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Evaluation of Vitamin D Receptor (VDR) Expression and Telomerase Activity after Treatment with the Biofield Energy Treated Proprietary Test Formulation in Unpredictable Chronic Stress (UCS)-Induced Sprague Dawley Rats

Trivedi MK[1] and Jana S[2]*

[1]Trivedi Global, Inc, Henderson, USA

[2]Trivedi Science Research Laboratory Pvt Ltd, Thane (W), India

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

Research Article

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Abstract

Vitamin D receptor (VDR) expression and telomerase enzyme activity was performed in the unpredictable chronic stress (UCS) model for the evaluation of Consciousness Energy Healing Treatment (the Trivedi Effect®) on a novel test formulation in male Sprague Dawley (SD) rats using ELISA assay. A unique test formulation 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) was formulated. The constituents of the test formulation were divided into two parts; one section was defined as the untreated test formulation, while the other portion of the test formulation and three group of animals were received Biofield Energy Healing Treatment by a renowned Biofield Energy Healer, Mr. Mahendra Kumar Trivedi. VDR in liver showed an increased expression in the Biofield Energy Treated Test formulation to the untreated rats (G5), G7 (15-days pre-treatment of Biofield Energy Treated Test formulation), and G8 (15 days pre-treatment of Biofield Energy Treated test formulation to the Biofield Energy Treatment *per se* rats) groups by 16.7%, 75.7%, and 14.3%, respectively as compared with the untreated test formulation group (G4). Similarly, the VDR expression in kidney was increased by 22.3% and 13.6% in the G6 (Biofield Energy Treatment *per se* to the rats) and G8 groups, respectively, as compared with the G4. However, the VDR expression in heart was altered by 20.2%, 26%, and 28.2% in the G5, G7, and G8 groups respectively, as compared with the G4. The telomerase activity in the brain was significantly increased by 16.9%, 116%, 258.6% ($p \leq 0.01$), 148.1%, and 54.7% in the G5, G6, G7, G8, and G9 (untreated test formulation to the Biofield Energy Treatment *per se* to the rats) groups respectively, as compared with the G4. Similarly, telomerase activity in heart was also increased by 11.1% in the G8 group as compared with the G4. Overall, the experimental data suggested significance effect of Biofield Energy *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 Test formulation groups (*viz*. G6, G7, G8, and G9) as compared to the disease control and untreated test formulation groups.

Keywords: Biofield Treatment; Vitamin D receptor; Telomerase assay; The Trivedi Effect®; Unpredictable Chronic Stress; ELISA

Abbreviations: PTSD: Post-Traumatic Stress Disorder; SPS: Single-Prolonged Stress; UCS: Unpredictable Chronic Stress; VDR: Vitamin D Receptor; CAM: Complementary and Alternative Medicine; NCCIH: National Centre of Complementary and Integrative Health; CPCSEA: Committee for the Purpose of Control and Supervision of Experiments on Animals.

Introduction

Different level of stress can results in severe anxiety disorder, which might lead in psychological trauma with altered metabolic functions. Post-traumatic stress disorder (PTSD), single-prolonged stress (SPS), and unpredictable chronic stress (UCS) animal models has been extensively developed and predicts the original trauma, avoidance of stimuli associated with the trauma and many more [1-3]. UCS results in brain alterations that have been related with various neuropsychiatric disorders, such as Alzheimer's disease, schizophrenia, depression, and cognitive decline. Scientific data suggested a strong correlation with induced UCS and strong positive relationship with vitamin D signaling and cognitive function [4]. Neurotransmission, neuroprotection and neuro-immunomodulation functions are strongly correlated with vitamin D regulation [4,5], along with calcium and phosphorous regulation with skeletal mineralization [6]. Vitamin D receptor (VDR) action was mediated with the presence of vitamin D. VDR, a ligand-activated transcription factor is [7] measured as a nuclear receptor, which is ubiquitously expressed in most of the organs or tissues, including adipose tissue, bone, muscle [8] cerebral cortex, and hippocampus [9]. VDR expression can be altered due to age-related genetic variations, altered cognitive functioning, and depressive symptoms [10]. Besides, neurodegenerative dementia, anxiety and motor disorders [11,12], have been linked due to the absence of VDR suggestive essential role of VDR [13]. Similarly, UCS, stressful life experience, and many other stresses have been related to alter life expectancy, health, and biomarkers of cellular senescence, such as telomerase activity [14].

Thus, a novel test formulation was designed that would improve the VDR activity and telomerase assay in presence of UCS animal model. The test formulation was the combination of different minerals (Zn, Fe, Cu, Se, Ca, Mg), vitamins (C, E, B6, B12, D3), and nutraceuticals (β-carotene, Ginseng, CBD). This formulation is designed for overall alteration of bone health especially VDR and telomerase activity. All the minerals and vitamins used in the test formulation have significant functional role to provide vital physiological actions [15,16]. Besides, biological importance of cannabidiol as novel anti-inflammatory and other disorders has been widely reported [17,18], while ginseng extract is regarded as the one of the best immune booster for overall immunity [19,20]. Thus, the present study aimed to evaluate the effect of unpredictable chronic stress (UCS) on VDR activity and telomerase assay of male Sprague Dawley rats in presence of novel test formulation, which was treated with Biofield Energy Treatment (a Complementary and Alternative Medicine, CAM) by a renowned Biofield Energy Healer.

Biofield Energy Healing Treatment, was used as one of the best available alternative treatment approach with significant clinical benefits against many disorders, and regarded as one of the best Complementary and Alternative Medicine (CAM) treatment approach [21-26]. 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 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. In this study, the authors sought 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 VDR and telomerase activity in presence of UCS using standard ELISA assay.

Material and Methods

Chemicals and Reagents

The test formulation contained constituents such as 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 B12), calcium chloride, vitamin E (Alpha-Tocopherol), cholecalciferol (vitamin D3), 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 VDR and telomerase, specific ELISA kits were used for detection the level of vitamin D receptor and telomerase, which were procured from CUSABIO, USA.

Study Design

The current experiment was designed to fulfill 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 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).

Maintenance of Animal

Randomly breed male Sprague Dawley (SD) rats with body weight ranges from 200 to 300 gm were used in this study. The animals were purchased from M/s. 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 was treated with the Trivedi Effect® - Energy of Consciousness Healing Treatment (Biofield Energy Treatment) by a renowned Biofield Energy Healer, Mr. Mahendra Kumar Trivedi under laboratory conditions for ~3 minutes. The novel test formulation was consisted of zinc chloride, iron (II) sulfate, copper chloride, vitamin B_6, vitamin B_{12}, vitamin D_3, sodium selenate, calcium chloride, ascorbic acid, vitamin E, beta carotene, Panax ginseng extract, cannabidiol isolate, and magnesium (II) gluconate. Besides, three group of animals 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 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 has not 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. Dosing for groups G7 and G8 were initiated 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 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 8^{th} 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 VDR level in the liver, kidney, and heart, telomerase assay in the brain and heart during final calculations.

Estimation of VDR (Liver, kidney, and heart) and Telomerase Assay (Brain and heart)

The serum from all the animals groups after experimental period was subjected for the estimation of level of VDR in liver, kidney, and heart, while telomerase assay was performed in the brain and heart. 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 antigen and antibody in sandwich manner assay.

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 VDR (Vitamin D Receptor) in Liver

The level of VDR in liver was measured in all the experimental groups and was graphically presented in the Figure 1. The level of liver VDR in the unpredictable chronic stress group (G2) was 236.75 ± 22.92 ng/mL, which was increased by 41.6% as compared to the normal control (G1, 167.20 ± 9.71 ng/mL). However, the imipramine treatment (G3) group showed a decreased liver VDR level by 27.4% (171.96 ± 35.89 ng/mL) as compared to the G2. Untreated test formulation to the untreated rats (G4) showed a decreased liver VDR level by 50.75% (116.60 ± 7.13 ng/mL) as compared to the G2. Biofield Energy Treated test formulation to the untreated rats (G5) showed decreased liver VDR level by 42.54% (136.04 ± 12.19 ng/mL) as compared to the G2. Biofield Energy Treatment to the rats (G6) showed decreased liver VDR level as 111.82 ± 12.40 ng/mL by 52.77% and 4.1% as compared to the G2 and G4 groups, respectively. 15 days pre-treatment of Biofield Energy Treated test formulation (G7) showed decreased liver VDR level (204.91 ± 50.62 ng/mL) by 13.45% as compared to the G2. 15 days pre-treatment of Biofield Energy Treated test formulation to the Biofield Energy Treated rats (G8) decreased liver VDR level (133.30 ± 12.25 ng/mL) by 43.69% as compared to the G2. Untreated test formulation to the Biofield Energy Treated rats (G9) showed significantly decreased liver VDR level (127.02 ± 11.84 ng/mL) by 46.35% as compared to G2. Further, the expression of VDR was increased by 16.67%, 75.74%, 14.33% and 8.93% in the G5, G7, G8, and G9 groups, respectively as compared to the untreated test formulation (G4) group. VDR plays a vital role in the mineral-ion homeostasis, while its deficiency would results in various liver metabolic diseases [37]. Thus, the experimental data suggested that Biofield Energy Healing Treatment *per se* and the test formulation plays a significant role in VDR activity in liver.

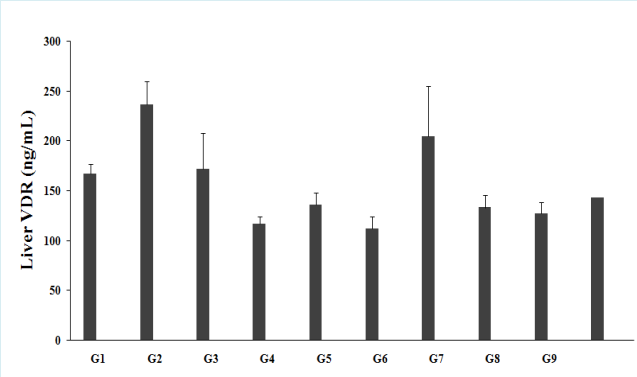

Figure 1: Effect of the test formulation on the level of liver VDR activity in Sprague Dawley rats. G: Group; G1: Normal control; G2: Disease control (UCS: Unpredictable chronic stress + 0.5% CMC); G3: Reference item (UCS + Imipramine hydrochloride 30 mg/kg); G4: (UCS + Untreated test formulation); G5: (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).

Estimation of VDR (Vitamin D receptor) in Kidney

The level of VDR in kidney was measured in all the experimental groups and was graphically presented in the Figure 2. The level of kidney VDR in the unpredictable chronic stress group (G2) was 35.61 ± 0.92 ng/mL, which was significantly ($p \leq 0.01$) decreased by 15.74% as compared with the normal control (G1, 42.26 ± 1.4 ng/mL). Imipramine treatment (G3) increased kidney VDR level (42.40 ± 6.9 ng/mL) by 19.1% as compared to the G2. Untreated test formulation to the untreated rats (G4) decreased kidney VDR level (30.33 ± 2.77 ng/mL) by 14.82% as compared to the G2. Biofield Energy Treated test formulation to the untreated rats (G5) showed a decreased kidney VDR level (32.24 ± 2.4 ng/mL) as compared to G2, while it was increased by 6.3% as compared to the G4. Biofield Energy Treatment to the rats (G6) showed increased kidney VDR level (37.08 ± 1.4 ng/mL) by 4.1% and 22.3% as compared to G2 and G4 groups, respectively. 15 days pre-treatment of Biofield Energy Treated test formulation (G7) showed a decreased kidney

VDR level (33.06 ± 1.3 ng/mL) as compared to G2, while it was increased by 9% as compared to the G4. 15 days pre-treatment of Biofield Energy Treated test formulation to the Biofield treated rats (G8) showed a decreased kidney VDR level (34.45 ± 3.9 ng/mL) as compared to and increased with the percentage change of 13.6%, when compared to G4. Untreated test formulation to the Biofield Energy Treated rats (G9) showed a decreased kidney VDR level (24.27 ± 1.2 ng/mL) by 31.8% and 20% as compared to the G2 and G4 groups, respectively. VDR is one of the best biomarker and therapeutic target for various kidney diseases; it is present in more than 30 classic and non-classical tissues such as intestine, kidney, cartilage, bone, activated B, and T lymphocytes [38]. Vitamin D deficiency significantly affects the VDR activity that results in kidney disease pathogenesis, as they are present in the proximal and distal tubular epithelial cells, podocytes, and collecting duct epithelial cells [39,40]. Thus, the experimental data suggested that Biofield Energy Healing Treatment *per se* and the test formulation play a significant role in VDR activity in kidney, which directly improved various kidney diseases.

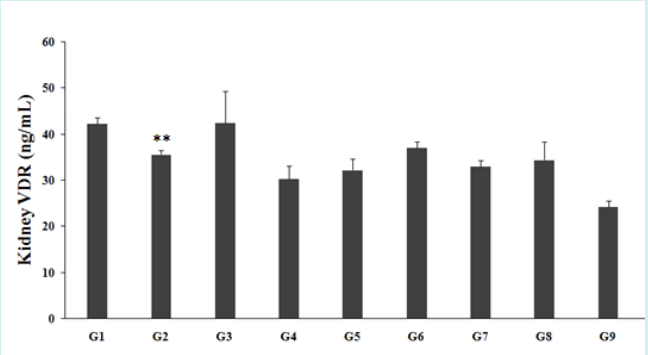

Figure 2: Effect of the test formulation on the level of kidney VDR activity in Sprague Dawley rats. G: Group; G1: Normal control; G2: Disease control (UCS: Unpredictable chronic stress + 0.5% CMC); G3: Reference item (UCS + Imipramine hydrochloride 30 mg/kg); G4: (UCS + Untreated test formulation); 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.

Estimation of VDR (Vitamin D receptor) in Heart

The level of VDR in heart was measured in all the experimental groups and was graphically presented in the Figure 3. The level of heart VDR level in the unpredictable chronic stress (G2) was 64.78 ± 6.16 ng/mL, which was significantly increased by 49.6% as comparison with the normal control (G1, 43.31 ± 3.58 ng/mL). However, imipramine treatment (G3) showed decreased heart VDR level (62.40 ± 1.55 ng/mL) by 3.7% as compared to the G2. Untreated test formulation to the untreated rats (G4) showed decreased heart VDR level (56.51 ± 4.05 ng/mL) by 12.8% as compared to the G2. G5 group showed a decreased heart VDR level (45.08 ± 4.78 ng/mL) by 30.4% and 20.2% as compared to the G2 and G4 groups, respectively. G6 group showed a decreased heart VDR level (54.07 ± 1.95 ng/mL) by 16.5% and 4.3% as compared to the G2 and G4 groups, respectively. G7 group showed a decreased heart VDR level (41.81 ± 2.59 ng/mL) by 35.5% and 26% as compared to the G2 and G4 groups, respectively. G8 group showed a significantly decreased heart VDR level (40.58 ± 2.50 ng/mL) by 37.4% and 28.2% as compared to the G2 and G4 groups, respectively. G9 group showed a decreased heart VDR level (58.16 ± 6.98 ng/mL) by 10.2% as compared to the G2 group. VDR are abundantly present in every cells of the cardiovascular system, with biological importance in managing the regulating blood pressure, cardiac hypertrophy and fibrosis, and controlling atherosclerosis [41-43]. Thus, the experimental data suggested that Biofield Energy Healing Treatment *per se* and the test formulation altered the VDR activity in cardiovascular system.

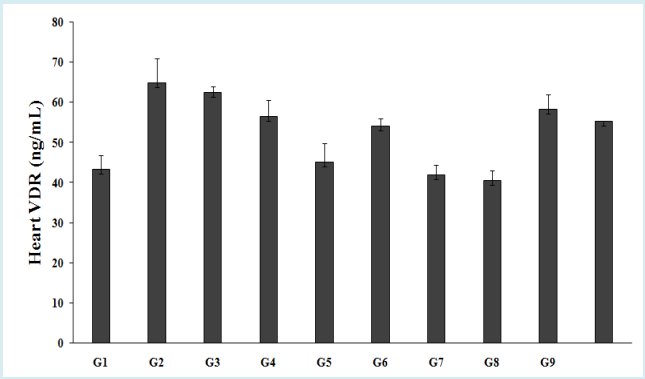

Figure 3: Effect of the test formulation on the level of heart VDR activity in Sprague Dawley rats. G: Group; G1: Normal control; G2: Disease control (UCS: Unpredictable chronic stress + 0.5% CMC); G3: Reference item (UCS + Imipramine hydrochloride 30 mg/kg); G4: (UCS + Untreated test formulation); G5: (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).

Estimation of Telomerase in Brain

The telomerase activity in brain was measured in all the experimental groups and was graphically presented in the Figure 4. The level of brain telomerase activity in unpredictable chronic stress (G2) was 0.367 ± 0.133, as compared to the normal control (G1, 0.269 ± 0.048). Imipramine treatment (G3) showed decreased value by 61.9% (0.140 ± 0.037) as compared to the G2. The untreated test formulation to the untreated rats (G4) showed a decreased value of brain telomerase by 60.2% (0.146 ± 0.040) as compared to the G2. However, the G5, G6, G8, and G9 groups showed a significant decreased telomerase activity by 53.5%, 14.1%, 1.4%, and 38.5%, respectively as compared to the G2. However, the telomerase activity in the G5, G6, G7, G8, and G9 groups were significantly increased by 16.9%, 116%, 258.6% ($p \leq 0.01$), 148.1%, and 54.7%, respectively as compared to the G4 group. Chronic or long term stress contributes to various forms of diseases, which results in huge damage to telomeres, the protective non-coding segments on the ends of chromosomes. Stress and altered telomerase activity results in stressed, depressed, anxious, or previously traumatized conditions, which hampers the overall activity of telomeres [44,45]. In conclusion, the present data suggested that Biofield Energy Healing Treatment *per se* and the test formulation play a significant role in telomerase assay in nervous system, which directly improved various stress disorders.

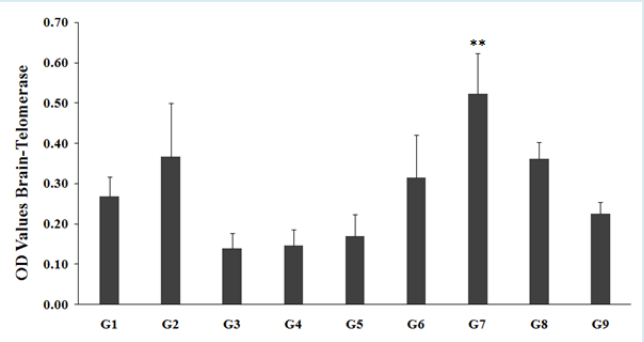

Figure 4: Effect of the test formulation on the level of brain telomerase assay in Sprague Dawley rats. G: Group; G1: Normal control; G2: Disease control (UCS: Unpredictable chronic stress + 0.5% CMC); G3: Reference item (UCS + Imipramine hydrochloride 30 mg/kg); G4: (UCS + Untreated test formulation); 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.* G4.

Estimation of Telomerase in Heart

The telomerase activity in heart was measured in all the experimental groups and was graphically presented in the Figure 5. The level of heart telomerase activity in unpredictable chronic stress (G2) was 0.146 ± 0.010, as compared to the normal control (G1, 0.177 ± 0.010). Imipramine treatment (G3) showed changed value as 0.155 ± 0.008 as compared to the G2. Untreated test formulation to the untreated rats (G4) showed an increased value of heart telomerase by 12.5% (0.165 ± 0.020) as compared to the G2. However, G5, G7, and G8 groups showed an increased heart telomerase activity by 4%, 2.9%, and 11.1%, respectively as compared to the G2. However, the heart telomerase activity in the G5, G6, G7, G8, and G9 groups were decreased by 7.6%, 16.7%, 8.5%, 1.2%, and 17%, respectively as compared to the G4. The role of telomerase in heart has been reported scientifically in the aging process and their association with cardiovascular diseases, besides telomere length is used as the biomarker of various coronary artery diseases [46,47].

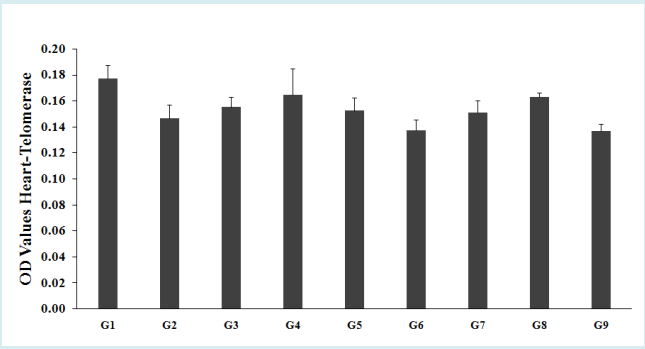

Figure 5: Effect of the test formulation on the level of heart telomerase assay in Sprague Dawley rats. G: Group; G1: Normal control; G2: Disease control (UCS: Unpredictable chronic stress + 0.5% CMC); G3: Reference item (UCS + Imipramine hydrochloride 30 mg/kg); G4: (UCS + Untreated test formulation); 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).

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 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 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 evaluates the effect of test formulation on the level of VDR and telomerase assay in presence of unpredictable chronic stress (UCS) animal model. The data revealed the significance role of Biofield Energy Treated test formulation and Biofield Energy *per se* on the animal stress level using various standard VDR and telomerase assay as compared with the other groups. VDR assay in liver results in an increased activity by 75.7% in the G7 group as compared with the untreated test formulation group (G4). Likewise, expression of VDR in kidney showed increased activity by 22.3% in the G6 group as compared with the G4. Though, the VDR expression in heart was changed in the G5, G7, and G8 groups by 20.2%, 26%, and 28.2% respectively, as compared with the G4. Besides, telomerase assay in the brain was significantly increased expression by 116%, 258.6% ($p \leq 0.01$), 148.1%, and 54.7% in the G6, G7, G8, and G9 groups, respectively as compared with the G4. On the other hand, the telomerase activity in heart was increased by 11.1% in the G8 group as compared with the G4. Biofield Energy Healing Treatment (the Trivedi Effect®) *per se* showed the best results with respect to the 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 to improve the overall health and quality of life in human. This therapy might also reduce the severity of any type of acute/chronic disease (autoimmune-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 such as many thyroid disorders. 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, and in the improvement of overall health and quality of life.

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References

1. Liberzon I, Young EA (1997) Effects of stress and glucocorticoids on CNS oxytocin receptor binding. Psychoneuroendocrinology 22(6): 411-422.

2. Khan S, Liberzon I (2004) Topiramate attenuates exaggerated acoustic startle in an animal model of PTSD. Psychopharmacology (Berl) 172(2): 225-229.

3. Iwamoto Y, Morinobu S, Takahashi T, Yamawaki S (2007) Single prolonged stress increases contextual freezing and the expression of glycine transporter 1 and vesicle-associated membrane protein 2 mRNA in the hippocampus of rats. Prog Neuropsychopharmacol Biol Psychiatry 31(3): 642-651.

4. Buell JS, Dawson Hughes B (2008) Vitamin D and neurocognitive dysfunction: Preventing 'D'ecline? Mol Aspects Med 29(6): 415-422.

5. Kalueff AV, Tuohimaa P (2007) Neurosteroid hormone vitamin D and its utility in clinical nutrition. Curr Opin Clin Nutr Metab Care 10(1): 12-19.

6. Bartoccini E, Marini F, Damaskopoulou E, Lazzarini R, Cataldi S, et al. (2011) Nuclear lipid microdomains regulate nuclear vitamin D_3 uptake and influence embryonic hippocampal cell differentiation. Mol Biol Cell 22(17): 3022-3031.

7. Pike JW, Meyer MB (2010) The vitamin D receptor: new paradigms for the regulation of gene expression by

1,25-dihydroxyvitamin D_3. Endocrinol Metab Clin North Am 39(2): 255-269.

8. Freeman MR, Cinar B, Kim J, Nishit KM, Dolores Di Vizio, et al. (2007) Transit of hormonal and EGF receptor-dependent signals through cholesterol-rich membranes. Steroids 72(2): 210-217.

9. Marini F, Bartoccini E, Cascianelli G (2010) Effect of 1alpha, 25-dihydroxyvitamin D_3 in embryonic hippocampal cells. Hippocampus 20(6): 696-705.

10. Kuningas M, Mooijaart SP, Jolles J, Slagboom PE, Westendorp RG, et al. (2009) VDR gene variants associate with cognitive function and depressive symptoms in old age. Neurobiol Aging 30(3): 466-473.

11. Burne TH, Johnston AN, McGrath JJ, Mackay Sim A (2006) Swimming behaviour and post-swimming activity in Vitamin D receptor knockout mice. Brain Res Bull 69(1): 74-78.

12. Kalueff AV, Lou YR, Laaksi I, Tuohimaa P (2004) Increased anxiety in mice lacking vitamin D receptor gene. Neuroreport 15(8): 1271-1274.

13. Sutherland MK, Somerville MJ, Yoong LK, Bergeron C, Haussler MR, et al. (1992) Reduction of vitamin D hormone receptor mRNA levels in Alzheimer as compared to Huntington hippocampus: Correlation with calbindin-28k mRNA levels. Brain Res Mol Brain Res 13(3): 239-250.

14. Beery AK, Lin J, Biddle JS, Francis DD, Blackburn EH, et al. (2012) Chronic stress elevates telomerase activity in rats. Biol Lett 8(6): 1063-1066.

15. Kennedy DO (2016) B Vitamins and the brain: Mechanisms, dose and efficacy--A review. Nutrients 8(2): 68.

16. Wilson DW, Nash P, Buttar HS, Keith Griffiths, Ram Singh, et al. (2017) The role of food antioxidants, benefits of functional foods, and influence of feeding habits on the health of the older person: An overview. Antioxidants (Basel) 6(4): 81.

17. Peres FF, Lima AC, Hallak JEC, Crippa JA, Silva RH, et al. (2018) Cannabidiol as a promising strategy to treat and prevent movement disorders?. Front Pharmacol 9: 482.

18. Nagarkatti P, Pandey R, Rieder SA, Hegde VL, Nagarkatti M (2009) Cannabinoids as novel anti-inflammatory drugs. Future Med Chem 1(7): 1333-1349.

19. Kang S, Min H (2012) Ginseng, the 'Immunity Boost': The effects of *Panax ginseng* on immune system. J Ginseng Res 36(4): 354-368.

20. Yang Y, Ren C, Zhang Y, Wu X (2017) Ginseng: An nonnegligible natural remedy for healthy aging. Aging Dis 8(6): 708-720.

21. Tabish SA (2008) Complementary and alternative healthcare: Is it evidence-based?. Int J Health Sci (Qassim) 2(1): V-IX.

22. Barner JC, Bohman TM, Brown CM, Richards KM (2010) Use of complementary and alternative medicine for treatment among African-Americans: A multivariate analysis. Res Social Adm Pharm 6(3): 196-208.

23. Astin JA, Shapiro SK, Eisenberg DM, Forys KL (2003) Mind-body medicine: State of the science, implications for practice. J Am Board Fam Pract 16(2): 131-147.

24. Maizes V, Rakel D, Niemiec C (2009) Integrative medicine and patient-centered care. Explore (NY) 5(5): 277-289.

25. Bischof M, Del Giudice E (2013) Communication and the emergence of collective behavior in living organisms: A quantum approach. Mol Biol Int 2013: 987549.

26. Cassidy CM (2004) What does it mean to practice an energy medicine?. J Altern Complement Med 10(1): 79-81.

27. Barnes PM, Bloom B, Nahin RL (2008) Complementary and alternative medicine use among adults and children: United States, 2007. Natl Health Stat Report 12: 1-23.

28. Fan KW (2005) National center for complementary and alternative medicine website. J Med Libr Assoc 93(3): 410-412.

29. Wisneski L, Anderson L (2009) The Scientific Basis of Integrative Medicine. Boca Raton, FL: CRC Press 205.

30. Trivedi MK, Alice B, Dahryn T, Snehasis J (2021) Effect of consciousness energy healing treatment on the metal profile and properties of tellurium. Eng Technol Open Acc 3(5): 555623.

31. Trivedi MK, Alice B, Dahryn T, Snehasis J (2021) Consciousness energy healing treatment impacted the isotopic abundance ratio of 6-Mercaptopurine (6-MP). Nov Appro Drug Des Dev 5(5): 555673.

32. Trivedi MK, Branton A, Trivedi D, Nayak G, Mondal SC, et al. (2015) Morphological characterization, quality, yield and DNA fingerprinting of biofield energy treated alphonso mango (*Mangifera indica* L.). Journal of Food and Nutrition Sciences 3(6): 245-250.

33. Trivedi MK, Jana S (2021) Anti-aging activity of biofield energy treated novel proprietary test formulation by assessment of vital biomarkers in cerebrospinal fluid (CSF) in Sprague Dawley rats. On J Neur Br Disord 5(2): 2021.

34. Trivedi MK, Snehasis J (2021) Evaluation of biofield energy healing treatment based proprietary test formulation on gut health potential in colon cancer cell line (HT-29). J Pharmacol Clin Res 8(4): 555743.

35. Trivedi MK, Branton A, Trivedi D, Jana S (2021) Isotopic abundance ratio analysis of consciousness energy healing treated folic acid. Food Nutr Current Res 4(2): 290-295.

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

37. Zúñiga S, Firrincieli D, Housset C, Chignard N (2011) Vitamin D and the vitamin D receptor in liver pathophysiology. Clin Res Hepatol Gastroenterol 35(4): 295-302.

38. Haussler MR, Whitfield GK, Haussler CA, Hsieh JC, Thompson PD, et al. (1998) The nuclear vitamin D receptor: Biological and molecular regulatory properties revealed. J Bone Miner Res 13(3): 325-349.

39. Kumar R, Schaefer J, Grande JP, Roche PC (1994) Immunolocalization of calcitriol receptor, 24-hydroxylase cytochrome P-450, and calbindin D28k in human kidney. Am J Physiol 266(32): 477-485.

40. Wang Y, Borchert ML, Deluca HF (2012) Identification of the vitamin D receptor in various cells of the mouse kidney. Kidney Int 81(10): 993-1001.

41. Gardner DG, Chen S, Glenn DJ (2013) Vitamin D and the heart. Am J Physiol Regul Integr Comp Physiol 305(9): 969-977.

42. Gonzalez Parra E, Rojas Rivera J, Tuñón J, Praga M, Ortiz A, et al. (2012) Vitamin D receptor activation and cardiovascular disease. Nephrol Dial Transplant Suppl 4: 17-21.

43. Nigwekar SU, Thadhani R (2011) Vitamin D receptor activation: Cardiovascular and renal implications. Kidney Int Suppl 3(5): 427-430.

44. Mathur MB, Epel E, Kind S, Desai M, Parks CG, et al. (2016) Perceived stress and telomere length: A systematic review, meta-analysis, and methodologic considerations for advancing the field. Brain Behav Immun 54: 158-169.

45. Kiecolt Glaser JK, Glaser R (2010) Psychological stress, telomeres, and telomerase. Brain Behav Immun 24(4): 529-530.

46. Bhattacharyya J, Mihara K, Bhattacharjee D, Mukherjee M (2017) Telomere length as a potential biomarker of coronary artery disease. Indian J Med Res 145(6): 730-737.

47. Yeh JK, Wang CY (2016) Telomeres and telomerase in cardiovascular diseases. Genes (Basel) 7(9): 58.