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An Evaluation of Vitamin D Receptor (VDR), Telomerase and Citrate Synthase in Various Tissues after Treatment with the Biofield Energy Healing based Test Formulation in Vitamin D_3 Deficiency Diet (VDD) Induced Animal Model

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Abstract

The study was evaluated the impact of Consciousness Energy Healing Treatment/Blessing (the Trivedi Effect®) on test formulation in rats under the influence of vitamin D_3 deficiency diet (VDD). The following mechanistic biomarkers were estimated such as vitamin D receptor (VDR), telomerase, and citrate synthase enzymes. The test formulation's ingredient was divided into two parts; one part was defined as untreated test formulation, while the other parts and the animals provided Biofield Energy Healing/Blessing Treatment by Mr. Mahendra Kumar Trivedi. The expression of VDR in liver was significant increased by 74.91%, 100.66%, and 64.25% in the G5 (VDD + Biofield Energy Treated test formulation), G6 (VDD + Biofield Energy Treatment *per se* to animals from day -15), and G7 (VDD + Biofield Energy Treated test formulation from day -15) groups, respectively as compared with the disease control (G2) group. Similarly, the expression of VDR in adipose tissue was significant increased by 268.6% ($p \leq 0.001$), 163.13% ($p \leq 0.001$), 92.42%, and 63.25% in G5, G6, G7, and G9 groups, respectively, than G4. However, the level of liver telomerase was reported to be increased by 59.09% ($p \leq 0.001$), 68.18% ($p \leq 0.001$), and 68.18% ($p \leq 0.001$) in the G6, G7, and G8, respectively as compared to the G4. Similarly, the telomerase level in the thigh muscle was significant increased by 15.79% in the G7 group as compared with the G4 group. Besides, the level of citrate synthase was significantly increased by 5.89% in the G8 group as compared to the G2 group. Altogether, the Biofield Treated/Blessed test formulation and Biofield Treatment *per se* to the animals could be an effective approach to improve the functioning of VDR (liver and adipose tissue), telomerase enzyme (liver and thigh muscle), and mitochondrial activity in terms of citrate synthase (substrate of kreb's cycle). Therefore, the findings could be used against bone-related disorders (osteoporosis, fracture, scoliosis, and bone cancer), deficiency of vitamin D_3, and mitochondrial dysfunction in associate with age-related and neurodegeneration disorders such as Alzheimer's disease, dementias, brain cancer, epilepsy and Parkinson's. The data showed a significant reduction of disease progression and all other disease-related complications/symptoms in the preventive treatment groups *viz.* G6, G7, G8, and G9.

Keywords: Calcitriol; Biofield Treatment; Vitamin D Receptor; The Trivedi Effect®; Citrate synthase; Vitamin D_3 deficiency diet

Introduction

A biomarker acts as an indicator in various pathological and biological processes. The mechanistic biomarker is used to guide the clinical management of different diseases [1]. Telomerase is a reverse transcriptase enzyme. It can restore DNA known as telomeres, otherwise shortened the telomere length, during cell division *via* mitosis. In humans, skeletal muscle telomere lengths remain stable from ages 23-74 years, hence muscular tissues are selected for the estimation of telomerase enzyme [2]. Besides, in human liver, hepatocytes show no age-related telomere shortening effect, hence liver tissue is also selected in this study for the estimation of telomerase enzyme level [3]. Studies on rats found that the deficiency in vitamin D receptor (VDR) may develop high blood pressure that are due to the activation of the renin-angiotensin-aldosterone system, as vitamin D acts as a negative regulator of renin synthesis [4]. Several biochemical measures of mitochondrial components are used as biomarkers of mitochondrial content and muscle oxidative capacity [5]. Citrate synthase activity is a validated biomarker for mitochondrial density in skeletal muscle. It is also used as a biochemical marker of the skeletal muscle oxidative adaptation [6].

Both pre-clinical and clinical trials have been focused to develop a novel proprietary test formulation that could be useful to maintain the overall health and quality of life. However, so far, no such novel herbal-based test formulation was designed that can improve the overall organ health using cell-based standard assay. With this respect, the formulation was designed as per best scientific literature, which is the best optimizable/possible combination of different minerals (zinc, selenium, copper, iron, magnesium, and calcium), vitamins (vit. C, vit. B12, vit. E, vit. D3, and vit. B6), cannabidiol isolate, *Panax ginseng* extract, and β-carotene. The novel proprietary test formulation is designed for maintain of overall health and quality of life in both healthy and pathologic conditions. Minerals and vitamins are included in the test formulation possesses excellent physiological support [7-9]. Apart from, *Panax ginseng* is one of the best medicinal plants that improve cognitive, mental, and immunomodulatory potential [10,11]. Cannabidiol (CBD) isolates has been widely reported in many pharmacological functions [12,13].

Biofield Therapy was reported in multiple scientific studies and clinical trials for its beneficial effects on cervical cancer patients [14], massage therapy [15]. 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 mental, physical, 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 *viz.* Reiki, Qi Gong, Tai Chi, chiropractic/ osteopathic manipulation, deep breathing, yoga, meditation, massage, homeopathy, progressive relaxation, guided imagery, special diets, acupressure, acupuncture, relaxation techniques, healing touch, hypnotherapy, pilates, movement therapy, traditional Chinese herbs and medicines, Ayurvedic medicine, naturopathy, cranial sacral therapy, and aromatherapy.

Human Biofield Energy has subtle energy that has the capacity to work in an effective manner [16,17]. Biofield Energy Healing/Blessing Treatment (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 materials 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 various mechanistic biomarkers such as VDR and telomerase in liver tissues, VDR in adipose tissues, and telomerase in muscular tissues by ELISA assay under VDD diet and test formulation in male *Sprague Dawley* rats.

Materials and Methods

Chemicals

Cannabidiol isolate and *Panax ginseng* extract were obtained from Panacea Phytoextracts, India and Standard Hemp Company, USA, respectively. 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. Calcitriol, pyridoxine hydrochloride (vitamin B_6), magnesium (II) gluconate, zinc chloride, and β-carotene (retinol, provit A) were purchased from TCI, Japan. Ascorbic acid (vitamin C) and sodium selenate were obtained from Alfa Aesar, India.

Maintenance of animal

Male *Sprague Dawley* (SD) rats with body weight ranges (200 to 300 gm) were used in this experiment. The animals were obtained from M/s. Vivo Bio Tech, Hyderabad, India. Animals were randomly distributed into nine groups (n=6). The animals were maintained as per standard protocol throughout the experiment.

Consciousness energy healing strategies

Each ingredient of the novel test formulation was divided into two parts. One part of the test compound did not receive Biofield Energy Treatment/Blessing and were defined as the untreated or control sample; it was subjected by a "sham" healer. The "sham" healer did not have any knowledge about the Biofield Energy Treatment/Blessing. The second part of the test items and three group of animals were received the Biofield Energy Treatment/ Blessing by a renowned Biofield Energy Healer, Mr. Mahendra Kumar Trivedi under 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 blessing, the Biofield Treated/Blessed samples, animals, and untreated test samples were used as per the study plan.

Experimental procedure

The animals were randomized and grouped based on body weight after seven days of acclimatization. All the animals except G1 were fed with vitamin D_3 deficient diet (VDD) from day -12 to till the end of the experiment. To induce CYP24A1 expression, to accelerate the catabolism of endogenous vitamin D_3, the rats (Group G2 to G6) were received intraperitoneal injections of 40 ng of 19-nor-1,25-dihydroxyvitamin D2 (Paricalcitol) on days -12, -10, -8, -6, -4, -2, day 1, 3 and 5. Group G1 to G5 animals were dosed with respective formulations from Day 1 to till the end of the experiment. However, Group G6 were not dosed. Animals (50% of the animals from each group) were kept for overnight fasting on Day 56 (Tentative). However, remaining 50% animals were dosed with respective formulations and were kept for fasting on Day 57 (Tentative). After that, various tissues like liver, adipose, and thigh muscles were isolated, homogenized by standard in-house method for the estimation of VDR receptor, telomerase assay, and mitochondrial assay by ELISA method.

Preparation of tissue homogenate

About 100 mg of the liver and adipose tissue homogenate were prepared as per Trivedi et al. [34].

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Quantification of vitamin D receptor (VDR) using Q-PCR in bone (MG-63) cells

The human bone osteosarcoma (MG-63) cells were counted using the hemocytometer were plated at a density of 2 X 10^5 cells/well in 6-well plates followed by overnight incubation. The cells were then sera starved for 24 hours and treated with the test formulation/positive control at the non-cytotoxic concentrations. The untreated cells that served as control that did not receive any treatment and were maintained in cell growth medium only. The treated cells were incubated for 24 hours and VDR expression was determined by Q-PCR using VDR specific primers. Cells were harvested by scrapping and washed with PBS. The cell pellets obtained were analyzed for VDR gene expression using human VDR specific primers: Forward: 5'-GCTGACCTGGTCAGTTACAGCA-3', Reverse: 5'-CACGTCACTGACGCGGTACTT-3'. VDR gene expression was normalized using House-keeping (HK) reference. Relative quantification (RQ) of VDR gene in Biofield Energy Treated cells was calculated with respect to the untreated cells using Equation 8:

$$RQ = 2\text{-}N \quad \text{..... (8)}$$

Where, N is the relative Threshold Cycle (CT) value of treated sample with respect to the untreated sample.

Estimation of citrate synthase enzyme in muscle

Muscles were subjected for the estimation of citrate synthase enzyme using ELISA method as per manufacturer's recommended standard procedure.

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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

The effect of the test formulation on vitamin D receptors (VDRs)

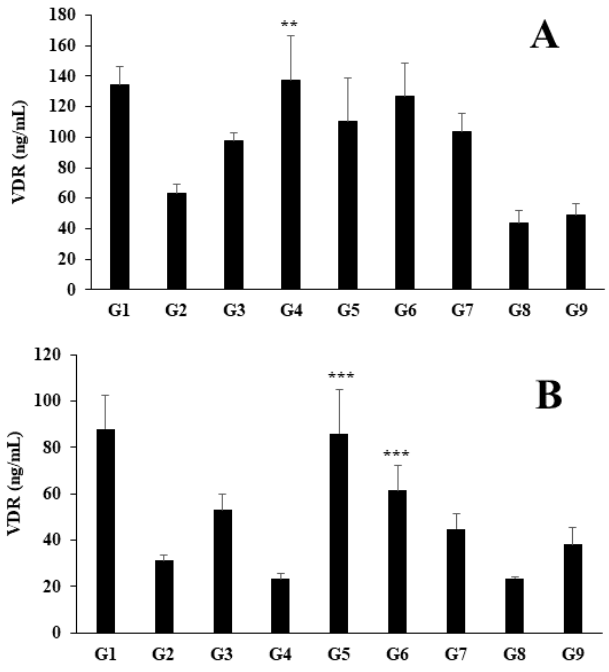

Figure 1: The effect of the test formulation on the level of vitamin D receptor (VDR) in A. liver homogenate and B. in adipose tissue of Sprague Dawley rats. G: Group; G1: Normal control (0.5% CMC); G2: Disease control (VDD: Vitamin D3 deficient diet + 0.5% CMC); G3: Reference item (VDD + Calcitriol); 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≤0.01 vs. G2 and ***p≤0.001 vs. G4.

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The human bone osteosarcoma cells (MG-63) were treated with the test formulation and the effect on VDR expression was determined using quantitative-polymerase chain reaction (Q-PCR) amplification. VDR-relative threshold cycle (VDR-CT) values were obtained from PCR amplification. Relative quantification (RQ) was calculated from the VDR-CT and house-keeping (HK)-CT values for MG-63 cells treated with test formulation and positive control is represented in Figure 1. The positive control (calcitriol) showed 54.93% increase of RQ of VDR as compared to the disease control group (G2) in the liver homogenate. The RQ of VDR was significantly increased by 117.39%, 74.91%, 100.66%, and 64.25% in the G4 group (VDD + untreated test formulation), G5 (VDD + Biofield Energy Treated test formulation), G6 (VDD + Biofield Energy Treatment *per se* to animals from day -15), and G7 (VDD + Biofield Energy Treated test formulation from day -15) groups, respectively as compared to the disease control (G2) group in the liver homogenate (Figure 1A).

The positive control (calcitriol) showed 70.12% increase of RQ of VDR as compared to the disease control group (G2) in the adipose tissue homogenate. The RQ of VDR was significantly increased by 176.84%, 97.62%, 44.52%, and 22.61% in the G5, G6, G7, and G9 (VDD + Biofield Energy Treatment *per se* animals plus untreated test formulation) groups, respectively as compared to the disease control (G2) group in adipose tissue homogenate. Moreover, the RQ of VDR was significantly increased by 268.6% ($p \leq 0.001$), 163.13% ($p \leq 0.001$), 92.42%, and 63.25% in the G5, G6, G7, and G9 groups, respectively as compared to the G4 group in adipose tissue homogenate (Figure 1B). The biologically active vitamin D metabolite is 1α, 25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), which functions as specific high-affinity ligand of the transcription factor of VDRs [35]. The active form of vitamin D [1α,25(OH) (2)D(3)] can binds and activates its specific nuclear receptor, *i.e.,* the vitamin D receptor (VDR). Thus, this activated VDR can prevents the release of calcium from its storage in bone to serum by stimulating intestinal calcium absorption and renal reabsorption [36]. Overall, the Biofield Energy Treated/Blessed test formulation has significantly increased the expression of VDRs, which might be helpful to bind more active vitamin D_3 metabolites and that ultimately can improve the more physiological functions of vitamin D and simultaneously improved bone cell growth and development.

Estimation of telomerase enzyme in liver and muscle

The telomerase activity in liver and muscles cells was measured in all the experimental groups and was graphically presented in the Figure 2. The level of liver telomerase activity was significantly increased by 52.38% in the positive control (calcitriol) group as compared to the disease control (G2) group. The level of telomerase activity in liver tissue was significantly increased by 4.76%, 23.81%, 66.67%, 76.19%, and 76.19% in the in the G4, G5, G6, G7, and G8 groups, respectively than G2. Further, the level of telomerase was significantly increased by 18.18%, 59.09% ($p \leq 0.001$), 68.18% ($p \leq 0.001$), and 68.18% ($p \leq 0.001$) in the G5, G6, G7 and G8 groups, respectively than G4 group in the liver (Figure 2A). Besides, the level of telomerase activity in thigh muscle was significantly increased by 5.26%, 5.26%, 15.79%, and 5.26%, in the G5, G6, G7, and G8 groups, respectively as compared to the G4 group (Figure 2B).

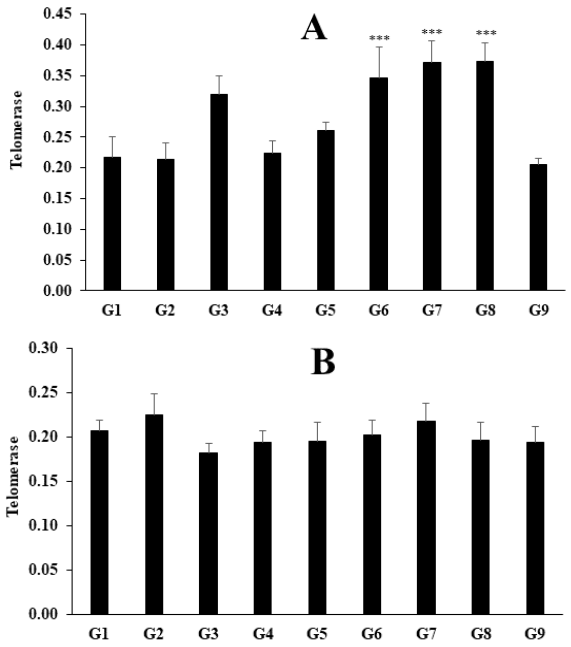

Figure 2: The effect of the test formulation on the level of telomerase enzyme in (A) liver and (B) thigh muscle of Sprague Dawley rats. The obtained data are presented as mean ± SEM (n=6). ***p≤0.001 vs. G4 group.

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Chronic or long term stress contributes to various forms of diseases, which results in huge damage to telomeres, 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 [37, 38]. In conclusion, the present data suggested that Biofield Energy Healing Treatment *per se* and the Biofield Energy Treated/Blessed test formulation play a significant role in telomerase assay in nervous system, which directly improved various stress disorders.

Estimation of citrate synthase enzyme in muscle

Mitochondria play a vital role in skeletal muscles functions and metabolic health. Citrate synthase is a key mitochondrial enzyme used as a biomarker of mitochondrial content and function in mammals [39]. The level of citrate synthase was significantly increased by 17.18% in the positive control (calcitriol) group as compared to the disease control (G2) group. Moreover, citrate synthase level was significantly increased by 4.36%, 3.85%, 5.89%, and 4.87% in the G5 (VDD + Biofield Energy Treated test formulation), G6 (VDD + Biofield Energy Treatment *per se* to animals 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) groups, respectively as compared to the disease control (G2) group. Further, Citrate synthase was increased by 4.09%, 3.58%, 5.63%, and 4.60% in the G5, G6, G8, and G9 groups, respectively as compared to the G4 group (Figure 3).

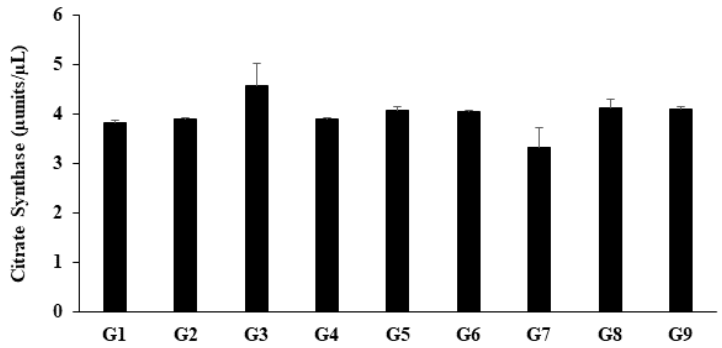

Figure 3: The effect of the proprietary test formulation on the level of citrate synthase in muscular tissues of Sprague Dawley rats. The obtained data are presented as mean ± SEM (n=6).

Here, four groups were considered as preventive maintenance groups such as G6, G7, G8, and G9. The current study data showed significant reduction of disease progression and all other disease-related symptoms/complications. Specifically, G6 group showed the desirable results as a preventive maintenance group. Altogether, it showed that the Biofield Energy Healing/Blessing Therapy was found to be most effective and beneficial to prevent and protect from different types of diseases in the rat model, which will ultimately improve the overall health and quality of life.

Conclusions

Based on the study outcomes, it was found that the expression of VDR liver tissue was significantly increased by 74.91%, 100.66%, and 64.25% in the G5, G6, and G7 groups, respectively than G2. The expression of VDR in adipose tissue was also significantly increased by 268.6%, 163.13%, 92.42%, and 63.25% in the G5, G6, G7, and G9 groups, respectively, as compared with the G4 group. Further, the level of liver telomerase was significantly increased by 59.09%, 68.18%, and 68.18% in the G6, G7, and G8, respectively as compared to the G4. The telomerase level in the thigh muscle was significant increased by 15.79% in the G7 group as compared with the G4 group. The level of citrate synthase was significantly increased by 5.89% in the G8 group as compared to the G2 group. Overall, results revealed that the Trivedi Effect® significantly increased the expression of vitamin D receptor (VDR), telomerase and mitochondrial enzyme functions by increasing cytochrome genes. This test formulation can be used against bone-related disorders (osteoporosis, fracture, scoliosis, and bone cancer), deficiency of vitamin D_3, and mitochondrial dysfunction in associate with age-related and neurodegeneration disorders such as Alzheimer's disease, dementias, brain cancer, epilepsy and Parkinson's.

It could also be used other disorders such as systemic lupus erythematosus, Addison disease, multiple sclerosis, myasthenia gravis, pernicious anemia, psoriasis, rheumatoid arthritis, Crohn's disease, as well as inflammatory disorders such as atherosclerosis, ulcerative colitis, hepatitis, dermatitis, and diverticulitis. However, Trivedi's Biofield Therapy can also be used in the prevention of different kinds of brain disorders, and in the improvement of overall health and quality of life. Trivedi's Biofield Therapy can also helped to reduce the disease progression and disease-related complications. Therefore, the Trivedi Effect® *per se* effect to the animals directly, which might be effective as extraplorative in

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healthy humans to sustain a good health, to boost overall health, to promote healthy aging and to increase the quality of life. In the presence of disease, the Biofield Energy therapy might reduce the severity of any acute/chronic disease (such as auto-immune related and inflammatory disorders) and / or slow the disease progression.

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