0.84

G1: Normal control; G2: Disease control; G3: Levamisole; G4: Biofield Energy Treated test formulation; G5: Untreated test formulation. TLC: Total leukocyte count, All the values are represented as mean ± SEM (n=8).

The lymphocytes are one of the major cells of immune system and are divided into T and B cells. They eliminate the antigen, either by releasing the antibodies (B cells), cytotoxic granules (cytotoxic T-cells) or directly by signaling to other cells of the immune system (helper T-cells). Thus, they are important for immune functions and regulate the accessory growth factors, which provide modalities to modify the immune response against infections [46]. The decreased level of neutrophils, eosinophils, and monocytes in the Biofield Energy Treated test formulation can be used against many diseases such as chronic inflammatory disease, acute infection, gout, rheumatoid arthritis, rheumatic fever, autoimmune disorders, etc. Overall, the data suggested that the Biofield Energy Treated test formulation significantly altered the concentrations of tested hematology profile, with an improved concentration of lymphocytes. Thus, it can be assumed that The Trivedi Effect®-Energy of Consciousness Healing Treatment has the capacity to improve the immunomodulatory potential of the test formulation with respect to altered hematological animal profile.

3.4. Measurement of Glucose and Lipid Biomarkers

Lipid profile analysis after oral administration of the Biofield Energy Treated and untreated test formulation are summarized in the Table 2. The biochemical parameters such as glucose, total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL), and very low density lipoprotein (VLDL) were analyzed and compared. The study result showed that the concentration of glucose was increased by 10.58%, but not significant in the Biofield Energy Treated test formulation (G4) compared with the disease control group (G2). However, the level of TC and LDL was increased by 25.61% and 31.84%, respectively in G4 group compared with the G2 group. Although, the level of triglycerides and VLDL was significantly decreased by 6.02% and 5.97%, respectively in the G4 group, while the level of HDL was significantly increased by 28.44% in the G4 group, compared with the disease control (G2) group. However, the level of HDL was decreased in the untreated test formulation (G5) by 6.28% compared with the G2, thus Biofield Energy Treated test formulation showed a significant improved lipid profile compared with the untreated test formulation, which can be used against many inflammatory and autoimmune disorders.

Table 2. *Lipid profile analysis after treatment with the test formulation on male rats.*

Group (G)	Glucose (mg/dL)	TC (mg/dL)	Triglyceride (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)
G1	107.80 ± 7.05	70.98 ± 3.62	51.95 ± 3.97	21.26 ± 1.09	39.36 ± 2.37	10.35 ± 0.80
G2	103.21 ± 6.39	74.00 ± 4.57	51.34 ± 5.74	22.15 ± 1.37	41.64 ± 2.53	10.21 ± 1.14
G3	118.80 ± 3.63	88.46 ± 3.89	44.45 ± 3.48	26.48 ± 1.15	53.14 ± 3.06	8.85 ± 0.69
G4	114.13 ± 7.73	92.95 ± 17.29	48.25 ± 3.87	28.45 ± 4.86	54.90 ± 12.47	9.60 ± 0.78
G5	111.60 ± 4.63	69.34 ± 2.88	41.04 ± 2.17	20.76 ± 0.87	40.39 ± 1.86	8.18 ± 0.44

G1: Normal control; G2: Disease control; G3: Levamisole; G4: Biofield Energy Treated test formulation; G5: Untreated test formulation. TLC: Total leukocyte count, All the values are represented as mean ± SEM (n=8). HDL: High density lipoprotein; LDL: Low density lipoprotein; VLDL: Very low density lipoprotein; mg/dL: Milligram per deciliter.

Overall, the Biofield Energy Treated test herbomineral formulation was observed with the beneficial effect on male SD rats for its lipid profile. It might be suggested that lipid profile was altered due the active constituents present in test formulation, but the Biofield Energy Treatment showed a significant improved lipid profile as compared with the untreated test formulation. Scientific literature suggests that the constituents present in test formulation such as ashwagandha, selenium, zinc, and magnesium have significant impact on lipid profile, serum cholesterol, LDL, HDL, etc. [47-50]. Besides, it was also reported that serum and liver lipid profile was altered when rats were exposed to extremely low frequency electromagnetic fields [51]. Thus, the Biofield Energy Healing Treatment might modulates the liver lipid profile after oral administration of the Biofield Energy Treated test formulation. Overall, the Biofield Energy Treated test herbomineral formulation can be used to improve the immunomodulatory activity by altering the animal lipid profile.

3.5. Measurement of Hepatic and Cardiac Biomarkers

The study results of hepatic biochemical markers such as serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGOT), alkaline phosphatase (ALP) and cardiac enzyme creatine kinase

myocardium band (CK-MB), and others biomarkers such as, total bilirubin, albumin, and globulin of different groups (G1 to G5) are summarized in Table 3. The level of SGOT, CK-MB, TP, TB, and G were significantly decreased by 6.27%, 14.97%, 2.85%, 16.66%, and 5.90%, respectively in the Biofield Energy Treated test formulation (G4) compared with the disease control (G2). However, levamisole treated group (G3) also showed a significant decreased CK-MB level by 14.97% compared with the disease control G2, group. Besides, no significant change was observed in G4 group in other tested parameters such as SGPT, A, and A/G with respect to G2 group.

Table 3. *Evaluation of hepatic biomarkers after treatment with the test formulation on male rats.*

Group (G)	TB (mg/dL)	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	CK-MB (U/L)	TP (g/dL)	A (g/dL)	G (g/dL)	A/G ratio
G1	0.10 ± 0.01	219.13 ± 10.39	43.11 ± 1.67	310.56 ± 13.06	178.08 ± 12.04	7.28 ± 0.08	3.65 ± 0.03	3.63 ± 0.05	1.01 ± 0.01
G2	0.12 ± 0.01	187.86 ± 6.86	34.14 ± 1.91	181.53 ± 5.73	155.75 ± 9.68	7.01 ± 0.07	3.45 ± 0.06	3.56 ± 0.04	0.97 ± 0.02
G3	0.10 ± 0.01	155.56 ± 10.82	35.34 ± 3.38	194.74 ± 8.68	128.15 ± 12.10	7.28 ± 0.06	3.61 ± 0.02	3.66 ± 0.05	0.99 ± 0.01
G4	0.10 ± 0.01	176.08 ± 13.09	34.70 ± 3.52	196.58 ± 11.63	132.43 ± 20.10	6.81 ± 0.09	3.46 ± 0.05	3.35 ± 0.08	1.04 ± 0.03
G5	0.10 ± 0.01	128.99 ± 6.71	22.94 ± 2.29	183.74 ± 9.09	84.10 ± 7.65	7.03 ± 0.11	3.61 ± 0.05	3.41 ± 0.09	1.06 ± 0.02

G1: Normal control; G2: Disease control; G3: Levamisole; G4: Biofield Energy Treated test formulation; G5: Untreated test formulation. All the values are represented as mean ± SEM (n=8). TB: Total bilirubin; SGOT: Serum glutamic oxaloacetic transaminase; SGPT: Serum glutamate-pyruvate transaminase; ALP: Alkaline phosphatase; CK-MB: Creatine kinase-myocardial band; TP: Total protein; A: Albumin; G: Globulin; A/G: Albumin/Globulin ratio; U/L: Unit per liter; mg/dL: Milligram per deciliter.

The tested liver biomarkers were significantly increased in the diseases control group (G2), while the toxicity of liver was significantly improved by the Biofield Energy Treated test formulation. Thus, it can be concluded that The Trivedi Effect®- Energy of Consciousness Healing Treatment can be used to improve the immunity profile by improving the level of important liver enzymes. These hepatic enzymes are the biomarkers for liver toxicity and suggest liver damage [52]. Besides, literature data reported that the constituents present in test formulation, such as ashwagandha, and minerals (*viz.* selenium, zinc, and magnesium) have protective activity for liver enzymes [53-56]. Therefore, it can be established that the Biofield Energy Treated test formulation could protect the liver toxicity and could help in regulating the immune function by altering the level of hepatic biomarkers.

3.6. Measurement of Sex Hormone-Testosterone

The results of serum testosterone level in male SD rats after oral administration of Biofield Energy Treated and untreated test formulation are shown in the Figure 3. Serum testosterone level was significantly altered after treatment compared with the normal control and disease control groups. The level of testosterone (in ng/dL) in disease control (G2), levamisole (G3), Biofield Energy Treated test formulation (G4), untreated test formulation (G5) was 173.50 ± 52.83, 142.25 ± 43.10, 409.00 ± 133.18, and 222.25 ± 58.61 ng/dL, respectively. The level of testosterone was significantly increased by 135.74% and 28.09% in the Biofield Energy Treated test formulation (G4) and untreated test formulation (G5) groups, respectively compared with the disease control group (G2). The effect of the Biofield Energy Healing Treatment on test formulation showed a significant improved sex hormone level concentration as compared with the untreated test formulation group.

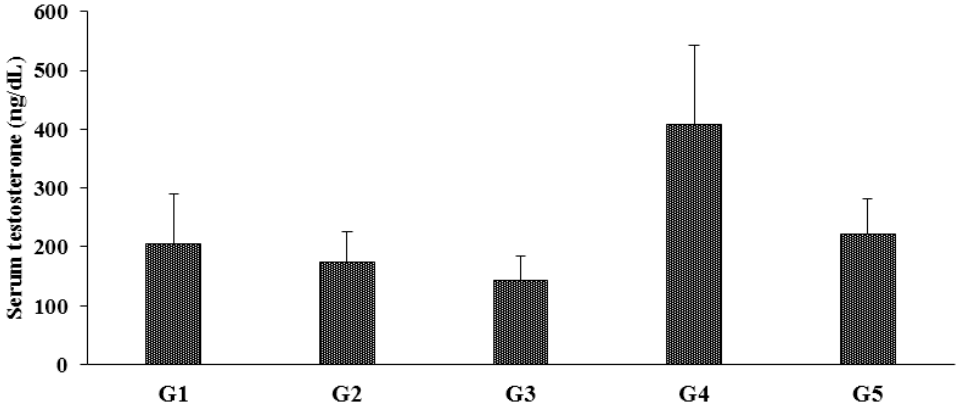

Figure 3. *The effect of the test formulation on the level of testosterone after treatment on various groups (G1 - G5) in male Sprague Dawley rats. G1: Normal control; G2: Disease control; G3: Levamisole; G4: Biofield Energy Treated test formulation; G5: Untreated test formulation. All the values are represented as mean ± SEM (n=8).*

It was reported that all the constituents present in the test formulation, *i.e.* ashwagandha, zinc, selenium, and magnesium are responsible for regulation of sex hormone, testosterone [57-59]. In addition, the minerals added in the herbomineral formulation like zinc and selenium are also used in various testicular disorders [60]. The study results

showed that the testosterone level after Biofield Energy Healing Treatment to the test formulation was significantly improved as compared with the untreated test formulation. This showed that The Trivedi Effect®-Energy of Consciousness Healing Treatment has the significant capacity to improve the sex hormone level, which suggested the use of test herbomineral formulation against many immunomodulatory and autoimmune disorders.

3.7. Measurement of Antioxidant Profile by ELISA Based Assay

The study results of the Biofield Energy Treated and untreated test formulation on the levels of various antioxidant enzymes such as SOD, LPO, and CAT in male SD rats are demonstrated in the Figure 4. The antioxidant biomarkers were evaluated in liver samples. The LPO level in Biofield Energy Treated test formulation (G4) and untreated test formulation (G5) was slightly decreased in both the groups, while in levamisole (G3) group, the LPO level was increased by 16.85% compared with the G2 group. However, the SOD level was significantly increased in the Biofield Energy Treated test formulation (G4) by 59.72% as compared with the G2 group. Similarly, the level of catalase (CAT) was increased in G4 group by 20.07%, while levamisole (G3) showed an increased catalase level by 43.64% as compared with the G2 group.

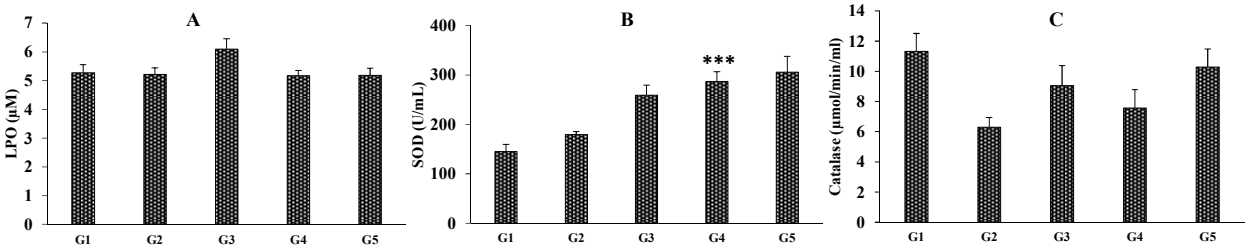

Figure 4. *Bar graphs showing activities of antioxidant enzymes (A) LPO, lipid peroxidase, (B) SOD, superoxide dismutase, and (C) catalase after treatment with the test formulation in male Sprague-Dawley rats. G1: Normal control; G2: Disease control; G3: Levamisole; G4: Biofield Energy Treated test formulation; G5: Untreated test formulation. LPO: Lipid peroxidation; SOD: Superoxide dismutase. All the values are represented as mean ± SEM (n=8).*

Overall, the data suggest that the level of free radicals was significantly reduced and the level of SOD, and catalase was significantly improved, which would be beneficial with respect to the anti-oxidative property. The level of free radicals are responsible for many inflammatory infections [61], and if this would decreased, it can be used as anti-inflammatory agent. This can be concluded that the Biofield Energy Treated test formulation showed a significant antioxidant activity and can be used to modulate the immune system against several inflammatory diseases.

4. Conclusions

The experimental results exhibited a significantly impact of The Trivedi Effect®- Energy of Consciousness Healing Treatment (Biofield Energy Healing Treatment) on test formulation with significant immunomodulatory action in male *Sprague Dawley* rats. The results of humoral immune biomarkers showed that the IgG level was increased by 6.10%, while IgM level remained unaltered in the Biofield Energy Treated test formulation (G4) group as compared with the disease control group (G2). In addition, the level of IgM was decreased by 13.03% in the untreated test formulation, thus the Biofield Energy Treated test formulation showed an improved results as compared with the untreated test formulation. Similarly, the cellular immune biomarkers (*i.e.* $CD4^+$ and $CD8^+$) levels were improved as compared with the disease control. The significant increase in the percentage of $CD4^+$ and $CD8^+$ levels were reported by 51.64% and 52.09%, in the Biofield Energy Treated test formulation (G4) compared with the G2 group. However, an improved cellular immune response was significant in the Biofield Energy Treated test formulation compared with the untreated test formulation. Besides, the blood differential test indicated a decreased level of TLC, neutrophils, lymphocytes, eosinophils, and monocytes by 7.38%, 19.35%, 21.14%, and 3.43%, respectively in the Biofield Energy Treated test formulation group (G4) compared with the disease control group (G2). However, the level of lymphocytes were increased by 38.87% in G4 compared with the G2 group. Biochemical analysis showed that the glucose concentration was altered (10.58%) and the level of LDL was increased by 31.84%, while VLDL and triglycerides were decreased by 5.97% and 6.02%, respectively in the Biofield Energy Treated test formulation (G4) group as compared with the disease control (G2) group. Although, the HDL level was significantly increased by 28.44% in G4 group compared with the G2 group. Further, the hepatic and cardiac biomarkers in serum sample were analyzed that showed decrease in the level of SGOT, CK-MB, TP, TB, and G by 6.27%, 14.97%, 2.85%, 16.66%, and 5.90%**,** respectively in the Biofield Energy Treated test formulation (G4) group compared with G2 group. The serum testosterone level was significantly increased by 135.74% in G4 group, while untreated test formulation showed only 28.09% increase in testosterone compared with the diseases control (G2) group. In addition, the antioxidant assay showed a significant decreased LPO level, while the level of SOD and catalase was significantly increased by 59.72% and 20.07%, respectively in G4 group compared with the G2 group.

Overall, the current experimental findings suggested that the Trivedi Effect®-Biofield Energy Healing Treatment done

remotely by the twenty Biofield Energy Healers, which enhanced the herbomineral test formulation's anti-inflammatory and immunomodulatory properties that can be used to improve the overall health. Thus, the Biofield Energy Treated test formulation may act as an effective anti-inflammatory and immunomodulatory product, and it can be used as a Complementary and Alternative Medicine (CAM) with a safe therapeutic index for various autoimmune disorders such as Lupus, Systemic Lupus Erythematosus, Fibromyalgia, Addison Disease, Hashimoto Thyroiditis, Celiac Disease (gluten-sensitive enteropathy), Multiple Sclerosis, Dermatomyositis, Graves' Disease, Myasthenia Gravis, Pernicious Anemia, Aplastic Anemia, Scleroderma, Psoriasis, Rheumatoid Arthritis, Reactive Arthritis, Type 1 Diabetes, Sjogren Syndrome, Crohn's Disease, Vasculitis, Vitiligo, Chronic Fatigue Syndrome and Alopecia Areata, as well as inflammatory disorders such as Irritable Bowel Syndrome (IBS), Asthma, Ulcerative Colitis, Alzheimer's Disease, Parkinson's Disease, Atherosclerosis, Dermatitis, Hepatitis, and Diverticulitis. Further, the Biofield Energy Healing Treated test formulation can also be used in the prevention of immune-mediated tissue damage in cases of organ transplants (for example heart transplants, kidney transplants and liver transplants), for anti-aging, stress prevention and management, and in the improvement of overall health and quality of life.

Abbreviations

Na-CMC: Sodium carboxymethyl cellulose; SD: *Sprague Dawley*; TC: Total cholesterol; TG: Triglycerides; LDL: Low Density Lipoprotein; HDL: High Density Lipoprotein; VLDL: Very Low Density Lipoprotein; ALP: Alkaline Phosphatase; SGOT: Serum glutamic oxaloacetic transaminase; SGPT: Serum glutamate-pyruvate transaminase; TLC: Total leukocyte count; DLC: Differential leukocyte count; CK-MB: Creatine kinase myocardium band; CAT: Catalase; SOD: Superoxide dismutase; LPO: Lipid peroxidation; CD: Cluster differentiation; NCCIH: National Center of Complementary and Integrative Health; CAM: Complementary and Alternative Medicine.

Acknowledgements

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

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