

# **Immunopotentiating Impact of The Trivedi Effect<sup>®</sup>-Biofield Energy Healing on Herbomineral Formulation After Oral Administration in Male *Sprague Dawley* Rats**

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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 Biofield Energy Healing (The Trivedi Effect<sup>®</sup>) 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 twenty renowned Biofield Energy Healers. The experimental parameters studies were humoral immune response (primary and secondary titre), delayed type hypersensitivity reaction, animal weight parameters, feed and water intake, histopathology, hematological and serum biochemistry. Humoral immune response showed the primary and secondary antibody titre values were significantly ( $p \leq 0.05$ ) increased by 5.33% and 112.5%, respectively in the Biofield Energy Treated test formulation (G4) group with respect to the disease control group (G2). Delayed type hypersensitivity results showed significant increase in paw edema by 88.24% ( $p \leq 0.05$ ) and 25% at 24 and 48 hours of duration, respectively in the Biofield Energy Treated test formulation (G4) group compared with the G2 group. Hematological studies showed a significant increase in the RBC count, hemoglobin, and packed cell volume (PCV), while the platelet count was increased by 8.40% in the Biofield Energy Treated test formulation group (G4) compared with the disease control group (G2). Biochemical analysis exhibited an increased level of calcium and phosphorus by 2.34% and 7.38%, respectively while serum uric acid was significantly decreased by 18.34% along with a slight increase in the magnesium, potassium, and sodium ions in the Biofield Energy Treated test formulation (G4) compared with the G2 group. In conclusion, the Biofield Energy Treated Test Formulation would be the powerful immunomodulatory product, which was found to be safe at the tested doses. Therefore, The Trivedi Effect<sup>®</sup>-Biofield Energy Healing based herbomineral formulation can be applied to potentiate the immune system that helps to fight against many infectious diseases. Overall, experimental data suggested 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®, Immunopotentiation, Herbomineral Formulation, Acquired Immunity, Paw Volume, Biochemistry

## 1. Introduction

Majority of the world's population depends upon the traditional medicine as the main source of treatment. In developed and developing countries alike, medicinal plant-derived drugs are continuously gaining popularity due to their natural origin and low side effects. Indigenous plants play an important role against various common ailments and chronic diseases [1]. Some medicinal plants are believed to be useful to strengthen the human immune system [2], while such plant based formulations play an important role with significant effect in the modern health care system [3]. Although, the human immune system works by destroying or eliminating the invading pathogens, but sometime this response was inadequate, which can lead to many autoimmune and stress related disorders [4, 5]. In this case, various immunomodulatory medicines are used, but they are reported with severe contrary effects and interactions [6]. In traditional medicine system, herbal formulation are claimed to induce paraimmunity, which is helpful to fight against infections [7]. Herbal formulations are extensively used to modulate the immune system, but the combination with minerals are highly recommended to be used for immunomodulatory action [8]. The significant outcomes of traditional natural medicine are due to its wide chemical and structural complexity that makes it an ideal candidate [9], compared with the modern medicine. 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. 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 an alternative therapies [10, 11] due to the presence of active molecule like withanolides [12]. Apart from its common attributes such as antibacterial, immunomodulatory and antitumor effects, many clinical and preclinical data have been available with respect to the immunomodulatory impact [12, 14]. The importance of minerals such as selenium, zinc, and magnesium to modulate the immune system has been well-defined [15].

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 [16]. 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 test herbomineral formulations, the Biofield Energy Healers

in this study have used Energy Medicine (Biofield Energy Healing Treatment) as a complementary and alternative approach to study the impact of Biofield Energy 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 [17], massage therapy [18], 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. However, as per the data of 2012 from the National Health Interview Survey (NHIS), which comprised that the highest percentage (17.7%) of the Americans used dietary supplement as complementary health approaches compared with other practices in past years. The National Center of Complementary and Integrative Health (NCCIH) has 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, cranial sacral therapy and applied prayer (as is common in all religions, like Christianity, Hinduism, Buddhism and Judaism). Human Biofield Energy has subtle energy that has the capacity to work in an effective manner [19]. Complementary and Alternative Medicine (CAM) therapies have been practiced worldwide with reported clinical benefits in different health disease profiles [20]. 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 research [21, 22], microbiology [23-26], genetics [27, 28], pharmaceutical science [29-32], agricultural science [33-36], and materials science [37-40].

The authors sought to evaluate the impact of the Biofield Energy Treatment (The Trivedi Effect®) on the test herbomineral formulation for immunomodulatory action with

respect to 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 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 hydrochloride 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 Bio Tech 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<sup>®</sup>) and coded as the Biofield Energy Treated formulation, while the second part of the test formulation did not receive any sort of treatment and was defined as the untreated test formulation. This Biofield Energy Treatment was provided through a group of twenty Biofield Energy Healers who participated in this study and performed the Biofield Energy Treatment remotely. Eighteen Biofield Energy Healers were remotely located in the U.S.A and two were located in Canada, while the test herbomineral formulation was located in the research laboratory of Dabur Research Foundation, New Delhi, India. This Biofield Energy Treatment was administered for 5 minutes through the Healer's unique Energy Transmission process remotely to the test formulation under laboratory conditions. None of the Biofield Energy Healers in this study visited the laboratory in

person, nor had any contact with the herbomineral samples. Further, the control group was treated with a "sham" healer for comparative purposes. The sham healer did not have any knowledge about the Biofield Energy Treatment. After that, the Biofield Energy treated and untreated samples were kept in similar sealed conditions and used for identification of immunological parameters.

### 2.4. Antigen (Sheep RBC, sRBC)

The fresh sheep blood was collected aseptically from the jugular vein of a healthy sheep and transferred immediately to the heparinized tube. The collected erythrocytes were separated from plasma by centrifugation (400 g,  $10^\circ\text{C}$ , 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 [41].

### 2.5. Experimental Procedure

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 ( $0.5 \times 10^9$  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  $\text{CO}_2$  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  $\mu\text{L}$  of serum was serially diluted with the 25  $\mu\text{L}$  of phosphate-buffered saline. The sRBC ( $0.025 \times 10^9$  cells) was added to each of these dilutions and incubated at  $37^\circ\text{C}$  for 1 hour. The rank of minimum dilution that exhibited hemagglutination was considered as an antibody

titre. The level of antibody titre on day 13<sup>th</sup> of the experiment was considered as the primary humoral immune response, while antibody titre on day 20<sup>th</sup> was considered as the secondary humoral immune response [42].

### 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 ( $0.5 \times 10^9$  cells) in the sub-plantar region. The increase in the paw thickness at 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 [43].

### 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 and 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) [44].

### 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 [45].

### 2.10. Clinical Sign and Symptoms

Animal clinical sign and symptoms were evaluated once daily throughout the experiment in accordance with in-house protocol [46] 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 testes 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 (1) mentioned below:-

$$\text{Relative organ weight} = \frac{\text{Absolute organ weight(g)}}{\text{weight of rat on sacrifice day(g)}} \times 100 \quad (1)$$

### 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. Effect of the Test Formulation on Humoral Immune Response

Humoral immune responses *i.e.* primary and secondary antibody titre values after administration of test formulation are presented in the Table 1. Cyclophosphamide group showed significant decreased primary and secondary antibody titre *i.e.*  $2.25 \pm 0.25$  and  $2.0 \pm 0.33$ , respectively in the disease control group (G2) with respect to normal control data. The primary ( $3.50 \pm 0.33$ ) and secondary antibody titre ( $2.5 \pm 0.33$ ) was slightly increased after administration of standard drug, levamisole (G3) with respect to the disease control (G2). However, the Biofield Energy Treated test formulation (G4) showed significant increase in the primary and secondary antibody titre values by 5.33% and 112.5% ( $p \leq 0.05$ ), respectively compared with the G2 group. Although, untreated test formulation (G5) showed increased secondary antibody titre by 106% (compared with the G2), thus it can be concluded that Biofield Energy Treatment on herbomineral formulation significantly improved the antibody titre values compared with the untreated test formulation.

**Table 1.** The effect of the test formulation on humoral immune response (haemagglutination titre) in male rats.

Groups	Primary HA titre	Secondary HA titre
G1	$7.50 \pm 0.50$	$7.5 \pm 0.50$
G2	$2.25 \pm 0.25$	$2.0 \pm 0.33$
G3	$3.50 \pm 0.33$	$2.5 \pm 0.33$
G4	$2.37 \pm 0.38$	$4.25 \pm 0.88^*$
G5	$4.50 \pm 0.50^{***}$	$4.12 \pm 0.67^*$

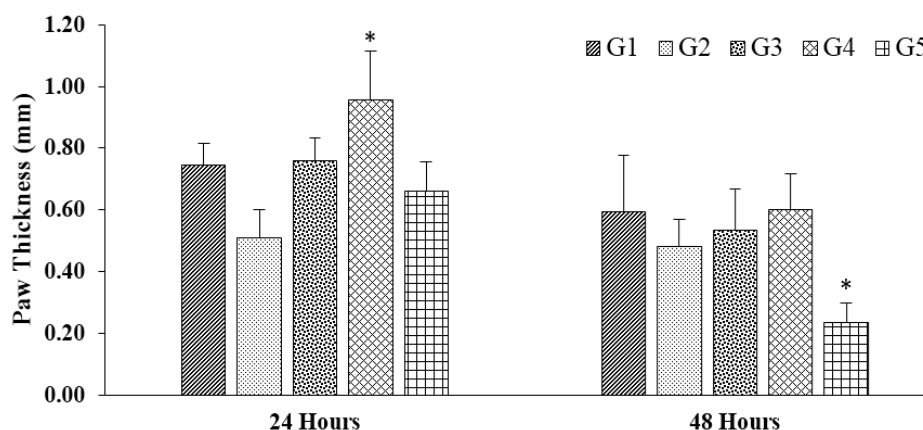
HA: Haemagglutination; G1: Normal Control; G2: Disease Control; G3: Levamisole; G4: Biofield Energy Treated test formulation; G5: Untreated test formulation. All values are expressed as the mean  $\pm$  SEM. \*\*\* $p \leq 0.001$ , \*  $p \leq 0.05$  compared with the normal control; (n = 8).

These experimental findings provided an important activity of Biofield Energy Treated test formulation about the influence on both the primary and secondary humoral immune responses in male rats. Humoral immune response was measured against sRBC by estimating the antibody titre, as it is suggested that at neutral pH, the red blood cells are having negative ions cloud, which makes the cells to repel from one another. This repulsive force is known as zeta potential. Further, due to its pentameric nature and size of cells, IgM may overcome the created electric barrier, and further results in cross-linking of red blood cells that lead to subsequent agglutination [47]. Overall, it can be concluded that primary and secondary humoral antibody titre values showed a significant increase in the experimental group treated with the Biofield Energy Treated test formulation when compared with the disease control group. Acquired immunity is highly dependent on humoral immune response and considered as an important parameter for immunomodulatory action and B-cells activation, which are the major portion of humoral immune system. B-cells functions and its activation are dependent upon the T-cell dependent mechanism [48, 49]. Overall, it can be concluded humoral immunity was significantly improved and Biofield Energy Treated test formulation has the significant capacity

to improve the humoral immune response (primary and secondary HA titre) compared with the untreated test formulation, which can be used against many autoimmune and anti-inflammatory disorders.

### 3.2. Estimation of Delayed Type Hypersensitivity

The results of rat paw thickness measurements at two time period *i.e.* 24 and 48 hours after administration of the Biofield Energy Treated test formulation against sRBC are presented in the Figure 1. Levamisole (G3) animal group showed increase in the paw thickness by 49.02% and 12.5% at 24 and 48 hours, respectively compared with the disease control group (G2). However, Biofield Energy Treated test formulation (G4) was also observed with significant increase in the paw thickness by 88.24% ( $p \leq 0.05$ ) and 25% at 24 and 48 hours, respectively compared with the disease control (G2). However, the delayed type hypersensitivity response in G4 was significantly higher compared with the untreated test formulation (G5) *i.e.* 29.41% increase in G5 compared with G2 at 24 hours. Besides, the paw thickness was decreased in the untreated test formulation (G5) by 50% compared with the G2 group at 48 hours.



**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. \* $p \leq 0.05$  compared with the disease control; ( $n = 8$ ).

Overall, it was concluded that Biofield Energy Treated test formulation significantly increase the inflammatory response against sRBC induced delayed-type hypersensitivity (DTH) response. Cell mediated immunity is carried out using effectors mechanism by T lymphocytes and their products such as lymphokines and this response are very important with respect to infection of foreign grafts and tumor immunity, infections and delayed type hypersensitivity reactions. DTH response are initiated between the anti-specific T-cells and the antigen that lead to lymphokines release, which may lead to affect the macrophage cells population [50]. The significant increase in the DTH responses in rats treated with the Biofield Energy Treated test formulation suggests inflammatory action.

Besides, the individual components present in test

formulation were reported to have increased paw thickness, such as ashwagandha [51] and the minerals was reported with increased DTH reactions [52]. However, Biofield Energy Treatment on the test formulation further significantly improved the immune response. Thus, it can be concluded that the Biofield Energy Treated test formulation can enhanced the cellular immune response compared with the untreated test formulation.

### 3.3. Effect of the Test Formulation on Hematological Parameters

The effect of oral administration of the Biofield Energy Treated test formulation with respect to the tested hematological parameters is shown in the Table 2. The RBC ( $10^6/\mu\text{L}$ ) and Hb (gm/dL) values in the Biofield Energy

Treated test formulation group (G4) was slightly increased *i.e.*  $8.96 \pm 0.11$  and  $15.63 \pm 0.27$  gm/dL, respectively compared with their respective disease control groups. However, the platelet count in G4 (*i.e.*  $806.25 \pm 108.74$  thousand/mm<sup>3</sup>) was increased by 8.40% compared with the

disease control group ( $743.75 \pm 42.72$  thousand/mm<sup>3</sup>). The analyzed parameters such as RBC, Hb, PCV, MCV, MCH, MCHC, platelet count, and RDW-CV did not record any statistically significant changes, but showed an increase level compared to the disease control group.

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

Group	RBC 10 <sup>6</sup> /μL	Hb gm/dL	PCV %	MCV fl	MCH pg	MCHC %	Platelet Count (thousand/mm <sup>3</sup> )	RDW-CV
G1	$9.36 \pm 0.11$	$16.50 \pm 0.24$	$54.95 \pm 0.78$	$58.79 \pm 0.45$	$17.58 \pm 0.13$	$29.99 \pm 0.11$	$856.25 \pm 101.08$	$0.14 \pm 0.00$
G2	$8.67 \pm 0.14$	$15.38 \pm 0.33$	$51.25 \pm 0.94$	$59.02 \pm 0.66$	$17.66 \pm 0.25$	$29.95 \pm 0.14$	$743.75 \pm 42.72$	$0.14 \pm 0.00$
G3	$8.84 \pm 0.22$	$15.59 \pm 0.45$	$52.30 \pm 1.71$	$59.18 \pm 0.61$	$17.58 \pm 0.13$	$29.78 \pm 0.17$	$762.5 \pm 58.06$	$0.13 \pm 0.00$
G4	$8.96 \pm 0.11$	$15.63 \pm 0.27$	$52.58 \pm 0.89$	$58.64 \pm 0.55$	$17.45 \pm 0.17$	$29.78 \pm 0.08$	$806.25 \pm 108.74$	$0.13 \pm 0.00$
G5	$9.24 \pm 0.16$	$16.33 \pm 0.33$	$54.54 \pm 1.08$	$59.09 \pm 0.45$	$17.61 \pm 0.15$	$29.89 \pm 0.14$	$875 \pm 89.64$	$0.14 \pm 0.00$

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).

Overall, the Biofield Energy Treated test formulation showed improve hematological profile with respect to the tested parameters such as platelet count, RBC, Hb, etc. However, the individual components of the test formulation are already reported in the literature with improved animal hematological profile such as ashwagandha, zinc, selenium, and magnesium with improved platelet count, red blood cell, Hb, etc. [53-55]. Thus, it can be concluded that the Biofield Energy Treated test formulation has the capacity to improve the animal blood profile, which can be used as immunomodulatory formulation against many autoimmune and inflammatory diseases.

### 3.4. Effect of the Test Formulation on Serum Biochemistry

The results of biochemical analysis after administration of

**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)	Calcium (mg/dL)	Phosphorus (mg/dL)	K <sup>+</sup> (mEq/L)	Na <sup>+</sup> (mEq/L)	Cl <sup>-</sup> (mEq/L)
G1	$4.44 \pm 0.05$	$30.68 \pm 0.96$	$0.32 \pm 0.01$	$1.81 \pm 0.13$	$10.30 \pm 0.06$	$8.84 \pm 0.94$	$5.03 \pm 0.12$	$150.64 \pm 0.70$	$106.13 \pm 1.38$
G2	$4.39 \pm 0.03$	$31.44 \pm 1.64$	$0.28 \pm 0.01$	$1.69 \pm 0.14$	$9.81 \pm 0.07$	$9.62 \pm 0.25$	$4.90 \pm 0.11$	$150.13 \pm 0.65$	$106.25 \pm 0.84$
G3	$4.45 \pm 0.04$	$34.71 \pm 1.62$	$0.32 \pm 0.00$	$1.94 \pm 0.21$	$10.39 \pm 0.05$	$10.53 \pm 0.18$	$5.09 \pm 0.10$	$151.04 \pm 1.03$	$105.25 \pm 0.86$
G4	$4.42 \pm 0.05$	$34.54 \pm 1.89$	$0.30 \pm 0.02$	$1.38 \pm 0.16$	$10.04 \pm 0.10$	$10.33 \pm 0.21$	$4.96 \pm 0.14$	$150.23 \pm 0.91$	$105.88 \pm 0.83$
G5	$4.38 \pm 0.03$	$29.15 \pm 1.81$	$0.27 \pm 0.01$	$1.34 \pm 0.11$	$10.06 \pm 0.09$	$10.11 \pm 0.20$	$5.00 \pm 0.09$	$150.91 \pm 0.52$	$105.75 \pm 1.50$

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).

Overall result suggested that the change in biochemical tested parameters after administration of the Biofield Energy Treated test formulation did not show any significant alterations, but the level of uric acid was significantly decreased compared with the disease control group. However, it was well documented that the increased serum uric acid could be responsible for gout and other pathological disease conditions [56]. Thus, the improved biochemical profile of animal suggest the importance of Biofield Energy Healing Treatment, which was done by renowned Biofield Energy Healers to the test formulation, which showed significant immunomodulatory function that could be used in various inflammatory diseases such as gout, arthritis, etc.

the test formulation are presented in Table 3. Data 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 (2.34%), phosphorus (7.38%), potassium, and sodium 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 18.34% in the G4 compared with G2. Overall, data suggest that biochemical profile was improved in the Biofield Energy Treated test formulation compared with the untreated test formulation group.

### 3.5. Effect of the Test Formulation on Body Weight and Organ to Body Weight Ratio

The results of animal weight parameters such as animal body weight, and respective organ weight obtained after oral administration of the test formulation are summarized in the Table 4. Initial and final weight in all the groups were altered, as final weight was increased in all the group but not statistically significant. The initial mean body weight was  $268.94 \pm 6.99$ ,  $270.18 \pm 6.75$ ,  $269.46 \pm 5.63$ ,  $270.10 \pm 5.52$ , and  $268.99 \pm 5.30$  gm from group G1 to G5 respectively. However, final body weight in all the group were increased *i.e.*  $351.86 \pm 14.67$ ,  $307.95 \pm 8.18$ ,  $291.58 \pm 7.30$ ,  $312.34 \pm 9.33$ , and  $318.13 \pm 7.05$  gm from group G1 to G5,



respectively. Thus, overall data of body weight analysis visualized no significant change in body weight with respect

to the disease control group, it suggest that the test formulation was found safe in all the tested animal groups.

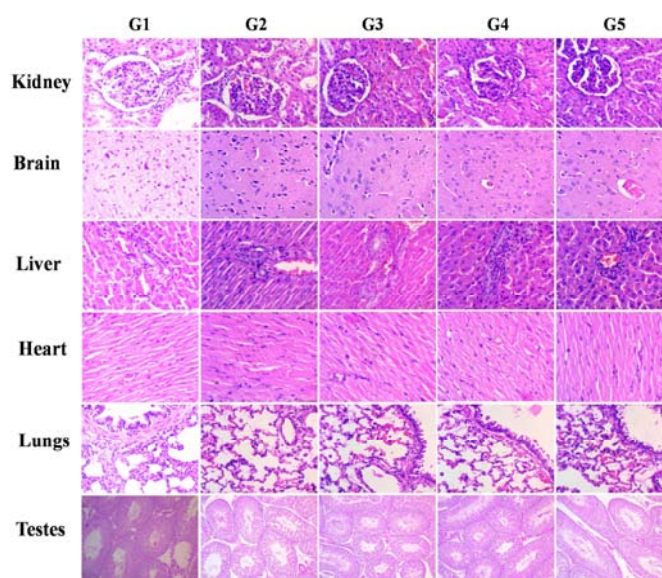
**Table 4.** Effect of the test formulation on organ weight parameters in male rats.

Relative weight (%)	G1	G2	G3	G4	G5
Liver	4.37 ± 0.09	4.42 ± 0.10	4.81 ± 0.16	4.51 ± 0.14	4.68 ± 0.20
Lungs	0.74 ± 0.07	0.95 ± 0.06	0.97 ± 0.06	0.91 ± 0.04	0.79 ± 0.07
Kidney	0.98 ± 0.02	1.01 ± 0.03	1.11 ± 0.03	1.02 ± 0.02	1.02 ± 0.02
Brain	0.62 ± 0.03	0.66 ± 0.02	0.66 ± 0.02	0.65 ± 0.02	0.64 ± 0.02
Heart	0.43 ± 0.01	0.45 ± 0.02	0.47 ± 0.02	0.43 ± 0.02	0.38 ± 0.01
Eyes	0.08 ± 0.00	0.09 ± 0.00	0.09 ± 0.00	0.08 ± 0.00	0.08 ± 0.01
Spleen	0.26 ± 0.01	0.21 ± 0.01	0.21 ± 0.02	0.22 ± 0.02	0.20 ± 0.01
Duodenum	0.24 ± 0.02	0.29 ± 0.02	0.36 ± 0.03	0.35 ± 0.03	0.38 ± 0.02
Jejunum	1.49 ± 0.09	1.35 ± 0.06	1.80 ± 0.05	1.89 ± 0.10	1.99 ± 0.14
Ileum	0.40 ± 0.05	0.31 ± 0.02	0.37 ± 0.03	0.39 ± 0.04	0.37 ± 0.03
Caecum	0.48 ± 0.03	0.50 ± 0.03	0.55 ± 0.05	0.63 ± 0.03	0.68 ± 0.07
Colon	0.33 ± 0.02	0.37 ± 0.01	0.44 ± 0.03	0.39 ± 0.04	0.39 ± 0.03
Rectum	0.15 ± 0.01	0.15 ± 0.01	0.21 ± 0.01	0.17 ± 0.01	0.17 ± 0.01
Testis	1.01 ± 0.05	1.07 ± 0.04	1.21 ± 0.03	1.13 ± 0.05	1.05 ± 0.03
Prostate	0.22 ± 0.03	0.20 ± 0.02	0.23 ± 0.02	0.23 ± 0.02	0.21 ± 0.02
Epididymis	0.30 ± 0.01	0.30 ± 0.01	0.36 ± 0.01	0.34 ± 0.02	0.33 ± 0.01
Vas Deference	0.07 ± 0.00	0.07 ± 0.00	0.07 ± 0.00	0.08 ± 0.01	0.06 ± 0.01
Pancreas	0.59 ± 0.05	0.48 ± 0.05	0.70 ± 0.07	0.62 ± 0.05	0.68 ± 0.07

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, in case of relative organ weight parameters no significant change was observed in 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 and disease control groups throughout the exposure period. It was expected that any unfavorable interaction of the Biofield Energy Treated test formulation with the major animal organs would cause directly results in cellular constriction and inflammation. However, these type of interaction was supposed to be reflected in the organ/body ratio and histopathological study [51]. Histopathological analysis of major organs is shown in the Figure 2. The

histopathological findings of kidney, brain, liver, heart, lungs, and testes suggest no abnormal change in organ histopathology at the tested dose, which conclude that the Biofield Energy Treated test formulation was found to be safe and non-toxic. However, reports also suggest that organ to body weight ratio is regarded as the useful index for the identification of swelling, atrophy, or hypertrophy after exposure of any test compound [57]. The relative organ weight index is regarded as important indicator to assess the deleterious effects of any test formulation. Altogether, the data of animal weight parameters suggest that no significant observations were reported in the body weight, relative organ weight, and histopathological analysis.



**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 results of animal feed and water intake are presented as mean values throughout the study period in the Table 5. Data showed no significant change was found in water and feed intake compared with the disease and normal control group. Feed intake mean values (in grams) in disease control group (G2) was  $24.82 \pm 1.51$  gm, while it was reported as  $21.40 \pm 0.98$ ,  $24.49 \pm 0.94$ , and  $25.35 \pm 1.05$  gm in the G3, G4, and G5 groups, respectively. However, the change with respect to the disease control group was found as non-significant. Similarly, in case of water intake parameters, the data suggested that maximum water intake values (in mL) in the disease control group (G2) was  $39.41 \pm 2.55$  mL, while it was  $46.58 \pm 3.03$ ,  $44.20 \pm 3.01$ , and  $43.26 \pm 3.05$  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 (gm)	Water Intake (mL)
G1	$27.82 \pm 1.52$	$43.98 \pm 3.17$
G2	$24.82 \pm 1.51$	$39.41 \pm 2.55$
G3	$21.40 \pm 0.98$	$46.58 \pm 3.03$
G4	$24.49 \pm 0.94$	$44.20 \pm 3.01$
G5	$25.35 \pm 1.05$	$43.26 \pm 3.05$

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).

Overall, the pattern of data suggest that the consumption of water and feed in all the tested group was regular and consistent throughout the study period. However, feed and water intake was considered as an important parameter with respect to the toxicity and safety profile of any new formulation. After studying these parameters, conclusion can be easily drawn with respect to any physiological and metabolic alteration in animals, so current study results directly reflects the safe nutritional status of the Biofield Energy Treated test formulation in animals. Throughout the study period, no animal was reported with overweight or loss in weight, this suggest that Biofield Energy Treatment did not produce any significant alterations in the appetite and was found as safe with respect to general health status and metabolic development.

## 4. Conclusions

On the basis of experimental observations, it can be concluded that The Trivedi Effect®-Biofield Energy Healing based herbomineral formulation has significant immunomodulatory potential compared with the untreated test formulation. Haemagglutination titre results showed an increase levels of primary and secondary antibody titre values by 5.33% and 112.5% ( $p \leq 0.05$ ), respectively in the Biofield Energy Treated test formulation group (G4) compared with the disease control group (G2), while the results were highly significant compared with the untreated

test formulation group (G5). Delayed-type hypersensitivity response was observed with significant increase in the paw thickness at 24 and 48 hours duration by 88.24% ( $p \leq 0.05$ ) and 25%, respectively in the Biofield Energy Treated test formulation group (G4), compared with the disease control group (G2). Hematological analysis showed an increase RBC count and Hb level, while the platelet count was significantly increased by 8.40% in the Biofield Energy Treated test formulation group (G4) compared with the disease control group (G2). Biochemical study results observed with significant decrease in the serum uric acid level by 18.34%, while increase level of magnesium, calcium, phosphorus, potassium, and sodium ions in the Biofield Energy Treated test formulation group compared with the disease control group. However, animal weight parameters such as body weight, relative organ weight, water intake, feed intake and histopathological findings observed no significant change in the Biofield Energy Treated test formulation group (G4), which recommend that the formulation was found to be safe, and did not have any deleterious effect. Overall, the change in the above weight parameters were consistent throughout the study, which suggest that the Biofield Energy Treated test formulation has safe nutritional status with respect to the physiological and metabolic changes.

Therefore, the current findings conclude 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.

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