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Investigation of Cardiac Biomarkers in Aorta 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

The aim of this study was to evaluate the effect of Biofield Energy Treated/Blessed Proprietary Test Formulation and Biofield Energy Treatment/Blessing *per se* on cardiac biomarkers in aorta homogenate on L-NAME and high fat diet (HFD)-induced cardiovascular disorders in Sprague Dawley rats. The functional aortic biomarkers such as inducible nitric oxide synthase (iNOS), angiotensin-II, C-reactive protein (CRP), cholesterol, troponin-1, and Na^+/K^+-ATPase were measured using standard ELISA assay. A test formulation was formulated including minerals (magnesium, zinc, copper, calcium, selenium, and iron), vitamins (ascorbic acid, pyridoxine HCl, vitamin B_9, cyanocobalamin, and cholecalciferol), *Panax ginseng* extract, β-carotene, and cannabidiol isolate. In this experiment, nine groups were assigned, in which four were preventive maintenance groups. The ingredients were used in this formulation divided into two parts; one was defined as the unblessed test formulation, while the other portion of the test formulation received Biofield Energy Healing Treatment/Blessing remotely for about 3 minutes by a renowned Biofield Energy Healer, Mr. Mahendra Kumar Trivedi. Among nine groups, three groups of animals were also received Blessing *per se* (at day -15). The results showed that the level of iNOS was significantly ($p \leq 0.001$) reduced by 32.4%, 27.8%, and 19.8% in the G5 (L-NAME + HFD + the Biofield Energy Treated test formulation), G6 (L-NAME + HFD + Biofield Energy Treatment *per se* to animals from day -15), and G7 (L-NAME + HFD + the Biofield Energy Treated test formulation from day -15) groups, respectively as compared to the disease control group (G2). Moreover, the level of angiotensin-II was significantly decreased by 33.6%, 47.2% ($p \leq 0.001$), 27.9%, 25.4%, and 22.3% in the G5, G6, G7, G8 (L-NAME + HFD + Biofield Energy Treatment *per se* plus the Biofield Energy Treated test formulation from day -15), and G9 (L-NAME + HFD + Biofield Energy Treatment *per se* animals plus the untreated test formulation) groups, respectively, as compared to the G2 group. The level of CRP was decreased by 26.1%, 25.7%, 15.8%, and 16.6% in the G5, G6, G7, and G9 groups, respectively as compared to the G2 group. Besides, the level of cholesterol was significantly ($p \leq 0.001$) decreased by 62.4%, 54.5%, 52%, 46.2%, and 64.1% in the G5, G6, G7, G8, and G9 groups, respectively as compared to the G2 group. The level of troponin-1 was significantly ($p \leq 0.001$) decreased by 42.8%, 42.5%, 36.7%, 28.9%, and 21.2% in the G5, G6, G7, G8, and G9 groups, respectively as compared to the G2 group. The level of Na^+/K^+-ATPase was decreased

by 14.8% in the G8 group as compared to the G4 group. Overall, the data suggested significance improvement of vital functional aortic biomarkers of the Biofield Energy Treated test formulation and Biofield Energy Treatment *per se* along with preventive measure on the animal with respect to various pathological conditions that might be beneficial various types of cardiovascular disorders. Therefore, the results showed the significant slowdown the cardiovascular diseases and its complications in the preventive Biofield Energy Treatment group *per se* and/or Biofield Energy Treated/Blessed Test formulation groups (*viz*. G6, G7, G8, and G9) as compared to the disease control group.

Keywords: Biofield Treatment; The Trivedi Effect®; High Fat Diet; Cardiovascular Disorders; iNOS; Angiotensin-II; Aorta; C-reactive protein; Troponin-I; Na^+/K^+-ATPase

Introduction

Cardiovascular diseases (CVDs) are the leading cause of death in adult population in the world. Nitric oxide (NO) is produced in almost all tissues and organs by 3 distinct NO synthase (NOS) isoforms (neuronal, inducible, and endothelial NOS), all the enzymes are expressed in the human cardiovascular system [1]. Abnormal generation of NO is considered as a major cause of coronary heart disease (CHD). It has been shown that endothelial dysfunction is characterized by reduced endothelial NO synthesis by constitutive NOS (cNOS) and increased systemic NO synthesis due to increased iNOS activity can leads to cardiovascular disorders [2]. Angiotensin II is considered one of the important mediator of the renin-angiotensin system (RAS). It has been reported that angiotensin-II plays a vital role for the pathophysiology of cardiovascular disorders such as hypertension, atherosclerosis, coronary heart disease, restenosis, and heart failure through the RAS [3,4]. C-reactive protein (CRP) seems to predict the risk of cardiovascular problems as well as cholesterol levels. A recent study reported that elevated levels of CRP is associated with three-times more risk of heart attack. CRP is one of the best possible marker of vascular inflammation and plays a vital role in promoting vascular inflammation, vessel damage and clinical cardiovascular disease [5,6]. 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 [7]. Cardiac troponins are considered the "gold standard" for diagnosing of myocardial damage in patients with chest pain [8]. There is great interest the use of high-sensitivity cardiac troponins for the development of CVDs and heart failure screening [9]. Based on the literatures reported that the concentration of Na^+/K^+-ATPase has been reduced by 40% in the heart failure patients [10]. Thus, in order to study the change in vital functional kidney biomarker 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 B_9, and cholecalciferol), and nutraceuticals (β-carotene, Ginseng, cannabidiol isolate (CBD)). All the minerals and vitamins used in the test formulation have significant functional role to provide vital 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 the one of the best immune booster for overall immunity [16]. The present study was aimed to evaluate the vital functional cardiac biomarker 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 materials science [23,24], agriculture science [25], antiaging [26], Gut health [27], nutraceuticals [28], pharmaceuticals [29], cardiac health [30], overall human health and wellness. In this study, the authors want to study the impact of the Biofield Energy Treatment (the Trivedi Effect®) on the given novel test formulation and Biofield Energy Treatment *per se* to the animals on

vital functional cardiac biomarkers on aorta in presence of L-NAME and High Fat Diet-Induced Cardiovascular Disorders in Sprague Dawley Rats using standard ELISA assay.

Material and Methods

Chemicals and Reagents

Atorvastatin, pyridoxine hydrochloride (vitamin B_6), zinc chloride, magnesium (II) gluconate, and β-carotene (retinol, provit A) were purchased from TCI, Japan. Copper chloride, cyanocobalamin (vitamin B_{12}), calcium chloride, vitamin E (Alpha-Tocopherol), cholecalciferol (vitamin D_3), 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. For the estimation of aortic biomarker panels specific ELISA kits were used such as for detection of inducible nitric oxide synthase (iNOS; CSB-E08325r), angiotensin-II (CSB-E04494r), C-reactive protein (CRP; CSB-E07922r), cholesterol, troponin-1 (CSB-E08594r), and Na^+/K^+-ATPase (CSB-EL002322RA) were procured from CUSABIO, 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 B_6, vitamin B_{12}, vitamin D_3, vitamin B_9, 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/ Blessing (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 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/Blessing. The Biofield Energy Treated 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 groups were divided as G1 as normal control (vehicle, 0.5% w/v CMC-Na); G2 as disease control (L-NAME + 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. The normal control animals' group (G1) was received 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), the animals were sacrifice, remove aorta, homogenate and subjected for the estimation of iNOS, angiotensin-II, CRP, cholesterol, troponin-1, and Na^+/K^+-ATPase.

Estimation of Different Biomarkers in Aorta Homogenate

The aorta homogenate from all the groups was subjected for the estimation of level of various vital biomarkers such as iNOS, angiotensin-II, CRP, cholesterol, troponin-1, and Na^+/K^+-ATPase. All the biomarker panel was estimation

using ELISA method as per manufacturer's recommended standard procedure. This was a quantitative method, and the principle was based on the binding of antigen and antibody in sandwich manner assay.

Statistical Analysis

The data were represented as mean ± 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 and Discussion

Estimation of Inos Aorta Homogenate

The effect of the test formulation and Biofield Energy Treatment *per se* on the expression of induced nitric oxide synthase (iNOS) is shown in Figure 1. The disease control (L-NAME + high fat diet (HFD) + 0.5% CMC) group (G2) showed value of iNOS as 16.96 ± 0.67 IU/mL, which was increased by 175.1% as compared with the normal control (G1, 6.16 ± 0.52 IU/mL). However, positive control (captopril + atorvastatin) treatment group (G3) showed decreased iNOS level by 38.4% *i.e.* 10.45 ± 1.01 IU/mL as compared to the G2 group. The expression of iNOS in aorta was significantly decreased by 14.3%, 32.4% ($p \leq 0.001$), 27.8% ($p \leq 0.001$), 19.8% ($p \leq 0.001$), 2.7%, and 7.7% in the G4 (L-NAME + HFD + 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 test formulation from day -15), and G9 (L-NAME + HFD + Biofield Energy Treatment *per se* animals plus the untreated test formulation) groups, respectively, as compared to the disease control group (G2). Further, the level of iNOS was reduced by 21.1%, 15.8%, and 6.5% in the G5, G6 and G7 groups, respectively as compared to the untreated test formulation (G4) group (Figure 1). Nitric oxide (NO) is the key endothelium-derived relaxing factor that maintain the vascular tone and reactivity. More generation of NO by the stimulation of iNOS have been proposed as a major mechanism of endothelial dysfunction, and that causes cardiovascular abnormalities [31,32]. Besides, iNOS is expressed due to the effects of proinflammatory cytokines and can release more NO than other isoform of nitric oxide synthase enzymes [2]. Overall, in this study the Biofield Energy Treated test formulation and Biofield Energy Treatment *per se* significantly reduced the level of iNOS, which was increased due to cardiovascular disease condition, induced by L-NAME and HFD, which could be beneficial in the cardiovascular patients.

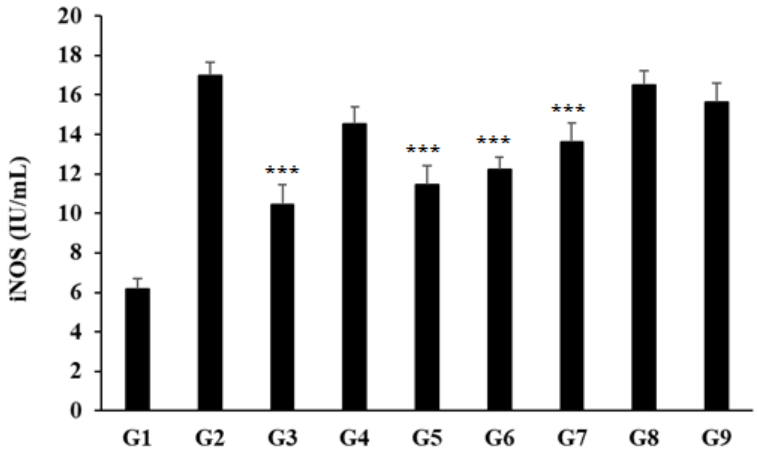

Figure 1: The effect of the test formulation on the level of inducible nitric oxide synthase (iNOS) on aorta homogenate in Sprague Dawley rats. G1 as normal control (vehicle, 0.5% w/v CMC-Na); G2 as disease control (L-NAME + high fat diet (HFD) + 0.5% CMC); G3 as reference item (L-NAME + HFD + Captopril + 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 ± SEM (n=7 to 9). ***$p \leq 0.001$ *vs.* Disease control (G2).

Estimation of Angiotensin-II in Aorta Homogenate

The level of angiotensin-II in aorta homogenate was measured and the data are shown in Figure 2. The disease control (L-NAME + high fat diet, HFD + 0.5% CMC) group (G2) showed the expression of angiotensin-II as 112.89 ± 11.20 pg/mL, which was increased by 54.9% as compared with the normal control (G1, 72.88 ± 11.80 pg/mL) group. While, in the positive control (captopril + atorvastatin) treatment (G3) the level of angiotensin-II was decreased by 48% *i.e.,* 58.75 ± 11.77 pg/mL. The level of angiotensin-II was significantly decreased by 9.5%, 33.6%, 47.2% ($p \leq 0.001$), 27.9%, 25.4%, and 22.3% in the G4 (L-NAME + HFD + 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 test formulation from day -15), and G9 (L-NAME + HFD + Biofield Energy Treatment *per se* animals plus the untreated test formulation) groups, respectively, as compared to the disease control group (G2). Moreover, the level of angiotensin-II was reduced by 26.7%, 41.6%, 20.3%, 17.5%, and 14.2% in the G5, G6, G7, G8, and G9 groups, respectively as compared to the untreated test formulation (G4) group (Figure 2). Based on the various research outcomes, it has been reported that angiotensin-II plays a vital role for the pathophysiology of cardiovascular disorders through the renin-angiotensin system (RAS) [3,4]. Overall, here the Biofield Energy Treated test formulation and Biofield Energy Treatment *per se* significantly reduced the level of angiotensin-II, which could be beneficial in the cardiovascular symptoms.

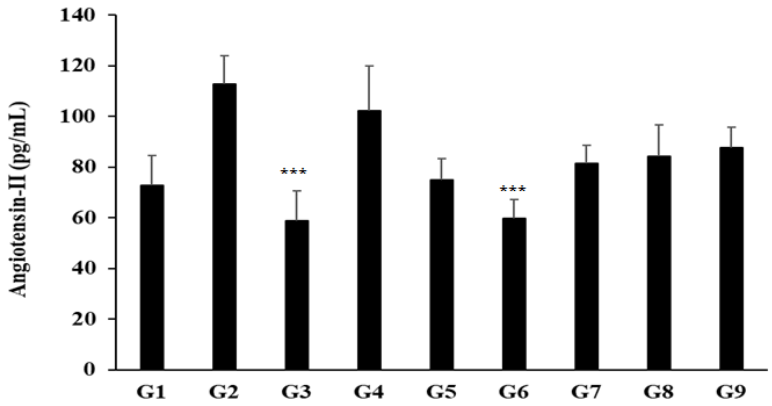

Figure 2: The effect of the test formulation on the level of angiotensin-II on aorta homogenate in Sprague Dawley rats. Values are presented as mean ± SEM (n=7 to 9). ***$p \leq 0.001$ *vs*. Disease control (G2).

Estimation of C - Reactive Protein (CRP) on Aorta Homogenate

The effect of the test formulation and Biofield Energy Treatment *per se* on the level of C-reactive protein (CRP) in aorta and the results are shown in Figure 3. The disease control (L-NAME + high fat diet, HFD + 0.5% CMC) group (G2) showed value of CRP as 1812.15 ± 245.72 ng/mL, which was increased by 196.2% as compared with the normal control (G1, 611.86 ± 93.97 ng/mL). Further, the positive control (captopril + atorvastatin) treatment (G3) showed significant ($p \leq 0.01$) decreased the level of kidney CRP by 39% *i.e.*, 1105.24 ± 95.14 ng/mL as compared to the G2 group. The level of CRP was decreased by 13.2%, 26.1%, 25.7%, 15.8%, 6.5%, and 16.6% in the G4 (L-NAME + HFD + 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 test formulation from day -15), and G9 (L-NAME + HFD + Biofield Energy Treatment *per se* animals plus the untreated test formulation) groups, respectively, as compared to the disease control group (G2). Similarly, CRP level was decreased by 14.9%, 14.4%, 3%, and 3.9% in the G7, G8, and G9 groups, respectively as compared to the untreated test formulation (G4) group (Figure 3). Inflammation plays a major role in the pathogenesis of cardiovascular disease [33]. In this context, CRP is playing an independent risk factor for cardiovascular patients and one of the best biomarker for detection of immune function alterations [34,35]. Therefore, in this experiment the Biofield Energy Treated test formulation and Biofield Energy Treatment *per se* reduced the level of CRP, which could be beneficial to improve the cardiovascular disease conditions.

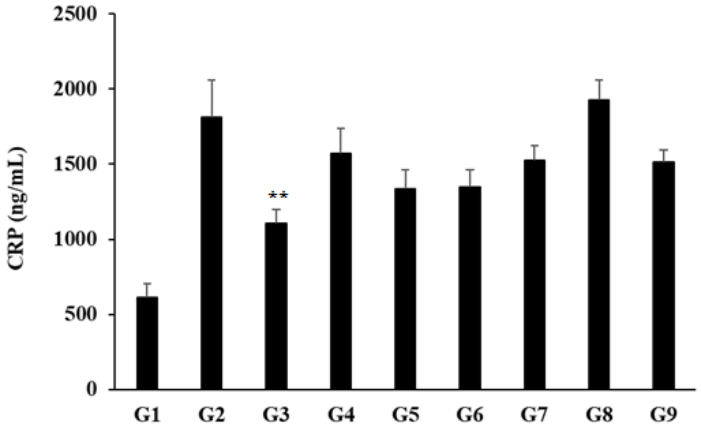

Figure 3: The effect of the test formulation on the level of aorta C-reactive protein (CRP) in Sprague Dawley rats. Values are presented as mean ± SEM (n=7 to 9). ***p*≤0.01 *vs.* Disease control (G2).

Estimation of Cholesterol in Aorta Tissue

The effect of the test formulation and Biofield Energy Treatment *per se* on the level of cholesterol in aorta tissue and the results are shown in Figure 4. The level of cholesterol in the disease control (L-NAME + high fat diet, HFD + 0.5% CMC) group (G2) was 23.49 ± 1.61 mU/mL, which was increased by 151.7% as compared with the normal control (G1, 9.33 ± 1.05 mU/mL). Further, the positive control (captopril + atorvastatin) treatment (G3) showed decreased level of cholesterol in aorta tissue by 76.7%, i.e., 5.47 ± 0.68 mU/mL as compared with the G2. The level of cholesterol was significantly (*p*≤0.001) decreased by 35.1%, 62.4%, 54.5%, 52%, 46.2%, and 64.1% in the G4 (L-NAME + HFD + 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 test formulation from day -15), and G9 (L-NAME + HFD + Biofield Energy Treatment *per se* animals plus the untreated test formulation) groups, respectively, as compared to the disease control group (G2). Similarly, CRP level was decreased by 42%, 29.9%, 26%, 17.1%, and 44.8% in the G5, G6, G7, G8, and G9 groups, respectively as compared to the untreated test formulation (G4) group (Figure 4). Cholesterol is a biological molecule essential component for cell membrane and function and synthesis of hormone and vitamin in mammals. Increased level of cholesterol leads to cardiovascular disorders like atherosclerosis [36]. Overall, in this experiment the Biofield Energy Treated test formulation and Biofield Energy Treatment *per se* significantly reduced the level of cholesterol, which could reduce the risks of cardiovascular risks.

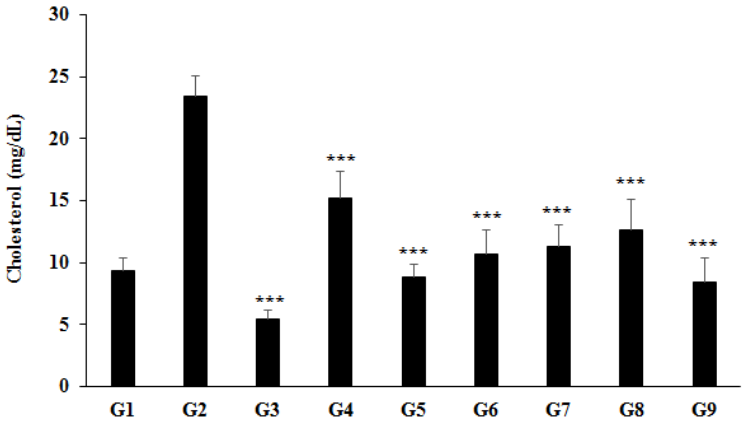

Figure 4: The effect of the test formulation on the level of cholesterol on aorta homogenate in Sprague Dawley rats. Values are presented as mean ± SEM (n=7 to 9). ****p*≤0.001 *vs.* Disease control (G2).

Estimation of In Troponin-1 Aorta Tissue

The effect of the test formulation and Biofield Energy Treatment *per se* on the level of troponin-1 in aorta tissue and the results are shown in Figure 5. The level of troponin-1 in the disease control (L-NAME + high fat diet, HFD + 0.5% CMC) group (G2) was 809.76 ± 75.78 mU/mL, which was increased by 83.2% as compared with the normal control (G1, 441.99 ± 96.08 mU/mL). Further, the positive control (captopril + atorvastatin) treatment (G3) showed decreased level of troponin-1 in aorta tissue by 50.7%, *i.e.,* 399.08 ± 53.50 mU/mL as compared with the G2. The level of troponin-1 was decreased by 33.9%, 42.8%, 42.5%, 36.7%, 28.9%, and 21.2% in the G4 (L-NAME + HFD + 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 test formulation from day -15), and G9 (L-NAME + HFD + Biofield Energy Treatment *per se* animals plus the untreated test formulation) groups, respectively, as compared to the disease control group (G2). Similarly, troponin-1 level was decreased by 13.5%, 13% and 4.2% in the G5, G6, and G7 groups, respectively as compared to the untreated test formulation (G4) group (Figure 5). Cardiac troponins are biomarkers mainly used to diagnose acute myocardial injury and cardiac infarction [37]. High level of troponins indicates acute myocardial infarction [38], coronary artery stenosis, microvascular lesions, silent plaque, rupture or subclinical myocardial fibrosis, and necrosis [39]. According to McLaurin et al. reported that some patients with chronic kidney disease have also high levels of troponin in the blood. Cardiac troponin I and creatine kinase-MB mass to rule out myocardial injury in hospitalized patients with renal insufficiency [40]. Overall, in this experiment the Biofield Energy Treated test formulation and Biofield Energy Treatment *per se* significantly reduced the level of troponin in aortic tissues, which could reduce the risks of cardiovascular diseases.

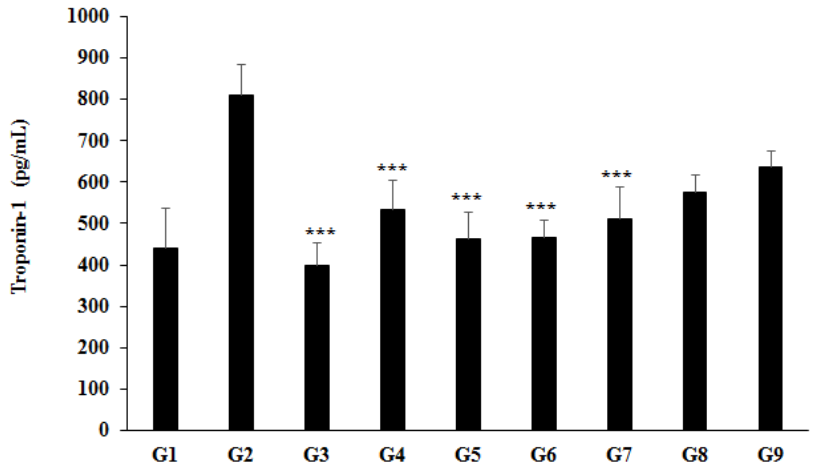

Figure 5: The effect of the test formulation on the level of cholesterol on aorta homogenate in Sprague Dawley rats. Values are presented as mean ± SEM (n=7 to 9). ***$p \leq 0.001$ *vs.* Disease control (G2).

Estimation of in Na^+/K^+-Atpase Aorta Tissue

The effect of the test formulation and Biofield Energy Treatment *per se* on the level of Na^+/K^+-ATPase in aorta tissue and the results are shown in Figure 6. The level of Na^+/K^+-ATPase in the disease control (L-NAME + high fat diet, HFD + 0.5% CMC) group (G2) was 447.61 ± 32.67 pg/mL, which was decreased by 28.3% as compared with the normal control (G1, 624.42 ± 84.56 pg/mL). Further, the positive control (captopril + atorvastatin) treatment (G3) showed level of Na^+/K^+-ATPase in aorta tissue as 419.56 ± 56.20 pg/mL. The level of Na^+/K^+-ATPase was increased by 4.7%, 7.7%, 14.8% and 3% 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 + Biofield Energy Treatment *per se* animals plus the untreated test formulation) groups, respectively, as compared to the untreated test formulation (G4) group (Figure 6). Based on the literature data, suggest that the level of Na^+/K^+-ATPase has been decreased in the heart failure patients, and simultaneously decrease the function of heart [41]. Overall, in this experiment the Biofield Energy

Treated test formulation and Biofield Energy Treatment *per se* significantly increased the level of Na^+/K^+-ATPase, which could reduce the risks of cardiovascular diseases.

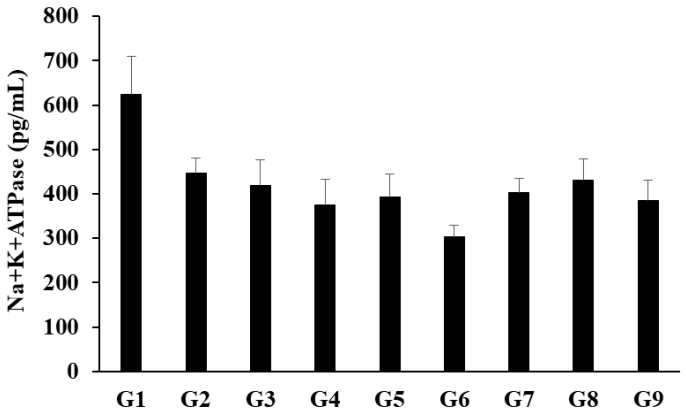

Figure 6: The effect of the test formulation on the level of Na^+/K^+-ATPase on aorta homogenate in Sprague Dawley rats. Values are presented as mean ± SEM (n=7 to 9).

This experimental, four preventive maintenance groups were used. These groups were G6, G7, G8, and G9. Results showed the significant slowdown of cardiovascular-related symptoms/complications and reduced the chances of disease susceptibility. Based on the findings, it suggests that the Biofield Energy Healing Therapy/Blessing was found to be most effective and benefited to prevent and protect from the occurrence of any type of diseases and that will ultimately improve the overall health and quality of life in human.

Conclusions

The level of iNOS was decreased by 32.4%, 27.8%, and 19.8% in the 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), groups, respectively as compared to the disease control group (G2). However, the level of angiotensin-II was significantly reduced by 33.6%, 47.2%, 27.9%, 25.4%, and 22.3% in the G5, G6, G7, G8, and G9 groups, respectively, as compared to the disease control group (G2). Additionally, the level of CRP was decreased by 26.1%, 25.7%, 15.8%, and 16.6% in the G5, G6, G7, G8, and G9 groups, respectively, than G2 group. On the other hand, estimation of cholesterol data showed that the level was decreased by 62.4%, 54.5%, 52%, 46.2%, and 64.1% in the G5, G6, G7, G8, and G9 groups, respectively than G2 group. The level of troponin-1 was decreased by 42.8%, 42.5%, 36.7%, 28.9%, and 21.2% in the G5, G6, G7, G8, and G9 groups, respectively as compared to the G2 group. Further, the level of Na^+/K^+-ATPase was decreased by 14.8% in the G8 group as compared to the G4 group. Altogether, the Biofield Energy Treated test formulation and Biofield Energy Healing Treatment (the Trivedi Effect®) *per se* showed significant results with respect to different aortic biomarkers 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-related complications of the overall animal's health. These data suggested that Biofield Energy Treatment *per se* and/or Biofield Energy Treated/Blessed Test formulation in combination would be the best treatment strategies to prevent and protect from the occurrence of any type of diseases. Thus, the Biofield Energy Treatment/ Blessing might act as a preventive maintenance therapy to maintain the overall health and quality of life in human. This therapy might also reduce the severity of various type of acute/chronic diseases like auto-immune, inflammatory, and many thyroid disorders. Overall, the data suggested the Biofield Energy Treated/Blessed test formulation and Biofield Energy Treatment *per se* in showed significant action on thyroid gland with respect to biomarkers, as a CAM. This test formulation also can be used against fibromyalgia, Addison disease, multiple sclerosis, myasthenia gravis, rheumatoid arthritis, aplastic anaemia, Crohn's disease, psoriasis, chronic fatigue syndrome, vitiligo, and alopecia areata, dermatitis, ulcerative colitis, hepatitis, mental disorders, diverticulitis, Parkinson's, and stroke in the improvement of overall health and quality of life.

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