

Protective Role of the Biofield Energy Treated Test Formulation on Vital Organs Function using Cell-Based Assays

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ABSTRACT

Dysfunction of vital organs is the main concern for human health. Therefore, it is necessary to homeostat the normal function of vital organs such as lungs, liver, brain and heart for better health. The aim of this study was to evaluate the effect of the Consciousness Energy Healing Treated test formulation on the function of vital organs in 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, Thomas Charles Slade, USA and were labeled as the Biofield Energy Treated (BT) test formulation/media. Cell viability data suggested that the tested formulation was safe and non-toxic in nature in six different cells. The experimental groups like the untreated medium (UT-Med) + Biofield Treated Test Item (BT-TI) and BT-Med + BT-TI groups showed 74.4% (at 10 µg/mL) and 73.7% (at 1 µg/mL) restoration of cell viability, respectively in human cardiac fibroblasts cells (HCF) compared to the UT-Med + UT-TI group. Moreover, the UT-Med + BT-TI and BT-Med + BT-TI groups showed 76.4% (at 10 µg/mL) and 87.5% (at 1 µg/mL) restoration of cell viability, respectively in human hepatoma cells (HepG2) compared to the untreated group. Furthermore, 209.5%, 757.8% and 836.2% 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 1 µg/mL compared to the untreated. Alkaline phosphatase (ALP) level was significantly increased by 71.7%, 71.9% and 56.7% in the UT-Med + BT-TI, BT-Med + UT-TI and BT-Med + BT-TI groups, respectively in human bone osteosarcoma cells (MG-63) at 50 µg/mL compared to the untreated. Additionally, the level of ALP was also significantly increased by 124.9% and 106.3% in the BT-Med + BT-TI group at 25 and 50 µg/mL, respectively 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 88.3% and 75.5% in the BT-Med + BT-TI group at 1 and 10 µg/mL, respectively; while 82.8% (at 0.1 µg/mL) in the UT-Med + BT-TI group as compared to the untreated in HCF cells. The percent protection of HepG2 (liver) cells (decreased of ALT activity) was significantly increased by 47.5%, 79.8% and 94% in the UT-Med + BT-TI, BT-Med + UT-TI and BT-Med + BT-TI groups, respectively at 1 µg/mL compared to the untreated in HepG2 cells. The percent protection of A549 (lungs) cells (increased of SOD activity) was significantly increased by 67.4%, 60.8% and 80.7% in the UT-Med + BT-TI, BT-Med + UT-TI and BT-Med + BT-TI groups, respectively at 10 µg/mL; while 137% (at 0.1 µg/mL) in the BT-Med + BT-TI group as compared to the untreated group. Serotonin level was significantly increased by 225.7% (at 1 µg/mL), 176.1% (at 25 µg/mL) and 175.7% (at 63 µg/mL) in the BT-Med + BT-TI group; while 317.9% (at 1 µg/mL) in the UT-Med + BT-TI group as compared to the untreated in human neuroblastoma cells (SH-SY5Y). The relative quantification (RQ) of vitamin D receptor (VDR) was significantly increased by 195.3% (at 1 µg/mL), 176.2% (at 10 µg/mL) and 194.7% (at 50 µg/mL) in the BT-Med + BT-TI group compared to the untreated group in MG-63 cells. Altogether data suggest that these results suggest that Biofield Energy Treated test formulation significantly protect the major organs viz. bones, heart, liver, lungs and brain and also improved their functions. Therefore, The Biofield Energy Treatment (The Trivedi Effect[®]) can be used as a complementary and alternative therapy against several disorders such as heart attack, heart failure, coronary artery disease, arrhythmias, congenital heart disease, cardiomyopathy,

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

Keywords: Cardiac health, Liver health, Brain health, Bone health, Lungs health, The Trivedi Effect[®], Consciousness energy healing, VDR receptor

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 D3, 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 immune 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[®], 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[®] 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

Zinc chloride, magnesium gluconate, β -carotene and calcitriol were purchased from TCI chemicals, Japan. Ferrous sulfate, vitamin B6, vitamin D3, 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). Silymarin and curcumin were obtained from Sanat Chemicals, India and quercetin obtained from Clearsynth, India. *Panax ginseng* extract obtained from panacea Phytoextracts, India. Sodium selenate and ascorbic acid were obtained from Alfa Aesar, 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, β -carotene, vitamin D3, 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, Thomas Charles Slade, USA, under laboratory conditions for ~3 min through healer's unique Biofield Energy Transmission process and was labeled as the Biofield Energy Treated (BT) test formulation/media. Further, the untreated group was treated with "sham" healer for comparison purpose. The "sham" healer did not have any knowledge about the

Biofield Energy Healing Treatment. Biofield Energy Healer was located in the USA; however the test items were located in the research laboratory of Dabur Research Foundation, New Delhi, India. Biofield Energy Healer in this experiment did not visit the laboratory, nor had any contact with the test samples. 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 and allow to 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 CO₂ incubator at 37°C, 5% CO₂ and 95% humidity and incubated for time period mentioned in **Table 1**. After incubation, the plates were taken out and 20 μ 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 μ 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:

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

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

The concentrations exhibiting percentage cytotoxicity <30% were 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)	3×10^4 cells/ well, 96-well plate	5 days
2	Ishikawa (Uterus)	3×10^4 cells/ well, 96-well plate	5 days
3	A549 (Lung)	10×10^4 cells/ well, 96-well plate	24 h
4	HepG2 (Liver)	1×10^4 cells/ well, 96-well plate	24 h
5	Human Cardiac fibroblasts (Heart)	1×10^4 cells/ well, 96-well plate	24 h
6	SH-SY5Y (Neuronal cell)	10×10^4 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:

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

Assessment of alkaline phosphatase (ALP) activity

The cells (human bone osteosarcoma cells-MG-63 and human endometrial adenocarcinoma cells-Ishikawa) were counted using an hemocytometer and plated in 24-well plates at the density corresponding to 1×10^4 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 CO₂ incubator at 37°C, 5% CO₂ 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 (MgCl₂) 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:

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

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 0.25×10^6 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.

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

Estimation of ALT in liver cells (HepG2)

The human hepatoma cells (HepG2) were counted and plated at the density of 5×10^4 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.

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

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 1×10^4 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:

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

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 10×10^4 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.

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

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 human bone osteosarcoma (MG-63) cells were counted using hemocytometer were plated at a density of 2×10^5 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 were 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'-CACGTCACCTGACGCGGTACTT-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:

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

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 Sigma Plot 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 test 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

Assessment of vital organs (heart, liver and lungs) function after administration of the formulation was examined in *in*

vitro cell-based assays under the influence of *tert*-butyl hydroperoxide (*t*-BHP) induced oxidative stress. *t*-BHP has been extensively used for the induction of oxidative stress in various cell-lines [41]. The cytoprotective activity of the Biofield Energy Treated 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 40.57%, 60.68% and 90.04% at 5, 10 and 25 μ g/mL, respectively compared to the *t*-BHP induced group. Besides, the test formulation showed 13.6% and 73.7% restoration of cell viability at 1 μ g/mL in the BT-Med + UT-TI and BT-Med + BT-TI groups, respectively as compared to the UT-Med + UT-TI group. Moreover, at 10 μ g/mL showed 74.4% and 28.8% restoration of cell viability in the UT-Med + BT-TI and BT-Med + BT-TI groups, respectively than UT-Med + UT-TI group. Further, at 25 μ g/mL showed 30.3% and 31% restoration of cell viability in the UT-Med + BT-TI and BT-Med + UT-TI groups, respectively 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 31.36%, 64.63% and 74.64% at 5, 10 and 25 μ g/mL, respectively compared to the *t*-BHP induced group. The test formulation showed 57.8% and 87.5% restoration of cell viability at 1 μ g/mL in the UT-Med + BT-TI and BT-Med + BT-TI groups, respectively as compared to the UT-Med + UT-TI group. Moreover, at 10 μ g/mL the UT-Med + BT-TI, BT-Med + UT-TI and BT-Med + BT-TI groups showed 76.4%, 41.1% and 49.6% restoration of cell viability, respectively than UT-Med + UT-TI group. Further, 38% and 12.3% cells were restored by UT-Med + BT-TI and BT-Med + BT-TI groups, respectively at 25 μ g/mL as compared to 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 30.80%, 69.33% and 72.22% at 5, 10 and 25 μ g/mL, respectively compared to the *t*-BHP induced group. Besides, the test formulation showed 53.4% restoration of cell viability at 0.1 μ g/mL in the BT-Med + BT-TI group compared to the UT-Med + UT-TI group. Moreover, at 1 μ g/mL the UT-Med + BT-TI, BT-Med + UT-TI and BT-Med + BT-TI groups showed 209.5%, 757.8% and 836.2% restoration of cell viability, respectively than UT-Med + UT-TI group. Additionally, at 10 μ g/mL the BT-Med + UT-TI and BT-Med + BT-TI groups showed 46.1%, 96% and 105.8% restoration of cell viability, respectively than UT-Med + UT-TI group. The test formulation showed 15.9%, 55.6% and 74.3% restoration of cell viability at 25 μ g/mL in the UT-Med + BT-TI, BT-Med + UT-TI, BT-Med + BT-TI groups, respectively compared to the UT-Med + UT-TI group. Further, the test formulation showed 58.4% and 35.5% restoration of cell viability at 63 μ g/mL in the BT-Med + UT-TI and BT-Med + BT-TI groups, respectively compared to the UWT-Med + UT-TI group (**Figure 1**). The 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®.

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

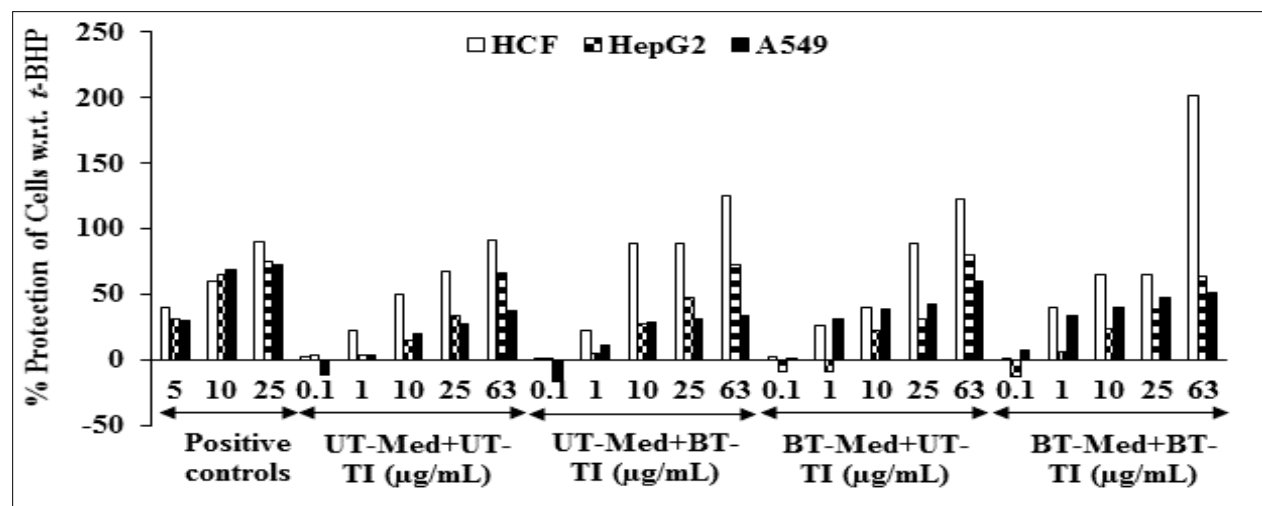


Figure 1. Assessment of cytoprotective effect of the test formulation in human cardiac fibroblasts cells (HCF), human hepatoma cells (HepG2) and adenocarcinomic human alveolar basal epithelial cells (A549) against *tert*-butyl hydroperoxide (*t*-BHP) induced damage. TMZ: Trimetazidine (µM), silymarin (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 experimental groups showed 34.8% increase the level of ALP in the BT-Med + BT-TI group with respect to the UT-Med + UT-TI group at 1 µg/mL. At 10 µg/mL, the percent ALP was significantly increased by 39.2% and 55.4% in the UT-Med + BT-TI and BT-Med + BT-TI groups, respectively compared to the UT-Med + UT-TI group. Moreover, the ALP level was significantly increased by 71.7%, 71.9% and 56.7% 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 at 50 µg/mL. Besides, the positive control naringenin showed 49.23%, 78.7% and 151.85% increase the level of ALP at 1, 5 and 10 nM, respectively in Ishikawa cells. ALP percent was significantly increased by 42.7% in the BT-Med + BT-

TI group compared to the UT-Med + UT-TI group at 10 µg/mL. Moreover, the experimental groups showed 62.1%, 32.3% and 124.9% increase the level of ALP in the UT-Med + BT-TI, BT-Med + UT-TI and BT-Med + BT-TI groups, respectively with respect to the UT-Med + UT-TI group at 25 µg/mL. At 50 µg/mL, the percent ALP was significantly increased by 42.6%, 54.3% and 106.3% 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 (**Figure 2**). For proper bone mineralization the ALP activity is essential and it is considered a useful biochemical marker for bone formation [42]. Thus, for the detection of bone specific biochemical marker in serum can be clinically useful in evaluating the progress of the bone healing process [43,44]. In this experiment, it was revealed that the Consciousness 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.

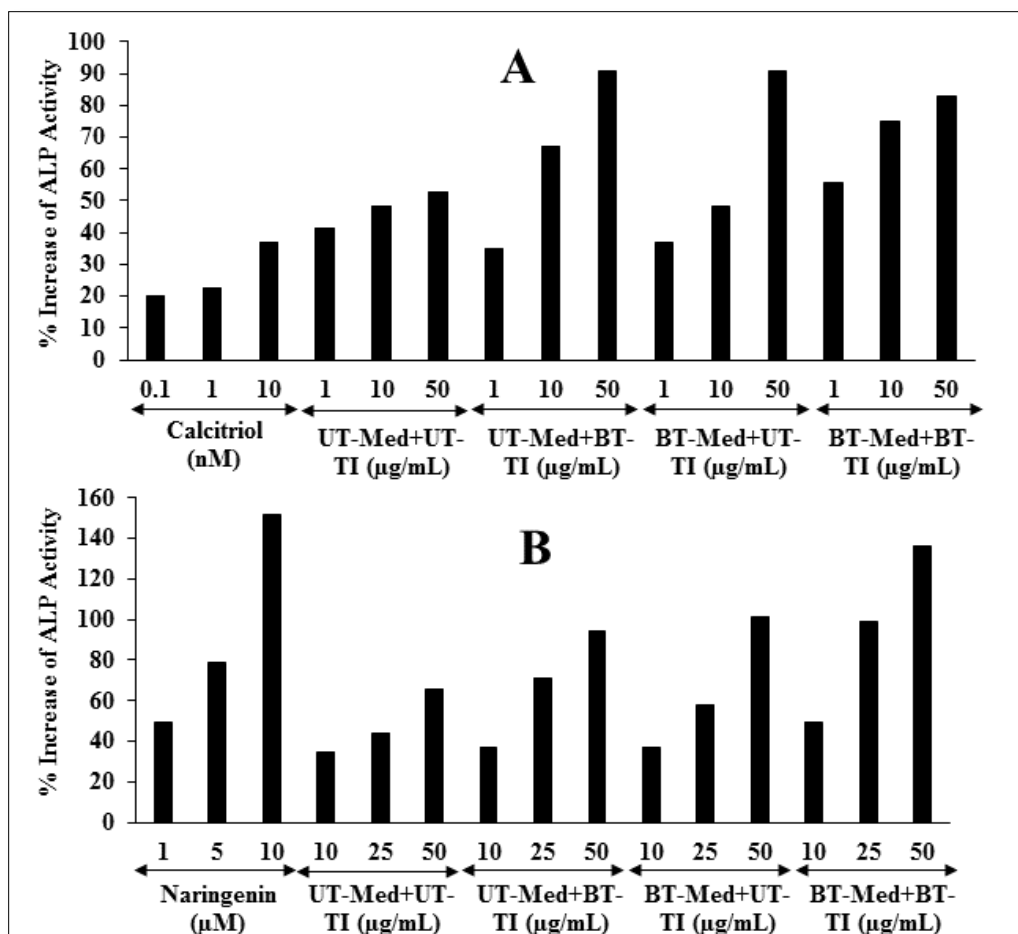


Figure 2. The effect of the test formulation on alkaline phosphatase (ALP) in A) Human bone osteosarcoma cells (MG-63) and B) Human endometrial adenocarcinoma cells (Ishikawa).

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

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

The lactate dehydrogenase (LDH) is normally present in the heart and skeletal muscle is responsible for anaerobic respiration of cells [45-47]. The effect of the test formulation on 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) 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 82.8% and 74.5% at 0.1 $\mu\text{g/mL}$ in the UT-Med + BT-TI and BT-Med + UT-TI groups, respectively as compared to the UT-Med + UT-TI group. Moreover, at 1 $\mu\text{g/mL}$, the percent protection

of HCF cells (decreased of LDH activity) was significantly increased by 56.1%, 30.2% and 88.3% 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, percent protection of HCF cells (decreased of LDH activity) was also significantly increased by 24.7%, 34% and 75.5% in the UT-Med + BT-TI, BT-Med + UT-TI and BT-Med + BT-TI groups, respectively at 10 $\mu\text{g/mL}$ as compared to the UT-Med + UT-TI group (**Figure 3**). Overall, data found that there was a significant reduction of LDH level after Biofield Energy Treatment and protect HCF cells, which might be helpful to resist against various pathological conditions like tissue injury, necrosis, hypoxia, hemolysis or malignancies. It also indicating that the heart cells acted normally under stress and anaerobic condition and improved overall heart function.

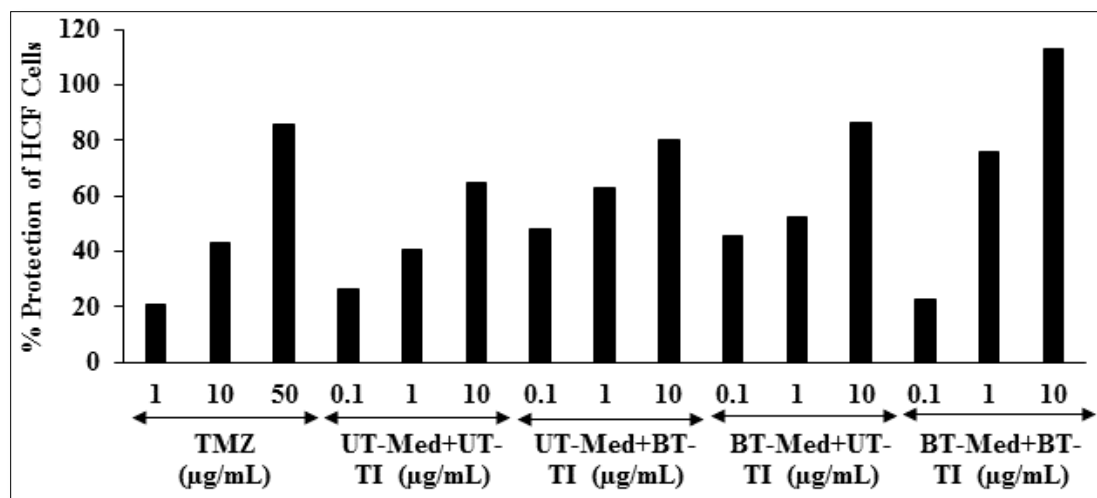


Figure 3. The effect of the test formulation on the percent protection of HCF cells in terms of decreased lactate dehydrogenase (LDH) activity against *tert*-butyl hydroperoxide (*t*-BHP) induced damage.

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 16.35%, 85.83% and 114.38% protection of HepG2 cells (decreased of ALT activity) at 1, 10 and 25 µL, respectively. The protection of HepG2 cells (decreased of ALT activity) was significantly increased by 47.5%, 79.8% and 94% at 1 µg/mL in the UT-Med + BT-TI, BT-Med + UT-TI and BT-Med + BT-TI groups, respectively as compared to the UT-Med + UT-TI group. Moreover, at 10 µg/mL, percent protection of HepG2 cells (decreased of

ALT activity) was increased by 35.3% and 25.5% in the 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 11%, 30.6% and 24.4% in the UT-Med + BT-TI, BT-Med + UT-TI and BT-Med + BT-TI groups, respectively at 25 µg/mL as compared to the UT-Med + UT-TI group (**Figure 4**). Increased level of ALT is directly proportional to the severity of the diseases like hepatocellular injury and death [48]. Thus, the elevation of ALT enzyme chances of liver disorders [49]. Here, the Biofield Energy Treatment significantly protects liver hepatocytes in terms of reducing the level of transaminases enzyme, ALT compared to the *t*-BHP inducing group.

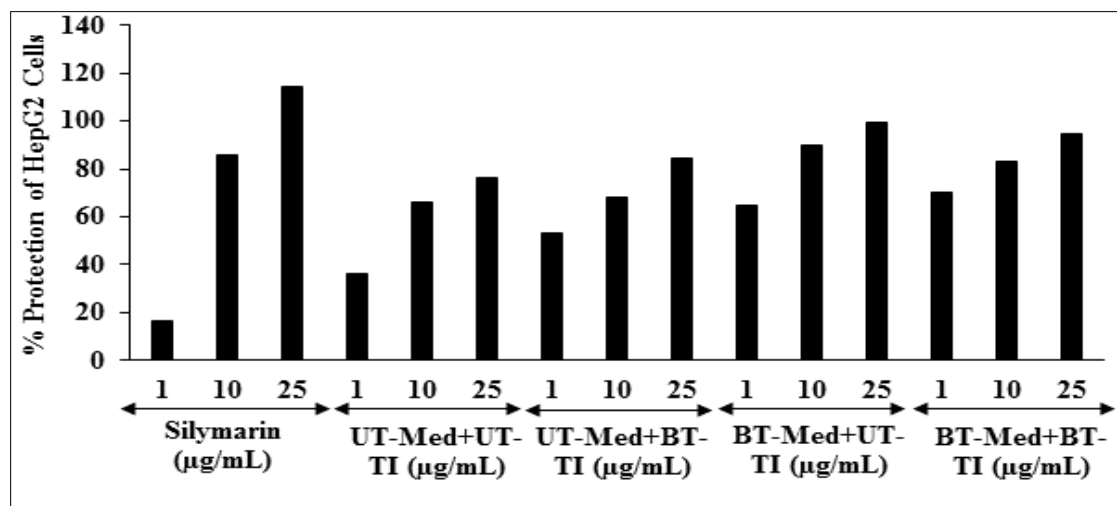


Figure 4. Effect of the test formulation on the percent protection of human liver cancer (HepG2) cells in terms of decreased alanine amino transaminase (ALT) activity under the stimulation of *tert*-butyl hydroperoxide (*t*-BHP).

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 (Quercetin), showed 28.12%, 52.82% and 740.04% protection of A549 (lungs) cells (increased of SOD activity) compared to the *t*-BHP group. The percent protection of A549 (lungs) cells (increased of SOD activity) was significantly increased by 35.4% and 137.6% at 0.1 $\mu\text{g/mL}$ in the BT-Med + UT-TI and BT-Med + BT-TI groups, respectively compared to the UT-Med + UT-TI group. Moreover, at 1 $\mu\text{g/mL}$, the percent protection of A549 (lungs) cells (increased of SOD activity) was significantly increased by 50.9% in the UT-Med + BT-TI as compared to the UT-Med + UT-TI group. Further, the percent protection of A549 (lungs) cells (increased of SOD activity) was significantly increased by 67.4%, 60.8% and 80.7% 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 (**Figure 5**). Respiratory disorders like asthma is a chronic inflammatory lung disease occurs due to an imbalance between the reducing and oxidizing systems with more oxidative state that causes less airflow, hyper reactivity and airway remodeling [50]. Both endogenous and exogenous reactive oxygen species (ROS), such as superoxide anion, hydroxyl radical and hydrogen peroxide and reactive nitrogen species, such as nitric oxide and peroxynitrite, play a major role in the airway inflammation. Superoxide dismutases (SODs) constitute a very important antioxidant defense against oxidative stress in the body. The enzyme acts as a good therapeutic agent against reactive oxygen species-mediated diseases [51]. Overall, data found that there was 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.

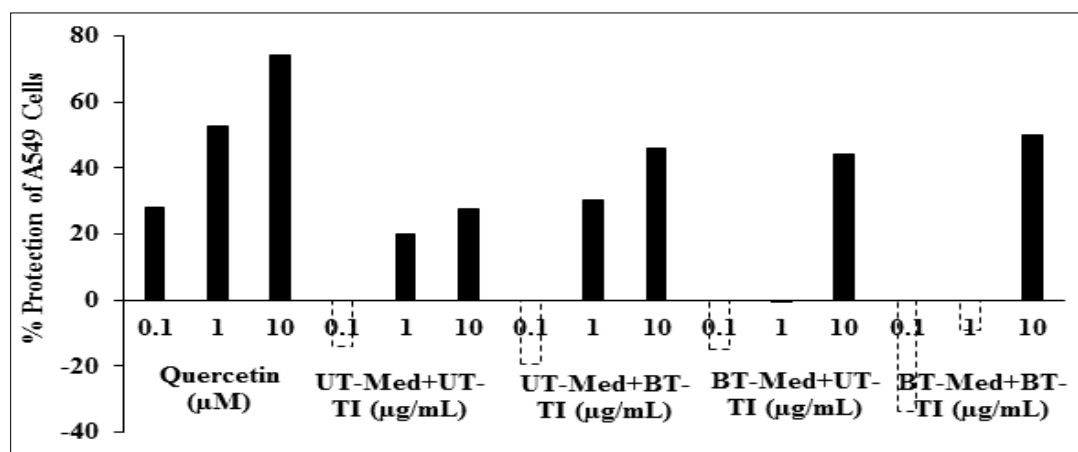


Figure 5. Effect of the test formulation on the percent protection of lungs cells (A549) in terms of increased SOD activity under the stimulation of *tert*-butyl hydroperoxide (*t*-BHP).

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 the test formulation on serotonin level in SH-SY5Y cells is shown in **Figure 6**. The positive control (curcumin), showed 112.82%, 127.18% and 160.21% increase the level of serotonin at 0.1, 1 and 5 μM , respectively as compared to the vehicle control (VC) group. The level of serotonin was significantly increased by 317.9%, 32% and 225.7% at 1 $\mu\text{g/mL}$ 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 25 $\mu\text{g/mL}$, 5-HT level was significantly increased by 116.3%, 28.6% and 176.1% 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. Further, the serotonin level was significantly increased by

113.3%, 130% and 175.7% 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 6**). Pain is very essential physiological phenomenon to avoid damage of vital organs for survival. The development of chronic pain is due to the maladaptive neuroplastic changes in the brain. 5-HT act as a modulator in the perception of experimental or chronic pain [52]. Serotonin is also involved in the regulation of emotion and behavior and the regulation of aggressive impulses [53]. Thus, the data suggested that Biofield Energy Healing Treated novel test 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.

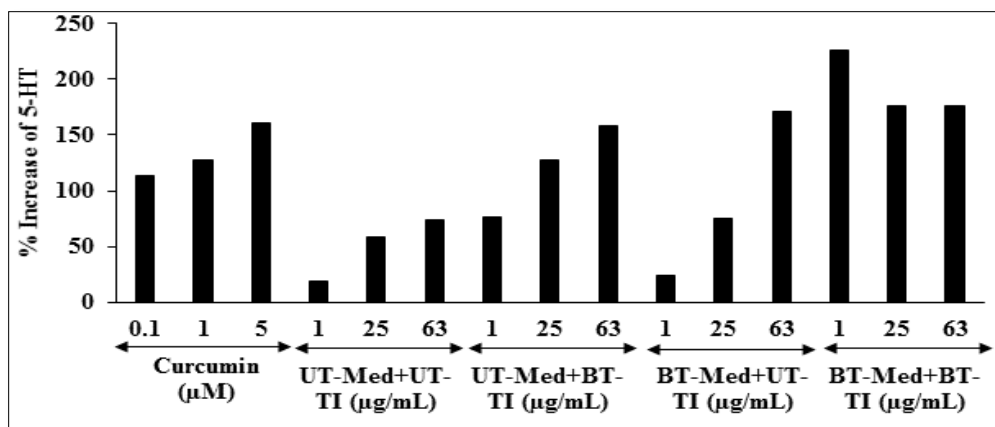


Figure 6. Effect of the test formulation on percent increase in 5-hydroxy tryptamine (5-HT) or serotonin in human neuroblastoma cells (SH-SY5Y).

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

Effect of test formulation on vitamin D receptors (VDRs)

Quantitative-polymerase chain reaction (Q-PCR) amplification was used for the determination of VDR expression after administration of the test formulation. VDR-relative threshold cycle (VDR-CT) values were obtained from Q-PCR amplification. Relative quantification (RQ) was calculated from the VDR-CT and house-keeping (HK)-CT values for MG-63 cells treated with the test formulation and positive control is represented in **Figure 7**. The RQ of VDR was significantly increased in a concentration-dependent manner by 22.26%, 46.41% and 171.32% in positive control group (calcitriol) at 1, 10 and 100 nM, respectively. The RQ of VDR was significantly increased by 195.3% and 134.8% in the 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 18%, 176.2% and 129% 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. VDR level was also significantly increased by 194.7% and 146.6% in the BT-Med + UT-TI and BT-Med + BT-TI groups, respectively at 50 μg/mL compared to the UT-Med + UT-TI group. The vitamin D receptor (VDR) can influence the pleiotropic activity of the active metabolite of vitamin D, i.e., 1, 25-dihydroxyvitamin D₃ (1, 25(OH)₂ D₃) such as orchestration of mineral homeostasis which is coordinated by the intestine, bone, kidney and parathyroid gland [54]. Expression of the VDR is regulated by external stimuli in a tissue-specific manner. Variety of studies have been reported the impact of VDR expression in T cells and found that VDR expression and activity plays an important role in both T cell development, differentiation and effector function [55]. Overall, the Biofield Energy Treated test formulation has significantly increased the expression of VDRs, which might be helpful to bind more active vitamin D₃ metabolites and that ultimately can improve the more physiological functions of vitamin D and simultaneously improved bone cell growth and development.

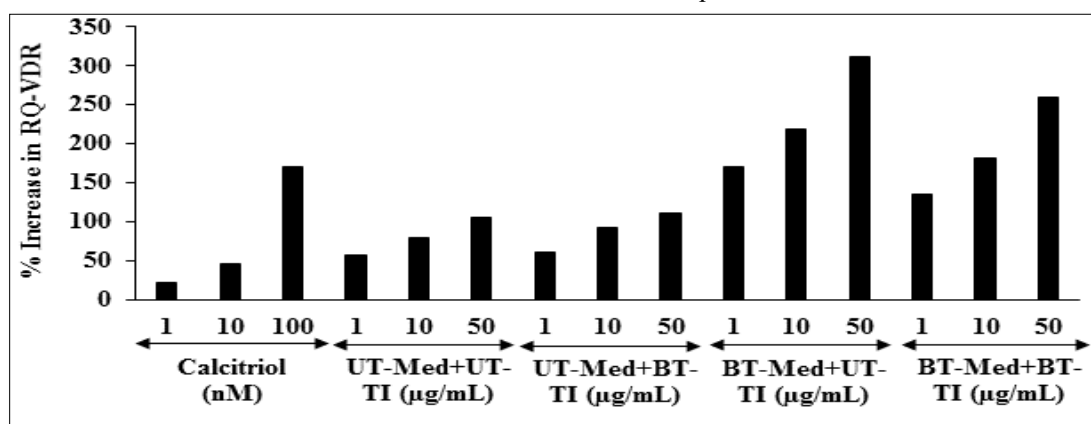


Figure 7. Effect of the test formulation on percent increase in relative quantification (RQ) of vitamin D receptors (VDRs) gene in human bone osteosarcoma cells (MG-63).

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

CONCLUSION

The study outcomes showed that the tested novel formulation was safe and non-toxic based on cell viability assay (MTT) in six different tested cells. The UT-Med + BT-TI group showed 74.4% 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 + BT-TI group showed and 87.5% (at 1 µg/mL) restoration of cell viability in human hepatoma cells (HepG2) compared to the untreated group. Besides, 209.5%, 757.8% and 836.2% 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 1 µg/mL as compared to the untreated group. Alkaline phosphatase (ALP) activity was significantly increased by 71.7% and 71.9% in the UT-Med + BT-TI and BT-Med + UT-TI groups, respectively at 50 µg/mL in human endometrial adenocarcinoma cells (Ishikawa). The percent protection of HCF cells (decreased of LDH activity) was significantly increased by 82.8% (at 0.1 µg/mL) and 88.3% (at 1 µg/mL) in the UT-Med + BT-TI and BT-Med + BT-TI groups, respectively as compared to the untreated group in HCF cells. The percent protection of HepG2 cells (decreased of ALT activity) was significantly increased by 79.8% and 94% in the BT-Med + UT-TI and BT-Med + BT-TI groups, respectively at 1 µg/mL compared to the untreated group in HepG2 cells. The percent protection of A549 (lungs) cells (increased of SOD activity) was significantly increased by 137% and 80.7% in the BT-Med + BT-TI group at 0.1 and 10 µg/mL, respectively compared to the untreated group in A549 cells. Serotonin level was significantly increased by 317.9% and 225.7% in the UT-Med + BT-TI and BT-Med + BT-TI groups, respectively at 1 µg/mL 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 195.3% (at 1 µg/mL), 176.2% (at 10 µg/mL) and 194.7% (at 50 µg/mL) in the BT-Med + BT-TI group compared to the untreated group in MG-63 cells. Taking everything into account, the Biofield Energy Treatment significantly improved heart, liver, bones, neuronal and lungs functional enzyme biomarkers and also protected hepatocyte, cardiomyocyte, pneumocyte, osteocytes and nerve cells from oxidative damage induced by *tert*-butyl hydroperoxide (*t*-BHP). Thus, it can be used as a complementary and alternative treatment for the prevention of various types of cardiac disorders (high blood pressure, congestive heart failure, stroke, peripheral artery disease, rheumatic heart disease, valvular heart disease, carditis, congenital heart disease and venous thrombosis, thromboembolic disease, etc.), hepatic disorders (cirrhosis, liver cancer, hemochromatosis, Wilson disease) and lungs disorders (Asthma, Chronic bronchitis, Emphysema, Cystic fibrosis, Pneumonia). Further, it could 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., kidney, liver and heart transplants), hormonal imbalance, aging and various inflammatory and immune-related disease conditions like Alzheimer's Disease (AD), Ulcerative Colitis (UC), Dermatitis, Asthma, Irritable Bowel Syndrome (IBS), Pernicious Anemia, Multiple Sclerosis, Aplastic Anemia, Hepatitis, Graves' Disease, Diabetes, Parkinson's Disease, Myasthenia Gravis, Atherosclerosis, Systemic Lupus Erythematosus (SLE), stress, etc., to improve overall health and Quality of Life.

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