

Antioxidant Activity of Biofield Treated Proprietary Formulation Supplemented with Vitamins and Minerals in D-Galactose Induced Aging Dysfunction in Sprague Dawley Rats

Trivedi MK¹ and Jana S^{2*}

¹Trivedi Global, Inc, Henderson, USA ²Trivedi 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

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Abstract

A proprietary formulation was designed that consist of minerals (zinc, magnesium, iron, and copper) and vitamins (pyridoxine HCl, cyanocobalamin, and cholecalciferol). The present study was aimed to evaluate the impact of Consciousness Energy Healing Treatment (the Trivedi Effect[®]) on a novel test formulation in male Sprague Dawley (SD) rats for antioxidant activity. The test formulation was divided into two parts. One part was denoted as the control without any Biofield Energy Treatment, while the other part was defined as the Biofield Energy Treated sample, which received the Biofield Energy Healing Treatment by renowned Biofield Energy Healer, Mr. Mahendra Kumar Trivedi. Additionally, three groups of animals were also received Biofield Energy Healing Treatment per se (day -15). The tissue lipid peroxidation data exhibited that the level of malondialdehyde (MDA) was altered by 20.86%, 12.92%, and 17.31% in the Biofield Energy Treated test formulation from day -15 (G7), Biofield Energy Treatment per se plus Biofield Energy Treated test formulation from day -15 (G8), and Biofield Energy Treatment per se animals plus untreated test formulation (G9) groups, respectively as compared to the untreated test formulation group (G4). Moreover, tissue myeloperoxidase (MPO) level was significantly reduced by 32.46%, 27.49%, 27.49%, and 23.71% in the Biofield Energy Treatment per se to animals from day -15 (G6), G7, G8, and G9 groups, respectively as compared to the G4 group. Antioxidant enzyme like SOD was significantly increased by 26.64%, 28.74%, 22.69%, 36.31%, and 36.11% in the Biofield Energy Treated test formulation (G5), G6, G7, G8, and G9, respectively compared to the disease control group (G2). Additionally, the level of catalase was significantly increased by 156.10%, 101.62%, 66.57%, and 179.17% in the G6, G7, G8, G9 groups, respectively compared to the G4 group. Further, LDH level was significantly reduced by 9.86% in the G9 group compared to the G4 group, while NO level was reduced by 11.42%, 10.76%, and 8.77% in the G5, G6, and G7 groups, respectively as compared to the G2 group. Altogether, results suggested that the Biofield Treated test formulation possess antioxidant potential and could be used to maintain bodies' oxidative stress, immunity and boost overall health and aging.

Keywords: Biofield Treatment; Antioxidant; The Trivedi Effect®; LDH; NO; Lipid peroxidation; Anti-Aging

Abbreviations: SD: Sprague Dawley; MDA: Malondialdehyde; MPO: Myeloperoxidase; ROS: Reactive Oxygen Species; HOCl: Hypochloric Acid; CAM: Complementary and Alternative Medicine; NHIS: National Health Interview Survey; NCCIH: National Center of Complementary and Integrative Health; SOD: Superoxide Dismutase; LDH: Lactate Dehydrogenase; NO: Nitric Oxide; LPO: Lipid Peroxidation; TBARS: Thiobarbituric Acid Reactive Species; SEM: Standard Error of Mean; PUFA: Polyunsaturated Fatty Acids.

Introduction

Oxidative stress is the primary cause for many diseases [1]. It has been well proven that reactive oxygen species (ROS) can directly causes oxidative injury to cells by damaging cell membranes, lipids, proteins, and nucleic acids in tissues [2]. The human has the excellent antioxidant defense system to protect the ROS. Decreased antioxidant system activities and increased ROS production leads to pathogenesis of many diseases like hypertension, atherosclerosis, diabetes, chronic renal disease, cancer, rheumatoid arthritis, ischemia/ reperfusion, chronic adenotonsillitis and aging [3, 4]. Antioxidant activity is considered as one of the vital property of any formulation or nutraceuticals. However, the high concentration of free radicals are very much accountable for abundant inflammatory infections [5]. Myeloperoxidase (MPO) is a pro-oxidant with antimicrobial activity. By the utilization of H₂O₂ it produced hypochloric acid (HOCl) and other toxic substances in neutrophil phagolysosomes. It also causes neurodegenerative disorders and atherosclerosis [6,7]. Aging causes morphological and metabolic changes in skeletal muscle followed by reduced ability and weakened athletic performance [8]. The current research work was designed with the aim of investigating the antioxidant and antiaging potentials of Biofield Energy Healing (The Trivedi Effect®) Treated test formulation supplemented with minerals and vitamins in Sprague Dawley rats. The newly formulated test formulation, which is a combination of multiple minerals such as iron sulfate, copper chloride, zinc chloride, magnesium (II) gluconate and vitamins like cholecalciferol (vitamin D₃), pyridoxine HCl (vitamin B₄), 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 of treatment against several disorders, among which Biofield Therapy (or Healing Modalities) is one approach that has been reported to have several benefits to enhance physical, mental, and emotional human wellness. Besides, as per National Health Interview Survey (NHIS) 2012, reported that the highest percentage of the Americans used the dietary supplement as complementary health approaches than conventional medicine therapy. The National Center of Complementary and Integrative Health (NCCIH) has recognized and accepted Biofield Energy Healing as a CAM health care approach in addition to other therapies, medicines and practices such as Qi Gong, Tai Chi, deep breathing, yoga, natural products, chiropractic/osteopathic manipulation, massage, meditation, special diets, progressive relaxation, homeopathy, guided imagery, acupuncture, acupressure, hypnotherapy, healing touch, relaxation techniques, pilates, movement therapy, rolfing structural integration,

mindfulness, traditional Chinese herbs and medicines, Ayurvedic medicine, aromatherapy, naturopathy, essential oils, Reiki, cranial sacral therapy and applied prayer (as is common in all religions, like Hinduism, Christianity, Judaism, and Buddhism).

Human Biofield Energy has subtle energy that can work effectively [13]. CAM therapies have been practiced worldwide with reported clinical benefits in different health disease profiles [14]. This energy can be harnessed and transmitted by individuals into living and nonliving things via the process of Biofield Energy Healing. Biofield Energy Treatment (the Trivedi Effect[®]) has been published in numerous peer-reviewed science journals with significant outcomes in many scientific fields such as cancer research [15,16], microbiology and biotechnology [17-19], pharmaceutical science [20-23], agricultural science [24-26], materials science [27-29], nutraceuticals [30,31], skin health [32,33], human health and wellness. The authors want to evaluate the impact of the Biofield Energy Healing Treatment (the Trivedi Effect[®]) on the test formulation for antioxidant action concerning lipid peroxidation biomarkers malondialdehyde (MDA) and myeloperoxidase (MPO) in liver, antioxidant enzyme superoxide dismutase (SOD), and catalase activity in brain, lactate dehydrogenase (LDH) in muscle, and nitric oxide (NO) in serum using standard 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 carboxymethyl cellulose (Na-CMC), and iron (II) sulfate were procured from Sigma-Aldrich, USA. D (+) galactose obtained from Amresco, LLC. Other chemicals used in this experiment were analytical grade procured from India.

Experimental Animals

Randomly breed male Sprague Dawley (SD) rats with body weight ranges from 240.48 to 428.27 gm were used in this study. The animals were purchased from M/s. National Institute of Biological, India. Animals were randomly divided into nine groups based on their body weights consist of ten animals of each group. They were kept 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 divided into two parts. One part of each ingredient was considered as control, where no Biofield Energy Treatment was provided. Another part of each ingredient and three group of animals were received Biofield Energy Treatment by Mr. Mahendra Kumar Trivedi (known as the Trivedi Effect®) under laboratory conditions for ~3 minutes. Biofield Energy Healer in this study never visited the laboratory (Dabur Research Foundation, New Delhi, India), nor had any contact with the test formulation. The energy transmission was done without touching the samples or animals. Similarly, the control samples were subjected by a "sham" healer under the same laboratory conditions for ~3 minutes. The "sham" healer did not have any knowledge about the Biofield Energy Treatment. After that, the Biofield Energy Treated samples were kept in the similar sealed condition and used as per the study plan. The Biofield Energy Treated animals were also is taken back to experimental room for further proceedings.

Experimental Procedure

days after acclimatization, animals Five were randomized and grouped based on body weight. Dosing for group G7 and G8 was also initiated on day -15 till end of the experiment. However, G1 to G6 and G9 animals were dosed from day 1 till the end of experiment. All the animals except G1 received D-Galactose, daily (500 mg/kg; i.p.) from day 1 to the end of the experiment. At the end of the experimental period, i.e., during 9th week, the animals were bled and the serum samples subjected for the estimation of nitric oxide (NO). Further, the animals were sacrificed and a portion of liver, brain, muscle samples were homogenized and stored in -80°C for estimation of anti-oxidant in liver homogenate (LPO and MPO), in brain homogenate (SOD and catalase), and in muscle homogenate (LDH) by ELISA method.

Antioxidant Assay Using ELISA Method

Tissue Lipid Peroxidation (LPO) in Liver Homogenate: Measurement of thiobarbituric acid reactive species (TBARS) levels is considered as an index of malondialdehyde (MDA) production [34]. This method depends on the formation of MDA as an end product of lipid peroxidation which reacts with TBARS, a pink chromogen, which can be measured spectrophotometrically at 532 nm, an MDA standard was used to construct a standard curve against which readings of the samples were plotted [35].

Tissue Myeloperoxidase (MPO) in Liver Homogenate: For MPO estimation, liver tissue (5%w/v) was homogenized in 0.5% hexadecyltrimethylammonium bromide (HTAB, Sigma-Aldrich, Co., St. Louis, MO, USA) with 50 mm potassium phosphate buffer, pH 6. The rest of the steps were performed as per in-house standard protocol. In addition, the homogenate was used for the estimation of myeloperoxidase (MPO) using Elisa kit (Cat No: k11-0575, Kinesisdx) through the colorimetric method as per manufacturer recommended standard procedure [36].

Estimation of Enzymic antioxidants - Superoxide dismutase (SOD) and Catalase (CAT) in Brain Homogenate: The brain homogenate was used as a matrix for the estimation of antioxidant enzymes by a colorimetric method with slight modification for SOD [37] and CAT [38]. Briefly, the formation of chromic acetate from dichromate and glacial acetic acid in the presence of hydrogen peroxide was measures colorimetrically at 570 nm. One enzyme unit was defined as the amount of enzyme which catalyzed the oxidation of 1 μ M H₂O₂ per minute under assay conditions [39].

Estimation of Lactate Dehydrogenase (LDH) in Muscle Homogenate

Lactate dehydrogenase (LDH) activity was determined spectrophotometrically with lactate as a substrate. The method used is based on that of Wacker WEC, et al. [40], with certain modifications [41].

Estimation of Nitric Oxide (NO) in Serum

Nitrite (NO_2-) and nitrate (NO_3-) are stable final products of NO metabolism and used as indirect markers of NO presence. Total NO concentration is commonly determined as a sum of nitrite and nitrate concentrations. NO concentration was determined using an indirect method based on measurement of nitrite concentration in serum according to Griess's reaction [42].

Statistical Analysis

The data were expressed as mean \pm standard error of mean (SEM) and subjected to statistical analysis using Sigma Plot (Version 11.0). Student's *t*-test was performed for comparison of the individual treatment group with control. The *p*≤0.05 was considered as statistically significant.

Results and Discussion

Measurement of Tissue Lipid Peroxidation (LPO)

Lipid peroxidation is the process in which the membrane bound enzymes, proteins, and receptors are inactivated through loss of cell membrane integrity [43]. In this reaction, the lipid containing polyunsaturated fatty acids (PUFA) are hydrolyzed into biologically active aldehydes and carbonyl compounds. Among these, the most important

is lipid peroxidation end product malondialdehyde (malondialdehyde, MDA) [44]. The effect of the test formulation on the lipid peroxidation in the liver tissue is shown in Figure 1. From the Figure 1, it was observed that the tissue (liver) MDA slightly increased in the disease control group (G2) compared to the normal control group (G1). Further, the level of MDA was altered by 3.45%, 8.24%, 20.86%, 12.92%, and 17.31% in the Biofield Energy Treated test formulation (G5), Biofield Energy Treatment *per se* to animals from day -15 (G6), Biofield Energy Treated test

formulation from day -15 (G7), Biofield Energy Treatment *per se* plus Biofield Energy Treated test formulation from day -15 (G8), and Biofield Energy Treatment *per se* animals plus untreated test formulation (G9) groups, respectively as compared to the untreated test formulation group (G4). After post-treatment with the test formulation the level of lipid peroxidation end product malondialdehyde (MDA) was significantly altered in the Biofield Energy Treatment groups compared to the disease control group, which might be due to The Trivedi Effect[®] - Conscious Energy Healing Treatment.



Figure 1: Lipid peroxide activity of the test formulation in male Sprague Dawley rats. Values are expressed as mean ± SEM, n=10 in each group. G: Group; G1: Normal control; G2: Disease control; G3: Reference item (resveratrol); G4: Untreated test formulation; G5: Biofield Energy Treated test formulation; G6: Biofield Energy Treatment *per se* to animals from day -15; G7: Biofield Energy Treated test formulation from day -15; G8: Biofield Energy Treatment *per se* plus Biofield Energy Treated test formulation from day -15; G8: Biofield Energy Treatment *per se* plus Biofield Energy Treated test formulation from day -15; G8: Biofield Energy Treatment *per se* plus Biofield Energy Treated test formulation from day -15; G8: Biofield Energy Treatment *per se* plus Biofield Energy Treated test formulation from day -15; G8: Biofield Energy Treatment *per se* plus Biofield Energy Treated test formulation from day -15; G8: Biofield Energy Treatment *per se* plus Biofield Energy Treated test formulation from day -15; G8: Biofield Energy Treatment *per se* plus Biofield Energy Treated test formulation from day -15; G8: Biofield Energy Treatment *per se* plus Biofield Energy Treated test formulation from day -15; G8: Biofield Energy Treatment *per se* plus Biofield Energy Treated test formulation from day -15; G8: Biofield Energy Treated test formulation from day -15; G8: Biofield Energy Treated test formulation from day -15; G8: Biofield Energy Treated test formulation from day -15; G8: Biofield Energy Treated test formulation from day -15; G8: Biofield Energy Treated test formulation from day -15; G8: Biofield Energy Treated test formulation from day -15; G8: Biofield Energy Treated test formulation from day -15; G8: Biofield Energy Treated test formulation from day -15; G8: Biofield Energy Treated test formulation from day -15; G8: Biofield Energy Treated test formulation from day -15; G8: Biofield Energy Treated test formulation from day -15; G8: Biofield Energy Treated test formulatice test formulation from day -15;

Tissue Myeloperoxidase (MPO) in Liver Homogenate

Myeloperoxidase (MPO) is a heme protein enzyme secreted by the activation of leukocytes that facilitates the conversion of hydrogen peroxide to hypochlorous acid and produces a reactive intermediate that promote lipid peroxidation [45]. The impact of Biofield Energy Treated test formulation on the activity of liver myeloperoxidase is shown in Figure 2. The level of MPO in the normal control (G1) and disease control (G2) groups was 4.35 ± 0.45 and 4.25 ± 0.46 ng/mL, respectively. Resveratrol (positive control) significantly reduced the level of MPO by 15.53% compared to the G2 group. Additionally, the MPO level was significantly reduced by 9.24%, 32.46%, 27.49%, 27.49%, and 23.71% in the Biofield Energy Treated test formulation

(G5), Biofield Energy Treatment per se to animals from day -15 (G6), Biofield Energy Treated test formulation from day -15 (G7), Biofield Energy Treatment per se plus Biofield Energy Treated test formulation from day -15 (G8), and Biofield Energy Treatment per se animals plus untreated test formulation (G9) groups, respectively as compared to the untreated test formulation group (G4). One study reported that during aging state there was an increased accumulation of lipid peroxidation end products, which indirectly indicated that oxidative stress and age are directly proportional [46,47]. Overall, the Biofield Treated groups more reduction of the MPO level compared to untreated test formulation group (G4), which can restrict or slowdown the process of lipid peroxidation. Thus, it would be beneficial for cardiovascular, kidney-related, and neurodegenerative, and inflammatory disease conditions.





Estimation of Antioxidant Enzymes - Superoxide dismutase (SOD) and Catalase (CAT)

The effect of the test formulation on the enzymic antioxidant level in the liver tissue is shown in Figure 3A and 3B. The level of SOD was significantly ($p \le 0.001$) reduced by 32.12% in the G2 group compared to the G1 group. However, the SOD level was increased significantly ($p \le 0.001$) by 37.16% in the positive control group (G3) compared to the G2 group. Further, SOD level was significantly increased by 57.31%, 26.64%, 28.74%, 22.69%, 36.31%, and 36.11% in the untreated test formulation (G4), Biofield Energy Treated test formulation (G5), Biofield Energy Treated test formulation from day -15 (G6), Biofield Energy Treatment *per se* to animals from day -15 (G7), Biofield Energy Treatment *per se* plus Biofield Energy Treatment *per se* animals plus untreated test formulation (G9) groups, respectively

compared to the G2 group (Figure 3A). Besides, the level of catalase was significantly ($p \le 0.001$) reduced by 63.56% in the G2 group compared to the G1 group. The positive control group significantly ($p \le 0.05$) increased by 113.61% of catalase enzyme compared to the G2 group. Further, the catalase level was significantly increased by 156.10%, 101.62%, 66.57%, and 179.17% in the G6, G7, G8, G9 groups, respectively compared to the G4 group (Figure 3B). Oxygen free radicals have been proposed to be involved in the process of aging. SOD and catalase are important for antioxidative defence [48,49]. From literature it was reported that an increased levels of both CuZn-SOD and catalase leads to increased maximum life span and further SOD plays an important role in longevity and degenerative disease [50]. Overall, Biofield Treated test formulation significantly improved the levels of antioxidant defenced enzymes compared to the untreated test formulation group.



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Figure 3B: Catalase of the test formulation in male Sprague Dawley rats. Values are expressed as mean \pm SEM, n=10 in each group. ### $p \le 0.001 vs$. G2; * $p \le 0.05 vs$. G2; *** $p \le 0.001 vs$. G4.

Lactate Dehydrogenase (LDH) in Muscle Homogenate

The effect of the test formulation on the level of lactate dehydrogenase (LDH) in muscle homogenate is shown in Figure 4. Lactate dehydrogenase is an enzyme that catalyzes the pyruvate-lactate reaction. Physical activity and age are among the factors affecting lactate levels and lactate dehydrogenase activity. Increased level of lactate and alterations of lactate dehydrogenase (LDH)-A/B mRNA-expression-ratio in brain is the hallmark of ageing [51,52]. Moreover, mitochondrial dysfunction also responsible for aging-related alterations in neuronal function [53]. The

level of LDH in muscle homogenate was observed 541.99 \pm 12.84, 457.42 \pm 15.66, and 512.74 \pm 17.43 ng/mL in the normal control (G1), disease control (G2), and positive control (G3) groups, respectively. Further, LDH level was significantly reduced by 4.09%, 7.65%, 6.37%, and 9.86% in the Biofield Energy Treatment *per se* to animals from day -15 (G6), Biofield Energy Treated test formulation from day -15 (G7), Biofield Energy Treatment *per se* plus Biofield Energy Treatment *per se* animals plus untreated test formulation (G9) groups, respectively compared to the untreated test formulation (G4) group.



Estimation of Nitric Oxide (NO) in Serum

Nitric oxide (NO) is normally produced by the influenced of endothelial (e) NO synthase (eNOS) and neuronal (n) NO

synthase nNOS, and plays a ubiquitous role in the body in controlling the function of every organ system [54]. NO has both anti-oxidant and pro-oxidative properties, while its level was increased in the inflammatory conditions [55,56].

It also plays an important role different chronic diseases [57]. The effect of test item on the level of nitric oxide (NO) in muscle homogenate is shown in Figure 5. The level of NO was increased by 14.83% in the disease control group (G2) as compared to the normal control (G1) group (5.26 \pm 0.32 μ M/mL). Positive control, resveratrol was significantly

($p \le 0.001$) reduced the NO level by 24.34% as compared to the G2 group. Further, NO level was reduced by 11.42%, 10.76%, 8.77%, and 4.30% in the G5, G6, G7, and G9 groups, respectively as compared to the disease control group (G2) group. Overall, Biofield Energy Treatment altered the level of NO in muscle homogenate.



Conclusion

Based on the study outcomes, the MPO level was significantly reduced by 32.46% (*p*≤0.05), 27.49%, 27.49%, and 23.71% in the G6, G7, G8, and G9 groups, respectively than the untreated test formulation (G4) group. SOD enzyme level was increased by 26.64%, 28.74%, 22.69%, 36.31%, and 36.11% in the G5, G6, G7, G8, and G9 groups, respectively compared to the disease control group (G2). Catalase enzyme was significantly increased by 156.10% ($p \le 0.001$), 101.62% (*p*≤0.05), 66.57%, and 179.17% in the G6, G7, G8, and G9 groups, respectively compared to the G4 group. LDH level was significantly reduced by 9.86% in the G9 group compared to the G4 group, while NO level was reduced by 11.42% and 10.76% in the G5 and G6 groups, respectively with respect to the G2 group. The current findings conclude that the Biofield Energy Treated Proprietary formulation possess antioxidant properties, which can be used to improve the overall health. Thus, the Biofield Treated test formulation can be used as a CAM for various autoimmune disorders such as Rheumatoid Arthritis, Myasthenia Gravis, Aplastic Anemia, Lupus Erythematosus, Addison Disease, Celiac Disease, Hashimoto Thyroiditis, Multiple Sclerosis, Dermatomyositis, Graves' Disease, Scleroderma, Psoriasis, Pernicious Anemia, Type 1 Diabetes, Crohn's Disease, Vasculitis, Vitiligo, Chronic Fatigue Syndrome and Alopecia Areata, a's well as inflammatory disorders such as Irritable Bowel Syndrome (IBS), Asthma, Ulcerative Colitis, Parkinson's Disease, Alzheimer's Disease, Dermatitis, Atherosclerosis, Hepatitis, and Diverticulitis.

Further, the Biofield Energy Healing Treated test formulation can also be used in the prevention of immune-mediated tissue damage in cases of organ transplants (for example heart transplants, kidney transplants, and liver transplants), for anti-aging, stress prevention and management, and in the improvement of overall health and quality of life.

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