



# Potential Role of the Trivedi Effect<sup>®</sup> - Biofield Energy Healing on Immunomodulatory Response of Herbomineral Formulation in Male *Sprague Dawley* Rats

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**Abstract:** A new proprietary herbomineral formulation was formulated, consisting of herbal root extract ashwagandha and minerals (zinc, magnesium, and selenium). The objective of this study was to evaluate the immunomodulatory effect of the Biofield Energy Healing (The Trivedi Effect<sup>®</sup>) Treatment on the test herbomineral formulation in male *Sprague Dawley* rats. The test formulation was divided into two parts; one was represented as control, while the other part was treated with the Biofield Energy Healing Treatment by eighteen renowned Biofield Energy Healers and defined as the Biofield Energy Treated formulation. Besides, one group of animals was also received Biofield Energy Treatment by same Biofield Energy Healers under similar conditions. The effect of the test formulation was monitored by an estimation of humoral immune response, delay type hypersensitivity, hematology, biochemistry, body weight, feed intake, relative organ weight, and histopathology in male *Sprague Dawley* rats. The primary antibody titre level was significantly increased by 36.36% in the Biofield Energy Treated formulation (G3); while decreased by 15.09% in the untreated test formulation (G4) compared to the disease control group (G2). The paw volume was significantly increased by 75% in the Biofield Energy Treated group *per se* at day -15 (G6) compared to the disease control. The level of red blood corpuscle (RBC) was significantly increased by 14.45% in the G6 group compared to the G2 group. The platelet count was significantly increased by 10.32% in the G3 group; while it was decreased by 5.71% in the G4 group compared with the G2 group. There was a significant elevation of serum phosphorus by 6.04% in the G3 group compared to the G2 group. The concentration of magnesium in serum was increased by 13.00% in the Biofield Energy Treated formulation (G3) compared to the disease control group (G2). The concentration of uric acid was significantly decreased by 8.05% in the Biofield Energy Treated formulation (G3) compared to the G2 group. Further, the change in body weight, feed consumption, organ to body weight ratio data, and histopathology examination did not suggest any statistical difference, which depicts that the Biofield Energy Treated test formulation was found to be safe. These data suggest that the Biofield Treated test formulation can be used for autoimmune and inflammatory diseases such as Rheumatoid arthritis, Alzheimer's disease, Atherosclerosis, Dermatitis, Diverticulitis, Diabetes, etc. along with stress prevention and management and anti-aging by improving overall health.

**Keywords:** Biofield Energy Healers, The Trivedi Effect<sup>®</sup>, Herbomineral Formulation, Immunomodulation, Humoral Immune Response, Histopathology, Stress Management, Anti-aging

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## 1. Introduction

In the last few years, there has been exponential growth reported in the herbal medicine sector. In developing and developed countries alike, medicinal plant-derived drugs are gaining popularity due to their eco-friendly nature and less side effects. Many traditional and complementary medicines currently in use are derived from medicinal plants, animals, and minerals, which are commonly used for the prevention and treatment of many diseases [1]. However, the use of traditional remedies has gained importance in cases when conventional medicine is ineffective for certain diseases. The traditional medicines are suitable candidates for new therapeutics due to their vast chemical diversity and various biological effects [2]. *Withania somnifera* (ashwagandha) is an important medicinal plant that belongs to the family *Solanaceae*. It is commonly known as Indian ginseng and is used for various treatments as alternative therapy [3]. Withanolides have been reported as major active constituents that are isolated from the root and leaves of the ashwagandha plant for biological activity [4]. Apart from its important antibacterial activity, several reports have demonstrated its potent immunomodulatory and anti-tumor activity [5]. Preclinical and clinical studies report that each of the active constituents of ashwagandha have shown immunomodulatory effects in various inflammatory diseases [6], but the mechanisms of anti-inflammatory/immunomodulation remained unknown. Selenium, zinc, copper, and magnesium, *etc.* are highly recommended trace elements due to their immunomodulatory impact [7, 8]. The coordinated interactions of these molecules with the immune cells may produce an appropriate immune response. Recently, we prepared a new proprietary herbomineral formulation, which consisted of a combination of the ashwagandha root extract and minerals (zinc, magnesium, and selenium). These ingredients of the test formulation possess significant anti-inflammatory, antioxidant, anti-infective, anti-viral and immune-modulating properties [5, 7, 9, 10], which plays a key role in protecting cells from oxidative stress. Based on the recent literature, it was reported that the herbomineral formulation had exhibited the level of phagocytic index and improved antibody titre to suggest a significant immunomodulatory response. Further, it was reported that the immunomodulatory effect was potentiated in the presence of minerals [11], which can be useful for immune-compromised patients, autoimmune disorders, cancer, anti-stress, anti-aging and in reducing the risk of cardiovascular diseases on long term basis. In recent years, Biofield Energy Treatment (The Trivedi Effect<sup>®</sup>) has been reported worldwide as an alternative treatment method which has

been known for its significant impact on various cancerous cells [12]. According to many scientific studies, Biofield Energy Healing has been reported to have significant outcomes that may prove to be a more cost effective alternative to other approaches [13]. Complementary and Alternative Medicines (CAM) are now rising as preferred method 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, Biofield Energy can exist in different forms such as kinetic, magnetic, potential, electrical, and electromagnetic. The human body has the power to produce low intensity electromagnetic signals known as the Biofield. Thus, a human has the ability to harness energy from the environment and transmit it to any living or nonliving object(s) around the globe. The objects always receive the energy and respond in a useful way. This process is known as Biofield Energy Healing Treatment (The Trivedi Effect<sup>®</sup>). Based on the literature data, Biofield Energy Treatment in terms of a CAM approach was practiced worldwide [14] in addition to herbal medicine. The National Center of Complementary and Integrative Health (NCCIH) has been recognized and accepted Biofield Energy Healing as a CAM health care approach in addition to other therapies, medicines and practices such as natural products, deep breathing, yoga, Tai Chi, Qi Gong, chiropractic/osteopathic manipulation, meditation, massage, special diets, homeopathy, progressive relaxation, guided imagery, acupressure, acupuncture, relaxation techniques, hypnotherapy, healing touch, movement therapy, pilates, rolfing structural integration, mindfulness, Ayurvedic medicine, traditional Chinese herbs and medicines, naturopathy, essential oils, aromatherapy, Reiki, and cranial sacral therapy. To this day, Biofield Energy Healing has had significant impact in the transformation of living organisms and nonliving materials including metals, polymers, ceramics, chemicals, and pharmaceutical compounds. Even further, Biofield Energy Healing Treatment (The Trivedi Effect<sup>®</sup>) has been published in numerous peer-reviewed science journals due to its significant impacts in the science fields of biotechnology [15-16], genetics [17-19], cancer [20-21], microbiology [22-26], materials science [27-30], and agriculture [31-34].

The authors of this study sought to evaluate the impact of Biofield Energy Treatment (The Trivedi Effect<sup>®</sup>) on the given herbomineral formulation, which might improve its immunomodulatory function in male *Sprague Dawley* rats with respect to the humoral immune response, hematological, biochemical, body weight, feed consumption, relative organ weight, and histopathology parameters.

## 2. Materials and Methods

### 2.1. Chemicals and Reagents

Pyrogallol and sodium carboxymethyl cellulose were purchased from Sigma Chemical Co. (St. Louis, MO). Ashwagandha (*Withania somnifera*) root extract powder was procured from Sanat Products Ltd., India. Zinc chloride and magnesium (II) gluconate hydrate were procured from TCI, Japan. Sodium selenate was procured from Alfa Aesar, U.S.A. Levamisole hydrochloride was procured from Sigma, U.S.A. All other chemicals used were of analytical grade available in India.

### 2.2. Laboratory Animals

*Sprague Dawley* (SD) male rats were purchased from M/s. Vivo Bio Tech Ltd., Hyderabad, India. All animals were maintained with 12 hours light and dark cycle during the 24 hours period along with a temperature control  $22 \pm 3^\circ\text{C}$ , humidity of 30% to 70%. The standard chow diet was procured from M/s. Golden feeds, Mehrauli, New Delhi, India, which was provided reverse osmosis filtered drinking water *ad libitum* to all the groups of animals. All the animal experimentation procedures were performed with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health. The approval of the Institutional Animal Ethics Committee was taken prior to animal experiments.

### 2.3. Biofield Energy Treatment Strategies

The herbomineral 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 to the test formulation through a group of eighteen Biofield Energy Healers who participated in this study and performed the Biofield Energy Treatment remotely. Eleven Biofield Energy Healers were remotely located in the U.S.A., four were remotely located in Canada, one in the UK, one in Russia and one in Ireland. 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 the laboratory conditions. Besides, one group of animals was also received Biofield Energy Treatment by the same Biofield Energy Healers under similar 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 test formulation

were returned in the similar sealed condition and kept in recommended storage condition.

### 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,  $10^\circ\text{C}$ , 10 minutes), washed twice with the normal saline and then further diluted in the normal saline and the samples were analyzed using Hematology analyzer (Abbott Model-CD-3700). Based on the number of erythrocytes, the samples were further diluted (using normal saline) before injecting to the rats [35].

### 2.5. Experimental Procedure

After 5 days of acclimatization, the animals were randomized and grouped based on the body weight. Normal control (G1) was received oral suspension of 0.5% carboxy methyl cellulose-sodium salt *via* gavage. The disease control group (G2) was received pyrogallol through intraperitoneal (*i.p.*) route at a dose of 100 mg/kg once daily for 7 days. The G3 and G4 animals were received the Biofield Energy Treated and untreated test formulations, respectively at 1105.005 mg/kg b.wt, *per oral* (*p.o.*). The G5 animals were received levamisole at a dose of 50 mg/kg *p.o.* G6 animals were received the Biofield Energy Treatment *per se* at day -15. Further, all the animals except normal control group (G1) received pyrogallol at a dose of 100 mg/kg through *i.p.* route once daily from day 1 to day 7. The animals were treated with the Biofield Energy Treated and untreated herbomineral formulations to the G3 and G4 animals respectively, 1 hour before pyrogallol challenge in the morning once daily for 22 days. The treatment was continued to all the tested groups (G1 to G6) with 5 mL/kg body weight dose volume. On day 7<sup>th</sup> and day 13<sup>th</sup>, all the animals in the G2-G6 except normal control (G1) were challenged with sheep red blood cells (sRBC) ( $0.5 \times 10^9/100$  gm; *i.p.*) as the antigenic material to sensitize them for immunological parameters. On the days 13<sup>th</sup> and 20<sup>th</sup>, blood sample was collected from retro-orbital plexus and subjected to hemagglutination test to evaluate the humoral immune response. On the same days, the animals were challenged with sRBC ( $0.5 \times 10^9$  cells/50  $\mu\text{L}$ /rat) in sub-planter region and on 22<sup>nd</sup> day (48 hours), paw volume was measured to evaluate cellular immune response. The body weight and food consumption were measured daily before treatment. On day 22, the animals were kept under fasting over night and on day 23; blood was collected again from retro-orbital plexus from each animal under isoflurane anaesthesia. Whole blood was analysed for haematological parameters and serum was analysed for serum biochemistry. 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

On day 13<sup>th</sup> and 20<sup>th</sup>, blood was withdrawn from the retro-orbital plexus of all antigenically challenged rats. Approximately 25  $\mu$ L of serum was serially diluted with 25  $\mu$ L of phosphate-buffered saline. The sRBC ( $0.025 \times 10^9$  cells) was added to each of these dilutions and incubated at 37°C for one 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 and the day 20<sup>th</sup> of the experiment was considered as the secondary humoral immune response [36].

## 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 ( $0.025 \times 10^9$  cells) in the sub-plantar region. The change in paw volume after 24 hours (on day 21) was assessed on digital plethysmometer (Pan Lab, 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

On day 23<sup>rd</sup> of the experiment, blood was collected from the retro-orbital plexus using heparinized and non-heparinized capillary tubes after 12 to 16 hours of fasting. The non-heparinized blood was kept as such from which serum was collected and further stored for biochemical analysis. The heparinized blood was directly subjected for the estimation of various hematological parameters using standard instrument. The various hematological parameters such as hemoglobin (Hb), red blood corpuscle (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. Further, the levels of magnesium, blood urea nitrogen (BUN), creatinine, uric acid, calcium, phosphorus, potassium, sodium, and chloride ion concentration were analyzed using an Hematology analyzer (Abbott Model-CD-3700) [37].

## 2.9. Determination of Body Weight and Feed Intake

Body weight and feed intake of all the animals were measured once daily before dosing. Briefly, the weight of daily feed supply and the left-over amount by the following day was 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 [38].

## 2.10. Clinical Signs and Symptoms

All the animals were observed for the clinical signs once

daily in accordance with in-house protocol [39]. Animals found in a moribund condition or enduring signs of severe distress was 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, heart, spleen, lungs, whole intestine, eyes, brain, testis, prostate, epididymis, and vas deference were excised, weighed, and observed various gross pathological lesions. These organs were trimmed off any adherent tissue and fat, as appropriate and were weighed in wet condition as soon as possible to avoid drying. The organ to body weight ratio was determined by comparing with the weight of each organ with the final body weight of each rat. Defined samples were placed in the neutral buffered formalin (10%) for histopathological examination as per standard in-house protocol.

## 2.12. Statistical Analysis

Data were expressed as mean  $\pm$  standard error of mean (SEM) and were subjected to one-way analysis of variance (ANOVA), and the *post-hoc* analysis was performed using Dunnett's test using Sigma Plot (11.0) statistical software. Statistical significance was considered at  $p < 0.05$ .

# 3. Results and Discussion

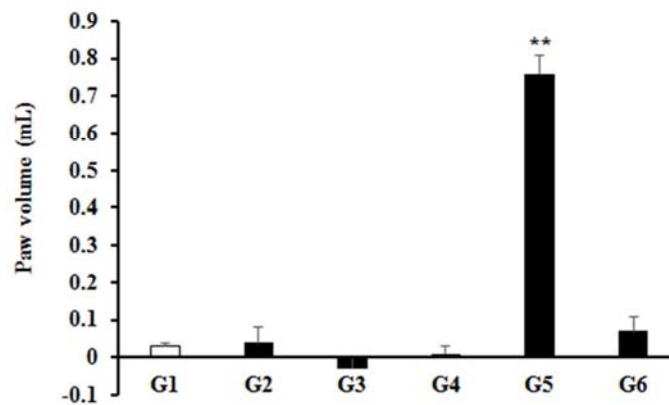
## 3.1. Determination of Humoral Immune Response

The results of primary and secondary humoral immune responses after administration of test formulation in male *Sprague Dawley* rats are summarized in the Table 1. Primary (on day 13<sup>th</sup>) and secondary (on day 20<sup>th</sup>) mean hemagglutination (HA) antibody titre values were increased in the disease control group (G2) compared to the normal control (G1). On day 13<sup>th</sup>, the disease control group (G2) showed significant higher titre value in the primary response, which was similar to the untreated group (G4), while in the Biofield Energy Treated formulation group (G3) the antibody titre level was significantly increased by 36.36% compared to the G2. The primary antibody titre level was decreased by 15.09% in the untreated test formulation group (G4) compared with the G2 group. The response of primary antibody titre in the levamisole group (G5) was decreased by 36.36% compared with the G2. Moreover, the primary HA titre was also decreased by 12.18% in the Biofield Energy Treatment group *per se* at day -15 (G6) compared to the G2. On day 20<sup>th</sup>, the secondary antibody titre level was reduced by 5.25% in the Biofield Energy Treated group (G3) compared to the G2; while it was decreased by 15.79% in the untreated test formulation (G4) compared to the G2. The secondary HA titre was decreased by 13.15% in the Biofield Energy Treated group *per se* at day -15 (G6) compared to the G2.

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

Group	Primary HA Titre	Secondary HA Titre
G1	1.00 ± 0.00	1.00 ± 0.00
G2	5.50 ± 1.2	25.33 ± 4.34
G3	7.5 ± 2.06	24.00 ± 3.58
G4	4.67 ± 0.67	21.33 ± 3.37
G5	3.50 ± 0.5	24.00 ± 3.58
G6	4.83 ± 1.11	22.00 ± 4.82

HA: Hemagglutination, All the values are expressed as the mean ± SEM; The primary responses of mean HA titre was recorded on day 13<sup>th</sup> and secondary response on day 20<sup>th</sup> of the experimental period. G1: Normal control; G2: Disease control (pyrogallol); G3: Biofield Energy Treated test formulation; G4: Untreated test formulation; G5: Reference item (levamisole); G6: Biofield Energy Treatment group *per se* at day -15.



**Figure 1.** Effect of the test formulation on rat paw volume (delayed-type hypersensitivity). G1: Normal control; G2: Disease control; G3: Biofield Energy Treated test formulation; G4: Untreated test formulation; G5: Reference item (levamisole); G6: Biofield Energy Treatment group *per se* at day -15. The values are represented as mean ± SEM (n=6). \*\* $p \leq 0.01$  vs disease control.

### 3.3. Determination of Hematological Parameters

The effect of the Biofield Energy Treated formulation on various hematological parameters is shown in the Table 2. The level of red blood corpuscle (RBC) was reduced by 4.52% in the disease control group (G2) compared to the normal control group (G1). Besides, RBC was significantly increased by 13.03%, 12.91%, and 14.45%, in the untreated test formulation group (G4), levamisole (G5), and Biofield Energy Treated group *per se* at day -15 (G6), respectively; while the level of RBC was decreased in the Biofield Energy Treated formulation (G3) compared to the disease control group (G2). The Biofield Energy Treatment group *per se* at day -15 (G6) showed better improvement in the formation of RBC compared to the untreated test formulation group (G4). Hemoglobin (Hb) was increased by 9.48% in the untreated test formulation group (G4); while reduced by 5.34% in the Biofield Energy Treated test formulation (G3) compared to the G2. Moreover, the level of Hb was significantly increased by 9.74% in the Biofield Energy Treatment group *per se* at day -15 (G6) compared to the disease control group (G2).

The platelet count was significantly increased by 10.32% in the Biofield Energy Treated group (G3); while platelet count was decreased by 5.71% in the untreated test formulation (G4) compared to the G2. Besides, the reduction of platelets was observed by 6.61% and 30.91% in the

### 3.2. Estimation of Delayed Type Hypersensitivity (Paw Volume)

The effects of the Biofield Energy Treated and untreated formulation on delayed type hypersensitivity (DTH) response are shown in Figure 1. The levamisole group (G5) showed significant ( $p < 0.01$ ) increase in the paw volume compared to the disease control (G2) group. The paw volume was significantly increased by 75% in the Biofield Energy Treatment group *per se* at day -15 (G6) compared to the disease control (G2).

levamisole (G5) and Biofield Energy Treatment group *per se* at day -15 (G6), respectively compared to the disease control (G2). Literature reported that ashwagandha prevented myelosuppression and increase the platelet count and body weight [40, 41]. The level of packed cell volume (PCV) was significantly increased by 21.61%, 17.29%, and 28.32% in the untreated test formulation (G4), levamisole (G5), and Biofield Energy Treatment group *per se* at day -15 (G6), respectively compared to the disease control (G2). However, the level of MCH was decreased by 3.97%, 9.00%, 15.25%, and 15.94% in the Biofield Energy Treated test formulation (G3), untreated test formulation group (G4), levamisole (G5), and Biofield Energy Treatment group *per se* at day -15 (G6), respectively compared to the disease control (G2).

The level of MCHC was significantly reduced by 9.30%, 13.85%, and 14.56% in the untreated test formulation (G4), levamisole (G5), and Biofield Energy Treatment group *per se* at day -15 (G6), respectively compared to the disease control (G2). The value of RDW-CV was increased by 8.33%, 25.00%, 41.67%, and 33.33% in the Biofield Energy Treated formulation (G3), untreated test formulation (G4), levamisole group (G5), and Biofield Energy Treatment group *per se* at day -15 (G6), respectively compared to the disease control (G2). It was reported that *W. somnifera* extract was non-toxic to human erythrocytes at different concentrations [42]. The present experimental results showed the Biofield Energy

Treated test formulation did not show any toxic effect on RBC, as no significant change was observed in different treatment groups with respect to the both normal control and

disease control. Besides, the minerals present in the herbomineral formulation were reported to be safe and good therapeutic effect [43].

**Table 2.** Evaluation of hematology parameters after treatment with the test formulation in male Sprague Dawley rats.

Group	RBC ( $10^6/\mu\text{L}$ )	Hb (gm/dL)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (%)	Platelet Count (thousand/ $\text{mm}^3$ )	RDW-CV
G1	8.84 ± 0.22	16.40 ± 0.37	49.12 ± 1.10	55.60 ± 0.63	18.55 ± 0.18	33.38 ± 0.14	1120.00 ± 83.03	0.12 ± 0.00
G2	8.44 ± 0.12	15.92 ± 0.12	47.60 ± 0.37	56.45 ± 0.77	18.88 ± 0.33	33.43 ± 0.20	961.00 ± 87.64	0.12 ± 0.00
G3	8.33 ± 0.29	15.07 ± 0.39	45.00 ± 1.18	54.17 ± 1.40	18.13 ± 0.51	33.48 ± 0.17	1060.17 ± 55.50	0.13 ± 0.00
G4	9.54 ± 0.55	17.43 ± 0.56	57.88 ± 3.54*	56.72 ± 0.52	17.18 ± 0.54	30.32 ± 1.02	906.17 ± 90.17	0.15 ± 0.01
G5	9.53 ± 0.28	16.10 ± 0.22	55.83 ± 0.88*	55.70 ± 0.47	16.00 ± 0.10	28.80 ± 0.19	897.50 ± 96.83	0.17 ± 0.02
G6	9.66 ± 0.46	17.47 ± 0.21	61.08 ± 0.66*	55.40 ± 0.61	15.87 ± 0.16**	28.55 ± 0.15*	664.00 ± 71.14*	0.16 ± 0.00

G1: Normal control; G2: Disease control; G3: Biofield Energy Treated test formulation; G4: Untreated test formulation; G5: Reference item (levamisole); G6: Biofield Energy Treatment group *per se* at day -15. The values are represented as mean ± SEM (n=6). \* $p \leq 0.05$  and \*\* $p \leq 0.01$  compared to the disease control. Hb: Hemoglobin; RBC: Red blood count; PCV: Packed cell volume; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; RDW-CV: Red cell distribution width and volume.

### 3.4. Effect of the Biofield Energy Treated Test Formulation on Serum Biochemistry

The effect of the Biofield Energy Treated test formulation on hematological parameters is shown in the Table 3. Among the ions estimated, there was a significant elevation of phosphorus by 6.04%, 20.11%, and 3.63% in the Biofield Energy Treated formulation (G3), untreated test formulation (G4), and levamisole (G5), respectively compared to the disease control (G2). Similarly, the level of potassium was increased by 1.70%, 15.15%, and 2.27% in the Biofield Energy Treated test formulation (G3), untreated formulation (G4), and levamisole (G5), respectively; while it was reduced by 11.36% in the Biofield Energy Treatment group *per se* at day -15 (G6) compared to the disease control (G2). The concentration of magnesium was increased by 13.00%, 30.67%, and 6.00% in the Biofield Energy Treated formulation (G3), untreated test formulation (G4), and levamisole group

(G5), respectively compared to the disease control (G2). In addition to, the serum concentration of BUN was reduced by 16.03% in the Biofield Energy Treatment group *per se* at day -15 (G6); while in the others tested groups it was increased compared to the disease control (G2). Moreover, the serum concentration of uric acid (UA) was significantly decreased by 8.05%, 26.83%, and 53.66% in the Biofield Energy Treated test formulation (G3), levamisole (G5), and Biofield Energy Treatment group *per se* at day -15 (G6), respectively compared with the G2 group. Here, the Biofield Energy Treated herbomineral formulation showed the better effect by reducing the UA than the untreated test formulation. These results might be due to the positive effect of the Biofield Energy Healing to the novel herbomineral formulation, which could be very helpful in immunocompromised patients. Besides, the levels of calcium, creatinine, sodium and chloride ions were altered in all the treated groups compared with the disease control.

**Table 3.** Estimation of biochemical parameters after treatment with the test formulation in male rats.

Group	Magnesium (mg/dL)	Blood Urea (mg/dL)	Creatinine (mg/dL)	Uric Acid (mg/dL)	Calcium (mg/dL)	Phosphorus (mg/dL)	Na <sup>+</sup> (Meq/L)	K <sup>+</sup> (mEq/L)	Cl <sup>-</sup> (mEq/L)
G1	3.01 ± 0.14	41.30 ± 0.66	0.52 ± 0.02	3.60 ± 0.25	10.68 ± 0.57	9.28 ± 0.21	150.67 ± 0.21	5.32 ± 0.11	102.83 ± 0.48
G2	3.00 ± 0.24	50.77 ± 2.96	0.48 ± 0.04	4.10 ± 0.77	10.38 ± 0.22	9.10 ± 0.50	151.00 ± 0.68	5.28 ± 0.29	102.83 ± 1.05
G3	3.39 ± 0.13	60.48 ± 4.84	0.53 ± 0.03	3.77 ± 0.70	10.35 ± 0.12	9.65 ± 0.34	152.83 ± 0.98	5.37 ± 0.16	103.00 ± 0.68
G4	3.92 ± 0.13	55.80 ± 3.62	0.47 ± 0.03	4.32 ± 0.48	10.63 ± 0.26	10.93 ± 0.43	150.07 ± 0.67	6.08 ± 0.38	101.33 ± 0.71
G5	3.18 ± 0.08	55.68 ± 3.00	0.50 ± 0.04	3.00 ± 0.52	10.13 ± 0.18	9.43 ± 0.14	150.83 ± 0.75	5.40 ± 0.16	102.67 ± 0.61
G6	2.95 ± 0.15	42.63 ± 3.35	0.58 ± 0.03	1.90 ± 0.46	10.45 ± 0.13	8.73 ± 0.25	153.33 ± 0.76	4.68 ± 0.07	104.17 ± 0.54

All values are presented as mean ± SEM (n=6). G represents as group; G1: Normal control; G2: Disease control; G3: Biofield Energy Treated test formulation; G4: Untreated test formulation; G5: Reference item (levamisole); G6: Biofield Energy Treatment group *per se* at day -15.

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

The effect of the Biofield Energy Treated test formulation administration on animal weight parameters in male rats was analyzed and presented in Table 4. The results reflect the change in body weight, as final weights were increased among all the tested groups. The mean body weight percentage difference in the Biofield Energy Treated test formulation group and untreated test formulation group did not have any significant difference compared with the disease control group. The Biofield Energy Treated test formulation group (G3)

showed slight reduction of feed consumption compared to the disease control, which might be due to physiological variation in male rats. It is assumed that the Biofield Treated formulation was safe and effective with respect to both consumption of feed and consequently change of body weight. The results suggest that no significant change throughout the experimental period in relative organ weight parameters like liver, lungs, kidneys, brain, heart, eyes, spleen, whole intestine, testis, prostate, epididymis, and vas deference compared to the normal and disease control groups (Table 4). The result of relative organ weight was slightly increased in the disease control group; while after treatment with the Biofield Energy

Treated test formulation, the organ weight reached to normal level similar to the control group.

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

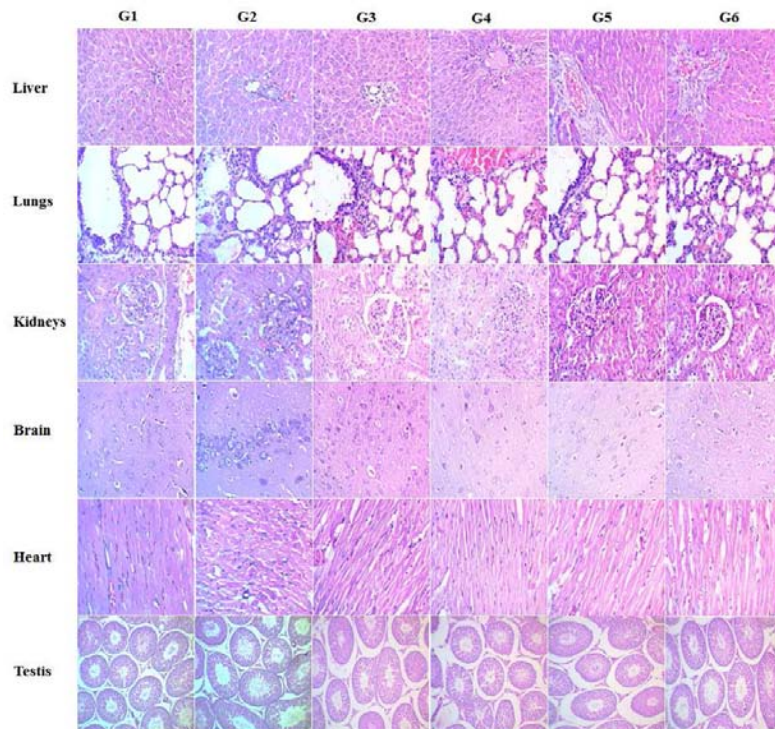
Relative organ weight (%)	G1	G2	G3	G4	G5	G6
Liver	3.60 ± 0.17	3.99 ± 0.19	3.56 ± 0.22	3.55 ± 0.09	3.92 ± 0.13	3.45 ± 0.12
Lungs	0.62 ± 0.03	0.71 ± 0.04	0.61 ± 0.04	0.61 ± 0.04	0.60 ± 0.02	0.54 ± 0.03
Kidneys	0.84 ± 0.02	0.91 ± 0.02	0.85 ± 0.06	0.80 ± 0.02	0.96 ± 0.03	0.83 ± 0.02
Brain	0.60 ± 0.01	0.59 ± 0.02	0.55 ± 0.02	0.58 ± 0.02	0.63 ± 0.03	0.60 ± 0.02
Heart	0.44 ± 0.03	0.40 ± 0.02	0.35 ± 0.01	0.36 ± 0.01	0.37 ± 0.02	0.37 ± 0.01
Eyes	0.08 ± 0.00	0.08 ± 0.02	0.09 ± 0.01	0.08 ± 0.01	0.09 ± 0.01	0.09 ± 0.01
Spleen	0.21 ± 0.01	0.29 ± 0.03	0.22 ± 0.01	0.20 ± 0.03	0.27 ± 0.02	0.28 ± 0.02
Whole intestine	5.47 ± 0.09	5.00 ± 0.24	6.64 ± 0.28	7.25 ± 0.40	6.44 ± 0.58	4.86 ± 0.22
Testis	0.96 ± 0.07	0.92 ± 0.04	0.92 ± 0.07	0.96 ± 0.05	1.01 ± 0.03	0.91 ± 0.10
Prostrate	0.27 ± 0.02	0.29 ± 0.03	0.22 ± 0.01	0.26 ± 0.03	0.25 ± 0.01	0.26 ± 0.02
Epididymis	0.38 ± 0.02	0.37 ± 0.03	0.36 ± 0.03	0.36 ± 0.04	0.42 ± 0.03	0.38 ± 0.03
Vas deference	0.07 ± 0.01	0.07 ± 0.00	0.07 ± 0.00	0.07 ± 0.00	0.07 ± 0.01	0.08 ± 0.01

Values are presented as mean ± SEM (n=6). G represents as group; G1: Normal control; G2: Disease control; G3: Biofield Energy Treated test formulation; G4: Untreated test formulation; G5: Reference item (levamisole); G6: Biofield Energy Treatment group *per se* at day -15.

The organ to body weight ratio is a useful index for the identification of swelling, atrophy or hypertrophy [44]. The increase in body weight or organ weight with the exposure of any compound in the animals during experiment suggest the hypertrophy, while decrease in the relative weight indicated the atrophy. The increase in body weight and organ-body ratio might be correlated with the sign of product toxicity, but the experimental results suggest that there was not much change in most of the vital organs, which depicts that the test formulation was non-toxic to the animals throughout the exposure period at a dose of 1105.005 mg/kg.

### 3.6. Histopathological Study

The effect of the Biofield Energy Treated test formulation on histopathological findings in male rats is shown in the Figure 2. No significant differences were observed either in gross or microscopic observation of the tested organs. Histopathological study results also suggest that no treatment-related histopathological findings were reported in all the experimental animals compared with the control group. The detailed histopathological images of microscopic sections of the organs are presented in Figure 2. Mild vacuolization in centrilobular hepatocytes was observed in few animals in the untreated group (G4). All other organs of animals were devoid of any microscopically changes (Figure 2).



**Figure 2.** Histopathological photomicrograph of major organs tested after Biofield Energy Treated test formulation in male *Sprague Dawley* rats. All the tissues were sectioned transversely and stained with hematoxylin and eosin. G1: Normal control; G2: Disease control; G3: Biofield Energy Treated test formulation; G4: Untreated test formulation; G5: Reference item (levamisole); G6: Biofield Energy Treatment group *per se* at day -15.

Biofield Energy Healing has been reported to be effective in cancer treatment by reducing the level of cytokines [45-47]. Besides, The National Center for Complementary/Alternative Medicine (NCCAM), 34% of adults in U.S. populations depends on some forms of complementary health approach, among which energy medicine is one of them. Complementary and Alternative Medicine (CAM) has several advantages instead of the current preferred treatment approach [48]. The new proprietary herbomineral formulation that might act as better immunomodulatory medicine in the future due to its anti-inflammatory and immunomodulatory effects. Therefore, it is assumed that the Biofield Energy Treated herbomineral formulation might be considered as a safe dietary supplement for boosting the immuno response.

#### 4. Conclusions

The primary antibody titre level was significantly increased by 36.36% in the Biofield Energy Treated test formulation group (G3) compared to the disease control group and also showed better result than the untreated test formulation (G4). The paw volume was significantly increased by 75% in the Biofield Energy Treatment group *per se* at day -15 (G6) compared to the disease control. The platelet count was significantly increased by 10.32% in the G3 group compared to the disease control group and also showed better response than the G4 group. Phosphorus and magnesium were significantly elevated in the G3 group by 6.04% and 13%, respectively compared to the disease control. Uric acid was significantly decreased by 8.05% in the G3 group compared to the disease control. The Biofield Energy Treated herbomineral formulation did not show any sign of toxicity as evidenced by mortality and clinical signs. Further, no treatment-related changes were observed in the Biofield Energy Treated formulation group with respect to the body weight and feed consumption during the experiment. The percentage of organ to body weight ratio data suggested that the Biofield Energy Treated test formulation was found to be safe with respect to the most of the vital organs toxicity. Overall, the Biofield Energy Treated herbomineral formulation showed better immune response without producing any adverse effect compared with the untreated test formulation. Thus, The Trivedi Effect<sup>®</sup> - Biofield Energy Healing administered remotely by the eighteen Biofield Energy Healers has significant capability to alter the immunomodulatory activity of the herbomineral formulation in male *Sprague Dawley* rats. It is then anticipated that the Biofield Energy Treated herbomineral formulation could be a more useful as immunomodulatory formulation for healthy human and in patients in the near future. Besides, it can also be utilized in various autoimmune disorders *viz.* Lupus, Addison Disease, Celiac Disease (gluten-sensitive enteropathy), Dermatomyositis, Graves' Disease, Hashimoto Thyroiditis, Multiple Sclerosis (MS), Myasthenia Gravis, Pernicious Anemia, Aplastic Anemia, Reactive Arthritis, Rheumatoid

Arthritis, Sjogren Syndrome, Systemic Lupus Erythematosus, Type 1 Diabetes, Alopecia Areata, Crohn's Disease, Fibromyalgia, Vitiligo, Psoriasis, Scleroderma, Chronic Fatigue Syndrome and Vasculitis, as well as inflammatory disorders such as Asthma, Ulcerative Colitis, Alzheimer's Disease, Atherosclerosis, Dermatitis, Diverticulitis, Hepatitis, Irritable Bowel Syndrome, Parkinson's Disease and stress etc. 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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