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Anti-Inflammatory and Anabolic Activity of the Biofield Treated Proprietary Test Formulation in Male Sprague Dawley Rats

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Abstract

The study was aimed to evaluate the anti-inflammatory potential of the Trivedi Effect® - Biofield Energy Healing Treatment on test formulation in male Sprague Dawley rats. Each ingredient of test formulation was divided in two portions. One part was denoted as the control without Biofield Treatment, while another part and three groups of animals were received Biofield Treatment by Mr. Mahendra Kumar Trivedi. Results showed that level of tumor necrosis factor-α (TNF-α) was significantly reduced by 11.37%, 11.19%, and 62.6% in Biofield Treated test formulation group (G5), Biofield Energy Treated test formulation at day -15 group (G7), and Biofield Treatment *per se* to animals plus Biofield Treated test formulation from day -15 groups (G8), respectively as compared to untreated test formulation (G4). Interleukin-1β (IL-1β) level was significantly decreased by 41.77% ($p \leq 0.001$), 28.76%, 64.44% ($p \leq 0.001$), and 75.65% ($p \leq 0.001$) in G6 (Biofield Energy Treatment *per se* at day -15), G7, G8, and Biofield Treatment *per se* (day -15) to animals plus untreated test formulation (G9) groups, respectively compared to G4. Moreover, IL-6 level was significantly reduced by 18.97%, 43.44% ($p \leq 0.05$), 17.43%, 15.37%, and 28.50% in the G5, G6, G7, G8, and G9 groups, respectively compared to G2. Besides, monocyte was significantly increased by 30.77% and 23.08% in the G6 and G7 groups, respectively; while neutrophil was increased by 21.52% in G8 as compared to G2. Further, eosinophil level was decreased by 20.67%, 33.33%, and 16.67% in G6, G8, and G9 groups, respectively compared to G4. Testosterone was significantly increased by 98.11% and 311.43% ($p \leq 0.001$) in G5 and G6 groups, respectively compared to G2. Overall, results suggested that Biofield Energy Treated test formulation and Biofield Energy Treatment *per se* to animals improved inflammation-related parameters and can be used for autoimmune and inflammatory disorders, stress management, and anti-aging by improving overall health.

Keywords: Inflammation; Biofield Energy Healing Treatment; Antiaging; The Trivedi Effect®; Hematology; Testosterone; Cytokine

Introduction

Overall health and quality of life (QoL) can be improved by maintaining the organic resistance of the body. It was reported that vitamins and minerals play a vital role in the immunomodulatory activities [1-3]. Vitamins and minerals are the major targeted product to modulate the immune system due to less toxic than conventional medicines against various types of infections [1,2]. It was well reported that different proinflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) and some hematology parameters were used for the diagnosis of various non-autoimmune diseases (hypertension, diabetes mellitus, arteriosclerosis etc) [3,4]. Inflammation is a normal physiological process that repairs the damaged tissues in response to either endogenous or exogenous aggressions. Aging is directly correlated with an increased levels of proinflammatory cytokines. Changes of

immune system due to aging, known as immunosenescence. High levels of IL-6 and TNF-α are associated with the increased risk of morbidity and mortality [5,6]. The new proprietary test formulation containing mixture of minerals (zinc chloride, magnesium gluconate hydrate, and ferrous sulphate, copper chloride) and vitamins (pyridoxine HCl, cyanocobalamin, and cholecalciferol). Individual constituent present in this formulation is commonly used as nutraceutical supplement [7-9]. The immunomodulatory agents have the ability to normalize or modulate pathophysiological processes [10,11]. Zinc plays major role in most of the biochemical reaction in living organism due to its enzyme catalyzing activity [12,13]. Magnesium reduces the production of inflammatory cytokine through activation of *NF*-κB pathways, which is a novel innate immunomodulatory mechanism [14].

Biofield Therapy or Healing Modalities is a type of Complementary and Alternative Medicine (CAM) therapies has been extensively used to enhance the mental, physical, and emotional human wellness. The National Center of Complementary and Integrative Health (NCCIH) has recognized and accepted Biofield Energy Healing as a CAM health care approach in addition to other therapies, medicines and practices such as Tai Chi, deep breathing, yoga, natural products, meditation, Qi Gong, massage, chiropractic/osteopathic, manipulation, progressive relaxation, special diets, guided imagery, homeopathy, acupressure, hypnotherapy, relaxation techniques, healing touch, acupuncture, movement therapy, rolfing structural integration, pilates, Ayurvedic medicine, mindfulness, essential oils, traditional Chinese herbs and medicines, Reiki, aromatherapy, naturopathy, cranial sacral therapy and applied prayer (as is common in all religions, like Christianity, Hinduism, Buddhism and Judaism). Human Biofield Energy has subtle energy that has the capacity to work in an effective manner [15]. Biofield Energy Healing Treatment has gained a holistic alternative and complementary medicine therapy that has significant impact on living organisms and nonliving materials without any adverse-effects and is a very cost-effective than conventional methods. Biofield Energy Treatment (the Trivedi Effect®) results has been published in numerous peer-reviewed science journals with significant outcomes in many scientific fields such as cancer research [16,17], microbiology [18-20], biotechnology [21,22], pharmaceutical science [23-26], agricultural science [27-29], materials science [30-32], nutraceuticals [33,34], skin health [35,36], human health and wellness. Based on the literature information and wide-spectrum effects of healing potential, the authors designed this experiment to evaluate the impact of the Biofield Energy Treatment (the Trivedi Effect®) on the test formulation for antiaging and anti-inflammatory activities with respect to proinflammatory cytokines, hematology, and testosterone using male Sprague Dawley rat model.

Materials and Methods

Chemicals and Reagents

Copper chloride, cholecalciferol (vitamin D_3), sodium carboxymethyl cellulose (Na-CMC), and iron (II) sulfate were procured from Sigma-Aldrich, USA. Pyridoxine hydrochloride (vitamin B_6), zinc chloride, cyanocobalamin (vitamin B_{12}), magnesium (II) gluconate, and resveratrol were purchased from TCI, Japan. D (+) Galactose obtained from Amresco, LLC. Rest of the chemicals used in this experiment were analytical grade procured from India.

Experimental Animals

Male Sprague Dawley (SD) rats with body weight ranges from 240.48 to 428.27 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 weight consist of ten 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 was received Biofield Energy Treatment by Mr. Mahendra Kumar Trivedi (known as the Trivedi Effect®) under laboratory conditions for ~3 minutes through the Healer's unique Energy Transmission process to the test formulation. The blessing/treatment was given to the test items/animals remotely without touching in the laboratory of Dabur Research Foundation, near New Delhi, India. Besides, three group of animals were also received Biofield Energy Treatment under laboratory conditions for ~3 minutes. 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 and used as per the study plan. The Biofield Energy Treated animals were also be taken back to experimental room for further proceedings.

Experimental Procedure

Five days after acclimatization, animals were randomized and grouped based on the body weight. Dosing for group

G7 and G8 was also initiated on day -15 till the end of the experiment. However, G1 to G6 and G9 animals were dosed from day 1 till the end of experiment. All the animals except G1 received D-Galactose, daily (500 mg/kg; i.p.) from day 1 to the end of the experiment. At the end of the experimental period, i.e., during 9th week, animals were bled and the blood and serum samples subjected for hematology and testosterone analysis, respectively. A portion of brain sample was homogenized and stored in -80°C for the estimation of cytokines such as TNF-α, IL-1β, and IL-6.

Estimation of Cytokines in Brain Homogenate

Rats brain tissues were isolated, homogenized, and centrifuged as per Zahr, et al. [37] with slight modification After that, the supernatants were collected and stored at −80°C for cytokines estimation such as TNF-*α, IL-1β, and IL-6.*

Hematology Parameters

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

Assessment of Testosterone

Testosterone was analyzed in serum using commercial kits. The percent change in the Biofield Energy Treated group was calculated compared to the vehicle treatment group.

Statistical Analysis

The data were expressed as mean ± standard error of mean (SEM) and subjected to statistical analysis using Sigma Plot (Version 11.0). Student's *t*-test was performed for comparison of the individual treatment group with control. The $p \leq 0.05$ was considered as statistically significant.

Results and Discussion

Estimation of Cytokines in Brain Homogenate

Estimation of Tumor Necrosis Factor Alpha (TNF-α): The effect of the test formulation on TNF-α in male Sprague Dawley rats is shown in Figure 1. The level of TNF-α in the normal control (G1) group was 12.29 ± 4.06 pg/mL and it was increased by 225.96% in the disease control (G2) group (40.06 ± 3.73 pg/mL). Positive control (resveratrol) group G3 reduced the level of TNF-α by 42.24% as compared to the G2 group. Further, level of TNF-α was reduced by 11.37%, 7.73%, 11.19%, and 62.6% in the Biofield Treated test formulation group (G5), Biofield Energy Treatment *per se* at day -15 group (G6), Biofield Energy Treated test formulation at day -15 group (G7), and Biofield Treatment *per se* to animals plus Biofield Treated test formulation from day -15 group (G8), respectively as compared to the untreated test formulation group (G4). Ageing is associated with an increased inflammatory activity in the blood, including increased circulating levels of TNF-α [38,39]. TNF-α is a multifunctional proinflammatory cytokine which may play a part in the pathogenesis of atherosclerosis.

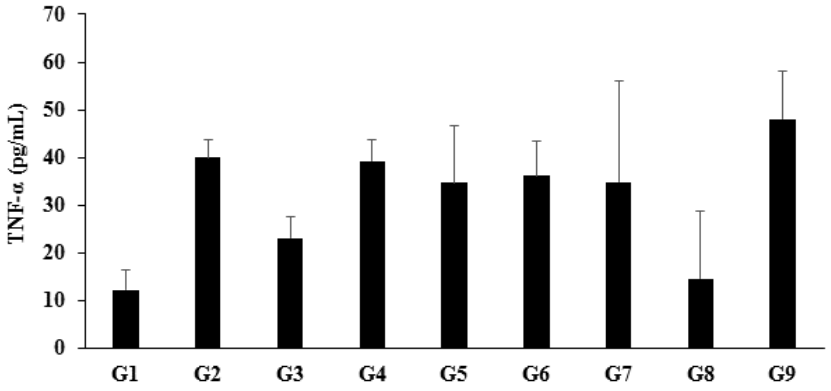

Figure 1: The effect of the test formulation on proinflammatory cytokine, tumor necrosis factor alpha (TNF-α) in male Sprague Dawley rats. G: Group; G1: Normal control; G2: Disease control; G3: Resveratrol; G4: Untreated test formulation; G5: Biofield Energy Treated test formulation; G6: Biofield Energy Treatment *per se* at day -15; and G7: Biofield Energy Treated test formulation at day -15. G8: Biofield Treatment *per se* to animals plus Biofield Treated test formulation from day -15; G9: Biofield Treatment *per se* (day -15) to animals plus untreated test formulation. All the values are represented as mean ± SEM (n=10).

Estimation of Interleukin-1β (IL-1β): The level of IL-1β after administration of the test formulation in male Sprague Dawley rats is shown in Figure 2. In the G2 group, the level of IL-1β was increased by 46.38% as compared to the normal control group (G1). Moreover, resveratrol (positive control) significantly reduced the level of IL-1β by 13.78% compared to the G2 group. Besides, the treatment groups showed significant reduction of the level of IL-1β by

6.68%, 41.77% (p<0.001), 28.76%, 64.44% (p<0.001), and 75.65% (p<0.001) in the G5, G6, G7, G8, and G9, respectively as compared to the untreated test formulation group (G4). From both preclinical and clinical studies data reported that IL-1β and IL-18 participate in fundamental inflammatory processes that increase during the aging process [40,41]. In this experiment, the Biofield Energy Treatment significantly reduced the level of proinflammatory cytokine IL-1β, which could be helpful for the management of aging-related disorders.

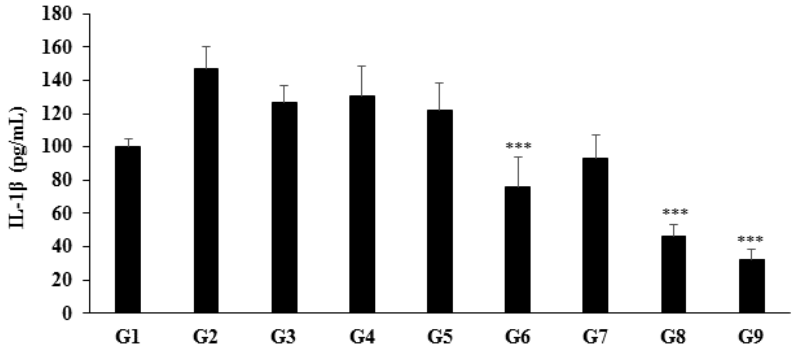

Figure 2: The effect of the test formulation on proinflammatory cytokine, interleukin-1β (IL-1β) in male Sprague Dawley rats. G: Group; G1: Normal control; G2: Disease control; G3: Resveratrol; G4: Untreated test formulation; G5: Biofield Energy Treated test formulation; G6: Biofield Energy Treatment *per se* at day -15; and G7: Biofield Energy Treated test formulation at day -15. G8: Biofield Treatment *per se* to animals plus Biofield Treated test formulation from day -15; G9: Biofield Treatment *per se* (day -15) to animals plus untreated test formulation. All the values are represented as mean ± SEM (n=10). ***p≤0.001 *vs.* untreated test formulation group (G4).

Estimation of Interleukin-6 (IL-6): The impact of the test formulation on the level of the interleukin-6 (IL-6) in male Sprague Dawley rats is shown in Figure 3. IL-6 level in the normal control group was 375.33 ± 60.13 pg/mL and it was increased by 29.31% in the disease control group (G2) induced by D-galactose (at 500 mg/kg *i.p.*). The positive control group (G3) showed 15.55% reduction of IL-6 as compared to the disease control (G2) group. Additionally, the treatment groups like G4, G5, G6, G7, G8, and G9 showed 5.06%, 18.97%, 43.44%, 17.43%, 15.37%, and 28.50% reduction of IL-6, respectively as compared to the G2 group. Further, the Biofield Treated test formulation and *per se* animal treatment groups G5, G6, and G9 showed 14.65%, 40.42%, and 24.69% reduction of the level of IL-6, respectively as compared to the untreated test formulation group (G4). Literature data reported that IL-6 is responsible for the pathogenesis of various age-associated diseases directly [42,43]. Here, the Biofield Treated test formulation had significantly reduced the level of IL-6, would be beneficial against age-related disorders.

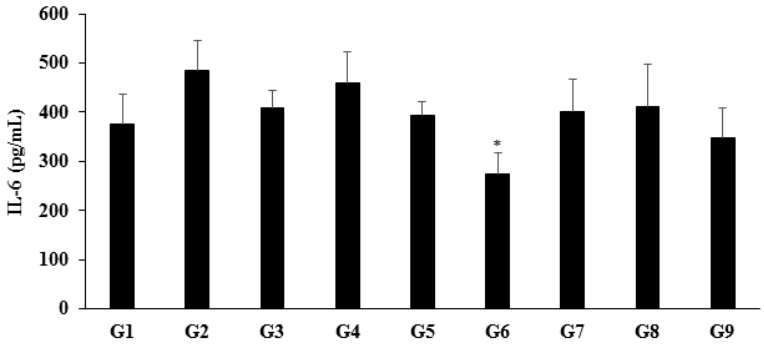

Figure 3: The effect of the test formulation on pro and anti-inflammatory cytokine, interleukin-6 (IL-6) in male Sprague Dawley rats. G: Group; G1: Normal control; G2: Disease control; G3: Resveratrol; G4: Untreated test formulation; G5: Biofield Energy Treated test formulation; G6: Biofield Energy Treatment *per se* at day -15; and G7: Biofield Energy Treated test formulation at day -15. G8: Biofield Treatment *per se* to animals plus Biofield Treated test formulation from day -15; G9: Biofield Treatment *per se* (day -15) to animals plus untreated test formulation. All the values are represented as mean ± SEM (n=10). *p≤0.05 *vs.* untreated test formulation group (G4).

Evaluation of Hematology Parameter

The hematology parameters such as total and differential leucocytes counts are shown in the Table 1. The disease control group (G2) showed reduction of neutrophils, eosinophils, and monocytes by 2.47%, 15.38%, and 10.47%, respectively compared to the normal control group (G1). The TLC was increased by 12.61% in the Biofield Energy Treated test formulation (G5) group compared to the disease control (G2). The level of neutrophils was significantly increased by 18.99%, 15.19%, and 21.52% in the Biofield Energy Treated test formulation (G5), Biofield Energy Treatment *per se* (G6) at day -15, and Biofield Treatment *per se* to animals plus Biofield Treated test formulation from day -15 (G8), respectively compared to the disease control group (G2). The level of lymphocytes was increased by 1.68% in the Biofield Energy Treated test formulation at day -15 (G7) group compared to the G2 group. The eosinophils level was decreased by 13.45%, 27.27%, and 9.09% in the G6, G8, and G9 groups, respectively as compared to the G2 group. Further, the level of eosinophils was reduced by 20.67%, 33.33%, and 16.67% in the G6, G8, and G9 groups, respectively as compared to the untreated test formulation (G4) group. The level of monocytes was significantly increased by 19.38%, 30.77% ($p \leq 0.05$), 23.08%, 11.69%, and 11.69% in the G5, G6, G7, G8, and G9, respectively compared to the disease control (G2). The positive control resveratrol showed 32% and 23.08% increase in the levels of eosinophils and monocytes, respectively compared to the disease control (G2). Jung, et al. [44], demonstrated the potential role of eosinophils as modulators of the intestinal immune system. Here, the Biofield Energy Treated test formulation showed reduction of the level of eosinophil to some extent compared to the both G2 and G4 groups. Another researcher reported the eosinophils and neutrophils show in the protective innate immune response [45]. Overall, the increased immune-related hematological parameters directly supports for the improvement of immunoresponse, and simultaneously it could protect various age-related disorders.

Group	TLC (10^3/mm^3)	Neutrophils (%)	Lymphocytes (%)	Eosinophils (%)	Monocyte (%)
G1	10.29 ± 0.49	20.25 ± 2.72	72.88 ± 2.35	3.25 ± 0.56	3.63 ± 0.42
G2	15.54 ± 1.56	19.75 ± 2.11	74.25 ± 1.79	2.75 ± 0.45	3.25 ± 0.31
G3	13.44 ± 0.75	18.13 ± 1.62	74.25 ± 1.51	3.63 ± 0.42	4.00 ± 0.19
G4	14.76 ± 0.66	16.88 ± 1.19	76.50 ± 1.70	3.00 ± 0.38	3.63 ± 0.32
G5	17.5 ± 0.1.39	23.50 ± 2.81	68.88 ± 2.82	3.75 ± 0.56	3.88 ± 0.23
G6	14.44 ± 1.21	22.75 ± 2.24	70.63 ± 2.39	2.38 ± 0.42	4.25 ± 0.16*
G7	14.31 ± 0.86	17.38 ± 2.07	75.50 ± 2.24	3.13 ± 0.40	4.00 ± 0.46
G8	14.36 ± 1.66	24.00 ± 5.20	70.38 ± 5.27	2.00 ± 0.42	3.63 ± 0.26
G9	13.23 ± 0.71	19.13 ± 2.45	74.75 ± 2.97	2.50 ± 0.27	3.63 ± 0.46

Table 1: The effect of the test formulation on hematological parameters.

Analysis of hematological profile like total and differential (5 parts) counts of white blood corpuscles of the test formulation in male Sprague Dawley rats. All the values are represented as mean ± SEM (n=10). G: Group; G1: Normal control; G2: Disease control; G3: Resveratrol; G4: Biofield Energy Treated test formulation; G5: Untreated test formulation; G6: Biofield Energy Treatment *per se* at day -15; and G7: Biofield Energy Treated test formulation at day -15. G8: Biofield Treatment *per se* to animals plus Biofield Treated test formulation from day -15; G9: Biofield Treatment *per se* (day -15) to animals plus untreated test formulation. All the values are represented as mean ± SEM (n=10). TLC: Total leukocyte count; %: Percentage. *$p \leq 0.05$ *vs.* G2.

Measurement of Testosterone

The effect of the test formulation on testosterone is shown in the Figure 4. The level of testosterone was significantly ($p \leq 0.001$) increased by 95.58% and 311.43% in the Biofield Energy Treatment *per se* at day -15 group (G6) as compared to the disease control group (G2) and untreated test formulation group (G4), respectively. Additionally, testosterone level was significantly increased by 98.11% and 15.93% in the Biofield Energy Treated test formulation (G5) and Biofield Treatment *per se* to animals plus Biofield Treated test formulation from day -15 (G8) groups, respectively as compared to the G4 group. Based on the scientific literature it was found that, supplementation of magnesium had increased the both free and total testosterone level [46]. Garcia, et al. [47], reported that zinc protected male sexual organ and simultaneously increased the level of serum testosterone in smoked in male rats which was due to antioxidant and stimulant effects of zinc. Overall, it is concluded that Biofield Energy Treatment on the combination of vitamins and minerals had remarkably improved the level of testosterone secretion, which might be due to the Trivedi Effect®.

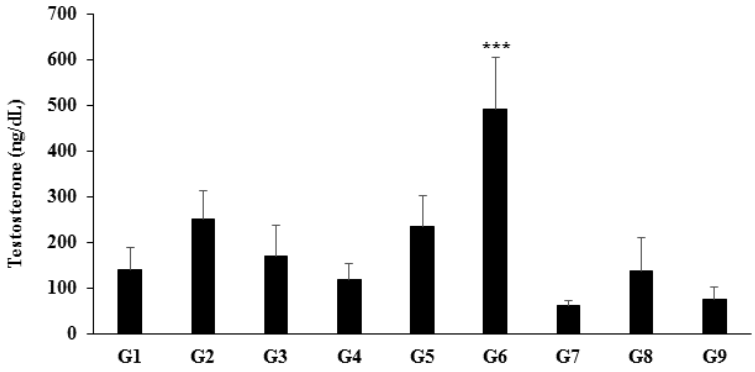

Figure 4: The effect of the test formulation on testosterone in male Sprague Dawley rats. G: Group; G1: Normal control; G2: Disease control; G3: Resveratrol; G4: Biofield Energy Treated test formulation; G5: Untreated test formulation; G6: Biofield Energy Treatment *per se* at day -15; and G7: Biofield Energy Treated test formulation at day -15. G8: Biofield Treatment *per se* to animals plus Biofield Treated test formulation from day -15; G9: Biofield Treatment *per se* (day -15) to animals plus untreated test formulation. All the values are represented as mean ± SEM (n=10). ***$p \leq 0.001$ *vs*. untreated test formulation group (G4).

Conclusion

Based on the current study findings, tumor necrosis factor-α (TNF-α) was significantly reduced by 62.6% in the Biofield Treatment *per se* to animals plus Biofield Treated test formulation from day -15 group (G8) group as compared to the untreated test formulation group (G4). Interleukin-1β (IL-1β) level was significantly decreased by 41.77%, 64.44%, and 75.65% in the Biofield Energy Treatment *per se* at day -15 (G6), G8, and Biofield Treatment *per se* (day -15) to animals plus untreated test formulation (G9) groups, respectively compared to the G4 group. Further, interleukin-6 (IL-6) level was significantly reduced by 43.44% and 28.50% in the G6 and G9 groups, respectively compared to the G2 group. Hematological parameters like monocytes was significantly increased by 30.77% and 23.08% in the G6 and G7 groups, respectively; while neutrophils was increased by 21.52% in the G8 group as compared to the G2 group. The eosinophils level was decreased by 20.67% and 33.33% in the G6 and G8 groups, respectively as compared to the G4 group. On the other hand, testosterone was significantly increased by 98.11% and 311.43% in the G5 and G6 groups, respectively as compared to the G2 group. All-inclusive, it can be inferred that the novel proprietary test formulation and animals *per se* after treatment with the Trivedi Effect®-Biofield Energy Healing by Biofield Energy Healers enhanced the test formulation's anti-inflammatory, immunomodulatory, and antiaging activities. Therefore, the Biofield Energy Treated test formulation and animals *per se* may act as an effective immunomodulatory and anti-inflammatory product, and it can be used as a Complementary and Alternative Medicine (CAM) in different autoimmune disorders *viz.* Addison Disease, Systemic Lupus Erythematosus, Dermatomyositis, Hashimoto Thyroiditis, Fibromyalgia, Celiac Disease (gluten-sensitive enteropathy), Scleroderma, Multiple Sclerosis, Myasthenia Gravis, Graves' Disease, Pernicious Anemia, Sjogren Syndrome, Aplastic Anemia, Vasculitis, Psoriasis, Rheumatoid Arthritis, Vitiligo, Reactive Arthritis, Crohn's Disease, Type 1 Diabetes, Chronic Fatigue Syndrome and Alopecia Areata, as well as inflammatory disorders *viz.* Ulcerative Colitis, Irritable Bowel Syndrome (IBS), Asthma, Dermatitis, Alzheimer's Disease, Hepatitis, Parkinson's Disease, Atherosclerosis, and Diverticulitis. In forward, the Biofield Treated test formulation can also be used in the prevention of inflammation-mediated tissue damage in cases of organ transplants (heart, kidney, and liver), anti-aging, stress prevention and management, and in the refinement of Quality of Life (QoL) and overall health.

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