

Immunological Effects of Biofield Energy Healing (The Trivedi Effect®) Based Novel Herbomineral Formulation After Oral Administration in Male *Sprague Dawley* Rats

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To cite this article:

Mahendra Kumar Trivedi, Alice Branton, Dahryn Trivedi, Gopal Nayak, William Dean Plikerd, Peter L. Surguy, Robert John Kock, Rolando Baptista Piedad, Russell Phillip Callas, Sakina A. Ansari, Sandra Lee Barrett, Sara Friedman, Steven Lee Christie, Su-Mei Chen Liu, Susan Elizabeth Starling, Susan Jones, Susan Mardis Allen, Susanne Kathrin Wasmus, Terry Ann Benczik, Thomas Charles Slade, Thomas Orban, Victoria L. Vannes, Victoria Margot Schlosser, Yusif Sarkis Yamin Albino, Mayank Gangwar, Snehasis Jana. Immunological Effects of Biofield Energy Healing (The Trivedi Effect®) Based Novel Herbomineral Formulation After Oral Administration in Male *Sprague Dawley* Rats. *Biomedical Sciences*. Vol. 3, No. 6, 2017, pp. 119-128. doi: 10.11648/j.bs.20170306.13

Received: October 30, 2017; **Accepted:** November 13, 2017; **Published:** December 5, 2017

Abstract: Herbomineral formulations have been used and accepted all around the world owing to its medicinal and therapeutic importance. The aim of the study was to evaluate the immunomodulatory potential of Biofield Energy Healing (The Trivedi Effect®) Treatment on the new proprietary 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 twenty renowned Biofield Energy Healers. The Biofield Energy Treated and untreated test formulation was evaluated using experimental parameters such as humoral immune response (primary and secondary antibody titre), delayed type hypersensitivity reaction, animal weight parameters, feed and water intake, histopathology, hematological and serum biochemistry were studied. Humoral immune data showed the primary and secondary antibody titre values were significantly ($p \leq 0.05$) increased by 105.88% and 260%, respectively in the Biofield Energy Treated Test formulation (G4) group with respect to the disease control group (G2). The delayed type hypersensitivity results showed a significant increased paw edema by 32.43% and 53.33% at 24 and 48 hours, respectively in the Biofield Energy Treated Test formulation (G4) group, while untreated test formulation group (G5) animals showed 13.33% increase in the paw edema (at 48 hour) compared with the G2 group. Hematological analysis showed a significant increase in the red blood cell count, while the platelet count was significantly increased by 27.78% in Biofield Energy Treated Test formulation (G4) group as compared with the G2 group. Biochemical analysis exhibited an increased level of calcium and phosphorus by 1.58% and 12.65%, respectively while serum uric acid was significantly decreased by 36.66% in the G4 group compared with the G2 group. In addition, animal weight parameters like body weight, relative organ weight, histopathology, water and feed intake data suggest that the Biofield Energy Treated herbomineral formulation was found safe, non-toxic, throughout the exposure period. In conclusion, the The Trivedi Effect®-Biofield Energy Healing based herbomineral formulation would be the powerful immunomodulatory product, which can be useful to modulate the immunity against many infectious diseases. Overall, experimental data suggests that the Biofield

Energy Treated test formulation can be used for autoimmune and inflammatory diseases, stress management and prevention, and anti-aging by improving overall health.

Keywords: Biofield Energy Healers, The Trivedi Effect®, Herbomineral Formulation, Paw Volume, Autoimmune Diseases, Inflammatory Diseases, Anti-aging

1. Introduction

Human dependence on plants and their originated products have been started since the origin of the human race, which has been considered as the best source of traditional medicine. Plants and plant based formulations have buildup the structure of traditional medicine therapies. These formulations are the base of patient care system and possess a divine and supernatural power of healing [1]. Against some common inflammatory, autoimmune, and chronic disorders, indigenous plants have played an important role [2]. Besides, their plant based formulation are reported to have important effect to modulate the human immune system [3, 4]. Although, many synthetic products are available worldwide for immune modulation, but they are somehow related to the toxicity [5], while herbomineral formulations claimed several benefits in many autoimmune disorders patients [6]. Thus, there is need to find out some relatively safe, effective, and economical formulation that can modulates the immune system. Besides, many scientific communities are looking at some traditional holistic medicine derived from medicinal plants and minerals. Therefore, herbomineral therapeutics is one of the most promising areas of treating many autoimmune and inflammatory disorders, cancer, stress management and prevention, and anti-aging by improving overall health. Several herbomineral preparations are available for immunomodulation with improved macrophage activity [7], immunopotential action [8], for type-II diabetes [9], and many more [10]. Herbomineral formulations are highly recommended to modulate the immune system compared with the herbal formulations [11]. Phyto-constituents and minerals plays a major role due to its wide chemical and structural complexity, which makes the herbomineral combination an idea product [12]. Hence, the authors of this study used a new proprietary herbomineral formulation with a combination of the herbal root extract ashwagandha and three minerals *viz.* zinc, magnesium, and selenium as a basis to investigate ways to improve its immunomodulatory activity. All the components of test formulation are reported with various pharmacological activities like ashwagandha root extract [13-16], and the minerals [17].

Scientific research has been reported that due to the combination of minerals, herbal medicines have been found to exhibit a high level of phagocytic index and improved antibody titre [18]. These 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 formulation, the Biofield Energy Healers in this

study have used Energy Medicine (Biofield Energy Healing Treatment) as a complementary and alternative approach to study the impact of Biofield Energy Treatment on the herbomineral formulation for its immunomodulatory potential in male *Sprague Dawley* rats.

In recent years, several scientific reports and clinical trials have revealed the useful effects of the Biofield Energy Treatment, which has shown enhanced immune function in cases of cervical cancer patients with therapeutic touch [19], massage therapy [20], etc. Complementary and Alternative Medicine (CAM) therapies are now rising as preferred models of treatment, among which Biofield Therapy (or Healing Modalities) is one approach that has been reported to have 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, Roling structural integration, mindfulness, Ayurvedic medicine, traditional Chinese herbs and medicines, naturopathy, essential oils, aromatherapy, Reiki, and cranial sacral therapy. The human Biofield Energy has subtle energy that has the capacity to work in an effective manner [21]. CAM therapies have been practiced worldwide with reported clinical benefits in different health disease profiles [22]. This energy can be harnessed and transmitted by individuals into living and non-living things *via* the process of Biofield Energy Healing. Biofield Energy Treatment (The Trivedi Effect®) has been published in numerous peer-reviewed science journals with significant outcomes in many scientific fields such as cancer and biotechnology research [23, 24], altered antimicrobial sensitivity of pathogenic microbes in microbiology [25-27], immunology [28-30], genetics [31, 32], nutraceuticals and pharmaceutical science [33-35], agricultural science [36-38], materials science [39-42], and human health and wellness.

The authors of this study want to evaluate the impact of the Biofield Energy Treatment (The Trivedi Effect®) on the given herbomineral formulation for immunomodulatory activity. The results were explored in term of humoral immune response, paw edema, hematological, biochemical parameters, and animal weight parameters.

2. Materials and Methods

2.1. Chemicals and Reagents

Cyclophosphamide and sodium carboxymethyl cellulose (CMC) were purchased from Sigma Chemical Co. (St. Louis, MO). *Withania somnifera* (Ashwagandha) root extract powder ($\geq 5\%$ 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 was procured from Sigma, USA. All other chemicals used in the experiment were of analytical grade available in India.

2.2. Laboratory Animals

A total number of 40 healthy male *Sprague Dawley* rats, weighing between 220 to 290 grams, were used for the study. The animals were purchased from M/s. Vivo Biotech Ltd., Hyderabad, India for this experiment. Standard normal rodent diet was procured from M/s. Golden feeds, Mehrauli, New Delhi, India and provided *ad libitum* to all the groups of animals during the experiment under controlled conditions with a temperature of $22 \pm 3^\circ\text{C}$, humidity of 30% to 70% and a 12-hour light/12-hour dark cycle. The animals were acclimatized for 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 Biofield Energy by renowned Biofield Energy Healers (also known as The Trivedi Effect[®]) and coded as the Biofield Energy Treated formulation, while the second part of the test formulation did not receive any sort of treatment and was defined as the untreated test formulation. This Biofield Energy Treatment was provided through a group of twenty Biofield Energy Healers who participated in this study and performed the Biofield Energy Treatment remotely. Eighteen Biofield Energy Healers were remotely located in the U. S. A and two were located in Canada, while the test herbomineral formulation was located in the research laboratory of Dabur Research Foundation, New Delhi, India. This Biofield Energy Treatment was administered for 5 minutes through the Healer's unique Energy Transmission process remotely to the test formulation under laboratory conditions. None of the Biofield Energy Healers in this study visited the laboratory in person, nor had any contact with the herbomineral samples. Further, the control group was treated with a "sham" healer for comparative purposes. The sham healer did not have any knowledge about the Biofield Energy Treatment. After that, the Biofield Energy treated and untreated samples were kept in similar sealed conditions and used for identification of

immunological parameters.

2.4. Antigen (Sheep RBC)

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 [43].

2.5. Experimental Procedure

A total of 40 animals were divided into five groups (G) in the study *i. e.* G1 to G5 with eight animals ($n=8$) in each group. After 5 days of acclimatization, the animals were grouped (G) based on the body weight. G1 (normal control) received oral suspension of 0.5% carboxy methyl cellulose-sodium salt *via* gavage. G2 served as a disease control (cyclophosphamide, 10 mg/kg, *p. o.*) and G3 group animals received levamisole (75 mg/kg; *p. o.*) from day 1 to day 22. G4 group animals received Biofield Energy Treated test formulation at a dose of 1105.005 mg/kg. G5 animals received of untreated test formulation at the same dose. However, during the treatment period all the animals except normal control (G1) were treated with cyclophosphamide (10 mg/kg, *p. o.*) daily, for initial 13 days. The treatment was continued to all the tested groups (G1 to G5) with 5 mL/kg body weight dose volume. Further, on day 7 and 13, all the groups (G1 to G5) received sRBC ($0.5 \times 10^9/100$ gm body weight; *i. p.*). On day 13 and 20, blood was withdrawn from retro orbital plexus under isoflurane anesthesia and the serum was separated for hemagglutination assay. On day 20, the animals were challenged with sRBC (0.5×10^9 cells/100 $\mu\text{L}/\text{rat}$) in right paw, while on day 21 and 22, the paw thickness was measured using micrometer (MITUTOYO, Japan). Body weight, food intake, and water intake were measured daily before the treatment. On day 22, the animals were kept under overnight fasting and on day 23 blood was withdrawn from retro orbital plexus under isoflurane anesthesia. 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

Approximately 25 μL of serum was serially diluted with the 25 μL of phosphate-buffered saline. The sRBC (0.025×10^9 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 [44].

2.7. Determination of 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 (0.5×10^9 cells) in the sub-plantar region. The increase in the paw thickness in 24 and 48 hours, *i. e.* on day 21 and 22 was assessed using a micrometer (MITUTOYO, Japan). The thickness of the left hind paw, injected similarly with normal saline, served as control. The mean percentage increase in paw thickness in comparison to control was considered as delayed type of hypersensitivity and as an index of cell-mediated immunity [45].

2.8. Determination of Hematological and Biochemical Parameters

After fasting for 12 to 16 hours, blood was collected from the retro-orbital plexus using heparinized or non-heparinized capillary tubes. One portion of the blood was kept in plain bottles from which serum was collected and stored for biochemical analysis. The other portion was directly subjected for the estimation of various hematological parameters using standard instruments. The levels of hemoglobin (Hb), red blood cell count (RBC), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and platelets were analyzed in the blood samples in all experimental groups. Further, the levels of magnesium, blood urea, creatinine, uric acid, calcium, phosphorus, potassium, sodium, and chloride ion concentration were analyzed using Hematology analyzer (Abbott Model-CD-3700) [46].

$$\text{Relative organ weight} = \text{Absolute organ weight (g)} / \text{weight of rat on sacrifice day (g)} \times 100$$

2.12. Statistical Analysis

Data were expressed as mean \pm 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. Estimation of Humoral Immune Response

Animal humoral immune responses were measured after administration of the test formulation. The results of humoral immune titre *i. e.* primary and secondary antibody titre values were evaluated and are presented in Table 1. Cyclophosphamide (G2, disease control group) showed significant decrease in the primary and secondary antibody titre values *i. e.* 4.25 ± 0.59 and 6.25 ± 1.71 , respectively with respect to the normal control data (G1). In addition, levamisole group (G3) showed increase in the primary (7.25 ± 1.51) and secondary titre (16.25 ± 7.60) with respect to the disease control (G2) group. However, biofield energy treated test formulation (G4) group was observed with significant increase in the primary and secondary antibody titre values

2.9. Determination of Body Weight, Water and Feed Intake

Body weight, water intake parameters and feed consumption of all the animals in various experimental groups were measured daily. Briefly, the weight of daily feed supply and the left-over 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. Similarly, the water intake was measured daily throughout the experiment period. All the data were reported from day 1 to day 23 as per study treatment regimen [47].

2.10. Clinical Sign and Symptoms

Animal clinical sign and symptoms were evaluated once daily throughout the experiment in accordance with in-house protocol [48] 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 uterus 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.

Relative organ weight was calculated using the formula mentioned below-

by 105.88% and 260% ($p \leq 0.01$), respectively compared with the G2 group animals. Although, untreated test formulation (G5) showed increase in the secondary antibody titre by 64%, while the primary antibody titre was slightly decreased by 11.76% compared with the G2 group animals. Therefore, it can be inferred that the Biofield Energy Treatment significantly improved the antibody titre values compared with the untreated test formulation.

Table 1. The effect of the test formulation on humoral immune response in male rats.

Groups	Primary HA titre	Secondary HA titre
G1	11.0 ± 1.46	12.0 ± 1.51
G2	$4.25 \pm 0.59^{***}$	$6.25 \pm 1.71^*$
G3	7.25 ± 1.51	16.25 ± 7.60
G4	8.75 ± 2.20	$22.5 \pm 4.66^{**}$
G5	3.75 ± 0.96	10.25 ± 3.75

HA: Haemagglutination; G1: Normal Control; G2: Disease Control; G3: Levamisole; G4: Biofield Energy Treated test formulation; G5: Untreated test formulation. All values are expressed as the mean \pm SEM. *** $p \leq 0.001$ and * $p \leq 0.05$ compared with normal control, while ** $p \leq 0.01$ compared with the disease control; (n = 8).

Humoral immunity modulation using Biofield Energy

Treated ashwagandha based herbal formulation could be a better alternative treatment strategy for various autoimmune disease conditions such as rheumatoid arthritis, multiple sclerosis, asthma, type 1 diabetes, etc. Antibody titre estimation using sheep RBC was considered as the standard method for estimation and it comprises RBCs that have negative ion charge cloud at neutral pH which makes them repel from one another. Further, because of the cell size and pentameric nature, cell immunity using immunoglobulins electric barrier can be overcome, which results subsequent agglutination due to cross-linking of RBCs [49]. This agglutination was measured, and study results concluded that the rate was significantly increased in both the primary and secondary humoral antibody titre values after administration of the Biofield Energy Treated herbomineral formulation. Besides, the results were very much comparable with the untreated test formulation, and the antibody titre was significantly increased due to Biofield Energy Healing Treatment. This can also be suggested that increased acquired immunity can induce the B-cells activation. B-cells functions and its activation depends upon the T-cell dependent mechanism [50, 51]. Therefore, Biofield Energy Treated herbomineral formulation might significantly improve the

immunity using B-cell activation mechanism.

3.2. Delayed-Type Hypersensitivity (DTH) Response

Rat paw thickness was measured at different time period *i.e.* at 24 and 48 hours after oral administration of the test formulation against sheep RBC. The DTH response (paw thickness) at different time interval *i.e.* 24 and 48 hours are presented in the Figure 1. Cyclophosphamide group (G2) showed decrease in the paw edema *i.e.* 0.37 ± 0.04 and 0.30 ± 0.06 mm at 24 and 48 hours, respectively. Levamisole (G3) group animal showed increase paw thickness by 67.57% and 6.66% at 24 and 48 hours, respectively compared with the G2 group animals. However, the Biofield Energy Treated test formulation (G4) group animals showed significant increase in the paw thickness by 32.43% and 53.33% at 24 and 48 hours, respectively compared with the disease control (G2). However, the response was significantly higher compared with the untreated test formulation group (G5) *i.e.* 13.33% increase in G5 compared with G2 at 48 hours. Besides, the paw thickness response was similar in the untreated and treated test formulation at 24 hours compared with the disease control group.

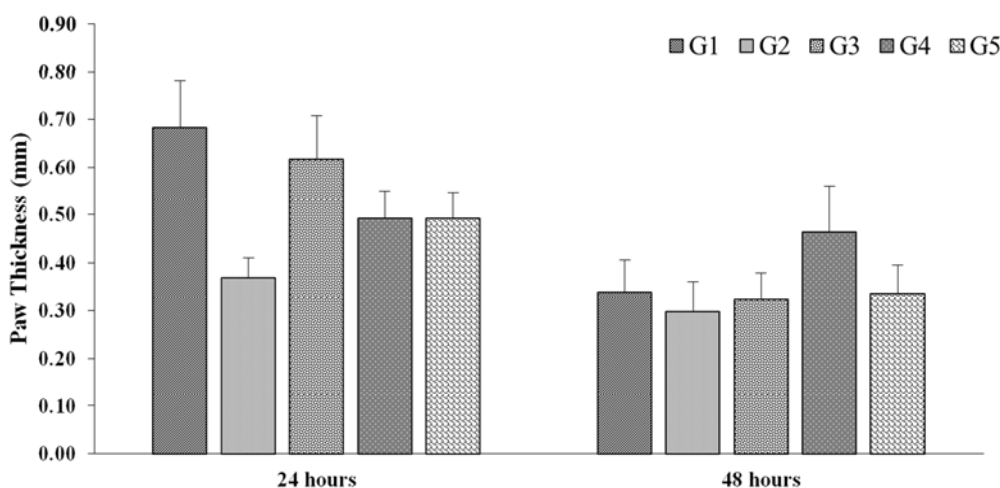


Figure 1. Effect of the test formulation on delayed-type hypersensitivity response in rats. G1: Normal control; G2: Disease control; G3: Levamisole; G4: Biofield Energy Treated test formulation; G5: Untreated test formulation. All values are expressed as the mean \pm SEM ($n = 8$).

DTH reactions are mostly initiated among the anti-specific T-cells and the antigen, which might lead to the lymphokines release that affect the macrophage cells [52]. T lymphocytes and lymphokines regulates the cell mediated immunity using effectors mechanism and this response are very important with respect to infection of foreign grafts and tumor immunity, infections and DTH reactions. The improved inflammatory response in the animals suggest inflammatory action of the Biofield Energy Treated test formulation. The inflammatory response of test formulation was due to *per se* effect of active constituents *i.e.* ashwagandha, zinc, magnesium and selenium [53, 54]. Moreover, Biofield Energy Healing further improves the cellular response of test formulation, which might be useful in against many autoimmune and inflammatory disorders.

3.3. Hematological Analysis

The effects of the Biofield Energy Treated and untreated test formulation with respect to major hematological parameters were analyzed and are shown in the Table 2. The RBC ($10^6/\mu\text{L}$) count in the Biofield Energy Treated test formulation group (G4) was slightly increased *i.e.* 9.44 ± 0.45 $10^6/\mu\text{L}$ compared with the disease control groups. However, significant increase in the platelet count was reported in G4 (*i.e.* 1006.25 ± 121.54 $10^6/\mu\text{L}$) was increased by 27.78% compared with the disease control (G2) group (787.50 ± 49.78 $10^6/\mu\text{L}$). However, other analyzed hematological parameters such as Hb, PCV, MCV, MCH, MCHC, and RDW-CV did not report with any statistically significant altered compared to the disease control group.

Table 2. Hematology profile after treatment with the test formulation in experimental rats.

Group	RBC $10^6/\mu\text{L}$	Hb Gm/dL	PCV%	MCV fl	MCH pg	MCHC%	Platelet Count (thousand/ mm^3)	RDW-CV
G1	9.93 \pm 0.13	17.29 \pm 0.23	64.24 \pm 0.94	64.74 \pm 0.42	17.36 \pm 0.21	26.90 \pm 0.32	975.75 \pm 102.57	0.15 \pm 0.00
G2	9.36 \pm 0.15	16.93 \pm 0.20	63.51 \pm 1.34	67.91 \pm 1.08	18.05 \pm 0.23	26.64 \pm 0.30	787.50 \pm 49.78	0.15 \pm 0.00
G3	8.99 \pm 0.16	15.95 \pm 0.28	60.39 \pm 1.11	67.29 \pm 0.79	17.70 \pm 0.19	26.38 \pm 0.22	831.25 \pm 50.83	0.16 \pm 0.00
G4	9.44 \pm 0.45	16.35 \pm 0.69	61.89 \pm 3.34	65.51 \pm 1.04	17.30 \pm 0.22	26.48 \pm 0.36	1006.25 \pm 121.54	0.16 \pm 0.00
G5	9.24 \pm 0.22	16.21 \pm 0.28	62.11 \pm 1.06	67.40 \pm 0.77	17.53 \pm 0.22	26.05 \pm 0.12	912.50 \pm 69.92	0.16 \pm 0.00

G1: Normal Control; G2: Disease Control; G3: Levamisole; G4: Biofield Energy Treated test formulation; G5: Untreated test formulation. 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 \pm SEM (n = 8).

The study data showed the Biofield Energy Treated test formulation improved hematological analysis. The individual components taken in the test formulation had reported with improved animal hematology parameters. Components such as ashwagandha, zinc, selenium, and magnesium were reported with improved platelet count, red blood cell, Hb, etc. [55-58]. Experimental results showed the herbomineral formulation improved the hematological animal profile, while Biofield Energy Healing Treatment on test formulation significantly improved the blood profile compared with the untreated test formulation. This suggest that the improved immunomodulatory activity of the Biofield Energy Treated herbomineral formulation can be used against various inflammatory and autoimmune diseases.

3.4. Biochemical Parameters

Biochemical analysis after oral administration of Biofield Energy Treated and untreated test formulation are presented in Table 3. Results suggest that the level of magnesium, blood urea, creatinine, uric acid, calcium, phosphorus, potassium, sodium, and chloride ion concentrations were changed in the Biofield Energy Treated test formulation, but not statistically significant. However, slight increase was reported in the level of magnesium, calcium (1.58%), phosphorus (12.65%), and potassium ions in the Biofield Energy Treated test formulation group (G4) compared with the disease control group (G2). Besides, the level of uric acid was significantly decreased by 36.66% in the G4 group compared with the G2 group.

Table 3. Estimation of biochemical parameters after the treatment with the test formulation in experimental rat.

Group	Magnesium (mg/dL)	Blood Urea (mg/dL)	Creatinine (mg/dL)	Uric Acid (mg/dL)
G1	6.15 \pm 0.03	35.30 \pm 2.81	0.42 \pm 0.09	1.55 \pm 0.42
G2	6.11 \pm 0.03	27.34 \pm 0.87	0.31 \pm 0.02	1.20 \pm 0.16
G3	6.17 \pm 0.02	33.20 \pm 1.89	0.52 \pm 0.09	1.03 \pm 0.17
G4	6.16 \pm 0.01	32.58 \pm 1.42	0.32 \pm 0.01	0.76 \pm 0.14
G5	6.13 \pm 0.01	33.44 \pm 2.79	0.34 \pm 0.02	0.70 \pm 0.15

Table 3. Continued.

Calcium (mg/dL)	Phosphorus (mg/dL)	K ⁺ (mEq/L)	Na ⁺ (mEq/L)	Cl ⁻ (mEq/L)
9.91 \pm 0.23	9.26 \pm 0.70	4.96 \pm 0.13	149.56 \pm 0.95	107.13 \pm 0.91
9.46 \pm 0.18	8.93 \pm 0.30	5.04 \pm 0.10	151.10 \pm 0.90	108.50 \pm 1.79
10.09 \pm 0.10	9.89 \pm 0.18	5.36 \pm 0.14	152.13 \pm 1.08	108.88 \pm 1.37
9.61 \pm 0.10	10.06 \pm 0.33	5.31 \pm 0.13	153.33 \pm 1.10	106.63 \pm 1.46
9.69 \pm 0.18	9.37 \pm 1.07	4.91 \pm 0.09	150.23 \pm 0.70	107.75 \pm 0.86

G1: Normal Control; G2: Disease Control; G3: Levamisole; G4: Biofield Energy Treated test formulation; G5: Untreated test formulation. All values are expressed as the mean \pm SEM (n = 8).

It can established that the change in analyzed biochemical parameters and decreased level of uric acid after oral administration of the Biofield Energy Treated test formulation might suggest the use of Biofield Energy Treated test formulation in autoimmune disorders. It was well documented that increased serum uric acid level might cause gout and other pathological implications [59]. Study data suggest the importance of Biofield Energy Healing Treatment on test formulation for its immunomodulatory action and many inflammatory diseases conditions.

3.5. Estimation of Animal Weight Parameters

Biofield Energy Treated and untreated herbomineral formulation was analyzed for animal weight parameters such as animal body weight and respective organ weight. The results of organ weight parameters are summarized in the Table 4. The study data suggest that the initial and final weight in all the group were altered according to the normal pattern in all the group. Overall, the data of animal body weight analysis envisaged no significant change in body weight with respect to the disease control group, it suggest that Biofield Energy Treated herbomineral formulation was found safe in all the tested animals.

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

Relative weight (%)	G1	G2	G3	G4	G5
Liver	4.01 ± 0.26	3.75 ± 0.06	4.70 ± 0.12	4.42 ± 0.19	3.98 ± 0.07
Lungs	0.60 ± 0.02	0.73 ± 0.06	0.91 ± 0.07	0.68 ± 0.05	0.80 ± 0.04
Kidney	0.90 ± 0.01	0.86 ± 0.03	1.02 ± 0.04	0.90 ± 0.03	0.85 ± 0.02
Brain	0.62 ± 0.03	0.65 ± 0.01	0.69 ± 0.02	0.64 ± 0.03	0.62 ± 0.02
Heart	0.41 ± 0.02	0.43 ± 0.01	0.43 ± 0.02	0.45 ± 0.02	0.40 ± 0.02
Eyes	0.08 ± 0.00	0.08 ± 0.00	0.09 ± 0.00	0.08 ± 0.00	0.08 ± 0.00
Spleen	0.23 ± 0.02	0.21 ± 0.01	0.20 ± 0.01	0.19 ± 0.01	0.19 ± 0.01
Duodenum	0.59 ± 0.03	0.56 ± 0.03	0.59 ± 0.03	0.59 ± 0.02	0.54 ± 0.01
Jejunum	0.28 ± 0.02	0.25 ± 0.02	0.35 ± 0.02	0.37 ± 0.02	0.40 ± 0.02
Ileum	1.41 ± 0.11	1.37 ± 0.04	1.56 ± 0.05	1.70 ± 0.05	1.77 ± 0.07
Caecum	0.34 ± 0.02	0.34 ± 0.03	0.40 ± 0.03	0.42 ± 0.02	0.47 ± 0.03
Colon	0.44 ± 0.03	0.47 ± 0.04	0.47 ± 0.05	0.56 ± 0.02	0.57 ± 0.03
Rectum	0.38 ± 0.02	0.31 ± 0.01	0.38 ± 0.03	0.38 ± 0.03	0.43 ± 0.02
Testis	0.15 ± 0.01	0.17 ± 0.01	0.18 ± 0.01	0.18 ± 0.01	0.21 ± 0.01
Prostrate	1.08 ± 0.05	1.15 ± 0.03	1.14 ± 0.02	1.08 ± 0.04	1.12 ± 0.03
Epididymis	0.21 ± 0.01	0.25 ± 0.02	0.19 ± 0.01	0.21 ± 0.02	0.18 ± 0.02
Vas Deference	0.35 ± 0.02	0.41 ± 0.01	0.39 ± 0.01	0.35 ± 0.01	0.36 ± 0.01
Pancreas	0.08 ± 0.00	0.09 ± 0.01	0.07 ± 0.00	0.07 ± 0.00	0.07 ± 0.00

G1: Normal Control; G2: Disease Control; G3: Levamisole; G4: Biofield Energy Treated test formulation; G5: Untreated test formulation. All values are expressed as the mean ± SEM (n = 8).

Similarly, the results of relative organ weight parameters suggest no significant change in tested organ weight throughout the experiment. The results were expressed 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 normal and disease control groups. The goal of the study with respect to organ weight parameter was to examine the oral toxic effect of test formulation, and it was expected that if the test formulation is toxic, it showed significant change in body weight along with organ weight data. The relative percentage change in organ weight showed no abnormal change, which reflect that the Biofield Energy Treated

herbomineral formulation did not have any direct cellular constriction and inflammation [60]. The results were also corroborated with the histopathological studies of the major organs such as kidney, brain, liver, heart, lungs, and testes. The data suggest no abnormal findings, which implement the safe animal profile with the test formulation. These parameters are important for the implications to know the toxic effect of any formulation. Besides, literature data suggest that organ to body weight ratio was observed as the valuable index for the documentation of swelling, atrophy, or hypertrophy after exposure of any test product [61]. With the above results, it can be concluded that the Biofield Energy Treated herbomineral formulation was found safe and non-toxic.

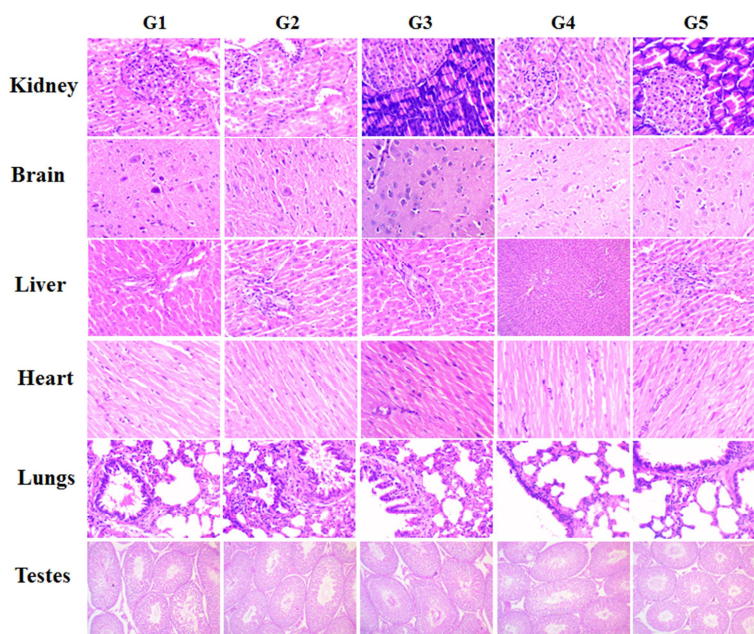


Figure 2. Histopathology of some major organs tested after treatment with the test formulation. G1: Normal Control; G2: Disease Control; G3: Levamisole; G4: Biofield Energy Treated test formulation; G5: Untreated test formulation.

3.6. Assessment of Animal Feed and Water Intake

The study results of animal feed and water intake after oral administration of the test formulation are presented in the Table 3. Results showed that no significant change was observed in animal water and feed intake compared with the disease and normal control groups. The results of feed intake data (in grams) in the disease control (G2) group was 24.28 ± 1.21 g, while it was reported as 20.46 ± 0.93 , 23.86 ± 1.17 , and 23.75 ± 0.93 g in G3, G4, and G5 groups, respectively. The change in feed intake values with respect to the disease control group was found as non-significant. Similarly, water intake values suggested no significant change and the values found in the disease control group (G2) was 37.97 ± 2.46 mL, while it was 44.95 ± 3.27 , 40.02 ± 3.22 , and 40.23 ± 2.77 mL in the G3, G4, and G5 groups, respectively.

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

Group	Feed Intake (g)	Water Intake (mL)
G1	25.87 ± 1.14	39.57 ± 2.33
G2	24.28 ± 1.21	37.97 ± 2.46
G3	20.46 ± 0.93	44.95 ± 3.27
G4	23.86 ± 1.17	40.02 ± 3.22
G5	23.75 ± 0.93	40.23 ± 2.77

G1: Normal Control; G2: Disease Control; G3: Levamisole; G4: Biofield Energy Treated test formulation; G5: Untreated test formulation. All values are expressed as the mean \pm SEM (n = 8).

The consumption of water and feed in all the tested group showed regular and consistent pattern during the experimental period. Feed and water intake parameters were considered as an important with respect to toxicity and safety profile of any formulation. This showed that the formulation was safe to the general health and did not alter the appetite or other metabolic development. Therefore, after evaluating the results, it can be concluded that the Biofield Energy Treated test formulation was found safe and did not show any abnormal physiological and metabolic changes in animals.

4. Conclusions

In conclusion, the data obtained from the present study revealed that the Biofield Energy Treated (The Trivedi Effect®) ashwagandha based herbomineral formulation possesses significant immunomodulatory activity compared with the untreated test formulation. Humoral immune response results suggest that the primary and secondary antibody titre values were significantly increased by 105.88% and 260% ($p \leq 0.01$), respectively in the Biofield Energy Treated test formulation (G4) group compared with the disease control (G2) group. However, untreated test formulation (G5) showed 64% increase in the secondary antibody titre and decrease in primary antibody titre compared with the G2 group. Delayed-type hypersensitivity (DTH) response in rats showed significant increase in the paw thickness by 32.43% and 53.33% ($p \leq 0.05$) at 24 and 48

hours, respectively in the G4 group compared with the disease control (G2) group. However, the percentage increase in the DTH response was higher in Biofield Energy Treated test formulation compared with the untreated test formulation (13.33%). Hematological study showed an increase in the RBC count, while the platelet count was significantly increased by 27.78% in the Biofield Energy Treated test formulation (G4) group compared with the disease control group (G2). Biochemical analysis showed significant decrease in the serum uric acid level by 36.66%, while the levels of magnesium, calcium, phosphorus, and potassium ions were increase in the Biofield Energy Treated test formulation (G4) group compared with the disease control group. In addition, the animal weight parameters such as body weight, relative organ weight, water intake, and feed intake were significantly correlated with the histopathological findings, which suggest no significant change in the Biofield Energy Treated test formulation group (G4). It mentioned that the test formulation was safe, non-toxic, and did not have any injurious effect.

Therefore, the current findings concluded that The Trivedi Effect®-Biofield Energy Healing administered remotely by the twenty Biofield Energy Healers enhanced the herbomineral test formulation's anti-inflammatory and immunomodulatory properties without any side effect, which can be used as a herbomineral product 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 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.

Acknowledgements

The authors are grateful to Dabur Research Foundation, Trivedi Science, Trivedi Global, Inc., and Trivedi Master Wellness for their support throughout the work.

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