# Investigation of Vital Organ Specific Biomarkers Using Cell-Based Assays after Treatment with the Biofield Energy Treated Test Formulation

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## Abstract

The present study aimed to determine the impact of the Biofield Energy Treated test formulation and different cell line mediums on vital organs function. Specific cell based assays were performed based on the vital organs function (bones, heart, liver, lungs, and brain). The test item (TI) and cell line media was divided into two parts; one was untreated (UT-TI) and other part received the Biofield Energy Treatment remotely by a renowned Biofield Energy Healer, James Jeffrey Peoples, USA and were labeled as the Biofield Energy Treated (BT) test formulation/media. The test formulation was tested against various activities using cell line assay in their specific medium (Med). The test formulation was tested for cell viability, and the results showed that the test formulation at tested concentrations was found non-toxic against all the cell lines. Cytoprotective action of the test formulation showed a significant restoration of cell viability by 48.3% (at 25.5 μg/mL), 9.3% (at 1 μg/mL), and 64.1% (at 25.5 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 48.3% (at 25.5 μg/mL), 9.3% (at 1 μg/mL), and 64.1% (at 25.5 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. However, cytoprotective activity in human hepatoma cells (HepG2) showed improved cell viability by 65.4% (at 1 μg/mL), 63.8% (at 1 μg/mL), and 39.4% (at 25.5 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 addition, cytoprotective activity in adenocarcinomic human alveolar basal epithelial cells (A549) showed improved cell viability by 28.4% (at 10 μg/mL), 101.7% (at 25.5 μg/mL), and 181.7% (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 test group. ALP activity in MG-63 cells was maximum increased by 88.1% at 50 μg/mL in the BT-Med + BT-TI group, while in Ishikawa cells showed maximum increased ALP activity by 433.3% and 136.1% at 0.1 and 10 μg/mL respectively, in the BT-Med + BT-TI group

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as compared to the untreated group. The maximum percent cellular protection of HCF (heart) cells (decreased of LDH activity) was significantly increased by 87.8% at 1 μg/mL in the UT-Med + BT-TI group, while BT-Med + UT-TI group showed increased protection by 40.1% at 10 μg/mL, and improved cellular protection by 70.1% at 1 μg/mL in the BT-Med + BT-TI group 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 by 35.6% (at 10 μg/mL), 19% (at 10 μg/mL), and 61.2% (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 the untreated test group. Cellular protection of A549 (lungs) cells (increased of SOD activity) in terms of percentage was increased by 102.3% (at 1 μg/mL), 10.3% (at 25.5 μg/mL), and 38.4% (at 10 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 23.7% (at 0.1 μg/mL), 36.8% (at 25 μg/mL), and 51.9% (at 25 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 471% (at 10 μg/mL), 318.9% (at 10 μg/mL), and 326.2% (at 50 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. In conclusion, 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

**Abbravations:** CAM: Complementary and Alternative Medicine; NCCIH: National Center of Complementary and Integrative Health; HCF: Human Cardiac Fibroblasts; TMZ: Trimetazidine; EDTA: Ethylenediaminetetraacetic Acid; HCF: Human Cardiac Fibroblasts; ALP: Alkaline Phosphatase; LDH: Lactate Dehydrogenase SOD: Superoxide Dismutase; TMZ: Trimetazidine ALP: Alkaline Phosphatase; LDH: Lactate Dehydrogenase; HCF: Human Cardiac Fibroblasts ROS: Reactive Oxygen Species; IBS: Irritable Bowel Syndrome; ALT: Alanine Amino Transferase.

## Introduction

Any form of diseases is one of the major reasons of regret in quality of life. Now-a-days, medicine is one of the immediate options using which the disease can be controlled or prevented or cured along with other complementary and alternative treatment approaches. The development of medicine and its discovery take longs time, which takes many successful preclinical and clinical trials [1,2]. In ancient times, the herbs were one of the most prevalent therapeutic approaches. However, with the huge development of vital nutrients such minerals and vitamins, their therapeutic values are known and developed along with their huge medicinal purposes and its frequent use [3]. Medicinal plants, minerals, and vitamins are equally efficient to check and control the diseased state and imbalance during the pathological process. Thus, herbomineral formulations are widely used against overall health improvement due to their prompt action, very small required doses, tastelessness, highly effectiveness against the incurable diseases and long shelf life compared with the chemical drug moiety. Mineral based test preparations are one of the preferred choices for health benefits as compare with the overall herbal test formulations. In general, mostly mineral preparation is required following raw materials such as use of Zinc, Magnesium, Iron, Mercury, Gold, Silver, Copper, Lead, Tin, Mica, Sulphur, etc. On the basis of recent scientific data, selected minerals, vitamins, and herbal product of the test formulation was selected, which would supposed to improve the overall organs health and quality of life. Thus,

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the novel test formulation is designed 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. All the constituents present in the formulation were selected based on their scientific data. Panax ginseng is considered as potent immunomodulator, role in mental and physical health, and was effective against many pathological conditions such as lung disorder, liver disorder, breast cancer, liver cancer, aging, muscle damage, and overall health [4-6]. Minerals and vitamins used in the test formulation were reported to have significant role in many pathological conditions [7-10]. The novel test formulation was tested against various standard specific cell lines for bone, liver, heart, mental, and bone health along with overall female reproductive functions [11-20]. Thus, the present study was aimed to detect the overall health status using novel test formulation after treatment with the Biofield Energy Treatment as one of the complementary and alternative medicine (CAM) treatment approach.

Biofield Energy Healing Therapy is one of the emerging CAM treatment approaches that aimed in building a scientific foundation with respect to the complex homeodynamic regulation of living systems. Biofield Energy Healing Modalities (as CAM) is supposed to be highly effective in order to enhance the physical, mental, and emotional human wellness without any invasive procedures, which improve the endogenous energy flows [21-23]. Biofield as a unifying energy healing therapy, which provides a new scientific research arena to focus on energy transmission in the body. National Center of Complementary and Integrative Health (NCCIH) has well defined, recognized, and accepted the Biofield Energy Healing therapies as a CAM health care approach along with other therapies such as external qigong, Johrei, Reiki, therapeutic touch, yoga, Qi Gong, polarity therapy, Tai Chi, pranic healing, deep breathing, 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. The Trivedi Effect® has been reported with significant results in the metal physicochemical properties, agriculture science, microbiology, biotechnology, and improved

bioavailability of many compounds, skin health, nutraceuticals, cancer science research, improved bone health, human health and wellness [25-40]. Due to the continued clinical and preclinical applications of the Biofield Energy Healing Treatment, the Biofield Energy Healing Treated test formulation was studied on the function of vital organs such as bones, heart, liver, lungs, and brain specific biomarkers in different standard cell-lines.

## Materials and Methods

### Chemicals and Reagents

Calcium chloride, ferrous sulfate, vitamin $B_6$, vitamin $B_{12}$, vitamin $D_3$, 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. Biofield Energy Healing was performed with the test formulation/media, while the untreated group was treated with a "sham" healer for comparison purposes, who did not have any knowledge about the Biofield Energy Healing Treatment. The rest constituents of the test formulation/media received Biofield Energy Treatment (The Trivedi Effect®) remotely by James Jeffrey Peoples, under standard laboratory conditions for ~3 minutes through healer's unique Biofield Energy Transmission process and were referred as the Biofield Energy Treated formulation/media. The Biofield Energy Healer was located in the USA, however the test formulation were located in the research laboratory of Dabur Research Foundation, New Delhi, India. Biofield

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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:

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

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) | $3 \times 10^4$ cells/ well, 96-well plate | 5 days |
| 2 | Ishikawa (Uterus) | $3 \times 10^4$ cells/ well, 96-well plate | 5 days |
| 3 | A549 (Lung) | $10 \times 10^4$ cells/ well, 96-well plate | 24 hours |
| 4 | HepG2 (Liver) | $1 \times 10^4$ cells/ well, 96-well plate | 24 hours |
| 5 | Human Cardiac fibroblasts (Heart) | $1 \times 10^4$ cells/ well, 96-well plate | 24 hours |
| 6 | SH-SY5Y (Neuronal cell) | $10 \times 10^4$ 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

Cytoprotective effect of the test formulation in various cells such as human cardiac fibroblasts-HCF; human hepatoma cells-HepG2; and adenocarcinomic human alveolar basal epithelial cells-A549 were counted and plated in suitable 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. The percentage protection corresponding to each treatment was calculated using equation 2:

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

### 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 $1 \times 10^4$ 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 ($MgCl_2$) 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:

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

Where, X = Absorbance of cells corresponding to positive control and test groups

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R = Absorbance of cells corresponding to baseline group (untreated cells)

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

HCF cells were counted and plated at the density of $0.25 \times 10^6$ cells/ well in 24-well plates in cardiac fibroblast specific mediumfollowed 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.

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

## Estimation of ALT in Liver Cells (HepG2)

The human hepatoma cells (HepG2) were counted and plated at the density of $5 \times 10^4$ cells/well in 48-well plates in DMEM media 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.

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

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

The adenocarcinomic human alveolar basal epithelial cells (A549) were counted and plated at the density of $1 \times 10^4$ cells/well in 24-well plates in DMEM followed by overnight incubation. The cells were then treated with the test formulation/positive control at the non-cytotoxic concentrations along with 100μM $t$-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 100μM 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:

% Increase in SOD activity = ((X-R)/R)*100............... (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 plated at the density of $10 \times 10^4$ cells/well in 96-well plates 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............... (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 test formulation on vitamin D receptor (VDR) activity in bone (MG-63) cells were counted using the hemocytometer at density $2 \times 10^5$ 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, 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

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Energy Treated cells was calculated with respect to the untreated cells using equation 8:

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

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

### Cell Viability Using MTT Assay

For the estimation of cell viability, MTT assay was analyzed and the results 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 non-cytotoxic test formulation concentration and the positive controls by MTT cell viability assay was resulted in less than 30% cytotoxicity or greater than 70% cell viability were considered as non-cytotoxic concentrations. Overall, 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 and were selected for other parameters.

### Evaluation of Cytoprotective Effect of the Test Formulation

The test formulation was initially screened for cytoprotective activity and the data was presented in terms of percentage cell 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 μg/mL, respectively as compared to the $t$-BHP induced group. Besides, the maximum restoration of cell viability among the tested groups by the test formulation was reported as 48.3% (at 25.5 μg/mL), 9.3% (at 1 μg/mL), and 64.1% (at 25.5 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 (UT-Med + UT-TI 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 65.4% and 43.5% at 1 and 25.5 μg/mL respectively, as compared to the untreated test group. Besides, the test formulation showed maximum restoration of cell viability by 63.8%, 20.6%, and 31.8% at 1, 10, and 25.5 μg/mL respectively, in the BT-Med + UT-TI group. Similarly, 39.4% improved cellular restoration was reported at 25.5 μg/mL 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 μg/mL, respectively compared to the $t$-BHP induced group. Besides, the test formulation showed maximum restoration of cell viability by 15% and 28.4% at 1 and 10 μg/mL respectively, in the UT-Med + BT-TI group. Similarly, 56.1%, 43.5%, 55.5%, and 101.7% improved cellular restoration was reported at 0.1, 1, 10, and 25.5 μg/mL respectively, at BT-Med + UT-TI groups as compared to the UT-Med + UT-TI group. However, 181.7%, 160.6%, 132.9%, and 78.1% improved cellular restoration was reported at 0.1, 1, 10 and 25.5 μg/mL respectively, at BT-Med + BT-TI groups as compared to the UT-Med + UT-TI group. For identification of cytoprotection of cell lines for any test formulation, using $tert$-butyl hydroperoxide ($t$-BHP) this method is considered as the gold standard test [41,42]. The data suggested that the test formulation and Biofield Energy treated media showed significant cellular protection against vital organs and their functioning $viz.$ heart, liver, and lungs. Oxidative stress is one of the vital factors for cell injuries, while cytoprotection is the tool to identify the nature and extend of cellular damage [41, 43-46]. Overall, it can be assumed that significant improved cellular restoration was reported due to Biofield Energy Treatment in all the three tested cell lines. Thus, experimental data revealed that Biofield Energy Healing Treatment (The Trivedi Effect®) significantly protects the $t$-BHP induced oxidative stress against in HCF, HepG2, and A549 cells with respect to the cardiotoxicity, hepatotoxicity, and lung cell toxicity. Therefore, the Biofield Energy Healing Treatment could be successfully used for the management of various pathological etiologies against cardiovascular, liver, and various lung diseases.
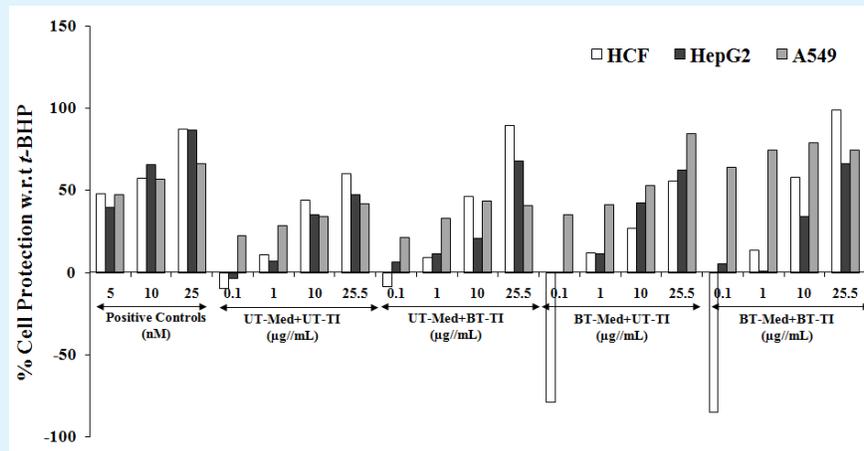
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**Figure 1:** Cytoprotective action of the test formulation in human cardiac fibroblasts cells (HCF), human hepatoma cells (HepG2), and adenocarcinomic human alveolar basal epithelial cells (A549) against *tert*-butyl hydroperoxide (*t*-BHP) induced 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

Estimation of ALP activity against two cell lines, MG-63 and Ishikawa cells were evaluated. The data suggested that in case of MG-63 cells, calcitriol (nM) was used as positive control 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 75.2% and 84.5% 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 84.5% and 87.8% at 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 79.8% and 88.1% at 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.
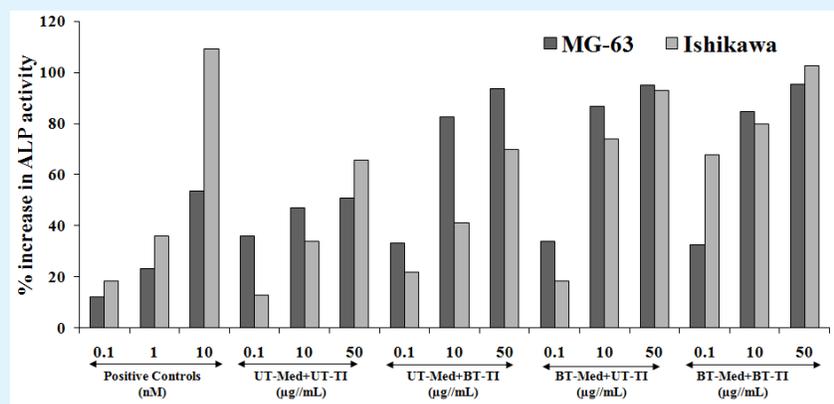


**Figure 2:** Alkaline phosphatase (ALP) activity in human bone osteosarcoma cells (MG-63) and human endometrial adenocarcinoma cells (Ishikawa) after treatment of the test formulation. Calcitriol and naringenin were used as positive control in MG-63 and Ishikawa cells, respectively. UT: Untreated; Med: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

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In the experimental tested groups, the ALP percent was significantly increased by 71.4%, 21.1%, and 6.4% 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 44.8%, 117.9%, and 41.6% 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 433.3%, 136.1%, and 56.4% 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. Overall, the ALP level was significantly increased and ALP is one of the significant bone health biomarker responsible for many bone related disorders [47,48]. Thus, the overall result showed significant improved ALP level after Biofield Energy Healing Treatment that signifies its application in low bone density and osteoporosis, osteogenesis imperfect and Paget's disease of bone that makes the bones brittle.

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

LDH activity was estimated in HCF cells and the data was represented by decreased LDH activity, which represents increased cellular protection of HCF cells. The effect of test formulation in different groups with respect to the percent protection of HCF cells in terms of decreased level of lactate dehydrogenase (LDH) activity is 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 87.8%, 7.6%, and 2.1% at 1, 10, and 25.5 μg/mL concentrations respectively, in the UT-Med + BT-TI group, while 6.4%, 40.1%, and 33.8% improved cellular protection (decreased of LDH activity) at 1, 10, and 25.5 μg/mL respectively in the BT-Med + UT-TI group, and 70.1%, 19.1%, and 11.9% 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. Thus, results showed significant increased cellular protection of HCF cells after treatment in various groups. LDH is present in all the living cells such as in blood cells, skeletal muscle, and heart muscle and is responsible for anaerobic cellular respiration, while it represents overall health of heart diseases and related tissues. LDH play a vital role in tissue injury, necrosis, hypoxia, hemolysis, or malignancies. 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 [49-51]. 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.
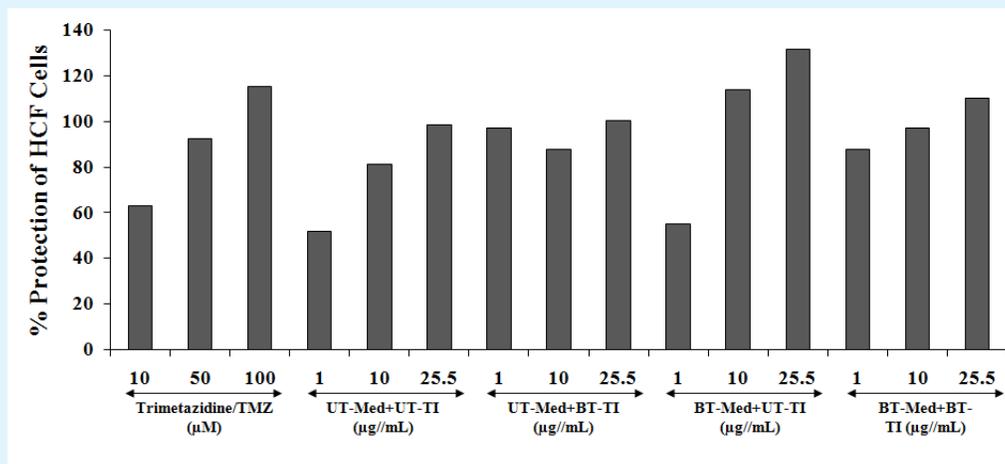


**Figure 3**: The effect of the test formulation on the percent protection of HCF cells in terms of decreased lactate dehydrogenase (LDH) activity against *tert*-butyl hydroperoxide (*t*-BHP) induced damage. TMZ: Trimetazidine; UT: Untreated; Med: Medium; BT: Biofield Treated; TI: Test item.

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### Estimation of Alanine Amino Transferase (ALT) Activity in Hepg2 Cells

ALT activity was examined in HepG2 cells and the results are presented in terms of decreased ALT activity (Figure 4), which represents increased cellular protection. The positive control, silymarin was selected in ALT activity and the data suggested increased percentage cellular protection (decreased ALT activity) by 40.2%, 63.4%, and 103.7% at 5, 10, and 25 µg/mL concentrations, respectively. Similarly, the test formulation groups showed improved cellular protection of HepG2 cells (decreased of ALT activity) by 35.6%, 30.2%, and 15.1% 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 19% at 10 µg/mL in the BT-Med + UT-TI group, and increased cellular protection of HepG2 cells (decreased of ALT activity) by 38.4%, 48.3%, and 61.2%

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 4). Thus, results showed significant increased cellular protection of HepG2 cells after treatment in various groups. ALP is one of the major enzymes of liver responsible for many physiological processes. Besides, ALP is also present in the kidney cells and heart muscles in small quantity. In addition, ALP has significant role in cellular energy production and have vital role in hepatocellular injury and death [52]. High level of ALT may be linked with the liver disorder or cellular damage with cellular injury [53]. Biofield Energy Treatment (The Trivedi Effect®) significantly protects the liver hepatocytes with reduced ALT enzyme as compared to the *t*-BHP inducing group, which can be useful in liver cancer, liver cirrhosis, hepatomegaly, liver failure, and hepatitis.
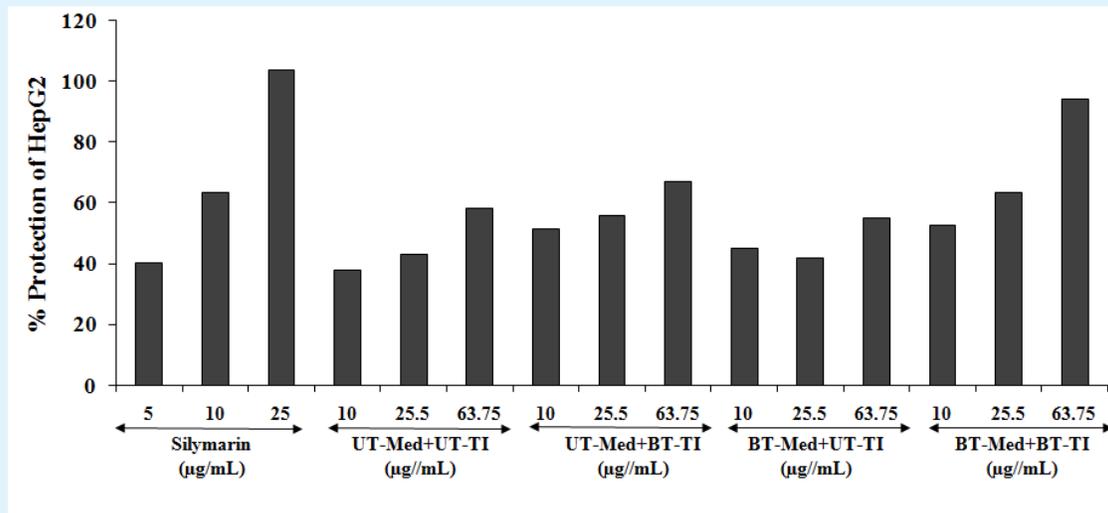


**Figure 4:** The effect of the test formulation on the percent protection of human liver cancer (HepG2) cells in terms of decreased alanine amino transaminase (ALT) activity under the stimulation of *tert*-butyl hydroperoxide (*t*-BHP). UT: Untreated; Med: Medium; BT: Biofield Treated; TI: Test item.

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

SOD activity was estimated in A549 cells in different test groups. SOD level is represented 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 µg/mL concentration respectively. However, the percent

protection of A549 (lungs) cells (increased of SOD activity) was significantly increased by 102.3% and 10.3% at 1 and 25.5 µg/mL respectively, in the UT-Med + BT-TI group, while increased SOD activity by 7.5% and 10.3% at 10 and 25.5 µg/mL respectively, in the BT-Med + UT-TI group, and increased SOD activity by 38.4% and 6.8% at 10 and 25.5 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 SOD enzyme activity after treatment in various groups. SOD is

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present in all the body cells, which play a vital role in body defense system. SOD represents high antioxidant activity that showed repair of the cellular damage affected by free radicals, reactive oxygen species (ROS) causing cell death [54]. Thus, experimental data showed that

Biofield Energy Treatment has significantly improved the SOD activity that can be used in various respiratory diseases such as pneumonia, asthma, pulmonary fibrosis, and lung cancer.
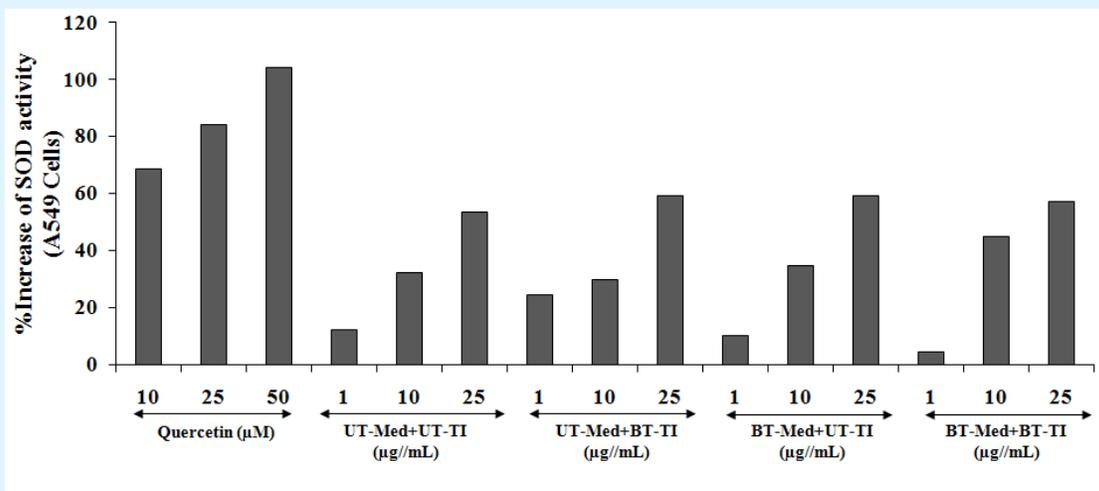
**Figure 5:** The effect of the test formulation on the percent protection of lungs cells (A549) in terms of increased SOD activity under the stimulation of *tert*-butyl hydroperoxide (*t*-BHP). UT: Untreated; Med: Medium; BT: Biofield Treated; TI: Test item. Data are expressed as mean ± SD of three 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.
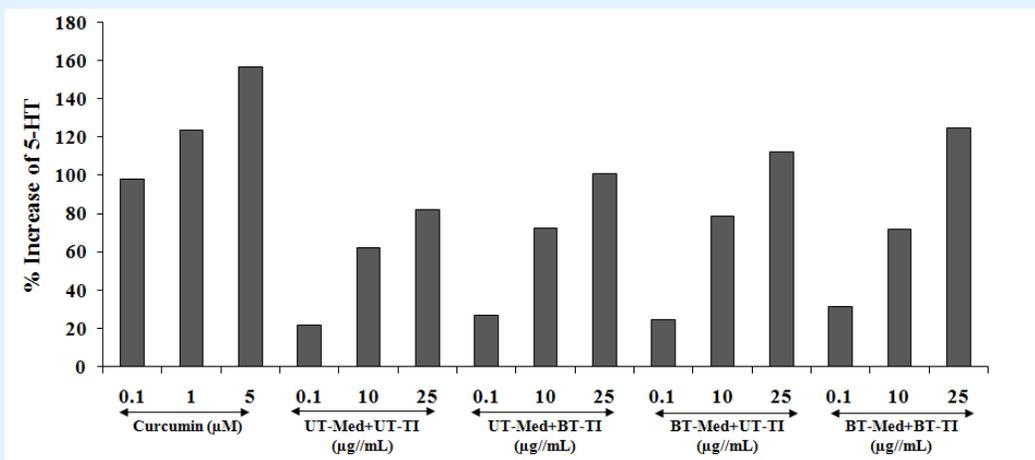
**Figure 6:** The effect of the test formulation on percent increase in 5-hydroxy tryptamine (5-HT) or serotonin in human neuroblastoma cells (SH-SY5Y). UT: Untreated; Med: Medium; BT: Biofield Treated; TI: Test item.

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The positive control, curcumin showed 98.2%, 123.5%, and 156.8% increase the level of serotonin at 0.1, 1, and 5 µg/mL respectively, compared to the vehicle control (VC) group. The data showed significant increased serotonin level by 23.7%, 17.2%, and 22.6% at 0.1, 10, and 25 µg/mL respectively, in the UT-Med + BT-TI, while significant increased serotonin by 13.7%, 26.7%, and 36.8% at 0.1, 10, and 25 µg/mL respectively, in the BT-Med + UT-TI, and 44.1%, 16.3%, and 51.9% 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 is one of the important neurotransmitter; it regulates the mood and social behavior, appetite and digestion, sleep, memory, and sexual desire and related functions. The presence of serotonin and its role has significant action 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 [55-58]. The data suggested significant improved level of serotonin after treatment with the Biofield Energy Healing Treated test formulation that would be highly useful against various neurodegenerative diseases and improved brain functioning.

## Effect of Test Formulation on Vitamin D Receptors (VDRs)

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-$C_T$ values 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 213.9%, 471%, and 283% in the UT-Med + BT-TI group at 1, 10, and 50 µg/mL respectively, while 318.9% and 212.5% increased RQ of VDR at 10 and 50 µg/mL respectively, in the BT-Med + UT-TI group, and increased RQ of VDR by 121%, 118.9%, and 326.2% at 1, 10 and 50 µg/mL respectively, in the BT-Med + BT-TI group as compared to the UT-Med + UT-TI group. Overall, the data concluded that VDR expression was significantly improved in MG-63 after treatment with the test formulation. Thus, results showed significant increased RQ-VRD expression in MG-63 cells and improved vitamin activity after treatment in various groups. Calcitriol 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 [59,60]. The results were well collaborated and can be concluded that after treatment the activity of VDR expression was significantly improved.
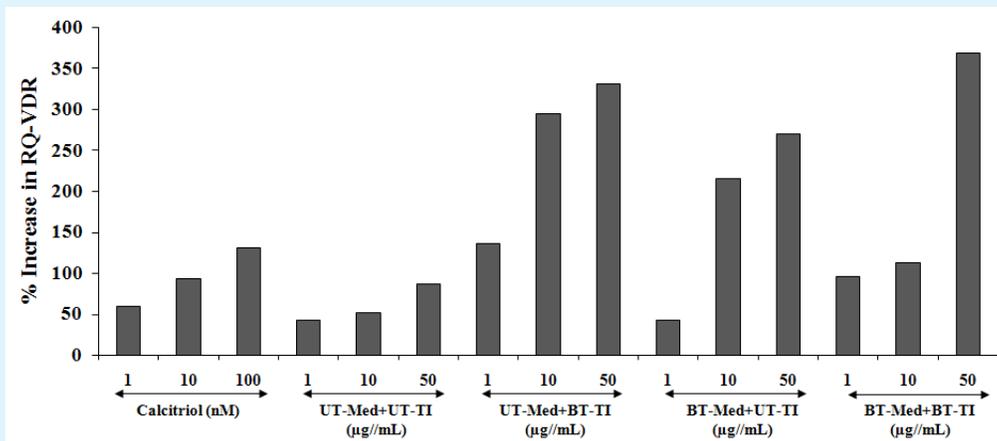


**Figure 7:** Effect of the test formulation on percent increase in relative quantification (RQ) of vitamin D receptors (VDRs) gene in human bone osteosarcoma cells (MG-63). UT: Untreated; Med: Medium; BT: Biofield Treated; TI: Test item.

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## Conclusions

MTT assay showed that the test formulation was found safe and non-toxic against 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 48.3% (at 25.5 µg/mL), 9.3% (at 1 µg/mL), and 64.1% (at 25.5 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, while in HepG2 cells the maximum restoration of cell viability by 65.4% (at 1 µg/mL), 63.8% (at 1 µg/mL), and 39.4% (at 25.5 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. Similarly, the test formulation in A549 cells showed maximum restoration of cell viability by 28.4% (at 10 µg/mL), 101.7% (at 25.5 µg/mL), and 181.7% (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 test group. ALP activity in MG-63 cells showed significantly increased ALP activity at 50 µg/mL by 84.5%, 87.8%, and 88.1% 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 by 71.4% (at 0.1 µg/mL), 117.9% (at 10 µg/mL), and 433.3% (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 test group. LDH activity was significantly decreased and the data was presented in terms of increased percentage cellular protection data, which showed maximum cellular protection by 87.8% (at 1 µg/mL), 40.1% (at 10 µg/mL), and 70.1% (at 1 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) by 35.6% (at 10 µg/mL), 19% (at 10 µg/mL), and 61.2% (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. SOD activity was significantly increased by 102.3% (at 1 µg/mL), 10.3% (at 25.5 µg/mL), and 38.4% (at 10 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 23.7% (at 0.1 µg/mL), 36.8% (at 25 µg/mL), and 51.9% (at 25 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 213.9%, 471%, and 283% in the UT-Med + BT-TI group at 1, 10, and 50 µg/mL respectively, while 318.9% and 212.5% increased RQ of VDR at 10 and 50 µg/mL respectively, in the BT-Med + UT-TI group, and increased RQ of VDR by 121%, 118.9%, and 326.2% at 1, 10, and 50 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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## Conflicts of Interest

The authors declare no conflicts of interest.

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