



Evaluation of Inflammatory Serum Biomarkers after Treatment with the Consciousness Energy Healing-Based 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 experiment was to evaluate the anti-inflammatory potential of Biofield Energy Treated/Blessed Proprietary Test Formulation and Biofield Energy Treatment *per se* to the animals on L-NAME and high fat diet (HFD)-induced cardiovascular disorders in Sprague Dawley rats using serum biomarkers. In this experiment various proinflammatory cytokines such as TNF- α , IL-6, IL-18, and IL-1 β , along with C - reactive protein (CRP) using ELISA assay. A test formulation was formulated including minerals (magnesium, zinc, copper, calcium, selenium, and iron), vitamins (ascorbic acid, pyridoxine HCl, vitamin B₉, vitamin B₁₂, and vitamin D₃), cannabidiol (CBD) isolate, Panax ginseng extract, and β -carotene. The constituents of the test formulation were divided into two parts; one part was denoted as the untreated, while the other part and three groups of animals received Biofield Energy Healing Treatment/Blessing remotely for about 3 minutes by a renowned Spiritual Energy Healer, Mr. Mahendra Kumar Trivedi. The cytokines estimation results showed that the level of tumour necrosis factor-alpha (TNF- α) was reduced by 12.72% in the G7 (L-NAME + HFD + the Biofield Energy Treated test formulation from day -15) group as compared to the disease control (L-NAME + HFD + 0.5% CMC) group (G2). Moreover, the level of interleukin-6 (IL-6) was reduced by 31.75%, 15.96%, 24.18%, and 24.11% 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), 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. Additionally, the level of interleukin-18 (IL-18) was decreased minimally in the treatment group as compared to the G2 group. The level of IL-1 β was decreased by 7.93%, 8.35%, 7.93%, 6.68%, and 6.68% in the G5, G6, G7, G8, and G9 groups, respectively as compared to the untreated test formulation (G4). Besides, the level of CRP was decreased by 33.25%, 25.78%, 15.63%, 42.12%, and 35.40% in the G5, G6, G7, G8, and G9 groups, respectively as compared to the G2 group. Overall, the data suggested significance anti-inflammatory potentials of the Biofield Energy Treated/Blessed test formulation and Biofield Energy Treatment *per se* along with preventive measure on the animal with respect to various inflammatory conditions that might be beneficial various types of cardiovascular disorders. Therefore, the results showed the significant slowdown the inflammation-related cardiovascular disease progression and its complications in the preventive treatment groups (*viz.* G6, G7, G8, and G9).

Keywords: Biofield Treatment; Inflammatory Biomarkers; Cytokines; The Trivedi Effect[®]; ELISA; High Fat Diet; Cardiovascular Disorders

Abbreviations: HFD: High Fat Diet; CRP: C - reactive protein; CBD: Cannabidiol; TNF- α : Tumour Necrosis Factor-Alpha; CAM: Complementary and Alternative Medicine; NCCIH: National Centre of Complementary and Integrative Health; SEM: Standard Error of Mean; RA: Rheumatoid Arthritis; CVD: Cardiovascular Disease.

Introduction

Cardiovascular diseases are the leading cause of death worldwide. Research in the last two decades explored that the inflammatory process is play a major role in the mechanism of different cardiac pathologies [1]. Several cytokines (TNF- α , TGF- β) and interleukins (IL-1, IL-4, IL-6, IL-8, and IL-18) are responsible for the development of various inflammatory cardiac pathologies such as ischemic heart disease, myocardial infarction, heart failure, and cardiomyopathies [2]. Proinflammatory cytokines affect nearly all tissues and organ systems. A considerable research has been focused on the role of proinflammatory cytokines, interleukins, and tumour necrosis factor (TNF), in the pathogenesis of sepsis and septic shock associated with congestive heart failure [3]. The cytokine hypothesis has been proposed by scientists based on the idea that the activation of the inflammatory immune system, which specifically involved proinflammatory cytokines release, further stimulates various neurochemical and neuroendocrine changes [4]. Overall, cytokine's role has been reported in various types of infections and the growth of malignant tumors, as the immune-stimulants and mediating the inflammatory response in a variety of human diseases [5,6].

Besides, 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 are associated with three-times more risk of heart attack. CRP is one of the best possible markers of vascular inflammation and plays a vital role in promoting vascular inflammation, vessel damage and clinical cardiovascular disease [7,8]. Thus, to study the change in serum cytokines and other inflammatory 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 B₉, 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 [9-11]. Besides, cannabidiol itself has wide range of pharmacological profile and has been reported to role in different disorders [12,13], while ginseng extract is regarded as the one of the best immune boosters for overall immunity [14]. The present study was aimed to evaluate the

anti-inflammatory potential of the Biofield Energy Treated Proprietary Test Formulation and Biofield Energy Treatment *per se* to the animals on L-NAME and high fat diet (HFD)-induced cardiovascular disorders in Sprague Dawley rats using serum biomarkers.

Biofield Energy Healing Treatment/Blessing has been reported with significant effects against various disorders, and defined as one of the best Complementary and Alternative Medicine (CAM) treatment approach [15-17]. National Center for Complementary/Alternative Medicine (NCCAM) recommended CAM with several clinical benefits as compared with the conventional treatment approach [18]. 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 [19,20]. The Trivedi Effect[®]-Consciousness Energy Healing/Blessing was scientifically reported on various disciplines such as in the materials science [21,22], agriculture science [23], antiaging [24], Gut health [25], nutraceuticals [26], pharmaceuticals [27], cardiac health [28], overall human health and wellness. In this study, the authors sought to study the impact of the Biofield Energy Treatment/Blessing (the Trivedi Effect[®]) on the given novel test formulation and Biofield Energy Treatment *per se* to the animals on serum cytokines 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

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. For the estimation of serum inflammatory biomarker panel, and cytokines estimation, specific ELISA kits were

used such as for detection of TNF- α , IL-6, IL-18, IL-1 β , and C-reactive protein (CRP), 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 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 or Blessing 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/Blessing Treatment (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 Healer was located in the USA; however the test formulation was located in the research laboratory of Dabur Research Foundation, New Delhi, India. 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/Blessed 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/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 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 receiving 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 high fat diet (HFD) throughout the experimental period. At the end of the experimental period (8 weeks treatment), the animals were sacrifice and blood was collected, and separate serum subjected for cytokines (TNF- α , IL-6, IL-18, IL-1 β) and other biomarker like C-reactive protein (CRP) estimation.

Preparation of Sample for the Estimation of Cytokines

With the continued treatment to the respective groups of 8th week of the experimental period, all the animals were individually subjected for blood collection using retro-orbital route and the blood was collected in the plain vial, which was used for the separation of serum in all the animals of different experimental groups. The serum from all the groups was stored at -20°C for further estimation. Alternatively, aliquot all the samples and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles, which may alter the level of cytokines during final calculations.

Estimation of Cytokine Levels and C-reactive Protein (CRP) in Serum

The serum from all the groups was subjected for the estimation of level of cytokines such as TNF- α , IL-6, IL-18, and IL-1 β , along with C-reactive protein (CRP). All the serum 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 \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 and Discussion

Estimation of Serum Tumor Necrosis Factor-Alpha (TNF- α)

Serum cytokine, TNF- α was estimated in the presence of the effect of the test formulation, which was measured in all the experimental groups and was 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 value of tumor necrosis factor-alpha (TNF- α) as 3.13 ± 0.07 pg/mL, which was increased by 4.74% as compared with the normal control (G1, 2.99 ± 0.06 pg/mL). However, positive control (captopril + atorvastatin) treatment (G3) showed the level of serum TNF- α i.e., 3.19 ± 0.17 pg/mL. The level of serum proinflammatory cytokine (TNF- α) was decreased by 7.27%, 5.45%, 5.98%, 12.72%, 6.26%, and 2.14% 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). On the other hand, the expression of TNF- α was reduced by 2.05%, 1.48%, 5.8%, 1.18%, and 5.62% in the G5, G6, G7, G8, and G9 groups, respectively as compared to the untreated test formulation (G4). TNF- α , a pro-inflammatory cytokine plays a wide role in the human body. These cells signaling protein (cytokine) significantly involved in systemic inflammation along with acute phase reaction. It mediates and regulates immune responses and inflammation. It also produces widespread deleterious effects when expressed in large amounts. It is produced in the heart by both cardiac myocytes and resident macrophages under conditions of cardiac stress and responsible for various cardiac diseases, elevated in congestive heart failure and potentiates heart failure [29]. TNF-alpha may also trigger and perpetuation of atherosclerosis. Treatment with biologic agents that inhibits TNF-alpha expression has various clinical benefits in inflammatory diseases such as rheumatoid arthritis (RA) and may be able to reduce cardiovascular risk [30]. Overall, in this experiment the Biofield Energy Treated test formulation and Biofield Energy Treatment *per se* reduced the level of TNF-alpha which might be helpful for the management of cardiovascular disorders.

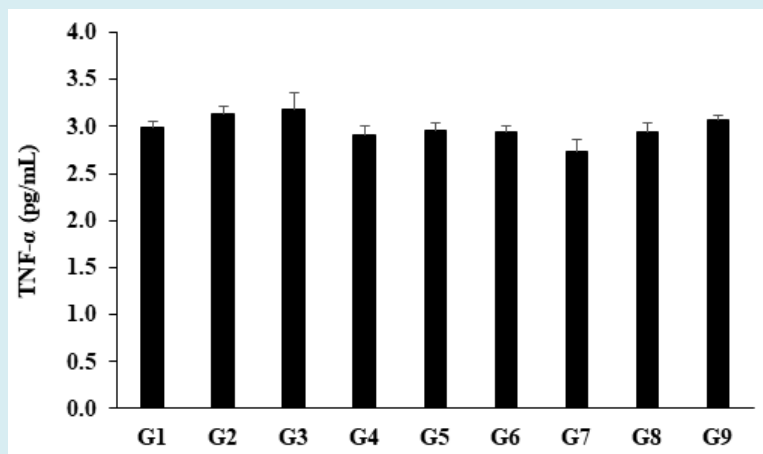
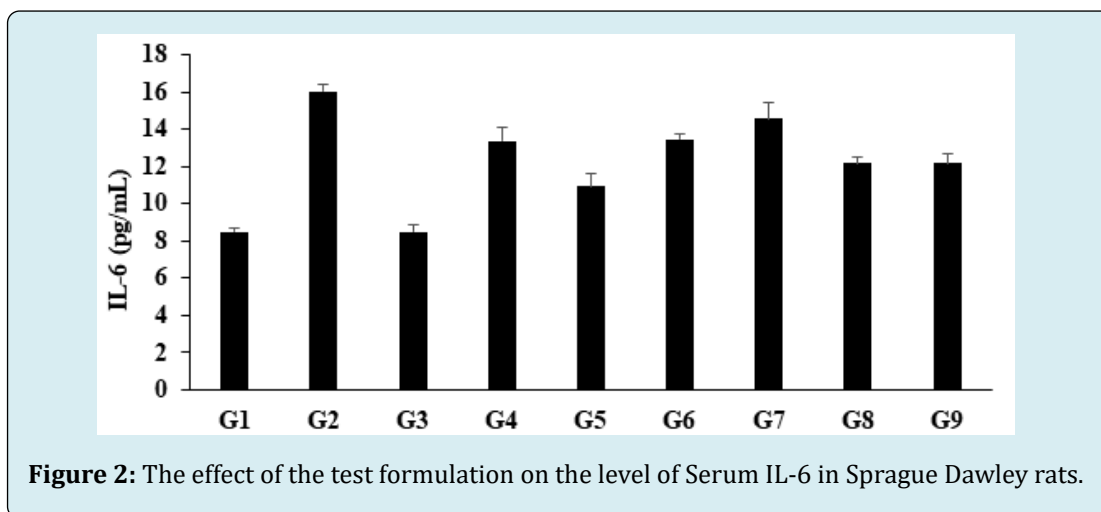


Figure 1: The effect of the test formulation on the level of serum TNF- α in Sprague Dawley rats. G1 as normal control (vehicle, 0.5% w/v CMC-Na); G2 as disease control (L-NAME + high fat diet (HFD) + 0.5% CMC); G3 as reference item (L-NAME + HFD + Captopril + Atorvastatin); G4 includes L-NAME + HFD 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=10).

Estimation of Serum IL-6

Stress induction was reported with high level of IL-6, thus, alteration and management in the level of cytokines has wide range of implicated in major depressive illness and its treatment. This is also regarded as the innate inflammatory response to many physical stressors (e.g, depression, traumatic stress, infection, inflammation) [31]. The effect of the test formulation and Biofield Energy Treatment *per se* was estimated using the level of serum IL-6, which was measured in all the experimental groups and was graphically presented in the Figure 2. The data suggested that the disease control (L-NAME + high fat diet (HFD) + 0.5% CMC) group (G2) showed value of IL-6 as 16.02 ± 0.39 pg/mL, which was increased by 88.87% as compared with the normal control (G1, 8.48 ± 0.23 pg/mL). However, positive control (captopril + atorvastatin) treatment group (G3) showed decreased serum IL-6 level by 47.01% i.e. 8.49 ± 0.37 pg/mL was compared to the G2. The level of proinflammatory cytokine interleukin-6 (IL-6) was decreased by 16.48%, 31.75%,

15.96%, 9.09%, 24.18%, and 24.11% 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 expression of IL-6 was reduced by 18.29%, 0.63%, 8.84%, 9.22%, and 9.14% in the G5, G6, G7, G8, and G9 groups, respectively as compared to the untreated test formulation (G4). Overall, in this study the Biofield Energy Treated test formulation and Biofield Energy Treatment *per se* significantly reduced the level of IL-6, which was increased due to cardiovascular disease condition, induced by L-NAME and HFD, which could be beneficial in the cardiovascular patients.



Estimation of Serum IL-18

Interleukin-18 (IL-18) is an independent predictor of cardiovascular event. The metabolic syndrome is a cluster of risk factors for cardiovascular disease (CVD), including obesity, hypertension, and elevated triglycerides [32]. There is increasing evidence that the metabolic syndrome is associated with a proinflammatory state. IL-18 is a potent proinflammatory cytokine that has been reported with metabolic syndrome and predict the development of type 2 diabetes [33], and atherosclerotic plaque [34]. The level of serum IL-18 was detected in all the experimental groups and was presented in Figure 3. The data suggested that disease control (L-NAME + high fat diet, HFD + 0.5% CMC) group (G2) showed value of IL-18 as 199.59 ± 3.47 pg/mL, which was increased by 8.23% as compared with the normal control

(G1, 184.40 ± 5.42 pg/mL) group. While the positive control (captopril + atorvastatin) treatment (G3) showed the level of IL-18 i.e., 213.83 ± 11.57 pg/mL. The level of interleukin-18 (IL-18) was decreased by 1.48%, 5.26%, and 4.63% in the G4 (L-NAME + HFD + untreated test formulation), G7 (L-NAME + HFD + the Biofield Energy Treated test formulation from day -15), and G8 (L-NAME + HFD + Biofield Energy Treatment *per se* plus the Biofield Energy Treated test formulation from day -15), groups, respectively, as compared to the disease control group (G2). Moreover, the level of IL-8 was reduced by 3.83% and 3.20% in the G7 and G8 groups, respectively as compared to the untreated test formulation (G4). Overall, here the Biofield Energy Treated/Blessed test formulation and Biofield Energy Treatment *per se* minimally reduced the level of IL-18, which could be beneficial in the cardiovascular symptoms.

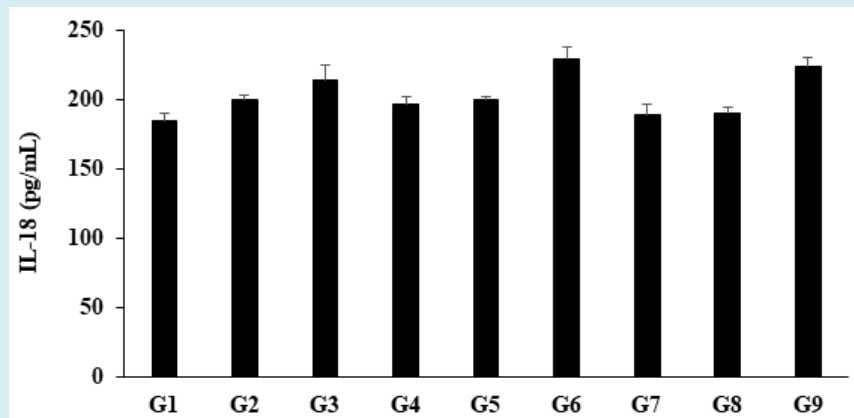


Figure 3: The effect of the test formulation on the level of serum IL-18 in Sprague Dawley rats.

Estimation of Serum IL-1 β

The effect of the test formulation and Biofield Energy Treatment *per se* was estimated using the level of serum IL-1 β , and the results were graphically presented in the Figure 4. The disease control (L-NAME + high fat diet, HFD + 0.5% CMC) group (G2) showed value of IL-1 β as 50.51 ± 0.92 pg/mL, which was increased by 8.88% as compared with the normal control (G1, 46.39 ± 0.48 pg/mL). Further, the positive control (captopril + atorvastatin) treatment (G3) showed decreased serum IL-1 β level by 2.88% i.e., 49.06 ± 0.55 pg/mL as compared to the G2 group. The level of IL-1 β was decreased by 6%, 6.42%, 6%, 4.72%, and 4.72% 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), 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, IL-1 β level was decreased by 7.93%, 8.35%, 7.93%, 6.68%, and 6.68% in the G5, G6, G7, G8, and G9 groups, respectively as compared to the untreated test formulation (G4). The experimental and clinical evidence reported that interleukin-1 beta (IL-1 β) plays both vascular and systemic contributor against conventional risk factors to atherosclerosis [35]. Another studies evidence in humans, suggests that IL-1 β plays a role in insulin resistance, both T2DM and pre-diabetic states [36]. Therefore, in this experiment the Biofield Energy Treated test formulation and Biofield Energy Treatment *per se* reduced the level of IL-1 β , which could be beneficial in the cardiovascular disease conditions.

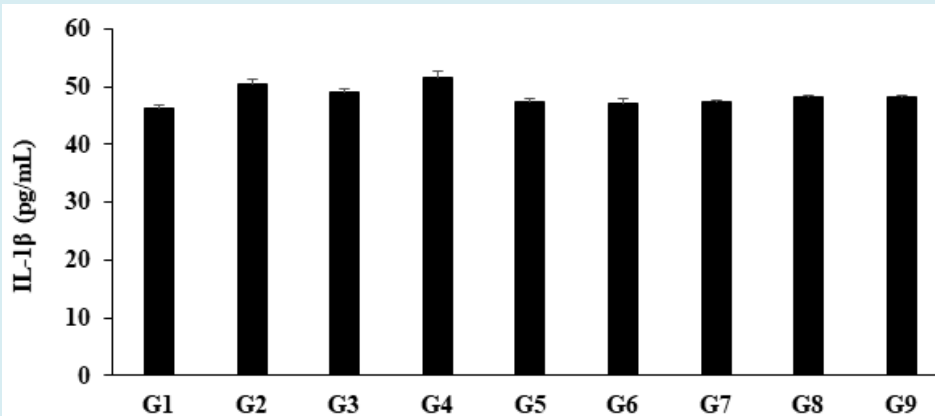


Figure 4: The effect of the test formulation on the level of serum IL-1 β in Sprague Dawley rats.

Estimation of Serum C-reactive Protein (CRP)

The effect of the test formulation and Biofield Energy Treatment *per se* was estimated using the level of serum C-reactive protein (CRP); the results were graphically presented in the Figure 5. The level of serum C-reactive protein (CRP) in the disease control (L-NAME + high fat diet, HFD + 0.5% CMC) group (G2) was 572.59 ± 22.78 ng/mL, which was increased by 339.16% as compared with the normal control (G1, 130.38 ± 8.03 ng/mL). Further, the positive control (captopril + atorvastatin) treatment (G3) showed decreased serum CRP level by 39.50%, 346.40 ± 22.87 ng/mL as compared with the G2. The level of CRP was decreased by 38.34%, 33.25%, 25.78%, 15.63%, 42.12%, and 35.40% 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). Besides, the level of CRP was decreased by 6.13% in the G8 group as compared to the untreated test formulation (G4). Inflammation plays a major role in the pathogenesis of cardiovascular disease [37]. In this context, the acute phase reactant C-reactive protein (CRP) is playing an independent risk factor for cardiovascular patients [38]. C-reactive protein (CRP) was reported is one of the best biomarkers for detection of immune function alterations [39]. Overall, in this experiment the Biofield Energy Treated/Blessed test formulation and Biofield Energy Treatment *per se* significantly reduced the level of CRP, which could be suppressed inflammatory conditions and simultaneously reduce the risks of cardiovascular diseases.

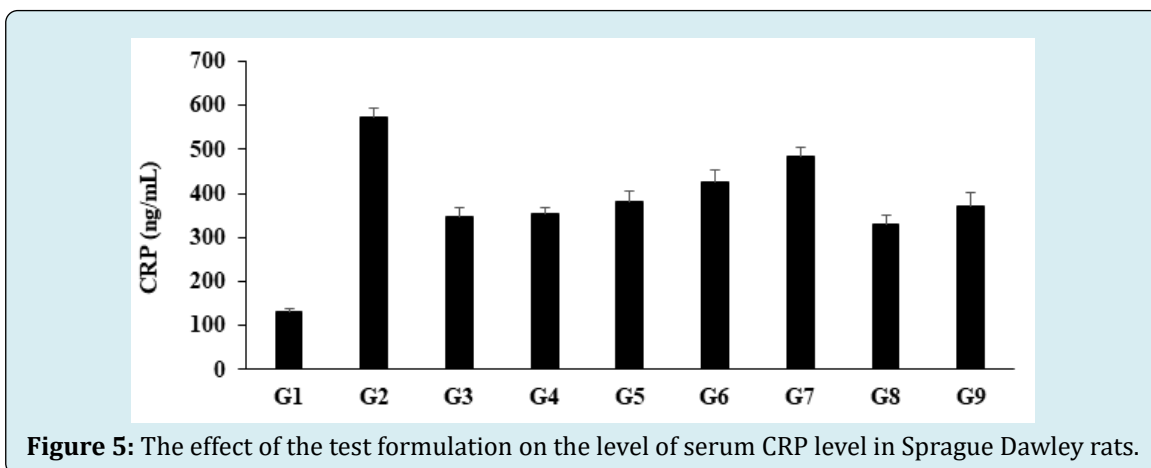


Figure 5: The effect of the test formulation on the level of serum CRP level in Sprague Dawley rats.

Experiment includes four preventive maintenance groups (G6, G7, G8 and G9). The findings showed the significant slowdown of cardiovascular-related symptoms and also reduced the chances of disease susceptibility. Based on the overall data, it suggests that Mr. Trivedi's Biofield Therapy was found to be most effective and benefited to protect from the manifestation of the existing ailments that will ultimately improve the overall health and quality of life in human.

Conclusion

The level of cytokines such as TNF- α , IL-6, IL-18, and IL-1 β , along with C-reactive protein (CRP) were estimated and compared with respect to untreated test formulation and other preventive measure groups. Serum TNF- α was decreased by 12.72% in the G7 (L-NAME + HFD + the Biofield Energy Treated test formulation from day -15) group as compared with the diseases control group, G2. However, the

level of serum IL-6 was significantly reduced by 31.75%, 15.96%, 24.18%, and 24.11% 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), G8 (L-NAME + HFD + Biofield Treatment *per se* plus the Biofield Energy Treated/Blessed 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). On the other hand, estimation of serum C-reactive protein (CRP) data showed that the level was significantly decreased by 33.25%, 25.78%, 15.63%, 42.12%, and 35.40% in the G5 to G9 groups, respectively than G2 group. Altogether, the Biofield Energy Treated/Blessed test formulation and Biofield Energy Healing Treatment/Blessing (the Trivedi Effect[®]) *per se* showed fruitful results with respect to different inflammatory biomarkers in the only blessing preventive maintenance group (G6) and other preventive maintenance groups (G7, G8, and G9) in L-NAME and High Fat Diet-Induced

cardiovascular disorders in rats. It could help to slow down the cardiovascular disease progression rate and disease-related complications and one of the alternative treatment approaches for the management of different types of diseases. Therefore, the Trivedi Effect® might act as a preventive maintenance therapy for the management of overall health and quality of life in human. Further, Mr. Trivedi's Biofield Energy Healing Therapy/Blessing could be utilized as a CAM approach for the management of multiple disorders viz. rheumatoid arthritis, myasthenia gravis, fibromyalgia, Addison disease, multiple sclerosis, aplastic anemia, psoriasis, Crohn's disease, vitiligo, ulcerative colitis, alopecia areata, dermatitis, hepatitis, diverticulitis, Parkinson's, and stroke, many more. This therapy might lower the chances of the severity of acute or chronic diseases progression rate and can be used both before and after the manifestation of any symptoms of disease in healthy and unhealthy/ill people alike.

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