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Evaluation of the Effects of Biofield Energy Healing Based Herbomineral Formulation on Various Biomarkers in Male *Sprague Dawley* Rats: Potential Role of the Trivedi Effect®

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Abstract: A new proprietary herbomineral formulation was formulated, consisting of essential ingredients *viz.* herbal root extract ashwagandha and minerals (zinc, magnesium, and selenium). The aim of the study was to evaluate the immunomodulatory potential of Energy of Consciousness Healing (The Trivedi Effect®) Treatment on the herbomineral formulation in male *Sprague Dawley* rats. The test formulation was divided into two parts. One part was denoted as the control without Biofield Energy Healing Treatment, while the other part was defined as the Biofield Energy Treated sample, which received the Biofield Energy Healing Treatment remotely from seven renowned Biofield Energy Healers. Additionally, one group of animals was also received Biofield Energy Treatment *per se* (day -15) by Biofield Energy Healers under similar conditions. The IgM, IgG, $CD4^+$, and $CD8^+$ were altered in the Biofield Energy Treated test formulation (G4) compared to the disease control (G2). TLC was significantly increased by 19.35% in the G4 compared to the G2. Neutrophil was significantly increased by 31.53%, 29.73%, 31.53%, and 33.84% in the G4, untreated test formulation (G5), G6, and G7, respectively compared to the G2. The levels of total cholesterol (TC), triglycerides (TG), and very low density lipoprotein (VLDL) were significantly lowered by 18.18% ($p \leq 0.01$), 46.33% ($p \leq 0.01$), and 46.41% ($p \leq 0.01$), respectively in the G4 compared to the G2. Additionally, the levels of TC, TG, low density lipoprotein (LDL), and VLDL were significantly reduced by 25.50% ($p \leq 0.001$), 96.39% ($p \leq 0.001$), 13.91%, and 55.08% ($p \leq 0.001$), respectively in the G6 compared to the G2. Alkaline phosphatase (ALP) was significantly reduced by 33.65% ($p \leq 0.001$) in the G4 compared to the G2. Moreover, the levels of serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), ALP, and creatine kinase myocardium band (CK-MB) were significantly decreased by 29.44% ($p \leq 0.05$), 25.54% ($p \leq 0.01$), 27.97% ($p \leq 0.01$), and 36.07% ($p \leq 0.01$), respectively in the G7 group compared to the G2. Testosterone was significantly increased by 83.68%, 305.90%, 1271.03% ($p < 0.001$) and 745.35% ($p < 0.05$) in the G4, G5, G6 and G7, respectively compared to the G2. The level of catalase enzyme was significantly increased by 57.91% ($p < 0.01$) and 98.51% ($p < 0.001$) in Biofield Energy Treatment *per se* at day -15 (G6) and Biofield Energy Treated test formulation at day -15 (G7), respectively compared to the G2. Overall, results suggested that the Biofield Energy Treated herbomineral formulation and Biofield Energy Treatment *per se* can be used for autoimmune and inflammatory diseases, stress management and prevention, and anti-aging by improving overall health.

Keywords: Biofield Energy Healing Treatment, Biofield Energy Healers, The Trivedi Effect®, Immunomodulation, Herbomineral Formulation, Antioxidant, Anti-aging, Inflammatory Disease and Stress Management

1. Introduction

An herbomineral products have been accepted worldwide against many health related disorders, due to their significant immunomodulatory potential. However, the action of herbominerals as an immune booster make it a unique compared with other available nutraceutical products. Overall, Quality of Life (QoL) can be improved by maintaining the organic resistance of the body. It was reported that secondary metabolites of plants extract and minerals play an important role in the immunomodulatory action [1-3]. An herbal medicines and minerals are the major targeted product to modulate the immune system due to its low toxicity profile compared with the synthetic drugs against infections [4, 5]. The main role of existed conventional medicine was to diagnose and treat the disease, but in 21st century the primary goal of nature-based herbomineral formulation is predictive and prevention of diseases. Biomarkers are the biological measurements, which can be utilized to predict the severity of disease and to recognize the forward detection of disease [6]. It was well established that the immune biomarkers were used for the early diagnosis and evaluation of target organ damage in most of non-autoimmune disease such as diabetes mellitus, hypertension, arteriosclerosis etc. [7, 8]. The early diagnosis of biomarker is a biologic indicator in clinical practice that indicate the risk of specific disorders, its progression, and the relative risk/benefit related with the specific therapy, plays a central role in the selection of the most effective treatment [9]. The new proprietary herbomineral formulation containing four ingredients; mixture of minerals (zinc chloride, magnesium gluconate hydrate, and sodium selenate) and an herbal extract (ashwagandha root extract). Each constituent of this formulation is commonly used as nutraceutical supplement [10-12]. The immunomodulatory agents have the ability to normalize or modulate pathophysiological processes [13, 14]. Various studies have reported anti-inflammatory, antiarthritic, antibiotic, antitumour immunomodulatory and central nervous system effects of withanolides [15, 16]. Zinc plays a major role in most of the biochemical reactions in living organism due to its enzyme catalyzing activity. Most of the enzymatic reaction are zinc dependent. It also plays a key role for regulation of sex hormones and in the immune system [17, 18]. Selenium also plays a major role for immunomodulation by alterations of cluster differentiation ($CD8^+$) lymphocyte function [19]. Magnesium reduces the production of inflammatory cytokine through activation of *NF-κB* pathways, which is a novel innate immunomodulatory mechanism [20]. It is observed that acute stress may enhance immune response, whereas chronic stress may suppress the immune system [21].

Scientific research has been reported that the combination of minerals and herbal medicines found to exhibit significant immunomodulatory action [22]. An herbomineral formulations can be used for better therapeutic effect in immune compromised patients that are affected by the 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 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 [23]. CAM therapies have been practiced worldwide with reported clinical benefits in different health disease profiles [24]. 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 conventional methods. Biofield Energy Treatment (The Trivedi Effect®) results has been published in numerous peer-reviewed science journals with significant outcomes in many scientific fields such as cancer research [25], microbiology [26-28], genetics [29, 30], pharmaceutics [31, 32], nutraceuticals [33], organic compounds [34, 35], agricultural science [36, 37], and changing the structure of the atom in relation to various metals, ceramics, polymers and chemicals in materials science [38-40]. In this study, the authors sought to explore the impact of the Biofield Energy Treatment (The Trivedi Effect®) on the given herbomineral formulation and Biofield Energy Treatment *per se* to the animals, which might improve the immunomodulatory function in cyclophosphamide induced immunosuppression in male *Sprague Dawley* rat model by identification of various immunity biomarkers.

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. Cyclophosphamide was used as inducing agent for immunosuppression was procured from Zydus Oncosciences India. Levamisole hydrochloride and sodium carboxymethyl cellulose (Na-CMC) were procured from Sigma-Aldrich, USA. All other chemicals used were of analytical grade available in India.

2.2. Experimental Animals

A total number of 56 healthy male *Sprague Dawley* rats, weighing between 220-250 grams, were used for the study (n=8, in each group). The animals were purchased from M/s. Vivo Bio Tech Ltd., Hyderabad, India. Standard rodent diet was procured from M/s. Golden feeds, Mehrauli, New Delhi, India and provided *ad libitum* to all the groups of animals during the experiment under controlled conditions with a temperature of 22 ± 3°C, humidity of 30% to 70% and a 12-hour light/12-hour dark cycle. The animals were acclimatized for 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 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 the 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 seven Biofield Energy Healers who participated in this study and performed the Biofield Energy Treatment remotely were located in the U.S.A., while the test herbomineral formulation was located in the research laboratory of Dabur Research Foundation, New Delhi, India. Additionally, one group of animals was also received the Biofield Energy Treatment *per se* by the Biofield Energy Healers under similar conditions. 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 purpose. 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)

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 ºC, 10 minutes), washed twice with the normal saline and then further diluted in saline, which were analyzed using Hematology analyzer (Abbott Model-CD-3700). Based on the number of erythrocytes, the samples were further diluted (using saline) before injecting to the rats [41].

2.5. Experimental Procedure

After seven days of acclimatization, the animals were grouped based on the body weight. A total of seven groups (G) were included *i.e.* G1 to G7 with eight animals (n=8) in each group. The animals were received cyclophosphamide in all the groups except G1 at a dose of 10 mg/kg in normal saline through intraperitoneal (*i.p.*) route 1 hour before administration of the test formulation, from day 1 to 13. However, G1, G2, and G6 group's animals were administered with vehicle (0.5% carboxy methyl cellulose-sodium) *via* oral gavage. The G3 group animals received reference item, levamisole at a dose of 75 mg/kg body weight. The G4 group animals received Biofield Energy Treated test formulation at 1105.005 mg/kg b.wt, per-oral (*p.o.*), and the G5 animals received the untreated test formulation at the same dose by oral route. Further, the G6 group animals received only Biofield Energy Treatment *per se* at day -15, without test formulation, while G7 group animals received Biofield Energy Treated test formulation at day -15. The freshly prepared suspensions of the Biofield Energy Treated and untreated test formulations were administered orally to the G4 and G5 groups, respectively at a dose of 1105.005 mg/kg from day 1 to day 25. However, Biofield Energy Treated test formulation was administered orally to the G7 group at same dose from day -15 to day 25. The treatment was continued to all the tested groups (G1 to G7) with 5 mL/kg body weight dose volume.

However, all the animals (G1-G7) were challenged with sheep red blood cells (sRBC) (0.5×10^9/100 gm; *i.p.*) on day 7 and 13, as the antigenic material to sensitize them for immunological studies. On day 13^{th} and 20^{th} the animals were bled and the samples were subjected to hemagglutination test for cellular ($CD4^+$ and $CD8^+$) and humoral (IgG and IgM) immune responses. On same day 20^{th}, the animals were challenged with sRBC (0.5×10^9 cells/50µL/rat) in sub-planter region and on day 21^{st} (24 hours) paw volume was measured to evaluate the cellular immune response. The body weight and feed consumption were measured daily before treatment. The animals were kept on overnight fasting on day 24, followed by blood collection from retro-orbital plexus under isoflurane anaesthesia and the samples were subjected for haematology analysis, serum for biochemistry and hormone estimation. A portion of liver samples were snap frozen and stored in -80°C for the estimation of anti-oxidant parameters (SOD, Catalase, and LPO). 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, 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 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 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 [42, 43].

2.9. Assessment of Sex Hormone

Testosterone was analyzed in serum using commercial kits. 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.

2.10. Assessment of Antioxidant Profile by ELISA Assay

Superoxide dismutase (SOD), catalase and lipid peroxidation (LPO) were analysed by ELISA assay using liver homogenate sample [44-46].

2.11. Statistical Analysis

The data were expressed as mean ± standard error of mean (SEM) and subjected to statistical analysis using SigmaPlot (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 effects of Biofield Energy Treated and untreated test formulation on the levels of IgM and IgG in male SD rats are shown in the Figure 1. The animals administered with untreated test formulation (G5) showed 14.27% increase in IgG compared to the G2 group; while it was altered in the rest of the tested groups. The level of IgM was reduced by 13.07%, 3.92% and 16.34% in the Biofield Energy Treated test formulation (G4), Biofield Energy Treatment *per se* at day -15 (G6) and Biofield Energy Treated test formulation at day -15 (G7), respectively compared to the G2 group. Yamada *et al.* [47], had reported that ashwagandha to enhance the immune function by increasing immunoglobulin production. In this experiment the Biofield Energy Treated test formulation showed slight increase the level of IgG compared to the disease control group. The data was supported with the literature [47, 48].

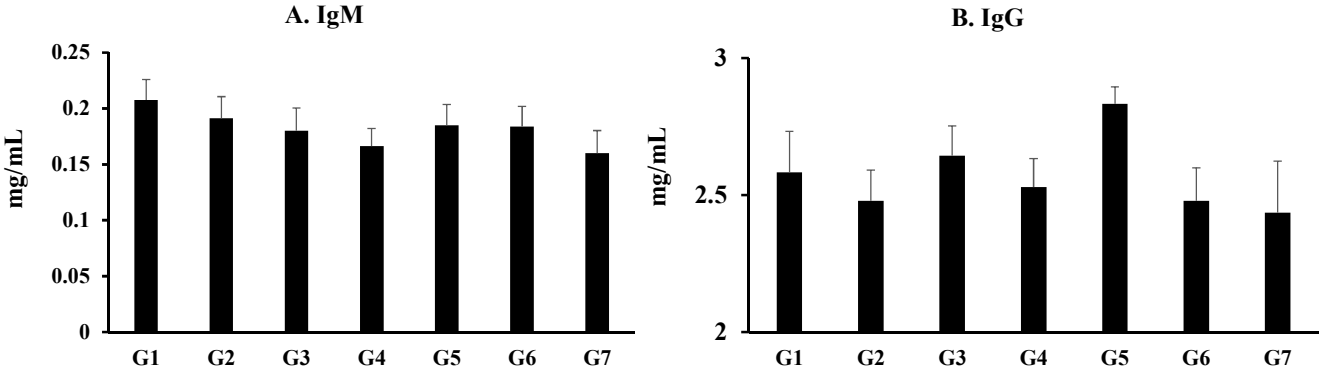

Figure 1. *The effect of the test formulation on immunoglobulins (A. IgM and B. IgG) after 24 consecutive days of treatment in male SD rats. G1: Normal control; G2: Disease control; G3: Levamisole; G4: Biofield Energy Treated test formulation; G5: Untreated test formulation; G6: Biofield Energy Treatment per se at day -15; and G7: Biofield Energy Treated test formulation at day -15. All the values are represented as mean ± SEM (n=8).*

3.2. Measurement of Cellular Responses

The effect of the test formulation on the levels of $CD4^+$ and $CD8^+$ in male SD rats is depicted in the Figure 2. The CD markers such as percentage of $CD4^+$ and $CD8^+$ cells were analyzed using whole blood. No substantial changes of $CD4^+$ counts were observed across the groups, while the $CD8^+$ count was increased by 7.17% and 1.42% in the untreated test formulation (G5) and Biofield Energy Treatment *per se* (G6) respectively, compared to the disease control (G2).

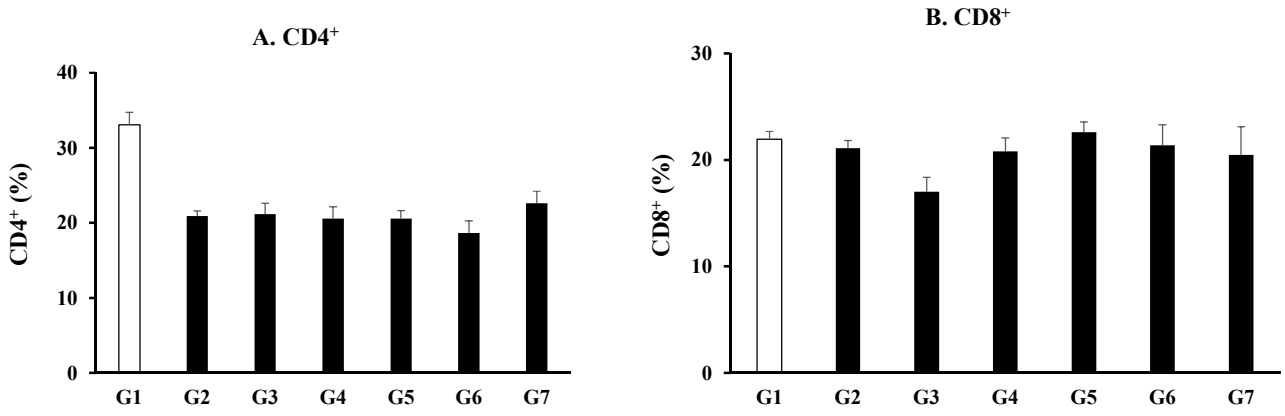

Figure 2. *The effect of the test formulation on immunoglobulin cellular biomarkers (A. CD4$^+$ and B. CD8$^+$) after 24 consecutive days of treatment in male SD rats. All the values are represented as mean ± SEM (n=8).*

3.3. Assessment of Hematology Parameters

The hematology parameters such as total and differential leucocytes counts are shown in the Table 1. The disease control group (G2) showed immunosuppression response was induced by cyclophosphamide as evident with the significant reduction of TLC, lymphocytes, and eosinophils by 29.43%, 14.23%, and 14.29%, respectively compared to the normal control group (G1). The TLC was increased by 19.35% and 2.25% in the Biofield Energy Treated test formulation (G4) and Biofield Energy Treated test formulation at day -15 (G7), respectively compared to the disease control (G2). The level of neutrophils was significantly increased by 31.53%, 29.73%, 31.53%, and 33.84% in the Biofield Energy Treated test formulation (G4), untreated test formulation (G5), Biofield Energy Treatment *per se* (G6) at day -15, and Biofield Energy Treated test formulation at day -15 (G7), respectively compared to the disease control group (G2). The level of lymphocytes was reduced minimally in all the treated groups (G3 - G7) compared to the disease control (G2). The eosinophils level was increased by 8.67%, 8.67%, 16.67%, and 14% in the Biofield Energy Treated test formulation (G4), untreated test formulation (G5), Biofield Energy Treated group *per se* (G6), and Biofield Energy Treated test formulation at day -15 (G7), respectively compared to the disease control (G2). The level of monocytes was decreased by 39.95% in the G4, G5, and G6; while increased by 8.68% in the Biofield Energy Treated test formulation group at day -15 (G7) compared to the disease control (G2). The positive control levamisole showed 26.13% and 16.67% increase in the levels of neutrophils and eosinophils, respectively compared to the disease control (G2). Eosinophils play the role of a beneficial modulatory element or an innocent bystander. Jung *et al.* [49], demonstrated that an increasing number of experimental observations indicated that eosinophils have multifunctional leukocytes that are involved in diverse inflammatory and physiologic immune responses. They have found the potential role of eosinophils as a modulators of the intestinal immune system [40]. This study therefore demonstrated that the Biofield Energy Treated test formulation (G4) showed an enhancement of the level of eosinophil to some extent compared to the G2 group. Another researcher reported the eosinophils and neutrophils show in the protective innate immune response [50]. Besides, the level of neutrophil was significantly increased by 31.53% in the G4 group compared to the G2 group.

Table 1. *Effect of the test formulation on hematological parameters.*

Group	TLC (10^3/mm^3)	Neutrophils (%)	Lymphocytes (%)	Eosinophils (%)	Monocyte (%)
G1	11.35 ± 1.01	17.88 ± 2.27	77.38 ± 2.93	1.75 ± 0.49	3.00 ± 0.89
G2	8.01 ± 1.09	27.75 ± 3.27	66.38 ± 4.03	1.50 ± 0.19	4.38 ± 1.13
G3	7.25 ± 0.52	35.00 ± 4.67	59.63 ± 5.03	1.75 ± 0.25	3.63 ± 0.50
G4	9.56 ± 1.29	36.50 ± 4.18	59.25 ± 4.17	1.63 ± 0.18	2.63 ± 0.18
G5	6.21 ± 0.72	36.00 ± 2.18	59.75 ± 2.23	1.63 ± 0.18	2.63 ± 0.18
G6	6.21 ± 0.66	36.50 ± 1.94	59.13 ± 2.00	1.75 ± 0.25	2.63 ± 0.26
G7	8.19 ± 0.62	37.14 ± 3.25	57.14 ± 3.40	1.71 ± 0.42	4.00 ± 0.98

Analysis of hematological profile like total and differential (5 parts) counts of white blood corpuscles after consecutive 24 days of treatment of the test formulation in male SD rats. All the values are represented as mean ± SEM (n=8). G: Group; G1: Normal control; G2: Disease control; G3: Levamisole; G4: Biofield Energy Treated test formulation; G5: Untreated test formulation; G6: Biofield Energy Treatment *per se* at day -15; and G7: Biofield Energy Treated test formulation at day -15. TLC: Total leukocyte count; %: Percentage

3.4. Measurement of Glucose and Lipid Biomarkers

The biochemical parameters *i.e.* glucose, lipid profile such as total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL), and very low density lipoprotein (VLDL) are shown in the Table 2. The disease control group (G2) showed an increased level of LDL by 2.27% compared to the normal control group (G1).

The levels of TC, TG, LDL, and VLDL were significantly reduced by 19.34% ($p \leq 0.01$), 21.94%, 20.85% ($p \leq 0.01$), and 22.15%, respectively in the Biofield Energy Treated test formulation group (G4) compared to the disease control (G2). Besides, the levels of TC, TG, LDL, and VLDL were remarkably reduced by 18.18% ($p \leq 0.01$), 46.33% ($p \leq 0.01$), 7.05%, and 46.41% ($p \leq 0.01$), respectively in the untreated test formulation group (G5) compared to the disease control (G2). Moreover, the levels of TC, TG, LDL, and VLDL were significantly reduced by 25.50% ($p \leq 0.001$), 96.39% ($p \leq 0.001$), 13.91%, and 55.08% ($p \leq 0.001$), respectively in the Biofield Energy Treated test formulation at day -15 (G7) compared to the disease control (G2). The positive control levamisole showed 17.84%, 8.09%, 21.89%, and 7.63% increased the levels of TC, TG, LDL, and VLDL, respectively compared to the disease control (G2). The levels of HDL and glucose were altered in all the tested groups compared to the disease control (G2).

Table 2. *Effect of the test formulation on lipid biomarkers.*

Group	Glucose (mg/dL)	Total Cholesterol (mg/dL)	Triglyceride (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)
G1	126.63 ± 8.07	91.48 ± 4.75	102.96 ± 6.62	27.39 ± 1.43	43.49 ± 3.66	20.57 ± 1.32
G2	117.06 ± 6.03	89.40 ± 3.80	87.10 ± 11.03	26.78 ± 1.14	45.08 ± 1.99	17.43 ± 2.21
G3	154.61 ± 23.44	105.35 ± 5.26	94.15 ± 4.18	31.58 ± 1.58	54.95 ± 3.58	18.76 ± 0.80
G4	147.10 ± 10.57	72.11 ± 2.98**	67.99 ± 8.52	21.62 ± 0.89	35.68 ± 1.64**	13.57 ± 1.70
G5	124.74 ± 10.43	73.15 ± 3.92**	46.75 ± 4.85**	21.91 ± 1.18	41.90 ± 2.17	9.34 ± 0.97**
G6	203.23 ± 22.16	86.48 ± 3.75	88.81 ± 22.15	25.90 ± 1.12	42.73 ± 3.11	17.77 ± 4.43
G7	127.73 ± 8.83	66.60 ± 3.69***	39.14 ± 4.31***	19.94 ± 1.11	38.81 ± 2.33	7.83 ± 0.86***

Analysis of lipid profile after consecutive 24 days of treatment of the test formulation in male SD rats. All the values are represented as mean ± SEM (n=8). Group; G1: Normal control; G2: Disease control; G3: Levamisole; G4: Biofield Energy Treated test formulation; G5: Untreated test formulation; G6: Biofield Energy Treatment *per se* at day -15; and G7: Biofield Energy Treated test formulation at day -15. HDL: High density lipoprotein; LDL: Low density lipoprotein; VLDL: Very low density lipoprotein; mg/dL: Milligram per deciliter; **$p \leq 0.01$ and ***$p \leq 0.001$ (compared to the disease control).

3.5. Measurement of Hepatic and Cardiac Biomarkers

The effect of the test formulation on the hepatic and cardiac biomarkers are shown in the Table 3. The level of ALP was significantly reduced by 33.65% ($p \leq 0.001$) in the Biofield Energy Treated test formulation group (G4) compared to the disease control (G2). The SGOT, ALP, and CK-MB were significantly decreased by 10.74%, 29.85% ($p \leq 0.05$), and 15.32%, respectively in the Biofield Energy Treatment *per se* at day -15 (G6) compared to the G2 group. Moreover, the levels of SGOT, SGPT, ALP, and CK-MB were significantly decreased by 29.44% ($p \leq 0.05$), 25.54% ($p \leq 0.01$), 27.97% ($p \leq 0.01$), and 36.07% ($p \leq 0.01$), respectively in the Biofield Energy Treated test formulation at day -15 (G7) compared to the G2 group. The ratio of A/G was reduced minimally in the levamisole group (G3) compared to the G2 group. Rest of the biochemical parameters like total bilirubin, total protein, albumin, and globulin did not show any significant changes in all the tested groups compared to the G2 group. Alteration of immune functions are associated with the elevation of liver enzymes in various disorders. The Biofield Energy Treated test formulation group (G4) showed decreased level of ALP by 33.65% compared to the G2 group, which would be beneficial in immunodeficiency patients. Thus, it is assumed that the reduction of ALP might be due to the effect of the Biofield Energy Healing Treatment to the test formulation. It had been shown that ashwagandha reduced the level of ALP level in rats. The current data were also corroborated with the reported literature [51].

Table 3. *Effect of the test formulation on hepatic and cardiac biomarkers in male rats.*

Group	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	CK-MB (U/L)	Tot. BL (mg/dL)	Tot. Prot. (g/dL)	A (g/dL)	G (g/dL)	A/G ratio
G1	201.68 ± 13.31	48.91 ± 3.79	306.88 ± 28.44	828.19 ± 82.35	0.13 ± 0.01	8.17 ± 0.06	3.82 ± 0.03	4.35 ± 0.05	0.88 ± 0.01
G2	170.58 ± 10.68	42.79 ± 3.15	305.05 ± 21.10	825.93 ± 61.10	0.14 ± 0.01	7.81 ± 0.17	3.78 ± 0.03	4.03 ± 0.16	0.95 ± 0.04
G3	164.26 ± 10.57	52.31 ± 1.73	243.66 ± 9.83*	719.13 ± 73.17	0.14 ± 0.01	8.06 ± 0.23	3.73 ± 0.06	4.96 ± 0.58	0.80 ± 0.06
G4	197.01 ± 27.31	44.44 ± 6.93	202.41 ± 14.16***	969.43 ± 86.79	0.15 ± 0.02	7.62 ± 0.15	3.70 ± 0.04	3.92 ± 0.13	0.95 ± 0.03
G5	178.90 ± 9.87	38.42 ± 1.65	209.50 ± 19.20**	809.99 ± 65.30	0.11 ± 0.00	7.72 ± 0.16	3.71 ± 0.07	4.01 ± 0.19	0.94 ± 0.05
G6	152.26 ± 9.94	44.70 ± 2.06	213.98 ± 24.93*	699.43 ± 109.63	0.13 ± 0.01	8.13 ± 0.16	3.73 ± 0.05	4.40 ± 0.12	0.85 ± 0.02
G7	120.36 ± 8.69**	31.86 ± 2.97*	219.74 ± 6.26**	528.01 ± 73.26**	0.14 ± 0.01	7.44 ± 0.12	3.71 ± 0.05	3.76 ± 0.07	0.99 ± 0.01

Analysis of the hepatic and cardiac biomarkers after consecutive 24 days of treatment of the test formulation in male SD rats. All the values are represented as mean ± SEM (n=8). Group; G1: Normal control; G2: Disease control; G3: Levamisole; G4: Biofield Energy Treated test formulation; G5: Untreated test formulation; G6: Biofield Energy Treatment *per se* at day -15; and G7: Biofield Energy Treated test formulation at day -15. 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; *$p \leq 0.05$, **$p \leq 0.01$, and ***$p \leq 0.001$ denoted as statistically significant as compared to the disease control.

3.6. Measurement of Sex Hormone

The effect of the test formulation on testosterone is shown in the Figure 3. An increasing trend of the testosterone was observed across the tested groups compared to the disease control (G2). The level of testosterone was significantly increased by 83.68%, 305.98%, 1271.03% ($p<0.001$) and

745.35% (p<0.05), in the G4, G5, G6, and G7, respectively compared to the G2 group. Ashwagandha (*Withania somnifera*) has been described in traditional Indian Ayurvedic medicine as an aphrodisiac that can be used to treat male sexual dysfunction and infertility. From the scientific literature it was evidenced that ashwagandha root extract has increased in sperm concentration, ejaculate volume, and motile sperm count and an increase the serum level of testosterone [52]. Cinar *et al.* [53], reported that the supplementation of magnesium had increased the both free and total testosterone level. Garcia *et al.* [54], reported that zinc protected male sexual organ and simultaneously increased the level of serum testosterone in smoked in male rats which was due to due to antioxidant and stimulant effects of zinc. Researcher had found an ameliorative potential of sodium selenate and zinc sulfate on intensive-swimming-induced testicular disorders, and significantly reduced the plasma level of testosterone in mature male rats [55].

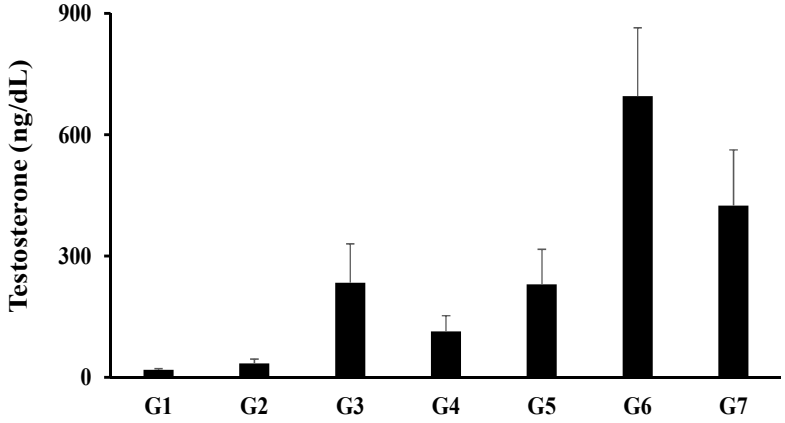

Figure 3. *The effect of the test formulation on testosterone after consecutive 24 days of treatment in male SD rats. G1: Normal control; G2: Disease control; G3: Levamisole; G4: Biofield Energy Treated test formulation; G5: Untreated test formulation; G6: Biofield Energy Treatment per se at day -15; and G7: Biofield Energy Treated test formulation at day -15. All the values are represented as mean ± SEM (n=8), *p≤0.05 and **p≤0.01 (compared to the disease control).*

3.7. Measurement of Antioxidant Profile by ELISA

The effect of the test formulation on various antioxidant enzymes like SOD, LPO, and CAT in male SD rats is demonstrated in the Figure 4. The level of LPO was significantly reduced by 11.98% and 24.15% in the Biofield Energy Treatment *per se* at day -15 (G6) and Biofield Energy Treated test formulation at day -15 (G7), respectively compared to the disease control (G2). No positive trend was observed in case of SOD. The level of CAT enzyme was significantly increased by 57.91% (p<0.01) and 98.51% p<0.001) in the G6 and G7, respectively compared to the disease control Group (G2).

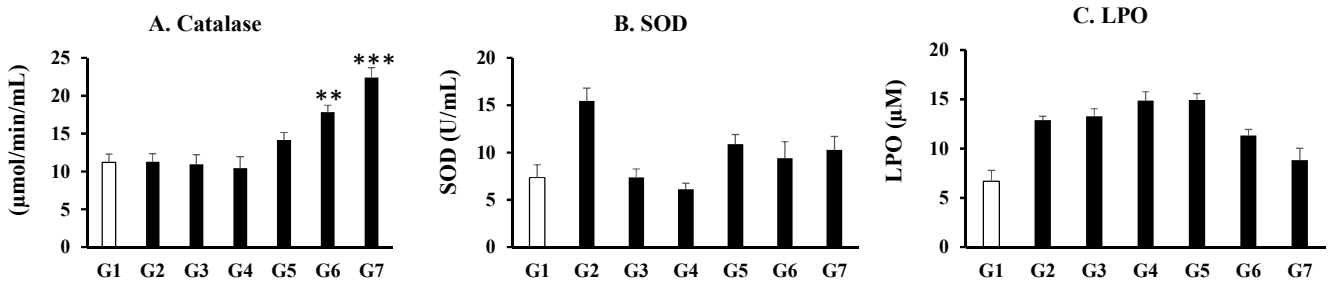

Figure 4. *The effect of the test formulation on anti-oxidative markers (A. CAT, B. SOD, and C. LPO) after 24 consecutive days of treatment in male SD rats. All the values are represented as mean ± SEM (n=8); **p<0.01 and ***p<0.001 (compared to the disease control).*

4. Conclusions

Based on the current study findings, the IgM level was significantly increased by 13.07% and 16.34% in the Biofield Energy Treated test formulation (G4) and Biofield Energy Treated test formulation at day -15 (G7), respectively compared to the disease control group (G2). The expression of the cellular biomarkers like $CD4^+$ and $CD8^+$ was altered minimally in the Biofield Energy Treated test formulation group compared to the disease control. The Biofield Energy Treated test formulation group (G4) showed significant increase the levels of TLC (19.35%) and neutrophils (31.53%), and reduce the levels of lipid biomarkers like TC, TG, and VLDL by 18.18%, 46.33%, and 46.41%, respectively compared to the disease control group (G2). Moreover, the levels of SGOT, SGPT, ALP, and CK-MB were significantly decreased by 29.44%, 25.54%, 27.97%, and 36.07%, respectively in the G7 group compared to the G2 group. The level of the hepatic enzyme like ALP was significantly reduced by 33.65% in the G4 group compared to the G2 group. Further, testosterone was significantly

increased by 83.68% and 745.35% in the G4 and G7, respectively compared to the G2 group. The level of catalase was significantly increased by 57.91% and 98.51% in the Biofield Energy Treatment group *per se* at day -15 (G6) and G7, respectively compared to the G2 group.

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

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 glutamic pyruvic 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

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