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Research Article

Mechanistic Biomarker Estimation in Kidney, Lungs, and Artery after Treatment with the Biofield Energy Healing Based Test Formulation in Vitamin D_3 Deficiency Diet (VDD) Induced Animal Model

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

[1]Trivedi Global, Inc., USA

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

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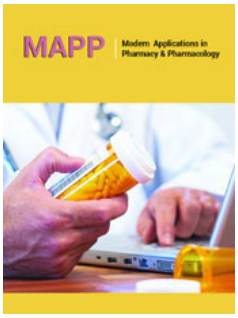

***Corresponding author:** Snehasis Jana, Trivedi Science Research Laboratory Pvt. Ltd., Thane (W), Maharashtra, India

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Abstract

This present study evaluated the impact of Consciousness Energy Healing/Blessing Treatment (the Trivedi Effect®) on a novel test formulation in male *Sprague Dawley* (SD) rats, fed with Vitamin D_3 Deficiency Diet (VDD) for the estimation of mechanistic biomarkers such as endothelin-1 and nitric oxide (NO) in kidney, lungs, and artery tissues. A novel proprietary test formulation was formulated including minerals (magnesium, zinc, copper, calcium, selenium, and iron), vitamins (ascorbic acid, pyridoxine HCl, alpha tocopherol, cyanocobalamin, and cholecalciferol), *Panax ginseng* extract, β-carotene, and cannabidiol isolate. The novel test formulation was divided into two parts; one part was defined as untreated test formulation, while the other of test formulation and three group of animals received Biofield Energy Healing Treatment/Blessing by a renowned Biofield Energy Healer, Mr. Mahendra Kumar Trivedi. Kidney endothelin-1 level was significantly increased by 33.9%, 94.6% (p<0.001), 19.2%, and 14.6% in Biofield Energy Treated/Blessed test formulation group (G5), Biofield Energy Treatment *per se* (G6), 15 days pre-treatment of Biofield Energy Treated test formulation (G7), and untreated test formulation to the Biofield Energy Treated animals (G9) groups, respectively as compared with the untreated group (G4); while 66.9% (p<0.001) in the G6 group as compared to the G2 group. Similarly, the level of NO in kidney was significant increased by 10.6% and 10.4% in the G7 and G8 groups, respectively as compared with the G4. However, lungs endothelin-1 was reported to be increased by 62.7%, 18.8%, 26.9%, and 35.5% in the G6, G7, G8, and G9 groups, respectively as compared to the G2. Similarly, the lungs NO level was significantly increased by 18.4% and 54.3% in the G6 and G7 groups, respectively, as compared with the G4. The level of endothelin-1 in artery was increased by 13.6%, 16.7%, and 28.2% in G5, G6, and G7 groups, respectively as compared with the G2 group. Similarly, the level of No in artery was increased by 107.4%, 65.4%, 71.9%, 59.7%, and 37.1% in the G5, G6, G7, G8, and G9 groups, respectively as compared with the G2 group. Altogether, the Biofield Treated test formulation and Biofield Energy Treatment *per se* significantly used as an effective approach to improve the functioning of endothelin-1 and NO in kidney, lungs and artery that can be used against many disorders such as Alzheimer's disease, dementias, brain cancer, epilepsy and other seizure disorders, mental disorders, and Parkinson's. Overall, the results showed the significant slowdown the disease progression and disease related all other complications/symptoms in the preventive Biofield Energy Treatment group *per se* and/or Biofield Energy Treated Test formulation groups (*viz*. G6, G7, G8, and G9) comparatively with the disease group.

Keywords: Biofield treatment; Mechanistic biomarkers; The trivedi effect®; Endothelin-1; Vitamin D_3 deficiency diet; Calcitriol

Introduction

A biomarker could be considered as a characteristic that that acts as an indicator and could be measured effectively regarding the normal or pathologic biological processes. Its use has also been evident as the response indicator to any therapy. In this regard, the mechanistic biomarker is used as a concept, which is based on the idea that the biomarker testing results can be used further to guide the clinical management of disease. The mechanistic biomarker is basically used in the clinical diagnosis of symptomatic disease such as, the presence of infectious diseases could be detected by analysing the antibodies directed against specific pathogens (e.g., HIV or hepatitis virus); diagnosis of certain cancers by detecting the specific genetic aberrations (including myelodysplastic syndrome and chronic myelocytic leukemia) [1]. Endothelin 1 (ET-1), also known as Preproendothelin-1 (PPET1), is a potent

vasoconstrictor produced by vascular endothelial cells [2]. In animals, the vitamin D deficiency has been reported to result in the cardiovascular consequence i.e., hypertension. Some research studies on rats reported that the deficiency in Vitamin D Receptor (VDR) may develop hypertension in them. It could be associated with the activation of the renin-angiotensin-aldosterone system, as vitamin D acts as a negative regulator of renin synthesis. The levels of endothelin-1 were estimated in kidney, lung and artery [3]. Besides, nitric Oxide is involved in the nociceptive process as an important neurotransmitter, where it is mainly related with the development of central sensitization in the dorsal horn of the spinal cord. The studies reported that vitamin D could inhibit the synthesis of Nitric Oxide Synthase (iNOS) (responsible for producing nitric oxide), in macrophages that activates microglia and astrocytes at the protein as well as m-RNA levels [4,5]. Inhibition of iNOS can be a potential mechanism for reducing pain and neuronal damage after injury or in diseases such as Parkinson's disease, ischemia, and Acquired Immune-Deficiency Syndrome (AIDS). In our explanatory model, there was reduced NO production in the vitamin D_3 deficient rats that results over time in arterial stiffening and increased pulse pressure. It further leads to altered collagen and elastin content in the aorta by long-term increases in mechanical strain as well as to cardiac functional impairment by chronically increased afterload at older ages. Thus, the levels of NO were estimated in kidney, lung and artery [6].

Thus, some standard mechanistic biomarkers of healthy ageing correlating with the overall health are the current utility as surrogate endpoints of research. Various pre-clinical and clinical trials have been focused to develop a novel formulation that works to improve the overall health. However, there is no such novel herbal-based test formulation was designed that can improve the overall organ health using cell based standard assays. There is currently no universally accepted test formulation, which improve the organ health biomarkers. With this respect, the novel test formulation was designed on the basis of best scientific literature, which is the combination of different minerals (selenium, zinc, iron, calcium, copper, and magnesium), vitamins (cyanocobalamin, ascorbic acid, pyridoxine HCl, alpha tocopherol, and cholecalciferol), β-carotene, cannabidiol isolate, and panax ginseng extract. This formulation is designed for overall health against many pathological bone health conditions. Minerals and vitamins present in the test formulation provide significant physiological support [7-9]. In addition, *panax ginseng* is one of the best reported medicinal plants that improve mental, physical abilities, cognitive health, and is potent immunomodulator [10,11], while biological importance of cannabidiol as has been widely reported in many pharmacological actions [12,13]. The test formulation and the animals *per se* were treated with the Biofield Energy by Biofield Energy Healer and were studied for mechanistic parameters.

In recent years, Biofield Energy Treatment was reported in several scientific reports and clinical trials for the useful effects in case of cervical cancer patients [14], massage therapy [15], and many more. Complementary and Alternative Medicine (CAM) therapies have been reported that Biofield Therapies (or Healing Modalities) as one of the best preferred models of treatment with several benefits to enhance physical, mental and emotional human wellness. National Centre of Complementary and Integrative Health (NCCIH) has been recognized and accepted Biofield Energy Healing as a CAM along with other therapies such as deep breathing, yoga, Tai Chi, Qi Gong, chiropractic/osteopathic manipulation, meditation, massage, special diets, homeopathy, progressive relaxation, guided imagery, acupressure, acupuncture, relaxation techniques, hypnotherapy, healing touch, movement therapy, pilates, Ayurvedic medicine, traditional Chinese herbs and medicines, naturopathy, essential oils, aromatherapy, Reiki, and cranial sacral therapy. Human Biofield Energy has subtle energy that has the capacity to work in an effective manner [16,17]. Biofield Energy Healing Treatment/Blessing (the Trivedi Effect®) results has been published in numerous peer-reviewed science journals with significant outcomes in many scientific fields on various models in the metal science [18,19], agriculture science [20], microbiology [21,22], biotechnology [23,24], and improved bioavailability of various compounds [25,26], skin health [27,28], nutraceuticals [29], cancer research [30], bone health [31-33], overall human health and wellness. The present study was designed to study the mechanistic biomarkers such endothelin-1 and nitric oxide in kidney, lungs, and artery using ELISA assay in male *Sprague Dawley* rats in presence of VDD diet and novel test formulation.

Materials and Methods

Chemicals and reagents

Pyridoxine hydrochloride (vitamin B_6), calcitriol, 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, 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. For the estimation of mechanistic biomarkers, specific ELISA kits were used such as for detection of endothelin-1 and nitric oxide, which were procured from CUSABIO, USA and MyBioSource, USA respectively.

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 throughout the experiment.

Consciousness energy healing strategies

Each ingredient of the test formulation was divided into two parts. One part of the test compound was not received any sort of

treatment and were defined as the untreated or control sample. The second part of the test formulation was treated with the Biofield Energy Treatment/Blessing 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 by Mr. Trivedi under similar laboratory conditions for ~3 minutes. The Blessing/Treatment was given to the test items remotely without touching in the laboratory of Dabur Research Foundation, near New Delhi, India. 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 energy treatment, under the same laboratory conditions. The "sham" healer did not have any knowledge about the Biofield Energy Treatment. 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. Dosing for groups G7 and G8 were initiated on day -15 and continued till end of the experiment. However, G1 to G6 and G9 groups were dosed from day 1 till the end of experiment. All the animals except G1 group received Vitamin D_3 Deficient Diet (VDD) daily to the end of the experiment. Three weeks after the initiation of induction of VDD, all the groups were dose with the respective formulations.

Preparation of tissue homogenate

About 100mg of the artery, lung tissue, and kidney homogenate was rinsed with 1X PBS, homogenized in 1mL of 1X PBS and stored overnight at -20 °C. After two freeze-thaw cycles were performed to break the cell membranes, the homogenates were centrifuged for 5 minutes at 5000*g*, at 2 to 8 °C. The supernatant was removed and assayed immediately. Alternatively, aliquot and store samples at -20 °C or -80 °C. Centrifuge the sample again after thawing before the assay. Avoid repeated freeze-thaw cycles.

Estimation of mechanistic biomarkers from artery, lungs, and kidney (endothelin-1 and nitric oxide)

Artery, lungs tissue, and kidney homogenate was subjected for the estimation of level of endothelin-1 and nitric oxide. All the mechanistic biomarkers estimation was performed using ELISA method as per manufacturer's recommended standard procedure. This was a quantitative method, and the principle was based on the quantitative method.

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

Effect of test formulation in kidney endothelin-1 and NO level

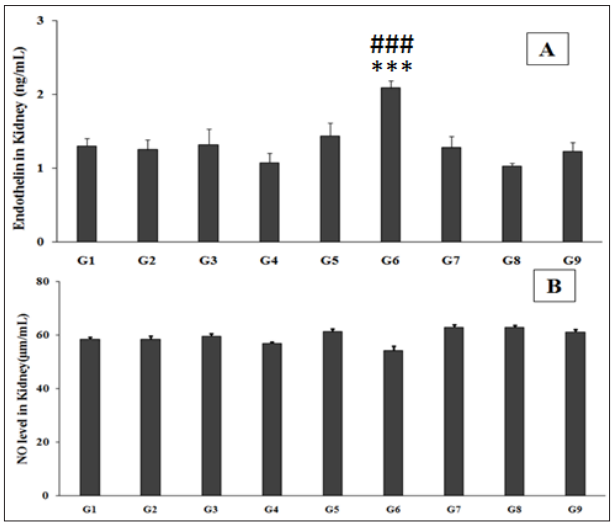

Figure 1: Effect of the test formulation on the level of (A) endothelin-1 and (B) NO in kidney homogenate of Sprague Dawley rats. G: Group; G1: Normal control (0.5% CMC); G2: Disease control (VDD: Vitamin D_3 deficient diet + 0.5% CMC); G3: Reference item (VDD + Calcitriol); G4: (VDD + untreated test formulation); G5: (VDD + Biofield Energy Treated test formulation); G6: (VDD + Biofield Energy Treatment *per se* to animals from day -15; G7: (VDD + Biofield Energy Treated test formulation from day -15); G8: (VDD + Biofield Energy Treatment *per se* plus Biofield Energy Treated test formulation from day -15), and G9: (VDD + Biofield Energy Treatment *per se* animals plus untreated test formulation). Values are presented as mean±SEM (n=6). ***$p \leq 0.001$ *vs*. G2 and ###$p \leq 0.001$ *vs*. G4.

Endothelin-1 is considered as a potent vasoconstrictor and it plays vital role in the regulation of volume homeostasis in normal physiologic as well as pathologic conditions. Research studies suggest that endothelin-1 exerts different biologic effects in the kidney, such as inhibiting sodium and water reabsorption and constricting renal vasculature, which may lead to glomerular and tubular damage [34,35]. Besides, NO is a free radical that also plays important role in various physiological processes. Its presence causes vasodilatation, and it prevents the proliferation of vascular smooth muscle and inhibits the adhesion and aggregation of blood platelets [36]. The level of kidney endothelin-1 and NO was calculated and presented in Figure 1. Kidney endothelin-1 level of vitamin D_3 deficient diet (G2) group was 1.25±0.14ng/mL, which was decreased by 4% as compared to the normal control (G1, 1.31±0.10ng/mL). Calcitriol treatment (G3), showed a significant increased kidney endothelin-1 level (1.32±0.21ng/mL) by 5.6% as compared to the G2. The untreated test formulation in the untreated animals (G4) showed significantly decreased the kidney endothelin-1 level (1.07±0.13ng/mL) by 14.2% as compared to the G2. However, Biofield Energy Treated test formulation group (G5) showed significantly increased the kidney endothelin-1 level (1.44±0.18ng/mL) by 33.9% as compared with the G4 group. Biofield Energy Treatment *per se* to the animals group (G6), significantly (p<0.001) increased kidney endothelin-1 level (2.09±0.10ng/mL) by 66.9% as compared to G2, while 94.6% improved level as compared to the G4. Similarly, 15 days pre-treatment of Biofield Energy Treated test formulation (G7) showed a significant increased endothelin-1 level (1.28±0.15ng/mL) by 19.2% as compared to the G4. However, 15 days pre-treatment of Biofield Energy Treated test formulation to the Biofield Energy Treated animals (G8) showed decreased endothelin-1 level (1.03±0.04ng/mL) by 4.2% as compared to the G4. Untreated test formulation to the Biofield Energy Treated animals (G9), significantly increased the kidney endothelin-1 level (1.23 ±0.12ng/mL) by 14.6% as compared to the G4.

Besides, the test formulation showed significant impact on the kidney NO level. It was observed that the test formulation groups G5, G7, G8, and G9 showed significant increased (p<0.05) in the kidney NO level by 7.5%, 10.6%, 10.4%, and 7.4%, respectively; while G6 showed 4.9% decrease (54.10±1.66µm/mL) in NO level, as compared with the G4 group (56.52±0.60µm/mL). Overall, our results revealed significant improved kidney endothelin-1 and NO level in all the experimental test groups as compared to G4. This suggested that Biofield Energy Treated/Blessed Test formulation and Biofield Energy *per se* in normal physiologic as well as various pathologic conditions of kidney.

Effect of test formulation in lung endothelin-1 and NO level

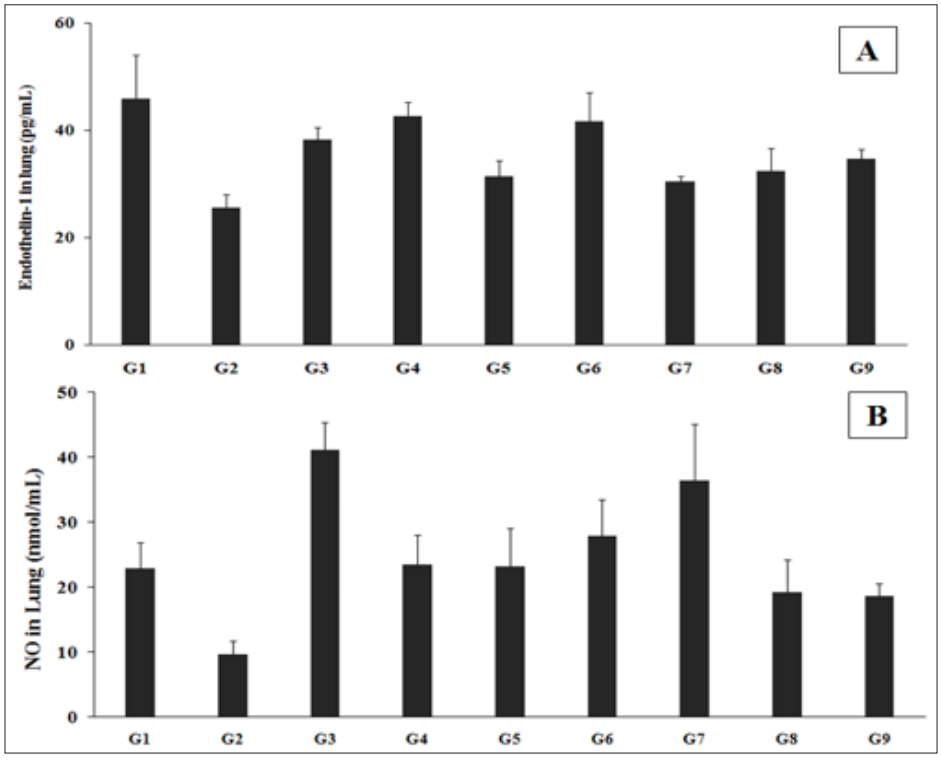

Figure 2: Effect of the test formulation on the level of (A) endothelin-1 and (B) NO in lung homogenate of Sprague Dawley rats. G: Group; G1: Normal control (0.5% CMC); G2: Disease control (VDD: Vitamin D_3 deficient diet + 0.5% CMC); G3: Reference item (VDD + Calcitriol); G4: (VDD + untreated test formulation); G5: (VDD + Biofield Energy Treated test formulation); G6: (VDD + Biofield Energy Treatment *per se* to animals from day -15; G7: (VDD + Biofield Energy Treated test formulation from day -15); G8: (VDD + Biofield Energy Treatment *per se* plus Biofield Energy Treated test formulation from day -15), and G9: (VDD + Biofield Energy Treatment *per se* animals plus untreated test formulation). Values are presented as mean±SEM (n=6).

In pulmonary system, endothelin-1 may cause broncho-constriction, along with increased cytokine production, bronchial reactivity to inhaled antigens as well as the influx of inflammatory cells, which may lead to airway edema and remodelling. However, endothelin-1 can also act as a vasodilator in the pulmonary circulation depending upon the presence of NO. The generation of NO or opening of ATP-sensitive potassium channels may cause hyperpolarization mediated by endothelin receptors on pulmonary endothelium that further results in vasodilation [37,38]. The level of lung endothelin-1 and NO was calculated and presented in Figure 2. Lung endothelin-1 level of vitamin D_3 deficient diet (G2) group was 25.59±2.37pg/mL, which was significantly decreased by 44.2% as compared to the normal control (G1, 454.88±8.22pg/mL). Calcitriol treatment (G3), showed a significant increased lung endothelin-1 level (38.31±2.15pg/mL) by 49.7% as compared to the G2. The untreated test formulation in the untreated animals (G4) showed significantly increased the lung endothelin-1 level (42.60±2.65pg/mL) by 66.4% as compared to the G2. Besides, the Biofield Energy Treated test formulation group (G5) showed significantly increased the lung endothelin-1 level (31.43±2.98pg/mL) by 22.8% as compared with the G2 group. Similarly, the other treated groups G6, G7, G8, and G9 showed a significantly increased endothelin-1 level by 62.7%, 18.8%, 26.9%, and 35.5%, respectively as compared to the G2. The impact of the Biofield energy Treated test formulation was also observed on the lung NO level. The results showed that the test formulation groups G5, G6, G7, G8, and G9 showed significant increase in the lung NO level by 139.5%, 187.8%, 275.1%, 98.5%, and 93.1%, respectively, as compared with the G2 group (9.70±2.09nmol/mL). However, lungs NO level was significantly improved by 18.4% and 54.3% in G6 and G7 groups respectively, as compared with the G4. Overall, our results revealed significant improved lung endothelin-1 and NO level in all the experimental test groups as compared to G4. This suggested that Biofield Energy Treated/Blessed Test formulation and Biofield Energy *per se* in normal physiologic as well as various pulmonary pathologic conditions.

Effect of test formulation in artery endothelin-1 and NO level

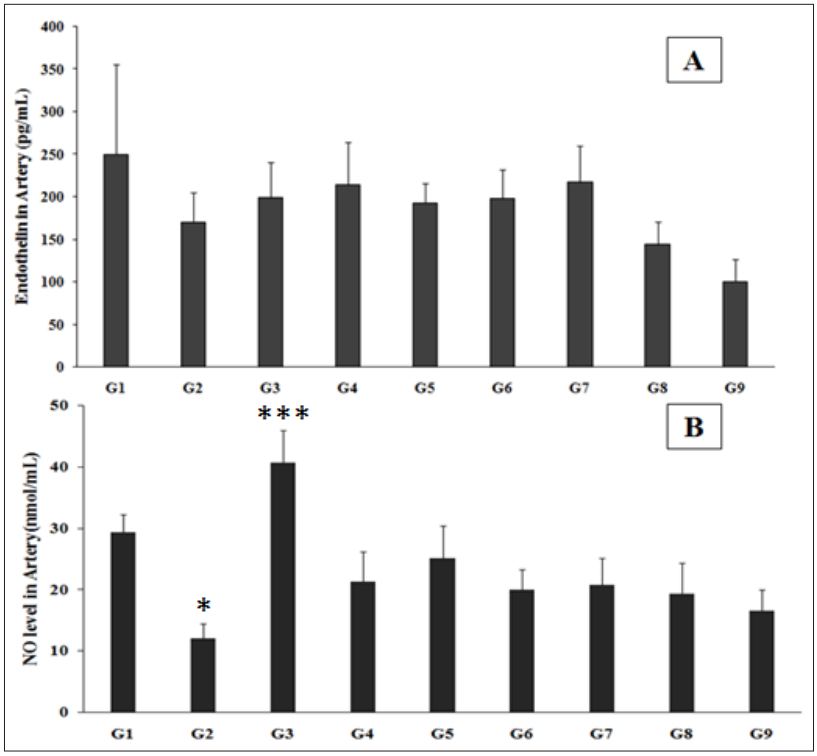

Figure 3: Effect of the test formulation on the level of (A) endothelin-1 and (B) NO in artery homogenate of Sprague Dawley rats. G: Group; G1: Normal control (0.5% CMC); G2: Disease control (VDD: Vitamin D3 deficient diet + 0.5% CMC); G3: Reference item (VDD + Calcitriol); G4: (VDD + untreated test formulation); G5: (VDD + Biofield Energy Treated test formulation); G6: (VDD + Biofield Energy Treatment *per se* to animals from day -15; G7: (VDD + Biofield Energy Treated test formulation from day -15); G8: (VDD + Biofield Energy Treatment *per se* plus Biofield Energy Treated test formulation from day -15), and G9: (VDD + Biofield Energy Treatment *per se* animals plus untreated test formulation). Values are presented as mean±SEM (n=6). *$p \leq 0.05$ *vs.* G1 and ***$p \leq 0.001$ *vs.* G2.

The level of artery endothelin-1 and NO was calculated and presented in Figure 3. Endothelin-1 level in the artery in vitamin D_3 deficient diet (G2) was 169.64±35.00pg/mL, significantly decreased by 32.2% as compared to the normal control (G1, 250.23±105.70pg/mL). Calcitriol treatment (G3), showed significant increased the endothelin-1 level (198.80±42.25pg/mL)

by 17.2% as compared to the G2. Untreated test formulation group (G4) showed significantly increased the artery endothelin-1 level (213.98±50.20pg/mL) by 26.1% as compared to the G2. However, the experimental test groups such as G5, G6, G8, and G9 showed decreased level of artery endothelin-1 by 9.9%, 7.5%, 32.7%, and 53% respectively as compared with the G4, while lung endothelin-1 was increased by 13.6%, 16.7%, and 28.2% in G5, G6, and G7 groups respectively as compared with the G2 group. NO level in the artery with vitamin D_3 Deficient diet (G2) was 12.09±2.36nmol/mL, significantly decreased (p<0.05) by 58.8% as compared with the normal control (G1, 29.32±2.98nmol/mL). Calcitriol treatment (G3) showed significantly increased (p<0.001) the artery NO level (40.71±5.24nmol/mL) by 236.6% as compared with the G2. Similarly, the level of NO in artery was significantly increased by 17.8% in G5 group as compared with the G4 group. However, as compared with the disease control G2 the level of artery NO was also reported to be increased by 107.4%, 65.4%, 71.9%, 59.7%, and 37.1% in the G5, G6, G7, G8, and G9 groups respectively. The vascular tone is regulated by the vascular endothelium *via* the production of vasoactive substances, such as NO as a vasodilator and endothelin-1 as a vasoconstrictor. Endothelin-1 acts by increasing the sympathetic nervous system activity and therefore, considered as the most powerful vaso-constricting agent. It may increase the arterial blood pressure, while reduces the renal plasma flow and glomerular filtration, and therefore decreases the natriuresis and dieresis [39]. NO and endothelin-1 play their role as natural counterparts in the vascular function, and the imbalance between these two mediators is considered as endothelial dysfunction characteristic that may symbolises the progression of vascular disease [40].

Overall, our results revealed significant improved artery endothelin-1 and NO level in all the experimental test groups as compared to G4. This suggested that Biofield Energy Treated Test formulation and Biofield Energy *per se* in normal physiologic as well as various cardiac functions. Thus, the present research plan defined four groups, which were considered as preventive maintenance groups *viz.* G6, G7, G8, and G9, where the Biofield Energy Treatment *per se* and/or Biofield Energy Treated/Blessed Test formulation in combination was used as preventive maintenance group with respect to improved animal behaviour. The results showed the significant slowdown of the disease progression, disease related all other complications and also reduced the chances of disease susceptibility in these groups. Specifically, group G6 (preventive Biofield Energy Treatment group *per se*) showed the best results as a prophylactic/preventive treatment group compared to the other groups in terms of locomotors activity, knee joint, and overall behaviour with muscle coordination. Based on the overall data, it suggests that the Biofield Energy Healing Therapy was found to be most effective and benefited in order to prevent and protect from the occurrence of any type of bone related diseases in rat model. It indicated that Biofield Energy Treatment/Blessing can act as a preventive maintenance therapy to slowdown the disease progression and disease related complications of the existing aliments that will ultimately improve the overall health and quality of life in human.

Conclusion

Endothelin 1 (ET-1), also known as Preproendothelin-1 (PPET1), is a potent vasoconstrictor and nitric oxide is an important neurotransmitter involved in the nociceptive process. Both are important mechanistic biomarkers. Based on the current findings, it can be concluded that the endothelin-1 and NO in kidney, lungs, and artery were significantly improved in the Biofield Energy Treated test formulation group. The kidney endothelin-1 level was significantly increased by 33.9%, 94.6%, 19.2%, and 14.6% in the groups G5, G6, G7, and G9 groups respectively, as compared with the G4. The level of kidney NO in the groups G5, G7, G8, and G9 showed significant increased level by 7.5%, 10.6%, 10.4%, and 7.4% respectively, as compared with the G4 group. Endothelin-1 level was found to be increased by 62.7%, 18.8%, 26.9%, and 35.5% in the G6, G7, G8, and G9, respectively as compared to the G2. Similarly, the lungs NO level was significant increased by 18.4% and 54.3% in G6 and G7 groups respectively, as compared with the G4. Endothelin-1 level in the artery tissue was increased by 13.6%, 16.7%, and 28.2% in G5, G6, and G7 groups respectively, as compared with the G2 group. Similarly, the level of NO in artery was increased by 107.4%, 65.4%, 71.9%, 59.7%, and 37.1% in the G5, G6, G7, G8, and G9 groups, respectively as compared with the G2 group. Overall, our results revealed statistically significant change in the endothelin-1 and NO level in the kidney, lungs, and artery, which the significant role of Biofield Energy Treated/Blessed test formulation and Biofield Energy Treatment *per se*. Therefore, the Trivedi Effect®-Biofield Energy Healing/Blessing Treatment enhanced the overall mechanistic biomarkers of the animals throughout the exposure period. Overall, it can be concluded that Biofield Energy Healing Treatment (the Trivedi Effect®) *per se* showed best results with respect to different efficacy and biomarker parameters in the preventive treatment approach (-15 days) as compared to the other preventive maintenance groups (G7, G8, and G9) in rat model study. It also helped to slow down the disease progression and disease related complications of the overall animal's health. This test formulation can also be used against other disorders such as systemic lupus erythematosus, fibromyalgia, Addison disease, multiple sclerosis, myasthenia gravis, pernicious anemia, aplastic anemia, Psoriasis, rheumatoid arthritis, Crohn's disease, vitiligo, chronic fatigue syndrome and alopecia Areata, as well as inflammatory disorders such as ulcerative colitis, atherosclerosis, dermatitis, hepatitis, and diverticulitis. However, Biofield Energy Healing Treated test formulation and Biofield Energy Healing Treatment *per se* can also be used in the prevention of brain disorders such as Alzheimer's disease, dementias, brain cancer, epilepsy and other seizure disorders, 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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