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An Investigation of The Trivedi Effect®-Energy of Consciousness Healing Treatment to Modulate the Immunomodulatory Effect of Herbomineral Formulation in Male *Sprague Dawley* Rats

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Abstract: Herbomineral formulations are used world-wide for various therapeutic purposes. More than 80% of the world population relies on natural herbal and mineral remedies as medicines for their health care. A new proprietary herbomineral formulation was created, consisting of the herbal root extract ashwagandha and minerals (zinc, magnesium, and selenium). The aim of the study was to evaluate the immunomodulatory potential of the Trivedi Effect®- Energy of Consciousness Healing Treatment (Biofield Energy Healing Treatment) on the herbomineral formulation in male *Sprague Dawley* rats. The test formulation was divided into two parts. One part was denoted as the control without any Biofield Energy Treatment, while the other part was defined as the Biofield Energy Treated sample, which received the Biofield Energy Healing Treatment remotely from eighteen renowned Biofield Energy Healers. The immunomodulatory effect of the Biofield Energy Treated and the untreated test formulations were studied to determine any alterations in the animal humoral immune response, paw volume, hematological study, serum biochemistry parameters, animal weight parameters, feed intake and histopathology analysis. Humoral immune response data of the reference standard (levamisole) showed a significant increase in secondary antibody titre value ($p \leq 0.05$), while the Biofield Treated test formulation group (G3) exhibited a significant increase in the secondary antibody titre by 115.34% compared with the disease control group (G2). A delayed type hypersensitivity (DTH) reaction showed that the paw volume was significantly decreased by 114.28% in the Biofield Energy Treated test formulation group (G3) with respect to the G2 group. The platelets count was increased by 61.55% in the Biofield Treated test formulation group (G3) compared to the G2 group. Moreover, the administration of the Biofield Treated herbomineral formulation (G3) group exhibited a decrease in the level of creatinine (9.62%), and uric acid (14.40%), while the level of potassium ion concentration was incresaed by 77.43% 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. Overall, The Trivedi Effect®- Energy of Consciousness Healing Treatment (Biofield Energy Healing Treatment) significantly modulates the immunological parameters of the herbomineral formulation compared with the untreated test formulation. These data suggest that the Biofield Treated test formulation can be used for autoimmune and inflammatory diseases along with stress management and anti-aging by improving overall health.

Keywords: Biofield Energy Healing Treatment, Biofield Energy Healers, Consciousness Energy Healing Treatment, Herbomineral Formulation, Immune-Modulation, Humoral Immune Response, Delayed Type Hypersensitivity, Stress Management, Anti-Aging

1. Introduction

Immunomodulators are classified as responders to the immune system. Immune system dysfunction leads to many infective and autoimmune diseases such as arthritis, ulcerative colitis, asthma, and cardiovascular diseases. Medicinal plants and minerals have been reported for significant immunomodulatory action [1]. Some of these herbal formulations are believed to improve the health by maintaining the body's self-defense mechanism against various infections by re-establishing the equilibrium of human body. Today, most of the traditional medicines are derived from the medicinal plants, minerals, and organic matter [2]. Medicinal plants are the ideal candidates for any new therapeutic formulation due to their broad biological activities, wide chemical diversity, structural complexity, and minimal toxic effects [3]. Medicinal plants and minerals have designed the strong basis of health care throughout the world. However, the use of traditional herbal and mineral remedies has gained importance due to being widely perceived as natural, safe, and non-toxic, while conventional medicines are found ineffective against many diseases. Herbal and traditional medicine are considered suitable candidates for new therapeutics due to their vast chemical diversity and various biological effects. Based on the literature, a new proprietary herbomineral formulation was formulated with a combination of the herb ashwagandha root extract and three minerals *viz.* zinc, magnesium, and selenium. All the ingredients of this formulation in this present study possess important immune modulating properties such as ashwagandha (*Withania somnifera*), also known as Indian ginseng used as alternative therapy for many pharmacological activities [4, 5]. The roots and leaves extracts of ashwagandha were reported to have significant roles as immunomodulatory, cancer or tumor treatments [6] due to the presence of active constituents like withanolides. Based on the recent literature, the immunomodulatory activity of ashwagandha has been reported in many pre-clinical and clinical studies [7]. Besides herbal medicine, selenium, zinc, copper, and magnesium are widely recommended trace elements because of their strong role in immunomodulation [8-10].

The scientific research has documented that the presence of minerals and herbal medicines have been found to exhibit a high level of phagocytic index with an improved antibody titre [11]. These formulations can be used for better therapeutic effect in immune compromised patients affected with cardiovascular diseases, age, and stress related diseases, cancer, and autoimmune disorders. The Biofield Energy Healers in this study have used the Trivedi Effect®- Energy of Consciousness Healing Treatment (Biofield Energy Healing Treatment) as a complementary and alternative approach to study the impact of Biofield Treatment on the herbomineral formulation for its immunomodulatory potential in male *Sprague Dawley* rats.

Based on the recent literature, many scientific and clinical trial reports have exhibited significant results of the Biofield Energy Treatment with enhanced immune function in cervical cancer patients with the therapeutic touch [12], massage therapy [13], etc. Complementary and Alternative Medicines (CAM) 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 Complementary and Alternative Medicine (CAM) health care approach in addition to other therapies, medicines and practices such as natural products, deep breathing, yoga, Tai Chi, Qi Gong, chiropractic/osteopathic manipulation, meditation, massage, special diets, homeopathy, progressive relaxation, guided imagery, acupressure, acupuncture, relaxation techniques, hypnotherapy, healing touch, movement therapy, pilates, Rolfing structural integration, mindfulness, Ayurvedic medicine, traditional Chinese herbs and medicines, naturopathy, essential oils, aromatherapy, Reiki, and cranial sacral therapy. Human Biofield Energy has subtle energy that has the capacity to work in an effective manner [14]. Scientific reports suggest that CAM therapies have been practiced worldwide with reported clinical benefits in different health disease profiles [15]. Biofield Energy Healing Treatment (The Trivedi Effect®) has had significant impact in the transformation of living organisms and nonliving materials including cancer research [16, 17], altered antimicrobial sensitivity of pathogenic microbes in microbiology [18-21], genetics [22, 23], altered the physical and chemical properties of pharmaceutical compounds [24-27], improved overall growth and yield of plants in agricultural science [28-31], and altered the structure of the atom in many metals, ceramics, polymers and chemicals in materials science [32-35].

The authors sought to evaluate the impact of The Trivedi Effect®-Energy of Consciousness Healing Treatment (Biofield Energy Healing Treatment) on the test herbomineral formulation, which might improve the

immunomodulatory function in male *Sprague Dawley* rats.

2. Material and Methods

2.1. Chemicals and Reagents

Pyrogallol and carboxymethyl cellulose sodium were purchased from Sigma Chemical Co. (St. Louis, MO). Ashwagandha (*Withania somnifera*) root extract powder (≥5% of total withanolides) was procured from Sanat Products Ltd., India. Zinc chloride and magnesium (II) gluconate hydrate were procured from TCI, Japan. Sodium selenate procured from Alfa Aesar, USA. Levamisole hydrochloride was procured from Sigma, U. S. A. All other chemicals used were of analytical grade available locally in India.

2.2. Laboratory Animals

A total number of 30 healthy male *Sprague Dawley* rats, weighing between 150-250 grams, were used for the study. Rodent laboratory diet and drinking tap water were provided *ad libitum* under controlled conditions with a temperature of 22 ± 3°C, humidity of 30% to 70% and a 12-hour light/12-hour dark cycle. The animals were acclimatized for minimum period of 5 days prior to the experiment, and all were accessed once daily for clinical signs, behaviors, morbidity and mortality. All the procedures were in strict accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health. The approval of the Institutional Animal Ethics Committee that was obtained prior to carrying out the animal experiment.

2.3. Energy of Consciousness Treatment Strategies

The test formulation was divided into two parts. One part of the test formulation was treated with Biofield Energy by renowned Biofield Healers (also known as The Trivedi Effect®) and coded as the Biofield Energy Treated formulation, while the second part of the test formulation did not receive any sort of treatment and was defined as the untreated test formulation. The Trivedi Effect®- Energy of Consciousness Healing Treatment (Biofield Energy Healing Treatment) was provided through a group of 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, two in Finland, and one of which was remotely located in Albania, while the test herbomineral formulation was located in the research laboratory of Dabur Research Foundation near New Delhi in Ghaziabad, India. This Biofield 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. Further, 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 and the samples were analyzed using a Hematology analyzer (Abbott Model-CD-3700). Based on the number of erythrocytes the samples were further diluted (using saline) before injecting to the rat [36].

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 (disease control) group animals received pyrogallol at a dose of 100 mg/kg through intraperitoneal (*i.p.*) route once daily for 7 days. G3 group animals received the Biofield Treated test formulation (1105.005 mg/kg b. wt,) per oral (*p.o.*). G4 group animals received untreated test formulation at the same concentration orally, while G5 group animals received levamisole at a dose of 50 mg/kg *p.o.* from day 1 to day 22. All the animals except normal control group received pyrogallol at a dose of 100 mg/kg through *i.p.* route once daily from day 1 to 7. The animals were treated with the Biofield Treated and untreated herbomineral formulation to the Group 3 and 4 animals respectively, 1 hour before pyrogallol challenge in the morning once daily for 22 days. On day 7 and 13, all the animals in Group 2 to 5 except normal control were challenged with sheep red blood cells (sRBC) (0.5×10^9/100 gm; *i.p.*), as the antigenic material to sensitize them for immunological parameters. On day 13th and 20th, blood was collected from retro orbital plexus and subjected to hemagglutination test to evaluate the humoral immune response. On same day 20th, the animals were challenged with sheep RBC (0.5×10^9 cells/50µL/rat) in sub-planter region and on 22nd (48 hours) day paw volume was measured to evaluate the cellular immune response. The treatment was continued to all the tested groups (G1 to G5) with 5 mL/kg body weight as dose volume. The body weight and food consumption were measured daily before the treatment. On day 22, the animals were kept under fasting over night and on day 23; blood was collected again from the retro-orbital plexus from each animal under anaesthetic condition using isoflurane. Whole blood was analysed for haematological parameters and the serum was analysed for biochemistry parameters. 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

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

2.7. Determination of Paw Volume (Delayed Type Hypersensitivity)

The cellular immune response was assayed by the footpad reaction method. The edema was induced in the right paw of rats by injecting sRBC (0.025 x 10^9 cells) in the subplantar region. The increase in the paw volume after 24 hours, *i.e.* on day 21 was assessed on digital plethysmometer (Pan Lab, Spain). The mean percentage increase in paw volume was considered as a 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

After fasting for 12 to 16 hours on day 23^{rd} of the experiment, 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 level of hemoglobin (Hb), red blood 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 level of magnesium, blood urea, creatinine, uric acid, calcium, phosphorus, potassium, and sodium and chloride ion concentration were analyzed using Hematology analyzer (Abbott Model-CD-3700) [38].

2.9. Determination of Body Weight and Feed Intake

Body weight and food consumption of all the animals in various experimental groups were measured once daily. Briefly, the weight of the daily feed supply and the left-over feed 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 [39].

2.10. Clinical Sign and Symptoms

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

2.11. Measurement of Relative Organ Weight and Histopathology

At the end of the experiment, rats were dissected and the whole liver, kidneys, hearts, spleens, lungs, and the 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.

2.12. Statistical Analysis

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

3. Results and Discussion

3.1. Evaluation of Humoral Immune Response

The results of primary and secondary humoral immune response in terms of HA (haemagglutination) titre after administration of the test formulation are summarized in Table 1. The mean value of the primary antibody titre significantly increased compared with the normal control. However, the primary HA titre was decreased significantly after administration of the Biofield Treated test formulation, an almost similar effect was observed with standard drug, levamisole. The animals in the Biofield Treated test formulation group (G3) showed primary HA titre value as 29.33 ± 7.64, which was almost double to the value obtained in untreated test formulation (G4). The value of primary antibody titre in levamisole group (G5) showed value as 22.67 ± 9.1. Similarly, the secondary antibody titre values in the Biofield Treated test formulation showed higher titre value as 18.67 ± 2.67 compared with the untreated test formulation (14.67 ± 1.33). The levamisole group (G5) showed a secondary titre value as 25.33 ± 4.34, while the values in treated, untreated, and levamisole groups exhibited significant effect ($p \leq 0.05$) when compared with the normal control (G1) group.

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

S. No	Groups	Primary HA titre	Secondary HA titre
1.	Disease control	50.67 ± 16.74	8.67 ± 4.67
2.	Biofield Treated test formulation	29.33 ± 7.64	18.67 ± 2.67*
3.	Untreated test formulation	50.67 ± 8.68*	14.67 ± 1.33*
4.	Levamisole	22.67 ± 9.10	25.33 ± 4.34*

Values are expressed as the mean ± SEM (n = 6). *$p \leq 0.05$ compared with the disease control.

Overall, the Biofield Treated test formulation group (G3) showed an increased secondary antibody titre level by 115.34% compared to the disease control group (G2) and also showed better results in comparison with the untreated test formulation (G4). Further, the Biofield Treated test formulation group (G3) showed a decreased primary antibody titre level by 42.12% compared to the disease control (G2). In the untreated test formulation group (G4), the primary antibody titre levels was unchanged compared to the disease control. The above findings suggest that the test formulation exhibited a potent immunomodulatory effect on the humoral mediated immunity with an enhanced antibody synthesis. The increase in antibody titre values in the Biofield Treated and untreated test formulations clearly indicated the humoral immunity modulation. This might involve the production of specific antibodies (immunoglobulins) by lymphatic or plasma cells after sensitization to the specific antigens [41]. Thus, the Biofield Treated herbomineral formulation may augment the body's immunity and can enhance the capacity against bacterial and viral infections, and lead to an improved immune response in the body. When compared with the untreated test formulation, the Biofield Treated herbomineral formulation showed a better immunosuppression effect. In the case of the secondary antibody, the Biofield Treated test formulation has enhanced the immune response in better way compared with the untreated test formulation. This suggests that Biofield Energy Treatment might alter some characteristic properties of the combination product which are responsible for immunological changes.

3.2. Estimation of Delayed Type Hypersensitivity (Paw Volume)

The effect of the Biofield Treated test formulation with respect to the delayed type hypersensitivity reaction in male rats were measured and are presented in the Figure 1. The results suggest that the mean paw edema volume (in mL) in the G1, G2, G3, G4, and G5 group was 00.03 ± 0.01, 00.14 ± 0.07, 00.01 ± 0.02, -00.02 ± 0.04, and 00.62 ± 0.09 mL, respectively. The levamisole group (G5) showed increased paw volume by 342.85% compared with the disease control group (G2). However, the Biofield Treated test formulation (G3) showed a significant decrease in paw volume by 114.28% with respect to the G2 group. Hence, it might be suggested that the Biofield Treated formulation, showed an immunomodulatory effect with respect to the significant altered rat paw volume. The significant change might be due to the active constituents present in the test formulation and further, the effect was potentiated by the Biofield Energy Healing Treatment as compared with the untreated test formulation. Thus, it can be concluded that the constituents present in the formulation are responsible for the delayed type hyper sensitivity reaction, however the the Trivedi Effect®- Energy of Consciousness Healing Treatment (Biofield Energy Healing Treatment) enhanced the immune response compared with the untreated test formulation.

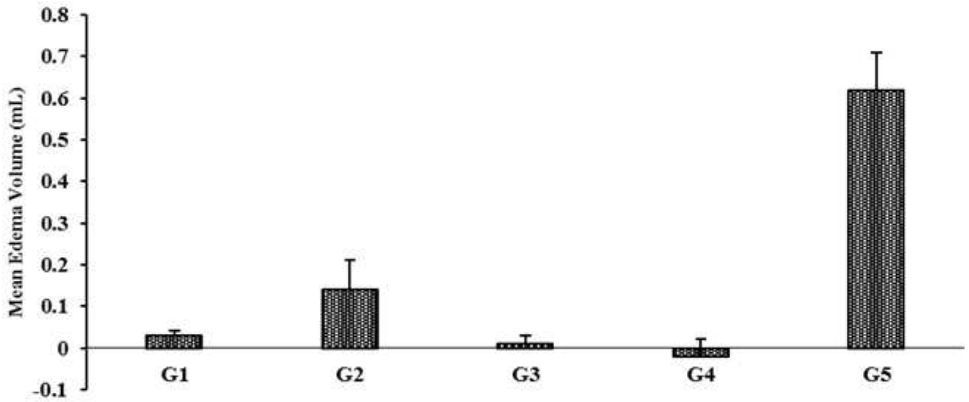

Figure 1. *Effect of the test formulation on rat paw volume (delayed-type hypersensitivity) in male Sprague Dawley rats. G1: Normal Control; G2: Disease Control: G3: Biofield Energy Treated test formulation; G4: Untreated test formulation; G5: Levamisole. All values are expressed as the mean ± SEM (n = 6).*

3.3. Assessment of Hematology Profile

The Biofield Treated and untreated test formulations in experimental animals showed altered hematological parameters but a statistically significant difference was not observed among different groups with respect to the control group. Administration of the Biofield Treated test formulation in our experimental design did not lead to unfavorable hematological changes (Table 2). All the tested parameters such as RBC, Hb, PCV, MCV, MCH, and MCHC showed slight alterations in values with respect to the normal and disease control groups. The RBC level was slightly increased in all tested groups compared with the control group (8.84 ± 0.22 $10^6/\mu$ L), but was not statistically significant. However, the Biofield Treated test formulation showed an increase in red blood cell count (9.47 ± 0.29 $10^6/\mu$ L) compared with the untreated test formulation (9.28 ± 0.21 $10^6/\mu$ L). In a study of mice, it was reported that ashwagandha (one of the constituents of test formulation) prevented myelosuppression and an increase in the hemoglobin concentration, red blood cell count, white blood cell count, platelet count, and body weight [42]. Our

experimental results suggest that the RBC and platelet count were significantly increased in the Biofield Energy Treated formulation compared with the untreated test formulation.

The platelet count in the vehicle control group (G1) was reported as 1120.00 ± 83.03 thousand per millimeter cubic, while in the disease control group (G2) showed a decreased platelet count as 726.60 ± 158.97 thousand per millimeter cubic. Further, the Biofield Treated test formulation showed that the platelet level was increased and normalized similar to the normal group as 1173.83 ± 201.63 thousand per millimeter cubic, while untreated group showed value as 963.67 ± 94.48 thousand per millimeter cubic, similar to levamisole group 914.67 ± 136.42 thousand per millimeter cubic. The animals treated with the Biofield Energy Treated test formulation showed an increased platelet count by 61.55% compared with the untreated test formulation (32.62%) and disease control group. So, it can be concluded that the Biofield Treated test formulation showed a better effect compared with the untreated test formulation. A study of the ashwagandha extract reported there was a significant increase in white blood cell and platelet counts [43]. The experimental results suggested that Biofield Treated test formulation can be used to improve the platelet count, while the Biofield Treated test formulation was proved as better efficacy than the untreated test formulation.

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

Group	RBC ($10^6/\mu$ L)	Hb (Gm/dL)	PCV (%)	MCV (fl)
1	8.84 ± 0.22	16.40 ± 0.37	49.12 ± 1.10	55.60 ± 0.63
2	9.51 ± 0.31	17.40 ± 0.20	60.13 ± 0.79	57.63 ± 0.90
3	9.47 ± 0.29	16.34 ± 0.32	55.03 ± 1.07	57.23 ± 0.80
4	9.28 ± 0.21	16.43 ± 0.33	56.73 ± 1.14	55.28 ± 0.70
5	9.22 ± 0.27	16.05 ± 0.52	55.18 ± 1.65	58.00 ± 0.88

Table 2. *Continued.*

Group	MCH (pg)	MCHC (%)	Platelet Count (thousand/mm^3)	RDW-CV
1	18.55 ± 0.18	33.38 ± 0.14	1120.00 ± 83.03	0.12 ± 0.00
2	16.62 ± 0.25	28.90 ± 0.07	726.60 ± 158.97	0.15 ± 0.00
3	16.92 ± 0.26	29.62 ± 0.05	1173.83 ± 201.63	0.15 ± 0.00
4	15.95 ± 0.20	28.92 ± 0.07	963.67 ± 94.48	0.15 ± 0.00
5	16.80 ± 0.23	29.03 ± 0.26	914.67 ± 136.42	0.15 ± 0.00

G1: Normal control; G2: Disease control; G3: Biofield Treated test formulation; G4: Untreated test formulation; and G5: Levamisole. The values are represented as mean ± SEM, n=6 animals in each group.

It was reported that *W. somnifera* root extract was non-toxic to the human erythrocytes, as literature data suggests no hemolysis effects at different concentrations [44]. Our experimental results also suggest that the Biofield Treated test formulation did not have any toxic effect on RBC, as no significant change was observed in different groups with respect to the normal control and disease control. Besides, the minerals present in the Biofield Treated test formulation were reported to be safe and have good therapeutic effect [45]. Overall, it can be concluded that the Biofield Treated test formulation did not show any direct or indirect effect *i.e.* no hemolytic effect. The Biofield Treated test formulation group showed an elevation of MCHC compared to the disease control. In parameters such as MCHC and platelet count; the Biofield Treated test formulation group exhibited increase level but non-significant.

3.4. Assessment of Biochemical Parameters Profile

The effect of the Biofield Treated test formulation on hematology and biochemical parameters such as the level of magnesium, blood urea nitrogen, creatinine, uric acid, calcium, phosphorus, potassium, sodium, and chloride ion concentrations were evaluated and are reported in Table 3. Serum biochemistry profile results suggested an elevation in the level of serum phosphorus, while significant increased level of sodium and chloride ions in the disease control group was observed as compared to the normal control. The change in concentrations of the tested parameters did not show any significant alteration with respect to the normal and disease control group. However, the Biofield Treated test formulation showed a slight decrease in the level of all parameters in comparison with the normal control group. It was also observed that the concentration of the tested parameters reported in the Biofield Treated group was slightly lower than the untreated test formulation except in case of potassium ion. The level of potassium ion in the Biofield Treated test formulation group showed a significant increase in level ($p \leq 0.05$) as 9.28 ± 0.21 mEq/L compared with the normal control group (5.32 ± 0.11 mEq/L). The uric acid level in treated, untreated and levamisole group showed significant change ($p \leq 0.05$) in values compared with the normal control. However, overall results suggest that the change in biochemical tested parameters after administration of the test formulation did not show any significant alterations compared with the disease control group.

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)
1	3.01 ± 0.14	41.30 ± 0.66	0.52 ± 0.02	3.60 ± 0.25
2	3.03 ± 0.13	35.92 ± 2.63	0.52 ± 0.02	2.78 ± 0.47
3	2.97 ± 0.12	36.80 ± 2.22	0.47 ± 0.04	2.43 ± 0.30*
4	3.40 ± 0.12	46.82 ± 2.16	0.53 ± 0.02	2.43 ± 0.27*
5	3.48 ± 0.09*	40.23 ± 3.37	0.47 ± 0.03	2.32 ± 0.21*

Table 3. *Continued.*

Calcium (mg/dL)	Phosphorus (mg/dL)	K^+ (mEq/l)	Na^+ (Meq/L)	Cl^- (mEq/L)
10.68 ± 0.57	9.28 ± 0.21	5.32 ± 0.11	150.67 ± 0.21	102.83 ± 0.48
10.68 ± 0.19	9.45 ± 0.33	5.23 ± 0.20	160.67 ± 1.74	114.17 ± 0.75
10.04 ± 0.20	9.11 ± 0.23	9.28 ± 0.21*	147.60 ± 0.95	104.78 ± 0.83
11.43 ± 0.19	10.55 ± 0.18*	5.55 ± 0.13	159.00 ± 0.68*	110.50 ± 0.62*
11.20 ± 0.16	10.42 ± 0.15*	5.62 ± 0.10	158.83 ± 0.54*	112.83 ± 1.89*

G1: Normal control; G2: Disease control; G3: Biofield Treated test formulation; G4: Untreated test formulation; and G5: Levamisole. The values are represented as mean ± SEM, n=6 male animals in each group. *$p \leq 0.05$ compared with the disease control group.

3.5. Analysis of Body Weight and Relative Organ Weight Parameters

The effect of the test formulation on the animal weight parameters were compared with the rats' respective initial mean body weights and are presented in Table 4. From this, based on the final body weight, the relative organ weight ratio as percentage was calculated in all the groups. The results showed the final body weights were increased between all initial values of the same groups. But the mean percentage difference in Biofield Treated and untreated test formulation group was not significant compared with the disease control group. Similarly, no significant change in organ weight was observed throughout the experiment in terms of the relative organ weight of the liver, kidney, lungs, spleen, heart, eyes, testis, brain, prostate, epididymis, intestine and vas deference with respect to the normal and disease control group throughout the experimental period.

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

Relative weight (%)	G1	G2	G3	G4	G5
Liver	3.60 ± 0.17	3.82 ± 0.22	4.04 ± 0.15	4.10 ± 0.16	4.38 ± 0.15
Kidney	0.84 ± 0.02	0.95 ± 0.03	0.90 ± 0.02	0.90 ± 0.02	1.07 ± 0.05
Lungs	0.62 ± 0.03	0.63 ± 0.03	0.59 ± 0.03	0.79 ± 0.07	0.81 ± 0.06
Spleen	0.21 ± 0.01	0.27 ± 0.02	0.22 ± 0.01	0.24 ± 0.01	0.23 ± 0.01
Eyes	0.08 ± 0.00	0.08 ± 0.01	0.08 ± 0.00	0.09 ± 0.01	0.09 ± 0.01
Heart	0.44 ± 0.03	0.39 ± 0.02	0.40 ± 0.02	0.39 ± 0.01	0.44 ± 0.02
Testis	0.96 ± 0.07	1.04 ± 0.04	1.01 ± 0.05	0.95 ± 0.03	1.10 ± 0.06
Brain	0.60 ± 0.01	0.62 ± 0.02	0.57 ± 0.03	0.59 ± 0.05	0.66 ± 0.03
Prostrate	0.27 ± 0.02	0.20 ± 0.02	0.24 ± 0.02	0.22 ± 0.02	0.39 ± 0.15
Epididymis	0.38 ± 0.02	0.39 ± 0.02	0.34 ± 0.01	0.33 ± 0.01	0.39 ± 0.03
Vas deference	0.07 ± 0.01	0.09 ± 0.01	0.12 ± 0.05	0.07 ± 0.00	0.08 ± 0.01
Whole intestine	5.47 ± 0.09	4.51 ± 0.21	6.28 ± 0.22	6.34 ± 0.38	4.81 ± 0.12

G1: Normal control; G2: Disease control; G3: Biofield Treated test formulation; G4: Untreated test formulation; and G5: Levamisole. The values are presented as mean ± SEM.

The organ to body weight ratio is a useful index for the identification of swelling, atrophy or hypertrophy [46]. The increase in body weight or organ weight with the exposure of any compound in the animals during experiment suggests the hypertrophy, while decreases in the relative organ 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 the alterations were not statistically significant, which depicts that the Biofield Treated test formulation was non-toxic to the animals throughout the exposure period at the tested dose.

3.6. Effect of the Biofield Treated Test Formulation on Feed Intake

The feed intake of the rats was measured throughout the experiment and compared with different groups. The results suggest that throughout the study period compared with the normal control group, no statistically significant change was observed (Figure 2). However, the animals in the levamisole group showed a slight decrease in mean feed intake (22.14 ± 1.31 gm) compared with the normal (23.63 ± 0.82 gm) and disease control groups (24.26 ± 1.09 gm). Overall, the effect of the Biofield Treated and untreated test formulations did

not show any significant change in feed intake in male animals. This indicated that the Biofield Treated test formulation possessed the ability to manage blood glucose level, as well as controlling muscle wasting, which resulted in a similar pattern of alteration in body weight and feed intake in all the animals in tested groups.

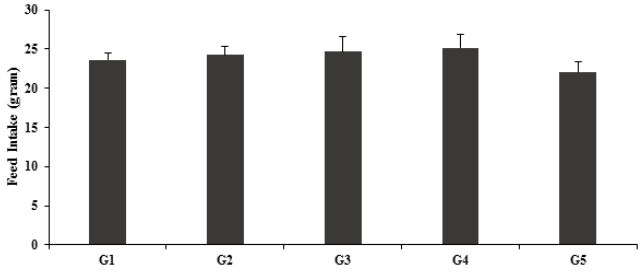

Figure 2. *The effect of the test formulation on feed intake of male rats. G1: Normal control; G2: Disease control; G3: Biofield Treated test formulation; G4: Untreated test formulation; and G5: Levamisole. All values are presented as mean ± SEM (n=6).*

3.7. Histopathological Analysis

A histopathological study also showed that no treatment-related histopathological findings were reported in all the experimental animals compared with the control groups. The detailed histopathological images of microscopic sections of the organs are presented in Figure 3. The analysis of all the groups suggest that there was no treatment related abnormal features in the Biofield Treated test formulation group (G3) and the untreated test formulation (G4). Overall, the histopathological data suggests no abnormal signs of gross examination in the tested animal tissues, so the Biofield Treated herbomineral formulation was found to be safe without any toxic effects.

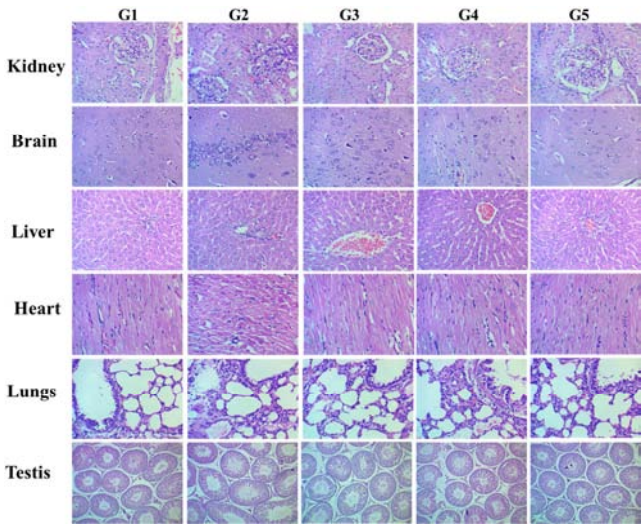

Figure 3. *Histopathological cross sections of major male rat organs tested after treatment with the test formulation. G1: Normal control; G2: Disease control; G3: Biofield Energy Treated test formulation; G4: Untreated test formulation; and G5: Levamisole.*

Altogether, the study results suggest a siginificant effect of the Biofield Energy Treatment on the test formulation, which might be due to the electromagnetic field of the Biofield Energy Healers during the treatment. Biofield Energy has been reported to be an effective in cancer treatment by reducing the level of cytokines [47-49]. Besides, according to the NCCAM, 34% of adults in U. S. population depend upon some kind of complementary health approach, among which Biofield Energy Medicine is one of them. Complementary and Alternative Medicine (CAM) has several advantages instead of the current preferred allopathic treatment approach [50]. The Biofield Treated test formulation is a natural proprietary herbomineral formulation that might result in a better immunomodulatory medicine in future. However, the overall effect of the formulation after the Biofield Treatment was better compared to the untreated formulation, which was be due to The Trivedi Effect®- Energy of Consciousness Healing Treatment (Biofield Energy Healing Treatment) that might alter the mechanism of action of the individual constituents present in the test formulation. Thus, it is assumed that the Biofield Treated herbomineral formulation might be considered as a safe supplementary therapy for immunomodulation.

4. Conclusions

Based on these results, it was concluded that The Trivedi Effect®- Energy of Consciousness Healing Treatment (Biofield Energy Healing Treatment) on the herbomineral formulation was found to be safe without having any toxicity profile. Further, no treatment-related changes were observed in the Biofield Treated groups with the test item during the course of the experiment. The Biofield Treated test formulation group (G3) animals showed an increased secondary antibody titre level by 115.34% compared to the disease control group (G2). The delayed type hypersensitivity reaction was measured in male rats and data suggest a significant decrease in rat paw volume by 114.28% in the Biofield Treated test formulation group (G3) with respect to the disease control group (G2). The Biofield Treated formulation group (G3) exhibited improved hematological parameters such as MCHC and platelets (61.55%) compared to the disease control (G2) group. However, the administration of the Biofield Energy Treated test formulation showed a significant decrease in the level of creatinine and uric acid by 9.62% and 14.40% respectively, while significantly improved levels of potassium ions concentration by 77.43% compared to the disease control group (G2). The percentage of organ to body weight ratio data suggest that the Biofield Energy Treated test formulation was safe with respect to the most of the vital organs regarding toxicity. No treatment related gross lesion or microscopic findings were observed in any of the organ from the treatment groups.

Therefore, The Trivedi Effect®- Energy of Consciousness Healing Treatment (Biofield Energy Healing Treatment) administered remotely by the eighteen Biofield Energy Healers enhanced the herbomineral test formulation's anti-inflammatory and immunomodulatory properties without any side effects, which can be used as a an 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 like 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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