

## The Potential Health Benefits of the Consciousness Energy Treated Novel Test Formulation on Various Functional Enzyme Biomarkers

Shirley Theresa Holmlund<sup>1</sup>, Mahendra Kumar Trivedi<sup>1</sup>, Alice Branton<sup>1</sup>, Dahryn Trivedi<sup>1</sup>, Gopal Nayak<sup>1</sup>, Sambhu Charan Mondal<sup>2</sup> and Snehasis Jana<sup>2\*</sup>

<sup>1</sup>Trivedi Global, Inc., Henderson, Nevada, USA

<sup>2</sup>Trivedi Science Research Laboratory Pvt. Ltd., Thane-West, Maharashtra, India.

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### ABSTRACT

The study objective was to investigate the effect of the Consciousness Energy Treated test formulation on vital organs like bones, heart, liver, lungs and brain using various cell-based assays. The test formulation and the cell media was divided into two parts; one untreated (UT) and other part received the Biofield Energy Treatment remotely by a renowned Biofield Energy Healer, Shirley Theresa Holmlund, Canada and was labeled as the Biofield Energy Treated (BT) test formulation/media. Cell viability data suggested that the test formulation was safe and non-toxic in six different cells. The Biofield Energy Treated Medium (BT-Med) + Biofield Treated Test Item (BT-TI) group showed 115.6% and 53.3% restoration of viable cells at 10 and 25  $\mu\text{g/mL}$ , respectively in human cardiac fibroblasts cells (HCF) compared to the UT-Med + UT-TI group. Moreover, the BT-Med + UT-TI group showed 113.5% and 73.5% restoration of cell viability at 0.1 and 1  $\mu\text{g/mL}$ , respectively in human hepatoma cells (HepG2) compared to the untreated group. Furthermore, 101.1%, 829.8% and 698.9% restoration of cell viability was observed in adenocarcinomic human alveolar basal epithelial cells (A549) by UT-Med + BT-TI, BT-Med + UT-TI and BT-Med + BT-TI groups, respectively at 10  $\mu\text{g/mL}$  compared to the untreated. The alkaline phosphatase (ALP) level was significantly increased by 97.9% and 69.7% in the UT-Med + BT-TI and BT-Med + BT-TI groups, respectively at 50  $\mu\text{g/mL}$  in human bone osteosarcoma cells (MG-63) compared to the untreated. Additionally, the level of ALP was significantly increased by 58.2% in the BT-Med + BT-TI group at 1  $\mu\text{g/mL}$  in human endometrial adenocarcinoma cells (Ishikawa) compared to the untreated. The percent protection of HCF (heart) cells (decreased of LDH activity) was significantly increased by 67.4% (at 0.1  $\mu\text{g/mL}$ ), 80.4% (at 0.1  $\mu\text{g/mL}$ ) and 119.8% (at 10  $\mu\text{g/mL}$ ) in the UT-Med + BT-TI, BT-Med + UT-TI, BT-Med + BT-TI groups, respectively compared to the untreated. The percent protection of HepG2 (liver) cells (decreased of ALT activity) was significantly increased by 57.6% and 82.5% in the BT-Med + UT-TI group at 25 and 63  $\mu\text{g/mL}$ , respectively; while 123.9% at 10  $\mu\text{g/mL}$  in the BT-Med + BT-TI group compared to untreated. The percent protection of A549 (lungs) cells (increased of SOD activity) was significantly increased by 53.6% and 59% in the UT-Med + BT-TI and BT-Med + BT-TI groups, respectively at 10  $\mu\text{g/mL}$  compared to the untreated. Serotonin level was significantly increased by 85.3% in the UT-Med + BT-TI and BT-Med + BT-TI groups at 0.1  $\mu\text{g/mL}$  as compared to untreated in human neuroblastoma cells (SH-SY5Y). The relative quantification (RQ) of vitamin D receptor (VDR) was significantly increased by 245.9% and 211.5% at 10 and 50  $\mu\text{g/mL}$ , respectively in the UT-Med + BT-TI group; while 174.3% (at 10  $\mu\text{g/mL}$ ) in the BT-Med + UT-TI group as compared to the untreated in MG-63 cells. Overall, these results suggest that Biofield Energy Treated test formulation has significantly improved the bones, heart, liver, lungs and brain functional enzymes biomarkers. Altogether data suggest that the Biofield Energy Treatment (The Trivedi Effect<sup>®</sup>) can be useful to protect and maintain the normal function of each vital organ such as lungs, liver, heart, brain, and bones. Therefore, The Trivedi Effect<sup>®</sup> can be used as a complementary and alternative therapy against several disorders such as heart attack, coronary artery disease, heart failure, arrhythmias, congenital heart disease, cardiomyopathy, Wilson disease,

**Corresponding author:** Snehasis Jana, Trivedi Science Research Laboratory Pvt. Ltd., Thane-West, Maharashtra, India, E-mail: publication@trivedieffect.com

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hemochromatosis, cirrhosis, liver cancer, pneumonia, emphysema, chronic bronchitis, asthma, osteoporosis, cystic fibrosis, etc.

**Keywords:** Biofield energy treatment, The Trivedi effect<sup>®</sup>, Cardiac health, Bone health, Liver health, VDR receptor, Lungs health, Brain health

## INTRODUCTION

Bones, heart, liver, lungs and brain disorders are the major concern of human overall health across the globe. The World Health Organization (WHO) estimates, in 2016, ~17.5 million people die due to cardiovascular (heart) disorders, ~3.5 million people die due to lungs disorders, ~1.3 million people die due to liver disorders around the globe each year [1]. Moreover, ~1.2 million people most frequently diagnosed adult-onset brain disorders in each year in the USA. [2]. Three main criteria to keep a healthy heart include the opening blood vessels, strengthening the heart muscle and controlling free radical damage by antioxidants [3]. The release of liver mitochondrial enzymes is considered strong evidence for hepatic (liver) necrosis, which is associated with an increased production of reactive oxygen species (ROS) that leads to hepatic lipid peroxidation [4-6]. Oxidative stress in the respiratory system increases the production of mediators of pulmonary inflammation and initiate or promote mechanisms of carcinogenesis [7]. The lung is one of the major organs, which is highly exposed by various oxidants, i.e., endogenous and exogenous oxidants (cigarette smoke, mineral dust, ozone and radiation). These oxidants produce free radicals, while reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced by phagocytes as well as by alveolar, polymorph nuclear, bronchial and different endothelial cells [8]. However, the role of oxidative stress in the pathogenesis of lung diseases has been widely reported such as asthma, chronic obstructive pulmonary disease (COPD), lung malignancies and parenchymal lung diseases like idiopathic pulmonary fibrosis and lung granulomatous diseases [9]. Serotonin (5-hydroxytryptamine, 5-HT) is among the brain's neuromodulators responsible for behavior and understanding [10]. Apart from medicines, non-pharmacologic methods that can increase serotonin by increasing recognition and happiness and well-being. These factors can protect against mental and physical disorders [11]. There is currently no universally accepted test formulation, which improve the organ health biomarkers. With this respect, the novel test formulation was designed on the basis of best scientific literature, which is the combination of herbal products viz. *Panax ginseng* extract and beta carotene, minerals viz. calcium chloride, magnesium gluconate, zinc chloride, sodium selenate, ferrous sulfate, and vitamins viz. vitamin B12, vitamin D<sub>3</sub>, ascorbic acid and vitamin B6. This formulation is designed for overall functioning of the organs that can results in improved overall health conditions against many pathological conditions such as lung disorder, liver

disorder, breast cancer, liver cancer, aging, muscle damage, and overall health. Minerals and vitamins present in the test formulation provide significant functional support to all the vital organs [12-14]. In addition, *Panax ginseng* is one of the best reported medicinal plants that improve mental, physical abilities, cognitive health and is potent immuno modulator [15,16].

Various study data suggested the effect of Energy Therapy in cancer patients through therapeutic touch [17]; massage therapy [18], etc. Complementary and Alternative Medicine (CAM) therapies are preferred model of treatment, among which Biofield Therapy (or Healing Modalities) is one approach to enhance emotional, mental, physical and human wellness. The National Center of Complementary and Integrative Health (NCCIH) has recognized and allowed Biofield Energy Healing as a CAM approach in addition to other therapies and medicines such as natural products, chiropractic/osteopathic manipulation, Qi Gong, deep breathing, Tai Chi, yoga, meditation, massage, special diets, healing touch, relaxation techniques, traditional Chinese herbs and medicines, naturopathy, movement therapy, homeopathy, progressive relaxation, guided imagery, pilates, acupuncture, acupressure, Reiki, rolfing structural integration, hypnotherapy, Ayurvedic medicine, mindfulness, essential oils, aromatherapy, and cranial sacral therapy. The Human Biofield Energy has subtle energy that has the capacity to work in an effective manner [19]. CAM therapies have been practiced worldwide with reported clinical benefits in different health disease profiles [20]. This energy can be harnessed and transmitted by the practitioners into living and non-living things via the process of Biofield Energy Healing. The Biofield Energy Treatment, the Trivedi Effect<sup>®</sup>, has been reported to have a significant impact in the field of cancer research [21,22], materials science [23,24], microbiology [25,26], agriculture [27,28], nutraceuticals [29,30] and biotechnology [31,32]. Further, the Trivedi Effect<sup>®</sup> also significantly improved bioavailability of various low bioavailable compounds [33-35], an improved overall skin health [36,37], bone health [38-40], human health and wellness. Based on the excellent outcomes of the Biofield Energy Therapy in wide spectrum of areas, the authors intend to see the impact of the Biofield Energy Healing Treated test formulation on the function of vital organs such as bones, heart, liver, lungs and brain specific biomarkers in different cell-lines.

## MATERIALS AND METHODS

### Chemicals and reagents

Ferrous sulfate, vitamin B6, vitamin D<sub>3</sub>, vitamin B12, calcium chloride, naringenin, trimetazidine (TMZ), 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) and ethylenediaminetetraacetic acid (EDTA) were obtained from Sigma Chemical Co. (St. Louis, MO). Zinc chloride, magnesium gluconate,  $\beta$ -carotene and calcitriol were purchased from TCI chemicals, Japan. *Panax ginseng* extract obtained from Panacea Phytoextracts, India. Sodium selenate and ascorbic acid were obtained from Alfa Aesar, India. Silymarin and curcumin were obtained from Sanat Chemicals, India and quercetin obtained from Clearsynth, India. Reverse Transcription Kit, RNeasy Mini Kit and Syber Green PCR kits were procured from Quagen, India. All the other chemicals used in this experiment were analytical grade procured from India.

### Biofield energy healing strategy

The test formulation was the combination of eleven ingredients viz. calcium chloride, *Panax ginseng* extract, vitamin B12,  $\beta$ -carotene, vitamin D<sub>3</sub>, zinc chloride, magnesium gluconate, sodium selenate, ferrous sulfate, ascorbic acid and vitamin B6. The test formulation and the cell media was divided into two parts; one untreated (UT) and other part received the Biofield Energy Treatment remotely by a renowned Biofield Energy Healer, Shirley Theresa Holmlund, under laboratory conditions for ~3 min through healer's unique Biofield Energy Transmission process and were labeled as the Biofield Energy Treated (BT) test formulation/media. Further, the untreated group was treated with a "sham" healer for comparison purposes. The "sham" healer did not have any knowledge about the

Biofield Energy Healing Treatment. The Biofield Energy Healer was located in the Canada; however the test items were located in the research laboratory of Dabur Research Foundation, New Delhi, India. Biofield Energy Healer did not visit the laboratory, nor had any contact with the test samples. After that, the Biofield Energy Treated and untreated test items were kept in similar sealed conditions and used for the study as per the study plan.

### Assessment of cell viability using MTT assay

Cells were counted using hemocytometer and plated in 96-well plates at the specific density described in **Table 1**. The cells were then incubated overnight under growth conditions to allow cell recovery and exponential growth. Following overnight incubation, cells were treated with different concentrations of test formulations (BT/UT). Following respective treatments, cells were incubated in a CO<sub>2</sub> incubator at 37°C, 5% CO<sub>2</sub> and 95% humidity and incubated for time period mentioned in **Table 1**. After incubation, the plates were taken out and 20  $\mu$ L of 5 mg/mL of MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide solution was added to all the wells followed by additional incubation for 3 h at 37°C. The supernatant was aspirated and 150  $\mu$ L 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 of TI was calculated using Equation 1:

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

Where, X=Absorbance of treated cells; R=Absorbance of untreated cells

The concentrations exhibiting percentage cytotoxicity <30% was considered as non-cytotoxic [41].

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

| 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 h       |
| 4      | HepG2 (Liver)                     | $1 \times 10^4$ cells/well, 96-well plate  | 24 h       |
| 5      | Human Cardiac fibroblasts (Heart) | $1 \times 10^4$ cells/well, 96-well plate  | 24 h       |
| 6      | SH-SY5Y (Neuronal cell)           | $10 \times 10^4$ cells/well, 96-well plate | 24 h       |

### Evaluation of the cytoprotective effect of the formulation

Cells (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. The cells were then treated with the test items/positive control at the non-cytotoxic concentrations for 24 h. After 24 h, oxidative

stress was given to the cells using 10 mM *t*-BHP for 3.5 h. The untreated cells served as a control that did not receive any treatment and was maintained in cell growth medium only. Cells treated with 10 mM of *t*-BHP alone served as negative control. After 3.5 h 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:

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

#### Assessment of alkaline phosphatase (ALP) activity

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 h in CO<sub>2</sub> incubator at 37°C, 5% CO<sub>2</sub> and 95% humidity. After 48 h of incubation, the plates were taken out and processed for the measurement of ALP enzyme activity. The cells were washed with 1x PBS and lysed by freeze-thaw method, i.e., incubation at -80°C for 20 min followed by incubation at 37°C for 10 min. To the lysed cells, 50 µL of substrate solution, i.e., 5 mM of *p*-nitrophenyl phosphate (*p*NPP) in 1 M diethanolamine and 0.24 mM magnesium chloride (MgCl<sub>2</sub>) solution (pH 10.4) was added to all the wells followed by incubation for 1 h 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:

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

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)

The human cardiac fibroblasts (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 medium followed by overnight incubation. The cells were then treated with the test formulation/positive control at the non-cytotoxic concentrations for 24 h. After 24 h, oxidative stress was given to the cells using 10 mM *t*-BHP for 3.5 h. The untreated cells were served as control that 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 h 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:

$$\% \text{ Increase} = \frac{[\text{LDH activity}_{\text{sample}} - \text{LDH activity}_{t\text{-BHP}}]}{[\text{LDH activity}_{\text{untreated}} - \text{LDH activity}_{t\text{-BHP}}]} \times 100 \quad (4)$$

#### 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 h. After 24 h, oxidative stress was given to the cells using 400 µM *t*-BHP for 3.5 h. 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 h 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:

$$\% \text{ Increase} = \frac{[\text{ALT activity}_{\text{sample}} - \text{ALT activity}_{t\text{-BHP}}]}{[\text{ALT activity}_{\text{untreated}} - \text{ALT activity}_{t\text{-BHP}}]} \times 100 \quad (5)$$

#### 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 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 h 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:

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

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 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 items/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 h. Serotonin release was determined by ELISA as per manufacturer's protocol. The percent increase in serotonin levels was calculated using Equation 7:

$$[(X-R)/R] \times 100 \quad (7)$$

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 human bone osteosarcoma (MG-63) cells were counted using the hemocytometer were plated at a density of  $2 \times 10^5$  cells/well in 6-well plates followed by overnight incubation. The cells were then sera starved for 24 h and treated with the test formulation/positive control at the non-cytotoxic concentrations. The untreated cells that served as control that did not receive any treatment and were maintained in cell growth medium only. The treated cells were incubated for 24 h and VDR expression was determined by Q-PCR 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'-CACGTCAGTACGCGGTACTT-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:

$$RQ = 2^{-N} \dots \dots \dots (8)$$

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

### STATISTICAL ANALYSIS

All the values were represented as Mean  $\pm$  SD (standard deviation) of three independent experiments. The statistical analysis was performed using SigmaPlot statistical software (v11.0). For two groups 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

Determination of non-cytotoxic concentration of the formulation and positive controls by MTT cell viability assay was used in terms of percent viable cells in six (6) 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 formulation and positive controls were safe and non-toxic at the tested concentrations in six different cell lines and selected for other parameters analysis.

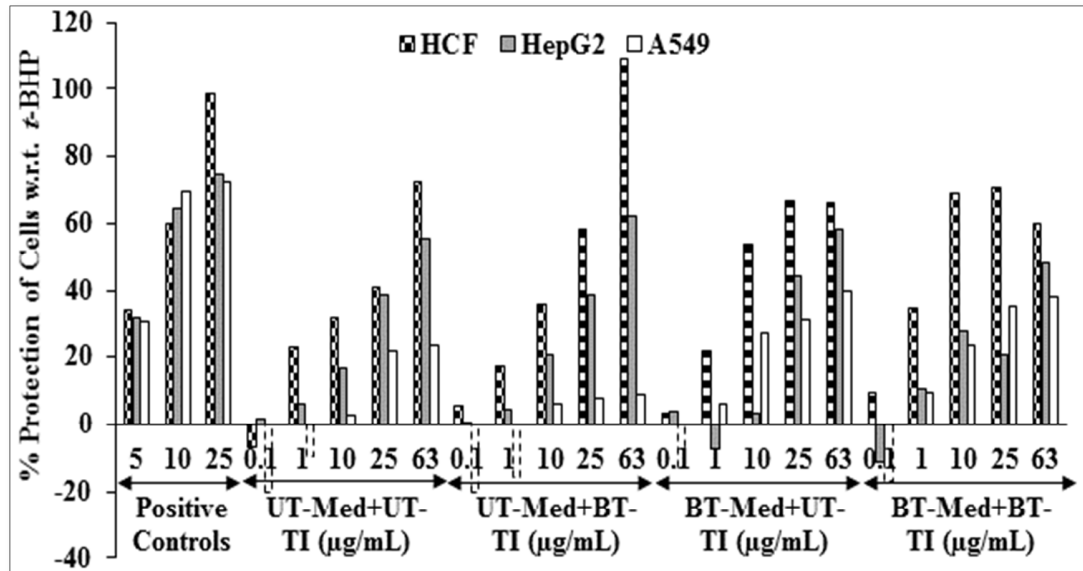
### Evaluation of cytoprotective effect of the test formulation

The cytoprotective activity of the novel proprietary test formulation on vital organs like liver, heart and lungs was examined in *in vitro* cell-based assay under the stimulation

of *tert*-butyl hydroperoxide (*t*-BHP) induced oxidative stress. *t*-BHP has been routinely used for the induction of oxidative stress in various cells [42]. The cytoprotective activity of the test formulation on the restoration of cell viability was determined against *t*-BHP induced cell damage and the result is shown in **Figure 1**. Trimetazidine (TMZ) was used as positive control in human cardiac fibroblasts cells (HCF) and showed, restoration of cell viability by 34.01%, 60.04% and 98.31% at 5, 10 and 25  $\mu$ g/mL, respectively compared to the *t*-BHP induced group. Besides, the test formulation showed 52.7% restoration of cell viability at 1  $\mu$ g/mL in the BT-Med + BT-TI group as compared to the UT-Med + UT-TI group. Moreover, at 10  $\mu$ g/mL the UT-Med + BT-TI, BT-Med + UT-TI and BT-Med + BT-TI groups showed 11.6%, 67.5% and 115.6% restoration of cell viability, respectively than UT-Med + UT-TI group. Additionally, the test formulation showed 41.9%, 62% and 71.8% restoration of cell viability at 25  $\mu$ g/mL in the UT-Med + BT-TI, BT-Med + UT-TI and BT-Med + BT-TI groups, respectively as compared to the UT-Med + UT-TI group. Further, at 63  $\mu$ g/mL the test formulation showed 51.2% restoration of cell viability in the UT-Med + BT-TI group than UT-Med + UT-TI group (**Figure 1**). Silymarin was used as positive control in human hepatoma cells (HepG2) resulted, restoration of cell viability by 31.63%, 64.63% and 74.64% at 5, 10 and 25  $\mu$ g/mL, respectively compared to the *t*-BHP induced group. The test formulation showed 113.5% restoration of cell viability at 0.1  $\mu$ g/mL in the BT-Med + UT-TI group as compared to the UT-Med + UT-TI group. Moreover, at 1  $\mu$ g/mL the BT-Med + UT-TI group showed 73.5% restoration of cell viability than UT-Med + UT-TI group. The test formulation showed 20.7% and 66.3% restoration of cell viability at 10  $\mu$ g/mL in the UT-Med + BT-TI and BT-Med + BT-TI groups, respectively as compared to the UT-Med + UT-TI group (**Figure 1**). Quercetin was used as positive control in adenocarcinomic human alveolar basal epithelial cells (A549) resulted, restoration of cell viability by 30.8%, 69.33% and 72.22% at 5, 10 and 25  $\mu$ g/mL, respectively compared to the *t*-BHP induced group. Besides, the test formulation showed 60% restoration of cell viability at 1  $\mu$ g/mL in the UT-Med + BT-TI group compared to the UT-Med + UT-TI group. Moreover, at 10  $\mu$ g/mL the UT-Med + BT-TI, BT-Med + UT-TI and BT-Med + BT-TI groups showed 101.1%, 829.8% and 698.9% restoration of cell viability, respectively than UT-Med + UT-TI group. Additionally, the test formulation showed 46.2% and 63.3% restoration of cell viability at 25  $\mu$ g/mL in the BT-Med + UT-TI and BT-Med + BT-TI groups, respectively as compared to the UT-Med + UT-TI group. Further, the test formulation showed 67.6% and 62.8% restoration of cell viability at 63  $\mu$ g/mL in the BT-Med + UT-TI and BT-Med + BT-TI groups, respectively compared to the UT-Med + UT-TI group (**Figure 1**). Oxidative stress is linked with a wide variety of inflammatory and metabolic disease conditions. Besides, cumulative damage of cells by free radicals inadequately

neutralized by antioxidants [43]. The study results suggest that Biofield Treatment has significantly protects *t*-BHP induced cardiotoxicity, hepatotoxicity and lung cell toxicity which could be due to The Trivedi Effect®-Biofield Energy

Healing as free radical scavenging activity. Therefore, Biofield Energy Healing Treatment could be used for the management of cardiovascular, liver and various lung disorders.



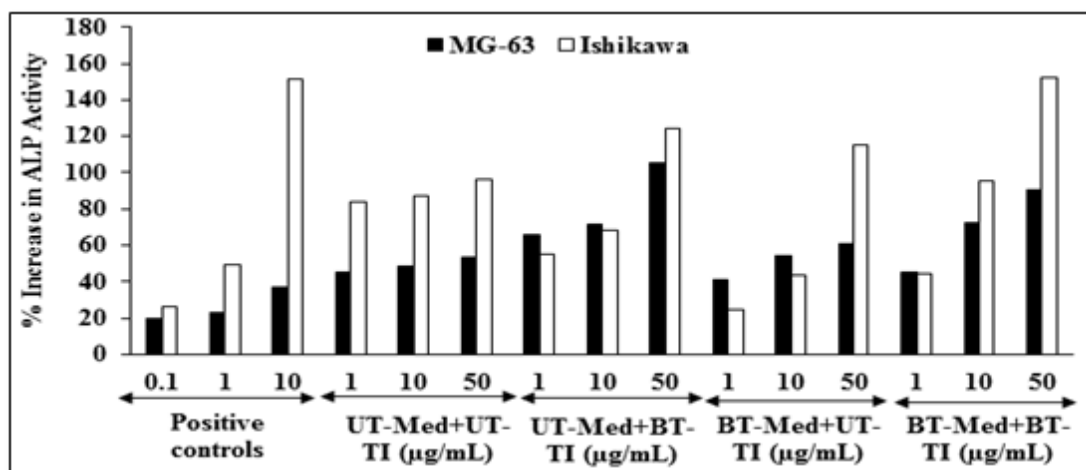
**Figure 1.** Assessment of cytoprotective effect of the test formulation in human cardiac fibroblasts cells (HCF), human hepatoma cells (HepG2) and adenocarcinomic human alveolar basal epithelial cells (A549) against *tert*-butyl hydroperoxide (*t*-BHP) induced damage. TMZ: Trimetazidine (μM), silymarin (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

#### Assessment of alkaline phosphatase (ALP) activity

The effect of the test formulation on bone-specific alkaline phosphatase level is shown in **Figure 2**. The positive control, calcitriol showed 20.03%, 22.71% and 36.75% increase the level of ALP at 0.1, 1 and 10 nM, respectively in MG-63 cells. The UT-Med + BT-TI group showed 44.9% increase the level of ALP in with respect to the UT-Med + UT-TI group at 1 μg/mL. At 10 μg/mL, the percent ALP was significantly increased by 47.8%, 12.4% and 48.1% in the UT-Med + BT-TI, BT-Med + UT-TI and BT-Med + BT-TI groups, respectively compared to the UT-Med + UT-TI group. Further, the percent ALP was significantly increased by 97.9%, 13.7% and 69.7% in the UT-Med + BT-TI, BT-Med + UT-TI and BT-Med + BT-TI groups, respectively at 50 μg/mL compared to the UT-Med + UT-TI group (**Figure**

**2**). Besides, the positive control, naringenin showed 25.93%, 49.23% and 151.85% increase the level of ALP at 0.1, 1 and 10 nM, respectively in Ishikawa cells. ALP percent was significantly increased by 29%, 19% and 58.2% in the UT-Med + BT-TI, BT-Med + UT-TI, BT-Med + BT-TI groups, respectively compared to the UT-Med + UT-TI group at 50 μg/mL (**Figure 2**). Numerous experimental data reported that lower level of serum alkaline phosphatase (ALP) can improve the bone mineral density (BMD) [43]. Thus, for the detection of bone specific biochemical marker in serum can be clinically useful in evaluating the progress of the bone healing process [44]. In this experiment, the level of ALP was revealed that the Biofield Energy Healing Treated test formulation significantly increased the level of ALP expression, which might be very helpful to the patients suffering from various bone-related disorders.



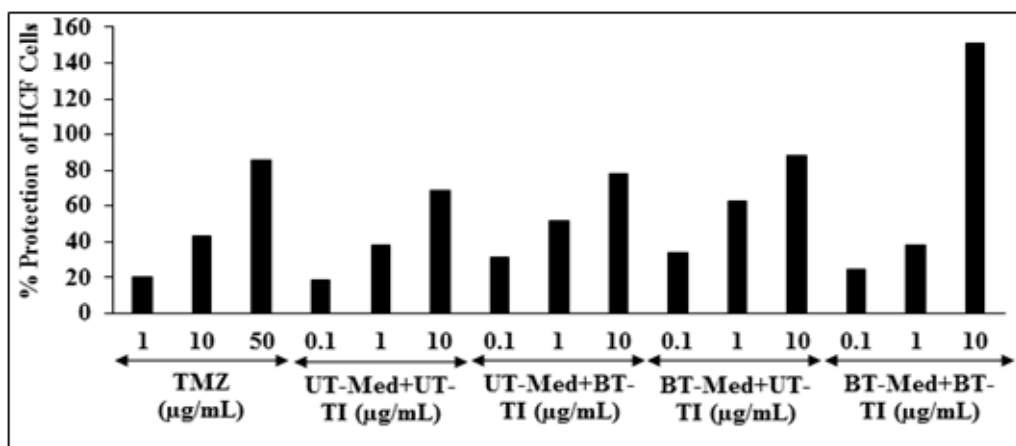
**Figure 2.** The effect of the test formulation on alkaline phosphatase (ALP) in A. Human bone osteosarcoma cells (MG-63) and B. Human endometrial adenocarcinoma cells (Ishikawa). Calcitriol and naringenin were used as positive control in Mg-63 and Ishikawa cells, respectively.

UT: Untreated; Med: Medium; BT: Biofield Treated; TI: Test Item

#### Estimation of lactate dehydrogenase (LDH) activity in human cardiac fibroblasts (HCF)

The effect of the test items on the percent protection of HCF cells in terms of decreased level of lactate dehydrogenase (LDH) activity is shown in **Figure 3**. The positive control, trimetazidine (TMZ) exhibited 20.53%, 43.08% and 85.86% protection of HCF cells (decreased of LDH activity) compared to the *t*-BHP group. The percent protection of HCF cells (decreased of LDH activity) was significantly increased by 67.4%, 80.4% and 28.9% in the UT-Med + BT-TI, BT-Med + UT-TI and BT-Med + BT-TI groups, respectively at 0.1 μg/mL as compared to the UT-Med + UT-TI group. Moreover, at 1 μg/mL, the percent protection of HCF cells (decreased of LDH activity) was significantly increased by 36.4% and 65.6% in the UT-Med + BT-TI and BT-Med + UT-TI groups, respectively as compared to the

UT-Med + UT-TI group. Further, percent protection of HCF cells (decreased of LDH activity) was also significantly increased by 13.8%, 28.2%, and 119.8% in the UT-Med + BT-TI, BT-Med + UT-TI and BT-Med + BT-TI groups, respectively at 10 μg/mL as compared to the UT-Med + UT-TI group (**Figure 3**). The lactate dehydrogenase (LDH) isoenzymes in serum was used for the late diagnosis of myocardial infarction [45], prognosis and management of certain tumors [46]. The study results found that there was a significant reduction of LDH level after Biofield Energy Treatment and protect heart cells, which might be helpful to resist against various pathological conditions like tissue injury, necrosis, hemolysis or malignancies, hypoxia, etc. It also indicating that the heart cells acted normally under stress and anaerobic condition and improved overall heart function.



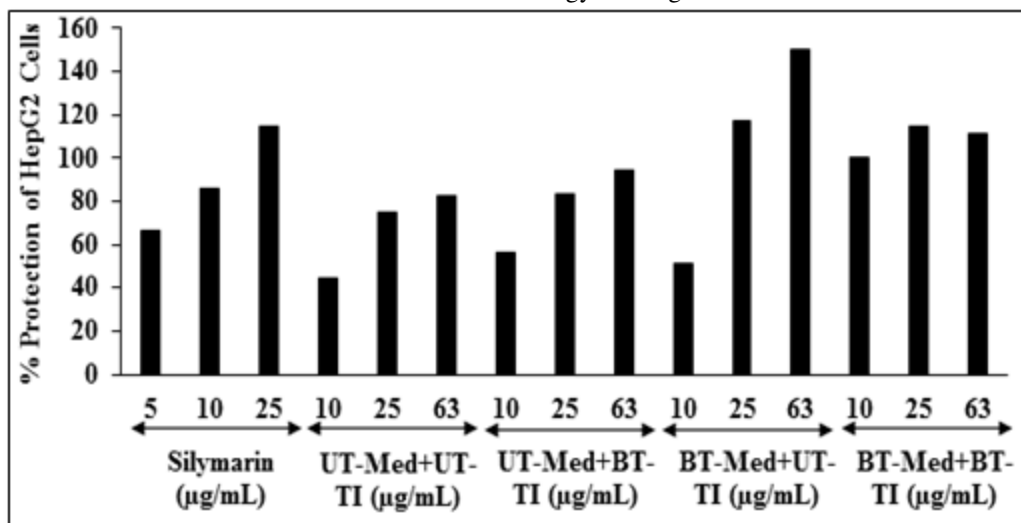
**Figure 3.** The effect of the test formulation on the percent protection of HCF cells in terms of decreased lactate dehydrogenase (LDH) activity against *tert*-butyl hydroperoxide (*t*-BHP) induced damage.

TMZ: Trimetazidine; UT: Untreated; Med: Medium; BT: Biofield Treated; TI: Test Item

### Estimation of alanine amino transferase (ALT) activity in HepG2 cells

The effect of the test formulation on protection of HepG2 cells in terms of decrease alanine amino transferase (ALT) activity is shown in **Figure 4**. The positive control, silymarin exhibited 66.35%, 85.83% and 114.38% protection of HepG2 cells (decreased of ALT activity) at 5, 10 and 25  $\mu\text{g/mL}$ , respectively as compared to the *tert*-butyl hydroperoxide (*t*-BHP). The protection of HepG2 cells (decreased of ALT activity) was significantly increased by 26.6%, 15% and 123.9% at 10  $\mu\text{g/mL}$  in the UT-Med + BT-TI, BT-Med + UT-TI and BT-Med + BT-TI groups, respectively as compared to the UT-Med + UT-TI group. Moreover, at 25  $\mu\text{g/mL}$  percent protection of HepG2 cells (decreased of ALT activity) was significantly increased by 11.3%, 57.6% and 54% in the UT-Med + BT-TI, BT-Med +

UT-TI and BT-Med + BT-TI groups, respectively as compared to the UT-Med + UT-TI group. Further, protection of HepG2 cells (decreased of ALT activity) was also significantly increased by 15.3%, 82.5% and 35% in the UT-Med + BT-TI, BT-Med + UT-TI and BT-Med + BT-TI groups, respectively at 63  $\mu\text{g/mL}$  as compared to the UT-Med + UT-TI group (**Figure 4**). Abnormal levels of liver enzyme like ALT cause liver damage or change in bile flow capacity by either accompanying biochemical picture in a patient with symptoms or signs [47]. This enzyme can catalyze the reversible transformation of  $\alpha$ -ketoacids into amino acids and play as a predictor of mortality independent of liver disease [48]. Here, the Biofield Energy Treatment significantly protect liver hepatocytes in terms of reducing the level of transaminases enzyme, ALT compared to the *t*-BHP inducing group, which might be due to Consciousness Energy Healing Treatment to the test formulation.



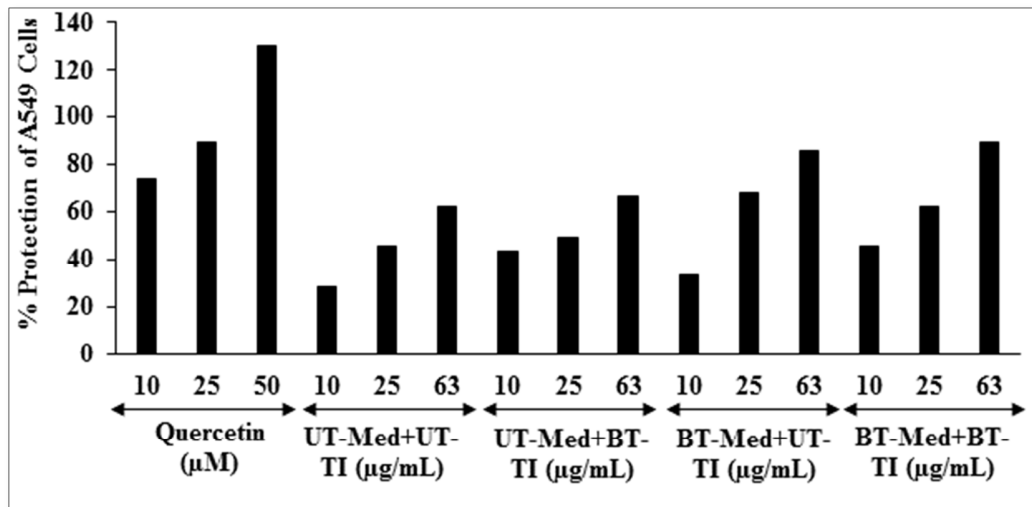
**Figure 4.** Effect of the test formulation on the percent protection of human liver cancer (HepG2) cells in terms of decreased alanine amino transaminase (ALT) activity under the stimulation of *tert*-butyl hydroperoxide (*t*-BHP).

UT: Untreated; Med: Medium; BT: Biofield Treated; TI: Test Item

### Estimation of superoxide dismutase (SOD) activity in adenocarcinomic human alveolar basal epithelial cells (A549)

The effect of the test formulation on the protection of lungs cells (A549) in terms of increased super oxide dismutase (SOD) activity is shown in **Figure 5**. The positive control, showed 74.04%, 89.75% and 129.89% protection of A549 (lungs) cells (increased of SOD activity) at 10, 25 and 63  $\mu\text{M}$ , respectively compared to the *t*-BHP group. The percent protection of A549 (lungs) cells (increased of SOD activity) was significantly increased by 53.6%, 17.4% and 59% at 10  $\mu\text{g/mL}$  in the UT-Med + BT-TI, BT-Med + UT-TI and BT-Med + BT-TI groups, respectively compared to the UT-Med + UT-TI group. Moreover, at 63  $\mu\text{g/mL}$ , the percent protection of A549 (lungs) cells (increased of SOD activity) was significantly increased by 6.7%, 38.1% and 44.1% in

the UT-Med + BT-TI, BT-Med + UT-TI and BT-Med + BT-TI groups, respectively as compared to the UT-Med + UT-TI group (**Figure 5**). Cells contain a large number of antioxidants like superoxide dismutase (SOD), catalase, glutathione peroxidase etc. to prevent or repair the damage of cells caused by ROS, as well as to regulate redox-sensitive signaling pathways [49]. Another, researcher reported that pulmonary superoxide dismutase (SOD) activity become more active in aged peoples and plays an important role against various respiratory disorders such as asthma, chronic obstructive pulmonary disease (COPD), lung malignancies, etc. [50]. Data found a significant increased SOD level after Biofield Energy Treatment in A549 cells, which might be helpful to resist against various pathological conditions like oxidative stress and related adverse effect. It also indicating that the lung cells acted normally and improved overall respiratory activities.



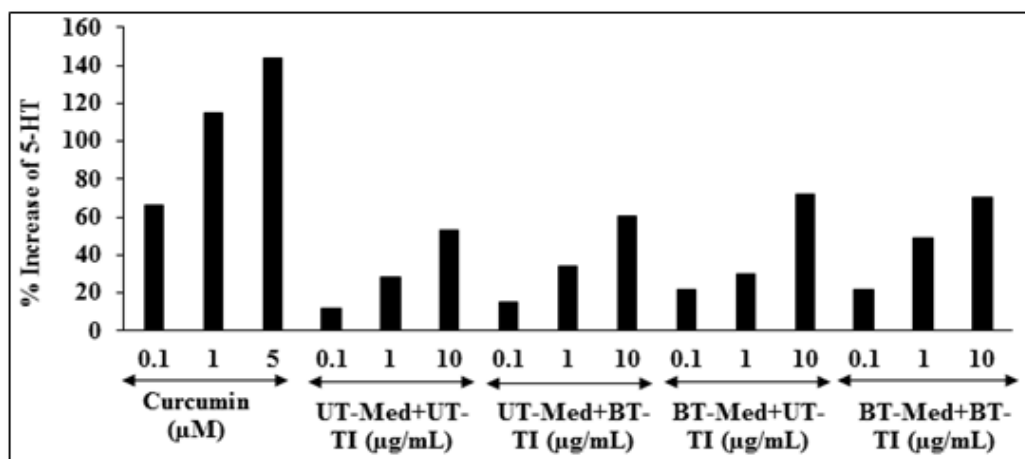
**Figure 5.** Effect of the test formulation on the percent protection of lungs cells (A549) in terms of increased SOD activity under the stimulation of *tert*-butyl hydroperoxide (*t*-BHP).

UT: Untreated; Med: Medium; BT: Biofield Treated; TI: Test Item

#### Effect of test formulation on serotonin in human neuroblastoma (SH-SY5Y) cells

The effect of test formulation on serotonin level is shown in **Figure 6**. The positive control showed 66.33%, 115.13% and 143.41% increase the level of serotonin. The level of serotonin was significantly increased by 33.3%, 85.3% and 85.3% in the UT-Med + BT-TI, BT-Med + UT-TI, BT-Med + BT-TI groups, respectively at 0.1 μg/mL compared to the UT-Med + UT-TI group. Moreover, at 1 μg/mL, 5-HT level was significantly increased by 21.9%, 7.7% and 74.2% in the UT-Med + BT-TI, BT-Med + UT-TI and BT-Med + BT-TI groups, respectively as compared to the UT-Med + UT-TI group. Further, the serotonin level was significantly increased by 13.8%, 35.8% and 33.6% in the UT-Med + BT-

TI, BT-Med + UT-TI and BT-Med + BT-TI groups, respectively at 10 μg/mL as compared to the UT-Med + UT-TI group (**Figure 6**). Serotonin (5-HT) is a neurotransmitter responsible for stress, anxiety, aggressive behavior, and many more [51]. Recent studies reported that brain endothelium is the specific target for serotonin and actively involved in the regulation of the blood-brain barrier (BBB) permeability and the cerebral blood flow *via* receptor-mediated mechanisms [52]. Thus, this experimental data suggested that Biofield Energy Healing Treated novel formulation significantly improved the serotonin level, which would be highly useful against various neurodegenerative diseases and other age-related disorders and improved the normal functioning of the brain tissues.



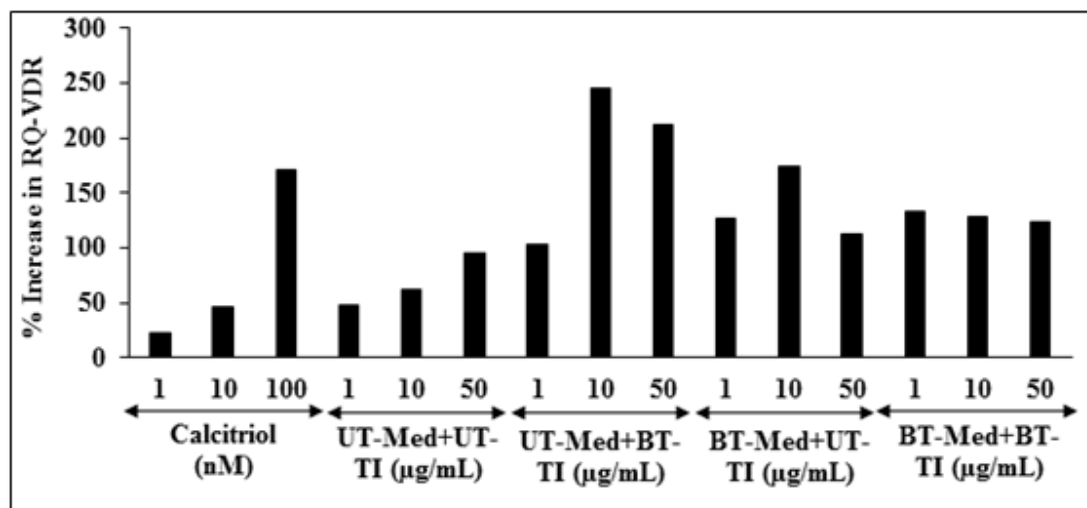
**Figure 6.** Effect of the test formulation on percent increase in 5-hydroxy tryptamine (5-HT) or serotonin in human neuroblastoma cells (SH-SY5Y).

UT: Untreated; Med: Medium; BT: Biofield Treated; TI: Test Item

#### Effect of test formulation on vitamin D receptors (VDRs)

Human bone osteosarcoma cells (MG-63) were treated with the test formulation and the effect on vitamin D receptor (VDR) expression was determined using quantitative-polymerase chain reaction (Q-PCR) amplification. VDR-relative threshold cycle (VDR-CT) values were obtained from PCR amplification. Relative quantification (RQ) of VDR was calculated from the VDR-CT and house-keeping (HK)-CT values for MG-63 cells treated with test formulation and positive control is shown in **Figure 7**. The positive control (calcitriol) showed 22.26%, 46.41% and 171.32% increase of RQ of VDR at 1, 10 and 100 nM, respectively. Moreover, RQ-VDR expression was significantly increased by 103.5%, 126.9% and 133% in the UT-Med + BT-TI, BT-Med + UT-TI and BT-Med + BT-TI groups, respectively at 1 µg/mL compared to the UT-Med + UT-TI group. Additionally, at 10 µg/mL the VDR level was significantly increased by 245.9%, 174.3% and 128.7% in the UT-Med + BT-TI, BT-Med + UT-TI, and BT-Med +

BT-TI groups, respectively compared to the UT-Med + UT-TI group. Further, VDR level was also significantly increased by 211.5%, 112.7% and 123.9% in the UT-Med + BT-TI, BT-Med + UT-TI and BT-Med + BT-TI groups, respectively at 50 µg/mL compared to the UT-Med + UT-TI group. Vitamin D can enhance calcium absorption in the small intestine and stimulates production of active metabolites  $1, 25[\text{OH}]_2 \text{D}_3$  in the kidney [53]. The hormone then interacts with the vitamin D receptor (VDR) in intestinal cells and complexes with the retinoic acid x receptor (RXR) in the nucleus [54]. This complex binds to the vitamin-D-responsive element (VDRE) of the calcium channel which increases uptake of calcium into the cells and increases the absorption of calcium [55]. Overall, the Consciousness Energy Treated test formulation has excellently increased the expression of VDRs, which might be helpful to bind more active vitamin  $\text{D}_3$  metabolites and that ultimately can improve the more physiological functions of vitamin D and simultaneously improved bone cell growth and development.



**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

The study findings showed that the tested novel test formulation was safe and non-toxic based on the MTT cell viability assay in six tested cells. The treatment group like BT-Med + BT-TI showed 115.6% restoration of cell viability at 10 µg/mL in human cardiac fibroblasts cells (HCF) compared to the UT-Med + UT-TI group. Moreover, the BT-Med + UT-TI group showed 113.5% and 73.5% restoration of cell viability at 0.1 and 1 µg/mL, respectively in human hepatoma cells (HepG2) compared to the untreated group. Additionally, 101.1%, 829.8%, and 698.9% restoration of cell viability at 10 µg/mL in adenocarcinomic human alveolar basal epithelial cells (A549) compared to the

untreated group. Alkaline phosphatase (ALP) activity was significantly increased by 97.9% and 69.7% in the UT-Med + BT TI and BT-Med + BT TI groups, respectively at 50 µg/mL compared to the untreated in human bone osteosarcoma cells (MG-63). Moreover, ALP activity was significantly increased by 58.2% in the BT-Med + BT-TI group at 1 µg/mL than untreated group. The percent protection of HCF cells (decreased of LDH activity) was significantly increased by 80.4% (at 0.1 µg/mL) and 119.8% (at 10 µg/mL) in the BT-Med + UT-TI and BT-Med + BT-TI groups, respectively compared to the untreated group. The percent protection of HepG2 cells (decreased of ALT activity) was significantly increased by 82.5% (at 63 µg/mL)

and 123.9% (at 10 µg/mL) in the BT-Med + UT-TI and BT-Med + BT-TI groups, respectively compared to the untreated group. The percent protection of A549 (lungs) cells (increased of SOD activity) was significantly increased by 59% in the BT-Med + BT-TI group at 10 µg/mL compared to the untreated group. The serotonin level was significantly increased by 85.3% at 0.1 µg/mL in the UT-Med + BT-TI and BT-Med + BT-TI groups as compared to the untreated group in human neuroblastoma cells (SH-SY5Y). The relative quantification (RQ) of vitamin D receptors (VDRs) level was significantly increased by 245.9% and 211.5% at 10 and 50 µg/mL, respectively in the UT-Med + BT-TI group; while 174.3% (at 10 µg/mL) in the BT-Med + UT-TI group compared to the untreated group in MG-63 cells. In conclusion, The Biofield Energy Treatment significantly improved heart, liver, bones, neuronal and lungs functional enzymes biomarkers and also protected cardiomyocyte, hepatocyte, osteocytes, pneumocyte and nerve cells from oxidative damage induced by *tert*-butyl hydroperoxide (*t*-BHP). Thus, results suggested that Biofield Energy Treatment can be used as a complementary and alternative treatment for the prevention of various types of cardiac disorders (peripheral artery disease, high blood pressure, congenital heart disease, stroke, congestive heart failure, rheumatic heart disease, carditis, valvular heart disease, thromboembolic disease and venous thrombosis, etc.), hepatic disorders (cirrhosis, Wilson disease, liver cancer, hemochromatosis), and lungs disorders (Asthma, Emphysema, Chronic bronchitis, Pneumonia, Cystic fibrosis). Further, it can be useful to improve cell-to-cell messaging, normal cell growth and differentiation, cell cycling and proliferation, neurotransmission, skin health, hormonal balance, immune and cardiovascular functions. Moreover, it can also be utilized in organ transplants (*i.e.*, liver, kidney, and heart transplants), aging, hormonal imbalance and various inflammatory and immune-related disease conditions like Alzheimer's Disease (AD), Dermatitis, Asthma, Ulcerative Colitis (UC), Hashimoto Thyroiditis, Pernicious Anemia, Sjogren Syndrome, Aplastic Anemia, Multiple Sclerosis, Hepatitis, Graves' Disease, Irritable Bowel Syndrome (IBS), Dermatomyositis, Diabetes, Myasthenia Gravis, Atherosclerosis, Parkinson's Disease, Systemic, etc., to Lupus Erythematosus (SLE), stress, improve overall health and Quality of Life.

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#### CONFLICT OF INTEREST

Authors declare no conflict of interest.

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