

Evaluation of Cardiac Performance after Treatment with the Biofield Energy Treated Proprietary Test Formulation on L-NAME and High Fat Diet-Induced Cardiovascular Disorders in Sprague Dawley Rats

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Abstract

Study was aimed to evaluate effect of Biofield Treated Proprietary Formulation and Biofield Treatment *per se* on cardiac performance on N^G-nitro-L-arginine methyl ester hydrochloride (L-NAME) and high fat diet (HFD)-induced cardiovascular disorders in Sprague Dawley rats. Nine groups were assigned, in

which four were preventive maintenance groups. The constituents of test formulation were divided into two parts; one section was defined as the untreated test formulation, while the other portion of the test formulation and three groups of animals received Biofield Energy Healing Treatment remotely for about 3 minutes by a renowned Biofield Energy Healer, Mr. Mahendra Kumar Trivedi. Systolic blood pressure (SBP) was significantly ($p \leq 0.001$) decreased by 13.39%, 14.65%, 17.74%, 14.36%, and 14.69% in the G5, G6, G7, G8, and G9 groups, respectively as compared to disease control (G2) group. Diastolic blood pressure (DBP) was significantly ($p \leq 0.001$) reduced by 25.95%, 24.41%, 30.79%, 24.67%, and 25.23% in G5, G6, G7, G8, and G9 groups, respectively than G2. Heart rate (HR) was significantly ($p \leq 0.05$) reduced by 6.58%, 8.06%, and 6.99% in G7, G8, and G9 groups, respectively than G2. Total leucocyte count (TLC) count was increased by 12.8% and 17.45% in G5 and G8 groups, respectively than G2 group. Neutrophils and lymphocytes were increased by 60.11% (G8) and 11.49% (G5), respectively than G2. Eosinophils were reduced by 11.11%, 20%, and 15% in G6, G7, and G9 groups, respectively than G2. Total cholesterol was significantly decreased by 22.64% ($p \leq 0.05$), 15.78%, 25.56% ($p \leq 0.05$), 22.56%, and

34.27% in G5, G6, G7, G8, and G9 groups, respectively than G2. Triglyceride was significantly ($p \leq 0.001$) reduced by 34.55%, 43.29%, 55.23%, 28.57%, and 37.28% in G5, G6, G7, G8, and G9 groups, respectively than G2. VLDL level was also significantly ($p \leq 0.001$) reduced by 80.81%, 83.61%, 86.82%, 79.19%, and 81.63% in G5, G6, G7, G8, and G9 group, respectively; while LDL was reduced by 20.32% (G9) group than G2. Atherogenic index (AI) was significantly ($p \leq 0.001$) decreased by 78.36%, 83.21%, 84.68%, 74.06%, and 72.98% in the G5, G6, G7, G8, and G9 groups, respectively than G2. The level of uric acid (UA) was significantly ($p \leq 0.001$) decreased by 57.51%, 52.36%, 45.49%, 43.78%, and 40.77% in the G5, G6, G7, G8, and G9 groups respectively, as compared with the G2 group. Serum glutamate pyruvate transaminases (SGPT) was significantly ($p \leq 0.001$) decreased by 45.96%, 48.01%, 37.19%, 37.69%, and 42.93% in the G5, G6, G7, G8, and G9 groups, respectively than G2. Creatine kinase myocardial band (CK-MB) level was significantly reduced by 10.19%, 21.97% ($p \leq 0.01$), 10.47%, and 16.94% in the G5, G6, G7, and G9 groups, than G2. Overall, the data suggested significance improvement of heart-related hematology, hepatic, and lipid parameters with respect to various pathological conditions that might be beneficial various types of cardiovascular disorders. Therefore, the results showed the significant slowdown the cardiovascular disease progression and its complications/symptoms in the preventive treatment groups *viz.* G6, G7, G8, and G9.

Introduction

High blood pressure is one of the most important risk factors for cardiovascular diseases (CVDs), which is the leading cause of mortality. About 54% of strokes and 47% of coronary heart diseases has been occurs in the worldwide due to high blood pressure [1]. There are various normal physiological parameters, which denote the healthy status of an individual, if present within the normal range. However, the presence of some agents/compounds indicates the physiological uncomfoting of the individual that might results due to an operation of different energy consuming mechanisms within the body

in the process of maintaining the homeostasis and thereby involves numerous biomarkers [2, 3]. Moreover, the changes in the body due to oxidative stress involves various physiological and endocrine alterations, along with disturbances in the biochemical and metabolic systems [4, 5]. Furthermore, such alterations consequently affect the important system of the body such as cardiovascular, renal, and CNS, followed by the hepato-biliary and pancreatic systems [6, 7]. Similarly, imbalance in the metabolic parameters such as, total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), cholesterol, glucose, triglycerides (TG), *etc.* There are many risk factors associated with CVDs such as abnormal blood lipid and sugar levels, obesity, smoking, and high blood pressure. Cholesterol plays the detrimental roles in the pathogenesis of atherosclerosis and CVDs [8]. Besides, the physiological parameters involved in the health and function of various vital organs like liver enzymes (SGOT, SGPT), kidney markers (uric acid, albumin); and in the heart like atherogenic index (AI), creatine kinase-myocardial band (CKMB) *etc.* have been enumerated [9, 10]. Thus, in order to study the change in vital function of heart in presence of L-NAME and High Fat Diet (HFD)-induced cardiovascular disorders in Sprague Dawley rats, a novel test formulation was designed with the combination of vital minerals (selenium, zinc, iron, calcium, copper, and magnesium), essential vitamins (cyanocobalamin, ascorbic acid, pyridoxine HCl, vitamin B₉, and cholecalciferol), and nutraceuticals (β -carotene, Ginseng, cannabidiol isolate (CBD)). All the minerals and vitamins incorporate in this test formulation have significant physiological roles [11-13]. Besides, cannabidiol itself has wide range of pharmacological profile and has been reported to role in different disorders [14, 15], while ginseng extract is regarded as one of the best immune boosters for overall immunity [16]. The present study was aimed to evaluate the various physiological parameters related to the heart on the Biofield Energy Treated Proprietary test formulation and Biofield Energy Treatment *per se* to the animals under L-NAME and high

fat diet (HFD)-induced cardiovascular disorders in Sprague Dawley rats.

Biofield Energy Healing Treatment has been reported with significant effects against various disorders, and defined as one of the best Complementary and Alternative Medicine (CAM) treatment approach [17-19]. National Center for Complementary/Alternative Medicine (NCCAM) recommended CAM with several clinical benefits as compared with the conventional treatment approach [20]. National Centre of Complementary and Integrative Health (NCCIH) accepted Biofield Energy Healing as a CAM health care approach in addition to other therapies such as deep breathing, natural products, Tai Chi, yoga, therapeutic touch, Johrei, Reiki, pranic healing, chiropractic/osteopathic manipulation, guided imagery, meditation, massage, homeopathy, hypnotherapy, special diets, relaxation techniques, movement therapy, mindfulness, Ayurvedic medicine, traditional Chinese herbs and medicines in biological systems [21, 22]. The Trivedi Effect®-Consciousness Energy Healing was scientifically reported on various disciplines such as in the nutraceuticals [23], agriculture science [24], cardiac health [25], materials science [26, 27], antiaging [28], Gut health [29], pharmaceuticals [30], overall human health and wellness. In this study, the authors want to study the impact of the Biofield Energy Treatment (the Trivedi Effect®) *per se* and Biofield Energy Treated test formulation on blood pressure, hematology, hepatic, cardiac, and lipid profile along with change in organ weight in presence of L-NAME and High Fat Diet-Induced Cardiovascular Disorders in Sprague Dawley Rats using standard methods.

Material and Methods

Chemicals and Reagents

Pyridoxine hydrochloride (vitamin B₆), atorvastatin, zinc chloride, magnesium (II) gluconate, and β-carotene (retinol, provit A) were purchased from TCI, Japan. Copper chloride, cyanocobalamin (vitamin B₁₂), calcium chloride, vitamin E (Alpha-Tocopherol),

cholecalciferol (vitamin D₃), iron (II) sulfate, captopril, L-NAME, and sodium carboxymethyl cellulose (Na-CMC) were procured from Sigma-Aldrich, USA. Ascorbic acid (vitamin C) and sodium selenate were obtained from Alfa Aesar, India. Cannabidiol isolate and *Panax ginseng* extract were obtained from Panacea Phytoextracts, India and Standard Hemp Company, USA, respectively. Standard normal chow diet and high fat diet were purchased from Altromin, USA and Research Diets, USA.

Maintenance of Animal

Randomly breed male Sprague Dawley (SD) rats with body weight ranges from 200 to 300 gm were used in this study. The animals were purchased from M/s. HYLASCO Biotechnology (India) Pvt. Ltd., India. Animals were randomly divided into nine groups based on their body weights consist of 15 animals of each group (at the time of induction period) and 10 animals of each group (at the time of treatment period). 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 novel test formulation was consisted of zinc chloride, iron (II) sulfate, copper chloride, vitamin B₆, vitamin B₁₂, vitamin D₃, vitamin B₉, sodium selenate, calcium chloride, ascorbic acid, beta carotene, *Panax ginseng* extract, cannabidiol and magnesium (II) gluconate. Each ingredient of the novel test formulation was divided into two parts. One part of the test compound did not receive any sort of treatment and were defined as the untreated or control sample. The second part of the test formulation was treated with the Trivedi Effect® - Energy of Consciousness Healing Treatment/ Blessing (Biofield Energy Treatment) by a renowned Biofield Energy Healer, Mr. Mahendra Kumar Trivedi under laboratory conditions for ~3 minutes. Besides, three group of animals also received Biofield Energy Healing Treatment (known as the Trivedi Effect®) by Mr.

Mahendra Kumar Trivedi under similar laboratory conditions for ~3 minutes. The Biofield Energy Healing Treatment/ Blessing (prayer) was done remotely, for about 3 minutes *via* online web-conferencing platform. After that, the Biofield Energy Treated/Treated samples was kept in the similar sealed condition and used as per the study plan. In the same manner, the control test formulation group was subjected to “sham” healer for ~3 minutes treatment, under the same laboratory conditions. The “sham” healer did not have any knowledge about the Biofield Energy Treatment/ Healing. The Biofield Energy Treated/Blessed animals were also taken back to experimental room for further proceedings.

Experimental Procedure

Seven days after acclimatization, animals were randomized and grouped based on the body weight. The test formulation was prepared freshly prior to dosing and administered to the animals using an oral intubation needle attached to an appropriately graduated disposable syringe. The dose volume was 10 mL/kg in morning and evening based on body weight. The experimental animals were divided into nine (9) different groups are shown in Table 1.

The normal control animals’ group (G1) was

receive normal drinking water and a normal diet throughout the experimental period. The animals in groups G2-G9 were received L-NAME (20 mg/kg, *i.p.*) and a HFD throughout the experimental period. At the end of the experimental period (8 weeks treatment), recorded systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR). After that, all the animals were individually subjected for blood collection using retro-orbital route to the experimental purpose such as hematology and biochemistry. The organs were isolated and recorded as wet weight.

Measurement of Blood Pressure and Heart Rate

Animals were subjected for the measurement of systolic and diastolic blood pressure and heart rate in conscious rats through the tail-cuff method. Each animal was introduced into a restrainer and kept in a quiet and warm environment for 10 minutes. The rat tails were cleaned/dilated using a cotton soaked in hot water. A rubber cuff (proximally) and a photoelectric sensor of pulsations (more distally) were placed around the tail. The sensor was connected to an amplifier and pulsations were recorded on a Non-Invasive Blood Pressure Amplifier, BioPac Inc.

Assessment of Hematology Parameters

Hematological parameters such as total

Table 1. Details description of study design with various components in different groups.

G1	Normal Control	Vehicle	L-NAME + HFD	
G2	Disease Control			
G3	Positive Control			Captopril + Atorvastatin
G4	Untreated Proprietary Formulation			Untreated Test formulation
G5	Treated Proprietary Formulation			Treated Test formulation
G6	Biofield Treatment/Blessing <i>per se</i> (Day -15)			Only Blessing
G7	Biofield Treated Proprietary Formulation (Day -15)			Treated Test formulation
G8	Biofield Treatment/Blessing <i>per se</i> & Biofield Treated/Blessed Proprietary Product (Day -15)			Blessing + Treated Test formulation
G9	Biofield Treatment/Blessing <i>per se</i> & Untreated Proprietary Product (Day -15)			Blessing + Untreated Test formulation

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.

Assessment of Hepatic and Cardiac Biomarkers

The test formulation was tested for important hepatic and cardiac biomarkers such as albumin (g/dl), alkaline phosphatase (U/L), bilirubin total (mg/dL), SGOT (U/L), SGPT (U/L), total protein (mg/dL), globulin (mg/dL), A/G ratio, and creatine kinase-MB (U/L) were analyzed using serum by Biochemistry Analyzer, Spectralab A- plus, Italy.

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 intake of feed was recorded in every three days interval throughout 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 [31]. All the data were reported through the study treatment regimen.

Relative organ weight was calculated as per

Equation 1.

$$\text{Relative organ weight} = \text{Absolute organ} \frac{\text{weight(g)}}{\text{weight}} \text{of rat on sacrifice day} \dots \dots \dots (1)$$

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 behaviour in animals was recorded with the time of onset and disappearance.

Statistical Analysis

The data were represented as mean \pm standard error of mean (SEM) and subjected to statistical analysis using Sigma-Plot statistical software (Version 11.0). For multiple comparison One-way analysis of variance (ANOVA) followed by post-hoc analysis by Dunnett's test and for between two groups comparison Student's *t*-test was performed. The $p \leq 0.05$ was considered as statistically significant.

Results

Measurement of Blood Pressure and Heart Rate

Blood pressure and heart rate were measured after treatment with the test formulation and the data were graphically presented in the Figure 1. The data suggested that the disease control (L-NAME + high fat diet (HFD) + 0.5% CMC) group (G2) showed systolic blood pressure (SBP) as 179.68 ± 2.49 mm of Hg, which was increased by 63.32% as compared with the normal control (G1, 110 ± 2.89 mm of Hg). However, positive control (captopril + atorvastatin) treatment (G3) showed the level of SBP *i.e.* 130.8 ± 1.19 mm of Hg, which was significantly ($p \leq 0.001$) decreased by 27.20% as compared to the G2 group. The level of SBP was significantly ($p \leq 0.001$) decreased by 12.60%, 13.39%, 14.65%, 17.74%, 14.36%, and 14.69% in the G4 (L-NAME + HFD along with untreated test formulation), G5 (L-NAME + HFD + the Biofield Energy Treated test formulation), G6 (L-NAME + HFD + Biofield Energy Treatment *per se* to animals from day -15), G7 (L-NAME + HFD + the Biofield Energy Treated test formulation from day -15), G8

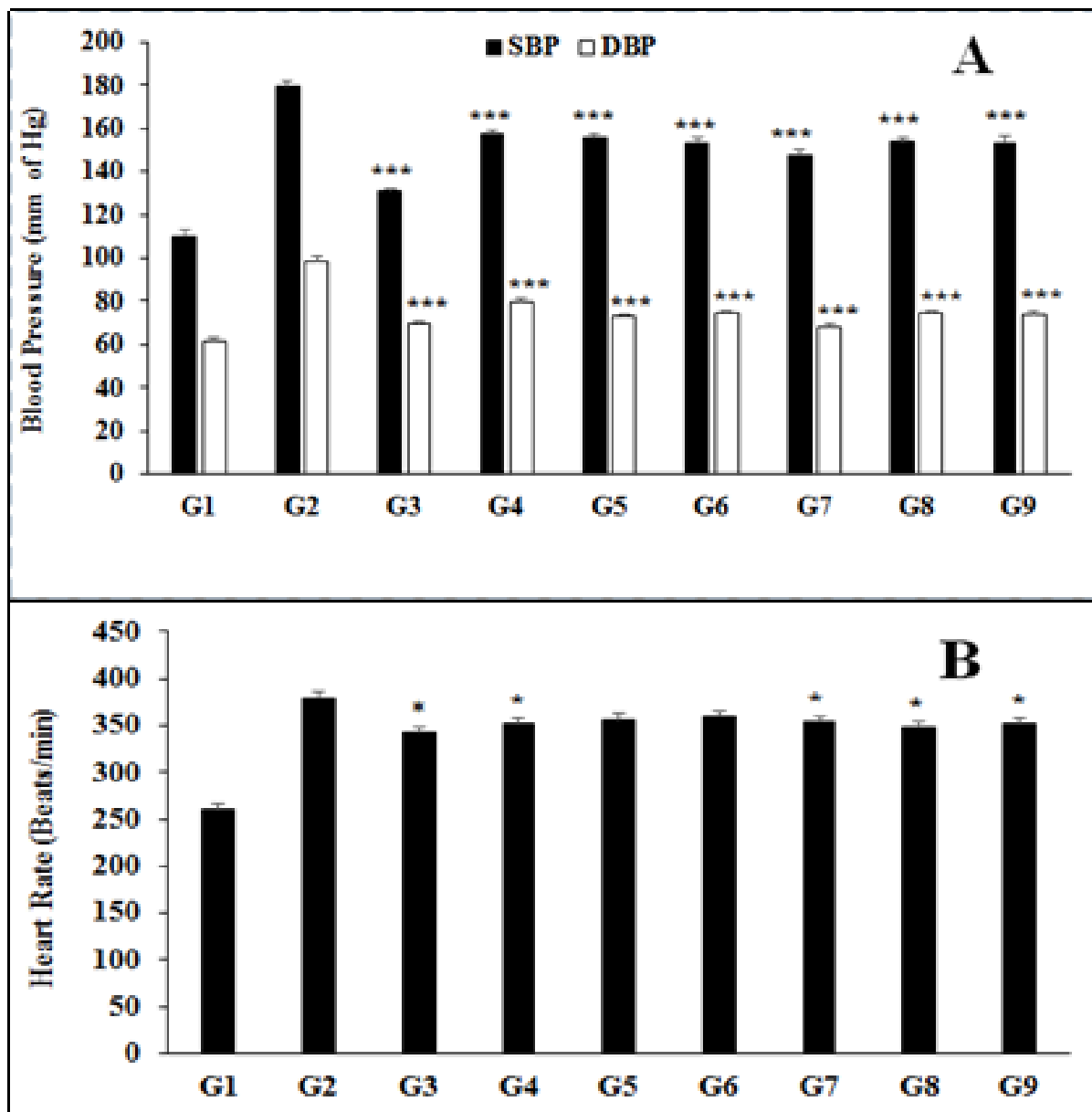


Figure 1. The effect of the test formulation on A. blood pressure (systolic and diastolic) and B. heart rate in Sprague Dawley rats. G1 as normal control (vehicle, 0.5% w/v CMC-Na); G2 as disease control (L-NAME + high fat diet (HFD) + 0.5% CMC); G3 as reference item (L-NAME + HFD + Captopril + Atorvastatin); G4 includes L-NAME + HFD along with untreated test formulation; G5 as L-NAME + HFD along with the Biofield Energy Treated test formulation; G6 group includes L-NAME + HFD along with Biofield Energy Treatment *per se* to animals from day -15; G7 as L-NAME + HFD along with the Biofield Energy Treated test formulation from day -15; G8 group includes L-NAME + HFD along with Biofield Energy Treatment *per se* plus the Biofield Energy Treated test formulation from day -15, and G9 group denoted L-NAME + HFD along with Biofield Energy Treatment *per se* animals plus the untreated test formulation. Values are presented as mean \pm SEM (n=7 to 9). * $p \leq 0.05$ and *** $p \leq 0.001$ vs. Disease control (G2).

(L-NAME + HFD + Biofield Energy Treatment *per se* plus the Biofield Energy Treated test formulation from day -15), and G9 (L-NAME + HFD along with Biofield Energy Treatment *per se* animals plus the untreated test formulation) groups, respectively, as compared to the disease control (G2) group (Figure 1A). Moreover, the level of diastolic blood pressure (DBP) was significantly ($p \leq 0.001$) reduced by 19.73%, 25.95%, 24.41%, 30.79%, 24.67%, and 25.23% in the G4, G5, G6, G7, G8, and G9 groups, respectively as compared to the G2 group. On the other hand, DBP was reduced by 7.75%, 5.83%, 13.79%, 6.16%, and 6.86% in the G5, G6, G7, G8, and G9 groups, respectively as compared to the untreated test formulation (G4) group (Figure 1A). Besides, heart rate (HR) was significantly reduced by 7.01% ($p \leq 0.05$), 6.02%, 4.86%, 6.58% ($p \leq 0.05$), 8.06% ($p \leq 0.05$), and 6.99% ($p \leq 0.05$) in the G4, G5, G6, G7, G8, and G9 groups, respectively as compared to the disease control (G2) group (Figure 1B).

*G2 as disease control (L-NAME + high fat diet (HFD) + 0.5% CMC); G3 as reference item (L-NAME + HFD + Captopril + Atorvastatin); G4 includes L-NAME + HFD along with untreated test formulation; G5 as L-NAME + HFD along with the Biofield Energy Treated test formulation; G6 group includes L-NAME + HFD along with Biofield Energy Treatment per se to animals from day -15; G7 as L-NAME + HFD along with the Biofield Energy Treated test formulation from day -15; G8 group includes L-NAME + HFD along with Biofield Energy Treatment per se plus the Biofield Energy Treated test formulation from day -15, and G9 group denoted L-NAME + HFD along with Biofield Energy Treatment per se animals plus the untreated test formulation. Values are presented as mean \pm SEM (n=7 to 9). * $p \leq 0.05$ and *** $p \leq 0.001$ vs. Disease control (G2).*

Evaluation of Haematological Parameters

The experimental results of the Biofield Energy Healing Treatment *per se* and Biofield Energy Treated test formulation on important haematology profile in different groups are summarized in Table 2. The study results suggest that Biofield Energy Treated test formulation

showed an improved animal hematology profile compared with the untreated test formulation group. The hematology parameters such as total leucocyte count (TLC) count was increased by 12.8%, 2.34%, 17.45%, and 7.38% in the G5 (L-NAME + HFD + the Biofield Energy Treated test formulation), G7 (L-NAME + HFD + the Biofield Energy Treated test formulation from day -15), G8 (L-NAME + HFD + Biofield Energy Treatment *per se* plus the Biofield Energy Treated test formulation from day -15), and G9 (L-NAME + HFD along with Biofield Energy Treatment *per se* animals plus the untreated test formulation) groups, respectively as compared with the G4 group. The level of neutrophil was increased by 11.70%, 60.11%, and 16.49% in the G5, G8, and G9 groups, respectively as compared with the G4 group. The levels of lymphocytes were increased by 11.49%, 6.42%, and 3.89% in the G5, G7, and G9 groups, respectively as compared with the G4 group. The level of monocyte was increased by 16.36%, 30.91%, and 12.73% in the G5, G8, and G9 groups, respectively, as compared with the G4 group. Similarly, level of eosinophil was reduced by 25%, 11.11%, 20%, and 15% in the G4, G6, G7, and G9 groups, respectively as compared with the G2 group.

Measurement of Glucose and Lipid Biomarkers

Lipid biomarker analysis was performed after treatment with the Biofield Energy Treated and untreated test formulations are summarized in the Table 3. The analyzed glucose and lipid biomarkers were tested such as total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL), and very low-density lipoprotein (VLDL). The experimental data showed that the level of total cholesterol was significantly decreased by 24.75% ($p \leq 0.05$), 22.64% ($p \leq 0.05$), 15.78%, 25.56% ($p \leq 0.05$), 22.56%, and 34.27% in the G4 (L-NAME + HFD along with untreated test formulation), G5 (L-NAME + HFD + the Biofield Energy Treated test formulation), G6 (L-NAME + HFD + Biofield Energy Treatment *per se* to animals from day -15), G7 (L-NAME + HFD + the Biofield Energy Treated test formulation from day -15), G8 (L-NAME + HFD + Biofield Energy Treatment *per se* plus the Biofield Energy Treated

Table 2. The effect of the Biofield Energy Treated test formulation and Biofield Energy Treatment *per se* on the level of haematological parameters in male Sprague Dawley rats.

Group (G)	TLC (X10 ³ /mm ³)	Neutrophils (X10 ³ /mm ³)	Lymphocytes (X10 ³ /mm ³)	Monocyte (X10 ³ /mm ³)	Eosinophils (X10 ³ /mm ³)
1	9.89 ± 0.44	2.07 ± 0.24	6.88 ± 0.47	0.75 ± 0.07	0.15 ± 0.01
2	10.39 ± 0.65	2.10 ± 0.12	7.36 ± 0.65	0.69 ± 0.05	0.20 ± 0.02
3	9.60 ± 0.50	1.67 ± 0.18	7.07 ± 0.48	0.71 ± 0.10	0.19 ± 0.02
4	8.54 ± 0.56	1.88 ± 0.15	5.92 ± 0.44	0.55 ± 0.08	0.15 ± 0.02
5	9.58 ± 0.89	2.10 ± 0.15	6.60 ± 0.78	0.64 ± 0.08	0.24 ± 0.04
6	8.36 ± 0.49	1.77 ± 0.23	5.84 ± 0.27	0.53 ± 0.06	0.18 ± 0.02
7	8.74 ± 0.65	1.77 ± 0.14	6.30 ± 0.55	0.49 ± 0.04	0.16 ± 0.02
8	10.03 ± 0.45	3.01 ± 0.24**	6.04 ± 0.31	0.72 ± 0.03	0.21 ± 0.02
9	9.17 ± 0.39	2.19 ± 0.21	6.15 ± 0.31	0.62 ± 0.07	0.17 ± 0.01

Values are presented as mean ± SEM (n=7 to 9). ***p*≤0.01 vs. untreated test formulation group (G4), TLC: Total leucocyte count

Table 3. Lipid profile analysis after treatment with the test formulation on male rats.

Group (G)	Total Cholesterol (mg/dL)	Triglyceride (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)	Glucose (mg/dL)
1	62.50 ± 4.38	95.09 ± 7.9	38.16 ± 3.14	7.93 ± 1.03	18.9 ± 1.64	79.71 ± 2.78
2	107.09 ± 10.25	432.89 ± 57.06	64.93 ± 7.91	8.12 ± 1.53	86.5 ± 11.39	97.91 ± 9.41
3	82.92 ± 3.68	58.35 ± 8.03	52.65 ± 2.86	11.32 ± 0.85	11.70 ± 1.63	97.94 ± 3.86
4	80.58 ± 7.30*	126.99 ± 16.29***	51.43 ± 6.14	7.20 ± 1.06	25.40 ± 3.26***	125.62 ± 3.67
5	82.84 ± 5.10*	83.11 ± 9.91***	54.20 ± 4.23	7.4 ± 0.73	16.6 ± 1.97***	119.92 ± 3.2
6	90.19 ± 6.53	72.02 ± 10.11***	57.68 ± 5.46	8.55 ± 0.84	14.44 ± 2.08***	94.10 ± 4.49
7	79.68 ± 5.08*	56.85 ± 4.95***	50.79 ± 4.01	8.04 ± 0.95	11.4 ± 0.97***	120.4 ± 5.92
8	82.93 ± 6.2	90.71 ± 12.34***	49.49 ± 4.87	7.67 ± 0.53	18 ± 2.45***	103.41 ± 6.68
9	70.39 ± 4.3	79.65 ± 10.10***	42.33 ± 3.63	6.47 ± 0.70	15.89 ± 1.98***	111.02 ± 6.05

Values are presented as mean ± SEM (n=7 to 9). **p*≤0.05 vs. G2. HDL: High density lipoprotein, LDL: Low density lipoprotein, VLDL: Very low density lipoprotein

test formulation from day -15), and G9 (L-NAME + HFD along with Biofield Energy Treatment *per se* animals plus the untreated test formulation) groups, respectively, as compared to the disease control (G2) group. Additionally, the level of total cholesterol was reduced by 12.65% in the G9 group as compared to the untreated test formulation group (G4). However, the level of triglyceride was reduced by 70.66%, 80.80%, 83.36%, 86.87%, 79.05%, and 81.60% in the G4, G5, G6, G7, G8, and G9 group, respectively as compared with the G2 group. Moreover, the level of triglyceride was significantly ($p \leq 0.001$) reduced by 34.55%, 43.29%, 55.23%, 28.57%, and 37.28% in the G5, G6, G7, G8, and G9 groups, respectively as compared to the G4 group. Similarly, LDL was reduced by 11.33%, 8.87%, 5.54%, and 20.32% in the G4, G5, G8, and G9 group, respectively as compared with the G2 group, while decreased by 10.14% in the G9 group as compared to the G4 group. VLDL level was also significantly ($p \leq 0.001$) reduced by 70.64%, 80.81%, 83.61%, 86.82%, 79.19%, and 81.63% in the G4, G5, G6, G7, G8, and G9 group, respectively as compared with the G2 group. The level of VLDL was decrease by 34.65%, 43.15%, 55.12%, 29.13%, and 37.44% in the G5, G6, G7, G8, and G9 groups, respectively as compared to the G4 group. Besides, level of good cholesterol HDL was

increased by 12.15% in the G6 group as compared to the G4 group.

Evaluation of Hepatic and Cardiac Biomarkers

The effects of the test formulation on hepatic and cardiac biomarkers are shown in Table 4. The level of obesity and coronary heart disease biomarker like atherogenic index (AI) was significantly ($p \leq 0.001$) decreased in the experiment by 64.92%, 78.36%, 83.21%, 84.68%, 74.06%, and 72.98% in the G4, G5, G6, G7, G8, and G9 groups respectively, as compared with the G2 group. Moreover, the level of AI was reduced by 38.31%, 52.11%, 56.32%, 26.05%, and 22.99% in the G5, G6, G7, G8, and G9 groups, respectively as compared to the G4 group. Similarly, the level of uric acid (UA) was significantly ($p \leq 0.001$) decreased in the experiment by 48.49%, 57.51%, 52.36%, 45.49%, 43.78%, and 40.77% in the G4, G5, G6, G7, G8, and G9 groups respectively, as compared with the G2 group. The level of SGPT was significantly ($p \leq 0.001$) decreased by 42.35%, 45.96%, 48.01%, 37.19%, 37.69%, and 42.93% in the G4, G5, G6, G7, G8, and G9 groups respectively, as compared with the G2 group. However, CK-MB level was significantly reduced by 7.97%, 10.19%, 21.97% ($p \leq 0.01$), 10.47%, 4.94%, and 16.94% in the G4, G5, G6, G7, G8, and G9 groups, respectively as compared with the G2.

Table 4. Evaluation of hepatic and cardiac biomarkers after treatment with the test formulation on male Sprague Dawley rats.

Group (G)	Albumin (g/dL)	Atherogenic Index (AI)	Uric acid (mg/dL)	SGOT (U/L)	SGPT (U/L)	Creatine Kinase-MB (U/L)
1	3.09 ± 0.00	2.57 ± 0.41	1.19 ± 0.20	208.04 ± 7.14	38.39 ± 1.62	167.78 ± 12.19
2	3.15 ± 0.03	7.44 ± 1.04	2.33 ± 0.18	219.09 ± 10.06	85.95 ± 6.01	247.75 ± 11.20
3	3.05 ± 0.03	1.11 ± 0.14	1.12 ± 0.08	219.63 ± 11.74	52.99 ± 5.94	208.88 ± 11.92
4	3.00 ± 0.03	2.61 ± 0.35***	1.20 ± 0.12***	224.72 ± 12.5	49.55 ± 2.32***	228.00 ± 16.85
5	3.03 ± 0.03	1.61 ± 0.22***	0.99 ± 0.07***	217.85 ± 11.8	46.45 ± 2.12***	222.50 ± 20.77
6	2.98 ± 0.02	1.25 ± 0.14***	1.11 ± 0.08***	201.57 ± 12.55	44.68 ± 4.11***	193.33 ± 13.59**
7	3.03 ± 0.02	1.14 ± 0.10***	1.27 ± 0.09***	243.94 ± 5.49	53.98 ± 4.52***	221.80 ± 10.43
8	3.03 ± 0.02	1.93 ± 0.25***	1.31 ± 0.10***	256.92 ± 7.24	53.55 ± 3.08***	235.50 ± 15.26
9	3.08 ± 0.04	2.01 ± 0.36***	1.38 ± 0.17***	247.67 ± 17.24	49.05 ± 4.24***	205.78 ± 26.11

Values are presented as mean ± SEM (n=7 to 9). ** $p \leq 0.01$ and *** $p \leq 0.001$ vs. G2 group. SGPT: Serum glutamate pyruvate transaminases, SGOT: Serum glutamic oxaloacetic transaminase.

Table 5. The effect of the test formulation on organ weight parameters of male Sprague Dawley rats.

Relative weight (%)	G1	G2	G3	G4	G5	G6	G7	G8	G9
Liver	3.01 ± 0.08	3.04 ± 0.14	2.84 ± 0.11	3.13 ± 0.10	3.02 ± 0.10	2.67 ± 0.11	3.19 ± 0.10	3.05 ± 0.10	2.83 ± 0.14
Lungs	0.42 ± 0.02	0.40 ± 0.02	0.42 ± 0.02	0.40 ± 0.03	0.41 ± 0.03	0.39 ± 0.03	0.37 ± 0.02	0.41 ± 0.01	0.42 ± 0.03
Kidney	0.65 ± 0.02	0.56 ± 0.02	0.67 ± 0.07	0.63 ± 0.03	0.63 ± 0.03	0.58 ± 0.03	0.71 ± 0.03	0.68 ± 0.02	0.65 ± 0.03
Heart	0.28 ± 0.01	0.26 ± 0.01	0.26 ± 0.01	0.27 ± 0.01	0.27 ± 0.02	0.26 ± 0.01	0.27 ± 0.01	0.26 ± 0.01	0.31 ± 0.01
Brain	0.41 ± 0.01	0.31 ± 0.01	0.35 ± 0.01	0.34 ± 0.03	0.34 ± 0.01	0.34 ± 0.01	0.34 ± 0.01	0.32 ± 0.01	0.36 ± 0.01
Spleen	0.15 ± 0.01	0.13 ± 0.01	0.12 ± 0.00	0.12 ± 0.01	0.12 ± 0.00	0.11 ± 0.00	0.13 ± 0.00	0.13 ± 0.00	0.12 ± 0.01
Pancreas	0.39 ± 0.03	0.26 ± 0.03	0.32 ± 0.03	0.34 ± 0.03	0.29 ± 0.03	0.24 ± 0.03	0.30 ± 0.03	0.24 ± 0.02	0.23 ± 0.03
Thymus	0.09 ± 0.01	0.08 ± 0.00	0.07 ± 0.00	0.08 ± 0.01	0.09 ± 0.01	0.10 ± 0.01	0.09 ± 0.01	0.09 ± 0.00	0.08 ± 0.00
Adrenal Gland	0.02 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00
Eye	0.06 ± 0.00	0.05 ± 0.00	0.06 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.05 ± 0.00	0.06 ± 0.00
Testis	0.66 ± 0.02	0.5 ± 0.01	0.6 ± 0.02	0.54 ± 0.02	0.50 ± 0.03	0.53 ± 0.02	0.56 ± 0.02	0.54 ± 0.02	0.56 ± 0.03
Prostate	0.18 ± 0.01	0.21 ± 0.02	0.2 ± 0.02	0.22 ± 0.02	0.20 ± 0.02	0.23 ± 0.01	0.19 ± 0.01	0.18 ± 0.01	0.17 ± 0.01
Epididymis	0.33 ± 0.02	0.25 ± 0.15	0.28 ± 0.02	0.25 ± 0.01	0.24 ± 0.01	0.30 ± 0.02	0.27 ± 0.01	0.25 ± 0.02	0.26 ± 0.01
Inguinal fat	2.36 ± 0.14	4.93 ± 0.15	3.08 ± 0.31	4.45 ± 0.49	4.13 ± 0.23	4.08 ± 0.19	3.65 ± 0.45	4.99 ± 0.72	3.87 ± 0.38
Epidymal fat	1.48 ± 0.12	2.84 ± 0.11	2.45 ± 0.24	2.26 ± 0.02	2.27 ± 0.21	2.79 ± 0.09	2.43 ± 0.17	2.82 ± 0.16	2.71 ± 0.18
Vas deferens	0.09 ± 0.01	0.07 ± 0.00	0.09 ± 0.01	0.09 ± 0.01	0.08 ± 0.00	0.09 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.07 ± 0.01

Values are presented as mean ± SEM (n=7 to 9).

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

Animal weight parameters, feed intake, and relative organ weight parameters were studied, and it was found that change in animal weight, daily feed intake, and organ weight were found to be normal and no significant changes were observed throughout the experimental period. 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. The results of animal tested organ weight parameters with respect to body weight are summarized in the Table 5. Thus, the relative organ weight parameters as summarized did not found with any significant change in the organ weight throughout the experiment suggested that the test formulation was found to be safe for the treatment. Overall, the animal weight data, relative organ weight, and feed intake data suggested no significant changes were reported as compared with untreated and disease control group (G2), it suggests that Biofield Energy Treated test formulation and Biofield Energy Treatment *per se* were found safe in all the tested animals.

Discussion

Biofield Energy Treatment/Blessing significantly reduced the elevated blood pressure (SBP, DBP) and HR to some extent as compared to disease control group. Mr. Trivedi's Biofield Energy improved the major immune blood markers. T and B cells are the major cells of lymphocytes that functions to eliminate the antigen, either by releasing the antibodies (B cells), cytotoxic granules or directly by signalling to other immune cells. Several growth factors are regulated in response to any infections, which was governed by the immune system [32]. Similarly, reduced level of neutrophils and monocytes can be correlated in presence of many chronic inflammatory diseases such as gout, rheumatoid arthritis, rheumatic fever, etc., which can be increased by any form of chronic stress factors. Overall, the Biofield Energy Treated test formulation significantly improved the concentrations of TLC, lymphocytes, and neutrophils

in hematology profile assay, which suggest that the Trivedi Effect® has the capacity to improve the immunomodulatory potential of the test formulation.

The test formulation components such as vitamins, minerals, and important extracts have been reported with significant improved lipid profile, serum cholesterol, LDL, HDL, etc. [33-35]. Overall, the Biofield Energy Healing Treatment *per se* and Biofield Energy Treated test formulation has significantly improved the lipid profile, which might be helpful in the cardiovascular disorders. The test formulation components have been reported to have significant action on hepatic enzymes [36-38], thus the test formulation have showed improved hepatic and cardiac biomarkers after treatment with the Biofield Energy Treatment *per se* as preventive maintenance. Therefore, it can be concluded that the Trivedi Effect®-Biofield Energy Healing can be used to improve the immunity profile by improving hepatic and cardiac enzymes and could be advantageous for the management of cardiovascular disorders.

In this research plan, four groups were considered as preventive maintenance groups. These groups were G6 (Biofield Energy Treatment *per se* to animals at -15 days), G7 (Biofield Energy Treated test formulation from day -15), G8 (Biofield Energy Treatment *per se* to animals along with Biofield Treated test formulation from day -15), and G9 (Biofield treatment *per se* at -15 days to animals with untreated test formulation). The results showed the significant slowdown of the disease progression, cardiovascular disease-related all other symptoms/complications and also reduced the chances of disease susceptibility in these groups. Based on the overall data, it suggests that the Biofield Energy Healing Therapy was found to be most effective and benefited in order to prevent and protect from the occurrence of any type of diseases in rat model. It indicated that this therapy can act as a preventive maintenance therapy to prevent the occurrence of the disease, slowdown the disease progression and disease-related complications of the existing ailments that will ultimately improve the overall

health and quality of life in human.

Conclusions

Based on the experimental findings it was stated that SBP was significantly decreased by 13.39% to 17.74% in the treatment groups than disease control (G2) group. Similarly, DBP was also significantly reduced by 24.41% to 30.79% in treatment groups than G2. The levels of TLC count and neutrophils were increased by 17.45% and 60.11%, respectively in the G8 groups, respectively than G2. Lipid parameters were significantly reduced such as total cholesterol (upto 34%), triglyceride (upto 55%), and VLDL (upto 86%) than G2 group. Atherogenic index (AI) was significantly decreased (upto 84%) than G2 group. Besides, uric acid (UA), SGPT, and CK-MB were significantly decreased upto 57.51%, 48.01%, and 21.97%, respectively in different treatment groups than G2. Altogether, the Biofield Energy Treated test formulation and Biofield Energy Healing Treatment (the Trivedi Effect®) *per se* showed significant results with respect to different parameters related to heart in the preventive maintenance group *per se* (G6), as well as other preventive maintenance groups (G7, G8, and G9) in L-NAME and High Fat Diet-Induced cardiovascular disorders rat model study. It also helped to slowdown the cardiovascular disease progression and disease-related complications of the overall animal's health. These data suggested that Biofield Energy Treatment *per se* and/or Biofield Energy Treated Test formulation in combination would be the best treatment strategies in order to prevent and protect from the occurrence of any type of diseases. Therefore, the Biofield Energy Treatment might act as a preventive maintenance therapy to maintain good health, or full restoration of health or improve the overall health and quality of life in human.

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Abbreviation

L-NAME: N^G-nitro-L-arginine methyl ester hydrochloride, HFD: High fat diet, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, HR: Heart rate, HDL: High density lipoprotein, LDL: Low density lipoprotein, VLDL: Very low density lipoprotein, AI: Atherogenic index, UA: Uric acid, SGPT: Serum glutamate pyruvate transaminases, SGOT: Serum glutamic oxaloacetic transaminase, ALP: Alkaline phosphatase, CK-MB: Creatine kinase myocardial band, CVDs: Cardiovascular diseases, TG: Triglycerides, TC: Total cholesterol, CAM: Complementary and alternative medicine, NCCAM: National center for complementary/alternative medicine, NCCIH: National centre of complementary and integrative health, CMC: Carboxy methyl cellulose, TLC: Total leucocyte count

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