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Immunomodulatory Potential of New Classical Herbomineral Formulation in Experimental Animals: Impact of Biofield Energy Healing Treatment

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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 Consciousness Energy Healing 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 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 seven renowned Biofield Energy Healers. Additionally, one group of animals also received Biofield Energy Treatment *per se* (day -15) by Biofield Energy Healers under similar conditions. The test formulation was evaluated for immunological parameters *viz.* humoral immune analysis, paw volume, hematological study, biochemistry, body weight, feed and water intake, and histopathology analysis were performed. The humoral immune response showed a significant increased primary and secondary antibody titre values by 26% and 150.4%, respectively in Biofield Energy Treatment *per se* group (G6, day -15), while primary and secondary antibody titre values were increased by 100.0% and 110.4%, respectively in the Biofield Energy Treated test formulation (G7, day -15) group compared with the disease control group (G2). The results of delayed type hypersensitivity showed a significant increase in the paw volume by 94.44% in the Biofield Energy Treated test formulation (G4) group with respect to the G2 group. However, a significant increase in paw volume by 61.11% and 44.44% was found in G6 and G7 group, respectively with respect to the G2 group. Hematological parameters showed a significant (p<0.001) increased platelet count by 85.6% in the G4 group, while 39.15% and 35.22% increase in the G6 and G7 groups, respectively compared with the G2 group. In biochemical analysis, a significant increase in calcium and phosphorus level were found in the G4 group compared with the G2 group. Animal weight parameters suggests that there were no treatment-related changes in any group, organ to body weight ratio, feed and water intake. The data described that the Biofield Energy Treated test formulation was found to be safe without any side effect during the course of the experiment. These data suggests that the Biofield Energy Treated test formulation and Biofield Energy Treatment *per se* can be used for autoimmune and inflammatory diseases, enhancing stress management and prevention, and anti-aging by improving overall health.

Keywords: Biofield Energy Healers, The Trivedi Effect®, Autoimmunity, Ant-aging, Alzheimer's Disease

1. Introduction

In traditional system of medicine, herbal formulations have been widely used for modulating the immune system [1]. Several synthetic immunomodulatory agents are used for autoimmune diseases, anti-inflammatory disorders, and antiaging, but mostly associated with some adverse effect and drug interactions [2-4]. Herbal formulations are extensively used in the indigenous system of medicine with significant clinical outcomes due to its high safety, better therapeutic action and few associated side effects [5]. Polyherbal and herbomineral formulations are used and prescribed for many autoimmune and anti-inflammatory disorders. Medicinal plant and minerals (Herbomineral based therapy) are used in combination for many immunomodulatory action [6]. Additionally, holistic medicine/integrative medicine addresses not only entire body, but the mind and spirit as well. Most of the traditional medicines are derived from medicinal plants, minerals, and organic matter [7]. The biological activities of traditional medicines are due to its wide chemical diversity, structural complexity, and broad spectrum activities, which referred as an ideal candidates for new therapeutics formulation. According to World Health Organization (WHO), medicinal plants are the major target of most of the pharmaceutical companies for new formulations due to the presence of one or more active phytoconstituents [8].

With this aspect, the authors of this study used a herbomineral formulation as a basis to investigate ways to improve the immunomodulatory activity. The test formulation containing four components with the combination of the herbal root extract ashwagandha and three minerals *viz.* zinc, magnesium, and selenium. Each constituent of the test formulation is reported for important pharmacological activities, such as ashwagandha (*Withania somnifera*) that belongs to the family *Solanaceae*, commonly used as alternative therapies due to the presence of active molecules like withanolides [9]. Apart from its common attributes such as antibacterial, immunomodulatory and cancer or tumor treatment, many clinical and preclinical data have been available with respect to the immunomodulatory impact [10-11]. The importance of minerals such as selenium, zinc, and magnesium to modulate the immune system has been well-defined [12].

The literature data found that the combination of minerals, herbal medicines exhibited a high level of phagocytic index and an improved antibody titre [13]. These 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 Consciousness Energy1 Healing Treatment (Biofield Energy Healing Treatment) as a complementary and alternative approach to study the impact of Biofield Energy Treatment on the herbomineral formulation for its immunomodulatory potential in male *Sprague Dawley* rats.

In recent years, scientific reports and clinical trials on Biofield Energy Treatments showed the useful effects and enhanced immune function in cases of cervical cancer patients with therapeutic touch [14], massage therapy [15], etc. Amidst many Complementary and Alternative Medicine (CAM) therapies, there have been an extensive number of scientific reports showed that the 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, cranial sacral therapy. The Human Biofield Energy has subtle energy that has the capacity to work in an effective manner [16]. CAM therapies have been practiced worldwide with reported clinical benefits in different health disease profiles [17]. 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 [18], microbiology [19-21], biotechnology [22], genetics [23, 24], pharmaceutics [25, 26], nutraceuticals [27], organic compounds [28, 29], agricultural science [30-32], and changing the structure of the atom in relation to various metals, ceramics, polymers and chemicals in materials science [33-35].

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 with respect to the antibody titre, delayed type hypersensitivity reaction, body weight change, feed consumption, hematological parameters, and serum biochemistry using standard assays.

2. Materials and Methods

2.1. Chemicals and Reagents

Cyclophosphamide and carboxymethyl cellulose sodium were purchased from Sigma Chemical Co. (St. Louis, MO). *Withania somnifera* (Ashwagandha) root extract powder (≥5% of total withanolides) was procured from Sanat

Products Ltd., India. Zinc chloride and magnesium (II) gluconate hydrate were procured from TCI, Japan. Sodium selenate was procured from Alfa Aesar, USA. Levamisole hydrochloride was procured from Sigma, USA. All other chemicals used were of analytical grade available in India.

2.2. Laboratory 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 (IAEC/35/372 & 374) that was obtained prior to carrying out the animal experiment.

2.3. Energy of Consciousness 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. The Trivedi Effect®- Energy of Consciousness Healing Treatment (Biofield Energy Healing Treatment) was provided through a group of seven Biofield Energy Healers who participated in this study and performed the Biofield Energy Treatment remotely. Six Biofield Energy Healers were remotely located in the U.S.A. and one was located in Canada, while the test herbomineral formulation was located in the research laboratory of Dabur Research Foundation, New Delhi, India. Additionally, one group of animals 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*, 10ºC, 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 [36].

2.5. Treatment Regimen

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 salt) *via* oral gavage. G3 group animals received reference item, levamisole at a dose of 75 mg/kg body weight. G4 group animals received Biofield Energy Treated test formulation (1105.005 mg/kg b.wt, *p.o.*), and G5 animals received the untreated test formulation at the same dose by oral route. Further, G6 group animals received 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 13th and 20th the animals were bled and the samples were subjected to hemagglutination test for humoral immune response. On same day 20th, the animals were challenged with sRBC (0.5×10^9 cells/50µL/rat) in sub-planter region and on day 21st (24 hours) paw volume was measured to evaluate the cellular immune response. The body weight and food 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 and serum for biochemistry. At the end of the study, animals were euthanized by CO_2 asphyxiation as per in-house approved standard protocol. Different organs of all animals were excised, weighed and preserved for histopathological analysis.

2.6. Determination of Humoral Immune Response

On day 13 and 20, blood was withdrawn from the retro-

orbital plexus of all antigenically challenged rats. Approximately 25 µL of serum was serially diluted with the 25 µL of phosphate-buffered saline. The sRBC (0.025 x 10^9 cells) was added to each of these dilutions and incubated at 37°C for 1 hour. The rank of minimum dilution that exhibited hemagglutination was considered as an antibody titre. The level of antibody titre on day 13th of the experiment was considered as the primary humoral immune response, while antibody titre on day 20th was considered as the secondary humoral immune response [37].

2.7. Determination of Paw Volume (Delayed Type Hypersensitivity)

The cellular immune response was assayed by the footpad reaction method. The edema was induced in the right paw of rats by injecting sRBC (0.025 x 10^9 cells) in the sub-plantar region. The increase in the paw volume after 24 hours (on day 21) was assessed on digital plethysmometer (PanLab, Spain). The mean percentage change in paw volume was considered as delayed type of hypersensitivity and as an index of cell-mediated immunity. The volume of the left hind paw, injected similarly with phosphate-buffered saline, served as control.

2.8. Determination of Hematological and Biochemical Parameters

The blood was collected from the retro-orbital plexus using heparinized and non-heparinized capillary tubes. One portion of the blood (non-heparinized capillary tubes) was kept as such for the serum collection and stored for biochemical analysis. The other portion (heparinized tubes) was directly subjected for the estimation of various hematological parameters using standard instruments. The level of hemoglobin (Hb), red cell blood count (RBC), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW-CV), and platelets were analyzed with blood samples. Further, the levels of magnesium, blood urea, creatinine, uric acid, calcium, phosphorus, potassium, sodium, and chloride ions concentration were analyzed using a Hematology analyzer (Abbott Model-CD-3700) [38].

2.9. Determination of Body Weight and Feed Intake

Body weight and feed consumption of all the animals in various experimental groups were measured daily. Briefly, the weight of the daily feed supply and the left-over feed by the following days were recorded and the difference was taken as the daily feed intake. The average of the feed intake was computed for every three days of the experimental period [39].

2.10. Clinical Sign and Symptoms

The animal clinical signs and symptoms were evaluated once daily throughout the experiment in accordance with in-house protocol [40] with slight modification. Animals found in a moribund condition or enduring signs of severe distress were humanely euthanized. Abnormal findings were recorded with the time of onset and disappearance.

2.11. Measurement of Relative Organ Weight and Histopathology

At the end of the experiment, rats were dissected and the whole liver, kidneys, hearts, spleens, lungs, and testis were excised, freed of fat, blotted with clean tissue paper, and then weighed. The organ to body weight ratio was determined by comparing the weight of each organ with the final body weight of each rat. Defined samples were placed in 10% neutral buffered formalin for histopathological examination.

2.12. Statistical Analysis

Data were expressed as mean ± standard error of mean (SEM) and were subjected to Student's *t*-test. Statistical significance was considered at $p \leq 0.05$.

3. Results and Discussion

3.1. Effect of Test Formulation on Humoral Immune Response

The effect of the test formulation on primary and secondary humoral titre values in animals is summarized in Table 1. The mean value of primary haemagglutination (HA) antibody titre in a disease control group was significantly ($p \leq 0.001$) decreased (00.50 ± 0.27) compared with the normal control (13.00 ± 2.10). However, the primary antibody titre was slightly increased (*i.e.* 00.88 ± 0.52) after administration of standard drug, levamisole (G3) with respect to the disease control (G2). The animals in the Biofield Energy Treated test formulation group (G4) showed a decreased value of primary antibody titre as 00.13 ± 0.13 with respect to the disease control group (G2), while the primary antibody titre in the untreated test formulation group (G5) was 00.50 ± 0.50. The primary antibody titre was 00.63 ± 0.50 in the G6 group (Biofield Energy Treatment *per se* day -15) and found to be higher than the G2 group. Similarly, the primary antibody titre was significantly increased by 100% (01.00 ± 0.53) in G7 group as compared with the G2 group. Overall, the primary antibody titre was increased by 76%, 26%, and 100% in the G3, G6 and G7 group, respectively while decreased by 74% in the G4 group compared with the disease control group (G2).

Similarly, the secondary HA antibody titre in case of the reference, levamisole (G3) showed a higher titre as 03.50 ± 1.18 compared with the disease control (01.25 ± 0.65). The mean value of secondary antibody titre in a disease control group was significantly ($p \leq 0.001$) decreased (1.25 ± 0.65) compared with the normal control (15.50 ± 2.87). However, G4 group showed a significant decrease in secondary titre by 88.8% (00.14 ± 0.13) compared with the disease control group. The secondary antibody titre in untreated test formulation (G5) group, G6, and G7 were 01.63 ± 0.46, 03.13 ± 1.16 and 02.63 ± 1.27, respectively. Overall, the

values of secondary antibody titre were increased by 180%, 30.4%, 150.4%, and 110.4% in G3, G5, G6, and G7 group respectively, while decreased by 88.8% in the G4 group compared with the disease control group (G2).

Table 1. *Effect of the test formulation on humoral immune response in male rats.*

Group (G)	Primary HA titre	Secondary HA titre
G1	13.00 ± 2.10	15.50 ± 2.87
G2	00.50 ± 0.27***	01.25 ± 0.65***
G3	00.88 ± 0.52	03.50 ± 1.18
G4	00.13 ± 0.13	00.14 ± 0.13
G5	00.50 ± 0.50	01.63 ± 0.46
G6	00.63 ± 0.50	03.13 ± 1.16
G7	01.00 ± 0.53	02.63 ± 1.27

HA: Haemagglutination; G1: Normal Control; G2: Disease Control: G3: Levamisole; G4: Biofield Energy Treated test formulation; G5: Untreated test formulation; G6: Biofield Energy Treatment *per se* (day -15); G7: Biofield Energy Treated test formulation (day -15). All values are expressed as the mean ± SEM. ***$p \leq 0.001$ compared with the normal control (n = 8).

These experimental findings provided a valuable information about the influence of Biofield Energy Healing Treatment on both the primary and secondary humoral immune responses in male rats. It is presumed that the Biofield Energy Treatment *per se* and Biofield Treated test formulation possess potential immunomodulatory activities. It can be assumed that the altered primary and secondary titre values in different groups with respect to disease control might be due to the T-cell-dependent antigen [41]. Further, the significant increase of the antibody titre in the Biofield Energy Treated test formulation, Biofield Energy Treatment *per se* (day -15), and the Biofield Energy Treated test formulation (day -15) clearly indicates significant modulation of the humoral immunity due to the Biofield Energy Healing. It might be expected that alteration in antibody titre might be due to the production of specific antibodies (immunoglobulins) by lymphatic or plasma cells after sensitization to the specific antigens [42]. Thus, Biofield Energy Healing Treatment on day -15 and after inducing the disease in different groups, which suggest a significant capacity to prevent the disease condition. However, the test formulation constituents such as ashwagandha and minerals might be responsible to increase the antibody titre values as reported in the scientific literature [43]. However, experimental data suggest that the Biofield Energy Treated test formulation (day -15) showed a better response compared with the G2, while Biofield Energy Treatment *per se* also showed a significant increase in the secondary antibody titre with respect to the other groups.

3.2. Effect of the Test Formulation on Paw Volume (Delayed Type Hypersensitivity)

With respect to the cellular immune response, levamisole treated group showed an increase in the paw volume (Figure 1) along with all other groups such as G3, G4, G5, G6, and G7 compared with the disease control group (00.18 ± 0.04). The mean edema values in the G3, G4, G5, G6, and G7 groups were 00.32 ± 0.06, 00.35 ± 0.08, 00.32 ± 0.04, 00.29 ± 0.08, and 00.26 ± 0.07 mL, respectively. However, the Biofield Energy Treated test formulation (G4) group showed a better response and significant increased paw volume by 94.44% with respect to disease control group (G2). The Biofield Energy Treated test formulation (G7, day -15) and Biofield Energy Treatment *per se* (G6, day -15) showed an increased paw edema volume by 44.44% and 61.11%, respectively. This indicated that the Biofield Energy Treated test formulation and Biofield Energy Treatment *per se* (day -15) were significantly effective with respect to the stimulation of secondary immune response. According to the scientific literature, ashwagandha was reported with an increased animal paw volume [43], while mineral complex such as zinc was also reported with an increased delayed type hypersensitivity reaction [44]. Thus, it can be concluded with the data, that the constituents present in the formulation are responsible for delayed type hyper sensitivity reaction, but Biofield Energy Treatment (The Trivedi Effect®) further enhanced the cellular immune response compared with the untreated test formulation.

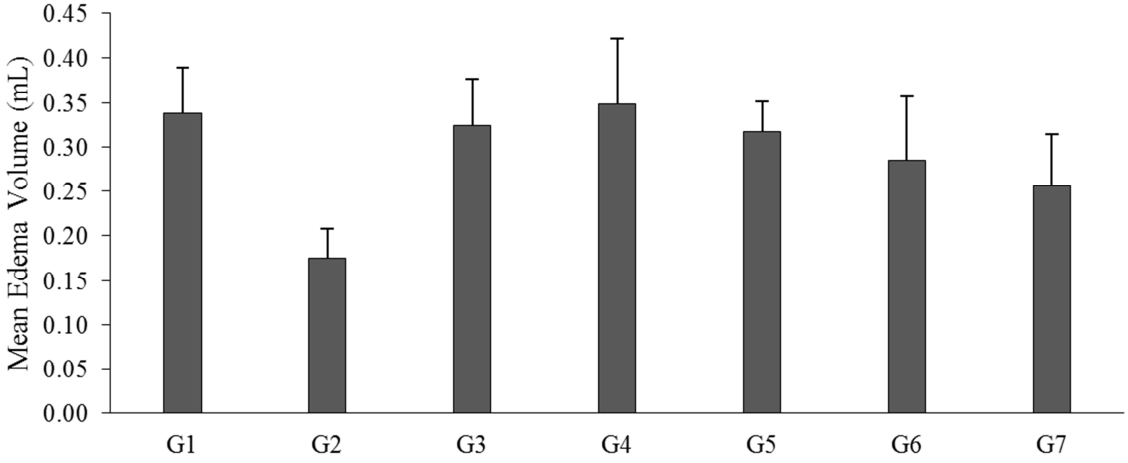

Figure 1. *The effect of the test formulation on paw volume (delayed-type hypersensitivity) in male rats. G1: Normal Control; G2: Disease Control: G3: Levamisole; G4: Biofield Energy Treated test formulation; G5: Untreated test formulation; G6: Biofield Energy Treatment per se (day -15); G7: Biofield Energy Treated test formulation (day -15). All values are expressed as the mean ± SEM (n = 8).*

3.3. Effect of the Test Formulation on Hematology Parameters

The results of the hematology profile analysis after administration of test formulation in experimental animals showed a slight alterations but no statistical significant difference among different group with respect to the disease control (G2) group. The results of the hematology profile of all the groups are summarized in the Table 2. All the tested parameters such as RBC, Hb, PCV, MCV, MCH, and MCHC showed a non-significant alteration with respect to the normal and disease control group. The RBC count was slightly increased in the Biofield Energy Treatment group *per se* day -15 (9.57 ± 0.19 $10^6/\mu$L) and Biofield Energy Treated test formulation group day -15 (G7) group (9.42 ± 0.16) compared with the disease control group (8.94 ± 0.17). However, the Biofield Treated test formulation group (G4) showed a significant (p<0.001) increase in the platelet count *i.e.* 1009.67 ± 115.43 thousand/mm^3 compared with the disease control group, 544.00 ± 77.92 thousand/mm^3. Overall, the platelet count was increased by 85.60% in the G4 compared with the G2 group. However, other experimental groups such as untreated test formulation (G5), Biofield Energy Treatment *per se* group (G6, day -15) and Biofield Energy Treated test formulation (G7, day -15) showed an increase in the platelet count by 21.07%, 39.15%, and 35.22% respectively, compared with the disease control (G2).

Table 2. *Hematology profile after treatment with the test formulation in male rat.*

Group	RBC $10^6/\mu$L	Hb gm/dL	PCV %	MCV fl	MCH pg	MCHC %	Platelet Count (thousand/mm^3)	RDW-CV
G1	9.59 ± 0.15	15.99 ± 0.19	56.13 ± 1.26	58.61 ± 1.35	16.79 ± 0.37	28.74 ± 0.64	685.00 ± 117.94	0.14 ± 0.01
G2	8.94 ± 0.17	15.38 ± 0.24	54.14 ± 1.75	60.59 ± 1.38	17.31 ± 0.18	28.75 ± 0.82	544.00 ± 77.92	0.15 ± 0.01
G3	8.50 ± 0.13	14.48 ± 0.24	51.76 ± 1.27	60.96 ± 1.17	17.14 ± 0.16	28.26 ± 0.71	643.75 ± 94.22	0.15 ± 0.00
G4	8.62 ± 0.47	15.78 ± 0.44	58.52 ± 1.78	64.13 ± 1.01	17.23 ± 0.30	26.93 ± 0.31	1009.67 ± 115.43***	0.16 ± 0.00
G5	9.72 ± 0.15	15.71 ± 0.16	58.88 ± 0.77	60.75 ± 1.41	16.14 ± 0.34	26.65 ± 0.14	658.63 ± 57.83	0.16 ± 0.00
G6	9.57 ± 0.19	15.49 ± 0.20	58.90 ± 0.96	61.78 ± 1.61	16.16 ± 0.36	26.26 ± 0.12	757.00 ± 72.98	0.17 ± 0.00
G7	9.42 ± 0.16	15.41 ± 0.40	57.05 ± 1.80	60.65 ± 1.67	16.31 ± 0.33	27.36 ± 0.26	735.63 ± 51.56	0.17 ± 0.00

G1: Normal Control; G2: Disease Control: G3: Levamisole; G4: Biofield Energy Treated test formulation; G5: Untreated test formulation; G6: Biofield Energy Treatment *per se* (day -15); G7: Biofield Energy Treated test formulation (day -15). RBC: Red blood cells, Hb: Hemoglobin; PCV: Packed cell volume; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; RDW-CV: Red cell distribution width - coefficient of variation. All values are expressed as the mean ± SEM. ***p<0.001 compared with the disease control, (n = 8).

The test formulation showed an improved hematological profile, it might be due to the components of the test formulation such as ashwagandha, zinc, selenium, and magnesium, which might be responsible for a significant improved platelet count. Scientific literature reported that ashwagandha treatment in mice increases the platelet count, red blood cell, white blood cell and hemoglobin concentration [45, 46]. In addition, ashwagandha root extract was non-toxic to the human erythrocytes [47]. The current findings showed no hemolysis at different tested concentrations of test herbomineral formulation. Further, it was also reported that selenium is significantly associated with an improved platelet count [48]. However, the zinc and magnesium present in the test formulation were also reported to be safe, important, and have good therapeutic action [49]. Our experimental results can be well collaborated with the existing literature results, significant improved blood profile might be due to the presence of components present in the test formulation. However, Biofield Energy Healing Treatment significantly increased the hematological profile in Biofield Energy Treated test formulation group compared with the untreated test formulation. Thus, it can be concluded that The Trivedi Effect®- Energy of Consciousness Healing Treatment (Biofield Energy Healing Treatment) has the significant capacity to improve the blood profile and can be used against many blood related autoimmune disorders, anti-inflammatory diseases and antiaging.

3.4. Effect of the Test Formulation on Biochemical Parameters

The serum biochemical parameters were examined after treatment with the test formulation in different tested groups such as the level of magnesium, blood urea, creatinine, uric acid, calcium, phosphorus, potassium, sodium, and chloride ion concentrations (Table 3). Among the tested ions, no significant change was observed in magnesium, creatinine, potassium, sodium and chloride levels in the tested groups compared with the disease control group. In case of blood urea and uric acid, the level was increased in all the tested group with respect to the disease control group. Besides, the level of calcium was reported to be slight increased as 12.23 ± 0.23, 12.14 ± 0.24, 12.38 ± 0.17, and 12.75 ± 0.11 mg/dL in the G4, G5, G6, and G7 groups respectively, compared with the disease control group (G2), 11.83 ± 0.09 mg/dL. Similar increased pattern was also reported in the phosphorus levels, *i.e.* 10.72 ± 0.48, 10.88 ± 0.25, 11.19 ± 0.23, and 11.30 ± 0.29 mg/dL in the G4, G5, G6, and G7 groups respectively, compared with the G2 group, 10.60 ± 0.60 mg/dL. Overall, the Biofield Energy Treated test formulation (G4) group showed an increase calcium and phosphorus levels by 3.38% and 1.13% respectively, while the Biofield Energy Treatment *per se* group (G6, day -15) showed an increased calcium and phosphorus levels by 4.65% and 5.67%, respectively compared with the disease control group.

However, overall result suggested that the change in biochemical parameters after administration of test formulation did not showed any significant alterations compared with the disease control group.

Table 3. *Effect of the test formulation on biochemical parameters in male rat.*

Group	Magnesium (mg/dL)	Blood Urea (mg/dL)	Creatinine (mg/dL)	Uric Acid (mg/dL)	Calcium (mg/dL)	Phosphorus (mg/dL)	K^+ (mEq/l)	Na^+ (Meq/l)	Cl^- (mEq/l)
G1	8.71 ± 0.62	37.79 ± 1.84	0.26 ± 0.03	0.91 ± 0.13	11.61 ± 0.17	9.48 ± 0.26	146.66 ± 0.94	4.65 ± 0.08	106.75 ± 1.06
G2	9.25 ± 0.06	39.23 ± 1.52	0.28 ± 0.02	0.90 ± 0.11	11.83 ± 0.09	10.60 ± 0.60	148.01 ± 0.95	4.93 ± 0.09	104.50 ± 1.94
G3	9.13 ± 0.06	57.41 ± 3.48	0.37 ± 0.02	1.28 ± 0.13	12.35 ± 0.13	10.96 ± 0.27	146.49 ± 1.09	4.90 ± 0.11	102.63 ± 1.12
G4	9.13 ± 0.12	53.18 ± 3.40	0.30 ± 0.00	2.13 ± 0.42	12.23 ± 0.23	10.72 ± 0.48	146.78 ± 1.29	4.67 ± 0.15	103.00 ± 1.03
G5	9.18 ± 0.02	51.20 ± 3.95	0.31 ± 0.02	1.41 ± 0.15	12.14 ± 0.24	10.88 ± 0.25	147.98 ± 1.18	4.98 ± 0.06	102.75 ± 1.78
G6	9.15 ± 0.04	58.05 ± 2.15	0.30 ± 0.02	1.83 ± 0.17	12.38 ± 0.17	11.19 ± 0.23	146.34 ± 1.00	4.70 ± 0.10	103.38 ± 1.76
G7	9.13 ± 0.04	66.59 ± 3.02	0.34 ± 0.03	2.20 ± 0.19	12.75 ± 0.11	11.30 ± 0.29	146.91 ± 0.85	4.99 ± 0.11	104.75 ± 1.24

G1: Normal Control; G2: Disease Control: G3: Levamisole; G4: Biofield Energy Treated test formulation; G5: Untreated test formulation; G6: Biofield Energy Treatment *per se* (day -15); G7: Biofield Energy Treated test formulation (day -15). All values are expressed as the mean ± SEM (n=8).

3.5. Effect of the Test Formulation on Animal Weight Parameters

The animal weight parameters such as body weight and the organ weight were compared with their respective initial mean animal body weight of rats after administration of test formulation (Table 4). The data showed the final body weights was increased with difference in the mean body weight from group G1 to G7. However, the final body weight in all the groups were changed but not significant with normal increase pattern, which suggests that the Biofield Energy Treated herbomineral formulation was found to be safe. Similarly, no significant change was observed in the tested organ weight throughout the experiment in terms of percentage relative organ weight of liver, lungs, kidney, brain, heart, eye, spleen, duodenum, jejunum, ileum, caecum, colon, rectum, testis, prostate, epididymis, vas deference, and pancreas with respect to the normal control throughout the exposure period. The relative organ weight in all the groups did not cause any significant alteration, it suggest that the Biofield Energy Treated test formulation and Biofield Energy Treatment *per se* (day -15) were found to be safe and non-toxic at all the tested concentrations.

Table 4. *Effect of the test formulation on weight parameters in male rats.*

Relative weight (%)	G1	G2	G3	G4	G5	G6	G7
Liver	3.62 ± 0.09	3.92 ± 0.04	5.11 ± 0.13	3.85 ± 0.18	4.33 ± 0.06	3.98 ± 0.12	4.25 ± 0.11
Lungs	0.89 ± 0.06	1.19 ± 0.06	1.05 ± 0.07	0.72 ± 0.09	0.78 ± 0.06	0.79 ± 0.05	0.94 ± 0.11
Kidney	0.86 ± 0.02	0.97 ± 0.02	1.06 ± 0.03	0.72 ± 0.04	0.87 ± 0.04	0.86 ± 0.03	0.86 ± 0.03
Brain	0.57 ± 0.02	0.43 ± 0.02	0.62 ± 0.02	0.52 ± 0.02	0.60 ± 0.02	0.59 ± 0.02	0.60 ± 0.03
Heart	0.40 ± 0.02	0.43 ± 0.04	0.42 ± 0.03	0.34 ± 0.02	0.40 ± 0.01	0.39 ± 0.02	0.40 ± 0.02
Eyes	0.09 ± 0.01	0.09 ± 0.01	0.10 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	0.09 ± 0.01
Spleen	0.26 ± 0.01	0.30 ± 0.01	0.23 ± 0.01	0.18 ± 0.01	0.22 ± 0.01	0.22 ± 0.01	0.22 ± 0.01
Duodenum	0.28 ± 0.05	0.30 ± 0.02	0.36 ± 0.04	0.29 ± 0.04	0.36 ± 0.02	0.29 ± 0.03	0.37 ± 0.05
Jejunum	2.02 ± 0.17	2.10 ± 0.08	2.21 ± 0.10	2.07 ± 0.20	2.45 ± 0.15	2.06 ± 0.08	2.34 ± 0.13
Ileum	0.29 ± 0.05	0.57 ± 0.02	0.33 ± 0.02	0.25 ± 0.20	0.35 ± 0.03	0.34 ± 0.04	0.31 ± 0.03
Caecum	0.59 ± 0.04	0.35 ± 0.04	0.59 ± 0.04	0.54 ± 0.04	0.59 ± 0.04	0.56 ± 0.07	0.58 ± 0.03
Colon	0.33 ± 0.02	0.15 ± 0.03	0.41 ± 0.03	0.28 ± 0.04	0.32 ± 0.02	0.31 ± 0.02	0.32 ± 0.03
Rectum	0.19 ± 0.02	0.15 ± 0.02	0.21 ± 0.03	0.15 ± 0.02	0.16 ± 0.01	0.18 ± 0.01	0.17 ± 0.01
Testis	0.96 ± 0.02	0.99 ± 0.05	1.03 ± 0.03	0.91 ± 0.04	0.99 ± 0.02	0.98 ± 0.02	1.01 ± 0.06
Prostrate	0.28 ± 0.01	0.41 ± 0.02	0.26 ± 0.02	0.23 ± 0.03	0.27 ± 0.02	0.30 ± 0.03	0.26 ± 0.01
Epididymis	0.39 ± 0.02	0.41 ± 0.02	0.41 ± 0.01	0.36 ± 0.01	0.46 ± 0.02	0.41 ± 0.02	0.42 ± 0.03
Vas deference	0.10 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	0.08 ± 0.00	0.09 ± 0.01	0.08 ± 0.01	0.09 ± 0.01
Pancreas	0.48 ± 0.02	0.45 ± 0.03	0.63 ± 0.06	0.43 ± 0.07	0.54 ± 0.06	0.51 ± 0.07	0.59 ± 0.05

G1: Normal Control; G2: Disease Control: G3: Levamisole; G4: Biofield Energy Treated test formulation; G5: Untreated test formulation; G6: Biofield Energy Treatment *per se* (day -15); G7: Biofield Energy Treated test formulation (day -15). All values are expressed as the mean ± SEM (n = 8).

Similarly, no significant change was observed in the tested organ weight throughout the experiment in terms of percentage relative organ weight of liver, lungs, kidney, brain, heart, eyes, spleen, duodenum, jejunum, ileum, caecum, colon, rectum, testis, prostate, epididymis, vas deference, and pancreas with respect to normal control throughout the exposure period. The relative organ weight in all the groups did not show any significant alteration, it suggest that the Biofield Energy Treated test formulation and Biofield Energy Treatment *per se* (day -15) were found safe and non-toxic at the tested concentrations.

Histopathological study analysis suggest that no treatment-related histopathological alterations were reported in all the experimental animals compared with the normal control animal groups. Overall, the data of animal body weight and tested organ weight, its relative percentage showed no significant change. The literature suggest that organ body weight ratio is regarded as the useful index for the identification of swelling, atrophy, or hypertrophy [50]. It was assumed that after exposure of any formulation, if body weight and organ weight were increased in

the animals, then it was regarded as hypertrophy, while decrease in the relative weight indicated the atrophy. However, an increase in body weight and organ-body ratio can be directly correlated with the product toxicity, but our experimental data showed no significant change that depict non-toxic nature of the Biofield Energy Treated test formulation throughout the exposure period.

3.6. Effect of the Test Formulation on Feed and Water Intake

The feed and water intake were measured throughout the experiment and compared with different groups. The results suggested no statistically significant change throughout the experimental period compared with the normal control group (Table 5). It was observed that the animal feed and water intake in the disease control group was slightly higher compared with the other tested groups. Feed intake data suggests the mean value in the disease control (G2) was 26.00 ± 2.09 gm, while it was reported as 20.28 ± 1.61, 19.41 ± 2.42, 21.44 ± 1.22, 23.42 ± 1.01, and 23.12 ± 1.86 gm in the G3, G4, G5, G6, and G7 groups, respectively. However, the change with respect to the disease control group was not statistically significant. Similarly, in case of water intake parameters, the data suggested that maximum water intake values (in mL) was in the disease control group (G2) *i.e.* 40.36 ± 2.52 mL, while it showed no significant change in other tested groups.

Besides, the pretreatment data (day -14) of initial feed consumed (in grams) in group G1, G2, G6, and G7 were reported as 22.93 ± 1.12, 25.44 ± 0.79, 21.94 ± 1.02, and 22.12 ± 1.00 gm respectively. However, the final (day -1) feed consumed (in grams) in all the groups were found non-significant. Similarly, the pretreatment data (day -14) of water intake (in mL) in group G1, G2, G6, and G7 were found as 42.25 ± 2.68, 43.75 ± 2.40, 38.63 ± 2.36, and 45.25 ± 2.53 mL, respectively. However, the final (day -1) water intake data (in mL) in group G1, G2, G6, and G7 were observed as 38.64 ± 2.65, 40.24 ± 2.89, 34.14 ± 1.74, and 35.16 ± 2.07 mL, respectively, which suggest no significant alteration among the tested groups.

Overall, the effect of the Biofield Energy Treated test formulation, untreated test formulation, and Biofield Energy Treatment *per se* (day -15) did not show any significant change in the feed and water intake in male animals.

Table 5. *The effect of the test formulation on the feed and water intake in male rats.*

Group	Feed Intake (gm)	Water Intake (mL)
G1	26.36 ± 1.14	39.59 ± 2.96
G2	26.00 ± 2.09	40.36 ± 2.52
G3	20.28 ± 1.61	39.66 ± 2.34
G4	19.41 ± 2.42	35.47 ± 4.08
G5	21.44 ± 1.22	36.82 ± 2.08
G6	23.42 ± 1.01	37.11 ± 1.93
G7	23.12 ± 1.86	39.59 ± 2.68

G1: Normal Control; G2: Disease Control: G3: Levamisole; G4: Biofield Energy Treated test formulation; G5: Untreated test formulation; G6: Biofield Energy Treatment *per se* (day -15); G7: Biofield Energy Treated test formulation (day -15). All values are expressed as the mean ± SEM (n = 8).

Overall, the study results of above experimental parameters suggested that the significant change in immunomodulatory parameters were due to the Biofield Energy Treatment *per se*, and Biofield Energy Treated test formulation with respect to the cellular and humoral immune responses along with improved hematological and biochemical parameters. It was suggested that The Trivedi Effect®- Energy of Consciousness Healing Treatment (Biofield Energy Healing Treatment) by the renowned Healing Practioners has the significant immunomodulatory action. These alteration might be due to the unique electromagnetic field or radiations of the Healers during the Biofield Energy Treatment. The Biofield Energy Treated test formulation is a new proprietary herbomineral formulation, which might be used to modulate the immune system and work as better approach in future against many autoimmune and antiinflammatory related disorders. However, the traditional medicines along with medicinal plants along with essential minerals can be considered as the powerful source of new drug moieties, and most of the world population depends upon the traditional medicine for health benefits in the developing world.

4. Conclusions

On the basis of experimental results, it can be concluded the significantly impact of The Trivedi Effect®-Energy of Consciousness Healing Treatment (Biofield Energy Healing Treatment) on test formulation exerts substantial anti-inflammatory and immunomodulatory potential without any adverse effect at all the tested concentrations. The humoral immune response data showed the primary and secondary antibody titre were increased by 26% and 150.4%, respectively in the Biofield Energy Treatment *per se* group (G6, day -15), while in the Biofield Energy Treated test formulation (G7, day -15) showed an increased primary and secondary antibody titre values by 100% and 110.4%, respectively compared with the disease control group (G2). Further, a delayed type hypersensitivity data suggests that the Biofield Energy Treated test formulation (G4) group showed a significant increased paw volume by 94.44% with respect to the G2 group. In addition, G6 and G7 groups showed a significant increased paw edema by 61.11% and 44.44%, respectively with respect to the G2 group. Hematology profile after treatment with the Biofield Energy Treated test formulation showed a significant (p<0.001) increase in the platelet count by 85.60% in the G4 group compared with the G2 group. However, the Biofield Energy Treatment *per se* (G6) and Biofield Energy Treated test formulation (G7) also showed an increase in the platelet count by 39.15% and 35.22%, respectively, compared with the G2 group. In serum biochemistry, a significant increase in calcium and phosphorus levels was reported, with respect to the disease control group. However, no treatment-related changes were observed in any group with respect to body weight, feed intake, and water intake data in the Biofield Energy Treated test formulation and Biofield Energy Treatment *per se* groups during the course of the experiment. Therefore, The Trivedi

Effect®- Energy of Consciousness Healing Treatment (Biofield Energy Healing Treatment) administered remotely by the seven Biofield Energy Healers enhanced the herbomineral test formulation's anti-inflammatory and immunomodulatory properties without any toxic effect to the animals throughout the exposure period. Thus, the Biofield Energy Treated test formulation and Biofield Energy Treatment *per se* in male rats showed an effective anti-inflammatory and immunomodulatory action, 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.

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Conflict of Interest

Authors declare no potential conflict of interest.

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