



Investigation of Immune Biomarkers Regulation by Biofield Energy Healing (The Trivedi Effect[®]) Based Herbomineral Formulation in Male *Sprague Dawley* Rats

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Abstract: Herbomineral formulations have been used worldwide against various chronic and degenerative diseases, due to its fewer side effects. 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 Biofield Energy Treated 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 biomarkers like alteration in immune biomarkers such as IgG and IgM, CD4⁺ and CD8⁺, hematology, lipid profile, hepatic enzymes, sex hormone, and antioxidant profile. The humoral immune response of IgM and IgG were altered by 4.54% and 9.49%, respectively in the Biofield Energy Treated test formulation (G4) group compared with the disease control (G2) group. On the other hand, the cellular immune response i.e. CD4⁺ and CD8⁺ counts were significantly increased by 56.21% and 73.6%, respectively in the G4 group compared with the G2. Hematology analysis showed an increased level of TLC and lymphocytes by 14.85% and 21.42%, respectively in the G4 group compared to the G2. Lipid profile analysis showed a significant decrease in the level of total cholesterol, triglycerides, LDL, and VLDL in the G4 group compared with the G2. Further, results suggests a significant decrease in the hepatic biomarkers such as SGOT, SGPT, and cardiac enzyme, CK-MB by 18.54%, 19.12%, and 26.23%, respectively in G4 group compared to the G2 group. However, the testosterone level was significantly decreased in G4 group and untreated test formulation (G5) groups by 53.87% and 26.25%, respectively compared with the G2 group. Additionally, the antioxidant assay data showed an altered level of tested enzymes such as LPO, SOD and CAT, in the G4 group compared with the G2 group. Therefore, it can be concluded that the Biofield Energy Treated test formulation showed a significant improved cellular and humoral immunity, hematological and biochemical profile as compared with the untreated test formulation. As a result, 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, The Trivedi Effect[®], Herbomineral Formulation, Autoimmune Diseases, Anti-aging, Testosterone, Anti-oxidation

1. Introduction

Herbomineral products have been used worldwide against various health related diseases and to boost the immunity. These products are considered as immune booster, which makes them unique as compared with the other nutraceutical products in market. Plant secondary metabolites and minerals in herbomineral formulations plays an important role to improve the overall quality of life (QoL) that makes resistance against various infectious inflammatory and autoimmune diseases [1, 2]. Minerals and medicinal plant extract are the major targeted constituents for any new immunomodulatory formulation due to its low toxicity profile [3]. According to the scientific literatures and as per the best medicinal activity of herbal extract, a new proprietary herbomineral formulation was created with a combination of the herb ashwagandha (*Withania somnifera*) root extract and three minerals viz. zinc, magnesium, and selenium. Each constituent of this test formulation is commonly used as a nutraceutical supplements [4-6]. Immunomodulatory products regulates the immune system either by suppression and stimulation of the cells or organs of the immune system. These formulations have the ability to normalize or modulate the pathophysiological processes [7, 8]. However, immunomodulation using natural products has been shown a continuing field of interest due to the action of primary or secondary metabolites that collectively work as safe nutraceuticals along with minerals [9]. Steroidal secondary metabolites of ashwagandha root powder are considered as Generally Recognized as Safe (GRAS) and used for their beneficial immunomodulatory potential [10, 11]. Withanolides present in ashwagandha has been reported for anti-inflammatory, anti-arthritis, antibiotic, anti-tumor immunomodulatory and action on central nervous system [12, 13]. In addition, the minerals such as zinc, magnesium, and selenium also have been reported to regulate the immune system [14, 15].

Scientific research has been reported that the combination of minerals and herbal medicines have been found to exhibit significant immunomodulatory action [16]. 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 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 research [19], microbiology [20-22], genetics and biotechnology [23, 24], pharmaceuticals [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 and chemicals in materials science [32-34], human health and wellness.

In this study, the authors sought to explore the impact of the Biofield Energy Healing Treatment (The Trivedi Effect[®]) on the test herbomineral formulation for its immunomodulatory properties using immune biomarkers such as 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

The constituents of test formulation *viz.* 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. 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 and was procured from Sigma-Aldrich, 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 $22 \pm 3^\circ\text{C}$, 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. 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 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 through a group of twenty Biofield Energy Healers who participated in this study and performed the Biofield Energy Treatment remotely. 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 a 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 ($0.5 \times 10^9/100$ 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 \pm standard error of mean (SEM) and subjected to statistical analysis using Sigma Plot software (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 levels of immunoglobulins (IgG and IgM) after treatment with test formulation are presented in Figure 1. The data suggest an increased levels of IgM and IgG in disease control group (G2) by 4.54% and 13.03%, respectively compared with the G1 group. However, Biofield Energy Treated test formulation (G4) group showed decrease level of IgM and IgG by 4.54% and 9.49%, respectively compared with the disease control group (G2). Moreover, the level of IgM was increased by 4.35% in G5 compared with the G2 group. Overall, it can be concluded that the Biofield Energy Healing Treatment altered the humoral immune response of herbomineral formulation with respect to untreated test

formulation.

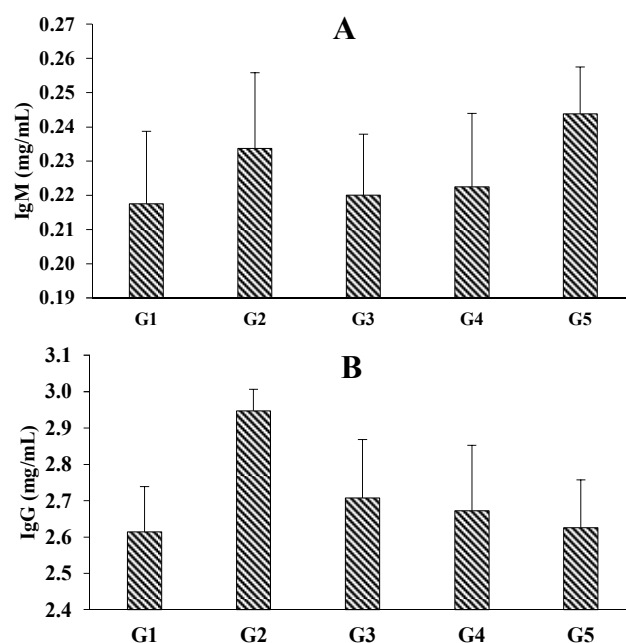


Figure 1. The effect of the test formulation on immunoglobulins, (A) IgM and (B) IgG after treatment on various groups (G1 – G5) in male SD rats. G1: Normal control; G2: Disease control; G3: Levamisole; G4: Biofield Energy Treated test formulation; G5: Untreated test formulation. All the values are represented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

IgG and IgM are considered as the major immunoglobulins and have an important role in complement activation, opsonization, neutralization of toxins, etc. The test formulation is the combination of ashwagandha root extract and the minerals, it might be suggested that the alteration in immunoglobulin production in different groups due to the Biofield Energy Healing Treatment or because of interactions between the active constituents. Literature data suggests that ashwagandha and the minerals such as zinc, selenium, and magnesium have significant effects on immunoglobulin production [41, 42].

3.2. Measurement of Cellular Responses

Cellular immune response was estimated by calculating the percentage of CD4⁺ and CD8⁺ and the results are presented in the Figure 2. The results showed the percentage of CD4⁺ in the Biofield Energy Treated test formulation (G4) group was significantly increased by 56.21%, while untreated test formulation group (G5) showed 9.07% increase of CD4⁺ with respect to G2 group. Similarly, the results of CD8⁺ showed a significant increase in the level by 73.6% in the Biofield Energy Treated test formulation (G4), while 14.17% increase in the untreated test formulation (G5) compared with the disease control (G2) group. This showed that Biofield Energy Healing Treatment significantly improved the cellular immune response compared with the untreated test formulation, which can be used against many inflammatory and autoimmune disorders.

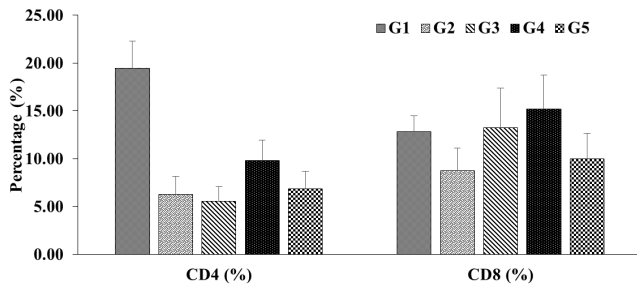


Figure 2. The effect of the test formulation on the ratio of cellular biomarkers ($CD4^+/CD8^+$ ration) after treatment on various groups (G1 – G5) in blood sample of male SD rats. G1: Normal control; G2: Disease control; G3: Levamisole; G4: Biofield Energy Treated test formulation; G5: Untreated test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

Cellular immunity is regulated with the strength of T-cells and they are categorized in two forms *i.e.* T4 and T8. Infection control and its spread is controlled by $CD4^+$ or T4 cells, which are known as helper cells. Besides, suppressor or killer cells (T8 cells or $CD8^+$) have the capacity to kill the infected cells or cancerous cells. Both are considered as the major part of immune system and regarded as the subpopulations of lymphocytes. Therefore, the strength of the cells will decide the immunity and the power to fight against numerous infection [43]. Thus, strong immunity will direct

the importance of T cell activation and proliferation [44]. The present study data concluded that Biofield Energy Healing (The Trivedi Effect[®]) Treatment has the capacity to significantly increased the number of $CD4^+$ and $CD8^+$ cells and Biofield Energy Treated test formulation can be used to improve the cellular immunity to fight against various infections.

3.3. Assessment of Hematology Parameters

The results of important hematology profile in different groups (G1 to G5) are summarized in Table 1. The study results suggests improved animal hematology profile compared with the disease control group. Hematology parameters such as TLC, lymphocytes, and eosinophils counts were increased by 14.85%, 21.42%, and 10.52%, respectively in the Biofield Energy Treated test formulation (G4) group compared with the disease control (G2) group. The concentration of TLC and eosinophils was decreased by 3.64% and 15.78% in the untreated test formulation (G5) compared with the G2 group. However, the concentration of neutrophils and monocytes were decreased in G4 group by 26.40% and 35.55%, respectively compared with the G2 group. It suggests that the Biofield Energy Healing Treatment has the capacity to improve the blood immunity related parameters.

Table 1. Hematology profile of rats after administration of the test formulation in Sprague Dawley rats.

Group (G)	TLC ($X10^3/mm^3$)	Neutrophils ($X10^3/mm^3$)	Lymphocytes ($X10^3/mm^3$)	Eosinophils ($X10^3/mm^3$)	Monocyte ($X10^3/mm^3$)
1	11.26 \pm 1.00	2.82 \pm 1.04	7.84 \pm 0.55	0.14 \pm 0.03	0.47 \pm 0.19
2	10.17 \pm 0.87	1.78 \pm 0.35	7.75 \pm 1.12	0.19 \pm 0.03	0.45 \pm 0.15
3	9.60 \pm 0.98	1.49 \pm 0.46	7.59 \pm 0.51	0.24 \pm 0.04	0.28 \pm 0.14
4	11.68 \pm 1.48	1.31 \pm 0.21	9.41 \pm 1.84	0.21 \pm 0.03	0.29 \pm 0.15
5	9.80 \pm 0.95	1.79 \pm 0.53	7.64 \pm 1.01	0.16 \pm 0.04	0.20 \pm 0.03

G: Group; 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 \pm SEM (n=8). All the values are represented as mean \pm SEM of independent experiment (n=8). TLC: Total leukocyte count.

In the blood, major immune system markers are the lymphocytes and eosinophils. Lymphocytes can be defined as T and B cells, which functions to eliminate the antigen, either by releasing the antibodies (B cells), cytotoxic granules or directly by signaling to other cells of the immune system. They are considered as an important immune function regulator against infections by regulating several growth factors [45]. Besides, the decreased level of neutrophils and monocytes might be used in many chronic inflammatory diseases, acute infection, gout, rheumatoid arthritis, rheumatic fever, *etc.* The study data concluded that the Biofield Energy Treated test formulation significantly improved the concentrations of TLC, lymphocytes, and eosinophils in hematology profile assay, which suggests that The Trivedi Effect[®] has the capacity to improve the immunomodulatory potential of test formulation with respect to altered hematological animal profile.

3.4. Measurement of Glucose and Lipid Biomarkers

Lipid profile analysis after treatment with the Biofield Energy Treated and untreated test herbomineral formulation are summarized in Table 2. The analyzed biochemical parameters were glucose, total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL), and very low density lipoprotein (VLDL). The study result showed that the concentration of glucose was increased by 11.44% and 8.06%, but not significant in the Biofield Energy Treated (G4) and untreated test formulation (G5) groups, respectively compared with the disease control group (G2). However, the level of TC, triglycerides, LDL, and VLDL was slightly decreased by 0.93%, 1.88%, 0.75%, and 1.81%, respectively in G4 group compared with the G2 group. While the level of HDL was slightly decreased in G4, compared with the disease control (G2) group. Therefore, the Biofield Energy Treated test formulation showed altered lipid profile, which might be

useful in immunomodulation.

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)
1	154.53 ± 13.41	69.31 ± 3.57	53.78 ± 7.78	20.75 ± 1.07	37.84 ± 1.95	10.73 ± 1.56
2	117.98 ± 7.81	68.65 ± 2.34	41.49 ± 3.40	20.55 ± 0.70	39.84 ± 1.43	8.26 ± 0.68
3	145.80 ± 9.94	94.71 ± 4.00	53.75 ± 4.49	28.36 ± 1.20	55.64 ± 2.84	10.71 ± 0.90
4	131.48 ± 13.45	68.01 ± 3.60	40.71 ± 3.70	20.36 ± 1.08	39.54 ± 1.89	8.11 ± 0.74
5	127.49 ± 9.86	63.79 ± 2.14	34.35 ± 2.44	19.08 ± 0.65	37.86 ± 1.46	6.83 ± 0.49

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.

Biofield Energy Healing Treatment has the capacity to alter the lipid profile, which suggest that Biofield Energy Treated test formulation can be used to improve the lipid profile. Literature data suggest that the constituents such as ashwagandha, selenium, zinc, and magnesium have significant impact on the lipid profile, serum cholesterol, LDL, HDL, etc. [46-49]. Therefore, Biofield Energy Healing Treatment modulates the liver lipid profile after oral administration of Biofield Energy Treated test formulation, which might suggests its role in immunomodulation and can be used against many autoimmune and inflammatory disorders.

3.5. Measurement of Hepatic and Cardiac Biomarkers

Hepatic biochemical markers such as serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), 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, SGPT, and CK-MB was significantly decreased by 18.54%, 19.12%, and 26.23%, respectively in the Biofield Energy Treated test formulation (G4) compared with the disease control (G2) group. Besides, no significant change was observed in the level of TB, TP, A, G and A/G.

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
1	0.09 ± 0.01	198.03 ± 12.19	41.35 ± 2.99	272.23 ± 10.99	179.83 ± 20.45	6.99 ± 0.15	3.46 ± 0.05	3.53 ± 0.12	0.99 ± 0.03
2	0.10 ± 0.01	166.19 ± 10.30	32.68 ± 1.99	209.25 ± 17.29	160.55 ± 11.47	6.65 ± 0.12	3.38 ± 0.06	3.28 ± 0.06	1.03 ± 0.01
3	0.10 ± 0.01	186.26 ± 9.73	44.40 ± 5.21	234.70 ± 17.36	156.54 ± 6.95	6.89 ± 0.10	3.50 ± 0.03	3.41 ± 0.09	1.03 ± 0.02
4	0.11 ± 0.01	135.38 ± 4.59	26.43 ± 1.76	217.05 ± 13.58	118.44 ± 8.88	6.55 ± 0.07	3.36 ± 0.04	3.19 ± 0.05	1.06 ± 0.02
5	0.11 ± 0.01	145.71 ± 10.74	28.63 ± 2.98	216.54 ± 6.45	113.46 ± 9.92	6.66 ± 0.05	3.36 ± 0.05	3.30 ± 0.05	1.02 ± 0.03

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.

Hepatic enzymes are the biomarkers for liver toxicity for any infection and suggest liver damage [50]. Besides, the scientific literature data suggests that all the individual components of the test formulation have significant effect against hepatic enzymes with protective effect [51-54]. Disease control group showed that the liver biomarkers were significantly increased, while the toxicity of liver enzymes was significantly decreased, while Biofield Energy Treated test formulation showed an improved liver health. Therefore, it can be concluded that The Trivedi Effect®-Biofield Energy Healing can be used to improve the immunity profile by improving the level of important liver enzymes.

3.6. Measurement of Sex Hormone-Testosterone

The serum testosterone level in male SD rats after oral administration of the Biofield Energy Treated and untreated test formulation are shown in the Figure 3. The study data suggests that the serum testosterone level was significantly altered after treatment compared with the normal control and disease control groups. The level of testosterone was significantly decreased by 53.87% and 26.25% in the Biofield Energy Treated test formulation (G4) and untreated test formulation (G5) groups, respectively compared with the disease control group (G2). This suggests that the Biofield Energy Healing Treatment has the capacity to regulate the sex hormone level. The effect of the Biofield Energy Healing

on test formulation (G4) was significant as compared with the untreated test formulation (G5) group.

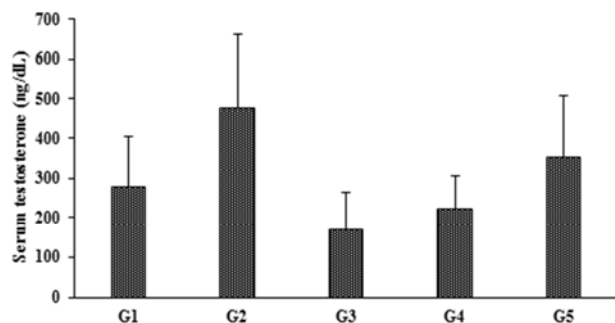


Figure 3. The effect of the test formulation on the level of testosterone after treatment on various groups (G1 - G5) in male Sprague Dawley rats. G1: Normal control; G2: Disease control; G3: Levamisole; G4: Biofield Energy Treated test formulation; G5: Untreated test formulation. All the values are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

Scientific literature data suggests that the constituents present in the test formulation has the significant capacity to regulate the testosterone level [55-57]. However, Biofield Energy Healing further downregulate the sex hormone level. Moreover, added minerals have been reported its application in testicular disorders [58]. The study showed regulation of

testosterone level after Biofield Energy Treatment on test formulation compared with the untreated test formulation. This showed that The Trivedi Effect[®] has the capacity to improve the level of sex hormone that can be implicated to use as immunomodulation.

3.7. Measurement of Antioxidant Profile by ELISA Based Assay

Antioxidant activity of the Biofield Energy Treated and untreated test formulation on antioxidant enzymes such as SOD, LPO, and CAT in male SD rats are demonstrated in the Figure 4. The antioxidant biomarkers such as SOD, LPO, and CAT were evaluated in the liver samples. The LPO levels in G4 and G5 groups were increased by 4.41% and 13.85%, respectively compared with the G2 group. However, the level of SOD was significantly increased in the untreated test formulation (G5) by 25.12% compared with the disease control, G2 group, while the level was slightly decreased by 4.6% in G4 compared with the G2 group. Similarly, the level of CAT was decreased in the G4 group by 29.78%, while levamisole (G3) showed an increased CAT by 11.72% compared with the G2 group.

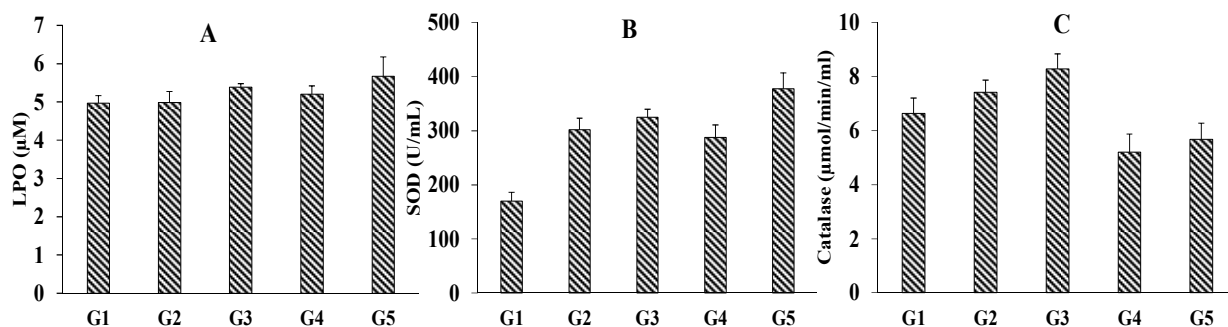


Figure 4. Bar graphs showing activities of antioxidant enzymes (A) LPO, lipid peroxidase, (B) SOD, superoxide dismutase, and (C) catalase after treatment with the test formulation in male Sprague-Dawley rats. G1: Normal control; G2: Disease control; G3: Levamisole; G4: Biofield Energy Treated test formulation;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

The antioxidant analysis suggests the effect of the Biofield Energy Healing Treatment on antioxidant enzymes (SOD, LPO, and CAT). These enzymes are reported to have beneficial role in anti-inflammatory property and responsible for many inflammatory infections [59]. The Trivedi Effect[®] has the capacity to alter the antioxidant enzymes level, which might be used against several inflammatory and autoimmune disease conditions.

4. Conclusions

In conclusion, the Biofield Energy Treated herbomineral formulation exhibited significant immunomodulatory action after oral administration in male *Sprague Dawley* rats. The results of humoral immune biomarkers showed a significant alterations in the level of IgG and IgM in the Biofield Energy Treated test formulation group compared with the disease control group (G2). In addition, the results of cellular

immune responses *i.e.* CD4⁺ and CD8⁺ were significant improved compared with the disease control group. The significant increase in the percentage of CD4⁺ and CD8⁺ level was found by 56.21% and 73.6%, respectively in the Biofield Energy Treated test formulation (G4) compared with the G2 group. However, the improved cellular immune response was highly significant *i.e.* CD4⁺ and CD8⁺ levels by 9.07% and 14.17%, respectively in the Biofield Energy Treated test formulation compared with the untreated test formulation (G5). The hematology parameters such as the level of TLC and lymphocytes were increased by 14.85% and 21.42%, respectively in the Biofield Energy Treated test formulation group (G4) compared with the disease control group (G2). Biochemical analysis showed that the glucose concentration was altered (11.44%), while levels of TC, triglycerides, LDL, and VLDL were decreased in the Biofield Energy Treated test formulation (G4) group compared with the disease control (G2) group. Further, the analysis of

hepatic and cardiac biomarkers in serum sample showed a significant decrease in the level of SGOT, SGPT, and CK-MB by 18.54%, 19.12%, and 26.23%, respectively in the Biofield Energy Treated test formulation (G4) compared with the disease control (G2) group. However, serum testosterone level was significantly decreased by 53.87% in G4 group, while untreated test formulation group showed decreased level by 26.25% as compared with the diseases control (G2) group. Further, the antioxidant profile showed a significant alteration in the level of LPO, SOD, and CAT in the G4 group compared with the G2 group.

Overall, the current experimental findings suggested the Trivedi Effect®-Biofield Energy Healing Treatment done remotely by the twenty Biofield Energy Healers 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 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 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.

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