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Identification of Endothelin-1 and Nitric Oxide Expression in Artery, Brain, and Kidney Tissues after Treatment with the Biofield Energy-Based Proprietary Formulation in Unpredictable Chronic Stress (UCS)-Induced Sprague Dawley Rats

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Abstract

Endothelin-1 and nitric oxide (NO) activities in artery, brain, and kidney tissues were performed in the unpredictable chronic stress (UCS) rat's model for the evaluation of Consciousness Energy Healing Treated (the Trivedi Effect®) novel proprietary test formulation in male Sprague Dawley (SD) rats using ELISA assay. The test formulation consisted of minerals (Zn, Fe, Cu, Se, Ca, Mg), vitamins (C, E, B_6, B_{12}, D_3), and nutraceuticals (β-carotene, ginseng, and cannabidiol isolate). The test formulation constituents were divided into two parts; one section was defined as the untreated test formulation, while the other portion of the test formulation and three groups of animals received Biofield Energy Healing Treatment/Blessing by a renowned Biofield Energy Healer, Mr. Mahendra Kumar Trivedi. Artery-tissue elasticity endothelin-1 was decreased by 58.1%, 41.7%, 51.3%, 52%, and 57.1% in the G5 (Biofield Energy Treated Test formulation to the untreated rats), G6 (Biofield Energy Treatment *per se* to the rats), G7 (15-days pre-treatment of Biofield Energy Treated Test formulation), G8 (15 days pre-treatment of Biofield Energy Treated Test formulation to the Biofield Energy Treatment *per se* rats), and G9 (untreated test formulation to the Biofield Energy Treatment *per se* to the rats) groups, respectively as compared with the disease control group (G2). Artery NO level was significantly decreased by 20.9%, 10%, and 30.1% ($p \leq 0.01$) in the G5, G6, and G9 groups, respectively as compared with the G2. Similarly, brain endothelin-1 level was significantly decreased by 30.1% ($p \leq 0.001$), 12.3%, 23.7% ($p \leq 0.001$), 43.4% ($p \leq 0.001$), and 43% ($p \leq 0.001$) in the G5, G6, G7, G8, and G9 groups, respectively as compared with the G2. Brain NO was increased by 12.1% and 21% in the G5 and G6 groups, respectively as compared with the G2. Endothelin-1 level in kidney was significantly decreased by 14.2% and 52.4% ($p \leq 0.001$) in the G8 and G9 groups, respectively as compared with the G2. Kidney NO level was significantly increased by 25%, 22.9%, 18.3%, 17.3%, and 23.4% in the G5, G6, G7, G8, and G9 groups respectively, as compared with the G4. Overall, the experimental data suggested significance effect of Biofield Energy/Blessing *per se* along with preventive measure on the animal with respect to various stress-related disorders. Overall, the results showed the significant slowdown the stress-related disease progression and its complications/symptoms in the preventive Biofield Energy Treatment group *per se* and/or Biofield Energy Treated/Blessed Test formulation groups (*viz.* G6, G7, G8, and G9) comparatively with the disease control group.

Keywords: Biofield Treatment, Endothelin-1; Nitric Oxide; Artery; The Trivedi Effect®; Unpredictable Chronic Stress; ELISA

Introduction

Chronic psychological stress has been known to accelerate the biological aging, and in this regard, the oxidative damage to the body is considered as a potential mediator of this process. According to the Free Radical Theory of Aging [1], the effect of oxidative damage get accumulated over time and thereby causes cellular aging, which became the main

contributor to the age-related disease. Such oxidative damage is known to be caused due to the unmitigated reactive oxygen species (ROS) by antioxidants. It is well known that ROS under healthy conditions is basically generated as a by-product of mitochondrial respiration [2], which acts to fuel the basic metabolic processes. However, the oxidative stress is considered as a state of cellular imbalance, in which there is excess ROS production than the neutralized ROS by the antioxidant mechanisms; thereby causing the oxidative damage to DNA, RNA and lipids, *etc* [3]. Various scientific studies reported that the chronic stress majorly acts as the contributor to depressive illness and could be considered to link it with peripheral vascular disease (PVD) [4,5]. The stress-induced depression may cause vascular dysfunction, up to some extent, by affecting the bioavailability of dilator metabolites such as nitric oxide (NO) [6-8]. There were various studies that indicated the relation between chronic psychological stress to the increased risk of atherosclerotic diseases, such as heart attack and stroke by leading the formation of plaque in the arteries. Besides, chronic stress affects the status of stress hormones in the body such as cortisol and catecholamine, which further affect the regulation of flow and blood pressure, and thereby, may cause the platelet drilling, endothelial injury, and hematopoietic stem cell proliferation [9,10]. On the other hand, the studies also reported the link of chronic stress to the macroscopic changes in certain brain areas, thereby causing the volume variations as well as the physical modifications to the network of neurons. Similarly, the animal studies indicated the impact of stress in the prefrontal cortex (PFC) and limbic system of the brain in terms of volume reductions of some structures, decreased spine density, and changes in the neuronal plasticity due to dendritic atrophy [11,12].

Besides, such impact of stress on the pathophysiology of the body is somewhat also found to be associated with the altered activity of the sympathetic/autonomic nervous system, inflammatory cytokines, the hypothalamic-pituitary-adrenal axis, and endothelin-A. Such changes may occur due to the increased glucocorticoid secretion, as well as the increased levels of inflammatory cytokines [13]. Therefore, these changes also suggest that there might be the pathologic link between stress, hypertension, and chronic kidney disease (CKD) as the neural mechanisms helps in regulating the sodium and water retention system, while the sympathetic nerves innervate all segments of the kidney [14-17]. Thus, in order to study the change after unpredictable chronic stress (UCS), endothelin-1 and NO activity in artery, brain, and kidney was estimated in presence of novel test formulation. The test formulation consist of vital minerals (selenium, zinc, iron, calcium, copper, and magnesium), essential vitamins (cyanocobalamin, ascorbic acid, pyridoxine HCl, alpha tocopherol, and cholecalciferol), and nutraceuticals *(β-carotene, Ginseng, (CBD)* cannabidiol isolate). Minerals and vitamins have been used to have significant functional physiological role [18-20]. Cannabidiol has wide range of potent biological activity [21,22], while ginseng is the best immune booster [23]. The test formulation was treated with the Biofield Energy Treatment (a Complementary and Alternative Medicine, CAM) by a renowned Biofield Energy Healer and tested for various biological properties.

Within the increasing escalating ground of CAM therapies, Biofield Energy Treatment is one of the emerging therapy with significant benefits. Biofield Energy Healing Treatment has been reported with significant effects against many disorders in form of Complementary and Alternative Medicine (CAM) approach [24-26]. National Center for Complementary/ Alternative Medicine (NCCAM) recommended CAM with several clinical benefits as compared with the conventional treatment approach [27]. 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 [28,29]. The Trivedi Effect®-Consciousness Energy Healing Treatment/Blessing was scientifically reported on various disciplines such as in the materials science [30,31], agriculture science [32], antiaging [33], gut health [34], nutraceuticals [35], pharmaceuticals [36], overall human health and wellness. Thus, the present study was aimed to evaluate the effect of the Biofield Treated Proprietary test formulation and Biofield Energy Treatment *per se* to the animals on the serum levels of endothelin-1 and NO in artery, brain, and kidney under the UCS-induced Sprague Dawley rats using standard ELISA assay.

Material and Methods

Chemicals and Reagents

The novel test formulation designed was constituted with pyridoxine hydrochloride (vitamin B_6), calcitriol, zinc chloride, magnesium (II) gluconate, and β-carotene (retinol, provit A), which were purchased from TCI, Japan. Copper chloride, cyanocobalamin (vitamin B_{12}), calcium chloride, vitamin E (alpha-tocopherol), cholecalciferol (vitamin D_3), iron (II) sulfate, 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. Imipramine

Hydrochloride was purchased from Sigma, USA. For the estimation of endothelin-1 and nitric oxide (NO), specific ELISA kits were used such as for detection, which were procured from CUSABIO and My Bio Source, USA respectively.

Study Design

The current experiment was designed to fulfil the study protocol, animals were assigned into nine (9) groups. G1: Normal control; G2: Disease control (UCS: Unpredictable chronic stress + 0.5% CMC); G3: Reference item (UCS + Imipramine hydrochloride 30 mg/kg); G4: (UCS + Untreated test formulation); G5: (UCS + Biofield Energy Treated/ Blessed test formulation); G6: (UCS + Biofield Energy Treatment *per se* to animals from day -15; G7: (UCS + Biofield Energy Treated test formulation from day -15); G8: (UCS + Biofield Energy Treatment/Blessing *per se* plus Biofield Energy Treated test formulation from day -15), and G9: (UCS + Biofield Energy Treatment *per se* animals plus untreated test formulation).

Maintenance of Animal

Randomly breed male Sprague Dawley (SD) rats with body weight ranges from 200 to 300gm were used in this study. The animals were purchased from M/s. Vivo Bio Tech, Hyderabad, India. Animals were randomly divided into nine groups based on their body weights consist of 6 animals of each group. 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 of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Govt. of India. The test facility is registered (registration no. 64/PO/br/s/99/CPCSEA) for animal experiments with the CPCSEA. The animals were procured using protocol approved by the Animal Ethics Committee (IAEC/41/505) and the husbandry conditions were maintained as per the recommendations of the CPCSEA.

Consciousness Energy Healing Strategies

The novel test formulation was subjected to Biofield Energy Healing Treatment, thus each ingredients were distributed into two parts. The test formulation one part constituents did not received any sort of treatment and was defined as the untreated or control sample. The second part of the test formulation and three group of animals were 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. The Biofield Energy Healer was located in the USA; however the test formulation were located in the research laboratory of Dabur Research Foundation, New Delhi, India. The energy transmission was done without touching the samples or animals. 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 under the same laboratory conditions. The "sham" healer did not have any knowledge about the Biofield Energy Treatment. The Biofield Energy Treated animals were also taken back to experimental room for further proceedings.

Experimental Test Procedure

For experimental procedure, animals were randomized and grouped based on the body weight seven days after acclimatization. Groups G7 and G8 were started dosing on day -15 and continued till end of the experiment. However, G1 to G5 and G9 groups were dosed from day 1 till the end of experiment. G6 group was not to be dosed with the test formulation. Body weight and clinical signs were taken daily throughout the experimental period. All the animals except G1 group received stress induced procedures such as stress procedures like sound stress, tilted cages and crowd stress, cold and warm water swim stress, food and water deprivation, stress due to change in the light and dark cycle were undergo seven different types of unpredictable stress procedures after scheduled dosing daily at specified interval to the end of the experiment for 8 weeks after the initiation of stress, which vary every week interval *i.e.* shuffling of stress type. During 4th week of the experimental period, all the animals were individually subjected for blood collection for the experimental purpose.

Preparation of Sample for ELISA Assay

With the continued stress treatment of 4th 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 endothelin-1 and NO in the artery, brain, and kidney during final calculations.

Estimation of Endothelin-1 and NO (Artery, Brain, and Kidney)

The serum from all the animals groups after experimental period was subjected for the estimation of level of endothelin-1 and NO in the artery, brain, and kidney was performed. The entire assay 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 protein and their specific antibody.

Statistical Analysis

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

Results and Discussion

Estimation of Artery Endothelin-1 and NO

The level of endothelin-1 and NO in artery was measured in all the experimental groups and was graphically presented in the Figures 1 & 2 respectively.

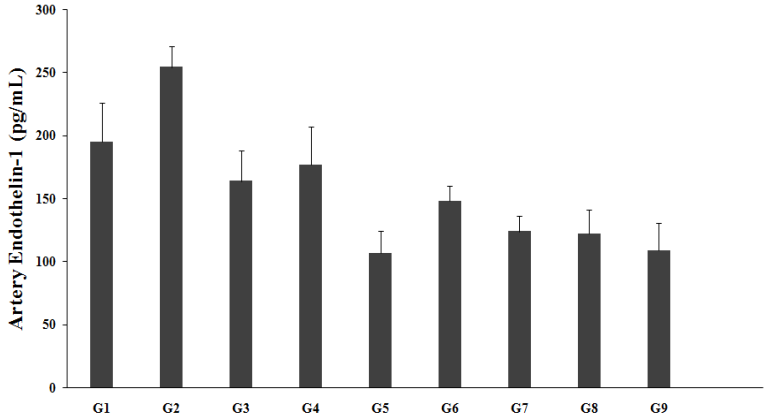

Figure 1: Effect of the test formulation on the level of artery endothelin-1 in Sprague Dawley rats. G: Group; G1: Normal control; G2: Disease control (UCS: Unpredictable chronic stress + 0.5% CMC); G3: Reference item (UCS + Imipramine hydrochloride 30 mg/kg); G4: (UCS + Untreated test formulation); G5: (UCS + Biofield Energy Treated test formulation); G6: (UCS + Biofield Energy Treatment *per se* to animals from day -15; G7: (UCS + Biofield Energy Treated test formulation from day -15); G8: (UCS + Biofield Energy Treatment *per se* plus Biofield Energy Treated test formulation from day -15), and G9: (UCS + Biofield Energy Treatment *per se* animals plus untreated test formulation). Values are presented as mean ± SEM (n=6).

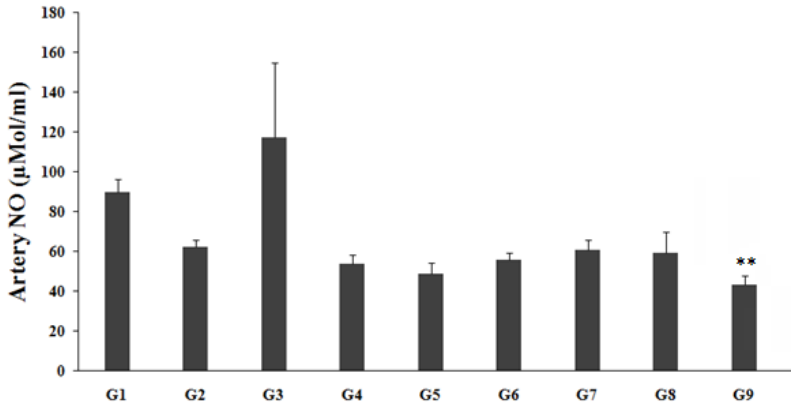

Figure 2: Effect of the test formulation on the level of artery nitric oxide (NO) in Sprague Dawley rats. G: Group; G1: Normal control; G2: Disease control (UCS: Unpredictable chronic stress + 0.5% CMC); G3: Reference item (UCS + Imipramine hydrochloride 30 mg/kg); G4: (UCS + Untreated test formulation); G5: (UCS + Biofield Energy Treated test formulation); G6: (UCS + Biofield Energy Treatment *per se* to animals from day -15; G7: (UCS + Biofield Energy Treated test formulation from day -15); G8: (UCS + Biofield Energy Treatment *per se* plus Biofield Energy Treated test formulation from day -15), and G9: (UCS + Biofield Energy Treatment *per se* animals plus untreated test formulation). Values are presented as mean ± SEM (n=6). $^{**}p \leq 0.01$ *vs.* G2.

Artery Endothelin-1: Endothelin-1 is a vasoactive and mitogenic polypeptide, synthesized and secreted by endothelial cells, which acts on specific endothelin receptor-A (ETA) on vascular smooth muscle, causing sustained powerful vasoconstriction. Endothelin-1 plays a central role in many vascular diseases such as pulmonary hypertension, vascular disease, angioplasty restenosis, allograft vasculopathy, atherosclerosis, ischemic heart disease, stroke, and vasculitis. Thus, it also participate in vascular damage to many heart diseases and elevates the blood pressure (BP). Stress may induce the production of endothelin-1 and treatment might regulate the level after Biofield Energy Treatment [37]. Artery-tissue elasticity endothelin-1 level in unpredictable chronic stress (G2) group was found to be increased by 30.5% (254.17 ± 16.3 pg/mL) as compared to the normal control (G1, 194.83 ± 30.8 pg/mL). Imipramine treatment (G3) group decreased endothelin-1 by 35.6% (163.73 ± 24.0 pg/mL) as compared to the G2. Untreated test formulation to the untreated rats (G4) showed decreased endothelin-1 level by 30.5% (176.53 ± 30.3 pg/mL) as compared to the G2. Biofield Energy Treated test formulation to the untreated rats (G5) showed a decreased endothelin-1 level (106.43 ± 17.7 pg/mL) by 58.1% and 39.7% as compared to the G2 and G4 groups, respectively. Biofield Energy per se to the rats (G6) significantly decreased endothelin-1 level (148.09 ± 11.8 pg/mL) by 41.7% and 16.1% as compared to the G2 and G4, respectively. 15 days pre-treatment of Biofield Energy Treated test formulation (G7) significantly decreased endothelin-1 level (123.72 ± 12.5 pg/mL) by 51.3% and 29.9% as compared to the G2 and G4, respectively. 15 days pre-treatment of Biofield Energy Treated test formulation to the Biofield Energy Treated rats (G8) significantly decreased endothelin-1 level (122.05 ± 19.1 pg/mL) by 52% and 30.9% as compared to the G2 and G4, respectively. Untreated test formulation to the Biofield Energy treated rats (G9) significantly decreased endothelin-1 level (108.99 ± 21.3 pg/mL) by 57.1% and 38.3% as compared to the G2 and G4, respectively. Thus, treatment significantly reduced the enhanced level of endothelin-1 level in artery, which is very useful for various cardiac diseases.

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Artery NO: NO has wide range of biological activities such as protection of vessels, vascular homeostasis, cell growth, and many more. Besides, it is the soluble gas synthesized by endothelium and showed an importance in coronary diseases such as hypertension and hypercholesterolemia. Different therapies were reported to improve the NO level and regulate the endothelial dysfunction by increasing the NO synthesis or protection of nitric oxide from oxidative inactivation [38,39]. NO level in the artery was measured in all the experimental groups are graphically presented in the Figure 2. Artery-tissue elasticity NO level (62.09 ± 3.5 µMol/mL) in the G2 was decreased by 30.6% as compared to the normal control (G1, 89.53 ± 6.9 µMol/mL). Imipramine (G3) showed increased NO level (117.3 ± 37.3 µMol/mL) by 88.6% as compared to G2. G4 group showed decreased NO level (53.79 ± 4.6 µMol/mL) by 13.4% as compared to G2. G6, G7, and G8 group showed an increased NO level in artery by 3.9%, 13.2%, and 10.2% groups, respectively as compared with the untreated test formulation (G4). Besides, G5 and G9 groups showed a decreased NO level by 8.7% and 19.3%, respectively as compared with the G4. In addition, G5, G6, G7, G8, and G9 groups showed a significant reduced value of artery NO by 20.9%, 10%, 1.9%, 4.5%, and 30.1% ($p \leq 0.01$) respectively, as compared with the G2.

Estimation of Brain Endothelin-1 and NO

Brain microvascular endothelial cells are another production source of high level of endothelin-1 during bacterial meningitis, which has high co-relationship with clinical abnormalities in cerebral blood flow. Thus, high level of endothelin-1 mediated the inflammatory sequence that potentiates different form of inflammation into the brain injured tissue [40]. Similarly, NO is one of the nervous system messenger, which plays a significant roles in many neurobiological processes. Thus, the regulation of brain endothelin-1 and NO was studies, which has a huge implication in nervous disorders [41]. The level of endothelin-1 and NO in brain was measured in all the experimental groups and are graphically presented in the Figures 3 & 4 respectively.

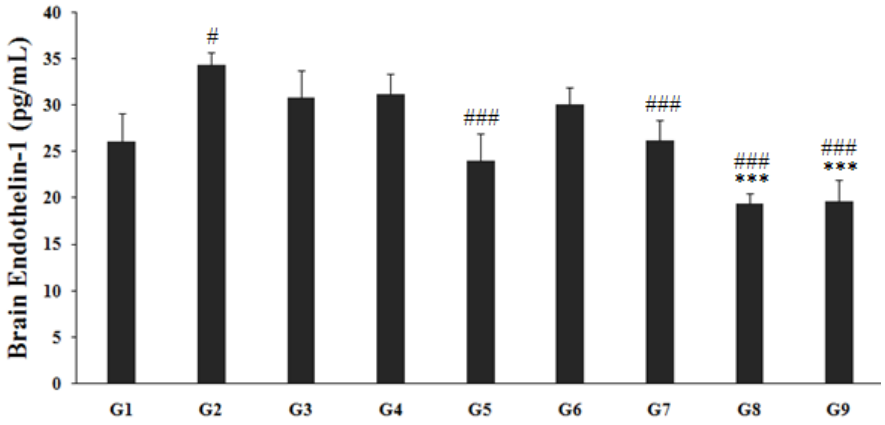

Figure 3: Effect of the test formulation on the level of brain endothelin-1 in Sprague Dawley rats. G: Group; G1: Normal control; G2: Disease control (UCS: Unpredictable chronic stress + 0.5% CMC); G3: Reference item (UCS + Imipramine hydrochloride 30 mg/kg); G4: (UCS + Untreated test formulation); G5: (UCS + Biofield Energy Treated test formulation); G6: (UCS + Biofield Energy Treatment *per se* to animals from day -15; G7: (UCS + Biofield Energy Treated test formulation from day -15); G8: (UCS + Biofield Energy Treatment *per se* plus Biofield Energy Treated test formulation from day -15), and G9: (UCS + Biofield Energy Treatment *per se* animals plus untreated test formulation). Values are presented as mean ± SEM (n=6). $^{\#}p\leq0.05$ *vs.* G1, $^{\#\#\#}p\leq0.001$ *vs.* G2, and $^{***}p\leq0.001$ *vs.* G4.

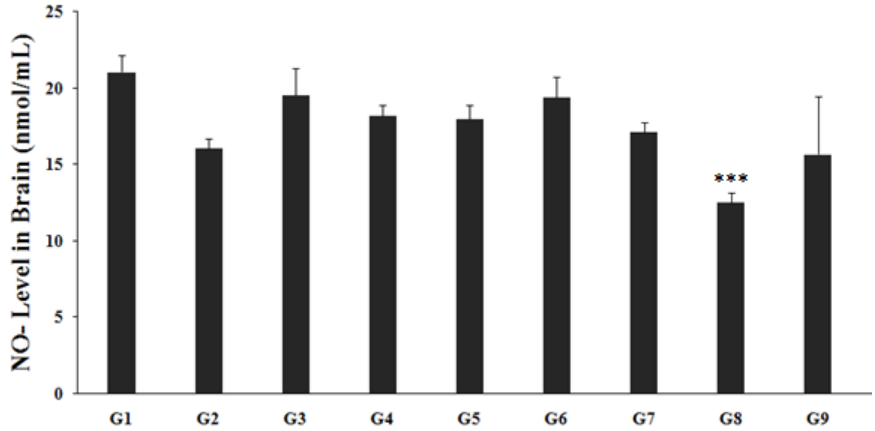

Figure 4: Effect of the test formulation on the level of brain nitric oxide (NO) in Sprague Dawley rats. G: Group; G1: Normal control; G2: Disease control (UCS: Unpredictable chronic stress + 0.5% CMC); G3: Reference item (UCS + Imipramine hydrochloride 30 mg/kg); G4: (UCS + Untreated test formulation); G5: (UCS + Biofield Energy Treated test formulation); G6: (UCS + Biofield Energy Treatment *per se* to animals from day -15; G7: (UCS + Biofield Energy Treated test formulation from day -15); G8: (UCS + Biofield Energy Treatment *per se* plus Biofield Energy Treated test formulation from day -15), and G9: (UCS + Biofield Energy Treatment *per se* animals plus untreated test formulation). Values are presented as mean ± SEM (n=6). $^{***}p\leq0.001$ *vs.* G4.

Brain Endothelin-1: The level of brain-tissue elasticity endothelin-1 in the G2 group was 34.37 ± 1.38 pg/mL, which was significantly ($p \leq 0.05$) increased by 31.7% as compared to the normal control (G1, 26.10 ± 3.00 pg/mL). The positive control group included imipramine treatment (G3) which showed a decreased endothelin-1 level (30.86 ± 2.90 pg/mL) by 10.2% as compared to the G2. G4 group results showed a decreased value of endothelin-1 (31.24 ± 2.17 pg/mL) by 9.1% as compared to the G2. However, experimentally controlled groups such as G5, G6, G7, G8, and G9 showed a significant decreased value of brain endothelin-1 level by 23.1%, 3.6%, 16%, 37.8% ($p \leq 0.001$), and 37.3% ($p \leq 0.001$), respectively as compared with the G4. Similarly, the level of endothelin-1 was also significantly reduced by 30.1% ($p \leq 0.001$), 12.3%, 23.7% ($p \leq 0.001$), 43.4% ($p \leq 0.001$), and 43% ($p \leq 0.001$) in the G5, G6, G7, G8, and G9 groups respectively, as compared with the G2.

Brain NO Level: In addition, brain NO level was estimated and the data suggested that the level was 16.02 ± 0.7 nmol/mL, which was decreased by 23.9% as compared to the normal control (G1, 21.05 ± 1.1 nmol/mL). Imipramine treatment (G3) showed increased NO level (19.50 ± 1.8 nmol/mL) by 21.7% as compared to the G2. Untreated test formulation to the untreated rats (G4) showed increased NO level (18.17 ± 0.7 nmol/mL) by 13.4% as compared to the G2. Experimental control group showed increased level of brain NO by 12.1%, 21%, and 6.9% in the G5, G6, and G7 groups, respectively as compared with the G2 group. Besides, G6 group showed increased value NO value of 6.7% as compared with the G4 group. However, other groups showed a significant reduced value of brain NO by 5.8%, 31% ($p \leq 0.001$), and 13.8% in the G7, G8, and G9 groups, respectively as compared with the G4 group; while G8 group showed 21.8% reduced as compared to the G2.

Estimation of Kidney Endothelin-1 and NO

Endothelin-1 has huge implication as physiological and pathophysiological kidney processes. Endothelin-1 has been reported its role in the development and progression of chronic kidney disease, also have implication in the glomerular and tubular damage, and Na^+/water homeostasis [42,43]. NO in kidney play many functioning aspect in renal haemodynamics, mediation of pressure-natriuresis, medullary perfusion, tubuloglomerular feedback, inhibition of tubular sodium reabsorption, and in renal sympathetic neural activity [44]. The level of endothelin-1 and NO in kidney was measured in all the experimental groups and are graphically presented in the Figures 5 & 6 respectively.

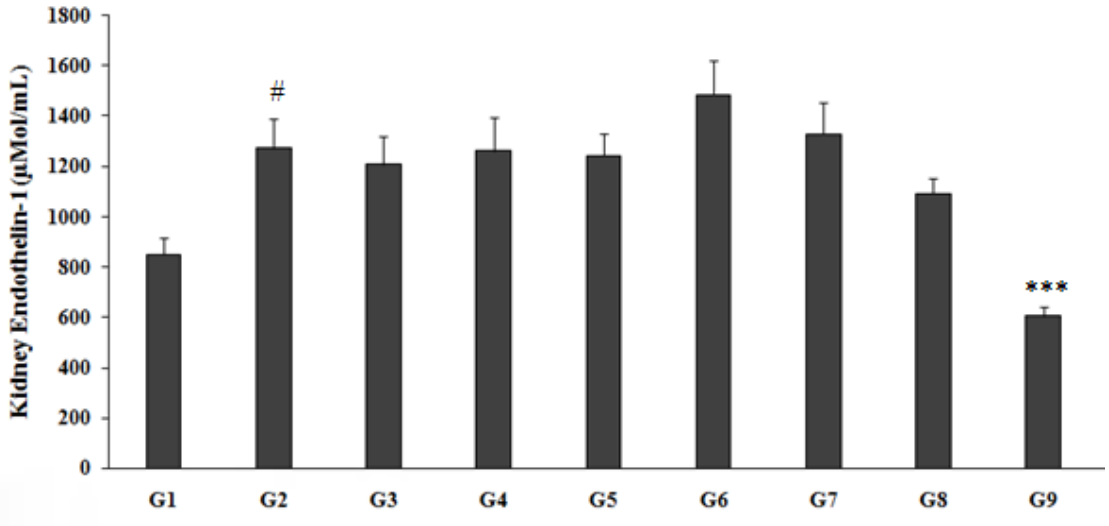

Figure 5: Effect of the test formulation on the level of kidney endothelin-1 in Sprague Dawley rats. G: Group; G1: Normal control; G2: Disease control (UCS: Unpredictable chronic stress + 0.5% CMC); G3: Reference item (UCS + Imipramine hydrochloride 30 mg/kg); G4: (UCS + Untreated test formulation); G5: (UCS + Biofield Energy Treated test formulation); G6: (UCS + Biofield Energy Treatment *per se* to animals from day -15; G7: (UCS + Biofield Energy Treated test formulation from day -15); G8: (UCS + Biofield Energy Treatment *per se* plus Biofield Energy Treated test formulation from day -15), and G9: (UCS + Biofield Energy Treatment *per se* animals plus untreated test formulation). Values are presented as mean ± SEM (n=6).

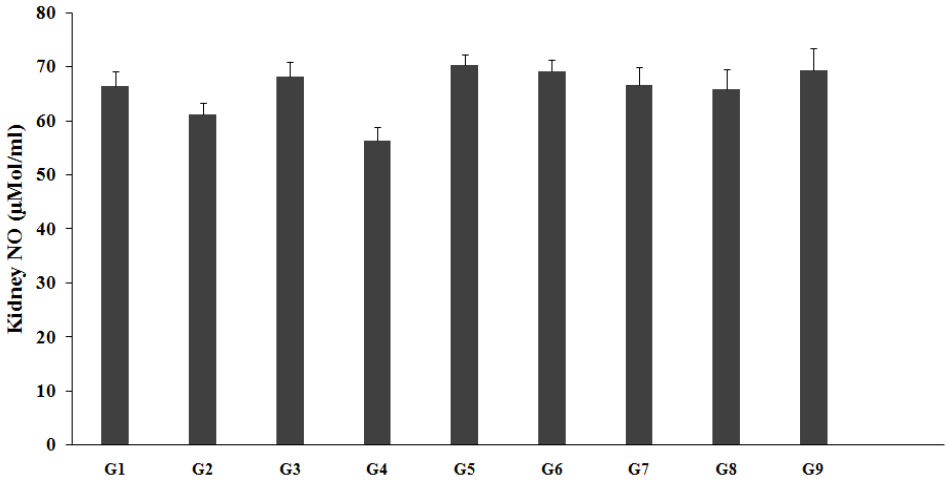

Figure 6: Effect of the test formulation on the level of kidney NO in Sprague Dawley rats. G: Group; G1: Normal control; G2: Disease control (UCS: Unpredictable chronic stress + 0.5% CMC); G3: Reference item (UCS + Imipramine hydrochloride 30 mg/kg); G4: (UCS + Untreated test formulation); G5: (UCS + Biofield Energy Treated test formulation); G6: (UCS + Biofield Energy Treatment *per se* to animals from day -15; G7: (UCS + Biofield Energy Treated test formulation from day -15); G8: (UCS + Biofield Energy Treatment *per se* plus Biofield Energy Treated test formulation from day -15), and G9: (UCS + Biofield Energy Treatment *per se* animals plus untreated test formulation). Values are presented as mean ± SEM (n=6).

Kidney Endothelin-1: The level of kidney-tissue elasticity endothelin-1 level was estimated in the unpredictable chronic stress (G2, 1272.01 ± 117.4 pg/mL), which was significantly ($p \leq 0.05$) increased by 50% as compared to the normal control (G1, 848.14 ± 68.5 pg/mL). Imipramine treatment (G3) showed decreased endothelin-1 level (1207.55 ± 109.2 pg/mL) by 5.1% as compared to the G2. Similarly, untreated test formulation to the untreated rats (G4) showed a decreased endothelin-1 level with value 1262.13 ± 131.8 pg/mL as compared to G2. G8 and G9 groups reported with significant decreased kidney endothelin-1 value by 14.2% and 52.4% ($p \leq 0.001$), respectively as compared with the G2. However, endothelin-1 level in kidney was significantly reduced by 13.5% and 52.1% in the G8 and G9 respectively, as compared with the G4. Other test groups showed a significant alteration in the kidney endothelin-1 value as compared with the untreated test formulation group.

Kidney NO Level: Kidney tissue elasticity NO level was tested and reported to be changed in the unpredictable chronic stress (G2, 61.12 ± 2.2 µMol/mL), which was decreased by 8% as compared to the normal control (G1, 66.46 ± 2.6 µMol/mL). Imipramine treatment (G3) showed increased NO level (68.18 ± 2.8 µMol/mL) by 11.6% as compared to the G2. The untreated test formulation group to the untreated rats (G4) showed decreased NO level as 56.22 ± 2.7 µMol/mL by 8% as compared to G2. On the contrary, after Biofield Energy Treatment, the level of kidney NO in experimental test groups were increased by 25%, 22.9%, 18.3%, 17.3%, and 23.4% in the G5, G6, G7, G8, and G9 groups, respectively as compared with the G4 group. Besides, the kidney NO level was also increased by 15%, 13.1%, 8.8%, 7.8%, and 13.5% in the G5, G6, G7, G8, and G9 groups, respectively as compared with the G2 group. Thus, the experimental data suggested that Biofield Energy Healing Treatment *per se* and the test formulation play a significant role in modulating the endothelin-1 and NO activity in brain, artery and kidney.

In this research plan, four groups were considered as preventive maintenance groups. These groups were G6 (Biofield Energy Treatment *per se* to animals at -15 days), G7 (Biofield Energy Treated test formulation from day -15), G8 (Biofield Energy Treatment *per se* to animals along with Biofield Treated (Blessed) test formulation from day -15), and G9 (Biofield treatment *per se* at -15 days to animals with untreated test formulation). The results showed the significant slowdown of the disease progression, stress-related all other symptoms/complications and also 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 benefited in order to prevent and protect from the occurrence of any type of diseases in rat model. It

indicated that this therapy can act as a preventive maintenance therapy to prevent the occurrence of the disease, slowdown the disease progression and disease-related complications of the existing ailments that will ultimately improve the overall health and quality of life in human.

Conclusion

The present study demonstrated a significant role of Biofield Energy Healing Treatment/Blessing to evaluate the test formulation activity on the level of endothelin-1 and nitric oxide levels in artery, brain, and kidney tissues in presence of unpredictable chronic stress (UCS) animal model. The data revealed the significance role of the Biofield Energy Treated/Blessed test formulation and Biofield Energy Treatment *per se* on the animal stress level using endothelin-1 and nitric oxide as compared with the other groups. Endothelin-1 level in artery was measured and reported to be significantly decreased by 58.1%, 41.7%, 51.3%, 52%, and 57.1% in the G5 (Biofield Energy Treated Test formulation to the untreated rats), G6 (Biofield Energy Treatment *per se* to the rats), G7 (15-days pre-treatment of Biofield Energy Treated Test formulation), G8 (15 days pre-treatment of Biofield Energy Treated Test formulation to the Biofield Energy Treatment *per se* rats), and G9 (untreated test formulation to the Biofield Energy Treatment *per se* to the rats) groups, respectively as compared with the disease control group (G2). Similarly, artery NO level was significantly decreased by 20.9% and 30.1% in the G5 and G9 groups, respectively as compared with the G2. However, the brain endothelin-1 level was significantly reduced by 30.1%, 43.4%, and 43% in the G5, G8, and G9 groups, respectively as compared with the G2. However, the brain NO level was increased by 21% in the G6 group as compared with the G2. Similarly, kidney endothelin-1 was significantly decreased by 52.4% in the G9 group as compared with the G2. However, the NO level in kidney was significantly increased by 25%, 22.9%, and 23.4% in the G5, G6, and G9 groups, respectively as compared with the G4. Biofield Energy Healing Treatment (the Trivedi Effect®) *per se* showed the best results with respect to different efficacy and biomarker parameters in the preventive maintenance group, G6 as compared to the other preventive maintenance groups (G7, G8, and G9) in rat model study. It also helped to slowdown the disease progression and disease-related complications of the overall animal's health. These data suggested that Biofield Energy Treatment *per se* and/or Biofield Energy Treated Test formulation in combination would be the best treatment strategies in order to prevent and protect from the occurrence of any type of diseases. Therefore, the Biofield Energy Treatment might act as a preventive maintenance therapy in order to maintain good health, or full restoration of health or improve the overall health and quality of life in human. This therapy might also reduce the severity of any type of acute/chronic diseases (auto-immune-related and inflammatory disorders) progression rate and can be used in both before and after the manifestation of any disease symptoms in healthy, unhealthy, and ill peoples. This test formulation also can be used against fibromyalgia, Addison disease, multiple sclerosis, myasthenia gravis, aplastic anemia, psoriasis, rheumatoid arthritis, Crohn's disease, vitiligo, chronic fatigue syndrome and alopecia areata, as well as various inflammatory disorders such as ulcerative colitis, dermatitis, hepatitis, diverticulitis, mental disorders, Parkinson's and other movement disorders, stroke and transient ischemic attack (TIA), and in the improvement of overall health and quality of life.

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