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Evaluation of Immune Biomarkers After Oral Administration of the Novel Herbomineral Formulation Treated with The Trivedi Effect® - Biofield Energy Healing 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 consisted of an ashwagandha root extract and minerals (zinc, magnesium, and selenium). The present study was aimed to evaluate the impact of the 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 Energy Treated sample, which received the Biofield Energy Healing Treatment remotely from twenty renowned Biofield Energy Healers. Biomarkers like immunoglobulins (IgG, IgM), cluster differentiation (CD4⁺, CD8⁺), superoxide dismutase (SOD), catalase (CAT), lipid peroxidation (LPO) were monitored. The level of IgM was increased by 4.76% in the Biofield Energy Treated test formulation (G4) compared to the disease control group (G2). The levels of CD4⁺ and CD8⁺ counts were significantly (p≤0.01) increased by 222.22% and 355.36% in the G4 group compared to the G2 group. The level of lymphocyte was increased by 5% and eosinophil count was significantly decreased by 75% in the G4 group compared to the G2 group. The lipid biomarkers such as total cholesterol (TC), low density lipoprotein (LDL), and very low density lipoprotein (VLDL) were significantly lowered by 9.70%, 6.67%, and 23.54%, respectively in the G4 group compared to the G2 group. The expression of SOD was reduced by 9.96% in the G4 group compared to the G2 group. Further, LPO expression was significantly reduced by 33.38% and 16.88% in the G4 and untreated test formulation (G5) groups, respectively compared to the G2 group. Therefore, it can be concluded that the Biofield Energy Treated test formulation showed significantly improved the cellular and humoral immunity, hematological and biochemical profile 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, Hematology, Hepatic Enzymes, Cardiac Biomarker, Anti-aging, Inflammatory Disease and Stress Management

1. Introduction

The newly designed proprietary herbomineral based formulation included four ingredients viz. a mixture of minerals (zinc chloride, magnesium gluconate hydrate, and sodium selenate) and an ashwagandha root extract. Various studies reported the role of dietary zinc as a nutritional immunomodulator. It plays a vital role in various biochemical reactions in living organisms due to its enzyme catalyzing activity [1, 2]. Literature cited that the immunomodulatory activity of magnesium through inhibition of inflammatory cytokines production, regulation of nuclear factor-κB (NF-κB) activation, and disease pathogenesis [3]. Selenium plays a major role for immunomodulation by the alteration of cluster differentiation (CD8+) lymphocyte function [4]. Various studies reported anti-inflammatory and immunomodulatory effects of ashwagandha [5, 6]. Biomarkers are the biological measurements, which can be utilized to predict the severity of disease [7]. It was well established that the immune biomarkers used for the early diagnosis and evaluation of the target organ damage in most of non-autoimmune disease such as diabetes mellitus, hypertension, arteriosclerosis, etc. [8, 9]. The diagnosis of a biomarker which is a biological indicator in clinical practice plays a central role in the selection of the most effective treatment [10].

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 Complementary and Alternative Medicine (CAM) health care approach in addition to other therapies, medicines and practices such as natural products, deep breathing, yoga, Tai Chi, Qi Gong, chiropractic/osteopathic manipulation, meditation, massage, special diets, homeopathy, progressive relaxation, guided imagery, acupressure, acupuncture, relaxation techniques, hypnotherapy, healing touch, movement therapy, pilates, rolfing structural integration, mindfulness, Ayurvedic medicine, traditional Chinese herbs and medicines, naturopathy, essential oils, aromatherapy, Reiki, and cranial sacral therapy. Human Biofield Energy has subtle energy that has the capacity to work in an effective manner [11]. Complementary and Alternate Medicine (CAM) therapies have been practiced worldwide with reported clinical benefits in different health disease profiles [12]. Biofield Energy Healing Treatment has gained rapid rapport as a holistic alternative and complementary medicine therapy that has the significant impact on living organisms and nonliving materials without any adverse effects in a most cost-effective manner than available conventional methods. The Biofield Energy Healing Treatment (The Trivedi Effect®) significant outcomes have been published in numerous peer-reviewed science journals in many scientific fields such as cancer research [13], microbiology [14-16], genetics [17, 18], pharmaceutics [19, 20], nutraceuticals [21], organic compounds [22, 23], agricultural science [24, 25], and changing the structure of the atom in relation to various metals, ceramics, polymers and chemicals in materials science [26-28]. 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

An 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. However, other common laboratory reagents used in this experiment were of analytical grade available in India.

2.2. Experimental Animals

Randomly breed male Sprague Dawley (SD) rats with body weight ranges between 237 to 286 gm were used in this experiment. The animals were purchased from M/s. Vivo Bio Tech Ltd., Hyderabad, India. Standard rodent diet was procured from M/s. Golden feeds, Mehruali, New Delhi, India and provided ad libitum to all the groups of animals during the experiment under controlled conditions with a temperature of 22 ± 3°C, humidity of 30% to 70% and a 12-hour light/12-hour dark cycle. The animals were acclimatized for the period of 5 days prior to the experiment, and all were accessed once daily for clinical signs, behaviors, morbidity and mortality. All the procedures were in strict accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health. The approval of the Institutional Animal Ethics Committee was obtained prior to carrying out the animal experiment.
2.3. 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. Thirteen Biofield Energy Healers were remotely located in the U.S.A., five were located in Canada, and two were located in Australia, while the test herbomineral formulation was located in the research laboratory of Dabur Research Foundation, New Delhi, India. This Biofield Energy Treatment was administered for 5 minutes through the Healer’s unique Energy Transmission process remotely to the test formulation under laboratory conditions. None of the Biofield Energy Healers in this study visited the laboratory in person, nor had any contact with the herbomineral samples. Further, the control group was treated with a “sham” healer for comparative purposes. The sham healer did not have any knowledge about the Biofield Energy Treatment. After that, the Biofield Energy Treated and untreated samples were kept in similar sealed conditions and used for identification of immunological parameters.

2.4. Antigen (Sheep RBC, sRBC)

The fresh sheep blood was collected aseptically from the jugular vein of a healthy sheep and transferred immediately to the heparinized tube. The collected erythrocytes were separated from plasma by centrifugation (400 g, 10°C, 10 minutes), washed twice with the normal saline and then further diluted in saline, which were analyzed using Hematology analyzer (Abbott Model-CD-3700). Based on the number of erythrocytes, the samples were further diluted (using saline) before injecting to the rat [29].

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 at 75 mg/kg per oral (p.o.). The G4 group animals were received Biofield Energy Treated test formulation at a dose of 1105.005 mg/kg. Similarly, the G5 animals were received untreated test formulation at the 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 the 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 at 0.5 X 10^9/100 gm body weight intraperitoneally (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 CO2 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 was 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) was estimated using Mini Vidas, Biomeric (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 and compared to the vehicle control group [30].

2.7. Assessment of Hematology Parameters

Hematological parameters such as total leucocyte count (TLC), and five parts differential leucocyte count (DLC) were analysed using an Hematology analyzer (Abbott Model-CD-3700) in blood samples [31].

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); serum glutamate-pyruvate transaminase (SGPT) were analysed using serum [32].

2.9. Measurement of Sex Hormone - Testosterone

Testosterone was analysed in serum using commercial kits. The mean value was calculated for each group with SEM.

2.10. Assessment of Antioxidant Profile by ELISA Assay

Antioxidants like SOD, CAT, and LPO were analysed using liver homogenate [33].

2.11. Statistical Analysis

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

3. Results and Discussion

3.1. Measurement of Humoral Immune Response

The levels of immunoglobulins (IgG and IgM) after treatment with the test formulation are presented in Figure 1. The level of IgM was increased by 4.76% in the Biofield Energy Treated test formulation group (G4) while unchanged in the untreated test formulation group (G5) compared to the G2 group. Besides, the level of IgG did not show any significant change in all the tested groups compared to the G2 group. 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 an 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 [34, 35].

![Figure 1. The effect of the test formulation on immunoglobulins (A. IgM and B. IgG) in male SD rats. G1: Normal control; G2: Disease control; G3: Levamisole; G4: Biofield Energy Treated test formulation; G5: Untreated test formulation. All the values are represented as mean ± SEM (n=8).](image)

3.2. Measurement of Cellular Immune Responses

The effect of the cellular immunomarkers (CD4+ and CD8+) after administration of the test formulation in male SD rats is shown in the Figure 2. The CD4+ is mainly produced from thymocytes and extra-thymic Th (helper T cells) lymphocytes responsible for cellular immune response [36]. The level of CD4+ count in the normal control group (G1) was 4.15 ± 1.99 and it was significantly reduced by 84.82% in the disease control group (0.63 ± 0.10). The reference item levamisole showed 1382.54% increased the CD4+ counts compared to the G2 group. Besides, the Biofield Energy Treated and untreated test formulations showed 222.22% and 233.33% increased the level of CD4+ counts, respectively compared to the G2 group. The effect of adaptive immune responses of CD8+ T-lymphocytes has been well established. Recently, CD8+ T cells showed the innate immune response, which was beneficial by controlling several types of bacterial infections [37]. The level of CD8+ counts in the normal control group (G1) was 2.36 ± 0.93 and it was significantly reduced by 76.27% in the disease control group (0.56 ± 0.12). The reference item levamisole showed 1083.93% increased the CD8+ counts compared to the G2 group. Besides, the Biofield Energy Treated and untreated test formulations showed 355.36% and 458.93% increased the level of CD8+ counts, respectively compared to the G2 group.

![Figure 2. The effect of the test formulation on cellular biomarkers (A. CD4+ and B. CD8+) in male SD rats. All the values are represented as mean ± SEM (n=8). G1: Normal control; G2: Disease control; G3: Levamisole; G4: Biofield Energy Treated test formulation; G5: Untreated test formulation. **p≤0.01 and ***p≤0.001 vs disease control.](image)

3.3. Assessment of Hematology Parameters

The results of hematology profile in different groups (G1 to G5) are summarized in Table 1. The level of lymphocytes was increased by 5% in the Biofield Energy Treated test formulation (G4) compared to the disease control group (G2). Additionally, the level of eosinophils was significantly increased by 5% in the Biofield Energy Treated test formulation (G4) compared to the disease control group (G2).
decreased by 75% in the G4 group compared to the G2 group. Monocytes was decreased significantly (p≤0.05) by 1.88% in the G4 group compared to the G2. Besides, levamisole showed an increment of TLC, neutrophils, and monocytes compared to the G2 group. It has been reported in the literature that eosinophils have the diverse inflammatory and physiologic immune responses and as a modulator of the intestinal immune system [38]. Another researcher reported that the eosinophils show a protective innate immune response [39]. The overall hematological findings indicated the increased the level of lymphocytes and decreased eosinophils due to the administration of the Biofield Energy Treated test formulation which might be beneficial in the immune-deficient persons to protect and/or improve the immunity to fight against infections.

### 3.4. Measurement of Glucose and Lipid Biomarkers

Glucose and others lipid parameters such as total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL), and very low density lipoprotein (VLDL) are depicted in Table 2. The level of glucose was increased by 8.72% in the Biofield Energy Treated test formulation (G4) compared to the disease control (G2). Further, TC was significantly reduced by 9.70% and 8.10% in the G4 and untreated test formulation (G5), respectively compared to the G2 group. The level of TG was significantly reduced by 23.15% and 15.88% in the G4 and G5, respectively compared to the G2 group. Overall, the reduced levels of all the lipid parameters in terms of TC, TG, LDL, and VLDL with respect to the untreated test formulation. It is assumed that this improvement of lipid profile could be due to The Trivedi Effect®. The level of HDL was significantly (p≤0.01) increased by 27.24% in the levamisole group compared to the disease control. Marken et al. reported that magnesium reduced the levels of TC, LDL, and VLDL [40]. Similarly, Lal et al. described the beneficial effect of magnesium on lipid profile [41]. Hereberg et al. demonstrated that long-term daily supplementation of selenium increased the serum triglyceride levels based on a randomized controlled trials [42]. Based on the current study findings and literature information it is assumed that the Biofield Energy Treated herbomineral formulation showed better response compared to the untreated test formulation group. Overall, the reduced levels of all the lipid parameters except HDL due to the Biofield Energy Treated test formulation could be beneficial in cardiovascular disorders.

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

<table>
<thead>
<tr>
<th>Group</th>
<th>TLC (10^3/mm³)</th>
<th>Neutrophils (%)</th>
<th>Lymphocytes (%)</th>
<th>Eosinophils (%)</th>
<th>Monocytes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>12.19 ± 0.92</td>
<td>17.25 ± 2.26</td>
<td>78.50 ± 3.47</td>
<td>1.50 ± 0.19</td>
<td>2.75 ± 1.19</td>
</tr>
<tr>
<td>G2</td>
<td>7.85 ± 0.89</td>
<td>31.63 ± 1.67</td>
<td>61.25 ± 2.74</td>
<td>2.50 ± 0.68</td>
<td>4.63 ± 0.73</td>
</tr>
<tr>
<td>G3</td>
<td>8.03 ± 0.41</td>
<td>37.25 ± 3.38</td>
<td>55.50 ± 4.00</td>
<td>2.13 ± 0.44</td>
<td>5.13 ± 1.04</td>
</tr>
<tr>
<td>G4</td>
<td>6.94 ± 0.46</td>
<td>29.50 ± 1.12</td>
<td>66.00 ± 1.15</td>
<td>1.75 ± 0.16</td>
<td>2.75 ± 0.31</td>
</tr>
<tr>
<td>G5</td>
<td>7.73 ± 0.50</td>
<td>30.25 ± 1.83</td>
<td>63.50 ± 2.44</td>
<td>2.25 ± 0.37</td>
<td>4.00 ± 0.93</td>
</tr>
</tbody>
</table>

G: Group; G1: Normal control; G2: Disease control; G3: Levamisole; G4: Biofield Energy Treated test formulation; G5: Untreated formulation. Analysis of hematological profile like total and differential (5 parts) counts of white blood corpuscles after consecutive 23 days of treatment of test formulation in male SD rats. All the values are represented as mean ± SEM (n=8). TLC: Total leukocyte count; %: Percentage; *: Percentage; **: Percentage vs control.

### Table 2. Effect of the test formulation on lipid biomarkers in male Sprague Dawley rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Glucose (mg/dL)</th>
<th>Total Cholesterol (mg/dL)</th>
<th>Triglyceride (mg/dL)</th>
<th>HDL (mg/dL)</th>
<th>LDL (mg/dL)</th>
<th>VLDL (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>109.73 ± 4.27</td>
<td>67.94 ± 3.76</td>
<td>54.56 ± 6.03</td>
<td>20.34 ± 1.13</td>
<td>36.74 ± 1.84</td>
<td>10.86 ± 1.21</td>
</tr>
<tr>
<td>G2</td>
<td>109.44 ± 5.69</td>
<td>76.78 ± 2.96</td>
<td>47.30 ± 5.71</td>
<td>22.98 ± 0.88</td>
<td>44.36 ± 1.47**</td>
<td>9.43 ± 1.14</td>
</tr>
<tr>
<td>G3</td>
<td>108.61 ± 6.14</td>
<td>97.17 ± 5.59</td>
<td>62.30 ± 5.01</td>
<td>29.24 ± 1.69**</td>
<td>51.51 ± 1.90</td>
<td>12.44 ± 1.00</td>
</tr>
<tr>
<td>G4</td>
<td>118.98 ± 9.30</td>
<td>69.33 ± 3.91</td>
<td>36.35 ± 2.64</td>
<td>20.75 ± 1.17</td>
<td>41.36 ± 2.41</td>
<td>7.21 ± 0.53</td>
</tr>
<tr>
<td>G5</td>
<td>109.90 ± 3.60</td>
<td>70.53 ± 3.40</td>
<td>39.79 ± 5.31</td>
<td>21.10 ± 1.02</td>
<td>42.74 ± 2.43</td>
<td>7.92 ± 1.06</td>
</tr>
</tbody>
</table>

G: Group; G1: Normal control; G2: Disease control; G3: Levamisole; G4: Biofield Energy Treated test formulation; G5: Untreated formulation. Analysis of lipid parameters after consecutive 23 days of treatment of the test formulation in male SD rats. 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; **: p<0.01 vs disease control and *p<0.01 vs normal control.

### 3.5. Measurement of Hepatic and Cardiac Biomarkers

The effect of the test formulation on various biochemical parameters and cardiac enzyme like creatine kinase myocardium band (CK-MB) is shown in Table 3. The level of hepatic enzymes like SGOT and SGPT in the untreated test formulation (G5) was significantly reduced by 22.46% (p≤0.05) and 32.23% (p≤0.01), respectively; while altered in the Biofield Energy Treated test formulation (G4) compared to the G2 group. The levels of ALP and CK-MB were altered minimally compared to the G2 group. Additionally, others biochemical parameters like total bilirubin, total protein, albumin, globulin even the ratio of albumin and globulin were unaffected with respect to the Biofield Energy Healing Treatment compared to the G2 group. It is assumed that the reduction of ALP value might be due to the Biofield Energy Healing (The Trivedi Effect®) Treatment.
The level of testosterone after oral administration of the test formulation in male SD rats is shown in the Figure 3. The level of testosterone in the normal control group was 218.75 ± 62.66 ng/dL and it was significantly increased by 159.49% in the disease control group (567.63 ± 169.28 ng/dL). The level of testosterone was decreased by 16.76% and 1.87% in the Biofield Energy Treated (G4) and untreated test formulation (G5), respectively compared to the G2 group. Besides, levamisole (G3) showed 70.42% reduction of testosterone level compared to the G2 group. Several literature reported that the high level of testosterone suppressed the immune system [43-45]. Rifé et al. described that testosterone regulate the immunosuppressive activity [46]. In molecular aspect, it has been reported that high level of testosterone reduced various transcription factors or regulatory proteins and enhanced expression of module 52 gene, which have correlation with the immune system. Thus ultimately accelerate the cell differentiation and suppression of immune response [47]. Overall, it was stated that testosterone level was high in the disease control, due to cyclophosphamide which was a well-known immunosuppressant. In this experiment, the Biofield Energy Treated test formulation group (G4) showed higher level of testosterone compared to the normal control group (G1). It is assumed that the Biofield Energy Treated test formulation could have the immunomodulatory activity.

3.6. Measurement of Sex Hormone-Testosterone

The effect of the test formulation on the level of various antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and lipid peroxidation (LPO) in male SD rats is shown in the Figure 4. CAT is an essential enzyme for innate immunity. Further, CAT can correlate between the stress and immune response. It can maintain the oxidation-reduction (redox) balance by removing the hydrogen peroxide (H₂O₂) of immune system [48]. The level of CAT in the normal control (G1) was 9.20 ± 0.97 µmol/min/mL and it was reduced by 8.91% in the disease control (G2; 8.38 ± 0.75 µmol/min/mL). The levamisole showed significant increment of CAT by 16.47% compared to the disease control (G2). The key role of antioxidant defense mechanism by CAT was due to the up-regulation of antimicrobial gene expression [49].

Due to macrophage activation, there was a massive release of cytokines and enzymes that shape the inflammatory response leading to increase the production of reactive oxygen species (ROS). Cu/Zn superoxide dismutase (SOD-1) is a vital enzyme responsible for the dismutation of superoxide radicals from cellular oxidative metabolism into hydrogen peroxide [50]. The level of SOD in the normal control (G1) was 286.21 ± 17.91 µmol/min/mL and it was reduced by 11.71% in the disease control (G2; 252.70 ± 18.15 µmol/min/mL). The levamisole showed significant increment of SOD by 1.86% as compared to the G2 group. The Biofield Energy Treated test formulation showed an inhibition of SOD by 16.47% compared to the disease control (G2). The Biofield Energy Treated test formulation showed an inhibition of SOD by 16.47% compared to the disease control (G2). The Biofield Energy Treated test formulation showed an inhibition of SOD by 16.47% compared to the disease control (G2). The Biofield Energy Treated test formulation showed an inhibition of SOD by 16.47% compared to the disease control (G2). The Biofield Energy Treated test formulation showed an inhibition of SOD by 16.47% compared to the disease control (G2).

Moreover, the level of LPO in the normal control (G1) was 5.11 ± 0.22 µmol/min/mL and it was significantly increased by 55.38% in the disease control (G2; 7.94 ± 2.77 µmol/min/mL). The innate immune responses and antioxidant/oxidant imbalance are the major determinants of various immune related disease in human (e.g., chagas disease). The antioxidant enzymes like LPO and others such as myeloperoxidase (MPO), malondialdehyde (MDA), and nitrite are excellent biomarkers for diagnosis of numerous diseases.

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

<table>
<thead>
<tr>
<th>Group</th>
<th>SGOT (U/L)</th>
<th>SGPT (U/L)</th>
<th>ALP (U/L)</th>
<th>CK-MB (U/L)</th>
<th>Tot. BL (mg/dL)</th>
<th>Tot. Prot. (g/dL)</th>
<th>A (g/dL)</th>
<th>G (g/dL)</th>
<th>A/G ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>177.71 ± 7.61</td>
<td>35.35 ± 1.60</td>
<td>288.98 ± 12.20</td>
<td>135.10 ± 11.07</td>
<td>0.09 ± 0.01</td>
<td>6.96 ± 0.10</td>
<td>3.61 ± 0.03</td>
<td>3.35 ± 0.08</td>
<td>0.10 ± 0.02</td>
</tr>
<tr>
<td>G2</td>
<td>155.84 ± 10.00</td>
<td>32.08 ± 2.40</td>
<td>196.09 ± 10.41</td>
<td>125.83 ± 10.29</td>
<td>0.10 ± 0.01</td>
<td>6.76 ± 0.18</td>
<td>3.50 ± 0.06</td>
<td>3.26 ± 0.12</td>
<td>1.08 ± 0.03</td>
</tr>
<tr>
<td>G3</td>
<td>197.81 ± 16.31</td>
<td>45.13 ± 6.55</td>
<td>191.68 ± 9.98</td>
<td>146.59 ± 15.49</td>
<td>2.12 ± 1.57</td>
<td>6.91 ± 1.00</td>
<td>3.55 ± 0.04</td>
<td>3.36 ± 0.07</td>
<td>1.06 ± 0.01</td>
</tr>
<tr>
<td>G4</td>
<td>160.09 ± 7.35</td>
<td>28.21 ± 2.29</td>
<td>194.83 ± 4.73</td>
<td>126.26 ± 11.17</td>
<td>0.12 ± 0.01</td>
<td>6.84 ± 0.07</td>
<td>3.58 ± 0.06</td>
<td>3.26 ± 0.05</td>
<td>1.10 ± 0.02</td>
</tr>
<tr>
<td>G5</td>
<td>120.84 ± 10.79</td>
<td>21.74 ± 1.45</td>
<td>194.74 ± 9.11</td>
<td>123.93 ± 51.07</td>
<td>0.11 ± 0.01</td>
<td>6.83 ± 0.08</td>
<td>3.58 ± 0.03</td>
<td>3.25 ± 0.07</td>
<td>1.10 ± 0.02</td>
</tr>
</tbody>
</table>

G: Group; G1: Normal control; G2: Disease control; G3: Levamisole; G4: Biofield Energy Treated test formulation; G5: Untreated formulation. Analysis of hepatic and cardiac biomarkers after treatment with the test formulation in male SD rats. All the values are represented as mean ± SEM (n=8). SGOT: Serum glutamic oxaloacetic transaminase; SGPT: Serum glutamate-pyruvate transaminase; ALP: Alkaline phosphatase; CK-MB: Creatine kinase-myoocardial 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<0.05 and **p<0.01 denoted as statistically significant as compared to the disease control.

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

3.7. Measurement of Antioxidant Profile by ELISA Assay

The effect of the test formulation on the levels of various antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and lipid peroxidation (LPO) in male SD rats is shown in the Figure 4. CAT is an essential enzyme for innate immunity. Further, CAT can correlate between the stress and immune response. It can maintain the oxidation-reduction (redox) balance by removing the hydrogen peroxide (H₂O₂) of immune system [48]. The level of CAT in the normal control (G1) was 9.20 ± 0.97 µmol/min/mL and it was reduced by 8.91% in the disease control (G2; 8.38 ± 0.75 µmol/min/mL). The levamisole showed significant increment of CAT by 16.47% compared to the disease control (G2). The key role of antioxidant defense mechanism by CAT was due to the up-regulation of antimicrobial gene expression [49].

Due to macrophage activation, there was a massive release of cytokines and enzymes that shape the inflammatory response leading to increase the production of reactive oxygen species (ROS). Cu/Zn superoxide dismutase (SOD-1) is a vital enzyme responsible for the dismutation of superoxide radicals from cellular oxidative metabolism into hydrogen peroxide [50]. The level of SOD in the normal control (G1) was 286.21 ± 17.91 µmol/min/mL and it was reduced by 11.71% in the disease control (G2; 252.70 ± 18.15 µmol/min/mL). The levamisole showed significant increment of SOD by 1.86% as compared to the G2 group. The Biofield Energy Treated test formulation showed an inhibition of SOD by 9.96% compared to the G2 group. Based on literature, it was reported that suppression of immune response by inhibits the release of various pro-inflammatory cytokines (TNF-α and VEGF) and metalloproteinase enzymes (MMP-2 and MMP-9) [51]. Overall, SOD data suggested that the Biofield Energy Treated test formulation could affect the immune response and pathologies.

Moreover, the level of LPO in the normal control (G1) was 5.11 ± 0.22 µmol/min/mL and it was significantly increased by 55.38% in the disease control (G2; 7.94 ± 2.77 µmol/min/mL). The innate immune responses and antioxidant/oxidant imbalance are the major determinants of various immune related disease in human (e.g., chagas disease). The antioxidant enzymes like LPO and others such as myeloperoxidase (MPO), malondialdehyde (MDA), and nitrite are excellent biomarkers for diagnosis of numerous diseases.
immune diseases [52]. The levamisole showed a significant reduction of LPO by 36.15% compared to the disease control (G2). Besides, the Biofield Energy Treated and untreated test formulation showed 33.38% and 16.88% reduction of LPO expression, respectively compared to the disease control. Lodi et al. reported that decreased level of LPO clearly demonstrate the anti peroxidative activity of Rubia cordifolia plant extract in renal tissue [53]. In this experiment, the Biofield Energy Treated test formulation also showed significant inhibition of LPO compared to both disease control (G2) and untreated test formulation (G5). It is presumed that the inhibitory effects of LPO by Biofield Energy Treated test formulation might be due to the free radical scavenging effect.

![Figure 4. The effect of the test formulation on anti-oxidative markers (A. CAT, B. SOD, and C. LPO) in male SD rats. All the values are represented as mean ± SEM (n=8).](image)

### 4. Conclusions

The current study findings suggested that the level of IgM was increased by 4.76% in the Biofield Energy Treated test formulation group (G4) compared to the disease control group (G2). The levels of CD4⁺ and CD8⁺ counts were significantly increased by 222.22% and 355.36%, respectively in the G4 group compared to G2 group. The lymphocyte was increased by 5% and eosinophil was significantly decreased by 75% in the G4 group compared to the G2 group. The lipid biomarkers such as total cholesterol (TC), low density lipoprotein (LDL), and very low density lipoprotein (VLDL) were significantly lowered by 9.70%, 6.67%, and 23.54%, respectively in the G4 group compared to G2 group. The level of LPO was significantly reduced by 33.38% in the G4 group compared to G2 group.

Overall, the current experimental findings suggested that The Trivedi Effect®-Biofield Energy Healing Treatment performed 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 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 Aloppecia 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 Treated test formulation can also be used in the prevention of immunemediated 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 leucocyte count; DLC: Differential leucocyte 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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### References


