

## Effect of Biofield Energy Healing Treatment on Rat Immunological Biomarkers in Vitamin D<sub>3</sub> Deficiency Diet (VDD) Induced Animal Model

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Received April 26, 2021; Revised June 14, 2021; Accepted June 17, 2021

### ABSTRACT

Combined test formulations including extract, minerals and vitamins are extensively used worldwide for wide range of disorders. A novel proprietary formulation was designed that consist of minerals (magnesium, zinc, copper, calcium, selenium, and iron), vitamins (ascorbic acid, pyridoxine HCl, alpha tocopherol, cyanocobalamin, and cholecalciferol), *Panax ginseng* extract,  $\beta$ -carotene, and cannabidiol isolate. This formulation was tested and evaluated for the impact of Consciousness Energy Healing/Blessing Treatment (the Trivedi Effect<sup>®</sup>) on a novel test formulation in male *Sprague Dawley* (SD) rats, fed with vitamin D<sub>3</sub> deficiency diet (VDD) for immunomodulatory activity. The novel test formulation was divided into two parts, one section was defined as the untreated test formulation, while the other part was defined as the Biofield Energy Treated/Blessed sample, which received the Biofield Energy Healing Treatment/Blessing by renowned Biofield Energy Healer, Mr. Mahendra Kumar Trivedi. Platelet counts were increased by 12.43% in the Biofield Energy Treated test formulation from day -15 (G7) group as compared with the G4 group. Total cholesterol was decreased by 15.01%, 12.34%, and 14.80% was reported in the G5, G6, and G9 groups, respectively as compared with the G4 group. The level of LDL was significantly reduced by 40.66% ( $p \leq 0.05$ ), 33.38% ( $p \leq 0.05$ ), 36.75% ( $p \leq 0.05$ ), 27.37%, and 20.54% in the test groups viz. G5, G6, G7, G8, and G9 groups, respectively as compared with the G4 group. VLDL was significantly decreased by 10.31% and 37.63% ( $p \leq 0.01$ ) in the G5 and G6 groups, respectively as compared with the G4. The level of triglycerides was significantly ( $p \leq 0.01$ ) reduced by 38.13% in the G6 group as compared with the G4 group. Calcium level was significantly ( $p \leq 0.001$ ) improved by 16.01%, 18.91%, and 14.7% in the G5, G7, and G8 groups, respectively as compared with the G4 group. Hormonal profile data suggested that testosterone was showed increased by 603.56%, 155.39%, 334.30%, 174.24%, and 224.39% in the G5, G6, G7, G8, and G9 groups, respectively while, serum corticosterone level was reported to be decreased by 13.94%, 23.61%, and 22.13% in the G6, G8, and G9 groups, respectively as compared with the G4 group. Organ histopathological analysis suggested that findings were spontaneous or incidental in nature, representing the normal physiological/metabolic or congenital changes encountered all the experimental groups. Altogether, the Biofield Treated test formulation and Biofield Energy Treatment *per se* significantly improved the immune-related parameters along with hormones like testosterone and corticosterone, which might be beneficial for the management of immune-compromised patients as well as to boost-up the immunity in healthy peoples. Overall, the results showed the significant slowdown the disease progression and disease-related all other complications/symptoms in the preventive Biofield Energy Treatment group *per se* and/or Biofield Energy Treated Test formulation groups (viz. G6, G7, G8, and G9) comparatively with the disease control group.

**Keywords:** Biofield Treatment, Immunomodulation, The Trivedi Effect<sup>®</sup>, Hematology, Biochemistry, Testosterone, Corticosterone, Vitamin D<sub>3</sub> deficiency diet, Calcitriol

### INTRODUCTION

Vitamin D deficiency (VDD) is still one of the major clinical concerns of public health, which is one of the preventable health concerns worldwide [1]. Most of the adult population has reported to have various metabolic diseases along with depression, cognitive outcomes, obesity, immune disorders, brain dysfunction, cancer, and many more, which are reported to be linked with VDD [2]. Most of the clinical data suggested that VDD significantly affects the brain function and its related diseases such existence of adverse cognitive outcomes and dementia in case of low vitamin D state. Besides, brain disorders VDD also affects the normal human

physiology such as some extent of hematology and biochemistry, which may lead to serious health concerns [3].

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**Citation:** Trivedi MK, Branton A, Trivedi D, & Jana S. (2021) Effect of Biofield Energy Healing Treatment on Rat Immunological Biomarkers in Vitamin D<sub>3</sub> Deficiency Diet (VDD) Induced Animal Model. J Immunol Res Ther, 7(1): 244-254.

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Thus, a novel test formulation was designed that would improve the immunomodulation using various hematological and biochemical characteristics. The test formulation was the combination of different minerals (selenium, zinc, iron, calcium, copper, and magnesium), vitamins (cyanocobalamin, ascorbic acid, pyridoxine HCl, alpha tocopherol, and cholecalciferol), cannabidiol isolate, and *Panax ginseng* extract. This formulation is designed for overall immunological health conditions. All the minerals and vitamins used in the test formulation have significant functional role to provide vital physiological role [4-6]. Besides, biological importance of cannabidiol as novel anti-inflammatory and other disorders has been widely reported [7,8], while ginseng extract is regarded as the one of the best immune boosters for overall immunity [9,10]. The present study was designed to study the immunological profile of male Sprague Dawley rats in presence of VDD diet and 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, one of the best CAM approach against various pathological conditions has been reported to be effective [11-13] and was accepted worldwide. The National Center for Complementary/Alternative Medicine (NCCAM) accepted the use and results of CAM in various forms of complementary health approach. More than 80% of the world population depends upon the some form of traditional medicine in the developing world as one of the best future scope [14]. Complementary and Alternative Medicine (CAM) has several advantages instead of the current preferred treatment approach [15]. 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 deep breathing, natural products, Tai Chi, yoga, therapeutic touch, Qi Gong, Johrei, Reiki, polarity therapy, pranic healing, chiropractic/osteopathic manipulation, guided imagery, meditation, massage, homeopathy, hypnotherapy, progressive relaxation, special diets, relaxation techniques, movement therapy, Pilates, mindfulness, Ayurvedic medicine, traditional Chinese herbs and medicines in biological systems [16,17]. The Trivedi Effect®-Consciousness Energy Healing therapy has been widely accepted worldwide in nonliving materials and living organisms. Consciousness Energy Healing therapy has been scientifically studies 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 [31-43], 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, which might improve the immunomodulatory function of blood-related parameters, serum biochemistry, hormones profile, and histopathological examination using standard assays.

## MATERIALS AND METHODS

### 1. Chemicals and Reagents

Pyridoxine hydrochloride (vitamin B<sub>6</sub>), calcitriol, zinc chloride, magnesium (II) gluconate, and  $\beta$ -carotene (retinol, provit A) were purchased from TCI, Japan. Copper chloride, cyanocobalamin (vitamin B<sub>12</sub>), calcium chloride, vitamin E (Alpha-Tocopherol), cholecalciferol (vitamin D<sub>3</sub>), iron (II) sulphate, 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.

### 2. 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 throughout the experiment.

### 3. Consciousness Energy Healing Strategies

Each ingredient of the novel test formulation was divided into two parts. One part of the test compound was not received any sort of treatment and defined as the untreated. The second part of the test formulation was treated with the Biofield Energy Treatment/Blessing by a renowned Biofield Energy Healer, Mr. Mahendra Kumar Trivedi under laboratory conditions for ~3 min. Besides, three group of animals also received Biofield Energy Healing Treatment/Blessing by Mr. Trivedi under similar laboratory conditions for ~3 min. The Blessing/Treatment was given to the test items/animals remotely in the laboratory of Dabur Research Foundation, near New Delhi, India. After that, the Biofield Energy Treated/Blessed samples was kept in the similar sealed condition and used as per the study plan. Similarly, the control test formulation was subjected to “sham” healer for ~3 min, under the same laboratory conditions. The “sham” healer not has any knowledge about the Biofield Energy Treatment/Blessing. The Biofield Energy Treated/Blessed animals were also taken back to experimental room for further proceedings.

#### 4. Experimental Procedure

Seven days after acclimatization, animals were randomized and grouped based on the body weight. Dosing for groups G7 and G8 were initiated on day -15 and continued till end of the experiment. However, G1 to G6 and G9 groups were dosed from day 1 till the end of experiment. All the animals except G1 group received vitamin D<sub>3</sub> deficient diet (VDD) daily to the end of the experiment. Three weeks after the initiation of induction of VDD, all the groups were dose with the respective formulations. At the end of 8<sup>th</sup> weeks after bleeding, blood from all the animals was collected from the retro-orbital plexus using capillary tube for hematology analysis. From one portion of blood, serum was isolated for the analysis of biochemical parameters. The selective vital organs were collected and weighed.

#### 5. Assessment of Hematology Parameters

After fasting for 12 to 16 hours on last day of the experiment, blood was collected from the retro-orbital plexus using heparinized and non-heparinized capillary tubes. The non-heparinized capillary tube of blood was kept in plain bottle from which serum was collected and further stored for biochemical analysis. The heparinized tube containing blood was directly subjected for the estimation of various hematological parameters using standard instrument. The various hematological parameters such as hemoglobin (Hb), red blood count (RBC), mean corpuscular volume (MCV), mean Corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), packed cell volume (PCV) and platelets were analyzed in the blood samples using auto hematology analyzer, Mid Ray BC-5000 VET.

#### 6. Assessment of Lipid Profile and Biochemical parameters

Glucose, total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL), high density lipoprotein (HDL), and very low-density lipoprotein (VLDL) were analyzed using serum by Biochemistry Analyzer, Mindray, Mispa Clinia. Further, the level of magnesium, blood urea nitrogen (BUN), creatinine, uric acid, calcium, phosphorus, potassium, sodium, and chloride ion concentration was analyzed in serum samples.

#### 7. Measurement of Hormones

The levels of testosterone and corticosterone were analyzed in serum in all the experimental groups using commercial kits.

#### 8. Measurement of Histopathological Examination

At the end of the experiment, rats were dissected and the whole liver, kidneys, hearts, spleens, lungs, whole intestine, etc. were excised, freed of fat, blotted with clean tissue paper, and then weighed. Thoracic aortae, calf muscle (gastrocnemius and soleus) and lungs embedded in paraffin

sections, stained with hematoxylin and eosin, and were examined histologically. Bones was decalcified by Gooding and Stewart's fluid for 5-10 days. The decalcified bone tissues were processed for paraffin embedding. Two slides were prepared with 3-6  $\mu$ M thick tissue sections from all the blocks. One slide was stained with hematoxylin and eosin stain for routine histopathological examination and another slide was stained with Safranin O for cartilage examination. All the sections were stained with the help of Automatic Linear Slide Stainer (Medimeas, MSS-AS) with hematoxylin and eosin staining method as per standard protocol.

#### 9. Clinical Sign and Symptoms

All the animals in different test groups were analyzed for various clinical signs and symptoms in accordance with in-house protocol. Abnormal behavior in animals was recorded with the time of onset and disappearance.

#### 10. Statistical Analysis

The data were represented as mean  $\pm$  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

#### 1. Effect of the Test Formulation on Animal Hematology Parameters

Clinical manifestation of vitamin D<sub>3</sub> deficiency includes exacerbated hematological imbalance leading to unexplained abnormalities. Hematology provides an insight into the diseased state of vitamin D<sub>3</sub> deficiency. The various components of blood disproportionately altered in different treatment groups and vitamin D<sub>3</sub> deficiency group. All the hematological data of the experiment was compiled in **Table 1**. The data suggested non-significant marginal increase in G2 in case of RBC count as compared to G1, whereas statistically significant decreased values were observed in the G3 and G7 as compared to G2. The values of hemoglobin in groups G5, G6, and G9 were increased by 1.72%, 2.05%, and 1.39%, respectively as compared with the untreated test formulation group, G4. Similarly, the platelet counts were increased by 12.43%, 4.64%, and 1.89% in the G7, G8, and G9 groups, respectively as compared with the G4 group. Non-significant increase in group G2 was found in RDW-SD level as compared to G1, while slight increase was observed in G3 as compared to the G2. No change was observed in all other treatment groups. However, no such significant changes were observed in some of the hematological parameters such as MCHC, MCV, MCH, and RDW-CV across all the treatment groups and vitamin D<sub>3</sub> deficiency group (G2). The present experimental data suggested significant change in the

hematology parameters in the Biofield Energy Treated test formulation, which showed a significant capacity to improve the blood immunity-related parameters. The improved hemoglobin and platelet count can be useful in many acute infections, gout, rheumatoid arthritis, chronic inflammatory diseases, rheumatic fever, etc. Minerals and vitamins play a

vital role to control the hematology profile [34,35]. Ginseng and cannabidiol has been reported to improve blood parameters [36,37]. However, Biofield Energy Treatment/Blessing significantly improved the blood profile as compared with the untreated test formulation.

**Table 1.** Evaluation of hematology parameters assessed after Biofield Energy Treatment on the test formulation in female Sprague Dawley rats.

| Group | RBC<br>(10 <sup>6</sup> /μL) | Hb<br>(gm/dL)   | PCV (%)         | MCV (fl)        | MCH<br>(pg)     | MCHC<br>(%)     | RDW-CV          | Platelet Count<br>(thousand/mm <sup>3</sup> ) |
|-------|------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------------------------------------|
| G1    | 9.26 ±<br>0.10               | 15.35 ±<br>0.27 | 39.18 ±<br>0.57 | 42.35 ±<br>0.74 | 16.59 ±<br>0.34 | 39.16 ±<br>0.22 | 15.13 ±<br>0.28 | 1273 ± 47.12                                  |
| G2    | 9.34 ±<br>0.18               | 15.78 ±<br>0.28 | 40.45 ±<br>0.74 | 43.43 ±<br>1.24 | 16.95 ±<br>0.48 | 39.04 ±<br>0.14 | 15.20 ±<br>0.45 | 1126.88 ± 57.13                               |
| G3    | 8.74 ±<br>0.14               | 15.63 ±<br>0.22 | 39.83 ±<br>0.57 | 45.61 ±<br>0.85 | 17.91 ±<br>0.35 | 39.24 ±<br>0.28 | 15.83 ±<br>0.44 | 1167.00 ± 53.56                               |
| G4    | 8.88 ±<br>0.16               | 15.26 ±<br>0.42 | 38.50 ±<br>0.82 | 43.14 ±<br>0.71 | 17.19 ±<br>0.33 | 39.61 ±<br>0.30 | 14.69 ±<br>0.39 | 1120.14 ± 40.37                               |
| G5    | 8.98 ±<br>0.21               | 15.53 ±<br>0.27 | 39.03 ±<br>0.74 | 43.58 ±<br>0.81 | 17.31 ±<br>0.31 | 39.75 ±<br>0.17 | 15.34 ±<br>0.27 | 1056.86 ± 52.31                               |
| G6    | 8.85 ±<br>0.13               | 15.58 ±<br>0.26 | 39.54 ±<br>0.57 | 44.70 ±<br>0.67 | 17.61 ±<br>0.24 | 39.41 ±<br>0.27 | 14.46 ±<br>0.23 | 1042.75 ± 26.53                               |
| G7    | 8.54 ±<br>0.14               | 14.80 ±<br>0.26 | 37.18 ±<br>0.73 | 43.54 ±<br>0.82 | 17.33 ±<br>0.34 | 39.83 ±<br>0.18 | 16.01 ±<br>0.30 | 1259.43 ± 92.12                               |
| G8    | 8.78 ±<br>0.12               | 15.36 ±<br>0.21 | 38.79 ±<br>0.68 | 42.96 ±<br>1.03 | 17.53 ±<br>0.14 | 39.60 ±<br>0.21 | 15.65 ±<br>0.27 | 1172.13 ± 36.94                               |
| G9    | 8.78 ±<br>0.11               | 15.48 ±<br>0.14 | 39.24 ±<br>0.38 | 44.70 ±<br>0.49 | 17.63 ±<br>0.18 | 39.46 ±<br>0.27 | 14.04 ±<br>0.19 | 1140.88 ± 85.80                               |

G: Group; G1: Normal control (0.5% CMC); G2: Disease control (VDD: Vitamin D<sub>3</sub> 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).

## 2. Effect of the Test Formulation on Animal Lipid Biochemical Profile

The effect of the test formulation on lipid profile were evaluated and presented in the **Table 2**. The level of total cholesterol was significantly reduced in the entire treated group such as significant decreased level of TC by 15.01%, 12.34%, 7.45%, 1.57%, and 14.80% was reported in the G5, G6, G7, G8, and G9 group respectively, as compared with the untreated G4 group. Similarly, the level of LDL was significantly reduced by 40.66%, 33.38%, 36.75%, 27.37%,

and 20.54% in the test groups viz. G5, G6, G7, G8, and G9 group respectively, as compared with the untreated G4 group. The level of triglycerides was also decreased by 9.53% and 38.13% in the group G5 and G6 respectively, as compared with the G4 group. VLDL is also considered as one of the types of bad cholesterol as it helps cholesterol to build up on the walls of arteries causing various cardiac diseases. The level of VLDL was also decreased by 10.31% and 37.63% in the group G5 and G6 groups, respectively, as compared with the G4 group. Similarly, zinc and magnesium supplementation were reported to have improved lipid

profile such as decreased total cholesterol and LDL level, while increased HDL, cholesterol, and triglycerides levels [38, 39]. Overall, the data suggested that Biofield Energy

Treated/Blessed test formulation and Biofield Energy *per se* was found to be significantly useful for improved lipid profile.

**Table 2.** Effect of the test formulation on the level of biochemical parameters for lipid profile analysis in male Sprague Dawley rats.

| Group (G) | Total Cholesterol (mg/dL) | Glucose (mg/dL) | HDL (mg/dL)  | LDL (mg/dL)   | Triglyceride (mg/dL) | VLDL (mg/dL)   |
|-----------|---------------------------|-----------------|--------------|---------------|----------------------|----------------|
| 1         | 109.84 ± 5.47             | 138.93 ± 7.29   | 67.85 ± 3.54 | 16.76 ± 1.36  | 121.98 ± 22.19       | 24.38 ± 4.49   |
| 2         | 122.38 ± 5.31             | 139.21 ± 8.46   | 75.74 ± 3.70 | 15.77 ± 1.14  | 141.23 ± 26.49       | 28.25 ± 5.35   |
| 3         | 109.03 ± 3.57             | 140.18 ± 10.63  | 72.45 ± 2.20 | 20.03 ± 1.43  | 151.01 ± 27.00       | 30.13 ± 5.39   |
| 4         | 119.16 ± 3.93             | 126.16 ± 10.19  | 73.66 ± 2.34 | 21.85 ± 1.66  | 120.69 ± 12.16       | 24.25 ± 2.37   |
| 5         | 101.27 ± 6.15             | 171.66 ± 13.33  | 63.53 ± 4.23 | 12.96 ± 1.21* | 109.20 ± 11.34       | 21.75 ± 2.27   |
| 6         | 104.45 ± 8.67             | 126.17 ± 4.03   | 63.38 ± 5.69 | 14.55 ± 2.42* | 74.68 ± 7.04**       | 15.13 ± 1.49** |
| 7         | 110.28 ± 4.17             | 154.63 ± 4.45   | 70.39 ± 3.33 | 13.82 ± 1.39* | 124.00 ± 10.80       | 24.63 ± 2.16   |
| 8         | 117.29 ± 10.82            | 153.99 ± 9.42   | 75.18 ± 7.53 | 15.87 ± 2.48  | 140.22 ± 19.27       | 28.13 ± 3.88   |
| 9         | 101.52 ± 5.68             | 133.58 ± 10.29  | 63.07 ± 3.83 | 17.36 ± 1.35  | 134.75 ± 14.25       | 27.00 ± 2.87   |

G: Group; G1: Normal control (0.5% CMC); G2: Disease control (VDD: Vitamin D<sub>3</sub> deficient diet + 0.5% CMC); G3: Reference item (VDD + Calcitriol); G4: (VDD + untreated test formulation); G5: (VDD + Biofield Energy Treated test formulation); G6: (VDD + Biofield Energy Treatment *per se* to animals from day -15; G7: (VDD + Biofield Energy Treated test formulation from day -15); G8: (VDD + Biofield Energy Treatment *per se* plus Biofield Energy Treated test formulation from day -15), and G9: (VDD + Biofield Energy Treatment *per se* animals plus untreated test formulation). Values are presented as mean ± SEM (n=6). \* $p \leq 0.05$  and \*\* $p \leq 0.01$  vs. G4.

### 3. Effect of the Test Formulation on Animal Hepatic Biochemical Parameters

The effect of the test formulation on hepatic biochemical parameters along with important ions were evaluated and presented in the **Table 3**. The level of calcium was significantly improved after treatment by 16.01%, 18.91%, and 14.70% in the G5, G7, and G8 groups, respectively as compared with the untreated G4 group. However, other ions concentrations in tested groups were also altered as compared with the disease control group. Similarly, hepatic parameters such as level of creatinine was significantly reduced by 3.38%, 6.39%, and 6.39% in the group G7, G8, and G9, respectively as compared with the untreated test formulation, G4 group. The level of uric acid was significantly decreased by 19.56%, 8.74%, 18.77%, and 13.56% in the G6, G7, G8, and G9 groups, respectively as compared with the G2 group. Besides, the levels of magnesium, creatinine, phosphorous, and ions such as sodium, potassium, and chloride were altered in all the tested groups to some extent but did not show any significant difference with respect to the G2 group. The test formulation constituents have been reported to have significant role on blood parameters [38-40], while Biofield Energy Treatment/Blessing significantly improved the biochemical profile as compared with the untreated test formulation.

### 4. Measurement of Hormones

The effect of the Biofield Energy Treated test formulation and Biofield Energy Treatment *per se* on the level of hormones such as testosterone and cortisone are presented in **Figure 1**. The experimental data suggested significant improved level of testosterone in the Biofield Energy Treated test formulation groups and Biofield Energy Treatment *per se* to the animals as compared to the disease control (G2) and untreated test formulation (G4) groups. Calcitriol treatment (G3), animals increased the serum testosterone level ( $48.33 \pm 10.69$  ng/dL) with percentage change of 13.53% as compared to the G2 group. The Biofield Energy Treated test formulation in the G5 group showed an increased serum testosterone level ( $164.83 \pm 78.70$  ng/dL) with percentage change of 287.19% as compared to the G2 group and 603.56% change as compared to the G4 group. G6 group showed an increased the serum testosterone level by 155.39% as compared to the G4 group. Similarly, the level of testosterone was increased by 334.30%, 174.24%, and 224.39% in the G7, G8, and G9 groups, respectively as compared with the untreated G4 group. Besides, corticosterone level was altered in the entire experimental test groups compared with G2 and G4 (**Figure 1B**). Calcitriol treatment (G3) significantly ( $p \leq 0.01$ ) decreased the serum corticosterone level ( $17.38 \pm 2.26$

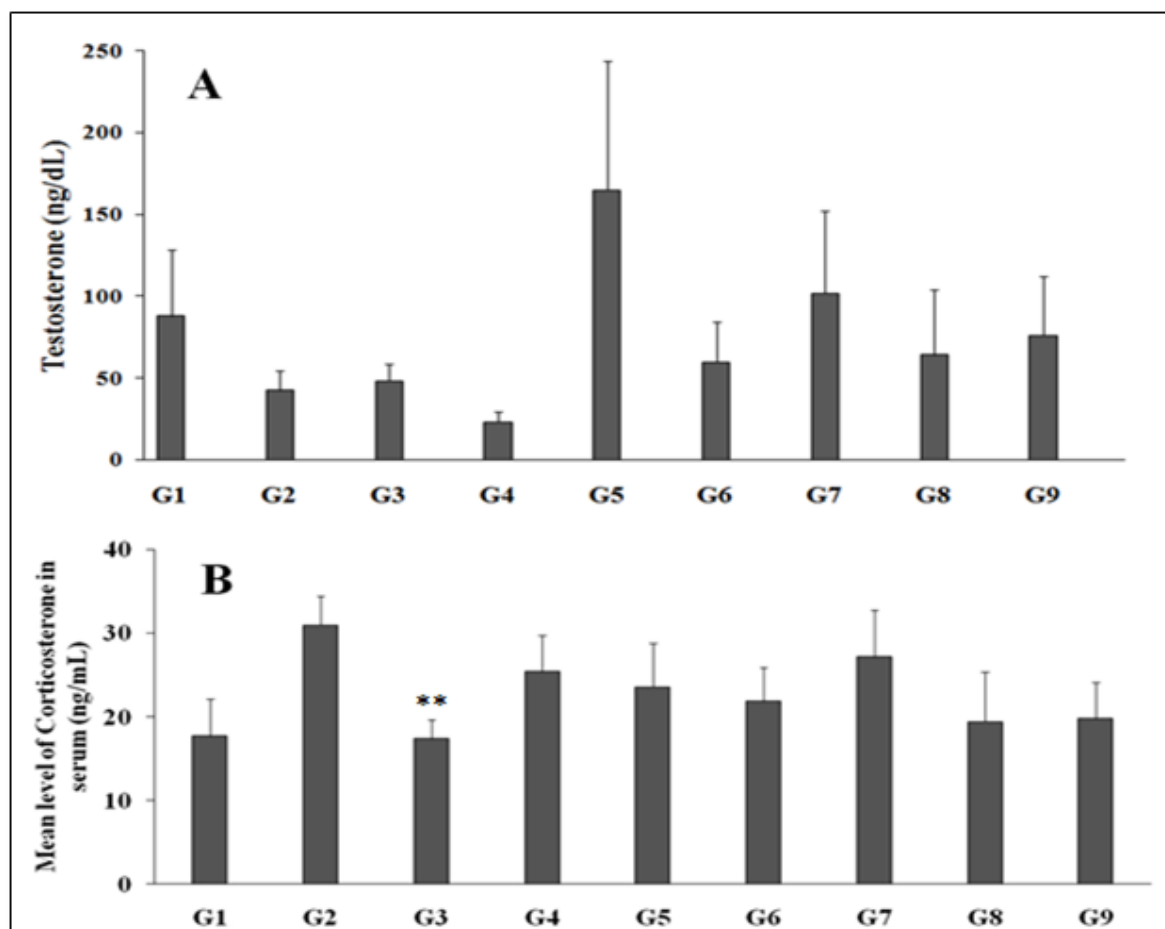
ng/mL) by 43.86% as compared to the G2. Similarly, the experimental test groups showed decreased level of serum corticosterone level by 7.06%, 13.94%, 23.61%, and 22.13% in the G5, G6, G8, and G9 groups respectively, as compared with the untreated group (G4). All the vital constituents present in the test formulation such as ginseng, cannabidiol,

minerals, and vitamins are reported to have significant role on hormones [41-43], while Biofield Energy Treatment/Blessing significantly improved the hormonal profile after treatment as compared with the untreated test formulation.

**Table 3.** Evaluation of hepatic biomarkers after treatment with the test formulation on male Sprague Dawley rats.

| Group | Calcium<br>(mg/dL) | Creatinine<br>(mg/dL) | Magnesium<br>(mg/dL) | Phosphorus<br>(mg/dL) | Uric<br>Acid<br>(mg/dL) | Blood<br>Urea<br>(mg/dL) | Na <sup>+</sup><br>(mmol/L) | K <sup>+</sup><br>(mmol/L) | Cl <sup>-</sup><br>(mmol/L) |
|-------|--------------------|-----------------------|----------------------|-----------------------|-------------------------|--------------------------|-----------------------------|----------------------------|-----------------------------|
| G1    | 10.35 ±<br>0.09    | 0.66 ± 0.01           | 1.75 ± 0.04          | 5.82 ± 0.07           | 0.87 ±<br>0.08          | 35.47 ±<br>1.99          | 142.98 ±<br>0.18            | 4.99 ±<br>0.07             | 95.44 ±<br>0.14             |
| G2    | 10.55 ±<br>0.08    | 0.66 ± 0.02           | 1.60 ± 0.05          | 5.51 ± 0.17           | 0.96 ±<br>0.03          | 31.99 ±<br>0.91          | 143.56 ±<br>0.24            | 4.86 ±<br>0.08             | 95.24 ±<br>0.23             |
| G3    | 11.63 ±<br>0.12    | 0.63 ± 0.02           | 1.29 ± 0.04          | 6.51 ± 0.15           | 1.02 ±<br>0.09          | 25.40 ±<br>0.79          | 137.31 ±<br>0.36            | 4.17 ±<br>0.12             | 94.04 ±<br>0.49             |
| G4    | 11.33 ±<br>0.07    | 0.67 ± 0.02           | 1.47 ± 0.05          | 6.67 ± 0.10           | 0.80 ±<br>0.08          | 39.91 ±<br>3.69          | 137.86 ±<br>0.29            | 4.50 ±<br>0.07             | 95.41 ±<br>0.33             |
| G5    | 13.14 ±<br>0.26*** | 0.68 ± 0.02           | 1.82 ± 0.05          | 6.58 ± 0.20           | 0.95 ±<br>0.06          | 34.47 ±<br>3.11          | 140.15 ±<br>0.45            | 4.51 ±<br>0.11             | 98.33 ±<br>0.57             |
| G6    | 10.55 ±<br>0.10    | 0.67 ± 0.01           | 1.61 ± 0.04          | 5.29 ± 0.10           | 0.77 ±<br>0.05          | 34.08 ±<br>1.50          | 143.84 ±<br>0.16            | 4.71 ±<br>0.09             | 95.59 ±<br>0.19             |
| G7    | 13.47 ±<br>0.34*** | 0.64 ± 0.02           | 1.78 ± 0.08          | 6.74 ± 0.19           | 0.88 ±<br>0.05          | 34.65 ±<br>2.27          | 140.34 ±<br>0.52            | 4.67 ±<br>0.12             | 100.15 ±<br>0.57            |
| G8    | 13.00 ±<br>0.16*** | 0.62 ± 0.01           | 1.65 ± 0.07          | 6.49 ± 0.14           | 0.78 ±<br>0.02          | 33.64 ±<br>2.43          | 140.69 ±<br>0.29            | 4.59 ±<br>0.04             | 99.51 ±<br>0.51             |
| G9    | 11.53 ±<br>0.21    | 0.62 ± 0.02           | 1.56 ± 0.06          | 6.45 ± 0.13           | 0.83 ±<br>0.07          | 36.17 ±<br>1.70          | 138.20 ±<br>0.32            | 4.54 ±<br>0.14             | 95.61 ±<br>0.20             |

G: Group; G1: Normal control (0.5% CMC); G2: Disease control (VDD: Vitamin D<sub>3</sub> 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≤0.001 vs. G4.



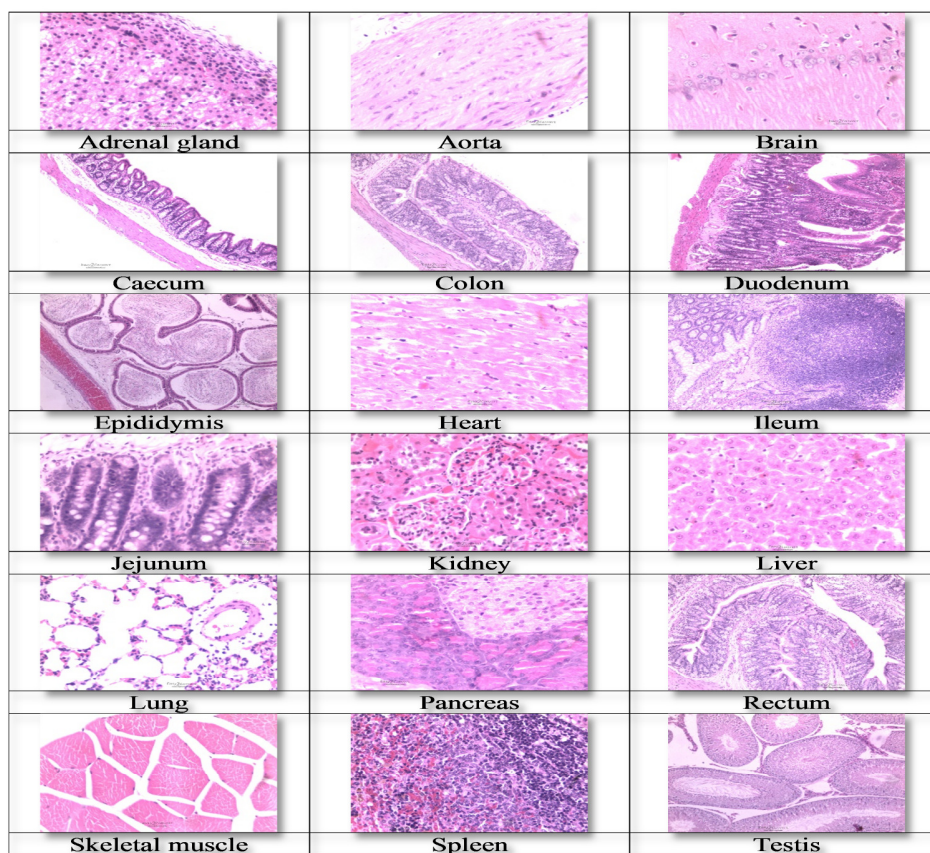
**Figure 1.** Effect of the test formulation on the level of hormonal parameters in serum (A) testosterone and (B) corticosterone in male Sprague Dawley rats. \*\* $p \leq 0.01$  vs. G2.

## 5. Assessment of Histopathological Examination

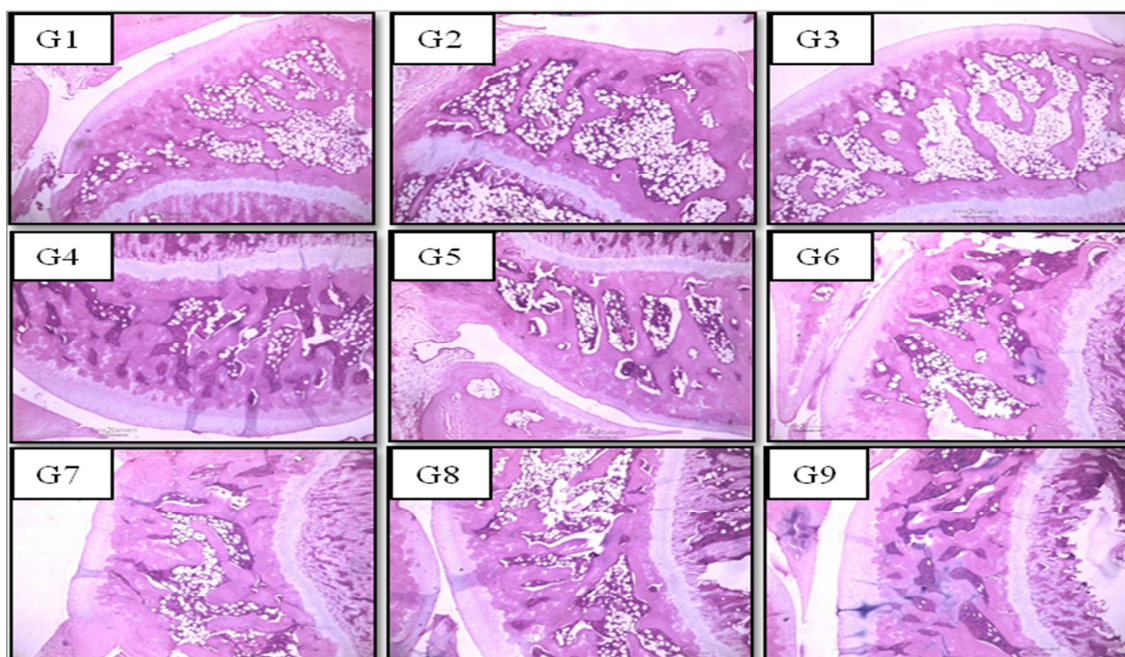
The effect of the test formulation on histopathological findings in male SD rats is shown in **Figure 2**. No significant differences were observed either in gross and microscopic observation of the tested organs. The pathological findings suggested that no Biofield Energy Healing related histopathological changes were observed as compared with the normal or disease control group.

Similarly, the histopathological findings of bone were evaluated for wide separation of bone trabeculae, wide epiphyseal growth plate, epiphyseal growth plate with irregular boundaries and disorderly alignment of chondrocytes by H&E staining for all the groups (**Figure 3**). Statistically significant increase in histopathological grading of bone was observed in all the parameters tested in vitamin D<sub>3</sub> deficiency group (G2) as compared to the G1 except wide epiphyseal growth plate. However, calcitriol treated group showed non-significant decreased pattern of

histopathological score in all the parameter tested except wide separation of bone trabeculae as compared to the vitamin D<sub>3</sub> deficiency group. In wide separation of bone trabeculae, statistically significant reduction in histopathological grading of bone was observed except G6. Other parameters such as, wide epiphyseal growth plate, epiphyseal growth plate with irregular boundaries, slight reduction was observed in all treatment groups as compared to the vitamin D<sub>3</sub> deficiency animals. In disorderly alignment of chondrocytes, significant reduction was found in the group G7, G8, and G9 as compared to the vitamin D<sub>3</sub> deficiency animals. Hence, all these findings could be considered as spontaneous or incidental in nature, representing the normal physiological/metabolic or congenital changes encountered in the tested animals. Overall, the data suggested that no toxicity and abnormal finding were observed in histopathological data, thus the treatment was found safe.



**Figure 2.** Represented histopathological photomicrograph of major organs of male Sprague Dawley rats. All the tissues were sectioned transversely and stained with hematoxylin and eosin.



**Figure 3.** Represented histopathological photomicrograph of bone tissue of male Sprague Dawley rats. All the tissues were sectioned transversely and stained with hematoxylin and eosin.

Thus, the present research plan defined four groups, which were considered as preventive maintenance groups *viz.* G6, G7, G8, and G9, where the Biofield Energy Treatment *per se* and/or Biofield Energy Treated Test formulation in combination was used as preventive maintenance group with respect to improved immunological parameters related to hematology and biochemistry. The results showed the significant slowdown of the disease progression, disease-related all other complications and also reduced the chances of disease susceptibility in these groups. Based on the overall data, it suggests that the Biofield Energy Healing Therapy was found to be most effective and benefited in order to prevent and protect from the occurrence of any type of bone-related-diseases in rat model. It indicated that Biofield Energy Treatment/Blessing can act as a preventive maintenance therapy to slowdown the disease progression and disease-related complications of the existing ailments that will ultimately improve the overall health and quality of life in human.

## CONCLUSIONS

Based on the present research study, blood parameters, supportive biochemical estimation using serum, hormones determination along with histopathology was studies, which suggested significantly improved overall immunity profile in the Biofield Energy Treated test formulation and Biofield Energy Treatment *per se*. The platelet count was significantly increased by 12.43%, in the G7 group as compared with the G4 group. Lipid profile analysis showed reduced the level of total cholesterol by 15.01%, 12.34%, and 14.80% in the G5, G6, and G9 groups, respectively as compared with the untreated G4 group. Similarly, LDL was significantly decreased by 40.66%, 33.38%, 36.75%, 27.37%, and 20.54% in the test groups *viz.* G5, G6, G7, G8, and G9 groups, respectively as compared with the untreated G4 group. Triglyceride was significantly decreased by 38.13% in the G6 group as compared with the G4 group. Serum biochemistry data suggested that calcium level was significantly improved by 16.01%, 18.91%, and 14.7% in the G5, G7, and G8 groups, respectively as compared with the untreated G4 group. Hormone profile data suggested that the level of testosterone was showed an increased by 603.56%, 155.39%, 334.30%, 174.24%, and 224.39% in the G5, G6, G7, G8, and G9 groups, respectively, as compared to the untreated G4 group. Similarly, decreased level of serum corticosterone level was reported by 13.94%, 23.61%, and 22.13% in the G6, G8, and G9 groups, respectively, as compared with the untreated group G4. Organ histopathological analysis suggested that findings was spontaneous or incidental in nature, representing the normal physiological/metabolic or congenital changes encountered all the experimental groups. Overall, the data suggested that no toxicity and abnormal finding were observed in histopathological data, thus the treatment was found safe. Overall, it can be concluded Biofield Energy Healing Treatment (the Trivedi Effect<sup>®</sup>) *per se* showed best results

with respect to different efficacy and biomarker parameters in the preventive treatment approach (-15 days) as compared to the other preventive maintenance groups (G7, G8, and G9) in rats. It also helped to slowdown the disease progression rate and disease-related complications. The data suggested that Biofield Energy Treatment *per se* and/or Biofield Energy Treated/Blessed Test formulation in combination would be one of the best treatment strategies in order to prevent from diseases. Therefore, the Biofield Energy Treatment might act as a preventive maintenance therapy in order to improve the overall health and quality of life. Thus, Mr. Trivedi's Biofield Therapy could be utilized for various autoimmune disorders such as systemic lupus erythematosus, fibromyalgia, Addison disease, Hashimoto thyroiditis, multiple sclerosis, dermatomyositis, myasthenia gravis, pernicious anemia, aplastic anemia, psoriasis, rheumatoid arthritis, Sjogren's syndrome, Crohn's disease, vitiligo, chronic fatigue syndrome and alopecia areata, as well as inflammatory disorders such as ulcerative colitis, Alzheimer's disease, Parkinson's disease, atherosclerosis, dermatitis, hepatitis, and diverticulitis.

## ACKNOWLEDGEMENTS

The authors are grateful to Dabur Research Foundation, Trivedi Science, Trivedi Global, Inc., and Trivedi Master Wellness for the assistance and support during the work.

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