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Impact of the Biofield Energy Healing Based Test Formulation on Various Health Biomarkers Using Cell-Based Assays

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Abstract

Various complementary approaches have been used against multiple organ dysfunction syndrome (MODS), which is the major contributor in high mortality among the healthcare centers. The aim of the present study was to determine the impact of the Biofield Energy Treatment on test formulation and different cell line medium related with vital organs functioning. Different organ based cell lines were used in the study for testing the effects of test formulation. The test item (TI) and specific cell line media (Med) was divided into two parts; one untreated (UT-TI) and other part received the Biofield Energy Treatment remotely by a renowned Biofield Energy Healer, Elizabeth Patric, USA and were labeled as the Biofield Energy Treated (BT) test formulation/media. MTT assay was used for cell viability assay, and the results showed that the test item was found non-toxic. Cytoprotective action of the test formulation showed a significant maximum restoration of cell viability by 129.7% (at 1 µg/mL), 28.4% (at 63.75 µg/mL), and 44.5% (at 10 µg/mL) in the UT-Med + BT-TI, BT-Med + UT-TI, and BT-Med + BT-TI groups respectively, as compared to the untreated test group in human cardiac fibroblasts cells (HCF) cells, while 53.4% (at 63.75 µg/mL), 14.5% (at 10 µg/mL), and 22.9% (at 25.5 µg/mL) improved cellular protection of human hepatoma cells (HepG2) cells in the UT-Med + BT-TI, BT-Med + UT-TI, and BT-Med + BT-TI groups respectively, as compared to the untreated test group. In addition, cytoprotective activity in adenocarcinoma human alveolar basal epithelial cells (A549) showed improved cell viability by 25.6% (at 25.5 µg/mL), 59.8% (at 10 µg/mL), and 26.1% (at 1 µg/mL) in the UT-Med + BT-TI, BT-Med + UT-TI, and BT-Med + BT-TI groups respectively, as compared to the untreated test group. ALP activity in MG-63 cells was maximum increased by 84.9% at 50 µg/mL in the BT-Med + BT-TI group, while in Ishikawa cells showed maximum increased ALP activity by 308.1% at 0.1 µg/mL in the BT-Med + BT-TI group as compared to the untreated group. The maximum percent cellular protection of HCF (heart) cells (decreased of LDH activity) was significantly increased by 76.7% (at 1 µg/mL), 44.3% (at 1 µg/mL), and 102.6% (at 10 µg/mL) in the UT-Med + BT-TI, BT-Med + UT-TI group, and BT-Med + BT-TI groups respectively, as compared to the untreated test group. Alanine amino transferase (ALT) in terms of percent protection of HepG2 (liver) cells (decreased of ALT activity) was reported at 63.75 µg/mL by 70.6%, 89.9%, and 76.6% in the UT-Med + BT-TI, BT-Med + UT-TI, BT-Med + BT-TI groups respectively, as compared to the untreated test group. Cellular protection of A549 (lungs) cells (increased of SOD activity) in terms of percentage was increased by 16.2% (at 10 µg/mL), 35.2% (at 63.75 µg/mL), and 17.7% (at 63.75 µg/mL) in the UT-Med + BT-TI, BT-Med + UT-TI, and BT-Med + BT-TI groups, respectively as compared to untreated group. Serotonin level was significantly increased by 14.6% (at 25 µg/mL), 41.2% (at 1 µg/mL), and 70.8% (at 10 µg/mL) in the UT-Med + BT-TI, BT-Med + UT-TI, and BT-Med + BT-TI groups, respectively compared to untreated test group in human neuroblastoma cells (SH-SY5Y). However, the relative quantification (RQ) of vitamin D receptor (VDR) was significantly increased by 166.8%, 266.4%, and 153.3% at 0.1 µg/mL in the UT-Med + BT-TI, BT-Med + UT-TI, and BT-Med + BT-TI groups, respectively as compared to the untreated in MG-63 cells. Thus, Biofield Energy Treated test formulation (The Trivedi Effect®) would be significantly useful for multiple organ health that can be used against coronary artery disease, arrhythmias, congenital heart disease, cardiomyopathy, cirrhosis, liver cancer, hemochromatosis, asthma, chronic bronchitis, cystic fibrosis, osteoporosis, etc.

Keywords: The Trivedi Effect®; Biofield Energy Treatment; Organ Health; Cardiac Health; Liver Health; Lungs Health; Multiple Organ Failure; Bone Health

Introduction

Herbal based test formulation/herbomineral formulations have been used in healthcare settings since time immemorial. National health care settings are now restoring the use and significance of herbal based medicines, phytonutrients or any form of nutraceuticals. This has continued to expand rapidly throughout the world against many health challenges [1]. The past decades suggested that natural therapies are now highly accepted among the public worldwide in both developing and developed countries. Nutraceuticals based formulation are now available in drug stores, food stores and also in supermarkets due to its high acceptance

rates. More than four billion populations in the developing world have accepted the herbal based formulations as their primary healthcare practices/needs [2]. Nutraceuticals are accepted as one of the balanced and moderate approach against wide range of diseases against individual healing. Besides, these nutraceuticals are free from most of the associated adverse effects as compared with the over-the-counter drugs along with excess cost. These are some of the reasons that explain the huge acceptance and blooming of the traditional medicine for overall health, which shows its presence in global drug market. In addition, the huge development of vital nutrients such as minerals and vitamins play an equal medicinal importance similar to herbal drugs [3]. Thus, mineral based test preparations are one of the preferred choices for overall health benefits as compared with phytomedicines. Thus, the novel test formulation was designed, which is composed of 11 important ingredients such as calcium chloride, magnesium gluconate, zinc chloride, sodium selenate, ferrous sulfate, vitamin B_{12}, vitamin D_3, ascorbic acid, vitamin B_6, panax ginseng extract, and beta carotene. These constituents are selected on the basis of recent scientific data in order to improve the overall organs health and quality of life. Most widely used herbal drug, Panax ginseng was used as one of the major section of the test formulation. It has been reported for its immunomodulatory action, improved mental and physical health, highly effective in case of lung disorder, liver disorder, breast cancer, liver cancer, aging, muscle damage, and overall health [4-6]. In addition, test formulation contained minerals and vitamins, which have very high biological activity [7-10]. The designed test formulation was used against specific organ health based cell lines (such as bone, liver, heart, mental, and bone health) along with overall female reproductive functions [11-20]. Thus, the present experiment was designed to evaluate the biological activity of test formulation using standard cell-line based assay after treatment with the Biofield Energy Treatment.

Biofield, also known as the Biological Field, which is a complex endogenously generated sphere of activity of energy living system [21]. Biofield Energy is engaged in the generation, maintenance, and also involved in the regulation of various biological dynamics and physiological functions. This is one of the best types of Complementary and Alternative Medicine (CAM) therapies that can improve enhance the physical, mental, and emotional human wellness [22,23]. 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 deep breathing, natural products, Tai Chi, yoga, Qi Gong, Johrei, Reiki, therapeutic touch, yoga, polarity therapy, pranic healing, chiropractic/osteopathic manipulation, guided imagery, meditation, massage, homeopathy, hypnotherapy, progressive relaxation, acupressure, acupuncture, special diets, relaxation techniques, Rolfing structural integration, healing touch, movement therapy, pilates, mindfulness, Ayurvedic medicine, traditional Chinese herbs and medicines in biological systems [24]. The Trivedi Effect®-Consciousness Energy Healing therapy as a Conventional biomedicine have been widely accepted worldwide in nonliving materials and living organisms. Consciousness Energy Healing therapy has been scientifically studies on various models in the metal science [25,26], agriculture science [27], microbiology [28,29], biotechnology [30,31], and improved bioavailability of various compounds [32,33], skin health [34,35], nutraceuticals [36], cancer research [37], bone health [38-40], overall human health and wellness. Due to the continued clinical and preclinical applications of Consciousness Energy Healing therapy, the test formulation and the specific cell line media was studied for the impact of the Biofield Energy Healing Treatment on the function of vital organs such as bones, heart, liver, lungs, and brain specific functional biomarkers in different standard cell-lines.

Materials and Methods

Chemicals and Reagents

Calcium chloride, ferrous sulfate, vitamin B6, vitamin B12, vitamin D3, naringenin, trimetazidine (TMZ), 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT), and ethylenediaminetetraacetic acid (EDTA) were procured from Sigma Chemical Co. (St. Louis, MO). Magnesium gluconate, zinc chloride, β-carotene, and calcitriol were procured from TCI chemicals, Japan. Panax ginseng extract was obtained from panacea Phytoextracts, India. Sodium selenate and ascorbic acid were procured from Alfa Aesar, India. Silymarin and curcumin were procured from Sanat Chemicals, India, while quercetin was purchased from Clearsynth, India. Reverse Transcription Kit, RNeasy Mini Kit, and Syber Green PCR kits were procured from Qiagen, India. All the other chemicals used in this experiment were analytical grade procured from India.

Biofield Energy Healing Treatment

The test formulation was the combination of eleven ingredients *viz*. calcium chloride, panax ginseng extract, vitamin B_{12}, β-carotene, vitamin D_3, zinc chloride, magnesium gluconate, sodium selenate, ferrous sulfate, ascorbic acid, and vitamin B_6. The test formulation/media was divided into two parts, one portion was considered as the untreated group, where no Biofield Energy Treatment was provided. However, the untreated group was treated by a "sham" healer for comparison purposes, who did not have any knowledge about the Biofield Energy Healing Treatment. Besides, the other portion of the test formulation/media received Biofield Energy Treatment (the Trivedi Effect®) remotely by Elizabeth Patric, under standard laboratory conditions for ~3 minutes through healer's unique Biofield Energy Transmission process and was referred as the Biofield Energy Treated formulation/media. Overall, there were four combinations of treatment groups for performing this experiment; a. untreated-test item/formulation (UNT-TI) + UNT-Medium (Med), b. UNT-TI + TRT-Med, c. TRT-TI + UNT-Med, and d. TRT-TI + TRT-Med. The Biofield Energy Healer was located in the USA; however the test formulation/media were located in the research laboratory of Dabur Research Foundation, New Delhi, India. Biofield Energy Healer in this experiment did not visit the laboratory, nor had any contact with the test samples. Further, Biofield Energy Treated and untreated test items were kept in similar sealed conditions and used for the study as per the study plan.

MTT Test for Cell Viability Assay

All the experimental cells used in this study were counted for cell viability using hemocytometer in 96-well plates at the specific density as mentioned in the Table 1. The cells were then incubated overnight under standard growth conditions to allow cell recovery and exponential growth. Following overnight incubation, cells were treated with different concentrations of test formulations (BT/UT). After respective treatments, the cells were incubated in a CO_2 incubator at 37 °C, 5% CO_2, and 95% humidity. After incubation, the plates were taken out and 20 µL of 5 mg/mL of MTT 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide solution was added to all the wells followed by additional incubation for 3 hours at 37 °C. The supernatant was aspirated and 150 µL of DMSO was added to each well to dissolve formazan crystals. The absorbance of each well was read at 540 nm using Synergy HT microplate reader. The percentage cytotoxicity at each tested concentration was calculated using Equation 1:

$$\% \text{ Cytotoxicity} = [(R-X)/R] * 100 \quad (1)$$

Where, X = Absorbance of treated cells; R = Absorbance of untreated cells
The concentrations exhibiting percentage cytotoxicity <30% was considered as non-cytotoxic [41].

S. No.	Cell Line	Plating	Time Point
1	MG-63 (Bone)	$3x10^4$ cells/ well, 96-well plate	5 days
2	Ishikawa (Uterus)	$3x10^4$ cells/ well, 96-well plate	5 days
3	A549 (Lung)	$10x10^4$ cells/ well, 96-well plate	24 hours
4	HepG2 (Liver)	$1x10^4$ cells/ well, 96-well plate	24 hours
5	Human Cardiac fibroblasts (Heart)	$1x10^4$ cells/ well, 96-well plate	24 hours
6	SH-SY5Y (Neuronal cell)	$10x10^4$ cells/ well, 96-well plate	24 hours

Table 1: Information related to six cell lines with their plating density and time-point

Cytoprotective Effect of the Test Formulation

The cytoprotective effect of the test formulation/Medium in various cells such as human cardiac fibroblasts-HCF; human hepatoma cells-HepG2; and adenocarcinomic human alveolar basal epithelial cells-A549 were counted and then plated in specific medium followed by overnight incubation. Further, the cells were then treated with the test items/positive control at the non-cytotoxic concentrations for 24 hours. After 24 hours, the oxidative stress using 10 mM *t*-BHP for 3.5 hours was given to the cells. The cells treated with 10 mM of *t*-BHP alone served as negative control. After 3.5 hours of incubation with *t*-BHP the above plates were taken out and cell viability was determined by MTT assay [41]. The percentage protection corresponding to each treatment was calculated using Equation 2:

$$\% \text{ Protection} = [(\text{Absorbance}_{\text{sample}} - \text{Absorbance}_{\text{t-BHP}})] * 100 / [\text{Absorbance}_{\text{untreated}} - \text{Absorbance}_{\text{t\_BHP}}] \quad (2)$$

Estimation of Alkaline Phosphatase (ALP) activity

For the estimation of ALP, the cells (human bone osteosarcoma cells-MG-63 and human endometrial adenocarcinoma cells-Ishikawa) were counted using a hemocytometer and plated in 24-well plates at the density corresponding to 1 X 10^4 cells/well in phenol-free DMEM supplemented with 10% CD-FBS. Following the respective treatments, the cells in the above plate were incubated for 48 hours in CO_2 incubator at 37 °C, 5% CO_2, and 95% humidity. After 48 hours of incubation, the plates were taken out and processed for the measurement of ALP enzyme activity. The cells were washed with 1 X PBS and lysed by freeze-thaw method *i.e.*, incubation at -80 °C for 20 minutes followed by incubation at 37 °C for 10 minutes. To the lysed cells, 50 µL of substrate solution *i.e.* 5 mM of *p*-nitrophenyl phosphate (*p*NPP) in 1M diethanolamine and 0.24 mM magnesium chloride (MgCl2) solution (pH 10.4) was added to all the wells followed by incubation for 1 hour at 37 °C. The absorbance of the above solution was read at 405 nm using Synergy HT microplate reader (Biotek, USA). The absorbance values obtained were normalized with substrate blank (*p*NPP solution alone) absorbance values. The percentage increase in ALP enzyme activity with respect to the untreated cells (baseline group) was calculated using Equation 3:

$$\% \text{ Increase in ALP} = \{(X-R)/R\} * 100 \quad (3)$$

Where, X = Absorbance of cells corresponding to positive control and test groups
R = Absorbance of cells corresponding to baseline group (untreated cells)

Estimation of Lactate Dehydrogenase (LDH) in Human Cardiac Fibroblasts (HCF) Cells

The HCF cells were counted and then plated at the density of 0.25 X 10^6 cells/well in 24-well plates in human cardiac fibroblast medium with 10% FBS followed by overnight incubation. The cells were then treated with the test formulation combinations/ positive control at the non-cytotoxic concentrations for 24 hours. After 24 hours, oxidative stress was given to the cells using 10 mM t-BHP for 3.5 hours. The untreated cells were served as control group, which did not receive any treatment and were maintained in cell growth medium only. Cells treated with 10 mM of *t*-BHP alone served as the negative control. After 3.5 hours of incubation

with *t*-BHP, the above plates were taken out and LDH activity was determined using LDH activity kit as per manufacturer's instructions. The percent increase in LDH activity was calculated using Equation 4.

$$\% \text{ Increase} = [(\text{LDH activity}_{\text{sample}} - \text{LDH activity}_{\text{t-BHP}})]^{*}100 / [\text{LDH activity}_{\text{untreated}} - \text{LDH activity}_{\text{t\_BHP}}] \quad (4)$$

Estimation of ALT in liver cells (HepG2)

The human hepatoma cells (HepG2) were counted and then plated at the density of 5 X 10^4 cells/well in 48-well plates in DMEM media with 10% FBS followed by overnight incubation. The cells were then treated with the test formulation/positive control at the non-cytotoxic concentrations for 24 hours. After 24 hours, oxidative stress was given to the cells using 400 µM t-BHP for 3.5 hours. The untreated cells served as control that did not receive any treatment and were maintained in cell growth medium only. Cells treated with 400 µM of *t*-BHP alone served as negative control. After 3.5 hours of incubation with *t*-BHP, the above plates were taken out and ALT activity was determined using ALT activity kit as per manufacturer's instructions. The percent increase in ALT activity was calculated using Equation 5.

$$\% \text{ Increase} = [(\text{ALT activity}_{\text{sample}} - \text{ALT activity}_{\text{t-BHP}})]^{*}100 / [\text{ALT activity}_{\text{untreated}} - \text{ALT activity}_{\text{t\_BHP}}] \quad (5)$$

Estimation of Superoxide Dismutase (SOD) in Lung (A549) Cells

The adenocarcinomic human alveolar basal epithelial cells (A549) were counted and then plated at the density of 1 X 10^4 cells/well in 24-well plates in DMEM medium with 10% FBS followed by overnight incubation. The cells were then treated with the test formulation/positive control at the non-cytotoxic concentrations along with 100 µM *t*-BHP to induce oxidative stress. The untreated cells served as control that did not receive any treatment and were maintained in cell growth medium only. Cells treated with 100 µM of *t*-BHP alone served as negative control. After 24 hours of incubation with *t*-BHP the above plates were taken out and SOD activity was determined using SOD activity kit as per manufacturer's instructions. The percent increase in SOD activity was calculated using Equation 6:

$$\% \text{ Increase in SOD activity} = ((X-R)/R)^{*}100 \quad (6)$$

Where, X = SOD activity corresponding to test item or positive control
R = SOD activity corresponding to Control group.

Estimation of Serotonin in Neuronal Cells (SH-SY5Y)

The human neuroblastoma (SH-SY5Y) cells were counted and then plated at the density of 10 X 10^4 cells/well in 96-well plates in DMEM/Ham's F12 with 10% FBS followed by overnight incubation. The cells were then treated with the test formulation/positive control at the non-cytotoxic concentrations. The untreated cells 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. Serotonin release was determined by ELISA as per manufacturer's protocol. The percent increase in serotonin levels was calculated using Equation 7:

$$[(X-R)/R]^{*}100 \quad (7)$$

Where, X = Serotonin levels corresponding to test item or positive control,
R = Serotonin levels corresponding to control group.

Effect of Test Formulation on Vitamin D Receptor (VDR) in Bone (MG-63) Cells

The effect of the Biofield Treated test formulation/medium on vitamin D receptor (VDR) activity was performed in bone (MG-63) cells. The cells were counted using the hemocytometer and then plated at density 2 X 10^5 cells/well in 6-well plates to Phenol-free DMEM with 10% FBS 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, while control group did not receive any treatment, which were maintained in cell growth medium only. The treated cells were incubated for 24 hours and VDR expression was determined by qPCR using VDR specific primers. Cells were harvested by scrapping and washed with PBS. 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:

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

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

Statistical analysis

All the values were represented as mean ± SD (standard deviation) of three independent experiments. The statistical analysis was performed using SigmaPlot statistical software (v11.0). For two group comparison, student's *t*-test was used. For multiple group comparison, one-way analysis of variance (ANOVA) was used followed by post-hoc analysis by Dunnett's test. Statistically significant values were set at the level of $p \leq 0.05$.

Results and Discussion

MTT Assay for Cell Viability

MTT assay was used initially for analyzing cell viability at various test concentrations. The initial results of MTT assay revealed that each cell line was found safe with respect to the tested concentrations of test formulation and were represented as percentage of cell viability. The criteria for selection of non-cytotoxic concentrations were less than 30% cytotoxicity or greater than 70% cell viability using MTT assay. The experimental data suggested that the overall percent cell viability in different cell-lines *viz*. MG-63, Ishikawa, A549, HepG2, HCF, and SH-SY5Y. Based on the percent cell viability data, it was observed that the test formulation and positive controls were found safe and non-toxic at the tested concentrations. These test concentrations of the formulation was used cell-based assays.

Evaluation of Cytoprotective Effect of the Test Formulation

Cytoprotective effect of the test formulation was screened and the data was presented in terms of percentage cellular protection against *t*-BHP induced cell damage (Figure 1). Trimetazidine (TMZ) was used as a positive control group in human cardiac fibroblasts cells (HCF) for cytoprotective effect which showed significant restoration of cell viability by 48%, 57.2%, and 87.2% at 5, 10 and 25 µM, respectively as compared to the *t*-BHP induced group. Besides, the restoration of cell viability among the tested groups by the test formulation was reported as 129.7%, 10.4%, and 26% at 1, 10, and 63.75 µg/mL respectively, in the UT-Med + BT-TI as compared with the untreated test group. Similarly, restoration of cell viability was increased in BT-Med + UT-TI group was 28.4% at 63.75 µg/mL, while increased cellular restoration was reported by 44.5%, 20.8%, and 38.9% at 10, 25.5, and 63.75 µg/mL respectively in the BT-Med + BT-TI group as compared with the untreated test group. Similarly, silymarin was used as positive control in HepG2 cells, which resulted in significant cellular restoration by 40%, 65.9%, and 86.6% at 5, 10 and 25 µg/mL, respectively as compared to the *t*-BHP induced group. Besides, test formulation groups such as in the UT-Med + BT-TI group showed increased cellular restoration by 21.9%, 37%, and 53.4% at 10, 25.5, and 63.75 µg/mL respectively, as compared to the untreated test group. Besides, the test formulation showed maximum restoration of cell viability by 14.5%, 5.8%, and 13.8% at 10, 25.5, and 63.75 µg/mL respectively, in the BT-Med + UT-TI group. Similarly, 5.7%, 22.9%, and 17.3% improved cellular restoration was reported at 10, 25.5, and 63.75 µg/mL respectively, at BT-Med + BT-TI groups as compared to the UT-Med + UT-TI group. In addition, quercetin was used as positive control in adenocarcinomic human alveolar basal epithelial cells (A549) resulted, restoration of cell viability by 56.8% and 66.4% at 10 and 25 µM, respectively compared to the *t*-BHP induced group. Besides, the test formulation showed maximum restoration of cell viability by 8%, 25.6%, and 10.3% at 1, 25.5, and 63.75 µg/mL respectively, in the UT-Med + BT-TI group. Similarly, 59.8%, 50.3%, and 35.1% improved cellular restoration was reported at 10, 25.5, and 63.75 µg/mL respectively, at BT-Med + UT-TI groups as compared to the UT-Med + UT-TI group. However, 26.1%, 13.3%, 22.4%, and 14% improved cellular restoration was reported at 1, 10, 25.5, and 63.75 µg/mL respectively, at BT-Med + BT-TI groups as compared to the UT-Med + UT-TI group. Using cell line assays, cytoprotection of various cells can be best determined using *tert*-butyl hydroperoxide (*t*-BHP) method [41,42]. The present data showed significant cellular protection after Biofield Energy Healing Treatment against vital organs and their functioning *viz*. heart, liver, and lungs. Cytoprotection results can be best defined for the cellular injuries and oxidative stress is one of the reasons to induce cell death [43-47]. Thus, Biofield Energy Healing Treatment (The Trivedi Effect®) can be assumed to manage the oxidative stress induced by various factors, which improved the cytoprotection in the tested cells. Therefore, the Biofield Energy Healing Treatment could be successfully used for the management of various pathological etiologies against cardiovascular, liver, and various lung diseases.

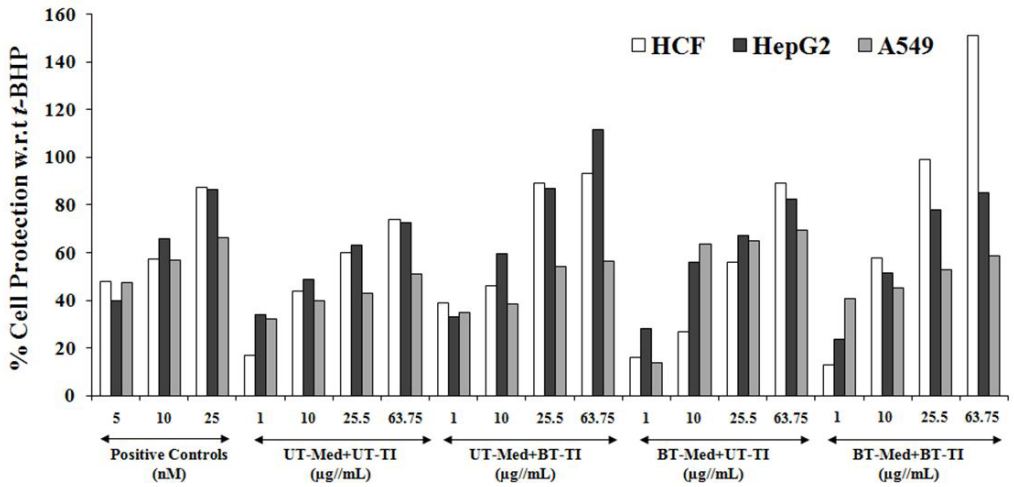

Figure 1: Cytoprotective action of the test formulation in human cardiac fibroblasts cells (HCF), human hepatoma cells (HepG2), and adenocarcinomic human alveolar basal epithelial cells (A549) against tert-butyl hydroperoxide (t-BHP) induced damage. Trimetazidine (µM), silymarin (µg/mL), and quercetin (µM) were used as positive control in HCF, HepG2, and A549 cells, respectively. UT: Untreated; Med: Medium; BT: Biofield Treated; TI: Test item

Estimation of Alkaline Phosphatase (ALP) Activity

ALP activity was tested for two cell lines *viz*. MG-63 and Ishikawa. Calcitriol (nM) was used as positive control in the MG-63 cells, and the results suggested significant increased ALP level by 12%, 23%, and 53.5% at 0.1, 1, and 10 nM respectively as presented in Figure 2. However, the experimental test groups showed increased ALP activity by 81.2% and 77.3% at 10 and 50 µg/mL respectively, in the UT-Med + BT-TI group as compared to the UT-Med + UT-TI group in MG-63 cells. In addition, ALP activity was increased by 8.1%, 80.8%, and 84.5% at 0.1, 10, and 50 µg/mL respectively, in the BT-Med + UT-TI group as compared to the UT-Med + UT-TI group. Similarly, ALP activity was increased by 8.6%, 78.1%, and 84.9% at 0.1, 10, and 50 µg/mL respectively, in the BT-Med + BT-TI group as compared to the UT-Med + UT-TI group. Similarly, naringenin was used as positive control for Ishikawa cells, and the data showed significant improved level of ALP by 18.3%, 35.8%, and 109.4% at 0.1, 1, and 10 nM respectively. In the experimental tested groups, the ALP percent was significantly increased by 64%, 29.4%, and 8.9% at 0.1, 10, and 50 µg/mL, respectively in the UT-Med + BT-TI group as compared to the UT-Med + UT-TI group. Similarly, ALP percent was significantly increased by 165.1%, 81.7%, and 95.3% at 0.1, 10, and 50 µg/mL, respectively in the BT-Med + UT-TI group as compared to the UT-Med + UT-TI group. However, ALP percent was significantly increased by 308.1%, 153.7%, and 108.9% at 0.1, 10, and 50 µg/mL, respectively in the BT-Med + BT-TI group as compared to the UT-Med + UT-TI group. Thus, the ALP level was significantly increased, which represents bone health biomarker of bone related disorders [48,49]. Overall, the experimental data suggested significant improved ALP level after Biofield Energy Healing Treatment that has important application in low bone density, osteoporosis, osteogenesis imperfect and Paget's disease of bone that makes the bones brittle.

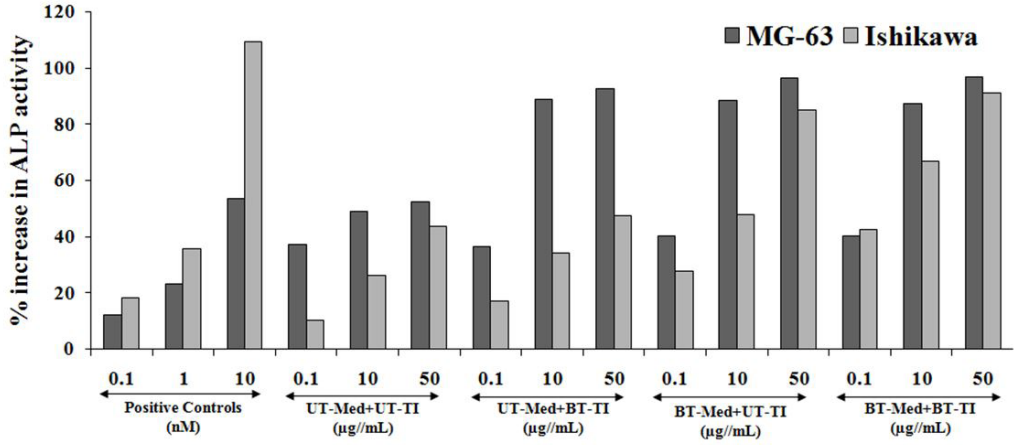

Figure 2: Alkaline phosphatase (ALP) activity in human bone osteosarcoma cells (MG-63) and human endometrial adenocarcinoma cells (Ishikawa) after treatment of the test formulation. Calcitriol and naringenin were used as positive control in MG-63 and Ishikawa cells, respectively. UT: Untreated; Med: Medium; BT: Biofield Treated; TI: Test item

Identification of Lactate Dehydrogenase (LDH) Activity in Human Cardiac Fibroblasts (HCF)

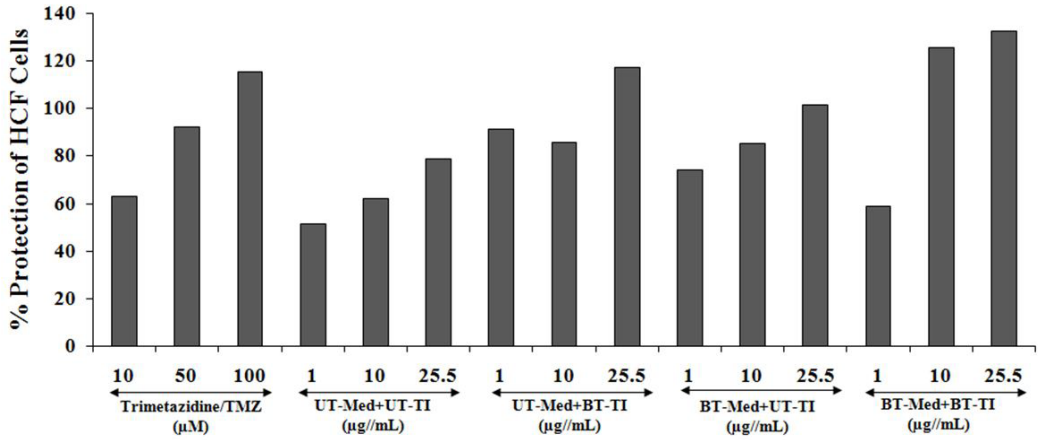

Figure 3: The effect of the test formulation on the percent protection of HCF cells in terms of decreased lactate dehydrogenase (LDH) activity against tert-butyl hydroperoxide (t-BHP) induced damage. TMZ: Trimetazidine; UT: Untreated; Med: Medium; BT: Biofield Treated; TI: Test item

LDH activity was evaluated in the HCF cells, while the results were concluded in terms of decreased LDH activity, which represents increased cellular protection of HCF cells. The results are presented in Figure 3. The positive control, trimetazidine (TMZ) showed 63.1%, 92.3%, and 115.2% increased cellular protection of HCF cells (decreased of LDH activity) at 10, 50, and 100 µM concentration

as compared to the t-BHP group. The test formulation showed maximum percent protection of HCF cells (decreased of LDH activity), which was significantly increased by 76.7%, 38.4%, and 48.8% at 1, 10, and 25.5 µg/mL concentrations respectively, in the UT-Med + BT-TI group, while 44.3%, 37.8%, and 29.06% improved cellular protection (decreased of LDH activity) at 1, 10, and 25.5 µg/mL respectively in the BT-Med + UT-TI group, and 14.2%, 102.6%, and 68.6% improved cellular protection (decreased of LDH activity) 1, 10, and 25.5 µg/mL respectively, in BT-Med + BT-TI group as compared to the UT-Med + UT-TI group. This suggested that improved cellular protection of HCF cells would be clinically useful in living cells of blood cells, skeletal muscle, and heart muscle, which can be beneficial for overall heart heath. LDH play a vital role in tissue injury, necrosis, hypoxia, hemolysis, or malignancies, while the HCF cells are used for the estimation of LDH activity as it plays a central role in the extracellular matrix maintenance of the normal heart functioning [50-52]. The present data concluded a significant reduction of LDH level after Biofield Energy Treatment and protection of the HCF cells, which would be useful in different pathological conditions.

Estimation of Alanine Amino Transferase (ALT) Activity in HepG2 Cells

ALT activity was tested in HepG2 cells and the results are presented in terms of decreased ALT activity (Figure 4), which showed increased cellular protection of HepG2 cells. The positive control, silymarin was selected in ALT activity and the data suggested increased percentage cellular protection (decreased ALT activity) by 56%, 85%, and 118.9% at 5, 10, and 25 µM concentrations, respectively. Similarly, the test formulation groups showed improved cellular protection of HepG2 cells (decreased of ALT activity) by 13.3%, 37.6%, and 70.6% at 10, 25.5, and 63.75 µg/mL respectively, in the UT-Med + BT-TI group, while increased cellular protection of HepG2 cells (decreased of ALT activity) by 26.7%, 55.5%, and 89.9% at 10, 25.5, and 63.75 µg/mL respectively, in the BT-Med + UT-TI group, and increased cellular protection of HepG2 cells (decreased of ALT activity) by 8.4% and 76.6% at 25.5 and 63.75 µg/mL respectively, in the BT-Med + BT-TI group as compared to the UT-Med + UT-TI group (Figure 4). ALT enzyme in liver has significant importance in many physiological processes. However, it is also present in the kidney cells and heart muscles. Besides, ALT has significant role in cellular energy production and have vital role in hepatocellular injury and death [53]. High level of ALT may be linked with the liver disorder or cellular damage with cellular injury [54]. Thus, results showed significant increased cellular protection of HepG2 cells after treatment Biofield Energy Treatment (The Trivedi Effect®), which significantly protects the liver hepatocytes that can be useful in liver cancer, liver cirrhosis, hepatomegaly, liver failure, and hepatitis.

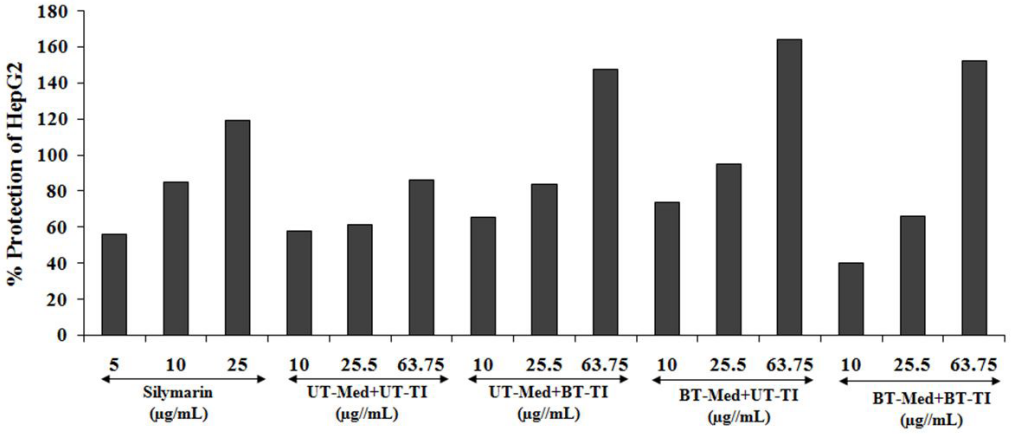

Figure 4: The effect of the test formulation on the percent protection of human liver cancer (HepG2) cells in terms of decreased alanine amino transaminase (ALT) activity under the stimulation of tert-butyl hydroperoxide (t-BHP). UT: Untreated; Med: Medium; BT: Biofield Treated; TI: Test item

Estimation of Superoxide Dismutase (Sod) Activity in Adenocarcinomic Human Alveolar Basal Epithelial Cells (A549)

SOD activity was evaluated in A549 cells in terms of increased cellular protection and the data was presented in Figure 5. The positive control, quercetin showed improved percentage increase in the SOD activity with respect to the *t*-BHP by 68.4%, 83.9%, and 104.2% at 10, 25, and 50 µM concentration respectively. However, the percent protection of A549 (lungs) cells (increased of SOD activity) was significantly increased by 16.2%, 7.7%, and 14.7% at 10, 25.5, and 63.75 µg/mL respectively, in the UT-Med + BT-TI group, while increased SOD activity by 35.2% and 7.2% at 10 and 63.75 µg/mL respectively, in the BT-Med + UT-TI group, and increased SOD activity by 14.1%, 6.2%, and 17.7% at 10, 25.5, and 63.75 µg/mL respectively, in the BT-Med + BT-TI group as compared to the UT-Med + UT-TI group (Figure 5). Thus, results showed significant increased cellular protection of A549 cells and improved level of SOD enzyme in various groups. SOD is present in almost all the body cell and act as the body defense system. SOD represents high antioxidant activity that showed repair of the cellular damage caused due to free radicals, reactive oxygen species (ROS), and many other factors causing cell death [55]. Thus, Biofield Energy Healing Treatment has significantly improved the SOD activity that can be used in various respiratory diseases such as pneumonia, asthma, pulmonary fibrosis, and lung cancer.

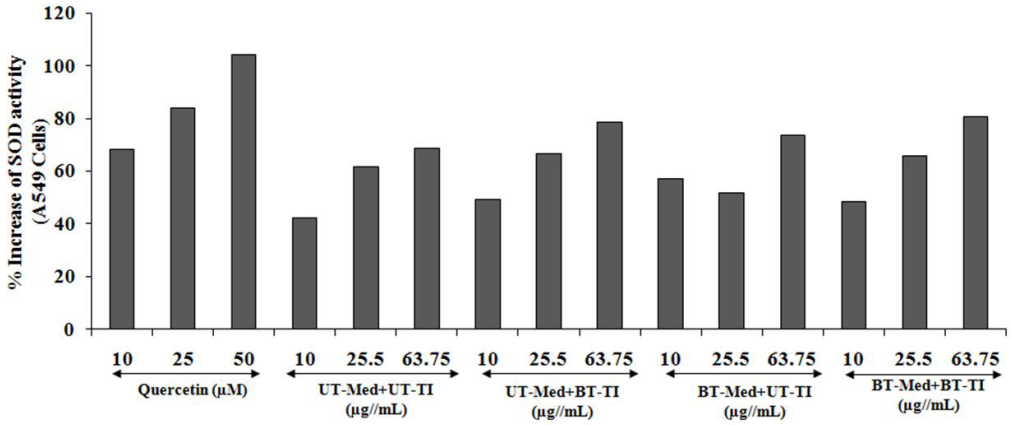

Figure 5: The effect of the test formulation on the percent protection of lungs cells (A549) in terms of increased SOD activity under the stimulation of tert-butyl hydroperoxide (t-BHP). UT: Untreated; Med: Medium; BT: Biofield Treated; TI: Test item. Data are expressed as mean ± SD of three independent experiments

Estimation of Serotonin Level in Human Neuroblastoma (SH-SY5Y) Cells

The change in serotonin level was estimated after treatment with the test formulation using standard cell based assay after 24 hours of treatment using ELISA method. Serotonin activity was reported and the effect of Biofield Energy Treated test formulation is presented in Figure 6. The positive control, curcumin showed 96.1%, 137.2%, and 169.6% increased the level of serotonin at 0.1, 1, and 5 µM respectively, compared to the vehicle control (VC) group. The data showed significant increased serotonin level by 14.6% at 25 µg/mL in the UT-Med + BT-TI, while significant increased serotonin by 41.2% and 9.6% at 1 and 25 µg/mL respectively, in the BT-Med + UT-TI, and 64%, 70.8%, and 58.4% improved serotonin level at 0.1, 10, and 25 µg/mL respectively, in the BT-Med + BT-TI group as compared to the UT-Med + UT-TI group (Figure 6). Serotonin regulates the mood and social behavior, appetite and digestion, sleep, memory, and sexual desire and related functions, an important neurotransmitter. It has important mechanism in the brain, bowels, and blood platelets. Serotonin imbalance results in many neuropsychiatric disorders such as emesis, irritable bowel syndrome (IBS), and pulmonary and systemic hypertension, Alzheimer's disease, cognitive health, loss of ability of thinking, migraine, depression, memory loss, etc. [56-59]. Biofield Energy Healing Treated test formulation can be used against various neurodegenerative diseases and improved brain functioning.

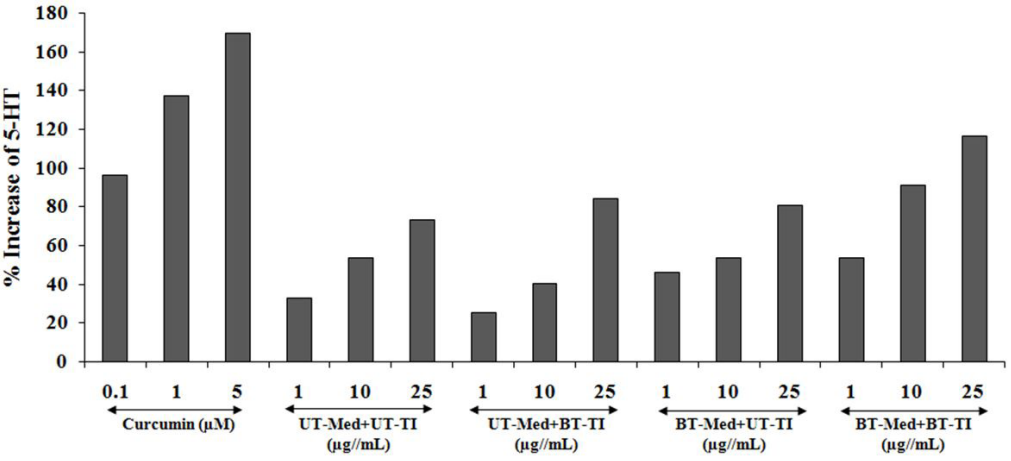

Figure 6: The effect of the test formulation on percent increase in 5-hydroxy tryptamine (5-HT) or serotonin in human neuroblastoma cells (SH-SY5Y). UT: Untreated; Med: Medium; BT: Biofield Treated; TI: Test item

Evaluation of vitamin D receptors (VDRs) activity

Human bone osteosarcoma cells (MG-63) was used for the estimation of VDR activity. The expression of VDRs was studies using the phenomenon of ligand binding through vitamin D active molecule that can be estimated using quantitative-polymerase chain reaction (qPCR) amplification. With the help of real time PCR, different VDR-relative threshold cycle (VDR-C_T) values were obtained after complete amplification cycles using specific primer probes. Relative quantification (RQ) was calculated from the VDR-C_T and house-keeping (HK)-C_T values in MG-63 cells. The VDR-C_T svalues of different experimental test groups are represented in Figure 7. Calcitriol, was used as a positive control and the RQ of VDR was found to be increased in concentration-dependent manner by 59.1%, 93.2%, and 131.3% at 1, 10, and 100 nM, respectively. The experimental test groups showed increased RQ of VDR expression by 166.8%, 43.1%, and 43.2% in the UT-Med + BT-TI group at 0.1, 1, and 10 µg/mL respectively, while 266.4%, 174.4%, and 178.4% increased RQ of VDR at 0.1, 1, and 10 µg/mL respectively, in the BT-Med +

UT-TI group, and increased RQ of VDR by 153.3%, 60.5%, and 85.6% at 0.1, 1, and 10 µg/mL respectively, in the BT-Med + BT-TI group as compared to the UT-Med + UT-TI group. The overall results showed significant increased RQ-VRD expression in MG-63 cells and improved vitamin activity after treatment in various groups. Calcitriol was used as positive control was reported to bind with the VDRs and extensively regulates the calcium homeostasis, immunity, overall cellular growth, bone growth, and differentiation. The results were well collaborated and can be concluded that after treatment the activity of VDR expression was significantly improved.

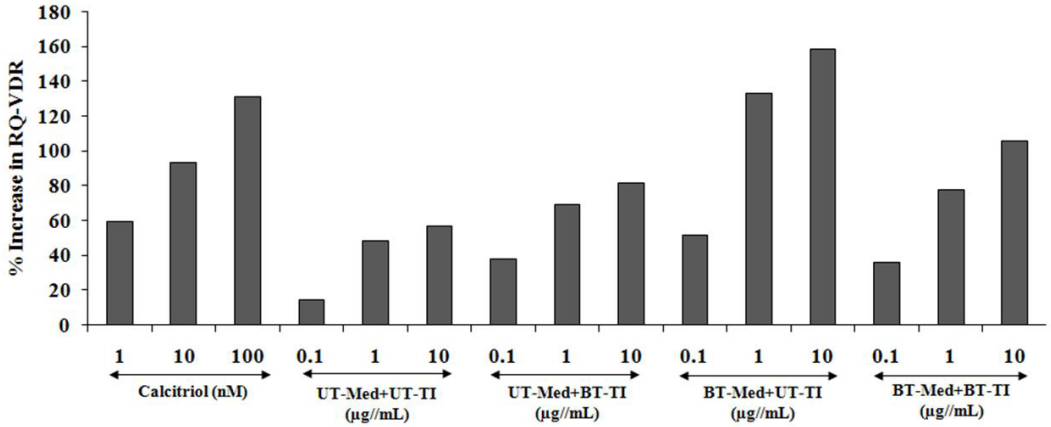

Figure 7: Effect of the test formulation on percent increase in relative quantification (RQ) of vitamin D receptors (VDRs) gene in human bone osteosarcoma cells (MG-63). UT: Untreated; Med: Medium; BT: Biofield Treated; TI: Test item

Conclusion

MTT assay showed that the test formulation was found safe all the tested cell lines. Cytoprotective activity against t-BHP induced cell damage was tested using human cardiac fibroblasts cells (HCF), which showed restoration of cell viability by 129.7% (at 1 µg/mL), 28.4% (at 63.75 µg/mL), and 44.5% (at 10 µg/mL) in the UT-Med + BT-TI, BT-Med + UT-TI, BT-Med + BT-TI groups respectively, as compared to the untreated test group, while in HepG2 cells the maximum restoration of cell viability by 53.4% (at 63.75 µg/mL), 14.5% (at 10 µg/mL), and 22.9% (at 25.5 µg/mL) in the UT-Med + BT-TI, BT-Med + UT-TI, BT-Med + BT-TI groups respectively, as compared to the untreated test group. Similarly, the test formulation in A549 cells showed maximum restoration of cell viability by 25.6% (at 25.5 µg/mL), 59.8% (at 10 µg/mL), and 26.1% (at 1 µg/mL) in the UT-Med + BT-TI, BT-Med + UT-TI, and BT-Med + BT-TI groups respectively, as compared to the untreated test group. ALP activity in MG-63 cells showed significantly increased ALP activity by 81.2% (at 10 µg/mL), 80.8% (at 10 µg/mL), and 84.9% (at 50 µg/mL) in the UT-Med + BT-TI, BT-Med + UT-TI group, and BT-Med + BT-TI groups respectively, as compared to the untreated test group. Similarly, ALP activity in Ishikawa cells with maximum cellular protection at 0.1 µg/mL by 64%, 165.1%, and 308.1% in the UT-Med + BT-TI, BT-Med + UT-TI, BT-Med + BT-TI groups respectively, as compared to the untreated test group. LDH data was presented in terms of increased percentage cellular protection data, which suggested significant decreased activity, which showed maximum cellular protection by 76.7% (at 1 µg/mL), 44.3% (at 1 µg/mL), and 102.6% (at 10 µg/mL) in the UT-Med + BT-TI, BT-Med + UT-TI group, and BT-Med + BT-TI groups respectively, as compared to the untreated test group. ALT activity was studied and data showed maximum improved cellular protection of HepG2 cells (decreased of ALT activity) at 63.75 µg/mL by 70.6%, 89.9%, and 76.6% in the UT-Med + BT-TI, BT-Med + UT-TI group, and BT-Med + BT-TI groups respectively, as compared with the untreated test group. SOD activity was significantly increased by 16.2% (at 10 µg/mL), 35.2% (at 63.75 µg/mL), and 17.7% (at 63.75 µg/mL) in the UT-Med + BT-TI, BT-Med + UT-TI group, and BT-Med + BT-TI groups respectively, as compared with the untreated test group. Serotonin level was significantly increased in SH-SY5Y cells by 14.6% (at 25 µg/mL), 41.2% (at 1 µg/mL), and 70.8% (at 10 µg/mL) in UT-Med + BT-TI, BT-Med + UT-TI, and BT-Med + BT-TI groups respectively, as compared with the untreated test group. However, VDR expression was tested in MG-63 cells, which showed increased RQ of VDR by 166.8%, 43.1%, and 43.2% in the UT-Med + BT-TI group at 0.1, 1, and 10 µg/mL respectively, while 266.4%, 174.4%, and 178.4% increased RQ of VDR at 0.1, 1, and 10 µg/mL respectively, in the BT-Med + UT-TI group, and increased RQ of VDR by 153.3%, 60.5%, and 85.6% at 0.1, 1, and 10 µg/mL respectively, in the BT-Med + BT-TI group as compared to the untreated test control group. Thus, Biofield Energy Treatment (The Trivedi Effect®) can be used for improving overall health such as significant role in cardiac disorders such as stroke, thromboembolic disease, congestive heart failure, congenital heart disease, peripheral artery disease, rheumatic heart disease, valvular heart disease, and venous thrombosis, etc. Besides, it would also protect against many hepatic disorders (cirrhosis, liver cancer, hemochromatosis, Wilson disease), lungs disorders (asthma, chronic bronchitis, emphysema, cystic fibrosis, and pneumonia), and many immune system related disorders. In addition, this novel test formulation can also be utilized for organ transplants (*i.e.*, kidney, liver, and heart transplants), hormonal imbalance, aging, and various inflammatory and immune-related disease conditions like Asthma, Aplastic Anemia, Graves' Disease, Hashimoto Thyroiditis, Multiple Sclerosis, Dermatitis, Diabetes, Parkinson's Disease, Myasthenia Gravis, Ulcerative Colitis (UC), Atherosclerosis, etc. to improve overall health and Quality of Life.

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