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Impact of Biofield Energy Treated (The Trivedi Effect®) Herbomineral Formulation on the Immune Biomarkers and Blood Related Parameters of Female *Sprague Dawley* Rats

Mahendra Kumar Trivedi[1], Alice Branton[1], Dahryn Trivedi[1], Gopal Nayak[1], Aileen Carol Lee[1], Aksana Hancharuk[1], Carola Marina Sand[1], Debra Jane Schnitzer[1], Rudina Thanasi[1], Eileen Mary Meagher[1], Faith Ann Pyka[1], Gary Richard Gerber[1], Johanna Catharina Stromsnas[1], Judith Marian Shapiro[1], Laura Nelson Streicher[1], Lorraine Marie Hachfeld[1], Matthew Charles Hornung[1], Patricia M. Rowe[1], Sally Jean Henderson[1], Sheila Maureen Benson[1], Shirley Theresa Holmlund[1], Stephen Phillip Salters[1], Mayank Gangwar[2], Snehasis Jana[2, *]

[1]Trivedi Global, Inc., Henderson, Nevada, USA

[2]Trivedi Science Research Laboratory Pvt. Ltd., Bhopal, Madhya Pradesh, India

Email address:

publication@trivedieffect.com (S. Jana)

[*]Corresponding author

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Abstract: The aim of the present study was to evaluate the immunomodulatory potential of Biofield Energy Treatment (The Trivedi Effect®) on a new proprietary compound composed of herbs and minerals (herbomineral formulation). The test formulation contains the mixture of the herbal root extract ashwagandha and the minerals (zinc, magnesium, and selenium). 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 and defined as the Biofield Energy Treated formulation. The immunomodulatory parameters *viz.* humoral immune response (IgG and IgM), cellular immune response ($CD4^+/CD8^+$), hematological parameters, lipid profile, hepatic enzymes, and sex hormones (progesterone and estrogen) in female *Sprague Dawley* rats were analyzed. The results of humoral immune response data showed decreased levels of IgG and IgM by 80.54% and 78.94% respectively, in the Biofield Energy Treated test formulation group (G3) compared with the disease control group (G2). Estimation of cellular immune response data revealed a significant ($p \leq 0.01$) increase in the ratio of $CD4^+/CD8^+$ by 161.71% in the G3 group, while it was increased by 100.50% in the untreated test formulation group (G4) compared with the G2 group. Thus, the humoral and cellular immune responses were significantly altered in the G3 as compared with the G4 group. The TLC and neutrophils counts were significantly increased by 11.34% and 1.34% respectively, in the G3 compared with the G2 group. Lipid profile data suggested that the Biofield Energy Treated test formulation showed improvement in the tested parameters compared with the untreated test formulation group. However, significant improvement was found in the tested hepatic biomarkers *i.e.* decreased levels of the SGOT, SGPT, and CK-MB by 14.28%, 8.54%, and 20.79% in the G3 group compared with the G2 group. The levels of total protein, alkaline phosphatase, albumin, and globulin were increased by 11.93%, 1.24%, 6.10%, and 20.74% respectively, in the G3 compared with the G2 group. Consequently, the levels of progesterone and estrogen were significantly increased by 199.86% and 50.19%, respectively in G3 with respect to the G2 group. In conclusion, overall data suggest that The Trivedi Effect®-Biofield Energy Healing Treatment on the herbomineral formulation can be used for autoimmune and inflammatory diseases, stress management, and anti-aging by improving overall health.

Keywords: Biofield Energy Healing Treatment, Biofield Energy Healers, The Trivedi Effect®, Herbomineral Formulation, Immune-Modulation, Autoimmune Diseases, Inflammatory Diseases, Anti-Aging

1. Introduction

The therapeutic properties of herbal formulations have been recognized and utilized worldwide since ancient times. Plant products and their extracts are used in both allopathic health care systems as well as complementary and alternative health care systems in order to improve the health and immune system [1, 2]. The herbal drugs in the traditional systems of medicine are widely used against many immunomodulatory activities and autoimmune disorders, but there are limited experimental studies based upon herbomineral formulations that combine herbs or plant extracts with minerals. Medicinal plants and minerals have been widely reported to have many healing properties including anti-inflammatory, anti-diabetic, anti-stress activities, autoimmune, antiaging, and many more. However, due to high toxicity and several adverse effects of the available synthetic immunomodulatory drugs, the interest has been shifted towards the use of Complementary and Alternative Medicine (CAM) that significantly modulates the immune system to fight against diseases [3, 4]. Many medicinal plants and minerals have been reported to boost the immunity that helps to improve the overall quality of life (QoL) by maintaining the organic body resistance. The pharmacological activities of the plants secondary metabolites and minerals have been found to be a relation with the immunostimulatory effect [5]. Immunomodulatory therapies have now been considered as an alternative to the conventional approach in many disease conditions. For the estimation of immunomodulatory effect, estimation of immune biomarkers have been considered as the gold standard. In general, medicinal plants and minerals serves as a therapeutic and safe alternative therapeutic approach with respect to the synthetic drugs. Based on the literature, a new proprietary herbomineral formulation was formulated with a combination of the *Withania somnifera* (ashwagandha) root extract and three minerals *viz.* zinc, magnesium, and selenium. Each constituent of the test formulation is commonly used in nutraceuticals and various herbal medicines for many important activities such as immune modulating properties, anti-inflammatory, antioxidant, anti-infective, antiaging, and anti-viral activities [6-9]. Ashwagandha biological activity is mainly reported due to the presence of withanolides, and it is used as complementary medicine in alternative therapy [10]. Apart from its common attributes such as antibacterial, immunomodulatory and antitumor effects, many clinical and preclinical data have been available with respect to the immunomodulatory potential [11]. The importance of minerals such as selenium, zinc, and magnesium to modulate the immune system and their synergistic impact with herbal drugs have been well-defined [7]. This formulation can be used for better therapeutic effect in immune compromised patients affected with cardiovascular diseases, aging and stress related diseases, cancer, and autoimmune disorders. Along with the herbomineral formulation, 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 the Biofield Energy Treatment on the herbomineral formulation for its immunomodulatory potential.

In recent years, Biofield Energy Treatment (The Trivedi Effect®) has been reported worldwide as an alternative treatment method which has been known for its significant impact on various cancerous cells [12]. According to many scientific studies, Biofield Energy Healing has been reported to have significant outcomes that may prove to be a more cost effective alternative approaches [13]. Amidst many CAM therapies, there have been an extensive number of scientific reports that use Biofield Energy Therapy (or Healing Modalities) as the preferred model of treatment, with several benefits to enhance the physical, mental, and emotional human wellness. Additionally, holistic medicine/integrative medicine addresses not only the entirety of the body, but the mind and spirit as well. The human body has the power to produce a low intensity electromagnetic signal known as the Biofield [14]. Thus, a human has the ability to harness energy from the environment (Life Force) and transmit it into living organisms and nonliving materials without any adverse effects and in a manner that is more cost-effective than more conventional methods. This process is known as Biofield Energy Healing Treatment (The Trivedi Effect®). Based on the literature data, Biofield Energy Treatment in terms of a CAM approach is practiced worldwide [15] in addition to herbal medicine. The National Center of Complementary and Integrative Health (NCCIH) has recognized and accepted Biofield Energy Healing as a holistic 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 a significant impact in the transformation of living organisms and nonliving materials. Even further, Biofield Energy Healing Treatment (The Trivedi Effect®) has been published in numerous peer-reviewed science journals in different fields such as cancer research [16], microbiology [17-19], genetics [20, 21], pharmaceutics [22, 23], agricultural [24, 25], materials science [26-28], biotechnology [29, 30],

nutraceuticals [31, 32], and human health and wellness.

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

2. Materials and Methods

2.1. Chemicals and Reagents

The chemicals such as pyrogallol and carboxymethyl cellulose sodium were purchased from Sigma Chemical Co. (St. Louis, MO). *Withania somnifera* (ashwagandha) root extract powder (≥5% of total withanolides) 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. Levamisole hydrochloride was procured from Sigma, USA. All other chemicals used in this experiment were of analytical grade available locally.

2.2. Laboratory Animals

A total number of 30 apparently healthy female *Sprague Dawley* rats, weighing between 150-250 grams, were used for the study. Rodent laboratory diet and drinking tap water were provided *ad libitum* 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 5 days prior to the experiment, and 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 that was obtained prior to carrying out the animal experiment.

2.3. Biofield Energy Treatment Strategies

The test formulation was divided into two parts, one part of the test formulation was treated with Biofield Energy by renowned Biofield 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 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, two in Finland, and one in Albania, while the test herbomineral formulation was located in the research laboratory of Dabur Research Foundation near New Delhi in Ghaziabad, India. This Biofield Energy Treatment was administered for 5 minutes through the Healers' 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 the untreated test samples were kept in similar sealed conditions and used for the study.

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 rats [33].

2.5. Experimental Procedure

After 5 days of acclimatization, the animals were grouped (G) based on the body weight. G1 (normal control) received oral suspension of 0.5% carboxy methyl cellulose-sodium salt *via* gavage. G2 (disease control) group animals received pyrogallol at a dose of 100 mg/kg through intraperitoneal (*i. p.*) route once daily for 7 days. G3 group animals received the Biofield Treated test formulation (1105.005 mg/kg b. wt, *p. o.*). G4 group animals received the untreated test formulation at the same dose orally, while the G5 group animals received levamisole at a dose of 50 mg/kg *p. o.* from day 1 to day 22. 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 7. The animals were treated with the Biofield Energy Treated and the untreated herbomineral formulation to the G3 and G4 animals respectively, 1 hour before pyrogallol challenge in the morning once daily for 22 days. On day 7 and 13, all the animals in G2 to G5 except normal control were challenged with sheep red blood cells (sRBC) (0.5 X 10^9/100 gm; *i.p.*), as the antigenic material to sensitize them for immunological parameters. On day 13^{th} and 20^{th}, blood was collected from retro orbital plexus and subjected to hemagglutination test to evaluate the immune response. On day 22^{nd}, the animals were kept under fasting overnight and on the next day, blood was collected again from retro orbital plexus from each animal under anaesthesia using isoflurane for haematological parameters and serum was analysed for biochemical examination. At the end of the study, animals were euthanized by CO_2 asphyxiation as per in-house approved standard protocol.

2.6. Assessment of Cellular and Humoral Responses

Humoral immune response identification includes IgG and IgM estimated using Mini Vidas, Biomeurix (French) from serum, using commercially available kits. Flow cytometry was used to evaluate the $CD4^+$ and $CD8^+$ cells count and its

ratio in blood as a measure of the cellular immune response. The mean values were calculated for each group. The percent change in the test formulation group was calculated as compared to 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 differential leukocyte count (DLC) such as, neutrophil, lymphocyte, monocyte, and eosinophil were evaluated using Hematology analyzer (Abbott Model-CD-3700).

2.8. Measurement of Lipid Profile and Hepatic Enzymes

Serum biochemistry parameters like 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

The level of progesterone and estrogen were analyzed in serum in all the experimental groups using commercial kits. The% change in hormones level was calculated and compared.

2.10. Statistical Analysis

All the data were expressed as mean ± standard error of mean (SEM) using Student's *t*-test to ascertain the statistical difference between the disease control and treated groups of the experiment. A probability level of $p \leq 0{\cdot}05$ was considered as statistically significant.

3. Results and Discussion

3.1. Measurement of Humoral Response

The effect of the test formulation on immunoglobulins (IgG and IgM) after administration is shown in the Figure 1 (A and B). The values of IgG (in g/L) in the normal control (G1), disease control (G2), Biofield Energy Treated test formulation (G3), untreated test formulation (G4) and levamisole (G5) were 2.43 ± 0.13, 2.21 ± 0.13, 0.43 ± 0.13, 2.02 ± 0.20, and 2.25 ± 0.26 g/L, respectively. Similarly, the level (in g/L) of IgM in G1, G2, G3, G4, and G5 were 0.19 ± 0.02, 0.19 ± 0.02, 0.04 ± 0.02, 0.23 ± 0.04, and 0.19 ± 0.02 g/L, respectively. Overall, the levels of IgG and IgM were significantly decreased by 80.54% and 78.94% respectively, in the Biofield Energy Treated test formulation group (G3) as compared to the G2 group. Besides, the level of IgG was increased by 1.81% in the levamisole group (G5), while the level of IgM was increased by 21.05% in the untreated test formulation group (G4) as compared with the disease control group.

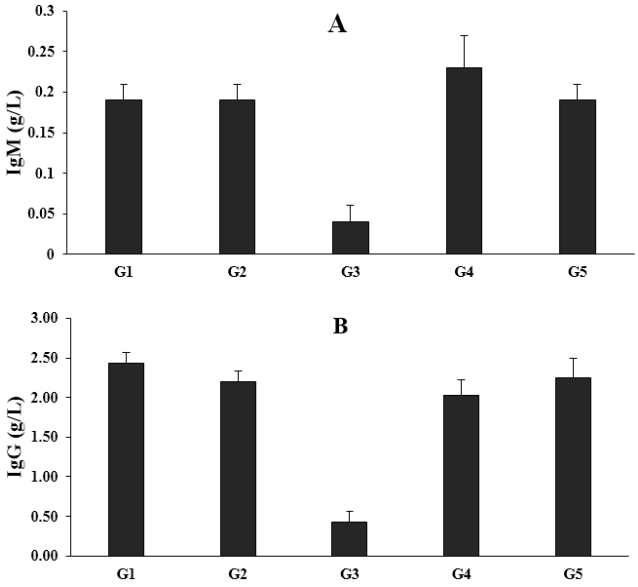

Figure 1. *Effect of the test formulation on (A) IgM and (B) IgG level. G1: Normal control; G2: Disease control (Pyrogallol); G3: Biofield Energy Treated test formulation; G4: Untreated test formulation; G5: Reference compound (Levamisole). All values are represented as mean ± SEM (n=6).*

Overall, the humoral immunity of animals after administration of the Biofield Energy Treated test formulation was altered, which might be due to the constituents of the test formulation such as ashwagandha [34, 35] and minerals such as zinc, selenium, and magnesium [36] have been reported for beneficial effect of immune function. Thus, the results exhibited significant alteration in the levels of IgG and IgM in the Biofield Energy Treated test formulation as compared with the disease control and untreated test formulation.

3.2. Measurement of Cellular Biomarkers

Cellular immune biomarkers results after oral administration of the test formulations are presented in the Figure 2, with respect to the ratio of $CD4^+/CD8^+$. The ratio of $CD4^+/CD8^+$ in the normal control (G1), disease control (G2), Biofield Energy Treated test formulation (G3), untreated test formulation (G4), and levamisole (G5) groups were 1.44, 3.97, 10.39, 7.96, and 6.07 respectively. The ratio ($CD4^+/CD8^+$) in the Biofield Energy Treated test formulation group was significantly increased by 161.71%, while the untreated test formulation showed 100.50% increase as compared with the disease control group. However, the levamisole group also showed significant increase by 52.89% as compared with the disease control group.

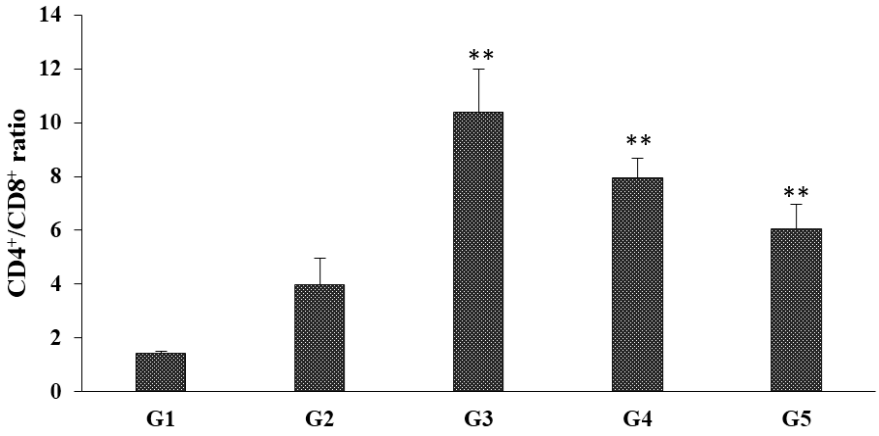

Figure 2. *The effects of the test formulations on the ratio of cellular biomarker ($CD4^+$ and $CD8^+$) after 24 consecutive days of treatment on various groups (G1 to G5) in female SD rats. All the values are represented as mean ± SEM (n=6). $^{**}p \leq 0.01$ compared with the disease control group.*

Natural killer (NK-cells), T and B-lymphocytes cells are classified as the lymphocytes, while $CD4^+$ and $CD8^+$ are directly correlated with the immune system of the body. The $CD4^+$ cells have the capacity to protect and fight against infections, while $CD8^+$ cells can kill cancer cells and other associated invaders. However, its ratio directly reflects the health of immune system and a higher ratio of $CD4^+/CD8^+$ suggest stronger immune system [37, 38]. Our results concluded that The Trivedi Effect® has the significant capability to improve the immune system that could help to fight against various infections. Overall, the Biofield Energy Treated test formulation showed immunomodulation potential, which was highly significant in comparison with the untreated test formulation against many autoimmune, anti-inflammatory and antiaging disorders.

3.3. Measurement of Hematology Parameters

The results of the hematology profile in the Biofield Energy Treated and the untreated test formulation groups are summarized in the Table 1. The results showed the hematology profile was increased in the levels of total leukocyte count (TLC) and neutrophils, while decreased levels in case of lymphocytes and monocytes in the Biofield Energy Treated test formulation as compared with the disease control group. The levels of TLC and neutrophils in the Biofield Energy Treated test formulation were reported as 9.82 ± 0.78 (thousand/mm^3) and 12.67 ± 0.76 (%), which suggest 11.34% and 1.34% increased levels of TLC and neutrophils, respectively as compared with the disease control group. However, the lymphocyte and monocyte levels were slightly decreased by 1.17% and 0.17% in the Biofield Energy Treated test formulation (G3) as compared with the disease control group.

Table 1. *Hematology profile of female Sprague Dawley rats after oral administration of the test formulation.*

Group (G)	TLC (thousand/mm^3)	Neutrophils (%)	Lymphocytes (%)	Eosinophils (%)	Monocyte (%)
1	6.68 ± 0.74	23.83 ± 1.72	72.00 ± 1.71	2.00 ± 0.37	2.17 ± 0.48
2	8.82 ± 0.71	11.33 ± 1.02	85.50 ± 1.09	1.17 ± 0.17	2.00 ± 0.26
3	9.82 ± 0.78	12.67 ± 0.76	84.33 ± 0.99	1.17 ± 0.17	1.83 ± 0.17
4	9.47 ± 1.23	15.33 ± 1.61	81.33 ± 1.67	1.33 ± 0.21	2.00 ± 0.26
5	6.18 ± 0.38	16.17 ± 1.68	80.67 ± 1.96	1.33 ± 0.21	1.83 ± 0.31

TLC: Total leukocyte count; G1: Normal control; G2: Disease control (Pyrogallol); G3: Biofield Energy Treated test formulation; G4: Untreated test formulation; G5: Reference compound (Levamisole). All values are represented as mean ± SEM (n=6).

Scientific literature data suggested that the hematological parameters can be improved by administration of various herbal extracts such as ashwagandha [39], wild mint [40], *Afzelia Africana* [41], and many more. However, the effects of important minerals like zinc [42], selenium [43], and magnesium [44] were widely reported with improved hematological and biochemical profiles. In the present experimental study, the test herbomineral formulation constituents might be responsible for the improved hematological activity. However, after the Biofield Energy Healing Treatment, the results were significantly improved as compared with the untreated test formulation. This suggests that The Trivedi Effect® has the capacity to improve the overall hematological profile of the test formulation against many inflammatory disorders.

3.4. Measurement of Lipid Profile

Analysis of lipid profile after treatment with the test formulation in female SD rats is tabulated in Table 2. The results showed an alteration in the tested parameters such as total cholesterol, triglycerides, HDL, LDL, VLDL in the Biofield Energy Treated test formulation group (G3) as compared with the disease control (G2) and untreated group (G4). Although, the total cholesterol, triglycerides, LDL, and

VLDL were increased by 5.80%, 17.09%, 6.65%, and 17.19% in the Biofield Energy Treated test formulation (G3) as compared with the G2 group. In case of the untreated test formulation, the levels of total cholesterol, triglycerides, LDL, and VLDL were significantly increased by 32.74%, 32.50%, 45.43%, and 32.40%, respectively as compared with the disease control group. The Levamisole group showed an increased level of total cholesterol, HDL, LDL and decreased levels of serum triglycerides and VLDL. Overall, data suggest that the Biofield Energy Treated test formulation showed a significant improved lipid profile as compared with the untreated test formulation.

Table 2. *Analysis of lipid profile parameters after treatment with the test formulation in the female Sprague Dawley rats.*

Group (G)	Total Cholesterol (mg/dL)	Triglyceride (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)
1	63.33 ± 4.82	39.68 ± 1.01	18.73 ± 1.22	36.67 ± 5.64	7.88 ± 0.21
2	65.48 ± 2.04	55.47 ± 8.80	13.53 ± 0.55	40.90 ± 1.99	11.05 ± 1.76
3	69.28 ± 6.12	64.95 ± 15.16	12.72 ± 1.08	43.62 ± 3.81	12.95 ± 3.03
4	86.92 ± 5.29	73.50 ± 9.74	12.80 ± 0.79	59.48± 4.32	14.63 ± 1.95
5	92.10 ± 7.12	44.18 ± 10.30	15.33 ± 1.12	67.95± 6.33	8.82 ± 2.05

HDL: High density lipoprotein; LDL: Low density lipoprotein; VLDL: Very low density lipoprotein; G1: Normal control; G2: Disease control (Pyrogallol); G3: Biofield Energy Treated test formulation; G4: Untreated test formulation; G5: Reference compound (Levamisole). All values are represented as mean ± SEM (n=6).

Many scientific reports suggest that minerals and herbal extracts have the capacity to alter the lipid profile in different ways. Ashwagandha, one of the major constituents of the test herbomineral formulation was reported to have a significant effect on lipid profile, while selenium supplementation is also reported to alter the serum cholesterol, LDL, HDL, etc. with no clinical adverse effects [45, 46]. Apart from selenium and ashwagandha, zinc and magnesium supplementation have been found to give beneficial results in terms of serum cholesterol and other lipid profile parameters [47, 48]. Therefore, it might be suggested that the altered lipid profile was due the presence of important constituents in the test formulation, however the Biofield Energy Treatment showed improved lipid profile as compared with the untreated test formulation. Biofield Energy Treatment includes the use of a low intensity electromagnetic field (the Biofield) by the Energy Healers, so the altered lipid profile activity might be due to the Biofield Energy transferred to the test formulation. A scientific study showed a beneficial effect of an extremely low frequency electromagnetic field on animal lipid profile by altering lipid metabolism [49]. Thus, it might be suggested, that the Biofield Energy Healing Treatment on the test formulation alters the lipid metabolism of animals, which would be useful against various inflammatory disease conditions.

3.5. Measurement of Hepatic Biomarkers

The major hepatic biomarkers were evaluated after treatment with the test formulation on female *Sprague Dawley* rats, and the results are presented in the Table 3. The Biofield Energy Treated test formulation showed a significant effect on all the major hepatic biomarkers as compared with the untreated test formulation. The total protein, alkaline phosphatase, albumin, and globulin levels were increased by 11.92%, 1.24%, 6.10%, and 20.74% respectively in the Biofield Energy Treated test formulation group. However, the levels were significantly decreased in SGOT (14.29%), SGPT (8.54%), and CK-MB (20.79%) in the Biofield Energy Treated test formulation group as compared with the disease control group. The SGPT level was increased by 33.64% in the untreated test formulation as compared with the disease control group. Thus, the Biofield Energy Treated test formulation showed much better results in terms of improved hepatic biomarkers as compared with the untreated test formulation.

Table 3. *Evaluation of major hepatic biomarkers after treatment with the test formulation in female Sprague Dawley rats.*

Group (G)	TB (mg/dL)	SGOT (U/L)	SGPT (U/L)	ALP (U/L)
1	0.11 ± 0.02	137.18 ± 13.73	32.33 ± 1.17	190.50 ± 23.19
2	0.22 ± 0.04	473.53 ± 74.24	59.40 ± 6.94	111.10 ± 21.59
3	0.21 ± 0.05	405.88 ± 70.90	54.33 ± 6.20	112.48 ± 26.66
4	0.22 ± 0.03	394.03 ± 41.19	79.38 ± 25.81	140.42 ± 17.01
5	0.15 ± 0.03	287.27 ± 29.15	37.02 ± 3.94	162.95 ± 25.68

Table 3. *Continued.*

Group (G)	CK-MB (U/L)	TP (g/dL)	A (g/dL)	G (g/dL)	A/G ratio
1	283.93 ± 70.60	4.93 ± 0.11	3.42 ± 0.03	1.52 ± 0.09	2.29 ± 0.31
2	1053.87 ± 90.55	5.45 ± 0.24	3.28 ± 0.04	2.17 ± 0.23	1.62 ± 0.48
3	834.80 ± 84.77	6.10 ± 0.50	3.48 ± 0.03	2.62 ± 0.49	1.61 ± 0.73
4	683.60 ± 75.05	6.60 ± 0.22	3.43 ± 0.04	3.17 ± 0.19	1.10 ± 0.13
5	1059.52 ± 202.07	5.65 ± 0.26	3.45 ± 0.08	2.20 ± 0.32	1.74 ± 0.61

SGOT: Serum glutamic oxaloacetic transaminase; SGPT: Serum glutamate-pyruvate transaminase; ALP: Alkaline phosphatase; CK-MB: Creatine kinase-myocardial band; TB: Total bilirubin; TP: Total protein; A: Albumin; G: Globulin; A/G: Albumin/Globulin ratio; G1: Normal control; G2: Disease control (Pyrogallol); G3: Biofield Energy Treated test formulation; G4: Untreated test formulation; G5: Reference compound (Levamisole). All values are represented as mean ± SEM (n=6).

The liver toxicity in the disease control group was increased after exposure with pyrogallol, data showed the levels of TB, SGOT, SGPT, ALP, TP, CK-MB, albumin, and globulin were increased as compared with the normal control. Further, the Biofield Energy Treated test formulation would protect the liver enzymes and found with decreased values of SGPT, SGOT, and CK-MB. The tested hepatic biomarkers describe the extent and type of liver damage and increased enzymes in the blood reflect damage [50]. Many scientific literature reports suggest the importance of minerals like zinc, its protective role on oxidative stress and improved liver enzymes activity [51] along with selenium [52] and magnesium [53]. Further, ashwagandha root extract has been reported with its protective activity on hepatic enzymes [54]. Thus, it might be suggested that the improved hepatic activity was due to the composition of the test formulation, however Biofield Energy Healing Treatment (The Trivedi Effect®) by Biofield Healers further improved the activity of herbomineral test formulation as compared with the untreated test formulation.

3.6. Measurement of Sex Hormones

The effect of the test formulation for the estimation of sex hormones *viz.* progesterone and estrogen is presented in the Figures 3 and 4, respectively. With respect to the normal and disease control data, there was a significant elevation of progesterone and estrogen levels in the Biofield Energy Treated test formulation (G3) as compared to the disease control (G2) and the untreated test formulation (G4). The values of progesterone in the normal control, disease control, Biofield Energy Treated test formulation, untreated test formulation, and levamisole were 13.47 ± 5.97, 14.43 ± 4.81, 43.27 ± 11.38, 26.89 ± 10.79, and 10.86 ± 2.99 ng/mL, respectively. Overall, the Biofield Energy Treated test formulation showed a significant increased level of progesterone by 199.86% as compared to the disease control group. In the untreated test formulation group (G4), the level of progesterone was increased by 86.34% as compared to the disease control group. The results suggest that the Biofield Energy Treated test formulation showed much better response as compared with the untreated test formulation.

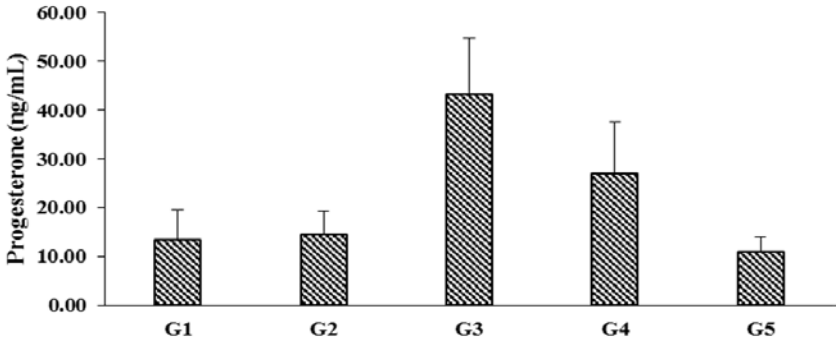

Figure 3. *Effect of the test formulation on the progesterone level. G1: Normal control; G2: Disease control (Pyrogallol); G3: Biofield Energy Treated test formulation; G4: Untreated test formulation; G5: Reference compound (Levamisole). All values are represented as mean ± SEM (n=6).*

Similarly, the level of estrogen in the Biofield Energy Treated test formulation (G3) group showed significant a response as compared with the untreated test formulation group (G5). The levels of estrogen in the normal control (G1), disease control (G2), Biofield Energy Treated test formulation (G3), untreated test formulation (G4), and levamisole (G5) were 405.03 ± 320.52, 28.81 ± 12.16, 43.27 ± 4.72, 26.89 ± 4.79, and 10.86 ± 5.17 pg/mL, respectively. The estrogen level was increased by 50.19% in the G3 group, while decrease the level by 6.66% in case of the untreated test formulation (G4) as compared to the disease control group (G2).

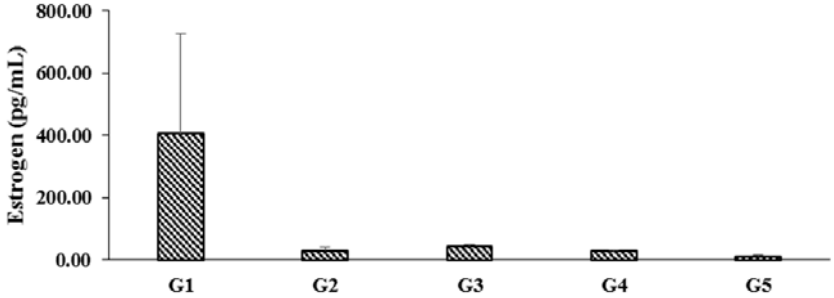

Figure 4. *Effects of the test formulation on estrogen level. G1: Normal control; G2: Disease control (Pyrogallol); G3: Biofield Energy Treated test formulation; G4: Untreated test formulation; G5: Reference compound (Levamisole). All values are represented as mean ± SEM (n=6).*

The herbomineral test formulation is the combination of minerals (zinc, selenium, and magnesium) and ashwagandha root extract, however various reports suggest that all the individual constituents have been reported with improved

levels of sex hormones. Zinc deficiency along with other minerals such as calcium, and magnesium were reported to affect the serum estrogen and progesterone levels, hence minerals play an important role for the regulation of sex hormones [55]. Besides, selenium has been reported to have significant relationship with the female hormones [56]. Ashwagandha root extract is highly significant to regulate the level of sex hormones along with improved immune response [57]. The experimental results of the Biofield Energy Treated test formulation support the existing literature, with improved levels of progesterone and estrogen. After the Biofield Energy Healing Treatment, the levels of both the sex hormones were significantly improved as compared with the untreated test formulation. Therefore, it can be assumed that the Biofield Energy Treatment might have the capacity to regulate and improve the sex hormones level as compared with the untreated test formulation, which can be used as an integrative health care approach against autoimmunity, anti-inflammatory and antiaging related disorders.

4. Conclusions

Based on the current study findings, the Biofield Energy Healing Treatment on the herbomineral formulation showed a significant increase in the humoral and cellular immune responses. The immunological profile suggested that the levels of IgG and IgM were significantly altered in the Biofield Energy Treated test formulation group compared with the disease control group. Further, the $CD4^+/CD8^+$ ratio was significantly ($p \leq 0.01$) increased by 161.71% in the Biofield Energy Treated test formulation group compared with the disease control group. The increased level of the $CD4^+/CD8^+$ ratio is directly related to improved immunity against many diseases. The levels of TLC and neutrophils were significantly increased by 11.34% and 1.34% respectively, in the Biofield Energy Treated test formulation group compared with the disease control group. Additionally, the lipid profile data suggest the levels of total cholesterol, triglycerides, HDL, LDL, and VLDL were significantly altered in the Biofield Energy Treated test formulation. Additionally, the hepatic profile was significantly altered in terms of total protein, alkaline phosphatase, albumin, and globulin level in the Biofield Energy Treated test formulation group, while the hepatic biomarkers like SGOT, SGPT, and cardiac biomarker, CK-MB were significantly decreased by 14.29%, 8.54%, and 20.79%, respectively with respect to the disease control group. Further, the level of sex hormones, *i. e.* progesterone and estrogen were significantly increased by 199.86% and 50.19% in the Biofield Energy Treated test formulation group, while the untreated test formulation showed decreased level of progesterone and estrogen by 86.34% and 6.66%, respectively compared with the disease control group. Therefore, the Trivedi Effect® Biofield Energy Healing administered remotely by the eighteen Biofield Energy Healers enhanced the herbomineral test formulation's anti-inflammatory and immunomodulatory properties in terms of improved cellular immune response, hematological profile, hepatic biomarkers, and increased sex hormone levels.

Overall, the Biofield Energy Treated test formulation can be used as a 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.

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