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Prevention of Aging and Improvement of Longevity and Life-Span in D-Galactose Induced Aging Rats After Treatment with the Biofield Energy Per Se and Biofield Treated Proprietary Test Formulation

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Abstract

The study was aimed to investigate the potential benefits of the Consciousness Energy Healing Treatment (the Trivedi Effect®) *per se* and Biofield Energy Healing treated novel test formulation in male *Sprague Dawley* rats for their antiaging activity by monitoring aging biomarkers such as brain-derived neurotrophic factor (BDNF), silent information regulator-1 (SIRT-1), and klotho protein. The test formulation was distributed into two parts. First part did not provide any Biofield Energy Treatment was denoted as the untreated sample, however the second part was received Biofield Energy Healing Treatment by a renowned Biofield Energy Healer, Mr. Mahendra Kumar Trivedi and defined as the Biofield Energy Treated sample. In this experiment, nine groups (n=10) were assigned, in which four were preventive maintenance groups. Among them, three groups of animals were also received Biofield Energy Healing Treatment *per se* (at day -15). BDNF was significantly increased by 25.83%, 19.35%, and 14.67% in the Biofield Energy Treated test formulation (G5), Biofield Energy Treatment *per se* at day -15 (G6), and Biofield Energy Treatment *per se* to animals plus Biofield Treated test formulation from day -15 (G8), respectively as compared to the disease control (G2) group. Moreover, expression of SIRT-1 protein was increased by 14.63% in the G5 group than the untreated test formulation (G4) group. Additionally, SIRT-1 activity was increased by 39.7%, 32.5%, 15.9%, and 136% in the G6, Biofield Energy Treated test formulation at day -15 (G7), G8, and Biofield Treatment *per se* (day -15) to animals plus untreated test formulation (G9) groups, respectively than the G4 group, while it was increased by 57.3% in the G9 group as compared to the G2 group. Besides, Klotho protein in kidney homogenate was significantly increased by 16.67% in the G5 group as compared to the G2 group. Altogether, the results showed a significant improvement of longevity mediators and antiaging biomarkers in the preventive maintenance groups. Therefore, results envisaged the significant slowdown of aging-related disorders and other complications 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 control group and could be utilized against various aging-related disorders like Alzheimer's disease, hypertension, osteoporosis, cataracts, type 2 diabetes, cancer, etc. along with it could be used to extend the life-span, stress and immune-related disorders.

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Introduction

Aging is the leading cause for the development of neurodegenerative disorders, and it simultaneously acts as a disease modifier [1]. SIRT1 stands for sirtuin (silent mating type information regulation 2 homolog) in yeast *(Sacaromysis cerevisiae)*. SIRT1 is an enzyme that deacetylates proteins that contribute to cellular regulation (reaction to stressors, longevity). Sirtuins proteins are enzymes present in organisms ranging from bacteria to plants, and animals [2]. SIRT1 also known as NAD-dependent deacetylase protein in humans that is encoded by the SIRT1 gene and regulates the expression of BDNF in the brain [3], and were accepted as evolutionarily conserved epigenetic mediators of longevity [4]. The key role of SIRT1 is to extend the life-span by regulating the response to some conditions such as fasting, caloric restriction and exercise. SIRT1 regulates many endocrine functions, protects organism from oxidative stress-related cellular events, promotes DNA stability, and decreases various age-related disorders, such as neurodegenerative disease, metabolic abnormalities, and cancer [5]. Brain-derived neurotrophic factor (BDNF) is responsible for the growth, promotion, and proliferation of cells in the hippocampus region and it is essential for long-term potentiation and memory function. In humans, with an increase in age and genetic polymorphism, the circulatory level of BDNF become decreased [6, 7]. Besides, reduced level of klotho protein concentration causes chronic kidney disease (CKD) and simultaneously higher risk of aging-related abnormalities [8]. The current study was performed with the aim of investigating the antiaging properties of Biofield Energy Healing *per se* (the Trivedi Effect®) and Biofield Treated test formulation supplemented with multiples minerals and vitamins in *Sprague Dawley* rats. The newly formulated test formulation, which was a combination of multiple minerals such as iron sulfate, copper chloride, zinc chloride, magnesium (II) gluconate and vitamins like cholecalciferol (vitamin D_3), pyridoxine HCl (vitamin B_6), and cyanocobalamin (vitamin B_{12}). Each component of this test formulation commonly used as nutraceutical supplement [9-12]. Complementary and Alternative Medicine (CAM) therapies are now considering as the first-line model for the treatment of various disorders. Biofield Energy Healing Therapy or Healing Modalities is one approach that has been reported to enhance the emotional, mental, physical, and human wellness benefits. The highest percentage of the Americans used the dietary supplement as complementary health approaches than conventional medical therapy as per the National Health Interview Survey (NHIS) 2012. The Biofield Energy Therapy comes under CAM approach, that has been recognized and well accepted by The National Center of Complementary and Integrative Health (NCCIH). It includes other healthcare approaches under CAM such as deep breathing, yoga, natural products, Qi Gong, Tai Chi, massage, chiropractic/osteopathic manipulation, special diets, meditation, progressive relaxation, guided imagery, homeopathy, acupressure, etc. Further, it also extended to acupuncture, hypnotherapy, relaxation techniques, pilates, healing touch, rolfing structural integration, movement therapy, traditional Chinese herbs and medicines, aromatherapy, naturopathy, mindfulness, Ayurvedic medicine, Reiki, cranial sacral therapy.

Human Biofield Energy has subtle energy that can work effectively [13]. CAM therapies have been practiced worldwide with reported great clinical benefits

in various disease profiles [14]. The inherent energy can be harnessed from the universe and transmitted by individuals into both living and non-living objects *via* the process of Biofield Energy Healing Treatment. The potential of Biofield Energy Healing Treatment (the Trivedi Effect®) has been already been contributes in numerous scientific journals in various fields such as skin health [15, 16], nutraceuticals [17, 18], agricultural science [19-21], microbiology and biotechnology [22-24], pharmaceutical science [25-28], cancer research [29, 30], materials science [31-33], human health and wellness. Based on the well-proven scientific literature on the impact of the Biofield Energy Healing Treatment (the Trivedi Effect®) in various fields, authors intend to conduct this experiment on the test formulation for anti-aging activity using standard antiaging biomarker assays.

Materials and Methods

Chemicals and Reagents

Pyridoxine hydrochloride (vitamin B_6), zinc chloride, cyanocobalamin (vitamin B_{12}), magnesium (II) gluconate, and resveratrol were purchased from TCI, Japan. Copper chloride, cholecalciferol (vitamin D_3), sodium carboxymethylcellulose (Na-CMC), and iron (II) sulfate were procured from Sigma-Aldrich, USA. D (+) galactose obtained from Amresco, LLC. Any other chemicals used in this study were analytical grade acquired from India.

Experimental Animals

Randomly breed male *Sprague Dawley* (SD) rats (n=10) with body weight ranges from 238 to 420 gm were used in this study. The animals were purchased from M/s. National Institute of Biologicals, India. Animals were randomly divided into nine groups based on their body weights consist of ten animals in 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

The test formulation was separated into two parts. The first part of each ingredient was considered as control, where no Biofield Energy Treatment was provided. The second part of each component was received Biofield Energy Treatment by Mr. Mahendra Kumar Trivedi (the Trivedi Effect®) under laboratory conditions for ~3 minutes through Mr. Trivedi's unique Energy Transmission process to the test formulation. The blessing/treatment was given to the test items/ animals remotely without touching in the laboratory of Dabur Research Foundation, near New Delhi, India. Similarly, the untreated test samples were subjected to "sham" healer under the same laboratory conditions for ~3 minutes. The "sham" healer did not have knowledge about the Biofield Energy Treatment. After that, the Biofield Energy Treated and untreated test samples were kept in the similar sealed condition and used as per the study plan. The Biofield Energy Treated animals were also be taken back to an experimental room for further proceedings.

Experimental Procedure

Five days after acclimatization, animals were randomized and grouped based on body weight. The test formulation was prepared freshly prior to dosing and administered to the animals using an oral intubation needle attached to an appropriately graduated disposable syringe. The dose volume was 5 mL/kg in morning and evening based on body weight. The experimental groups were divided as G1 as normal control (vehicle, 0.5% w/v CMC-Na); G2 as disease control (D (+) Galactose + 0.5% CMC); G3 as reference item (resveratrol, 200 mg/kg body weight); G4 includes D (+) Galactose along with untreated test formulation; G5 as D (+) Galactose along with the Biofield Energy Treated test formulation; G6 group includes D (+) Galactose along with Biofield Energy Treatment *per se* to animals from day -15; G7 as D (+) Galactose along with the Biofield Energy Treated test formulation from day -15; G8 group includes D (+) Galactose along with Biofield Energy Treatment *per se* plus the Biofield Energy Treated test formulation from day -15, and G9 group denoted D (+) Galactose along with Biofield Energy Treatment *per se* animals plus the untreated test formulation. Dosing for group G7 and G8 was also initiated on a day -15 till the end of the experiment. However, G1 to G6 and G9 animals were dosed from day 1 until the end of an experiment. All the animals

except G1 received D-Galactose, daily (500 mg/kg; *i.p.*) from day 1 to the end of the investigation. After completion of an experiment *i.e.,* during 9th week, the animals were sacrificed and a portion of the brain and kidney samples was homogenized and stored in -80°C for estimation of BDNF, SIRT-1 protein, and SIRT-1 activity in brain homogenate, and klotho protein in kidney homogenate by ELISA method.

Antiaging Biomarkers Assay Using ELISA Method

Estimation of Brain-Derived Neurotrophic Factor (BDNF) in Brain Homogenate

The expression of the brain-derived neurotrophic factor (BDNF) was quantified in rat's brain homogenate using human BDNF ELISA kits (Cloud-Clone Corp, USA; Cat. No. SEA011Ra) according to the manufacturer's instructions [34].

Estimation of the Level of SIRT-1 in Brain Homogenate

The level of sirtuin 1 (SIRT1) is a member of silent information regulator 2 was quantified in rats brain homogenate using Enzyme-linked Immunosorbent Assay Kit (Cloud-Clone Corp, USA; Cat. No. SEE912Ra) according to the manufacturer's instructions [35].

Assessment of SIRT-1 Activity in Brain Homogenate

The SIRT1 activity was determined with the help of universal SIRT activity assay colorimetric Kit (Abcam; Cat. No. ab156915) in rats brain homogenate in according to the manufacturer's instructions [36].

Estimation of Klotho Protein in Kidney Homogenate

The Klotho protein expression was determined using Rat Klotho ELISA Kit, CUSABIO, USA (Cat. No. CSB-E14958r) in rat's kidney homogenate in according to the manufacturer's instructions [37].

Statistical Analysis

The data were expressed as a mean ± standard error of the mean (SEM) and subjected to statistical analysis using Sigma Plot (Version 11.0). For multiple comparison one-way Analysis of Variance (ANOVA) followed by *post-hoc* analysis by Tukey's test. Between two group comparisons, Student's *t*-test was performed. The $p \leq 0.05$ (n=10) was considered as statistically significant.

Results and Discussion

Estimation of Brain-Derived Neurotrophic Factor (BDNF) in Brain Homogenate

The BDNF level was estimated in rat brain homogenate after administration of test formulation and the results obtained are shown in Figure 1. The normal control group (G1) showed 142.56 ± 12.78 pg/mL and it was significantly ($p \leq 0.05$) reduced by 30.58% in the aging control (G2) group (98.97 ± 11.35 pg/mL). The positive control, resveratrol (200 mg/kg) significantly ($p \leq 0.05$) increased the level of BDNF by 48.84% compared to the G2 group. Moreover, the level of BDNF was increased by 25.83%, 19.35%, 4.92%, 14.67%, and 5.43% in the Biofield Energy Treated test formulation (G5), Biofield Energy Treatment *per se* at day -15 (G6), Biofield Energy Treated test formulation at day -15 (G7), Biofield Treatment *per se* to animals plus Biofield Treated test formulation from day -15 (G8), and Biofield Treatment *per se* (day -15) to animals plus untreated test formulation group (G9) compared to the G2 group. High level of oxidative stress is the key initiative for the development of neurodegenerative disorders [38], leads to the reduction of BDNF expression. Reduced level of BDNF plays a vital role in the pathogenesis of neurodegenerative disorders, widely distributed neurotrophin in the central nervous system, and it also plays in synaptic plasticity and neuronal survival [39].

Estimation the Level of SIRT-1 Protein in Brain Homogenate

Sirtuin 1 (SIRT1) is a member of silent information regulator 2 in mammals responsible for age-related diseases, such as cancer, diabetes, metabolic diseases, cardiovascular disease, neurodegenerative diseases, osteoporosis and chronic obstructive pulmonary disease (COPD) [40]. Aging is defined as the dysfunction of organ functions. Nowadays, sirtuins is a famous molecule responsible for slow the aging process and thus reduced the chances of age-related disorders [41]. The impact of the test formulation on the expression of sirtuin 1 (SIRT1) is a member of silent information regulator 2 is shown in Figure 2. The expression of a SIRT-1 protein in the normal control group (G1) was 29.13 ± 4.31 ng/mL, and it was reduced by 29.21% in the aging control (G2) group (20.62 ± 3.21 ng/mL). The positive control group

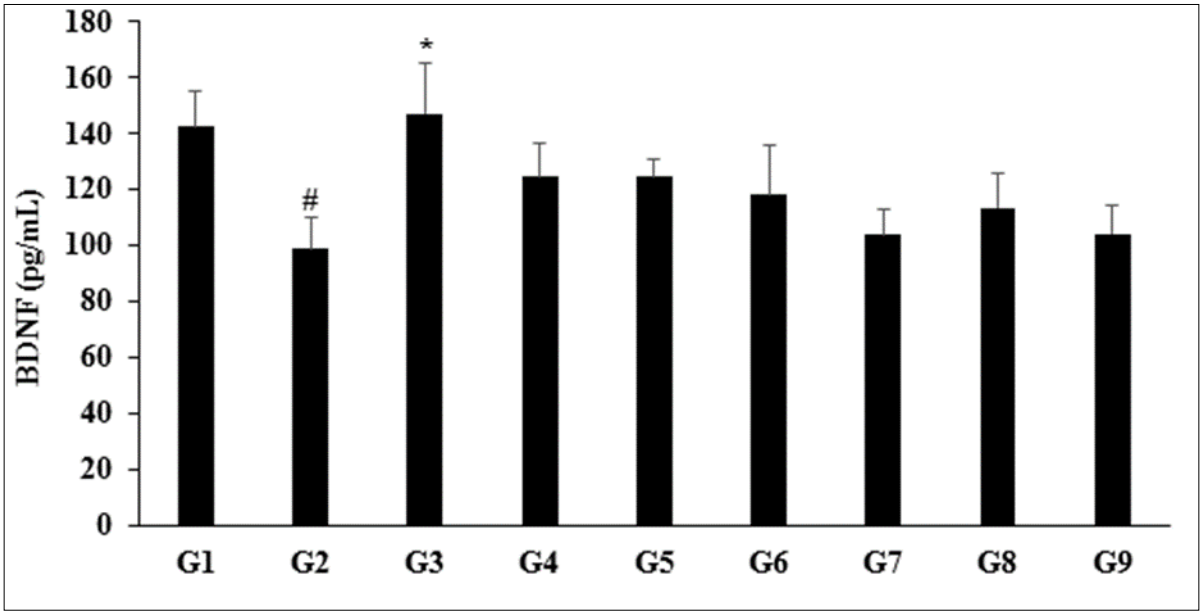

Figure 1. Representing the impact of the Biofield Treated and untreated test formulation on the level of the brain-derived neurotrophic factor (BDNF) in rat's brain homogenate. G: Group; G1: Normal control; G2: Aging control; G3: Resveratrol (200 mg/kg); G4: Untreated test formulation; G5: Biofield Energy Treated test formulation; G6: Biofield Energy Treatment *per se* at day -15; and G7: Biofield Energy Treated test formulation at day -15. G8: Biofield Treatment *per se* to animals plus Biofield Treated test formulation from the day -15; G9: Biofield Treatment *per se* (day -15) to animals plus untreated test formulation. The values are shown as mean ± SEM (n=10). $^{*}p \leq 0.05$ *vs.* G2 and $^{\#}p \leq 0.05$ *vs.* G1.

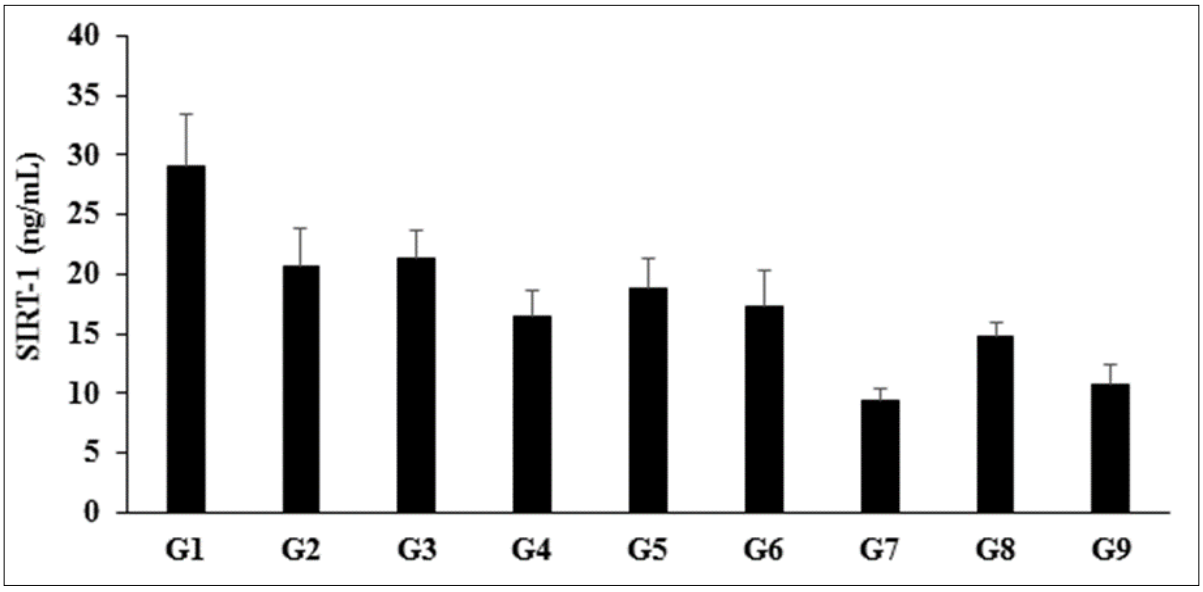

Figure 2. Representing the impact of the Biofield Treated and untreated test formulation on the level of the level of SIRT-1 protein in rat brain homogenate. G: Group; G1: Normal control; G2: Aging control; G3: Resveratrol (200 mg/kg); G4: Untreated test formulation; G5: Biofield Energy Treated test formulation; G6: Biofield Energy Treatment *per se* at day -15; and G7: Biofield Energy Treated test formulation at day -15. G8: Biofield Treatment *per se* to animals plus Biofield Treated test formulation from the day -15; G9: Biofield Treatment *per se* (day -15) to animals plus untreated test formulation. The values are shown as mean ± SEM (n=10).

(G3) showed 3.88% increased the level of SIRT-1 compared to the G2 group. Moreover, the expression of SIRT-1 was increased by 14.63% and 4.55% in the Biofield Energy Treated test formulation (G5) and Biofield Energy Treatment *per se* at day -15 (G6) groups, respectively compared to the untreated test formulation group (G4).

Assessment of SIRT-1 Activity in Brain Homogenate

The effect of the test formulation on SIRT-1 activity in rat brain homogenate as shown in Figure 3. The SIRT-1 activity in the normal control group was found 0.11 ± 0.03 OD/min/mg, and it was reduced by 18.18% in the aging control (G2) group (0.09 ± 0.03) OD/min/mg. SIRT-1 activity was increased by 16.8% in the positive control group as compared to the G2 group. The SIRT-1 activity was increased by 39.7%, 32.5%, 15.9%, and 136% in the Biofield Energy Treatment *per se* at day -15 (G6), Biofield Energy Treated test formulation at day -15 (G7), Biofield Treatment *per se* to animals plus Biofield Treated test formulation from day -15 (G8), and Biofield Treatment *per se* (day -15) to animals plus untreated test formulation group (G9) compared to the untreated test formulation (G4) group (Figure 3). Further, it was increased by 57.3% in the G9 group as compared to the disease control (G2) group. Overall, improvement of brain SIRT-1 activity which might be due to Biofield Energy Treatment. Sirtuin protein expression and their activity resembled life-span by regulating cellular metabolism and aging process [42].

Estimation of Klotho Protein in Kidney Homogenate

The impact of the test formulation on the expression of Klotho protein in kidney homogenate is shown in Figure 4. The level of Klotho protein in the normal control group was 664.79 ± 53.09 ng/mL and it was significantly ($p \leq 0.001$) decreased by 34.15% in the aging control (G2) group (437.75 ± 15.21 ng/mL). The positive control (resveratrol) showed 11.39% increased the level of Klotho protein expression as compared to the G2 group. Further, expression of Klotho protein was increased by 16.67% and 6.83% in the Biofield Energy Treated test formulation (G5) and Biofield Energy Treatment *per se* at day -15 (G6) groups, respectively compared to the aging control group (G2). Others Biofield Treatment groups have altered the level of kidney Klotho expression. Klotho gene is recognized as a putative aging-suppressor gene, has a great interest and provides more useful information of the aging process. Data obtained from one experiment in mice reported that the overexpression of the Klotho gene extends the life-span, and mutations to the klotho gene which shorten the life-span [43, 44].

Based on the literature, it has been stated that SIRT1 can regulates the expression of BDNF in the brain. It also mentioned that increased SIRT1 level can sometimes diminished the BDNF signaling [3]. However, in this experiment, authors found that 50% groups increased level of SIRT-1 can acclerate the level of BDNF and Klotho and 50% groups can suppressed the levels of Klotho only. As per literature, it has been stated that zinc/magnesium can suppressed the deacetylase activity of SIRT1 [45]. Thus, it could be due to the presence of minerals zinc/magnesium in the test proprietary formulation. In this research design, 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, complications and also reduced the chances of disease susceptibility in these groups. Based on the overall data, it suggests that the Biofield Energy Healing Therapy has improved antiaging biomarkers that could be effective and benefited in order to prevent and protect from the occurrence of diseases in rat model that correlate with longevity, life-span, overall health, and quality of life in human.

Conclusions

Results of the study revealed that brain-derived neurotrophic factor (BDNF) in brain homogenate was increased significantly by 25.83% and 19.35% in the Biofield Energy Treated test formulation (G5) and Biofield Energy Treatment *per se* at day -15 (G6), respectively compared to the aging control group (G2). Moreover, the SIRT-1 activity was increased by 39.7%, 32.5%, and 136% in the G6, G7, and G9 groups,

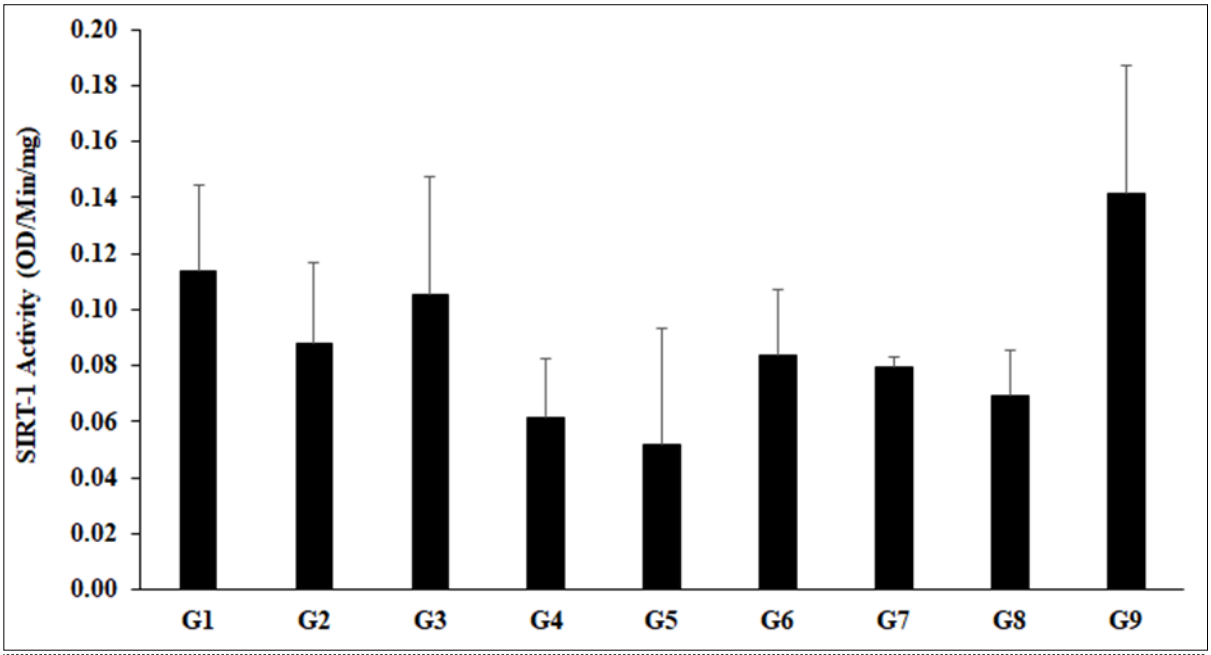

Figure 3. Showing the effect of the Biofield Energy Treated and untreated test formulation on the level of the level of SIRT-1 activity in rat brain homogenate. G: Group; G1: Normal control; G2: Aging control; G3: Resveratrol (200 mg/kg); G4: Untreated test formulation; G5: Biofield Energy Treated test formulation; G6: Biofield Energy Treatment *per se* at day -15; and G7: Biofield Energy Treated test formulation at day -15. G8: Biofield Treatment *per se* to animals plus Biofield Treated test formulation from the day -15; G9: Biofield Treatment *per se* (day -15) to animals plus untreated test formulation. The values are denoted as mean ± SEM (n=10).

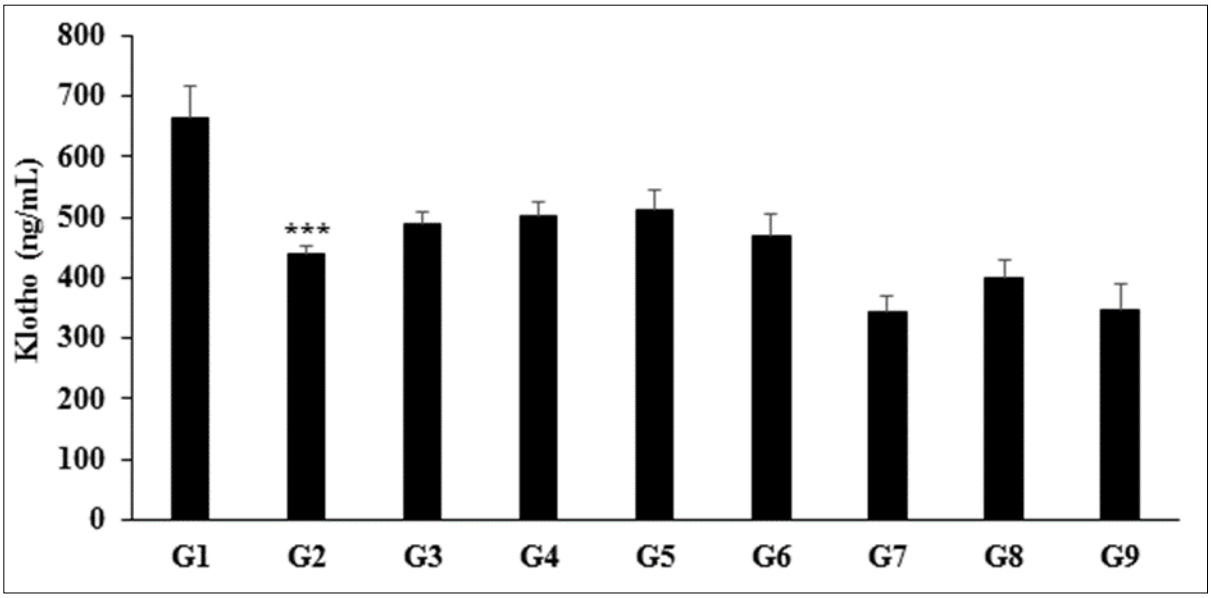

Figure 4. The potential of the Biofield Treated and untreated test formulation on the level of klotho protein in rat brain homogenate. G: Group; G1: Normal control; G2: Aging control; G3: Resveratrol (200 mg/kg); G4: Untreated test formulation; G5: Biofield Energy Treated test formulation; G6: Biofield Energy Treatment *per se* at day -15; and G7: Biofield Energy Treated test formulation at day -15. G8: Biofield Treatment *per se* to animals plus Biofield Treated test formulation from the day -15; G9: Biofield Treatment *per se* (day -15) to animals plus untreated test formulation. The values are cited as mean ± SEM (n=10). ***$p \leq 0.001$ *vs.* G1.

respectively compared to the G4 group, however it was increased by 57.3% in the G9 group as compared to the G2 group. Further, Klotho protein expression in kidney homogenate was increased by 16.67% in the G5 group compared to the G2 group. The current findings conclude that the Trivedi Effect®-Biofield Energy Healing Treatment has significantly enhanced the test formulation's anti-aging properties, which can be used to improve the overall health. Biofield Energy Healing Treatment (the Trivedi Effect®) also showed good results with respect to different efficacy and biomarker parameters in the preventive maintenance group (G6, 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. 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 disease (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. It can be used as a CAM with a safe therapeutic index for various autoimmune disorders such as Lupus Erythematosus, Systemic Fibromyalgia, Hashimoto Thyroiditis, Celiac Disease (gluten-sensitive enteropathy), Addison Disease, Dermatomyositis, Graves' Disease, Multiple Sclerosis, Myasthenia Gravis, Pernicious Anemia, Aplastic Anemia, Scleroderma, Rheumatoid Arthritis, Psoriasis, Reactive Arthritis, Type 1 Diabetes, Sjogren Syndrome, Vasculitis, Crohn's Disease, Chronic Fatigue Syndrome and Alopecia Areata, as well as Vitiligo, inflammatory disorders such as Irritable Bowel Syndrome (IBS), Asthma, Hepatitis, Parkinson's Disease, Ulcerative Colitis, Dermatitis, Alzheimer's Disease, Diverticulitis, and Atherosclerosis. Additionally, the Biofield Energy Healing Treated test formulation can be used in the prevention of immune-mediated tissue damage in cases of organ transplants patients like heart, kidney, and liver transplants, for anti-aging, stress, and in the improvement of overall health and quality of life.

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Conflict of Interest

No conflict of interest was declared by the authors.

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