

Effect of Biofield Energy Healing (The Trivedi Effect®) Based Novel Herbomineral Formulation on Immune Biomarkers After Oral Administration in Female *Sprague Dawley* Rats

Mahendra Kumar Trivedi¹, Alice Branton¹, Dahryn Trivedi¹, Gopal Nayak¹, Cathryn Dawn Nykvist¹, Celine Lavelle¹, Daniel Paul Przybylski¹, Dianne Heather Vincent¹, Dorothy Felger¹, Douglas Jay Konersman¹, Elizabeth Ann Feeney¹, Jay Anthony Prague¹, Joanne Lydia Starodub¹, Karan Rasdan¹, Karen Mie Strassman¹, Leonid Soboleff¹, Maire Mayne¹, Mary M. Keesee¹, Padmanabha Narayana Pillai¹, Pamela Clarkson Ansley¹, Ronald David Schmitz¹, Sharyn Marie Sodomora¹, Sambhu Charan Mondal², Snehasis Jana^{2,*}

¹Trivedi Global, Inc., Henderson, USA

²Trivedi Science Research Laboratory Pvt. Ltd., Bhopal, India

Email address:

publication@trivedieffect.com (S. Jana)

*Corresponding author

To cite this article:

Mahendra Kumar Trivedi, Alice Branton¹, Dahryn Trivedi, Gopal Nayak, Cathryn Dawn Nykvist, Celine Lavelle, Daniel Paul Przybylski, Dianne Heather Vincent, Dorothy Felger, Douglas Jay Konersman, Elizabeth Ann Feeney, Jay Anthony Prague, Joanne Lydia Starodub, Karan Rasdan, Karen Mie Strassman, Leonid Soboleff, Maire Mayne, Mary M. Keesee, Padmanabha Narayana Pillai, Pamela Clarkson Ansley, Ronald David Schmitz, Sharyn Marie Sodomora, Sambhu Charan Mondal, Snehasis Jana. Effect of Biofield Energy Healing (The Trivedi Effect®) Based Novel Herbomineral Formulation on Immune Biomarkers After Oral Administration in Female *Sprague Dawley* Rats. *European Journal of Clinical and Biomedical Sciences*. Vol. 3, No. 6, 2017, pp. 120-128. doi: 10.11648/j.ejcbcs.20170306.14

Received: October 30, 2017; **Accepted:** November 11, 2017; **Published:** December 5, 2017

Abstract: The herbomineral formulations usage have been increased world-wide in health care sector due to their high safety and better therapeutic action. A new proprietary herbomineral formulation was formulated with a mixture of the minerals (zinc, magnesium, and selenium) and the herbal root extract ashwagandha. The aim of the study was to evaluate the immunomodulatory potential of Biofield Energy Healing (The Trivedi Effect®) on the herbomineral formulation in female *Sprague Dawley* (SD) rats. 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 Energy Treated sample, which received the Biofield Energy Healing Treatment remotely from eighteen renowned Biofield Energy Healers. The Biofield Energy Treated group (G3) showed 6.39% increased the CD4⁺ count compared with the disease control (G2) group. The level of IgM was significantly increased by 23.08% and 46.15% in the G3 group and Biofield Energy Treatment group *per se* at day -15 (G6), respectively compared with the G2 group. The total leucocyte count (TLC) and neutrophil were significantly increased by 16.15% and 26.69% ($p \leq 0.05$), respectively in the G3 group compared with the G2 group. Further, the levels of TLC, eosinophil, and monocyte were significantly increased by 70.57% ($p \leq 0.01$), 33.33%, and 8.50%, respectively in the G6 group compared with the G2 group. Low density lipoprotein (LDL) was reduced by 6.24% in the G3 group; while triglyceride (TG) and LDL were reduced by 12.12% and 16.99%, respectively in the G6 group compared with the G2 group. The levels of serum glutamate-pyruvate transaminase (SGPT), alkaline phosphatase (ALP), and total bilirubin were significantly decreased by 20.26%, 32.98%, and 25.00%, respectively in the G3 group compared with the G2 group. Further, SGPT and total bilirubin were reduced by 9.02% and 16.67%, respectively in the G6 group compared with the G2 group. Progesterone was significantly increased by 59.85% in the G3 group, compared with the G2 group. The data suggests that the Biofield Energy Treated test formulation and Biofield Energy Treatment group *per se* at day -15 have shown a significant immunomodulatory potential. The overall results demonstrated that the Biofield Energy Treated test formulation showed better immune response compared

with the untreated test formulation. These data also suggest that the Biofield Energy Treated test formulation can be used for autoimmune and inflammatory diseases, stress management and anti-aging by improving overall health.

Keywords: Biofield Energy Healers, The Trivedi Effect[®], Herbomineral Formulation, Immune-Modulation, CD4⁺/CD8⁺ Count, Progesterone, Stress Management, Anti-aging

1. Introduction

A biomarker is a substance or activity that can be measured in the blood or urine sample and serves as a marker for a specific biological activity. It is a characteristic to measured and evaluate as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention [1]. In the developing and developed countries alike, medicinal plant-derived drugs are continuously gaining popularity due to their natural origin, high safety, better therapeutic response, and less side effects. Many traditional and complementary medicines are derived from medicinal plants, minerals, and organic matter, which are commonly used for the prevention and treatment of many diseases. The therapeutic properties of plant extracts have been recognized and utilized worldwide since ancient times [2, 3]. Scientific studies have identified the immunomodulatory properties of medicinal plants, which can be further potentiated with the addition of some minerals that regulate the immune cells. These types of formulations are well-defined as herbomineral formulations and are the major target of pharmaceutical companies as daily dietary supplements. However, the use of traditional remedies has gained importance in cases when conventional medicines are ineffective for certain diseases, besides having natural, safe, and non-toxic nature. An herbal and traditional medicine are suitable candidates for new therapeutics due to their vast chemical diversity and various biological effects [4]. Several marketed medicinal formulations are in use, but the serious concern is the safety issues, as most of them are associated with the side effects. This has developed lead research to characterize some natural compounds along with the essential minerals, which are always required in minute quantity to boost the immune system and retain low profile toxicity [5]. With this respect, a new proprietary herbomineral formulation was formulated with a combination of the herb *Withania somnifera* (ashwagandha) root extract and three minerals viz. zinc, magnesium, and selenium. All the individual components have been reported with significant biological activities such as antioxidant, anti-inflammatory, anti-viral, and immune modulating [6-8]. In general, ashwagandha plant has been reported for immunomodulatory activity, antitumor, antibacterial effects, role in cancer treatment, and many more with available pre-clinical and clinical reports [9, 10]. Besides, the important minerals such as selenium, zinc, copper, magnesium, etc. have been reported for beneficial role in immunomodulation [6].

According to the scientific studies and clinical trials, Biofield Energy Healing Treatment has been reported

worldwide as an alternative treatment method, which has been known for its significant impact on various cancerous cells [11]. According to many scientific studies, Biofield Energy Healing have been reported to have significant outcomes that may prove to be a more cost effective an alternative to other approaches [12]. The use of Complementary and Alternative Medicine (CAM) are now rising as preferred models of treatment, among which Biofield Therapy (or Healing Modalities) is one approach that has been reported to have several benefits to enhance physical, mental, and emotional human wellness. However, Biofield Energy can exist in different forms such as kinetic, magnetic, potential, electrical, and electromagnetic. The human body has the power to produce low intensity electromagnetic signals known as the Biofield. Thus, a human has the ability to harness energy from the environment and transmit it to any living or nonliving object (s) around the globe. The objects always receive the energy and respond in a useful way. This process is known as Biofield Energy Healing Treatment (The Trivedi Effect[®]). Based on the literature data, Biofield Energy Treatment in terms of a Complementary and Alternative Medicine (CAM) approach was practiced worldwide [13]. The National Center of Complementary and Integrative Health (NCCIH) has recognized and accepted Biofield Energy Healing as a Complementary and Alternative Medicine (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. To this day, Biofield Energy Healing has had significant impact in the transformation of living organisms and nonliving materials including metals, polymers, ceramics, chemicals, and pharmaceutical compounds. In addition, Biofield Energy Healing Treatment (The Trivedi Effect[®]) outcomes have been published in numerous peer-reviewed science journals due to its significant impacts in many scientific fields such as cancer research [14, 15], microbiology [16-19], genetics [20-22], pharmaceutical science [23-26], agricultural science [27-30], materials science [31-34], biotechnology, nutraceuticals, and human health and wellness. In this study, the authors evaluated the impact of Biofield Energy Healing (The Trivedi Effect[®]) Treatment on the designated herbomineral formulation for immunomodulatory potential with respect to

the cellular and humoral immune response, hematological parameters, lipid profile, hepatic enzymes, and sex hormone in female *Sprague Dawley* rats.

2. Materials and Methods

2.1. Chemicals and Reagents

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. Pyrogallol, levamisole hydrochloride, and sodium carboxymethyl cellulose (Na-CMC) were procured from Sigma Chemical Co. (St. Louis, MO). All the other chemicals used in this experiment were analytical grade procured from India.

2.2. Laboratory Animals

Randomly breed female *Sprague Dawley* (SD) rats were purchased from M/s. Vivo Bio Tech Ltd., Hyderabad, India. The animals were divided into six groups (n=6) based on their body weight. Illumination was controlled to give 12 hours light and 12 hours dark cycle during the 24-hours period alongwith a temperature of $22 \pm 3^\circ\text{C}$, humidity of 30% to 70%. Standard rodent diet was procured from M/s. Golden feeds, Mehrauli, New Delhi, India was provided *ad libitum* to all the groups of animals during the experiment. The animals were received reverse osmosis filtered drinking water *ad libitum* by a water dispensing bottle. 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 that was obtained prior to carrying out the animal experiment.

2.3. Biofield Energy Treatment Strategies

The herbomineral 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. This Biofield Energy Treatment was provided to the test formulation through a group of eighteen Biofield Energy Healers who participated in this study and performed the Biofield Energy Treatment remotely. Eleven Biofield Energy Healers were remotely located in the U. S. A., four were remotely located in Canada, one in the UK, one in Russia and one in Ireland. The test herbomineral formulation was located in the research laboratory of Dabur Research Foundation, New Delhi, India. This Biofield Treatment was administered for 5 minutes through the Healer's Unique Energy Transmission process remotely to the test formulation under the laboratory conditions. Besides, one group of animals was also received Biofield Energy Treatment by the same Biofield Energy Healers under similar 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 purpose. The sham healer did not have any knowledge about the Biofield Energy Treatment. After that, the Biofield Energy Treated and untreated test formulation were returned in the similar sealed condition and kept in recommended storage condition.

2.4. Antigen (Sheep RBC)

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 and the samples 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

After 5 days of acclimatization, the animals were randomized and grouped based on the body weight. Normal control (G1) was received oral suspension of 0.5% carboxy methyl cellulose-sodium salt *via* gavage. The disease control group (G2) was received pyrogallol through intraperitoneal (*i. p.*) route at a dose of 100 mg/kg once daily for 7 days. The G3 and G4 group animals were received the Biofield Energy Treated and untreated test formulations, respectively at 1105.005 mg/kg b. wt, *per oral* (*p. o.*). The G5 animals received levamisole at a dose of 50 mg/kg *p. o.* The G6 animals were received the Biofield Energy Treatment *per se* at day -15. Further, all the animals except normal control group (G1) received pyrogallol at a dose of 100 mg/kg through *i. p.* route once daily from day 1 to day 7. The animals were treated with the Biofield Energy Treated and untreated herbomineral formulations to the G3 and G4 animals respectively, 1 hour before pyrogallol challenge in the morning once daily for 22 days. On day 7th and 13th, all the animals in the G2 to G6 except G1 were challenged with sheep red blood cells (sRBC) ($0.5 \times 10^9/100 \text{ gm}$; *i. p.*), as the antigenic material to sensitize them for immunological parameters. The animals were kept on overnight fasting on day 22. The next day on day 23 the animals were bled and the samples were subjected for humoral immune response (IgG and IgM), cellular immune response (CD4^+ and CD8^+), hematology, biochemistry, and sex hormone (progesterone). After bleeding all the animals were euthanized using CO_2 asphyxiation followed by exsanguination.

2.6. Assessment of Cellular and Humoral Immune Responses

For humoral immune response, the immunoglobulins such as IgG and IgM were estimated using 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 treated group were calculated compared with the vehicle control group.

2.7. Measurement of Hematology Parameters

On 23rd day of the experiment, blood was collected from the retro-orbital plexus using capillary tubes and hematology parameters such as total leukocyte count (TLC) and five parts differential leukocyte count (DLC) were analyzed using Hematology analyzer (Abbott Model-CD-3700).

2.8. Measurement of Lipid Profile and Hepatic Enzymes

Serum biochemistry parameters such as 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 in the test formulation.

2.9. Measurement of Sex Hormone

Progesterone level was analyzed in serum using commercial kits. The mean value was calculated for each group with SEM.

2.10. Statistical Analysis

The data were expressed as mean \pm standard error of mean

(SEM) and subjected to statistical analysis using Sigma Plot (Version 11.0). Student's *t*-test was done with a comparison among control and treatment groups. The $p \leq 0.05$ was considered as statistically significant.

3. Results and Discussion

3.1. Measurement of Cellular Responses

The effects of cellular immunomarkers such as CD4⁺ and CD8⁺ after administration of the Biofield Energy Treated and untreated test formulations in female rats are shown in the Figure 1 (A and B). The level of CD4⁺ counts in the normal control group (G1) was 34.62 ± 0.56 and it was significantly ($p \leq 0.001$) reduced by 47.14% in the disease control (18.30 ± 0.95). The reference item levamisole showed significant ($p \leq 0.01$) 42.95% increase the CD4⁺ counts compared with the disease control (G2). Further, the CD4⁺ counts were increased by 6.39% and 20.60% in the Biofield Energy Treated (G3) and untreated test formulation (G4), respectively compared with the G2 (Figure 1A). The level of CD8⁺ counts in the normal control (G1) was 24.20 ± 0.61 and it was significantly reduced by 40.33% ($p \leq 0.001$) to the disease control (14.44 ± 0.69). The levamisole showed 48.34% ($p \leq 0.05$) increase the CD8⁺ counts compared with the G2 group. Moreover, the Biofield Energy Treated and untreated test formulations exhibited 0.28% and 13.43% increase the level of CD8⁺ counts, respectively compared with the G2 group (Figure 1B).

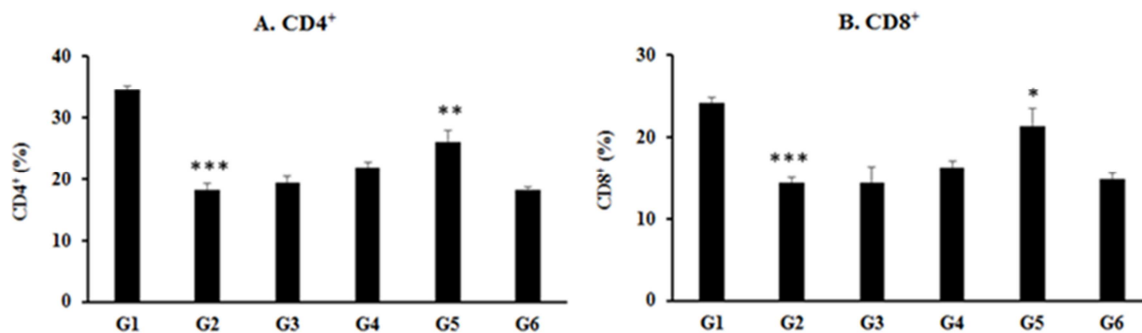


Figure 1. The effects of the Biofield Energy Treated and untreated test formulations on cellular immune-biomarkers (CD4⁺ and CD8⁺). G: Group; G1: Normal control; G2: Disease control; G3: Biofield Energy Treated test formulation; G4: Untreated test formulation; G5: Levamisole; G6: Biofield Energy Treatment group per se at day -15. All the values are represented as mean \pm SEM (n=6). * $p \leq 0.05$ and ** $p \leq 0.01$ vs G2 and *** $p \leq 0.001$ vs G1.

3.2. Measurement of Humoral Immune Responses

Antibody molecules are the product of B lymphocytes and plasma cells, are the central component to produce humoral immune responses. The IgG and IgM are the major immunoglobulins, which are involved in the complement activation, opsonization, neutralization of toxins, etc. The effect of the test formulation on IgM and IgG in female rats is shown in the Figures 2 and 3, respectively. The level of IgM was reduced by 31.58% in the disease control group (G2) compared with the normal control (G1). Further, IgM was increased by 23.08%, 23.08%, 7.69%, and 46.15% in the Biofield Energy Treated test formulation (G3), untreated test

formulation (G4), levamisole (G5), and Biofield Energy Treatment group per se at day -15 (G6) group, respectively compared with the G2 group (Figure 2). The level of IgG was increased by 12.41% in the G5 group compared with the G2 group (Figure 3).

In this experiment, the Biofield Energy Treated (G3) and Biofield Energy Treatment group per se at day -15 (G6) showed significant increase the level of IgM compared with the disease control group (G2). The enhanced level of IgM could be due to the treatment of Biofield Energy from renowned Biofield Energy Healers to the test formulation. The result could be due to presence of ashwagandha and other minerals like zinc. Deficiency of zinc induces the

production of a low-molecular-weight humoral factor [36]. As per literature it was well reported that ashwagandha enhance the immune function by increasing production of immunoglobulin. Zinc nutriture had influenced the balance between cell-mediated immunity (lymphocyte TH1 elements and functions) and humoral immunity (TH2 elements and functions) [37].

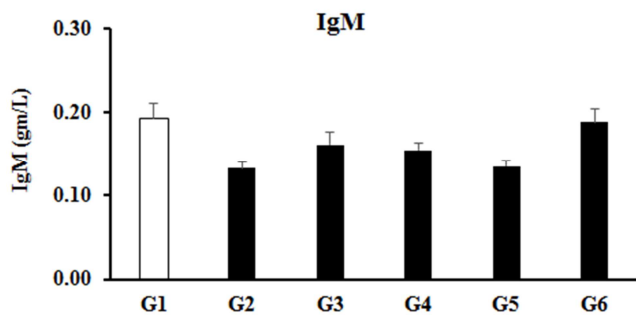


Figure 2. The effects of the Biofield Energy Treated and untreated test formulations of immunoglobulin (IgM). All the values are represented as mean \pm SEM (n=6).

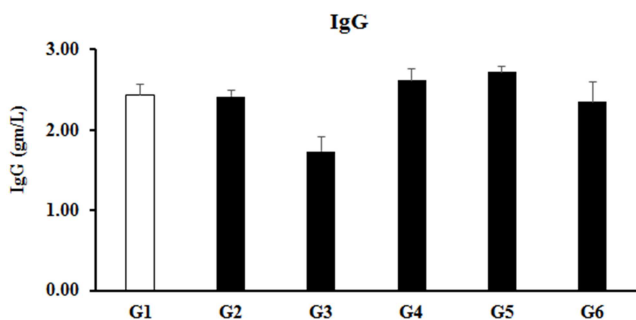


Figure 3. The effects of the Biofield Energy Treated and untreated test formulations of immunoglobulin (IgG). All the values are represented as mean \pm SEM (n=6).

3.3. Measurement of Hematology Parameters

The results of the hematology parameters such as total and differential leucocytes counts are shown in the Table 1.

Table 1. Effects of the Biofield Energy Treated and untreated test formulations on hematological parameters.

Group	TLC ($10^3/\text{mm}^3$)	Neutrophil (%)	Lymphocyte (%)	Eosinophil (%)	Monocyte (%)
G1	6.68 \pm 0.74	23.83 \pm 1.72	72.00 \pm 1.71	2.00 \pm 0.37	2.17 \pm 0.48
G2	5.98 \pm 0.42	17.50 \pm 1.69	79.17 \pm 1.76	1.50 \pm 0.22	2.00 \pm 0.52
G3	6.95 \pm 0.65	22.17 \pm 0.70*	74.33 \pm 1.38	1.50 \pm 0.34	2.00 \pm 0.52
G4	7.80 \pm 1.29	13.50 \pm 1.95	81.33 \pm 2.28	2.83 \pm 0.75	2.33 \pm 0.21
G5	6.23 \pm 1.44	11.67 \pm 4.11	84.00 \pm 4.86	2.50 \pm 1.22	2.00 \pm 0.89
G6	10.20 \pm 1.23*	13.00 \pm 1.29	82.83 \pm 1.05	2.00 \pm 0.45	2.17 \pm 0.31

Analysis of the hematological profile like total and differential (5 parts) counts of white blood corpuscles after consecutive 22 days treatment of the test formulation in female rats. All the values are represented as mean \pm SEM (n=6). TLC: Total leukocyte count; %: Percentage * $p \leq 0.05$ (compared to the disease control).

3.4. Measurement of Lipid Biomarkers

The effects of the Biofield Energy Treated and untreated test formulations on serum lipid profile are presented in the Table 2. Immunosuppression was induced by pyrogallol showed reduction of all the lipid parameters such as total

Immunosuppression induced by pyrogallol showed reduction of TLC, neutrophils, eosinophils, and monocytes by 10.48%, 25.00%, and 7.83%, respectively in the disease control group (G2) compared with the normal control group (G1). The TLC was increased by 16.15%, 4.18%, and 70.57% ($p \leq 0.05$), in the Biofield Energy Treated test formulation (G3) levamisole (G5) and Biofield Energy Treatment group *per se* at day -15 (G6) compared with the G2 group. The level of neutrophil was significantly ($p \leq 0.05$) increased by 26.69% in the G3 group, while it was reduced in the other groups compared with the G2 group. Galioto *et al.* demonstrated the innate and protective immune responses of neutrophil in mice model [38]. In this experiment, authors have found the increased level of neutrophil in the Biofield Energy Treated group (G3), while in other group did not raise the level of neutrophil. It is assumed that the increased level of neutrophil in the G3 group might be due to the effect of Healer's Biofield Energy Treatment to the test formulation. The level of lymphocyte was increased by 6.10% and 4.62% in the G5 and G6 groups, respectively compared with the G2 group. The eosinophil level was increased by 66.67% and 33.33% in the G5 and G6 groups, respectively; while the value was unchanged in the G3 group compared to the G2.

From the literature, it was reported that the increased level of eosinophil is destructive to the end-stage effector cells and have a role in parasitic infections and allergic reactions by the release of granule-derived cytotoxic proteins. Due to multifunctional activities of eosinophil it was demonstrated involvement in the diverse inflammatory and physiologic immune responses [39]. In this experiment, the elevated level of eosinophil in the Biofield Energy Treatment group *per se* at day -15 (G6) could be helpful to restore the immune response especially in the gastrointestinal tract. The study results demonstrated that G6 group animals showed significant elevation (33.33%) of eosinophil counts compared with the disease control group (G2). Further, the monocyte level was increased by 8.5% in the G6 group compared with the disease control (G2).

cholesterol (TC), triglyceride (TG), HDL, LDL, and VLDL by 17.37%, 12.98%, 3.47%, 25.51%, and 13.32%, respectively in the disease control group (G2) compared with the normal control group (G1). The levels of TG, HDL, LDL, and VLDL were reduced by 1.36%, 6.24%, 6.24%, and 1.68%, respectively in the Biofield Energy Treated group

(G3) compared with the G2. The levamisole (G5) showed 37.59%, 30.11%, 39.44%, and 30.24% increase the levels of TC, TG, LDL, and VLDL, respectively compared with the G2 group. There was a significant reduction of TC, TG, HDL, LDL, and VLDL by 12.12%, 2.34%, 5.16%, 16.99%, and 2.23%, respectively in the Biofield Energy Treatment group *per se* at day -15 (G6) compared with the G2 group. Udayakumar *et al.* demonstrated the hypolipidaemic effects

of *Withania somnifera* root extracts in rats by restoring the elevated levels of various lipid biomarkers which was induced by alloxan. In this experiment, the levels of various tested lipid biomarkers were suppressed in the Biofield Energy Treated test formulation (G3), which might be due to the effect of Biofield Energy Treatment to the test formulation. The findings were well corroborated with the literature [40].

Table 2. Effects of the Biofield Energy Treated and untreated test formulations on the lipid biomarkers in serum sample.

Group	Total Cholesterol (mg/dL)	Triglyceride (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)
G1	63.33 ± 4.82	39.68 ± 1.01	18.73 ± 1.22	36.67 ± 13.80	7.88 ± 0.50
G2	74.33 ± 8.83	44.83 ± 2.22	19.38 ± 0.56	46.02 ± 20.69	8.93 ± 1.10
G3	74.60 ± 4.64	44.22 ± 3.50	18.17 ± 1.23	43.15 ± 14.29	8.78 ± 1.71
G4	77.08 ± 2.80	52.93 ± 5.07	16.65 ± 1.23	49.87 ± 9.25	10.57 ± 2.49
G5	102.27 ± 5.85**	58.33 ± 2.79*	17.48 ± 1.87	64.17 ± 23.57	11.63 ± 1.36
G6	65.32 ± 5.02	43.78 ± 3.57	18.38 ± 0.92	38.20 ± 11.40	8.73 ± 1.76

All the values are represented as mean ± SEM (n=6). HDL: High density lipoprotein; LDL: Low density lipoprotein; VLDL: Very low density lipoprotein; mg/dL: Milligram per deciliter ** $p \leq 0.01$ and * $p \leq 0.05$ (compared to the disease control).

3.5. Measurement of Hepatic and Cardiac Biomarkers

The effect of the test formulation on the hepatic and cardiac parameters is depicted in the Table 3. Disease control group (G2) showed a significant increased the hepatic enzymes such as SGOT, SGPT, and ALP by 30.51%, 22.12%, and 4.73%, respectively compared with the normal control group (G1). The level of cardiac biomarker like CK-MB was significantly increased by 49.12% in the G2 group compared with the G1. The level of SGPT was significantly reduced by 20.26% and 9.02% in the Biofield Energy Treated test formulation (G3) and Biofield Energy Treatment group *per se* at day -15 (G6), respectively, compared with the G2 group. The level of ALP was significantly reduced by 32.98% ($p \leq 0.01$), 6.33%, and 3.86% in the G3 group, levamisole (G5), and G6 group, respectively compared with the G2 group. The CK-MB level was reduced by 1.94% in the G6 group compared with the G2 group. Moreover, the level of total bilirubin was significantly reduced by 25%, 16.67%, and 16.67% in the G3 group, untreated test formulation (G4), and G6 group, respectively compared with the G2 Group.

The Biofield Energy Treated test formulation group (G3) showed a protective effect on the hepatic biomarkers compared with the untreated test formulation

group (G4). The serum hepatic enzyme estimation is defined as the useful quantitative marker for the extent and type of damage to the hepatocellular damage. An increased level of enzymes reflects the structural damage of liver, which results an increased level of hepatic enzymes in the blood [41]. However, reports suggest that administration of ashwagandha has protective activity on the hepatic enzymes by normalizing the hepatic biomarkers [42]. Further, it was also reported that the minerals present in the test formulation have significance importance in liver protection, which could prevent the prognosis of liver disease by stabilizing the membrane activity and hepatic biomarkers [43]. In this experiment, the Biofield Energy Treated test formulation and Biofield Energy Treatment group *per se* at day -15 (G6) showed significant decreased the level of SGPT by 20.27% and 9.09%, respectively compared with the disease control (G2) which would be beneficial in most of the immunodeficiency patients. Thus, it is assumed that the reduction of SGPT value might be due to the effect of Biofield Energy Healing to the test formulation. From literature it was reported that ashwagandha reduced the level of SGPT level in rats [44]. This findings could be due to the presence of ashwagandha root extract and other minerals in the test formulation

Table 3. Effects of the Biofield Energy Treated and untreated test formulations on the hepatic and cardiac biomarkers in female rats.

Group	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	CK-MB (U/L)
G1	137.18 ± 13.73	32.33 ± 1.17	190.50 ± 23.19	283.93 ± 70.60
G2	179.03 ± 18.58	39.48 ± 4.13	199.52 ± 10.99	423.40 ± 51.91
G3	188.58 ± 9.66	31.48 ± 2.58	133.72 ± 24.42**	643.58 ± 63.45
G4	194.95 ± 6.60	39.83 ± 5.26	266.88 ± 42.83	516.47 ± 76.37
G5	205.23 ± 15.12	39.32 ± 5.19	186.90 ± 7.10	804.45 ± 64.07
G6	185.78 ± 32.96	35.92 ± 10.32	191.82 ± 9.35	415.17 ± 53.54

Table 3. Continued.

Group	Tot. BL (mg/dL)	Tot. Prot. (g/dL)	A (g/dL)	G (g/dL)	A/G ratio
G1	0.11 ± 0.02	4.93 ± 0.11	3.42 ± 0.03	1.52 ± 0.09	2.25 ± 0.13
G2	0.12 ± 0.02	5.22 ± 0.14	3.45 ± 0.04	1.77 ± 0.14	1.97 ± 0.13
G3	0.09 ± 0.01	5.33 ± 0.17	3.50 ± 0.06	1.83 ± 0.12	1.90 ± 0.11
G4	0.10 ± 0.01	5.30 ± 0.12	3.53 ± 0.04	1.77 ± 0.09	1.97 ± 0.10
G5	0.21 ± 0.11	5.42 ± 0.03	3.38 ± 0.03	2.03 ± 0.04	1.62 ± 0.07
G6	0.10 ± 0.01	5.00 ± 0.08	3.37 ± 0.02	1.63 ± 0.08	2.05 ± 0.12

All the values are represented as mean ± SEM (n=6). SGOT: Serum glutamic oxaloacetic transaminase; SGPT: Serum glutamate-pyruvate transaminase; ALP: Alkaline phosphatase; CK-MB: Creatine kinase-myocardial band; Tot. BL: Total bilirubin; Tot. Prot.: Total protein; A: Albumin; G: Globulin; A/G: Albumin/Globulin ratio; U/L: Unit per liter; mg/dL: Milligram per deciliter. * $p \leq 0.01$ (compared with the disease control).

3.6. Measurement of Sex Hormone

The effect of the Biofield Energy Treated and untreated test formulations on progesterone level in female rats are shown in the Figure 4. The level of progesterone was reduced by 19.38% in the disease control group (G2) compared with the normal control group (G1). The level of progesterone was significantly increased by 59.85%, 7.46%, and 117.50% ($p \leq 0.05$) in the Biofield Energy Treated (G3), untreated (G4), and levamisole group (G5), respectively compared with the G2 group. In this study resulted, the elevation of serum progesterone in the Biofield Energy Treated test formulation (G3), but the data was not statistically significant compared with the disease control group (G2). Scientific literature evidence that zinc is responsible for the gonadal function [45, 46]. Thus, the elevation of progesterone could be due to the Biofield Energy Treatment of the herbomineral test formulation, in which zinc is one of the component. Overall, it is presumed that the improvement of progesterone in female rats could be due to the Biofield Energy Treatment (The Trivedi Effect®) to the test formulation.

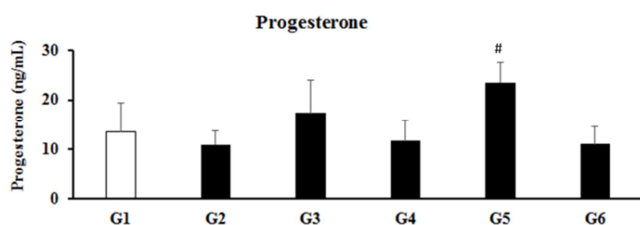


Figure 4. The effects of the Biofield Energy Treated and untreated test formulations of progesterone in female rats. All the values are represented as mean ± SEM (n=6). # $p \leq 0.05$ (compared to the normal control).

4. Conclusions

The current results indicated that, the level of CD4⁺ counts was increased by 6.39% in the Biofield Energy Treated test formulation group (G3) compared with the disease control group (G2). The level of IgM was increased by 23.08% and 46.15% in the G3 group and Biofield Energy Treatment group *per se* at day -15 group (G6), respectively compared with the G2 group. The levels of TLC and neutrophil were significantly increased by 16.15% and 26.69% ($p \leq 0.05$), respectively in the G3 group compared with the G2 group. Moreover, the levels of TLC and eosinophil were significantly increased by 70.57% ($p \leq 0.01$) and 33.33%,

respectively in the G6 group compared with the G2 group. There was a substantial reduction of the levels of TC, TG, HDL, LDL, and VLDL in the G6 group compared with the G2 group. Additionally, the levels of SGPT, ALP, and total bilirubin were significantly reduced by 20.26%, 32.98%, and 25.00%, respectively in the G3 group compared with the G2 group. The level of progesterone was significantly increased by 59.85% in the G3 group, compared with the G2 group. Therefore, it is assumed that the Biofield Energy Treated test formulation and Biofield Energy Treatment group *per se* at day -15 shown a significant impact on immune, cardiac, and lipid biomarkers in female *Sprague Dawley* rats. The Trivedi Effect®-Biofield Energy Healing Treatment administered remotely by the eighteen Biofield Energy Healers enhanced the herbomineral test formulation's anti-inflammatory and immunomodulatory properties without any adverse effect to the animals throughout the exposure period. Overall, it can be concluded that the novel Biofield Energy Treated herbomineral formulation showed better immunomodulatory action compared with the untreated test formulation against many infectious diseases *viz.* Lupus, Addison Disease, Celiac Disease (gluten-sensitive enteropathy), Dermatomyositis, Graves' Disease, Hashimoto Thyroiditis, Multiple Sclerosis, Myasthenia Gravis, Pernicious Anemia, Aplastic Anemia, Reactive Arthritis, Rheumatoid Arthritis, Sjogren Syndrome, Systemic Lupus Erythematosus, Type 1 Diabetes, Alopecia Areata, Crohn's Disease, Fibromyalgia, Vitiligo, Psoriasis, Scleroderma, Chronic Fatigue Syndrome and Vasculitis, as well as inflammatory disorders such as Asthma, Ulcerative Colitis, Alzheimer's Disease, Atherosclerosis, Dermatitis, Diverticulitis, Hepatitis, Irritable Bowel Syndrome, Parkinson's Disease, etc. 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 autoimmune disorders, anti-aging, stress prevention and management, and for 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.

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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