International Journal of Cell Science & Molecular Biology
ISSN: 2572-1100

Juniper Publishers
key to the Researchers

Research Article
Volume 7 Issue 1 - July 2021
DOI: 10.19080/IJCSMB.2021.07.555703

Int J cell Sci & mol biol
Copyright © All rights are reserved by Snehasis Jana

Cellular Biomarkers ($CD4^+$, $CD8^+$), Immunoglobulins, Testosterone, and Hematology Analysis of Biofield Energy Treated Proprietary Formulation on L-NAME and HFD-Induced Cardiovascular Disorders in Sprague Dawley Rats

Mahendra Kumar Trivedi[1], Alice Branton[1], Dahryn Trivedi[1] and Snehasis Jana[2]*

[1]*Trivedi Global, Inc., Henderson, USA*

[2]*Trivedi Science Research Laboratory Pvt. Ltd., India*

Submission: June 07, 2021; **Published:** July 06, 2021

***Corresponding author:** Snehasis Jana, Trivedi Science Research Laboratory Pvt. Ltd., Thane (W), Maharashtra, India

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 immune biomarkers and testosterone on Nω-Nitro-L-arginine Methyl Ester (L-NAME) and high fat diet (HFD)-induced cardiovascular disorders in male Sprague Dawley rats. 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 constituents of the test formulation were divided into two parts; one section was defined as the untreated test formulation, while the other part and three group of animals received Biofield Energy Healing Treatment/Blessing remotely for about 3 minutes by a renowned Spiritual Energy Healer, Mr. Mahendra Kumar Trivedi. The results showed that cluster of differentiation 4 (CD4+)-T-cell count was significantly increased by 11.45%, 20.02%, 14.18% ($p \leq 0.05$), 19.37% ($p \leq 0.05$), and 10.53% 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 along with Biofield Energy Treatment *per se* animals plus the untreated test formulation) groups, respectively as compared to the untreated test formulation (G4) group. Moreover, the $CD8^+$ T-cell count was increased by 13.82% in the G8 group as compared to the G2 group. There was an alteration of immunoglobulins in the treatment groups than G2. The level of testosterone was significantly increased by 55.53%, 161.66% ($p \leq 0.05$), 110.03% ($p \leq 0.05$), 93.71% ($p \leq 0.05$), and 102.05% ($p \leq 0.05$) in the G5, G6, G7, G8, and G9 groups, respectively as compared to the G2 group. Hematological parameter like platelet count was increased by 10.18%, 10.44%, 14.23%, and 14.44% in the G5, G7, G8, and G9 groups, respectively as compared to the G2 group. Additionally, RBC distribution width-variation coefficient (RDW-CV) was altered by 13.29%, 16.94%, 14.83%, and 11.55% in the G5, G6, G8, and G9 group respectively, as compared with the G2 group. Histopathological examination data did not show any drastic cellular changes in all the treatment groups. Overall, the data suggested that "Trivedi's Biofield" significantly improved the cellular immune biomarkers, testosterone, and hematology parameters in the preventive treatment groups with respect to various pathological conditions that might be beneficial various types of cardiovascular disorders. Therefore, the study outcomes could be slowdown the cardiovascular disease progression rate and its complications in the preventive treatment groups (*viz.* G6, G7, G8, and G9).

Keywords: CD Biomarker, Immunoglobulins, The Trivedi Effect®, Testosterone, High Fat Diet (HFD), Cardiovascular Disorders, Hematology, Histopathology

Abbreviation: CVDs: Cardiovascular Diseases; CHDs: Coronary Heart Diseases; Th: T-Helper; HFD: High Fat Diet; CBD: Cannabidiol Isolate; CAM: Complementary and Alternative Medicine; NCCAM: National Center for Complementary/Alternative Medicin; NCCIH: National Centre of Complementary and Integrative Health; SD: Sprague Dawley

Introduction

Cardiovascular diseases (CVDs) remains one of the most leading causes of morbidity and mortality in the World. About 54% of strokes and 47% of coronary heart diseases (CHDs) has been occurs in the worldwide due to stress or hypertension [1]. Dysregulation of the immune system and inflammatory pathways

are the leading mechanisms of CVDs, including heart failure, cardiomyopathies, and rhythm disorders. Immuno-inflammatory mechanisms play a vital role in modulating the CVDs [2]. The heart and the immune system are highly amalgamated through cytokines, hormones, and neurotransmitters. Alteration of this unite balance by physical or psychological stressors leads to inflammation, endothelial dysfunction, and tissue damage [3]. The $CD8^+$ T-cells and subsets of $CD4^+$ T-cells, which include T-helper (Th) cells and T-regulatory (T-reg) cells, cooperate with innate cells during the immune response [4]. One study reported that testosterone plays a vital role in organ reperfusion following vascular occlusion, which can impart cardio protection [5]. Thus, to study the change in vital cardiac function in presence of L-NAME and high fat diet (HFD)-induced cardiovascular disorders/model in Sprague Dawley rats, a novel proprietary 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 incorporate in this test formulation have significant physiological roles [6-8]. Besides, cannabidiol itself has wide range of pharmacological profile and has been reported to role in different disorders [9, 10], while ginseng extract is regarded as one of the best immune boosters for overall immunity [11]. 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 HFD-induced cardiovascular disorders in Sprague Dawley rats. 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 [12-14]. National Center for Complementary/Alternative Medicine (NCCAM) recommended CAM with several clinical benefits as compared with the conventional treatment approach [15]. 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 [16, 17]. The Trivedi Effect®-Consciousness Energy Healing/Blessing was scientifically reported on multiple fields such as nutraceuticals [18], agriculture science [19], cardiac health [20], materials science [21, 22], antiaging [23], Gut health [24], pharmaceuticals [25], overall human health and wellness. In this study, the authors need to evolve to understand the impact of the Biofield Energy Treatment/Blessing (the Trivedi Effect®) *per se* to the animals and Biofield Energy Treated/Blessed test formulation on cellular immune biomarkers ($CD4^+$ and $CD8^+$), immunoglobulins (IgA, IgE, IgG, and IgM), testosterone, hematology profile along with change in cellular structure by histopathology in presence of L-NAME and HFD-induced cardiovascular disorders in male Sprague Dawley (SD) rats.

Material and Methods

Chemicals and reagents

Pyridoxine hydrochloride (vitamin B_6), atorvastatin, 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.

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

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

How to cite this article: Mahendra K T, Alice B, Dahryn T, Snehasis J. Cellular Biomarkers ($CD4^+$, $CD8^+$), Immunoglobulins, Testosterone, and Hematology Analysis of Biofield Energy Treated Proprietary Formulation on L-NAME and HFD-Induced Cardiovascular Disorders in Sprague Dawley Rats. Int J Cell Sci & Mol Biol. 2021; 7(1): 555703. DOI: 10.19080/IJCSMB.2021.07.555703

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 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 HFD throughout the experimental period. At the end of the experimental period (8 weeks treatment), all the animals were individually subjected for blood collection using retro-orbital route for the analysis of cellular biomarkers ($CD4^+$ and $CD8^+$) and hematological parameters. From the collected blood, isolate serum for the estimation of humoral immune parameters like IgA, IgE, IgG, and IgM. All the surviving animals were euthanized by CO_2 asphyxiation followed by exsanguinations. Some vital organs were isolated, preserved in 10% neutral buffered formalin for histopathological examination.

Assessment of cellular and humoral immune responses

To study the humoral immune response, IgA, IgE, IgG, and IgM were estimated using Mini Vidas, Biomeurix (French) from serum, using commercially available kits as per manufacturer's instructions. Flow cytometry was used to evaluate the $CD4^+$ and $CD8^+$ cells in whole blood as a measure of the cellular immune response using Guava Flow Cytometer, Easy Cyte. The mean value was calculated for each group with SEM. The percent change in the Biofield Energy Treated group was calculated compared to the vehicle treatment group.

Estimation of testosterone

The level of testosterone was analyzed in serum in all the experimental groups using commercial kits. The values were calculated and presented as mean ± SEM.

Determination of hematological parameters

An aliquot of blood were directly subjected for the estimation of various hematological parameters using standard instrument. The various hematological parameters were measured such as hemoglobin (Hb), red blood count (RBC), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and platelets.

Measurement of histopathological analysis

At the end of the experiment, rats were dissected and various organs *viz*. whole liver, lungs, kidneys, brain, hearts, spleens, testis, and thymus were isolated and kept for histopathological examination. Defined samples were placed in 10% neutral buffered formalin for histopathological examination. Testes was fixed in modified Davidson fluid at least for 24 hours and followed by 70% alcohol at least for next 24 to 48 hour. After that, organ was washed in tap water for 10-15 minute and were transferred to 10% neutral buffer formalin or were processed directly as per the requirement. All organs from found dead (if any) animal(s) were collected. Organs from moribund animal(s) (if any) were collected at the discretion. Section was cut at an approximate thickness of 4 to 5 microns with the help of Microtome and sprayed on Flotation Workstation and collected on double frosted slide dried at room temperature or with the Slide Dryer if required. All the sections were stained with the help of hematoxylin and eosin staining method. The instruments details were incorporated in study report. Histopathological examination and reading was performed on all the preserved organs slides. The representative histopathological slide figure of each organ from each group was incorporated in the report.

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

Measurement of cellular responses

The test formulation was tested for cellular immune response, which was estimated by calculating the percentage of vital biomarkers such as $CD4^+$ and $CD8^+$ the results are presented in the (Figure 1). The levels of $CD4^+$ in the normal control (G1), disease control (G2), and positive control (G3) groups were 31.41 ± 0.89, 31.43 ± 1.33, and 30.37 ± 1.78%, respectively. However, the level of $CD4^+$ was significantly increased by 11.45%, 20.02%, 14.18% ($p \leq 0.05$), 19.37% ($p \leq 0.05$), and 10.53% in the G5 (L-NAME + HFD

How to cite this article: Mahendra K T, Alice B, Dahryn T, Snehasis J. Cellular Biomarkers ($CD4^+$, $CD8^+$), Immunoglobulins, Testosterone, and Hematology Analysis of Biofield Energy Treated Proprietary Formulation on L-NAME and HFD-Induced Cardiovascular Disorders in Sprague Dawley Rats. Int J Cell Sci & Mol Biol. 2021; 7(1): 555703. DOI: 10.19080/IJCSMB.2021.07.555703

+ 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 along with Biofield Energy Treatment *per se* animals plus the untreated test formulation) groups, respectively, as compared to the untreated test formulation (G4) group (Figure 1). Besides, the levels of $CD8^+$ in the normal control (G1), disease control (G2), and positive control (G3) groups were 26.51 ± 2.27, 31.04 ± 0.69, and 28.50 ± 1.70%, respectively. However, the level of $CD8^+$ was increased by 7.6%, 8.39%, 5.03%, 13.82%, and 7% in the G4 (L-NAME + HFD along with untreated test formulation), 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 to the disease control (G2) group (Figure 1). Based on the literature it was observed that lower baseline and proximal $CD4^+$ T-lymphocyte counts in myocardial infarction (MI) patients compared with patients who did not have MI. [26]. Another study data showed that major $CD4^+$ decline could be a marker of cardiovascular diseases [27]. High concentration of $CD8^+$ T cells were characterized by lowered the cytokine release from activated leucocytes [28]. Overall, it can be concluded that Biofield Energy Healing/Blessing (the Trivedi Effect®)-based test formulation showed a significant improved cellular immune response, which results in increased number of $CD4^+$ and $CD8^+$ cells count that would significantly improve the cellular immunity to fight against various infections against many inflammatory and autoimmune disorders.

Evaluation of humoral immune response

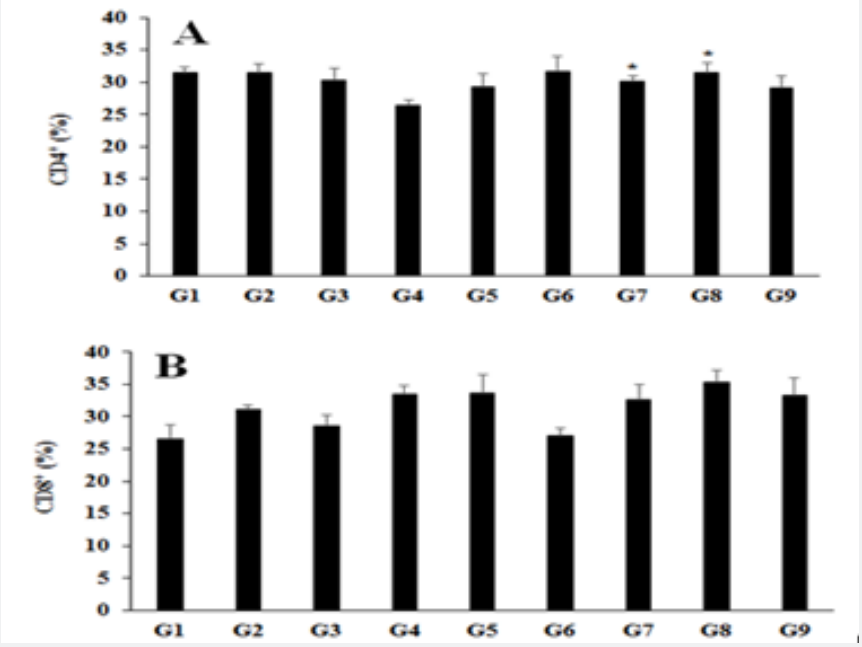

Figure 1: The effect of the test formulation on the cellular immune biomarkers A. Cluster of differentiation (CD4+) and B. CD8+ in blood sample of male SD rats on various groups (G1 – G9). G1 as normal control (vehicle, 0.5% w/v CMC-Na); G2 as disease control (L-NAME + high fat diet (HFD) + 0.5% CMC); 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≤0.05 vs. untreated test formulation group (G4).

Immunoglobulin's levels (IgE, IgA, IgG, and IgM) after treatment with the test formulation in different experimental groups are shown in (Figure 2). The data showed the IgE, IgA, IgG, and IgM levels in the disease control (L-NAME + HFD + 0.5% CMC) (G2) group was found to be 2.65 ± 0.2 IU/mL, 74.75 ± 1.44 mg/dL, 425 ± 29.87 mg/dL, and 16.63 ± 0.42 mg/dL, respectively. The positive control (captopril + atorvastatin) treatment (G3) was increased the levels of IgA and IgM by 3.34% and 1.5%, respectively as compared to the G2 group. The level of immunoglobulin E (IgE) was increased by 8.96% and 2.96% in the G5 (L-NAME + HFD + the Biofield Energy Treated test formulation) and G9 (L-NAME + HFD along with Biofield Energy Treatment *per se* animals plus the

How to cite this article: Mahendra K T, Alice B, Dahryn T, Snehasis J. Cellular Biomarkers ($CD4^+$, $CD8^+$), Immunoglobulins, Testosterone, and Hematology Analysis of Biofield Energy Treated Proprietary Formulation on L-NAME and HFD-Induced Cardiovascular Disorders in Sprague Dawley Rats. Int J Cell Sci & Mol Biol. 2021; 7(1): 555703. DOI: 10.19080/IJCSMB.2021.07.555703

untreated test formulation) groups, respectively, as compared to the disease control group (G2), while 9.38% and 3.35% increase in the G5 and G9 groups, as compared to the untreated test formulation group (G4). Moreover, the level of immunoglobulin E (IgG) was increased by 3.63% and 1.69% in the G5 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 untreated test formulation group (G4). Further, the level of immunoglobulin E (IgM) was increased by 3.73%, 1.47%, and 1.47% in the G4 (L-NAME + HFD along with untreated test formulation), G5, and G8 groups, respectively, as compared to the disease control group (G2). Khamis and co-workers reported that lower levels of total IgG and to some extent also lower levels of IgM are one of the risk factors for coronary heart diseases (CHDs) apart from traditional risk factors like smoking, hypertension, and hypercholesterolemia, etc. Peoples with high IgG levels have an almost 60% lower risk of CHDs than those in the lowest IgG level [29]. Another study reported that salivary IgG has inversely correlated with the CAD [30]. Overall, it can be concluded that the Biofield Energy Healing Treatment *per se* and Biofield Energy Treated test formulation marginally improved the humoral immune response with respect to the untreated test formulation as well as disease control group.

Estimation of testosterone

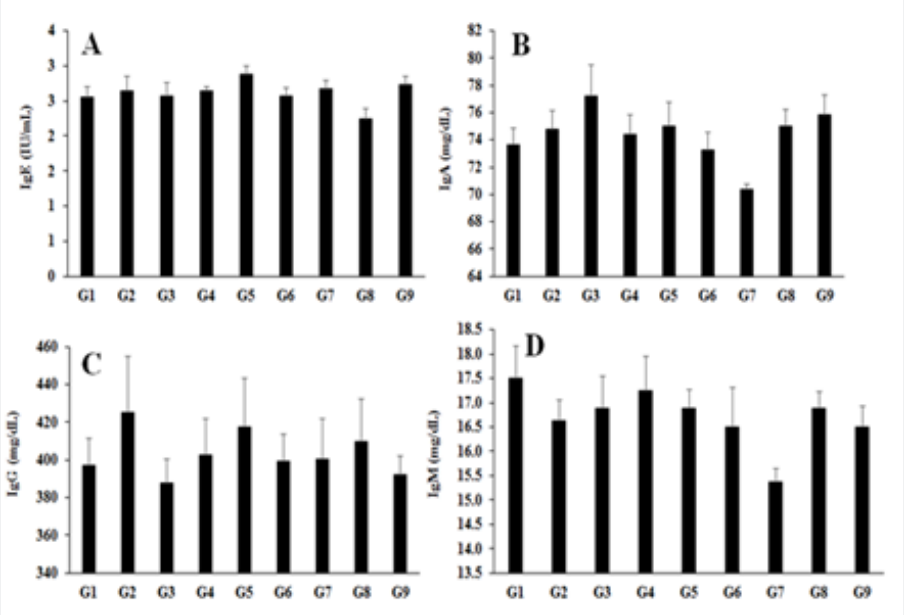

Figure 2: The effect of the test formulation on the immunoglobulin, (A) IgE, (B) IgA, (C) IgG, and (D) IgM in various test groups G1 to G9 in male Sprague Dawley rats.

The serum testosterone level was estimated in all the experimental groups in male SD rats after oral administration of the test formulation and the data was shown in (Figure 3,4). The level of testosterone was decreased by 38.95% in the disease control (G2) group (1.51 ± 0.09 ng/mL) as compared to the normal control (G1) group (2.47 ± 0.59 ng/mL). The positive control (captopril + atorvastatin) treatment (G3) was increased by 77.10% (2.67 ± 0.79 ng/mL) as compared to the G2 group. The level of testosterone was significantly increased by 59.87%, 55.53%, 161.66% ($p \leq 0.05$), 110.03% ($p \leq 0.05$), 93.71% ($p \leq 0.05$), and 102.05% ($p \leq 0.05$) 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 along with 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 along with Biofield Energy Treatment *per se* animals plus the untreated test formulation) groups, respectively, as compared to the disease control (G2) group. Moreover, the level of testosterone was increased by 63.95%, 31.60%, 21.37%, and 26.60% in the G6, G7, G8, and G9 groups, respectively, as compared to the untreated test formulation (G4) group. This suggests that the Biofield Energy Healing Treatment/ Blessing and the Blessed test formulation have significant capacity to improved testosterone. As per literature, testosterone can directly act on the blood vessels of the cardiovascular system and on the heart, as well as effects on risk factors for CVD. Most of heart failure patients with demonstrate a reduced serum level of testosterone [31]. Another couples of literatures reported that low levels of testosterone are associated with more atherosclerosis, coronary artery disease (CAD), and may increase the risk of developing metabolic syndrome and type 2 diabetes [32, 33]. In this experiment, the Biofield Treated/Blessed test formulation significantly improved the testosterone level, which could be helpful to reduce the risks of cardiovascular complications.

How to cite this article: Mahendra K T, Alice B, Dahryn T, Snehasis J. Cellular Biomarkers (CD4+, CD8+), Immunoglobulins, Testosterone, and Hematology Analysis of Biofield Energy Treated Proprietary Formulation on L-NAME and HFD-Induced Cardiovascular Disorders in Sprague Dawley Rats. Int J Cell Sci & Mol Biol. 2021; 7(1): 555703. DOI: 10.19080/IJCSMB.2021.07.555703

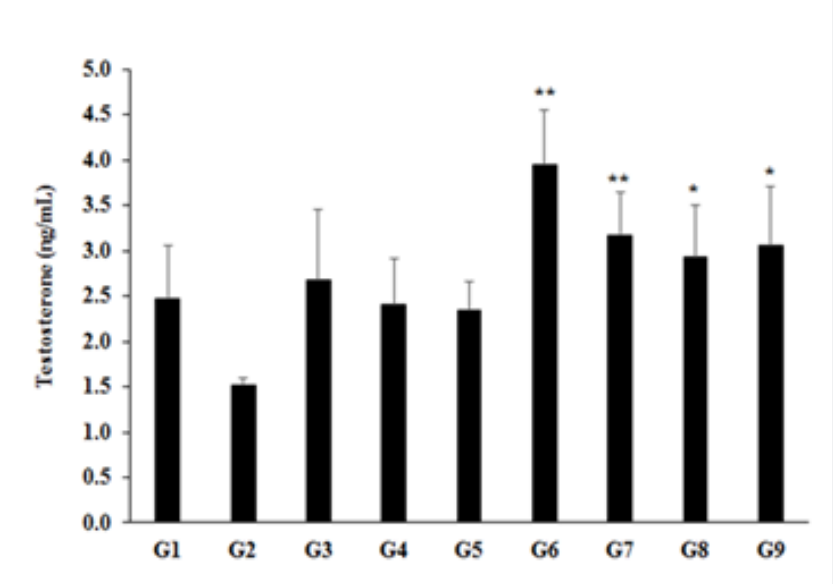

Figure 3: Evaluation of testosterone after treatment with the Biofield Treated test formulation and Biofield Energy Healing/Blessing on male Sprague Dawley rats. *$p \leq 0.05$ and **$p \leq 0.01$ *vs*. Disease control group (G2).

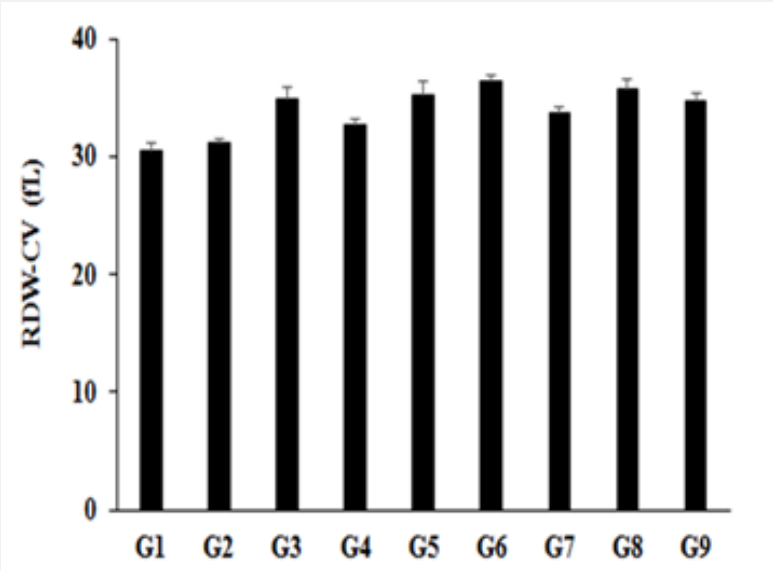

Figure 4: Evaluation of RDW-CV after treatment with the test formulation on male Sprague Dawley rats.

Evaluation of hematological parameters

The experimental results showed an important hematology profile in different groups (G1 to G9), which are summarized in (Table 1). The study showed that the Biofield Energy Treated/ Blessed test formulation showed an improved animal hematology profile compared with the disease control group. Hematology parameter such as RBC count and Hb were marginally increased in the G8 and G9 groups, respectively as compared with the G2 group. Platelet count was increased by 12.59%, 10.18%, 10.44%, 14.23%, and 14.44% in the G4, G5, G7, G8, and G9 groups, respectively as compared to the G2 group. Similarly, levels of calculated hematology parameters like HCT, MCV, MCH, and MCHC were altered but did not show any lucrative changes compared with the G2 group. According to Sloan et al. reported that high platelet counts are associated with less chances to cardiovascular risk [34]. Besides, low platelets count are associated with cancer [35]. However, RBC distribution width-variation coefficient (RDW-CV) was altered by 13.29%, 16.94%, 8.12%, 14.83%, and 11.55% in the G5, G6, G7, G8, and G9 group respectively, as compared with the G2 group. Moreover, the value of RDW-CV was altered by

How to cite this article: Mahendra K T, Alice B, Dahryn T, Snehasis J. Cellular Biomarkers (CD4+, CD8+), Immunoglobulins, Testosterone, and Hematology Analysis of Biofield Energy Treated Proprietary Formulation on L-NAME and HFD-Induced Cardiovascular Disorders in Sprague Dawley Rats. Int J Cell Sci & Mol Biol. 2021; 7(1): 555703. DOI: 10.19080/IJCSMB.2021.07.555703

8.02%, 11.51%, 3.09%, 9.49%, and 6.36% in the G5, G6, G7, G8, and G9 group respectively, as compared with the G4 group. As per existed literature indicated that RDW is a non-specific surrogate marker of prognosis in cardiovascular diseases especially in heart failure and stroke. It is also a surrogate marker in various forms of anaemia especially in iron deficiency that are known to be associated with a poorer prognosis in heart failure [36-38].

Table 1: Evaluation of hematology parameters assessed after Biofield Energy Treatment on the test formulation in male Sprague Dawley rats.

Group	RBC (10^6/ μ L)	Hb (gm/dL)	HCT (%)	MCV (fl)	MCH (pg)	MCHC (%)	Platelet Count (thousand/ mm^3)
G1	9.49 ± 0.43	16.74 ± 0.19	51.50 ± 2.09	54.34 ± 0.34	17.86 ± 0.53	32.81 ± 0.88	1271.9 ± 82.75
G2	8.92 ± 0.19	17.04 ± 0.18	47.05 ± 0.38	52.89 ± 0.93	19.17 ± 0.40	36.22 ± 0.22	1118.4 ± 79.96
G3	8.79 ± 0.12	16.4 ± 0.16	47.90 ± 0.58	54.52 ± 0.74	18.68 ± 0.21	34.31 ± 0.27	945.6 ± 142.74
G4	9.21 ± 0.07	17.18 ± 0.19	49.49 ± 0.49	53.78 ± 0.48	18.66 ± 0.19	34.76 ± 0.23	1259.25 ± 33.17
G5	9.21 ± 0.48	16.92 ± 0.32	50.77 ± 2.91	55.06 ± 0.66	18.55 ± 0.49	33.77 ± 0.93	1232.30 ± 40.65
G6	8.52 ± 0.12	15.74 ± 0.19	45.94 ± 0.57	53.96 ± 0.33	18.48 ± 0.14	34.26 ± 0.11	1157.70 ± 44.41
G7	8.52 ± 0.11	16.27 ± 0.22	47.43 ± 0.54	55.68 ± 0.27	19.07 ± 0.13	34.23 ± 0.17	1235.2 ± 31.51
G8	9.16 ± 0.19	17.34 ± 0.35	49.79 ± 0.96	54.47 ± 0.77	18.97 ± 0.30	34.83 ± 0.17	1277.5 ± 52.56
G9	9.16 ± 0.10	17.22 ± 0.24	49.7 ± 0.57	54.28 ± 0.47	18.84 ± 0.24	36.87 ± 2.24	1279.89 ± 58.91

Measurement of histopathological analysis

Change in cellular structure after repeated administration of Biofield Treated test formulation and exposure of Biofield Energy/ Blessing directly to the animals were evaluated by histopathology. The histopathological findings of vital tissues in male Sprague Dawley rats are shown in (Figure 5). Different vital organs like kidneys, brain, liver, heart, lungs, testis, spleen, and thymus were performed for histopathological analysis. Based on the detailed scoring it was found that the histopathological examination data did not show any drastic cellular changes/alteration that supports the nontoxic nature of the test formulation or per se Blessing. However, slight decreased cellularity in white pulp and cortex and diffuse in spleen and thymus were observed in few animals, that did not affect the study, which might be due to tissue handling or as a human error (Figure 5). Overall, the Biofield Energy Healing Treatment/Blessing and Biofield Energy Treated/Blessed test formulation has significantly improved the cellular immune biomarkers ($CD4^+$ and $CD8^+$), testosterone, and blood biomarkers, which might be helpful in the cardiovascular disorders. Experiment includes four preventive maintenance groups (G6, G7, G8, and G9). The findings showed the significant slowdown of cardiovascular-related symptoms and reduced the chances of disease susceptibility. It also suggests that Mr. Trivedi's Biofield Therapy was found to be most effective and benefited to protect from various disorders that will ultimately improve the overall health and quality of life in human.

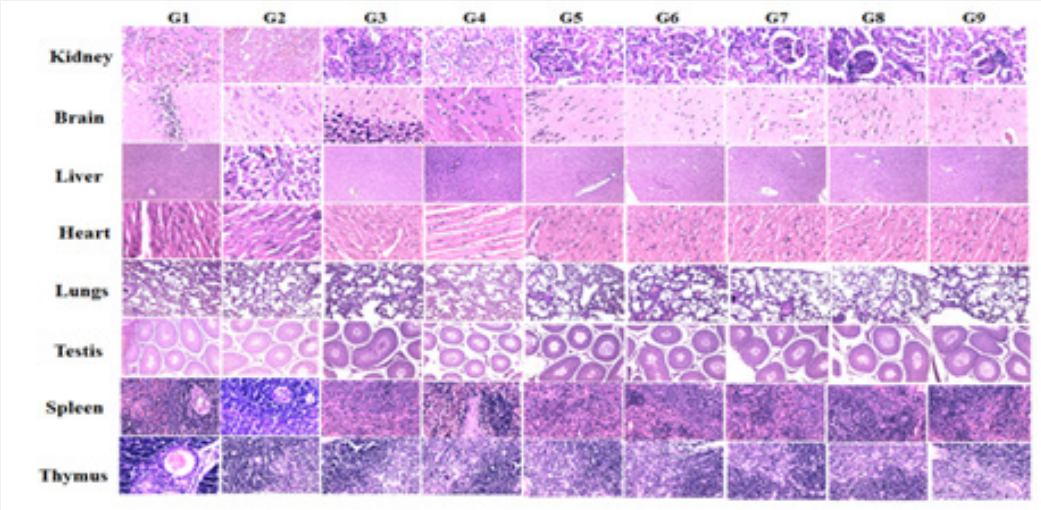

Figure 5: Histopathological photomicrograph of major organs after treatment with the Biofield Energy Treated/Blessed test formulation and Biofield Energy Treatment/Blessing to animals *per se* in male Sprague Dawley rats. All the tissues were sectioned transversely and stained with haematoxylin (H) and eosin (E).

How to cite this article: Mahendra K T, Alice B, Dahryn T, Snehasis J. Cellular Biomarkers ($CD4^+$, $CD8^+$), Immunoglobulins, Testosterone, and Hematology Analysis of Biofield Energy Treated Proprietary Formulation on L-NAME and HFD-Induced Cardiovascular Disorders in Sprague Dawley Rats. Int J Cell Sci & Mol Biol. 2021; 7(1): 555703. DOI: 10.19080/IJCSMB.2021.07.555703

Conclusion

Based on the experimental findings it was stated that $CD4^+$ count was significantly increased by 11.45%, 20.02%, 14.18% ($p \leq 0.05$), 19.37% ($p \leq 0.05$), and 10.53% in G5, G6, G7, G8, and G9 groups, respectively with respect to untreated test formulation (G4) group. Additionally, $CD8^+$ count was increased by 13.82% in the G8 group than G2 group. Testosterone level was significantly increased by 55.53%, 161.66%, 110.03%, 93.71%, and 102.05% in the G5, G6, G7, G8, and G9 groups, respectively as compared to the G2 group. Platelet count was increased by 10.18%, 10.44%, 14.23%, and 14.44% in G5, G7, G8, and G9 groups, respectively with respect to G2 group. Additionally, RDW-CV value was altered by 13.29%, 16.94%, 14.83%, and 11.55% in the G5, G6, G8, and G9 group respectively, as compared with the G2 group. Altogether, the Biofield Energy Treated/Blessed test formulation and Biofield Energy Healing Treatment/Blessing (the Trivedi Effect®) *per se* showed significant improvement of CD markers, testosterone, and hematology parameters in the preventive maintenance groups (G6, 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 rate and its complications. Therefore, the Biofield Energy Treatment/Blessing might use as preventive maintenance therapy to maintain good health and to improve quality of life. This therapy might decrease the severity of various type of acute/chronic diseases like auto-immune, inflammatory, and many thyroid disorders. Thus, his test formulation also can be used against fibromyalgia, Addison disease, multiple sclerosis, myasthenia gravis, rheumatoid arthritis, aplastic anemia, 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.

Acknowledgement

The authors are grateful to Dabur Research Foundation, Trivedi Science, Trivedi Global, Inc., and Trivedi Master Wellness for the assistance and support during the work.

References

1. Wu CY, Hu HY, Chou YJ, Huang N, Chou YC, et al. (2015) High blood pressure and all-cause and cardiovascular disease mortalities in community-dwelling older adults. Medicine (Baltimore) 94(47): e2160.
2. Lazzerini PE, Hamilton RM, Boutjdir M (2019) Editorial: Cardioimmunology: Inflammation and immunity in cardiovascular disease. Front Cardiovasc Med 6: 181.
3. Dal Lin C, Tona F, Osto E (2019) The crosstalk between the cardiovascular and the immune system. Vasc Biol 1(1): H83-H88.
4. Carrillo Salinas FJ, Ngwenyama N, Anastasiou M, Kaur K, Alcaide P (2019) Heart inflammation: Immune cell roles and roads to the heart. Am J Pathol 189(8): 1482-1494.
5. Tambo A, Roshan MHK, Pace NP (2016) Testosterone and cardiovascular disease. The Open Cardiovascular Medicine Journal 10: 1-10.
6. Byrne JH, Voogt M, Turner KM, Eyles DW, Mc Grath JJ, et al. (2013) The impact of adult vitamin D deficiency on behaviour and brain function in male Sprague-Dawley rats. PLoS One 8(8): e71593.
7. Rayman MP (2000) The importance of selenium to human health. Lancet 356: 233-241.
8. Beard JL, Connor JR (2003) Iron status and neural functioning. Ann Rev Nutr 23: 41-58.
9. Peres FF, Lima AC, Hallak JEC, Crippa JA, Silva RH, et al. (2018) Cannabidiol as a Promising Strategy to Treat and Prevent Movement Disorders. Front Pharmacol 9: 482.
10. Nagarkatti P, Pandey R, Rieder SA, Hegde VL, Nagarkatti M (2009) Cannabinoids as novel anti-inflammatory drugs. Future Med Chem 1(7): 1333-1349.
11. Kang S, Min H (2012) The 'Immunity Boost': The Effects of Panax ginseng on Immune System. J Ginseng Res 36(4): 354-368.
12. Maizes V, Rakel D, Niemiec C (2009) Integrative medicine and patient-centered care. Explore (NY) 5(5): 277-289.
13. Bischof M, Del Giudice E (2013) Communication and the emergence of collective behavior in living organisms: a quantum approach. Mol Biol Int 2013: 987549.
14. Cassidy CM (2004) What does it mean to practice an energy medicine. J Altern Complement Med 10(1): 79-81.
15. Barnes PM, Bloom B, Nahin RL (2008) Complementary and alternative medicine use among adults and children: United States, 2007. Natl Health Stat Report 12: 1-23.
16. Fan K wai (2005) National Center for Complementary and Alternative Medicine Website. J Med Libr Assoc 93: 410-412.
17. Wisneski L, Anderson L (2009) The Scientific Basis of Integrative Medicine. Boca Raton, FL: CRC Press 205.
18. Trivedi MK, Branton A, Trivedi D, Jana S (2021) Isotopic abundance ratio analysis of consciousness energy healing treated folic acid. Food Nutr Current Res 4(2): 290-295.
19. Trivedi MK, Branton A, Trivedi D, Nayak G, Mondal SC, Jana S (2015) Morphological characterization, quality, yield and DNA fingerprinting of biofield energy treated alphonso mango (*Mangifera indica* L.). Journal of Food and Nutrition Sciences 3: 245-250.
20. Trivedi MK, Jana S (2019) *In vitro* assessment of the biofield treated test item on cardiac function using rat cardiomyocytes cell line (H9c2) *via* multiparametric analysis. Journal of Hypertension and Cardiology 2(4): 1-12.
21. Trivedi MK, Branton A, Trivedi D, Jana S (2021) Effect of consciousness energy healing treatment on the metal profile and properties of tellurium. Eng Technol Open Acc 3(5): 555623.
22. Mahendra KT, Alice B, Dahryn T, Snehasis J (2021) Consciousness energy healing treatment impacted the isotopic abundance ratio of 6-Mercaptopurine (6-MP). Nov Appro Drug Des Dev 5(5): 555673.
23. Trivedi MK, Jana S (2021) Anti-aging activity of biofield energy treated novel proprietary test formulation by assessment of vital biomarkers in cerebrospinal fluid (CSF) in Sprague Dawley rats. On J Neur & Br Disord 5(2): 2021.
24. Trivedi MK, Jana S (2021) Evaluation of biofield energy healing treatment based proprietary test formulation on gut health potential in colon cancer cell line (HT-29). J Pharmacol Clin Res 8(4): 555743.

How to cite this article: Mahendra K T, Alice B, Dahryn T, Snehasis J. Cellular Biomarkers ($CD4^+$, $CD8^+$), Immunoglobulins, Testosterone, and Hematology Analysis of Biofield Energy Treated Proprietary Formulation on L-NAME and HFD-Induced Cardiovascular Disorders in Sprague Dawley Rats. Int J Cell Sci & Mol Biol. 2021; 7(1): 555703. DOI: 10.19080/IJCSMB.2021.07.555703

25. Trivedi MK, Branton A, Trivedi D, Jana S (2020) The consciousness energy healing treatment and its impact on the isotopic abundance ratio analysis of flutamide. Drug Des Int Prop Int J 3(5): 2020.

26. Lichtenstein KA, Armon C, Buchacz K, Chmiel JS, Buckner K, et al. (2010) Low CD4+ T cell count is a risk factor for cardiovascular disease events in the HIV outpatient study. Clinical Infectious Diseases 51(4): 435-447.

27. Helleberg M, Kronborg G, Larsen CS, Pedersen G, Pedersen C, et al. (2013) CD4 decline is associated with increased risk of cardiovascular disease, cancer, and death in virally suppressed patients with HIV. Clinical Infectious Diseases 57(2): 314-321.

28. Kolbus D, Ljungcrantz I, Andersson L, Hedblad B, Fredrikson GN, et al. (2013) Association between CD8+ T-cell subsets and cardiovascular disease. J Intern Med 274(1): 41-51.

29. Nilsson J (2016) Can antibodies protect us against cardiovascular disease. EBioMedicine 9: 29-30.

30. Janket S, Meurman JH, Baird AE, Qvarnström M, Nuutinen P, et al. (2010) Salivary immunoglobulins and prevalent coronary artery disease. J Dent Res 89(4): 389-394.

31. M Webb C, Collins P (2017) Role of testosterone in the treatment of cardiovascular disease. Eur Cardiol 12(2): 83-87.

32. Kloner RA, Carson C, Dobs A, Kopecky S, Mohler ER (2016) Testosterone and cardiovascular disease. J Am Coll Cardiol 67(5): 545-557.

33. Goodale T, Sadhu A, Petak S, Robbins R (2017) Testosterone and the heart. Methodist Debakey Cardiovasc J 13(2): 68-72.

34. Sloan A, Gona P, Johnson AD (2015) Cardiovascular correlates of platelet count and volume in the Framingham heart study. Ann Epidemiol 25(7): 492-498.

35. Vinholta PJ, Hvas AM, Frederiksen, Bathum L, Jørgensene MK, et al. (2016) Platelet count is associated with cardiovascular disease, cancer, and mortality: A population-based cohort study. Thromb Res 148: 136-142.

36. Ani C, Ovbiagele B (2009) Elevated red blood cell distribution width predicts mortality in persons with known stroke. J Neurol Sci 277: 103-108.

37. Demir A, Yarali N, Fisgin T (2002) Most reliable indices in differentiation between thalassemia trait and iron deficiency anemia. Pediatr Int 44: 612-616.

38. Danese E, Lippi G, Montagnana M (2015) Red blood cell distribution width and cardiovascular diseases. J Thorac Dis 7(10): E402-E411.

This work is licensed under Creative Commons Attribution 4.0 License
DOI:10.19080/IJCSMB.2021.06.555703

Your next submission with Juniper Publishers will reach you the below assets

- Quality Editorial service
- Swift Peer Review
- Reprints availability
- E-prints Service
- Manuscript Podcast for convenient understanding
- Global attainment for your research
- Manuscript accessibility in different formats

(Pdf, E-pub, Full Text, Audio)

- Unceasing customer service

Track the below URL for one-step submission

https://juniperpublishers.com/online-submission.php

How to cite this article: Mahendra K T, Alice B, Dahryn T, Snehasis J. Cellular Biomarkers (CD4+, CD8+), Immunoglobulins, Testosterone, and Hematology Analysis of Biofield Energy Treated Proprietary Formulation on L-NAME and HFD-Induced Cardiovascular Disorders in Sprague Dawley Rats. Int J Cell Sci & Mol Biol. 2021; 7(1): 555703. DOI: 10.19080/IJCSMB.2021.07.555703