

Evaluation of Anti-Oxidative Potential of Novel Proprietary Test Formulation: Influence of The Trivedi Effect®-Biofield Energy Healing Treatment

Mahendra Kumar Trivedi¹ and Snehasis Jana^{2*}

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

²Trivedi Science Research Laboratory Pvt. Ltd., Thane (W), Maharashtra, India.

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ABSTRACT

The study aim was to evaluate the antioxidant potential of Biofield Energy Healing Treatment (The Trivedi Effect®) on a novel proprietary test 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 test formulation. Additionally, three groups of animals were also received Biofield Energy Healing Treatment *per se* (day 15). The test formulation was evaluated for antioxidant enzymes, hematology, biochemistry, organ weight and histopathology analysis. The antioxidant results showed that the glutathione (GSH) level was significantly increased by 26.95%, 33.66%, 71.59%, 28.50% and 86.15% in the Biofield Energy Treated test formulation (G5), Biofield Energy Treatment *per se* to animals at day 15 (G6), Biofield Energy Treated test formulation from day 15 (G7), Biofield Energy Treatment *per se* to animals with the Biofield Energy Treated test formulation from day 15 (G8) and Biofield Energy Treatment *per se* to animals with untreated test formulation (G9) groups, respectively as compared to the disease control (G2) group. Antioxidant enzyme like glutathione peroxidase (GPx) level was significantly increased by 22.12%, 37.88%, 48.71% and 21.18% in G5, G7, G8 and G9 groups, respectively as compared to the G2. The level of myeloperoxidase (MPO) was decreased by 15.70%, 13.41%, 21.56% and 11.80% in G5, G6, G7 and G8 groups, respectively as compared to the G2. Hematology profile showed an improvement of total leukocyte count (TLC) level by 62.5%, 55.05%, 63.03% and 16.75% in the G6, G7, G8 and G9 groups, respectively as compared with the G2 group. Lipid profile data showed a significant reduction of triglycerides and very low density lipoprotein (VLDL) levels by 51.39% and 51.56%, respectively in the G8 group as compared with the G2 group. Hepatic biomarkers analysis showed decreased serum glutamate oxaloacetate transaminase (SGOT) level by 24.84%, 57.89% and 17.43% in the G5, G6 and G8 groups, respectively as compared with the G2 group. Further, the level of serum glutamate pyruvate transaminase (SGPT) was significantly decreased by 47.70% and 19.30% in the G6 and G8 groups, respectively compared with the G2 group. However, relative organ weight (%) and histopathology data suggested that there were no treatment-related changes in any group, which was found to be safe without any side-effect during the course of the experiment. These data suggested that the Biofield Energy Treated test formulation and The Trivedi Effect®-Consciousness Energy Healing Treatment *per se* can be used for improving the antioxidant enzymes levels that might be useful against many autoimmune and inflammatory diseases, stress management and prevention and act as anti-aging therapy by improving overall body's detoxification process.

Keywords: Consciousness energy healing, The Trivedi effect®, Immunomodulation, Nanocurcumin, Antioxidant, Hematology, Biochemistry

INTRODUCTION

Today, herbal based remedies are accepted worldwide and are back into the prominence. The use of such Complementary and Alternative Medicines (CAM) has become increasingly popular in the developed world [1,2]. For complementary therapies, plants or plant based constituents are always the key source of treatment strategy in various medicinal systems. In recent years, combination of herbal product (polyherbal) or single herbs has been used as curative substance in order to improve the health

Corresponding author: Snehasis Jana, Trivedi Science Research Laboratory Pvt. Ltd., Thane (W), Maharashtra, India, E-mail: publication@trivedieffect.com

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conditions. WHO data suggest that most of the populations worldwide are accepting the herbal materials (such as plant parts) or formulations, processed and finished herbal products for their basic healthcare needs. CAM are being used by more than 60% of the world's population, while these medicines are very much dominant over modern medicines in rural masses as well as developed primary health care countries [3]. Evidence-based medicines and research is receiving higher acceptance worldwide and National Center for Complementary and Alternative Medicine (NCCAM) has been inaugurated as the United States Federal Government's lead agency for conducting scientific research and practicing in the arena of medicine [4]. Thus, these herbo-mineral combinations are also used as nutritional supplements due to the presence of minerals and vitamins, while lack of nutritional supplements results in infectious and immunological diseases results in morbidity and mortality [5]. Although many synthetic immunomodulatory drugs are available worldwide for autoimmune diseases, anti-inflammatory disorders and anti-aging effect, but are mostly associated with adverse effects [6]. In order to develop novel test formulation for significant antioxidant activity, nanocurcumin, zinc chloride, magnesium (II) gluconate hydrate, sodium selenate, ascorbic acid (vitamin C), cholecalciferol (vitamin D3), iron (II) sulfate and copper chloride were used to formulate the novel formulation. All the active constituents of the novel formulation such as nanocurcumin, minerals and vitamins were reported to have significant antioxidant and immunological activities [7-9]. The novel proprietary formulation was treated with Biofield Energy Healing Treatment as CAM approach by a renowned Biofield Energy Healer and was evaluated for its antioxidant potential in male Sprague Dawley rats. Biofield Energy Healing Treatment as a CAM approach has been reported to have significant outcomes against various disease conditions. National Institute of Health (NIH) recommend and included various Energy therapies such as Reiki, Qi Gong, natural products, Tai Chi, deep breathing, yoga, chiropractic/osteopathic manipulation, massage, meditation, special diets, progressive relaxation, Ayurvedic medicine, homeopathy, guided imagery, acupuncture, acupressure, hypnotherapy, movement therapy, pilates, relaxation techniques, rolfing structural integration, cranial sacral therapy, mindfulness, healing touch, traditional Chinese herbs and medicines, naturopathy, essential oils, aromatherapy and applied prayer (as is common in all religions, like Christianity, Hinduism, Buddhism and Judaism) under CAM category that has been accepted by the most of the U.S. population with several advantages [10]. Every living organism possess some kind of unique energy that can be harness and transmit it into other living and non-living things by the process of Biofield Energy Healing by altered atomic/molecular weights through possible mediation of neutrinos [11]. Biofield Energy Healing Treatment (The Trivedi Effect®-Consciousness Energy Healing) have been

studied and reported with significant outcomes in various scientific disciplines such as microbiology with altered antimicrobial sensitivity against pathogenic microbes [12-15], genetics [16,17], skin health [18,19], agricultural science [20,21], immunity [22,23], pharmaceuticals [24,25] and materials science [26,27]. In the present study, the authors evaluated the impact of the Biofield Energy (The Trivedi Effect®-Consciousness Energy Healing) Treatment on the test formulation and Biofield Energy Treatment *per se* to the animals for its anti-oxidative potential, which might improve the immunomodulatory function, body's detoxification pathways, hematological parameters, serum biochemistry and organ histopathology using standard assays.

MATERIALS AND METHODS

Requirements

Iron sulfate, copper chloride, cholecalciferol, streptozotocin, cyclophosphamide and sodium carboxymethyl cellulose were obtained from Sigma Chemical Co. (St. Louis, MO). Nanocurcumin was purchased from Sanat Products Ltd., India. Quercetin dihydrate was procured from Central Drug House Pvt. Ltd., India. Magnesium (II) gluconate and zinc chloride were obtained from TCI, Japan. Sodium selenate and ascorbic acid were procured from Alfa Aesar, USA. All other chemicals used in this study were analytical grade available in India.

Laboratory animals

Randomly breed male *Sprague Dawley* (SD) rats with body weight ranges between 200-280 g were used in this experiment. The animals were purchased from M/s. Vivo Bio Tech Ltd., Hyderabad, India. Standard rodent diet was procured from M/s. Golden feeds, Mehrauli, New Delhi, India and provided *ad libitum* to all the groups of animals during the experiment under controlled conditions with a temperature of $22 \pm 3^\circ\text{C}$, humidity of 30% to 70% and a 12 h light/12 h dark cycle. The animals were acclimatized for the 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 was obtained prior to carrying out the animal experiment.

Study design

The animals were randomized and grouped according to their body weight. A total of nine groups (G) were included, i.e., Group 1 (G1) was served as a normal control (i.e., vehicle control) and G2 was served as a disease control; both the groups were received 0.5% Na-CMC, while G3 group animals received quercetin dihydrate (100 mg/kg; p.o.) as positive control. G4 group animals received untreated test formulation and G5 group animals received Biofield Energy

Treated test formulation at a dose of 624.12 mg/kg. Similarly, G6 group animals received Biofield Energy Treatment (15 days) *per se*, G7 animals received Biofield Energy Treated test formulation (15 days); G8 group defined as Biofield Energy Treated animals + Biofield Energy Treated test formulation (15 days) and G9 group denoted as Biofield Energy Treatment *per se* to animals plus untreated test formulation.

Biofield energy treatment strategies

The test formulation was divided into two parts. First part of each ingredient was considered as control, where no Biofield Energy Treatment was provided. Second part of each ingredient and three groups (G6, G8 and G9) of animals were received Biofield Energy Treatment (also known as The Trivedi Effect®-Consciousness Energy Healing) by a renowned Biofield Energy Healer, Mr. Mahendra Kumar Trivedi under laboratory conditions for ~3 min. The energy transmission was done without touching the samples and animals. Similarly, the control samples were subjected to “sham” healer under the same laboratory conditions for ~3 min. The “sham” healer did not aware 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 is taken back to the experimental room for further proceedings.

Experimental procedure

Five days after the acclimatization, animals were randomized and grouped based on body weight. After 15 days pre-study period the G6 group was received vehicle; while G7 and G8 groups were received the test formulation. The animals were fasted for 15-18 h and were injected with streptozotocin (STZ 45 mg/kg, i.p. single dose). After 1 week of post STZ injection, basal glucose levels (tail cut method) were measured for confirmation of diabetes (Day 1). The animals were treated with the test formulation/vehicle/positive control daily for up to 56 days. The body weight was recorded daily throughout the experimental period. On day 56, 50% of animal population was kept for overnight fasting and day 57 animals were bled and the samples subjected for hematology, biochemistry and electrolytes analysis. After bleeding, animals were humanely sacrificed to collect organ, i.e., liver. A portion of liver samples was weighed and transferred to the prescribed homogenizing buffer. The collected liver samples were homogenized and stored in -80°C for the estimation of various antioxidant parameters (GSH, GPx and MPO) using commercially available kit.

Antioxidant assay using ELISA method

Estimation of antioxidants - GSH and GPx: For the estimation of GSH, the liver sample was used, which is based on the reduction of 5, 5 dithiobis (2-nitrobenzoic acid) (DTNB) with reduced glutathione (GSH) to produce a

yellow compound. The reduced chromogen is directly proportional to the GSH concentration and its absorbance was measured at 405 nm by using a commercial kit (Item No: 703002, Cayman Chemicals) [28]. Liver tissues (GPx) enzyme activity was measured as IU/g tissue by the reaction between glutathione remaining after the action of GPx and 5, 5-dithiobis-(2-nitrobenzoic acid) to form a complex that absorbs maximally at 412 nm. The sample absorbance was measured at 405 nm by using a commercial kit (Item No: 703102, Cayman Chemicals) [29].

Anti-inflammatory marker, MPO: For MPO estimation, liver tissue (5%w/v) was homogenized in 0.5% hexadecyl trimethyl ammonium bromide (HTAB, Sigma-Aldrich, Co., St. Louis, MO, USA) with 50 mM potassium phosphate buffer, pH 6. The rest of the steps were performed as per in-house standard protocol. In addition, the homogenate was used for the estimation of myeloperoxidase (MPO) using Elisa kit (Cat No: k11-0575, Kinesisdx) through the colorimetric method as per manufacturer recommended standard procedure [30].

Measurement of hematology parameters

For the estimation of hematology, blood was withdrew from the retro-orbital plexus by capillary tubes and the hematology parameters such as differential leukocyte count (DLC), total leukocyte count (TLC), and lymphocyte, neutrophil, eosinophil and monocyte were evaluated using Hematology analyzer (Abbott Model-CD-3700) [31].

Measurement of hepatic enzymes and lipid profile

Serum biochemistry parameters *viz.* high density lipoprotein (HDL), total cholesterol (TC), low density lipoprotein (LDL), triglycerides (TG), very low density lipoprotein (VLDL), serum glutamic oxaloacetic transaminase (SGOT), alkaline phosphatase (ALP), serum glutamate-pyruvate transaminase (SGPT), creatine kinase-myocardial band (CK-MB), total protein (TP), total bilirubin (TB), albumin (A), globulin (G) and albumin/globulin ratio (A/G) were analyzed in the test formulations [31].

Clinical sign and symptoms

The animal clinical sign and symptoms were evaluated once daily throughout the experiment in accordance with in-house protocol with few modification [32]. Animals found in a moribund or even enduring signs of severe distress were humanely euthanized. Abnormal findings were noted with the time of onset and disappearance.

Measurement of organ weight and histopathology

After completion of the experiment, rats were dissected and the whole liver, kidneys, hearts, spleens, lungs and testis 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.

STATISTICAL ANALYSIS

Each experiment was carried out in eight independent assays and was represented as mean $\pm$ standard error of mean (SEM). Student's *t*-test was used to compare two groups to judge the statistical significance. For multiple group comparison, one-way analysis of variance (ANOVA) was used followed by post-hoc analysis using Dunnett's test. Statistically significant values were set at the level of $p \leq 0.05$.

RESULTS AND DISCUSSION

Effect of the test formulation on antioxidant parameters

Antioxidant activity of the novel test formulation was studied using ELISA method by estimating various enzymes such as antioxidants *viz.* GPx and GSH; and acute inflammatory marker *viz.* MPO. Liver homogenate of rat in various groups were used for the estimation of antioxidants enzymes and results are presented in **Figure 1**. The administration of novel test formulation and Biofield Energy Healing Treatment *per se* results in significant decrease in the content of enzymatic antioxidants (GPx) and non-enzymatic antioxidants (GSH) in cyclophosphamide (G2) group (**Figure 1A**). However, GSH was significantly increased by 26.95%, 33.66%, 71.59%, 28.50% and 86.15% in the G5, G6, G7, G8 and G9 groups, respectively as compared to the diseases control group G2. In addition, GPx level was increased by 22.12%, 7.06%, 37.88%, 48.71% and 21.18% in the G5, G6, G7, G8 and G9 groups, respectively as compared to the diseases control group G2 (**Figure 1B**). Acute inflammatory marker, MPO concentration was significantly decreased in the test formulation groups in comparison with the G2 group. The level of MPO was decreased by 15.70%, 13.41%, 21.56%, 11.80% and 8.46% in the G5, G6, G7, G8 and G9 groups, respectively as compared to the diseases control group G2 (**Figure 1C**). However, the level of MPO was decreased after Biofield Energy Healing treatment by 8.31%, 5.80%, 14.68% and 4.06% in the G5, G6, G7 and G8 groups, respectively as compared to the untreated test formulation group (G4). Antioxidant activity is considered as one of the vital property of any formulation or nutraceuticals. However, the high concentration of free radicals is very much accountable for abundant inflammatory infections [33]. Overall, the experimental data suggested that the novel test formulation has the significant antioxidant activity, which might help to minimize the inflammatory responses against wide range of inflammatory disease conditions.

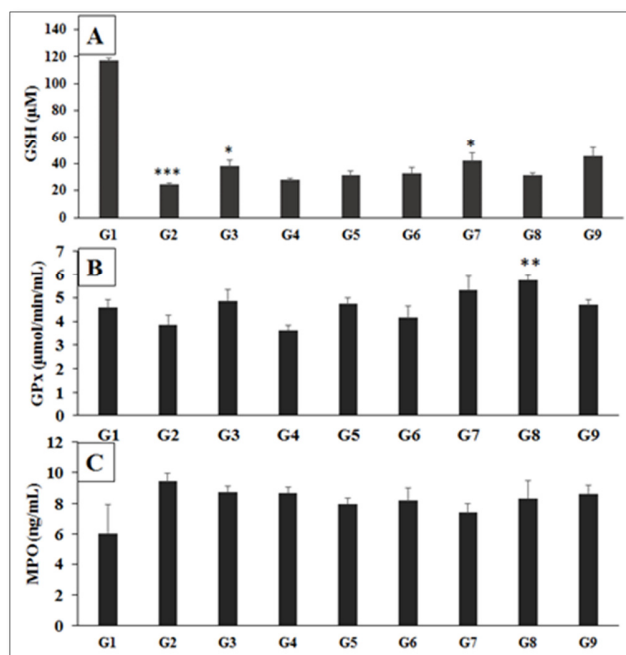


Figure 1. Effect of the Biofield Energy Treated test formulation on antioxidant profile using ELISA assay. (A) GSH, (B) GPx, and (C) MPO.

G1: Normal control; G2: Disease control; G3: Quercetin dihydrate; G4: Untreated test formulation; G5: Biofield Energy Treated test formulation; G6: Biofield Energy Treatment *per se* to animals (15 days); G7: Biofield Energy treated test formulation from day 15; G8: Biofield Energy Treatment *per se* to animals with Biofield Energy Treated test formulation from day 15; and, G9: Biofield Energy Treatment *per se* to animals with untreated test formulation. All values are expressed as the mean $\pm$ SEM. *** $p < 0.001$ and * $p < 0.05$ compared with the disease control ($n = 10$)

Analysis of hematological parameters

The experimental results of Biofield Energy Healing Based Test formulation and Biofield Energy Treatment *per se* showed significant change in blood profile of animals among different tested group with respect to the disease control (G2) group. The results of the hematology profile of all the groups are summarized in **Table 1**, which exhibited significant effect of the test formulation after Biofield Energy Healing Treatment. The TLC level was found to be increased by 62.5%, 55.05%, 63.03% and 16.75%, respectively in the G6, G7, G8 and G9 groups, respectively as compared with the G2 group. In addition, the level of neutrophils, monocytes, lymphocytes, etc. were altered after Biofield Energy Healing Treatment as compared with the untreated group. Thus, overall results showed that the blood profile was improved in the Biofield Energy Treated test formulation groups compared with the untreated test formulation. Many scientific reports support the beneficial role of herbal products along with minerals and vitamins

such as zinc, selenium and magnesium supplementation in order to improve the hematology parameters [34,35]. Thus, it can be suggested that the test formulation showed improved blood profile after treatment with the Biofield Energy Healing Treatment that can be assumed to have

significant capacity to improve the hematological activity of the formulated product against many blood related autoimmune disorders, anti-inflammatory diseases and anti-aging.

Table 1. Effect of the test formulation on the hematological parameters of male SD rats.

Group (G)	TLC (Thou/mm ³)	Neutrophils (%)	Lymphocytes (%)	Eosinophils (%)	Monocyte (%)
1	9.10 ± 0.44	17.50 ± 1.54	78.80 ± 1.65	1.50 ± 0.17	2.20 ± 0.42
2	3.76 ± 0.68	25.00 ± 1.21	69.13 ± 1.87	2.88 ± 0.88	3.00 ± 0.78
3	5.53 ± 0.85	24.50 ± 2.12	68.75 ± 2.58	2.75 ± 0.70	4.00 ± 1.00
4	4.60 ± 0.68	24.11 ± 2.06	70.78 ± 2.01	2.11 ± 0.65	4.11 ± 0.87
5	4.19 ± 0.48	23.75 ± 1.03	70.38 ± 1.48	2.00 ± 0.33	3.88 ± 0.90
6	6.11 ± 1.07	21.29 ± 1.63	76.00 ± 1.62	2.00 ± 0.53	2.14 ± 0.83
7	5.83 ± 1.22	21.71 ± 2.13	72.86 ± 2.85	2.57 ± 0.84	2.86 ± 0.63
8	6.13 ± 0.81	22.22 ± 2.09	72.11 ± 1.93	2.33 ± 0.41	3.33 ± 0.60
9	4.39 ± 0.48	26.00 ± 2.45	69.22 ± 2.75	2.00 ± 0.29	2.78 ± 0.40

TLC: Total Leukocyte Count, G: Group; G1: Normal Control; G2: Disease Control; G3: Quercetin Dihydrate; G4: Untreated Test Formulation; G5: Biofield Energy Treated Test Formulation; G6: Biofield Energy Treatment *per se* to Animals (15 days); G7: Biofield Energy Treated test Formulation from Day 15; G8: Biofield Energy Treatment *per se* to Animals with Biofield Energy Treated test Formulation from Day 15; and, G9: Biofield Energy Treatment *per se* to Animals with Untreated Test Formulation

Measurement of lipid profile

The effects of the Biofield Energy Treated and untreated test formulations along with Biofield Energy Treatment *per se* on animal serum lipid profile are presented in **Table 2**. Among the estimated parameters; significant decreased level of total cholesterol (87.80 ± 5.53 mg/dL), triglycerides (66.95 ± 20.02 mg/dL) and VLDL (13.38 ± 4.00 mg/dL) were found in the Biofield Treated formulation (G5) as compared with the disease control (G2) group. The level of total cholesterol, triglycerides and VLDL was significantly decreased by 7.14%, 42.70% and 42.69%, respectively in G5 group as compared with the G2 group. However, triglycerides and VLDL levels were significantly reduced by 51.39% and 51.56%, respectively in the G8 group, respectively as compared with the G2 group. With respect to serum lipids; there was a reduction in the VLDL levels in the Biofield Energy Treated test formulation and Biofield Energy Treated *per se* group as compared with the disease control and untreated test formulation groups. Scientific

literature suggested that the all the active constituents in the test formulation were reported with the beneficial effect on blood lipid profile. Individual ingredients such as nanocurcumin, minerals and vitamins have been reported for significant decreased level of triglycerides, serum cholesterol, LDL and VLDL levels. Major component of the formulation, nanocurcumin has been found to have beneficial role in improving the lipid profile [36]. Minerals such as selenium were reported to have beneficial role in lowering the serum total cholesterol and LDL along with improved humoral immunity [37]. Likewise, zinc and magnesium were found to have improved lipid profile such as decreased total cholesterol, triglycerides and LDL level, while increased HDL levels [38,39]. Overall, the results suggested that the Biofield Energy Treated test formulation groups and Biofield Energy Treatment *per se* showed significantly improved lipid profile as compared with the untreated test formulation, which can be used as better hypocholesterolemia agent.

Table 2. Effect of the Biofield Energy Treated test formulation on lipid profile of male SD rats.

Group (G)	Total Cholesterol (mg/dL)	Triglyceride (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)
1	90.88 ± 4.21	49.90 ± 3.06	27.40 ± 1.28	53.40 ± 2.84	9.94 ± 0.61
2	94.56 ± 13.22	116.86 ± 36.05	28.38 ± 4.00	45.63 ± 3.90	23.35 ± 7.20
3	106.39 ± 9.07	140.88 ± 49.77	30.50 ± 2.34	46.13 ± 7.76	28.15 ± 9.95
4	114.02 ± 10.45	150.74 ± 43.69	34.22 ± 3.04	49.22 ± 8.39	30.11 ± 8.73
5	87.80 ± 5.53	66.95 ± 20.02	25.75 ± 1.52	47.75 ± 4.78	13.38 ± 4.00
6	95.23 ± 11.34	130.26 ± 44.48	28.57 ± 3.40	40.43 ± 9.84	26.01 ± 8.90
7	103.24 ± 10.83	106.01 ± 41.07	30.86 ± 3.23	50.71 ± 8.71	21.19 ± 8.21
8	91.69 ± 7.75	56.80 ± 12.92	27.34 ± 2.27	51.00 ± 4.37	11.31 ± 2.58
9	106.82 ± 5.90	101.49 ± 34.05	32.00 ± 1.75	54.89 ± 5.00	20.27 ± 6.81

LDL: Low Density Lipoprotein; VLDL: Very Low Density Lipoprotein; HDL: High Density Lipoprotein; G: Group; G1: Normal Control; G2: Disease Control; G3: Quercetin Dihydrate; G4: Untreated Test Formulation; G5: Biofield Energy Treated Test Formulation; G6: Biofield Energy Treatment per se to Animals (15 days); G7: Biofield Energy Treated Test Formulation from Day 15; G8: Biofield Energy Treatment per se to Animals with Biofield Energy Treated Test Formulation from Day 15; and, G9: Biofield Energy Treatment per se to Animals with Untreated Test Formulation

Measurement of hepatic biomarkers

The effect of proprietary novel formulation on hepatic parameters is presented in **Table 3**. The data suggested that

the disease control (G2) group significant changed the level of hepatic biomarkers, which were standardized by quercetin dihydrate along with the Biofield Energy Treated Test formulation and Biofield Energy Treatment *per se* group.

Table 3. Effect of the Biofield Energy Treated test formulation on the hepatic biomarkers in male SD rats.

Group (G)	TB (mg/dL)	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	TP (g/dL)	A (g/dL)	G (g/dL)	A/G ratio	CK-MB (U/L)
1	0.11 ± 0.01	149.40 ± 9.67	26.84 ± 1.43	212.76 ± 9.60	6.41 ± 0.17	3.41 ± 0.05	2.96 ± 0.11	1.12 ± 0.03	186.08 ± 29.90
2	0.14 ± 0.02	492.24 ± 76.15	153.98 ± 31.10	1200.53 ± 160.31	4.48 ± 0.36	2.63 ± 0.15	1.81 ± 0.22	1.53 ± 0.17	186.28 ± 23.10
3	0.14 ± 0.03	722.63 ± 217.14	250.80 ± 84.02	1316.30 ± 166.78	5.28 ± 0.21	2.95 ± 0.11	2.28 ± 0.10	1.30 ± 0.04	249.04 ± 34.17
4	0.19 ± 0.04	317.44 ± 53.12	122.86 ± 20.27	1262.67 ± 103.52	4.84 ± 0.25	2.83 ± 0.10	1.98 ± 0.18	1.44 ± 0.11	250.49 ± 35.84
5	0.14 ± 0.02	369.95 ± 58.98	181.90 ± 34.90	1167.29 ± 109.04	4.76 ± 0.26	2.81 ± 0.13	1.90 ± 0.15	1.49 ± 0.09	299.69 ± 25.79
6	0.12 ± 0.01	207.24 ± 31.19	80.53 ± 11.45	1283.61 ± 129.91	4.96 ± 0.32	2.87 ± 0.11	2.07 ± 0.22	1.39 ± 0.10	225.74 ± 29.82
7	0.20 ± 0.05	637.34 ± 231.88	227.47 ± 67.70	1118.97 ± 142.79	5.11 ± 0.18	2.97 ± 0.06	2.10 ± 0.12	1.39 ± 0.06	241.90 ± 27.39

8	0.16 ±	406.44 ±	124.26 ±	1159.00 ±	5.06 ±	2.79 ±	2.20 ±	1.31 ±	260.51
	0.02	68.24	17.33	159.37	0.28	0.14	0.18	0.13	± 25.14
9	0.14 ±	515.90 ±	188.67 ±	1391.79 ±	5.00 ±	2.91 ±	2.02 ±	1.40 ±	245.49
	0.02	189.39	45.61	83.14	0.16	0.06	0.12	0.08	± 16.97

SGPT: Serum Glutamate-Pyruvate Transaminase; ALP: Alkaline Phosphatase; SGOT: Serum Glutamic Oxaloacetic Transaminase; CK-MB: Creatine Kinase-Myocardial Band; TP: Total Protein; TB: Total Bilirubin; A: Albumin; G: Globulin; A/G: Albumin/Globulin Ratio. G: Group; G1: Normal Control; G2: Disease Control; G3: Quercetin Dihydrate; G4: Untreated Test Formulation; G5: Biofield Energy Treated Test Formulation; G6: Biofield Energy Treatment *per se* to Animals (15 days); G7: Biofield Energy Treated Test Formulation from Day 15; G8: Biofield Energy Treatment *per se* to Animals with Biofield Energy Treated Test Formulation from Day 15; G9: Biofield Energy Treatment *per se* to the Animals with Untreated Test Formulation

The level of SGOT was reduced by 24.84%, 57.89% and 17.43% in the G5, G6 and G8 groups, respectively as compared with the G2 group. However, SGPT level was decreased by 47.70% and 19.30% in G6 and G8 groups, respectively as compared with G2 group. The level of ALP was decreased by 2.77%, 6.79% and 3.41% in the G5, G7 and G8 groups, respectively as compared with the G2. CK-MB level was reduced by 9.88% and 3.43% in G6 and G7 groups, respectively as compared with untreated test formulation (G4) group. The alteration in hepatic enzymes directly reflects the severity to the hepatocellular damage. An increase in liver enzymes in blood reflects the extent of damage, which will affect the liver function [40]. Scientific literature suggests that the constituents of test formulation such as nanocurcumin reported to have significant hepatic protection effect [41]. Similarly, minerals and vitamins present in the test formulation have significance liver protection action that helps to prevent the liver disease by stabilizing the membrane activity and hepatic biomarkers [42]. Therefore, it is concluded that Biofield Energy Healing Treatment *per se* and Biofield Energy Treated test formulation have significant capacity to protect the liver enzymes and can be used against many liver disorders.

Analysis of animal weight parameters

After treatment, all the animals in different groups were studied for their organ weight, which was compared with their initial body weight during experimental periods (Table 4). Overall, the experimental analysis data showed the final weights of tested organs showed no significant change in various groups from G1 to G9. The values were presented and compiled as organ to body weight ratio (expressed as relative organ weight in percentage). However, no significant change was observed in the tested organ weight throughout the experiment such as the organ weight of liver, lungs, kidneys, brain, heart, eyes, spleen, pancreas, thymus, small intestine, large intestine, testis, prostate, epididymis and vas deference with respect to the normal control and disease control group throughout the exposure period. In addition, the body weight of all the animals in various groups has been altered during the study period but not

significant, which suggested that the Biofield Energy Treated test formulation and Biofield Energy Treatment *per se* (day 15) were found to be safe and non-toxic during the exposure period.

Histopathological analysis was performed in all the groups after treatment and analysis suggested that no treatment-related changes were observed as compared with the normal control groups (Figure 2). Overall, the tested organ weight of all the animals was represented as relative organ weight (%) which suggested no significant change. Literature suggest that histopathological abnormalities such as swelling, atrophy or hypertrophy data can be used to understand the pathological conditions, which is the useful index to test any formulation for toxicity assay [43,44]. After treatment with any test formulation, if body weight and organ weight changed significantly then it suggested toxicity of the product. Atrophy refers to the decrease in organ weight, while increase in body or organ weight defined as hypertrophy in animals after exposure to the test formulation. However, data suggest that there was no significant change in all the treatment groups, which represent non-toxic and safe nature of the Biofield Treated test formulation and Biofield Energy Healing Treatment *per se* throughout the exposure period.

Table 4. Effect of the Biofield Energy Treated test formulation on relative organ weight (in percentage) parameters in male rats.

Relative organ weight (%)	G1	G2	G3	G4	G5	G6	G7	G8	G9
Liver	2.94 ± 0.44	4.56 ± 0.45	2.96 ± 0.73	5.36 ± 0.21	4.29 ± 0.30	4.83 ± 0.29	4.64 ± 0.41	4.81 ± 0.18	4.30 ± 0.50
Lungs	0.46 ± 0.01	0.68 ± 0.05	0.60 ± 0.05	0.63 ± 0.03	0.65 ± 0.04	0.57 ± 0.04	0.57 ± 0.03	0.69 ± 0.06	0.59 ± 0.02
Kidney	0.76 ± 0.02	1.36 ± 0.04	1.29 ± 0.03	1.36 ± 0.03	1.29 ± 0.05	1.26 ± 0.02	1.23 ± 0.07	1.39 ± 0.07	1.23 ± 0.03
Brain	0.46 ± 0.02	0.85 ± 0.11	0.82 ± 0.06	0.80 ± 0.03	0.81 ± 0.10	0.76 ± 0.03	0.79 ± 0.04	0.90 ± 0.04	0.79 ± 0.03
Heart	0.33 ± 0.01	0.42 ± 0.02	0.41 ± 0.02	0.45 ± 0.02	0.44 ± 0.02	0.43 ± 0.02	0.40 ± 0.02	0.40 ± 0.01	0.43 ± 0.01
Eyes	0.07 ± 0.00	0.14 ± 0.01	0.12 ± 0.01	0.12 ± 0.00	0.13 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.13 ± 0.01	0.12 ± 0.01
Spleen	0.18 ± 0.01	0.16 ± 0.01	0.16 ± 0.02	0.17 ± 0.01	0.15 ± 0.01	0.18 ± 0.02	0.16 ± 0.01	0.16 ± 0.01	0.17 ± 0.01
Pancreas	0.37 ± 0.02	0.35 ± 0.04	0.38 ± 0.02	0.34 ± 0.02	0.31 ± 0.03	0.35 ± 0.02	0.29 ± 0.03	0.33 ± 0.04	0.33 ± 0.04
Thymus	0.11 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.04 ± 0.00	0.08 ± 0.04	0.04 ± 0.00	0.08 ± 0.03	0.04 ± 0.00	0.05 ± 0.01
Small intestine	1.96 ± 0.06	6.51 ± 0.34	6.10 ± 0.44	6.64 ± 0.29	7.20 ± 0.53	5.84 ± 0.37	5.97 ± 0.73	6.22 ± 0.57	5.80 ± 0.31
Large intestine	1.46 ± 0.10	10.81 ± 1.03	7.81 ± 1.14	8.04 ± 0.73	8.08 ± 0.58	8.97 ± 1.01	10.29 ± 1.42	7.88 ± 0.54	7.93 ± 0.49
Testis	0.85 ± 0.03	0.78 ± 0.18	1.18 ± 0.10	1.28 ± 0.05	1.17 ± 0.07	1.28 ± 0.06	1.12 ± 0.08	1.20 ± 0.13	1.26 ± 0.04
Prostrate	0.16 ± 0.01	0.13 ± 0.01	0.16 ± 0.02	0.17 ± 0.02	0.14 ± 0.01	0.13 ± 0.02	0.15 ± 0.01	0.14 ± 0.01	0.17 ± 0.02
Epididymis	0.32 ± 0.01	0.27 ± 0.04	0.39 ± 0.04	0.39 ± 0.02	0.35 ± 0.03	0.42 ± 0.02	0.36 ± 0.03	0.39 ± 0.04	0.39 ± 0.02
Vas deference	0.07 ± 0.00	0.07 ± 0.01	0.08 ± 0.01	0.08 ± 0.00	0.08 ± 0.01	0.08 ± 0.00	0.07 ± 0.00	0.08 ± 0.00	0.08 ± 0.01

G1: Normal Control; G2: Disease Control; G3: Quercetin Dihydrate; G4: Untreated Test Formulation; G5: Biofield Energy Treated Test Formulation; G6: Biofield Energy Treatment per se to Animals (15 days); G7: Biofield Energy Treated Test Formulation from Day 15; G8: Biofield Energy Treatment per se to Animals with Biofield Energy Treated Test Formulation from Day 15; G9: Biofield Energy Treatment per se to Animals with the Untreated Test Formulation

Data are expressed as the mean ± SEM

Evaluation of histopathological examination

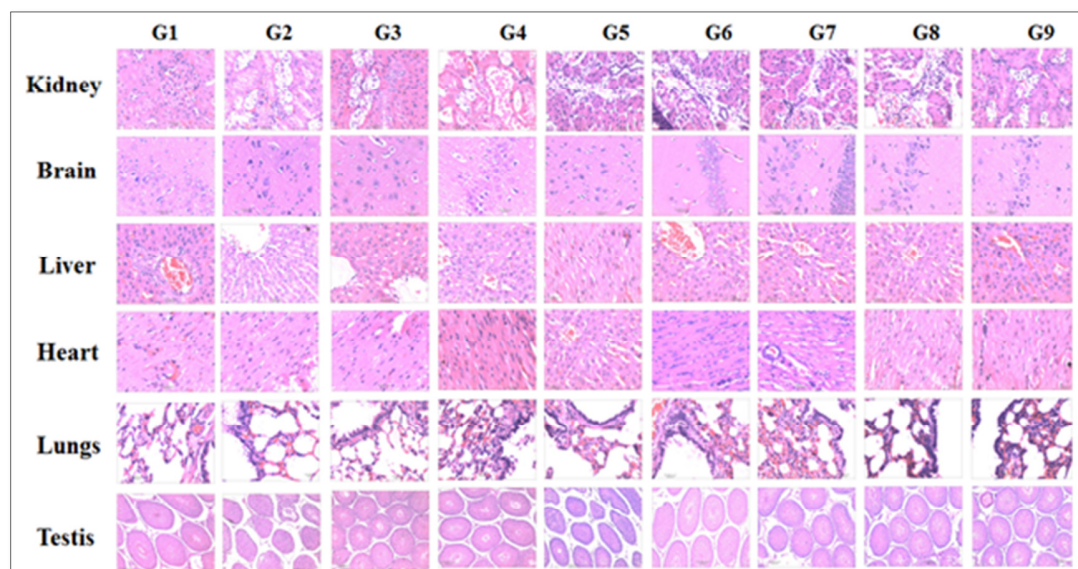


Figure 2. Histopathological photomicrograph of major organs tested after the Biofield Energy Treated test formulation in male Sprague Dawley rats. All the tissues were sectioned transversely and stained with hematoxylin and eosin. G1: Normal control; G2: Disease control; G3: Quercetin dihydrate; G4: Untreated test formulation; G5: Biofield Energy Treated test formulation; G6: Biofield Energy treatment *per se* to animals (15 days); G7: Biofield Energy Treated test formulation from day 15; G8: Biofield Energy Treatment *per se* to animals with Biofield Energy Treated test formulation from day 15; G9: Biofield Energy Treatment *per se* to animals with untreated test formulation.

Overall, the significant antioxidant activity, hematology and biochemistry parameters suggested that Biofield Energy Treatment *per se* and Biofield Energy Treated test formulation can be used to improve various inflammatory diseases. It was suggested that Biofield Energy Healing based test formulation and Biofield Energy Healing Treatment *per se* by a renowned Healing Practitioner has explained the significant antioxidant action. The Biofield Energy Treated novel proprietary formulation can be used to modulate the immune system and work as better approach in future against many autoimmune disorders.

CONCLUSION

Among tested antioxidants, GSH level was significantly increased by 26.95%, 33.66%, 71.59%, 28.50% and 86.15% in the G5, G6, G7, G8 and G9 group, respectively, as compared to the diseases control group G2. GPx level was increased by 22.12%, 7.06%, 37.88%, 48.71% and 21.18% in the G5, G6, G7, G8 and G9 groups, respectively as compared to the diseases control group G2. However, anti-inflammatory marker MPO was decreased by 15.70%, 13.41%, 21.56%, 11.80% and 8.46% in the G5, G6, G7, G8 and G9 groups, respectively, as compared to the diseases control group G2. Hematology data after treatment with the Biofield Energy Treated test formulation showed a significant increase in the TLC level by 62.5%, 55.05%, 63.03% and 16.75% in the G6, G7, G8 and G9 groups, respectively as compared with the G2 group. Lipid profile data showed that the total cholesterol, triglycerides and

VLDL were significantly decreased by 7.14%, 42.70% and 42.69%, respectively, in the G5 group as compared with the G2 group. Similarly, total cholesterol, triglycerides and VLDL levels were also reduced by 3.4%, 51.39% and 51.56% in the G8 group, respectively, as compared with the G2 group. Hepatic biomarker analysis revealed that SGOT level was reduced by 24.84%, 57.89% and 17.43% in G5, G6 and G8 groups, respectively, as compared with the G2 group. On the other hand, SGPT level was significantly decreased by 47.70% and 19.30% in the G6 and G8 groups, respectively as compared with G2 group. In addition, ALP level was decreased by 2.77%, 6.79% and 3.41% in the G5, G7 and G8 groups, respectively, as compared with the G2 group. However, no treatment-related changes were observed in any experimental treated group with respect to the relative organ weight (%) values in the Biofield Energy Treated test formulation and Biofield Energy Treatment *per se* groups throughout the experiment. Overall, the data suggested that The Trivedi Effect®-Consciousness Energy Healing Treatment enhanced the test formulation's antioxidant action. Thus, the Biofield Energy Treated test formulation and Biofield Energy Treatment *per se* in male SD rats showed significant antioxidant activity along with improved blood profile. Further, it can be used as a Complementary and Alternative Medicine (CAM) with a safe therapeutic index for various autoimmune disorders such as Lupus, Systemic Lupus Fibromyalgia, Erythematous, Hashimoto Thyroiditis, Addison Disease, Celiac Disease (gluten-sensitive enteropathy),

Dermatomyositis, Multiple Sclerosis, Graves' Disease, Pernicious Anemia, Myasthenia Gravis, Scleroderma, Aplastic Anemia, Psoriasis, Reactive Arthritis, Rheumatoid Arthritis, Sjogren Syndrome, Type 1 Diabetes, Vasculitis, Crohn's Disease, Chronic, Fatigue Syndrome Vitiligo 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 anti-aging, stress prevention and management and in the improvement of overall health and quality of life.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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