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Antiaging, Antistress, and Neuroprotective Potential of 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 study was planned to assess the antiaging, antistress, and neuroprotective potential of the Biofield Energy Treated/Blessed novel Proprietary Test Formulation and Biofield Energy Treatment per se to the animals on NG-nitro-L-arginine methyl ester A hydrochloride (L-NAME) and high fat diet (HFD)-induced cardiovascular model in Sprague Dawley rats using various functional biomarkers in cerebrospinal fluids (CSF). The functional biomarkers like klotho protein, dopamine, corticosterone, tao protein, and norepinephrine in CSF using ELISA assay for the assessment of antiaging, antistress, and neuroprotective activities. The test formulation was formulated including minerals (magnesium, zinc, copper, calcium, selenium, and iron), vitamins (ascorbic acid, pyridoxine HCl, vitamin B9, cholecalciferol, and cyanocobalamin), cannabidiol isolate, Panax ginseng extract, and β-carotene. The ingredients of the test formulation were divided into two parts; one part was denoted as the untreated and other part of the test formulation and three groups of animals received Blessing remotely for about 3 minutes by Mr. Mahendra Kumar Trivedi, a renowned Biofield Energy Healer. Klotho protein was significantly increased by 12.24%, 33.89%, 22.82%, 31.26%, and 21.66% 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/Blessed 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 (L-NAME + HFD + untreated test formulation) group (G4). Moreover, the level of dopamine was increased by 18.47% in the G6 group as compared to the G2 group. Corticosterone was significantly decreased by 95.96%, 93.61%, 94.68%, 97.96%, and 93.04% in the G5, G6, G7, G8, and G9 groups, respectively than G2 group. Additionally, the level of tao protein was increased by 12.16% in the G6 group as compared to the G2 group. Further, the level of norepinephrine was increased by 10% in the G7 group as compared to the G4 group. Overall, the data suggested a significant antiaging activity by increasing the levels of klotho protein, antistress activity by reducing the level of corticosterone, and neuroprotective activity by increasing the levels of dopamine, tao protein, and norepinephrine in CSF of the Biofield Energy Treated test formulation and Biofield Energy Treatment per se along with preventive measure on the animals that might be beneficial various types of cardiovascular disorders. Therefore, the results showed the significant slowdown the oxidative stress-related cardiovascular disease progression and its complications and/or symptoms in the preventive treatment group per se and/or Biofield Energy Treated/Blessed Test formulation groups (viz. G6, G7, G8, and G9).

Keywords: Biofield Treatment; Antiaging, Antistress; Neuroprotective; The Trivedi Effect®; ELISA; High Fat Diet; Cardiovascular Disorders

Introduction

Cardiovascular diseases (CVDs) are one of the leading causes of death worldwide [1]. World health organisation (WHO) reported that approximately 17.9 million people died due to CVDs per year, in which specifically more than 75% death occurs in the low- and middle-income countries, and 80% death are due to heart attacks and strokes [2]. Most aged peoples faced some sort of memory loss, which is supposed to be related with various brain aging biomarkers [3]. Mild cognitive impairment (MCI) and dementia are the common aging health issues, and the common risk factors for Alzheimer's disease, type 2 diabetes, hypertension,

obesity, and depression patients. According to the WHO, aged population number has been significantly increasing and would be doubled in 2050 [4]. Lifestyle modification is considered as one of the important factors to control the release of biomarkers like corticosterone, tau protein, klotho protein, etc., which are responsible for aging and memory loss [5]. Klotho protein is one the pleiotropic protein, which is associated with delaying the aging and enhances cognition [6,7]. However, its brain levels decrease with aging [8] naturally that result in decline in the cognitive ability. In addition, another important CSF biomarker for aging and cognitive health is the tau protein, also this is one of the best biochemical markers for Alzheimer's disease and play important role in cardiovascular system [9]. Different studies reported an important role of corticosteroid, dopamine, and norepinephrine on cardiovascular system. Therefore, in order to study the change in functional cerebrospinal fluids (CSF) biomarkers in presence of N^G-nitro-L-arginine methyl ester hydrochloride (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 (copper, selenium, iron, zinc, calcium, and magnesium), essential vitamins (pyridoxine HCl, vitamin B_9, cyanocobalamin, ascorbic acid, and cholecalciferol), and nutraceuticals (Ginseng, β-carotene, cannabidiol isolate (CBD)). All the minerals and vitamins used in the test formulation have significant functional role to provide vital physiological effects [10-12]. Besides, cannabidiol itself has wide range of pharmacological profile and has been reported to role in different disorders [13,14], while ginseng extract is regarded as the one of the best immune stimulants for overall immunity and antioxidative activity [15]. The present study was aimed to evaluate the antiaging, antistress, and neuroprotective potential of the Biofield Energy Treated Proprietary Test Formulation and Biofield Energy treatment *per se* to the animals on L-NAME and HFD-induced cardiovascular disorders in Sprague Dawley rats using various functional biomarkers in CSF.

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 [16-18]. National Center for Complementary/Alternative Medicine (NCCAM) recommended CAM with several clinical benefits as compared with the conventional treatment approach [19]. 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 [20,21]. The Trivedi Effect®-Consciousness Energy Healing was scientifically reported on various disciplines such as in the nutraceuticals [22], agriculture science [23], cardiac health [24], materials science [25,26], antiaging [27], Gut health [28], pharmaceuticals [29], overall human health and wellness. In this study, the authors wish 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 CSF biomarkers (klotho protein, dopamine, corticosterone, tao protein, and norepinephrine) in presence of L-NAME and HFD-induced cardiovascular disorders in Sprague Dawley Rats using standard ELISA assay.

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, 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), vitamin B9 (folic acid), 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 cerebrospinal fluids (CSF) biomarker panel (Klotho protein, Dopamine, Corticosterone, Tau Protein, Norepinephrine), specific ELISA kits were used, were procured from CUSABIO, USA.

Animals

The animals (male Sprague Dawley) were purchased from M/s. HYLASCO Biotechnology (India) Pvt. Ltd., India and 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 separately 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 tested 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 (n=10/per group) also received Biofield Energy Healing Treatment (known as the Trivedi Effect®) by Mr. Mahendra Kumar Trivedi under similar laboratory conditions for ~3 minutes. The Biofield Energy Healer was located in the USA, however the test formulation was located in the research laboratory of Dabur Research Foundation, New

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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 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 receive normal drinking water and a normal diet throughout the experimental period. The animals in groups G2-G9 were received L-NAME (20 mg/kg, *i.p.*) and a high fat diet (HFD) throughout the experimental period. At the end of the experimental period (8 weeks treatment), the animals were sacrifice and CSF were collected and subjected for biomarker estimation such as Klotho protein, Dopamine, Corticosterone, Tau Protein, Norepinephrine using suitable ELISA method.

Estimation of Klotho Protein, Dopamine, Corticosterone, Tau Protein, Norepinephrine in Cerebrospinal fluids (CSF)

The Klotho protein expression was determined using Rat Klotho ELISA Kit (Catalog Number CSB-E14958r) in rat's CSF in according to the manufacturer's instructions [30]. Others CSF biomarkers were also estimated using specific ELISA kits such as for dopamine (Cat. No. CSB-E08660r), corticosterone (CSB-E07014r), tau protein (CSB-E13729r), and norepinephrine (CSB-E07022r) 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.

Results and Discussion

Estimation of Klotho Protein in CSF

Klotho protein was measured in the cerebrospinal fluids (CSF) after treatment with the test formulation, and the data are shown in Figure 1. The level of klotho protein in the disease control (L-NAME + high fat diet (HFD) + 0.5% CMC) group (G2) was 399.87 ± 54.31 pg/mL, which was decreased by 20.78% as compared with the normal control (G1, 504.75 ± 58.11 pg/mL). Moreover, the positive control (captopril + atorvastatin) treatment (G3) showed the level of klotho *i.e.,* 383.31 ± 50.80 pg/mL. The level of klotho was increased by 11.00%, 32.40%, 21.46%, 29.80%, and 20.30% 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). Further, klotho protein expression was increased by 12.24%, 33.89%, 22.82%, 31.26%, and 21.66% in the G5, G6, G7, G8, and G9 groups, respectively as compared to the untreated test formulation group (G4) (Figure 1). Klotho is an antiaging protein mainly expressed abundantly in the heart and kidney tissues and responsible for cardioprotection. In the heart tissues, soluble Klotho (sKlotho) protects systolic dysfunction independently [30]. It acts by inhibiting the insulin/insulin-like growth factor-1 (IGF-1) signalling pathway that regulates oxidative stress and thus reduces the cell death. Further, a lot of evidence reported that Klotho deficiency leads to development of atherosclerosis, myocardial infarction, coronary artery disease, and left ventricular hypertrophy [31,32]. Therefore, an involvement of Klotho in the signalling pathways and in regulation of a proper cell metabolism could be a crucial factor in the cardiac and vascular protection. Overall, in this experiment the Biofield Energy Treated test formulation and Biofield Energy Treatment *per se* significantly increased the level of antiaging biomarker klotho in CSF, which might be helpful for the management of cardiovascular disorders. Thus, Biofield Energy Treatment would be the best alternative treatment approach to treat stress-induced cardiovascular dysfunctions by improving antiaging activity.

Estimation of Dopamine in CSF

The effect of the Biofield Energy Treated test formulation and Biofield Energy Treatment *per se* on the level of dopamine in CSF, is shown in Figure 2. The disease control (L-NAME + high fat diet (HFD) + 0.5% CMC) group (G2) showed the value of dopamine as 6.01 ± 0.3 ng/mL, which was increased by 66.15% as compared with the normal control (G1, 3.62 ± 0.3 ng/mL) group. However, positive control (captopril + atorvastatin) treatment group (G3) showed the level of dopamine in CSF *i.e.,* 3.37 ± 0.27 ng/mL as compared to the G2 group. The level of dopamine was increased by

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18.47% in the G6 (L-NAME + HFD + Biofield Energy Treatment *per se* to animals from day -15) group as compared to the untreated test formulation (G4) group (Figure 2). According to Varriale P (1999), reported that dopamine can protect heart from congestive heart failure (CHF) by stimulating β1-adrenergic receptor and α-adrenergic receptor stimulation [33]. Overall, in this study the Biofield Energy Treatment *per se* increased the brain motivation by increasing the level of neurotransmitter dopamine that could be beneficial in the cardiovascular patients related to CHF.

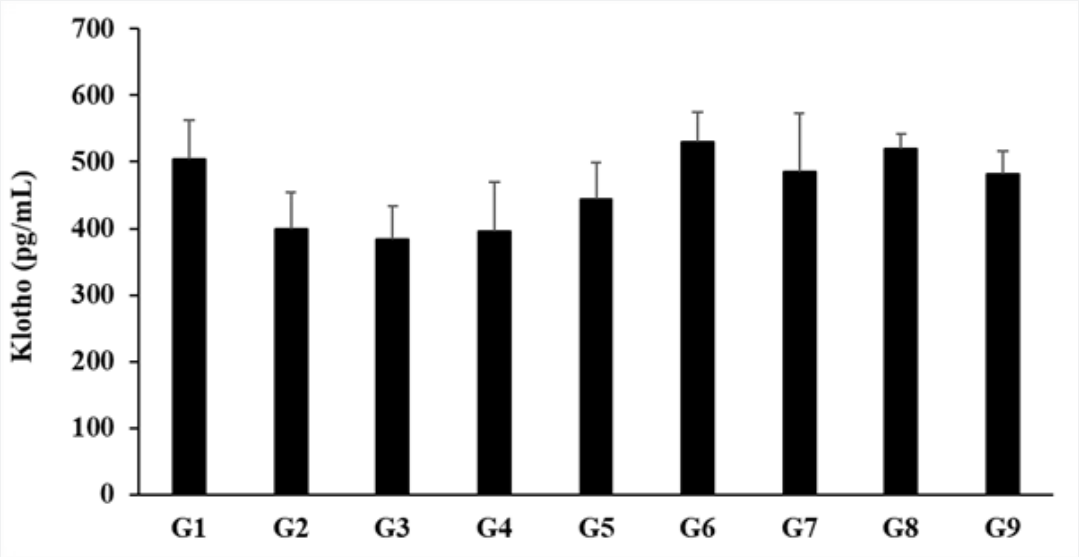

Figure 1: The effect of the test formulation on the level of klotho protein in CSF in Sprague Dawley rats. G: Group; G1 as normal control (vehicle, 0.5% w/v CMC-Na); G2 as disease control (L-NAME + high fat diet (HFD) + 0.5% CMC); G3 as reference item (L-NAME + HFD + Captopril + Atorvastatin); G4 includes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

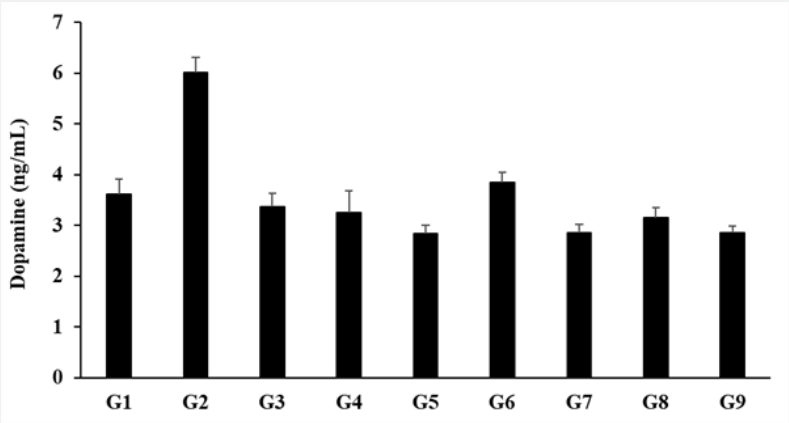

Figure 2: The effect of the test formulation on the level of dopamine in Sprague Dawley rats CSF.

Estimation of Corticosterone in CSF

The level of corticosterone in CSF was measured in all the experimental groups and the data are shown in Figure 3. The disease control (L-NAME + high fat diet, HFD + 0.5% CMC) group (G2) group showed the value of corticosterone as 1.2 ± 0.16 ng/mL, which was increased by 863.85% as compared with the normal control (G1, 0.12 ± 0.3 ng/mL) group. While the positive control (captopril + atorvastatin) treatment group (G3) decreased

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the level of corticosterone by 81.88% *i.e.,* 0.22 ± 0.04 ng/mL as compared to the G2 group. The level of corticosterone was significantly decreased by 87.07%, 95.96%, 93.61%, 94.68%, 97.96%, and 93.04% 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) (Figure 3). Corticosterone has both weak glucocorticoid and mineralocorticoid potencies in humans [34]. Cardiac fibrosis is a crucial factor for the development of heart failure. Inhibition of the mineralocorticoid receptor (MR) that attenuated cardiac fibrosis and reduce the chance of chronic heart failure [35]. Here, Biofield Energy Treatment/Blessing significantly reduced the stress hormone corticosterone in CSF, which might be due to inhibition of MR-receptor and could be beneficial in the cardiac patients.

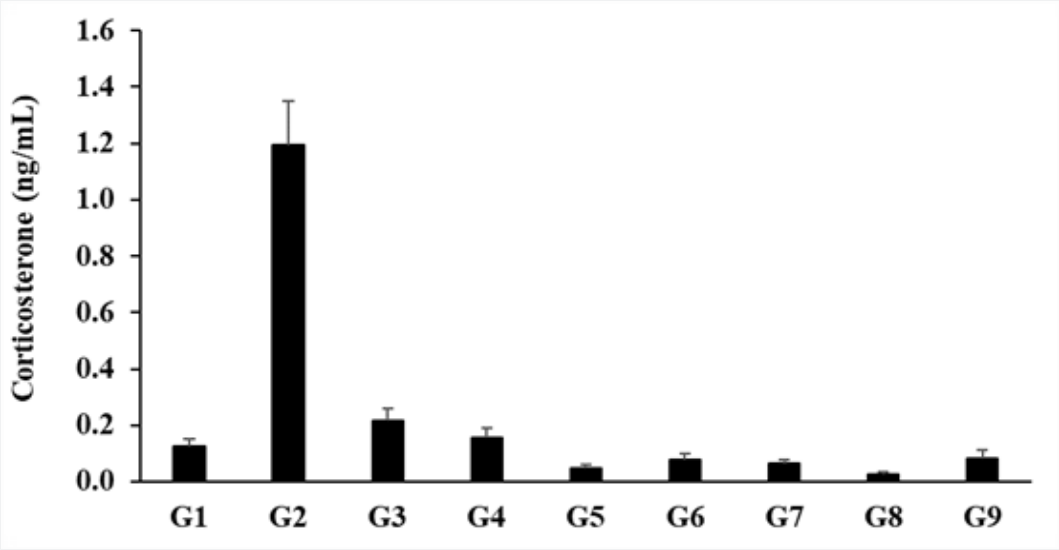

Figure 3: The effect of the test formulation on the level of corticosterone in Sprague Dawley rats CSF.

Estimation of Tao Protein in CSF

The effect of the test formulation and Biofield Energy Treatment *per se* was estimated by measuring the level of tao protein, and the results are shown in the Figure 4. The disease control (L-NAME + high fat diet, HFD + 0.5% CMC) group (G2) showed value of tao protein as 1985.03 ± 146.69 pg/mL, while it was found as 1483.69 ± 108.1 pg/mL in the positive control (captopril + atorvastatin) treatment (G3) group. The level of tao protein was increased by 12.16% in the G6 (L-NAME + HFD + Biofield Energy Treatment *per se* to animals from day -15) group as compared to the untreated test formulation (G4) group (Figure 4). The presence of tau protein in the cardiac tissue and its role in the cardiovascular system is confirmed by Betrie AH et al. [36], and reported that loss of tao protein in the heart leads to a deterioration in cardiovascular performance [36,37]. However, in this experiment the outcomes revealed that the Biofield Energy Treated test formulation and Biofield Energy Treatment *per se* to the animals directly increased the level of tao protein in CSF, which could be improved the cardiovascular performance.

Estimation of Norepinephrine (NE) in CSF

The effect of the Biofield Energy Treated test formulation and Biofield Energy Treatment *per se* on the level of norepinephrine/noradrenaline in CSF is shown in Figure 5. The level of norepinephrine in the disease control (L-NAME + high fat diet, HFD + 0.5% CMC) group (G2) was 154.36 ± 16.95 pg/mL, while in the positive control (captopril + atorvastatin) treatment (G3) 123.35 ± 17.63 pg/mL. The level of norepinephrine was increased by 10% in the G7 (L-NAME + HFD + the Biofield Energy Treated test formulation from day -15) group as compared to the untreated test formulation (G4) group (Figure 5). Elevated activities of the sympathetic nervous system the neurotransmitter, noradrenaline can release and acts on the renin-angiotensin-aldosterone system (RAAS), and plays a central role on cardiovascular systems [38]. Overall, here the Biofield Energy Treated test formulation has increased the level of norepinephrine in CSF sample, which could be improved the brain neurotransmitter-related physiological functions.

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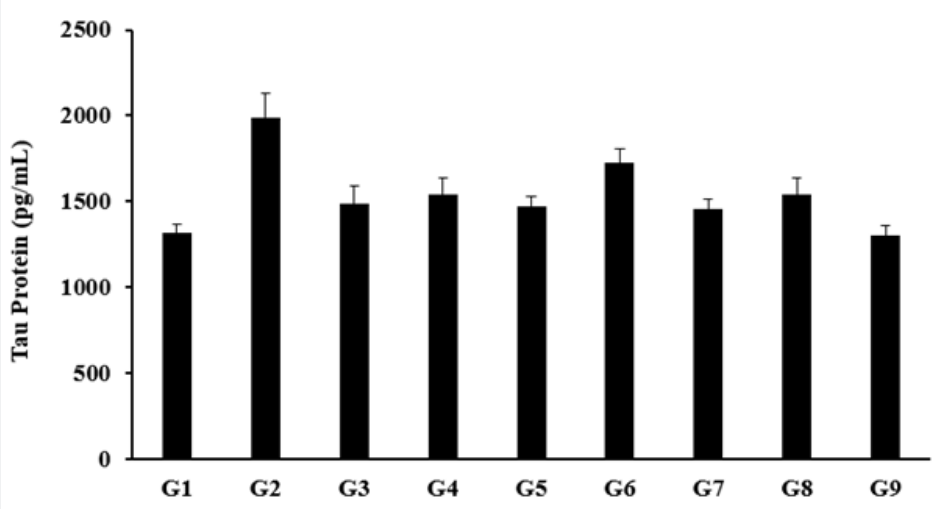

Figure 4: The effect of the test formulation on the level of Tao Protein in Sprague Dawley rats CSF.

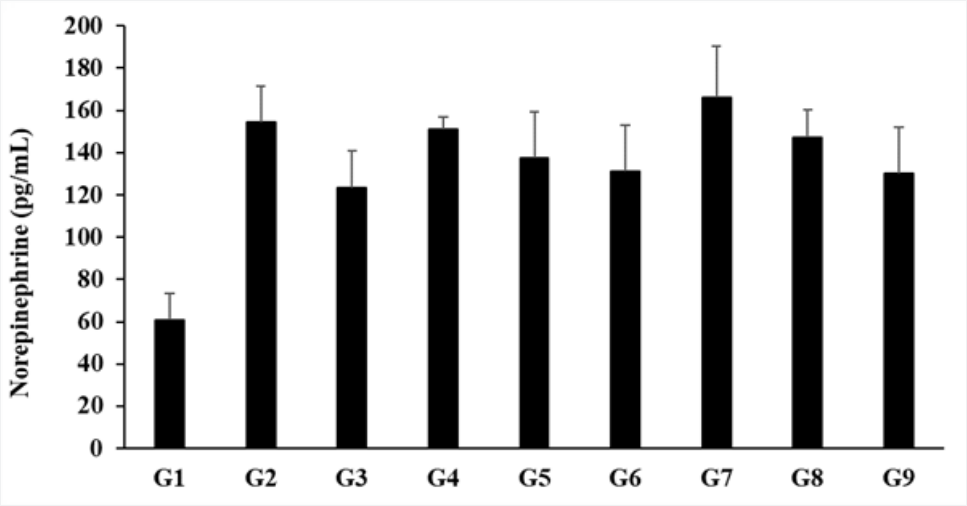

Figure 5: The effect of the test formulation on the level of norepinephrine (NE) level in Sprague Dawley rats CSF.

In this experiment, four preventive maintenance groups *viz.* G6, G7, G8, and G9 were considered. The results showed the significant slowdown of the disease progression, cardiovascular disease related all other symptoms/complications and reduced the chances of disease susceptibility in these groups. Specifically, group G6 (preventive Biofield Energy Treatment group *per se* at -15 days) showed the best results as a prophylactic/preventive treatment group compared to the other groups. Based on the overall data, it suggests that the Biofield Energy Healing Therapy/ Blessing was found to be most effective and beneficial to prevent the manifestation of diseases. It indicated that this therapy could act as a preventive maintenance therapy to prevent the occurrence of the disease, slowdown the disease progression and disease-related complications that will ultimately maintain the overall health and quality of life in human.

Conclusion

Based on the study outcomes it was observed that the level of klotho protein in cerebrospinal fluids (CSF) was significantly increased by 12.24%, 33.89%, 22.82%, 31.26%, and 21.66% in the 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 +

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Biofield Energy Treatment *per se* animals plus the untreated test formulation) groups, respectively, as compared to the (L-NAME + HFD + untreated test formulation) group (G4). Moreover, dopamine was increased by 18.47% in the G6 group as compared to the disease control (G2) group. Corticosterone was decreased by 95.96%, 93.61%, 94.68%, 97.96%, and 93.04% in the G5, G6, G7, G8, and G9 groups, respectively than G2 group. The level of tao protein was significantly increased by 12.16% in the G6 group as compared to the G4 group. Besides, the level of norepinephrine was increased by 10% in the G7 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 fruitful results with respect to different CSF biomarkers with antiaging, antistress, and neuroprotective activities in the preventive maintenance group, 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-related complications. Thus, the Blessing might act as a preventive maintenance therapy to maintain quality of life and overall health. "Biofield Therapy" might also reduce the severity of disease progression rate and manifestation of disease symptoms/disorders such as goitre, hyperthyroidism, hypothyroidism, Graves' disease. The test formulation can also be used against myasthenia gravis, Addison disease, psoriasis, multiple sclerosis, rheumatoid arthritis, aplastic anaemia, Crohn's disease, as well as various inflammatory disorders (ulcerative colitis, dermatitis, hepatitis), Parkinson's, stroke, etc.

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