Journal of Nutritional Dietetics & Probiotics

Research Article *Volume 4 Issue 1*

Immunomodulatory Action of a Proprietary Product Using TNBS (Tri Nitro Benzene Sulfonic Acid) - Induced Ulcerative Colitis in Sprague Dawley Rats

Trivedi MK[1] and Jana S[2]*

[1]Trivedi Global, Inc., Henderson, Nevada, USA

[2]Trivedi Science Research Laboratory Pvt. Ltd., Thane-West, Maharashtra, India

***Corresponding author:** Snehasis Jana, Trivedi Science Research Laboratory Pvt. Ltd., Thane-West, Maharashtra, India, Email: publication@trivedisrl.com

Received Date: February 06, 2021; **Published Date:** March 18, 2021

Abstract

Immunomodulatory activity of the novel formulation with respect to gut health using TNBS induced ulcerative colitis was studied in the present experiment. The action of Biofield Energy Healing (the Trivedi Effect®) based test formulation and Biofield Energy Treatment *per se* was studies. The test formulation consisted of essential ingredients *viz.* minerals (zinc, magnesium, iron, and copper) and vitamins (B_6, B_{12}, and D_3). Each ingredient of the test formulation was divided into two parts. One part was denoted as the control without any Biofield Energy Healing Treatment, while the other part was defined as the Biofield Energy Treated sample, which received the Biofield Energy Healing Treatment by a renowned Biofield Energy Healer, Mr. Mahendra Kumar Trivedi. Additionally, three group of animals also received Biofield Energy Treatment *per se* (day -15) under similar conditions. The immune biomarkers such as immunoglobulin M (IgM), IgG, IgA, IgE, biochemistry, and hematology parameters were evaluated. The level of IgE was significantly increased by 22.95%, 25.51%, and 48.57% ($p \leq 0.001$) in the G5, G6, and G7 groups, respectively as compared with the untreated test group (G4). IgM level was significantly ($p \leq 0.001$) increased by 12.16%, 20.27%, and 11.49% in the G7, G8, and G9 groups, respectively as compared with the G4 group. The level of IgG was significantly ($p \leq 0.001$) increased by 11.19% in the G9 group as compared with the G4 group. Hematology data suggested significant increased the levels of total leukocyte count (TLC) (25.95%; $p \leq 0.05$ in G9), neutrophils (65.26%, 59.62%, and 117.84% ($p \leq 0.05$) in G6, G7, and G9 respectively), and monocytes (61.97% in G9) were reported when compared with the G4 group. Further, the level of lymphocytes were significantly ($p \leq 0.001$) increased by 98.24%, 70.31%, 61.91%, and 108.20% in the G5, G6, G8, and G9 groups, respectively as compared to the disease control (G2) group. Serum glutamic-oxaloacetic transaminase (SGOT) or AST level was significantly reduced by 31.96% ($p \leq 0.05$), 23.24%, 23.44%, 24.45%, and 23.93% in the G5, G6, G7, G8, and G9 groups, respectively as compared with the G2 group. Moreover, serum glutamic pyruvic transaminase (SGPT) or ALT level was reduced by 22.63% and 23.44% in G6 group as compared to G2 and G6 groups, respectively. The level of alkaline phosphatase (ALP) was reduced by 28.42%, 21.50%, 17.91%, 17.00%, and 21.63% in the G5, G6, G7, G8, and G9 groups, respectively than G2 group. Creatine kinase-myocardial band (CK-MB) level was significantly ($p \leq 0.001$) decreased by 46.26%, 28.88%, 42.33%, 43.57%, and 43.97%, respectively in G5, G6, G7, G8, and G9 groups, respectively as compared with the G4 group. The level of testosterone was increased by 45.96% and 35.31% in the G6 group as compared with the G2 and G4 groups, respectively. In addition, animal body weight, feed intake, and relative organ weight data did not showed any abnormal findings with respect to the safe and non-toxic treatment strategies. In conclusion, the Trivedi Effect®-Biofield Energy Healing Treatment and the test formulation has the significant capacity for immunomodulatory effect, which supposed to be found effective against overall gut health and inflammation.

Keywords: Biofield Energy Healing Treatment; The Trivedi Effect®; Gut Heath; Immunomodulation; Hematology; Biochemistry

Trivedi MK and Jana S. Immunomodulatory Action of a Proprietary Product Using TNBS (Tri Nitro Benzene Sulfonic Acid) - Induced Ulcerative Colitis in Sprague Dawley Rats. J Nutri Diet Probiotics 2021, 4(1): 180028.

Abbreviations: TNBS: Tri-Nitro Benzene Sulfonic Acid; UC: Ulcerative Colitis; IBDs: Inflammatory Bowel Diseases; CAM: Complementary and Alternative Medicine; NCCIH: National Center of Complementary and Integrative Health; SD: Sprague Dawley; TLC: Total Leukocyte Count; DLC: Differential Leukocyte Counts; TC: Total Cholesterol; TG: Triglycerides; LDL: Low Density Lipoprotein; HDL: High Density Lipoprotein; VLDL: Very Low Density Lipoprotein; ALP: Alkaline Phosphatase; SGOT: Serum Glutamic Oxaloacetic Transaminase; SGPT: Serum Glutamate-Pyruvate Transaminase; SEM: Standard Error Of Mean; CK-MB: Creatine Kinase Myocardium Band; AD: Addison Disease; RA: Rheumatoid Arthritis; IBS: Irritable Bowel Syndrome; Asthma, UC: Ulcerative Colitis; AD: Alzheimer's Disease; PD: Parkinson's Disease; QoL: Quality of Life; DRF: Dabur Research Foundation.

Introduction

A novel test formulation was designed to regulate the gut health, which comprised of zinc chloride, ferrous sulphate, copper chloride (II-cupric), pyridoxine HCl (vitamin B_6), cyanocobalamin (vitamin B_{12}), magnesium (II) gluconate, and cholecalciferol (vitamin D_3). The test formulation was designed to study the immunomodulatory parameters such as immunoglobulins, some hematology profile, and biochemistry parameters using tri-nitro benzene sulfonic acid (TNBS)-induced ulcerative colitis (UC) animal model. Gut inflammation and inflammatory bowel diseases (IBDs) such as UC and Crohn's disease are the major occurring diseases worldwide with age group 15 to 40 years [1]. Besides, IBD has been reported to affect more than 1 million individuals in the United States and about 2.5 million individuals in Europe [2]. The colon is the major part affected in UC, while various parts of digestive system from mouth to anus are affected in Crohn's disease. These diseases are causing high burden to economic and significantly affecting and reducing the quality of life. Various factors have been proposed to affect the gut health such as dietary habits, psychosocial factors, and abnormalities in gastrointestinal motility, and many more [3]. These factors either alone or in combination are still insufficient to explain the symptoms of diseases of gut. Some common symptoms of IBDs include are pain, cramps or swelling in the tummy, recurring or bloody diarrhea, weight loss, and extreme tiredness. However, some symptoms are also weakly reported such as joint pain in arthritis, painful red eyes in case of iritis, painful red skin nodules in erythema nodosum, and jaundice in case of primary sclerosing cholangitis [4]. Apart from usual line of treatment, which are full of associated side effects; while, an alternative medicine treatments using minerals and vitamins can be much more useful as compared with the conventional mode of modern medicine. Minerals and vitamins are considered as the major targeted constituents for any new immunomodulatory formulation due to its low toxicity profile [5,6]. According to the scientific literatures, a new proprietary test formulation was formulated with a combination of the minerals and vitamins. Each constituents of this test formulation is commonly used for a nutraceutical supplement for many beneficial purposes [7-10]. In addition, the formulated test formulation was treated with the complementary medicine, Biofield Energy Treatment by a renowned Biofield Energy Healer and tested for gut health for its immunomodulatory potential in male Sprague Dawley rats.

Biofield Therapy (or Healing Modalities) is one of the best Complementary and Alternative Medicine (CAM) therapy approach, which has been reported with number of scientific reports as preferred models of treatment to enhance physical, mental and emotional human wellness [11-15]. National Center of Complementary and Integrative Health (NCCIH) has been recognized and accepted the Biofield Energy Healing Therapy 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) [16]. Human Biofield Energy has subtle energy that has the capacity to work in an effective manner [17]. Complementary and Alternative Medicine (CAM) therapies have been practiced worldwide with reported clinical benefits in different health disease profiles [18]. Biofield Energy Healing Treatment (the Trivedi Effect®) significant outcomes has been published in numerous peer-reviewed science journals in many scientific fields such as cancer research [19], microbiology [20-22], genetics [23,24], pharmaceutics [25,26], nutraceuticals [27], organic compounds [28,29], agricultural science [30,31], and changing the structure of the atom in relation to various metals, ceramics, polymers and chemicals in materials science [32-34].

In this study, the authors sought to explore the impact of the Biofield Energy Healing Treatment (the Trivedi Effect®) on the test formulation for its immunomodulatory properties with respect to gut health using immune biomarkers such as humoral and cellular immune responses, hematology, clinical biochemistry, and sex hormone in male Sprague Dawley (SD) rats.

Materials and Methods

Chemicals and Reagents

Copper chloride, cholecalciferol (vitamin D_3), sodium carboxymethyl cellulose (Na-CMC), sulphasalazine, and iron (II) sulfate were procured from Sigma-Aldrich, USA. Pyridoxine hydrochloride (vitamin B_6), zinc chloride, cyanocobalamin (vitamin B_{12}), and magnesium (II) gluconate were purchased from TCI, Japan. TNBS (Trinitro Benzene Sulphonic acid) was purchased from HiMedia, India. All the other chemicals used in this experiment were analytical grade procured from India.

Experimental Animals

Randomly breed male Sprague Dawley (SD) rats with body weight around 220 to 350 gm were used in this study. The animals were purchased from M/s. National Institute of Biologicals, India. Animals were randomly divided into nine groups based on their body weights consist of eight animals of each group. They were kept individually in sterilized polypropylene cages with stainless steel top grill having provision for holding pellet feed and drinking water bottle fitted with stainless steel sipper tube. The animals were maintained as per standard protocol throughout the experiment.

Consciousness Energy Healing Strategies

The test formulation was divided into two parts. One part of each ingredient was considered as control, where no Biofield Energy Treatment was provided. Another part of each ingredient received Biofield Energy Treatment by Mr. Mahendra Kumar Trivedi (known as the Trivedi Effect®) under laboratory conditions for ~3 minutes. In addition, three different test groups as per experimental protocol, the animals were also received Biofield Energy Treatment under laboratory conditions for ~3 minutes. The blessing/treatment was given to the test items/animals remotely without touching in the laboratory of Dabur Research Foundation, near New Delhi, India. Similarly, the control samples were subjected to "sham" healer under the same laboratory conditions for ~3 minutes. The "sham" healer did not have any knowledge about the Biofield Energy Treatment. After that, the Biofield Energy Treated samples were kept in the similar sealed condition. The Biofield Energy Treated animals were also taken back to the animal experimental room for further proceedings.

Experimental Test Groups

The gut health oxidative stress biomarkers experiment was divided into 9 animals groups from G1 to G9. G1 denoted as normal control with vehicle (0.5% CMC), G2 group defined as colitis control, with 50% TNBS in ethanol using intra colonic route, G3 group include reference compound i.e. sulfasalazine (250 mg/kg), G4 group included administration of untreated test formulation, G5 include Biofield Energy Treated test formulation, G6 group denoted as Biofield Energy Treatment *per se* to the animals (day -15) along with vehicle (0.5% CMC), G7 group defined as Biofield Energy Treated test formulation from day -15, G8 group included Biofield Energy Treatment *per se* to the animals along with Biofield Energy Treated test formulation from day -15, and G9 group animals were administered with the Biofield Energy Treatment *per se* (day -15) to the animals along with the untreated test formulation. 50% TNBS in ethanol was given to the entire test group except G1.

Detailed Experimental Procedure

After acclimatization, animals were randomized and grouped based on their body weight after five days. Out of total nine groups, groups G1, G2, and G6 were treated with 0.5% w/v CMC-Na in distilled water for 8 weeks (day 1 to 56). Group 2 is TNBS induced colitis group (50% TNBS in ethanol), while group 3 was treated orally with sulphasalazine (reference item) at a dose of 250 mg/kg body weight for 8 weeks. The freshly prepared suspensions of untreated and Biofield Energy Treated Test formulation were administered orally to the G4 and G5 groups at a dose of 130.525 mg/kg for 8 weeks. Similarly, group G7 and G8 group were treated with test formulation at a dose 130.525 mg/kg from the day of Biofield Energy Treatment (day -15 to 56), while in group G9, Biofield Energy Treated animals were treated with untreated test formulation for 8 weeks. Before the induction of experimental colitis, a short fasting preceded. The duration ranged from 12 to 18 hours, while the chronic colitis was induced by intra colonic administration of TNBS-50% ethanol in a total volume of 400 µL, at a dose of 10 mg/rat. TNBS was instilled by a suitable medical-grade polyurethane catheter for enteral feeding approximately 8 cm proximal to the anal verge. Accordingly, TNBS injection was given on day 1, 8, 15, 21, 27, 34, 40, 48, and 54. TNBS-50% ethanol was given to all the animals from G2 to G9. After the end of the experiment, blood from all the animals was collected from the retro-orbital plexus using capillary tube and the following tests were carried out for immunomodulatory action such as hematology parameters, clinical biochemistry, and testosterone level.

Assessment of Humoral Responses

Humoral immune response, IgA, IgG, IgE and IgM were estimated using Mini Vidas, Biomeurix (France) from serum, using commercially available kits. The mean value was calculated for each group with SEM. The percent change in the Biofield Energy Treated group was calculated compared

to the vehicle treatment group.

Assessment of Hematology Parameters

Hematological parameters such as total leukocyte count (TLC), and differential leukocyte counts (DLC), were analyzed using Hematology analyzer (Abbott Model-CD-3700) in blood samples.

Assessment of Lipid Profile and Hepatic Enzymes

Glucose, total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL), high density lipoprotein (HDL), very low density lipoprotein (VLDL), alkaline phosphatase (ALP), serum glutamic oxaloacetic transaminase (SGOT), and serum glutamate-pyruvate transaminase (SGPT) were analyzed using serum by Biochemistry Analyzer, Spectralab A– plus, Italy [35,36].

Assessment of Sex Hormone-Testosterone

Testosterone was analyzed in serum using commercial kits. The testosterone level in serum was estimated in animals and the data was presented as mean ± SEM using Mini Vidas, Biomeurix (France). The % change in treated group was calculated as compared to vehicle group. The mean value was calculated for each group with SEM.

Determination of Body Weight, Feed Intake, and Organ Weight Parameters

All the experimental animals were daily analyzed for their change in body weight, feed intake, and organ weight parameters, which was calculated by weighing the daily feed supply and the left-over amount that evaluate the average daily feed intake. The average of the feed intake was computed for every three days of the experimental period. After terminal bleeding, the animals were sacrificed and the following organs such as liver, lung, kidney, brain, heart, eye, pancreas, spleen, thymus, adrenal gland, intestine, intestine and reproductive organs, i.e., testis, prostate, epididymis and vas deferens were collected. These organs were trimmed off any adherent tissue and fat, as appropriate and weighed. The organ to body weight ratio percentage was identified by comparing the weight of each organ with the final body weight of individual rat. All the data were reported through the study treatment regimen. Relative organ weight was calculated using the formula mentioned below.

Relative organ weight = Absolute organ weight (g)/weight of rat on sacrifice day(g)X100

Clinical Sign and Symptoms

All the animals in different test groups were analyzed for various clinical sign and symptoms in accordance with in-house protocol. Abnormal behavior in animals was recorded with the time of onset and disappearance.

Statistical Analysis

The data were expressed as mean ± standard error of mean (SEM) and subjected to statistical analysis using SigmaPlot (Version 11.0). For between two groups comparison Student's *t*-test was performed, while multiple groups analysis one-way ANOVA was performed followed by post hoc analysis by Dunnett's test. The $p \leq 0.05$ was considered as statistically significant (n=8).

Results and Discussion

Measurement of Humoral Immune Response

For the estimation of immunoglobulins (IgM, IgG, IgA, and IgE) after treatment with the test formulation and Biofield Energy Healing Treatment, the data are presented in Figure 1 (A-D). Immunoglobulin's levels of rats treated with TNBS (G2) showed reduced level by 5.87% (IgM) and 3.29% (IgG), respectively, while increased pattern was reported by 8.91% (IgA) and 9.76% (IgE) as compared with the control (G1) group. In addition, animals treated with Biofield Energy Treatment per se, reference compound, different combination of Biofield Energy Treated and untreated test formulation showed significant change in the level of immunoglobulins. The levels of IgM, IgG, IgA, and IgE in the G5 group was significantly increased by 4.73%, 1.14%, 7.46%, and 22.95%, respectively as compared with the untreated test formulation (G4) group. The level of IgE in the G6 group was increased by 8.79% and 25.51% as compared to the G2 and G4 groups, respectively. Additionally, the level of IgG and IgA in G7 group was decreased by 8.08% and 8.96%, respectively; while IgM and IgE level was increased by 12.16% and 48.57%, respectively as compared with the G4 group. The level of IgM and IgA in G8 group was increased by 20.27% and 8.4%, respectively while IgG and IgE level was decreased by 2.14% and 12.91%, respectively as compared with the G4 group. However, level of IgM, IgG, and IgA in G9 group was increased by 11.49%, 11.19%, and 5.97%, respectively while IgE level was decreased by 20.59% (G9) as compared with the G4 group. The scientific data suggested that the level of immunoglobulin's in case of ulcerative colitis and chronic diseases condition vary significantly and its alteration vary among the specific immunoglobulins [37]. Serum immunoglobulins have been found to have important role in complement activation, opsonization, neutralization of toxins, etc. Overall, it can be concluded that the Biofield Energy Healing Treatment significantly altered the humoral immune response with respect to the untreated test formulation.

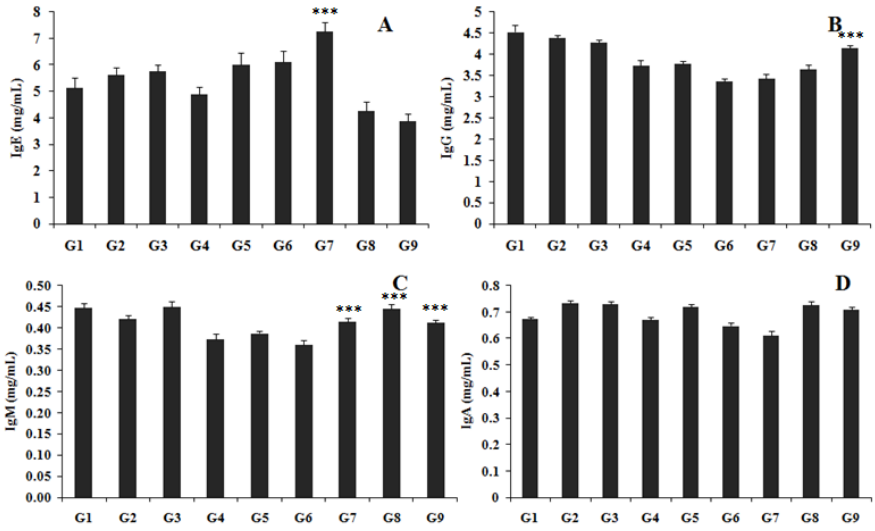

Figure 1: The effect of the test formulation on tested immunoglobulin, (A) IgE, (B) IgG, (C) IgM, and (D) IgA in various test groups G1 to G9 in male SD rats. G1: Normal control; G2: Disease control (50% TNBS in ethanol, (10 mg); 400 µL through intra-colonic route); G3: Sulphasalazine, 250 mg/kg; G4: Untreated test formulation; G5: Biofield Energy Treated test formulation; G6: Biofield Treatment *per se* to animals (-15 days); G7: Biofield Energy Treated test formulation (-15 day); G8: Biofield Energy Treatment *per se* to animals plus Biofield Energy Treated test formulation (-15 day); and G9: Biofield Energy Treatment *per se* to animals plus untreated test formulation. All the values are represented as mean ± SEM (n=8). ***$p \leq 0.001$ *vs.* G4.

Assessment of Hematology Parameters

The experimental data of hematology parameters in various groups (G1 to G9) are summarized in Table 1. The results suggested an improved animal hematology profile as compared with the disease control (G2) group. The tested hematology parameters such as TLC were found to be increased by 9.38% and 25.95% in the G5 and G9 groups, respectively as compared with the G4 group. Similarly, the level of neutrophils were increased by 59.15%, 65.26%, 59.62%, 26.76%, and 117.84% in the G5, G6, G7, G8, and G9 groups, respectively as compared with the G4 group. The level of eosinophils was significantly increased by 34.78% in G5 and G7 group as compare with the G4 group. Similarly, the level of monocytes was increased by 22.53% and 61.97% in the G5 and G9 groups as compare with the G4 group. Overall, the experimental data suggested that the Biofield Energy Healing Treatment has the significant capacity to improve the blood immunity related parameters.

Group (G)	TLC (X103/ mm3)	Neutrophils (X103/ mm3)	Lymphocytes (X103/ mm3)	Eosinophils (X103/ mm3)	Monocyte (X103/ mm3)
1	9.38 ± 0.43	2.24 ± 0.13	5.94 ± 0.47	0.41 ± 0.09	0.63 ± 0.05
2	12.38 ± 1.58	5.84 ± 1.51	5.12 ± 0.61	0.20 ± 0.02	0.99 ± 0.13
3	15.46 ± 0.62	3.05 ± 0.42	10.92 ± 0.41***	0.35 ± 0.05	0.90 ± 0.11
4	13.64 ± 0.97	2.13 ± 0.15	10.43 ± 0.85***	0.23 ± 0.02	0.71 ± 0.03
5	14.92 ± 0.91	3.39 ± 0.36	10.15 ± 0.94***	0.31 ± 0.07	0.87 ± 0.07
6	13.77 ± 1.31	3.52 ± 0.49	8.72 ± 0.82***	0.64 ± 0.10	0.70 ± 0.07
7	11.06 ± 0.78	3.40 ± 0.63	6.48 ± 0.35	0.31 ± 0.06	0.71 ± 0.06
8	12.17 ± 0.53	2.70 ± 0.36	8.29 ± 0.47***	0.25 ± 0.03	0.73 ± 0.08
9	17.18 ± 1.10*	4.64 ± 0.77*	10.66 ± 0.45***	0.42 ± 0.09	1.15 ± 0.07***

Table 1: Hematology profile of rats after administration of the test formulation in Sprague Dawley rats.

G1: Normal control; G2: Disease control; G3: Sulphasalazine, 250 mg/kg; G4: Untreated test formulation; G5: Biofield Energy Treated test formulation; G6: Biofield treatment *per se* to animals (-15 Days); G7: Biofield Energy Treated test formulation (-15 day); G8: Biofield Energy Treatment *per se* to animals plus Biofield Energy Treated test formulation (-15

day); and G9: Biofield Energy Treatment *per se* to animals plus untreated test formulation. TLC: Total leukocyte count, All the values are represented as mean ± SEM (n=8). All the values are represented as mean ± SEM of independent experiment (n=8). TLC: Total leukocyte count. #$p \leq 0.05$ and ###$p \leq 0.001$ *vs.* G4; ***$p \leq 0.001$ *vs.* G2.

The altered hematology profile might be used in many chronic inflammatory diseases, acute infection, gout, rheumatoid arthritis, rheumatic fever, etc. However, minerals and vitamins play a vital role to control the hematology profile [38-40]. The study data concluded that the Biofield Energy Treated (the Trivedi Effect®) test formulation significantly improved the concentrations of TLC, lymphocytes, and monocytes in hematology profile, which suggest immunomodulatory potential of the test formulation with respect to altered hematological animal profile.

Measurement of Hepatic and Cardiac Biomarkers

Hepatic and cardiac biochemical markers were tested for the test formulation and the results are tabulated in Table 2. The parameters used are serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP) and cardiac enzyme creatine kinase myocardium band (CK-MB), and others biomarkers such as, total bilirubin, albumin, and globulin of different groups (G1 to G9) are summarized and compared with their respective controls. The level of SGOT was significantly reduced by 31.96% ($p \leq 0.05$), 23.24%, 23.44%, 24.45%, and 23.93% in the G5, G6, G7, G8, and G9 groups, respectively as compared with the disease control (G2) group. Similarly, SGPT level was reduced by 22.63% and 23.44% in the G6 group as compared with the disease control (G2) and untreated test formulation (G4) groups, respectively. The level of ALP was also found to be reduced by 28.42%, 21.50%, 17.91%, 17.00%, and 21.63% in the G5, G6, G7, G8, and G9 groups, respectively as compared with the G2 group. Cardiac biomarker like creatine kinase-myocardial band (CK-MB) was significantly ($p \leq 0.001$) reduced by 46.26%, 28.88%, 42.33%, 43.57%, and 43.97% in the G5, G6, G7, G8, and G9 groups, respectively as compared with the G4 group. Besides, no significant change was observed in the level of TB, TP, A, G, and A/G in other experimental groups.

Group (G)	TB (mg/dL)	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	CK-MB (U/L)	TP (g/dL)	A (g/dL)	G (g/dL)	A/G ratio
1	0.15 ± 0.01	160.36 ± 5.75	46.78 ± 1.60	368.94 ± 16.02	96.14 ± 11.70	7.85 ± 0.09	3.44 ± 0.04	4.42 ± 0.11	0.72 ± 0.03
2	0.15 ± 0.02	217.20 ± 28.12	60.76 ± 6.18	398.31 ± 39.19	68.60 ± 16.23	6.64 ± 0.21	2.99 ± 0.09	3.65 ± 0.14	0.76 ± 0.03
3	0.17 ± 0.02	146.19 ± 7.82*	43.21 ± 2.75	321.81 ± 25.03	78.53 ± 7.70	7.43 ± 0.24	3.08 ± 0.03	4.35 ± 0.23	0.68 ± 0.04
4	0.13 ± 0.01	189.49 ± 13.99	61.41 ± 3.49	324.60 ± 43.23	127.90 ± 13.65	7.30 ± 0.11	3.29 ± 0.04	4.01 ± 0.11	0.78 ± 0.03
5	0.14 ± 0.01	147.78 ± 6.68*	63.53 ± 4.19	285.10 ± 20.97	68.74 ± 5.05***	7.33 ± 0.15	3.23 ± 0.06	4.10 ± 0.10	0.74 ± 0.02
6	0.16 ± 0.01	166.71 ± 11.11	47.01 ± 3.84	312.66 ± 16.41	90.96 ± 6.70***	7.21 ± 0.12	3.19 ± 0.05	4.03 ± 0.09	0.74 ± 0.02
7	0.13 ± 0.02	166.29 ± 5.98	62.41 ± 5.76	326.98 ± 23.41	73.76 ± 4.77***	7.46 ± 0.14	3.30 ± 0.05	4.16 ± 0.13	0.78 ± 0.03
8	0.12 ± 0.02	164.09 ± 23.77	64.54 ± 11.75	330.60 ± 17.72	72.18 ± 8.63***	7.20 ± 0.17	3.29 ± 0.05	3.91 ± 0.13	0.79 ± 0.02
9	0.15 ± 0.02	165.23 ± 13.33	59.60 ± 5.57	312.16 ± 23.71	71.66 ± 4.53***	7.59 ± 0.20	3.31 ± 0.06	4.28 ± 0.15	0.71 ± 0.02

Table 2: Evaluation of hepatic biomarkers after treatment with the test formulation on male rats.
G1: Normal control; G2: Disease control; G3: Sulphasalazine, 250 mg/kg; G4: Untreated test formulation; G5: Biofield Energy Treated test formulation; G6: Biofield treatment *per se* to animals (-15 days); G7: Biofield Energy Treated test formulation (-15 day); G8: Biofield Energy Treatment *per se* to animals plus Biofield Energy Treated test formulation (-15 day); and G9: Biofield Energy Treatment *per se* to animals plus untreated test formulation. All the values are represented as mean ± SEM (n=8). TB: Total bilirubin; SGOT: Serum glutamic oxaloacetic transaminase; SGPT: Serum glutamate-pyruvate transaminase; ALP: Alkaline phosphatase; CK-MB: Creatine kinase-myocardial band; TP: Total protein; A: Albumin; G: Globulin; A/G: Albumin/Globulin ratio; U/L: Unit per liter; mg/dL: Milligram per deciliter. *$p \leq 0.05$ *vs.* G2. ***$p \leq 0.001$ *vs.* G4.

Liver toxicity was measured by the hepatic biomarkers, and any high alteration in these enzymes results in infection and liver damage [41]. The experimental data suggested that the Biofield Energy Treated test formulation showed an improved liver health as reflected by many improved level of hepatic enzymes. Therefore, it can be concluded that the Trivedi Effect®-Biofield Energy Healing significantly improved the live health and its immunity profile.

Measurement of Sex Hormone-Testosterone

The level of testosterone after treatment with the test formulation in terms of percentage change is shown in the Figure 2. The study data suggest that the serum testosterone level was increased by 45.96% and 35.31% in G6 group as compared with the disease control (G2) and untreated test formulation (G4) groups, respectively. However, other

test groups showed a significant alteration after treatment compared with the normal control and disease control groups. Overall, the data showed that the Trivedi Effect®-Biofield Energy Healing has the power to regulate the level of sex hormone like testosterone.

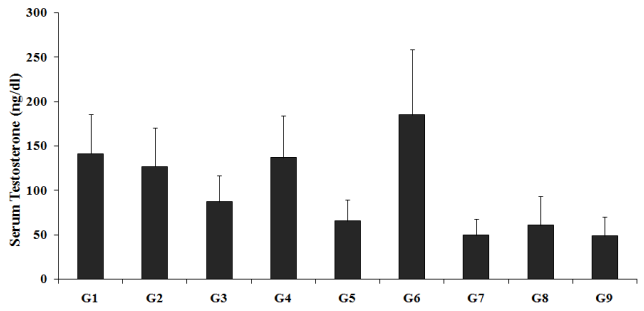

Figure 2: The effect of the test formulation on the level of testosterone after treatment on various groups (G1 – G9) in male Sprague Dawley rats. G1: Normal control; G2: Disease control; G3: Sulphasalazine, 250 mg/kg; G4: Untreated test formulation; G5: Biofield Energy Treated test formulation; G6: Biofield treatment *per se* to animals (-15 Days); G7: Biofield Energy Treated test formulation (-15 Day); G8: Biofield Energy Treatment *per se* to animals plus Biofield Energy Treated test formulation (-15 Day); and G9: Biofield Energy Treatment *per se* to animals plus untreated test formulation. All the values are represented as mean ± SEM (n=8).

Scientific data reported that the mineral, vitamins have been found to support the regulation of sex hormone [42]. However, as compared with the untreated test formulation, Biofield Energy Healing has further improved the level of testosterone. This signifies that the Trivedi Effect® can improve the sex hormone profile and also regulates the immune system.

Estimation of Animal Weight Parameters, Feed Intake, and Relative Organ Weight

The test formulation in whole experimental study was calculated and defined with respect to weight parameters, feed intake, and relative organ weight. The results of animal tested organ weight parameters are summarized in the Table 3. The change in animal weights is reported as per the normal physiological process. Thus, the relative organ weight parameters did not show any significant change in the tested organ weight throughout the experiment, suggested that the test formulation was found to be safe for the treatment. Organ to body weight ratio is the valuable index for any experimental test procedure with respect to the documentation of swelling, atrophy, or hypertrophy after exposure of test samples. Overall, the animal weight data, relative organ weight, and feed intake data suggested no significant abnormal change with respect to the disease control group, it suggest that the Biofield Energy Treated test formulation and Biofield Energy Treatment *per se* were found safe in all the tested animals.

Relative weight (%)	G1	G2	G3	G4	G5	G6	G7	G8	G9
Liver	2.5 ± 0.03	3.0 ± 0.19	2.4 ± 0.09	2.5 ± 0.05	2.3 ± 0.06	2.6 ± 0.09	2.6 ± 0.10	2.7 ± 0.15	2.7 ± 0.08
Lungs	0.5 ±0.03	0.54 ±0.04	0.52 ±0.02	0.54 ±0.04	0.50 ±0.03	0.50 ±0.01	0.48 ±0.01	0.47 ±0.01	0.61 ±0.08
Kidney	0.67 ±0.02	0.76 ±0.04	0.70 ±0.03	0.65 ±0.03	0.77 ±0.07	0.70 ±0.03	0.72 ±0.04	0.76 ±0.02	0.81 ±0.09
Brain	0.52 ±0.01	0.60 ±0.02	0.58 ±0.03	0.52 ±0.02	0.54 ±0.01	0.52 ±0.02	0.55 ±0.02	0.58 ±0.01	0.58 ±0.01
Heart	0.31 ±0.01	0.36 ±0.02	0.32 ±0.01	0.32 ±0.02	0.31 ±0.01	0.31 ±0.01	0.32 ±0.02	0.31 ±0.00	0.32 ±0.01
Eyes	0.07 ±0.00	0.08 ±0.01	0.09 ±0.00	0.08 ±0.00	0.08 ±0.00	0.08 ±0.00	0.08 ±0.01	0.08 ±0.01	0.09 ±0.00
Spleen	0.17 ±0.01	0.22 ±0.02	0.18 ±0.01	0.18 ±0.01	0.18 ±0.01	0.21 ±0.01	0.18 ±0.01	0.18 ±0.01	0.19 ±0.00
Pancreas	0.30 ±0.02	0.34 ±0.02	0.33 ±0.02	0.32 ±0.01	0.32 ±0.02	0.31 ±0.01	0.36 ±0.03	0.31 ±0.01	0.35 ±0.01
Thymus	0.08 ±0.01	0.08 ±0.01	0.07 ±0.01	0.09 ±0.01	0.08 ±0.00	0.08 ±0.01	0.07 ±0.01	0.08 ±0.01	0.09 ±0.01
Adrenal Gland	0.002 ±0.00	0.001 ±0.00	0.002 ±0.00	0.002 ±0.00	0.001 ±0.11	0.001 ±0.0	0.003 ±0.0	0.001 ±0.0	0.001 ±0.0
Intestine	3.11 ±0.07	3.93 ±0.08	3.39 ±0.15	3.39 ±0.15	3.29 ±0.02	3.25 ±0.04	3.65 ±0.22	3.64 ±0.38	3.65 ±0.25
Testis	0.035 ±0.04	0.046 ±0.05	0.032 ±0.03	0.032 ±0.03	0.021 ±0.01	0.038 ±0.04	0.06 ±0.06	0.037 ±0.04	0.055 ±0.06
Prostrate	0.01 ±0.01	0.010 ±0.01	0.008 ±0.01	0.008 ±0.01	0.008 ±0.01	0.009 ±0.01	0.016 ±0.02	0.011 ±0.01	0.007±0.01
Epididymis	0.015 ±0.01	0.036 ±0.04	0.014 ±0.01	0.014 ±0.01	0.013 ±0.01	0.019 ±0.02	0.044 ±0.04	0.016 ±0.02	0.021 ±0.02
Vas Deference	0.003 ±0.00	0.004 ±0.00	0.006 ±0.01	0.006 ±0.01	0.005 ±0.01	0.003 ±0.0	0.007 ±0.01	0.004 ±0.00	0.003 ±0.0

Table 3: The effect of the test formulation on organ weight parameters of male rats.

G1: Normal control; G2: Disease control; G3: Sulphasalazine, 250 mg/kg; G4: Untreated test formulation; G5: Biofield Energy Treated test formulation; G6: Biofield treatment *per se* to animals (-15 days); G7: Biofield Energy Treated test formulation (-15 day); G8: Biofield Energy Treatment *per se* to animals plus Biofield Energy Treated test formulation (-15 day); and G9: Biofield Energy Treatment *per se* to animals plus untreated test formulation.

Conclusion

The experimental data of gut health reavealed significant gut health activity of Biofield Energy (the Trivedi Effect®) based test formulation and Biofield Energy Treatment *per se* for their significant immunomodulatory activity. After treatmnent, cellular and humoral immune response was significantly changed in all the treatment groups when compared with the disease control and untreated test formulation groups. The level of test immunoglobulins such as IgM, IgG, and IgE was significantly increased by 20.27% in G8, 11.19% in G9, and 48.57% in G7 group, respectively as compared with the untreated test group (G4). Blood profile data showed that the TLC, neutrophils, and monocytes numbers were significantly increased by 25.95%, 117.84%, and 61.97%, respectively in the G9 group as compared to the G4 group. SGOT and SGPT levels were significantly decreased by 31.96% (G5) and 23.44% (G6), respectively as compared with the G2 group. In addition, ALP level was reduced by 28.42%, 21.50%, and 21.63% in the G5, G6, and G9 groups, respectively than G2 group. Moreover, CK-MB level was significantly decreased by 46.26%, 28.88%, 42.33%, 43.57%, and 43.97%, respectively in G5, G6, G7, G8, and G9 groups, respectively compared with the G4 group. However, testosterone level was increased by 45.96% and 35.31% in the G6 group as compared with the G2 and G4 groups, respectively. An experimental weight parameters such as body weight, organ weight, and feed intake data suggested normal changes, which suggest no toxicity profile of the test formulation. Thus, the present experiment suggested that the Trivedi Effect®-Biofield Energy Healing based novel test formulation significantly enhanced the test formulation's immunomodulatory activity. Therefore, the Biofield Energy Treated test formulation and Biofield Energy Treatment *per se* 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 Systemic Lupus Erythematosus (SLE), Fibromyalgia, Addison Disease (AD), Hashimoto Thyroiditis, Celiac Disease (gluten-sensitive enteropathy), Myasthenia Gravis, Pernicious Anemia, Aplastic Anemia, Dermatomyositis, Multiple Sclerosis, Chronic Fatigue Syndrome Graves' Disease, Scleroderma, Psoriasis, Sjogren Syndrome, Crohn's Disease, Vasculitis, Rheumatoid Arthritis (RA), Reactive Arthritis, Type 1 Diabetes, Vitiligo, and Alopecia Areata, as well as inflammatory disorders such as Irritable Bowel Syndrome (IBS), Asthma, Ulcerative Colitis (UC), Alzheimer's Disease (AD), Parkinson's Disease (PD), 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 and can be used as a stress prevention and management which include overall health and improved Quality of Life (QoL).

Acknowledgements

The authors are gratefully acknowledged to Trivedi science, Trivedi Global, Inc., and Trivedi master wellness and to Dabur Research Foundation (DRF), India for their support.

References

1. Miyoshi J, Chang EB (2016) The gut microbiota and inflammatory bowel diseases. Transl Res 179: 38-48.

2. Kaplan GG (2015) The global burden of IBD: From 2015 to 2025. Nat Rev Gastroenterol Hepatol 12(12): 720-727.

3. Kaser A, Zeissig S, Blumberg RS (2010) Inflammatory bowel disease. Annu Rev Immunol 28: 573-621.

4. Baumgart DC, Sandborn WJ (2007) Inflammatory bowel disease: Clinical aspects and established and evolving therapies. Lancet 369(9573): 1641-1657.

5. Maggini S, Wintergerst ES, Beveridge S, Hornig DH (2007) Selected vitamins and trace elements support immune function by strengthening epithelial barriers and cellular and humoral immune responses. Br J Nutr 98(1): 29-35.

6. Mora JR, Iwata M, von Andrian UH (2008) Vitamin effects on the immune system: Vitamins A and D take centre stage. Nat Rev Immunol 8(9): 685-698.

7. van Etten E, Mathieu C (2005) Immunoregulation by 1,25-dihydroxyvitamin D3: Basic concepts. J. Steroid Biochem Mol Biol 97(1-2): 93-101.

8. Wintergerst ES, Maggini S, Hornig DH (2007) Contribution of selected vitamins and trace elements to immune function. Ann Nutr Metab 51(4): 301-323.

9. Karacabey K, Ozdemir N (2012) The effect of nutritional elements on the immune system. J Obes Wt Loss Ther 2: 152.

10. Rosenberg IH (2007) Challenges and opportunities in the translation of the science of vitamins. Am J Clin Nutr 85(1): 325-327.

11. Jonas WB, Eisenberg D, Hufford D, Crawford C (2013)

The evolution of complementary and alternative medicine (CAM) in the USA over the last 20 years. Forsch Komplementmed 20(1): 65-72.

12. Morgan N, Irwin MR, Chung M, Wang C (2014) The effects of mind-body therapies on the immune system: Meta-analysis. PLoS One 9(7): e100903.

13. Anderson JG, Taylor AG (2011) Biofield therapies in cardiovascular disease management: A brief review. Holist Nurs Pract 25(4): 199-204.

14. Ernst E (2000) Prevalence of use of complementary/ alternative medicine: A systematic review. Bull of WHO 78(2): 252-257.

15. Wootton JC, Sparber A (2001) Surveys of complementary and alternative medicine: Part I: General trends and demographic groups. J Alt Comp Med 7: 195-208.

16. Koithan M (2009) Introducing complementary and alternative therapies. J Nurse Pract 5(1): 18-20.

17. Rubik B (1994) Manual healing methods. Alternative medicine: expanding medical horizons, Washington, DC, US Government Printing Office, NIH Publication pp: 94-66.

18. Cooper EL (2007) The immune system and complementary and alternative medicine. Evid Based Complement Alternat Med 4(1): 5-8.

19. Trivedi MK, Patil S, Shettigar H, Mondal SC, Jana S (2015) In vitro evaluation of biofield treatment on viral load against human immunodeficiency-1 and cytomegalo viruses. American Journal of Health Research 3(6): 338-343.

20. Trivedi MK, Patil S, Shettigar H, Mondal SC, Jana S (2015) In vitro evaluation of biofield treatment on *Enterobacter cloacae*: Impact on antimicrobial susceptibility and biotype. J Bacteriol Parasitol 6(5): 241.

21. Trivedi MK, Patil S, Shettigar H, Mondal SC, Jana S (2015) Evaluation of biofield modality on viral load of hepatitis B and C viruses. J Antivir Antiretrovir 7(3): 83-88.

22. Trivedi MK, Patil S, Shettigar H, Mondal SC, Jana S, et al. (2015) An impact of biofield treatment: Antimycobacterial susceptibility potential using BACTEC 460/MGIT-TB system. Mycobact Dis 5(4): 189.

23. Trivedi MK, Branton A, Trivedi D, Nayak G, Mondal SC, et al. (2015) Evaluation of antibiogram, genotype and phylogenetic analysis of biofield treated *Nocardia otitidis*. Biol Syst Open Access 4(2): 143.

24. Trivedi MK, Branton A, Trivedi D, Nayak G, Gangwar M, et al. (2015) Antibiogram, biochemical reactions, and genotypic pattern of biofield treated Pseudomonas aeruginosa. J Trop Dis 4: 181.

25. Trivedi MK, Patil S, Tallapragada RM (2013) Effect of bio field treatment on the physical and thermal characteristics of vanadium pentoxide powders. J Material Sci Eng S 1(11): 001.

26. Trivedi MK, Branton A, Trivedi D, Shettigar H, Bairwa K, et al. (2015) Fourier transform infrared and ultraviolet-visible spectroscopic characterization of biofield treated salicylic acid and sparfloxacin. Nat Prod Chem Res 3(5): 186.

27. Trivedi MK, Tallapragada RM, Branton A, Trivedi D, Nayak G, et al. (2015) Potential impact of biofield treatment on atomic and physical characteristics of magnesium. Vitam Miner 4(3): 129.

28. Trivedi MK, Branton A, Trivedi D, Nayak G, Sethi KK, et al. (2016) Gas chromatography-mass spectrometry based isotopic abundance ratio analysis of biofield energy treated methyl-2-napthylether (Nerolin). American Journal of Physical Chemistry 5(4): 80-86.

29. Trivedi MK, Branton A, Trivedi D, Nayak G, Panda P, et al. (2016) Gas chromatography-mass spectrometric analysis of isotopic abundance of 13C, 2H, and 18O in biofield energy treated p-tertiary butylphenol (PTBP). American Journal of Chemical Engineering 4(4): 78-86.

30. Trivedi MK, Branton A, Trivedi D, Nayak G, Mondal SC, et al. (2015) Evaluation of biochemical marker - glutathione and DNA fingerprinting of biofield energy treated *Oryza sativa*. American Journal of BioScience 3(6): 243-248.

31. Trivedi MK, Branton A, Trivedi D, Nayak G, Gangwar M, et al. (2016) Molecular analysis of biofield treated eggplant and watermelon crops. Adv Crop Sci Tech 4(1): 208.

32. Trivedi MK, Tallapragada RM, Branton A, Trivedi D, Nayak G, et al. (2015) Physical, atomic and thermal properties of biofield treated lithium powder. J Adv Chem Eng 5(4): 136.

33. Trivedi MK, Tallapragada RM, Branton A, Trivedi D, Nayak G, et al. (2015) Evaluation of biofield energy treatment on physical and thermal characteristics of selenium powder. Journal of Food and Nutrition Sciences 3(6): 223-228.

34. Trivedi MK, Tallapragada RM, Branton A, Trivedi D, Nayak G, et al. (2015) Physicochemical characterization of biofield energy treated calcium carbonate powder.

American Journal of Health Research 3(6): 368-375.

35. King EJ, Armstrong AR (1934) Estimation of alkaline phosphatase. Canad Med Assoc J 311: 152-156.

36. Folch J, Lees M, Sloane Stanley GH (1957) A simple method for the isolation and purification of total lipids from animal tissue. J Biol Chem 226(1): 497-509.

37. Sailer D, Ullmann B, Berg G (1976) Serum immunoglobulin levels in ulcerative colitis and Crohn's disease. Dtsch Med Wochenschr 101(33): 1214-1217.

38. Bunglavan SJ, Garg AK, Dass RS, Shrivastava S (2014) Effect of supplementation of different levels of selenium as nanoparticles/sodium selenite on blood biochemical profile and humoral immunity in male wistar rats. Vet World 7(12): 1075-1081.

39. Fox C, Ramsoomair D, Carter C (2001) Magnesium: Its proven and potential clinical significance. South Med J 94(12): 1195-1201.

40. Payahoo L, Ostadrahimi A, Mobasseri M, Bishak YK, Farrin N, et al. (2013) Effects of zinc supplementation on the anthropometric measurements, lipid profiles and fasting blood glucose in the healthy obese adults. Adv Pharm Bull 3(1): 161-165.

41. Giannini EG, Testa R, Savarino V (2005) Liver enzyme alteration: A guide for clinicians. CMAJ 172(3): 367-379.

42. Jana K, Samanta PK, Manna I, Ghosh P, Singh N, et al. (2008) Protective effect of sodium selenite and zinc sulfate on intensive swimming-induced testicular gamatogenic and steroidogenic disorders in mature male rats. Appl Physiol Nutr Metab 33(5): 903-914.