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Research Article

Impact of the Consciousness Energy Healing-based Novel Test Formulation on Human Organ Specific Biomarkers

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Abstract

The study was investigated to find out the impact of the Biofield Energy Treated test formulation on the function of vital organs *viz*. bones, heart, liver, lungs, and brain in various cell-based assays. The test formulation/test item (TI) and the cell media (Med) was divided into two parts; one untreated (UT) and other part received the Biofield Energy Treatment remotely by a renowned Biofield Energy Healer, Vicki Lynn Kindy, USA 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 nature in six different cells. The experimental groups of untreated medium (UT-Med) + Biofield Treated Test Item (BT-TI), BT-Med + UT-TI, and BT-Med + BT-TI groups showed 116.4% (at 1 µg/mL), 84.1% (at 63 µg/mL), and 98.5% (at 63 µg/mL) restoration of cell viability, respectively in human cardiac fibroblasts cells (HCF) compared to the UT-Med + UT-TI group. Moreover, UT-Med + BT-TI group showed 62.5%, 156.8%, and 82.4% restoration of cell viability at 0.1, 1, and 10 µg/mL, respectively in human hepatoma cells (HepG2) compared to untreated. Furthermore, 224.2% and 295.6% restoration of cell viability was observed in adenocarcinomic human alveolar basal epithelial cells (A549) by UT-Med + BT-TI group at 1 and 10 µg/mL, respectively compared to the untreated. The alkaline phosphatase (ALP) level was significantly increased by 85.3%, 72.2%, and 79.4% in the UT-Med + BT-TI, BT-Med + UT-TI, and BT-Med + BT-TI groups, respectively at 10 µg/mL in human bone osteosarcoma cells (MG-63) compared to the untreated. Additionally, the level of ALP was significantly increased by161% (at 0.1 µg/mL), 84.5% (at 25 µg/mL), and 63.9%(at 50 µg/mL) in the UT-Med + BT-TI, BT-Med + UT-TI, and BT-Med + BT-TI groups, 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 189.7% (at 0.01 µg/mL) and 51.7% (at10 µg/mL) in the UT-Med + BT-TI and BT-Med + BT-TI groups, respectively compared to the untreated in HCF cells. The percent protection of HepG2 (liver) cells (decreased of ALT activity) was significantly increased by 95.1% (at 25 µg/mL), 63.2% (at 1 µg/mL), and 112% (at 63 µg/mL) in the UT-Med + BT-TI, BT-Med + UT-TI, and BT-Med + BT-TI groups, respectively compared to untreated in HepG2 cells. The percent protection of A549 (lungs) cells (increased of SOD activity) was significantly increased by 36.9% and 44.1% in the BT-Med + UT-TI and BT-Med + BT-TI groups, respectively at 0.1 µg/mL compared to untreated in A549 cells. Serotonin level was significantly increased by 199.5%, 168.7%, and 83.9% in the BT-Med + BT-TI group at 0.1, 1, and 10 µg/mL, respectively as compared to untreated in human neuroblastoma cells (SH-SY5Y). The relative quantification (RQ) of vitamin D receptor (VDR) was significantly increased by 205.2% (at 10 µg/mL), 189% (at 0.01µg/mL), and 315.6% (at 10 µg/mL) in the UT-Med + BT-TI, BT-Med + UT-TI, and BT-Med + BT-TI groups, respectively compared to the untreated in MG-63 cells. Overall, these results suggest that Biofield Treated test formulation significantly improved the bones, heart, liver, lungs, and brain-related functional enzyme biomarkers. Altogether data suggest that the Biofield Energy Treatment (The Trivedi Effect®) 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® can be used as a complementary and alternative therapy against several disorders such as coronary artery disease, heart attack, heart failure, arrhythmias, congenital heart disease, cirrhosis, cardiomyopathy, liver cancer, Wilson disease, hemochromatosis, pneumonia, asthma, chronic bronchitis, emphysema, osteoporosis, cystic fibrosis, etc.

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

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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 B_{12}, vitamin D_3, ascorbic acid, and vitamin B_6. 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 immunomodulator [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

Ferrous sulfate, vitamin B_6, vitamin D_3, vitamin B_{12}, 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, β-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 B_{12}, β-carotene, vitamin D_3, zinc chloride, magnesium gluconate, sodium selenate, ferrous sulfate, ascorbic acid, and vitamin B_6. The test formulation and the cell media was divided into two parts; one part was untreated (UT) and other part received the Biofield Energy Treatment remotely by a renowned Biofield Energy Healer, Vicki Lynn Kindy, USA under laboratory conditions for ~3 minutes 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 by 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 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 to allow cell recovery

Table 1: Information related to six cell lines with their plating density and time-point [41]

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

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_2 incubator at 37°C, 5% CO_2, and 95% humidity and incubated for time period mentioned in Table 1. After incubation, the plates were taken out and 20 µL 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 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 of TI was calculated using Equation 1:

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

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

The concentrations exhibiting percentage cytotoxicity < 30% were considered as non-cytotoxic.

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 hours. After 24 hours, oxidative stress was given to the cells using 10mM *t*-BHP for 3.5 hours. The untreated cells served as a control that did not receive any treatment and was maintained in cell growth medium only. Cells treated with 10mM 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:

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

Assessment of alkaline phosphatase (ALP) activity

The cells (human bone osteosarcoma cells-MG-63 and human endometrial adenocarcinoma cells-Ishikawa) were counted using a hemocytometer and plated in 24-well plates at the density corresponding to 1 X 10^4 cells/well in phenol-free DMEM supplemented with 10% CD-FBS. Following the respective treatments, the cells in the above plate were incubated for 48 hours in a 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:

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

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 X 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/positive control at the non-cytotoxic concentrations for 24 hours. After 24 hours, oxidative stress was given to the cells using 10mM *t*-BHP for 3.5 hours. The untreated cells were served as control that did not receive any treatment and were maintained in cell growth medium only. Cells treated with 10mM 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.

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

Estimation of ALT in liver cells (HepG2)

The human hepatoma cells (HepG2) were counted and plated at the density of 5 X 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 400µM *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 400µM 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.

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

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 X 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:

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

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 X 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 hours. Serotonin release was determined by ELISA as per manufacturer's protocol. The percent increase in serotonin levels was calculated using Equation 7.

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

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

R = Serotonin levels corresponding to control group.

Effect of test formulation on vitamin D receptor (VDR) in bone (MG-63) cells

The human bone osteosarcoma (MG-63) cells were counted using the hemocytometer were plated at a density of 2 X10^5 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. 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 hours 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'-CACGTCACTGACGCGGTACTT-3'.VDR gene expression was normalized using House-keeping (HK) reference. Relative quantification (RQ) of VDR gene in Biofield Energy Treated cells was calculated with respect to the untreated cells using Equation 8:

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

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

Statistical analysis

All the values were represented as percentage. 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 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

For the evaluation of the vital organs *viz*. heart, liver, and lungs of the formulation 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 [41, 42]. 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 56.61%, 94.09%, and 102.29% at 5, 10, and 25 µg/mL, respectively compared to the *t*-BHP induced group. Besides, the test formulation showed 116.4%, 36.9%, and 70.8% restoration of cell viability at 1 µg/mL in the UT-Med + BT-TI, BT-Med + UT-TI, BT-Med + BT-TI groups, respectively as compared to the UT-Med + UT-TI group. Moreover, at 10 µg/mL the UT-Med + BT-TI and BT-Med + BT-TI group showed 10.3% and 32.1% restoration of cell viability, respectively than UT-Med + UT-TI group. Additionally, the test formulation showed 26% restoration of cell viability at 25 µg/mL in the BT-Med + UT-TI group as compared to the UT-Med + UT-TI group. Further, at 63 µg/mL the test formulation showed 35.5%, 84.1%, and 98.5% restoration of cell viability, respectively in the UT-Med + BT-TI, BT-Med + UT-TI, and BT-Med + BT-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 38.79%, 73.92%, and 81.74% at 5, 10 and 25 µg/ mL, respectively compared to the *t*-BHP induced group. Besides, the test formulation showed 62.5%, 156.8%,and 82.4% restoration of cell viability at 0.1,1, and 10 µg/mL in the UT-Med + BT-TI group as compared to the UT-Med + UT-TI group. Moreover, at 63 µg/mL the BT-Med + UT-TI group showed 51.5% restoration of cell viability

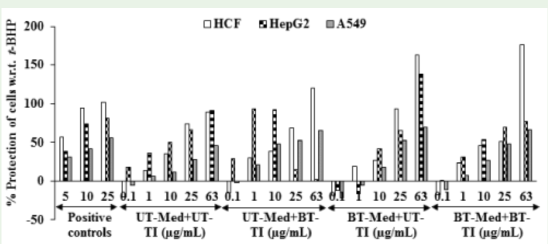

Figure 1: Assessment of cytoprotective effect of the test formulationin human cardiac fibroblasts cells (HCF), human hepatoma cells (HepG2), and adenocarcinomic human alveolar basal epithelial cells (A549) against *tert*-butyl hydroperoxide (*t*-BHP) induced damage.TMZ: Trimetazidine (µM), silymarin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

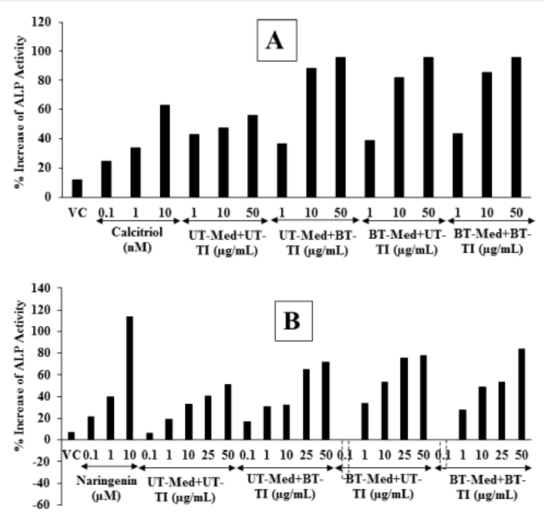

Figure 2: The effect of the test formulation on alkaline phosphatase (ALP) in A. Human bone osteosarcoma cells (MG-63) and B. Human endometrial adenocarcinoma cells (Ishikawa).Calcitriol and naringenin were used as positive control in Mg-63 and Ishikawa cells, respectively.VC: Vehicle control; UT: Untreated; Med: Medium; BT: Biofield Treated; TI: Test item.

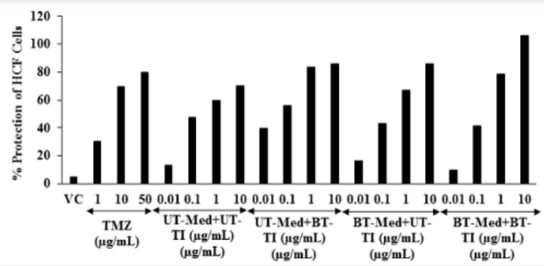

Figure 3: The effect of the test formulation on the percent protection of HCF cells in terms of decreasedlactate dehydrogenase (LDH) activity against *tert*-butyl hydroperoxide (*t*-BHP) induced damage. TMZ: Trimetazidine; VC: Vehicle control; UT: Untreated; Med: Medium; BT: Biofield Treated; TI: Test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

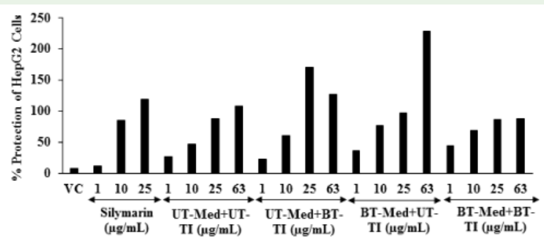

Figure 4: Effect of the test formulation on the percent protection of human liver cancer (HepG2) cells in terms of decreased alanine amino transaminase(ALT) activity under the stimulation of *tert*-butyl hydroperoxide (*t*-BHP). VC: Vehicle control; UT: Untreated; Med: Medium; BT: Biofield Treated; TI: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

than 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 31%, 41.93%, and 55.74% at 1, 10 and 25 µg/mL, respectively compared to the *t*-BHP induced group. Besides, the test formulation showed 328.3% and 113.8% restoration of cell viability at 0.1 µg/mL in the BT-Med + UT-TI and BT-Med + UT-TI groups, respectively compared to the UT-Med + UT-TI group. Moreover, at 1 µg/mL the UT-Med + BT-TI and BT-Med + BT-TI groups showed 224.2% and 21.7% restoration of cell viability, respectively than UT-Med + UT-TI group. Additionally, the test formulation showed 295.6%, 52.3%, and 115.5% restoration of cell viability at 10 µg/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. Further, the test formulation showed 85.4%, 84.2%, and 71.4% restoration of cell viability at 25 µg/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. Besides, at 63 µg/mL the test formulation exhibited 40.3%, 48.6%, and 43% restoration of cell viability 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 1). The cellular antioxidant capacity can reduce due to excess production of free radicles that leads to inflammation[43]. This excess level of free radicles can affect the normal functions of cell membrane, and ultimately altered the genetic materials and cause various age-related disorders such as autoimmune diseases, cardiovascular, diabetes, and cancer [44-46].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. Therefore, Biofield Energy Healing Treatment could be used for the management of cardiovascular, liver, and various lung disorders.

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 24.82%, 33.7%, and 62.95% increased the level of ALP at 0.1, 1, and 10 nM, respectively in MG-63 cells. Moreover, the experimental groups showed 85.3%, 72.2%, and 79.4% increased 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 10 µg/mL. At 50 µg/mL, the percent ALP was significantly increased by 70.4%, 70%, and 70.4% 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). Besides, the positive control naringen in showed 21.5%, 39.43%, and 113.64% increased the level of ALP at 0.1, 1, and 10 nM, respectively in Ishikawa cells. ALP percent was significantly increased by 161% in the UT-Med + BT-TI group compared to the UT-Med + UT-TI group at 0.1 µg/mL. Moreover, the experimental groups showed 58%, 41.1%, and 45.4% increased 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 1 µg/mL. At 10 µg/mL, the percent ALP was significantly increased by 61.5% and 49.2% in the 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 59.9%, 84.5%,and 31.2% in the UT-Med + BT-TI, BT-Med + UT-TI and BT-Med + BT-TI groups, respectively at 25 µg/mL compared to the UT-Med + UT-TI group. Additionally, the percent ALP was significantly increased by 39.9%, 51.5%, and 63.9% 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 (Figure 2). The ALP activity is essential for the bone mineralization and considered a useful biochemical marker for bone formation [47]. Thus, for the detection of bone specific biochemical marker in serum can be clinically useful in evaluating the progress of the bone healing process [48, 49]. 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.

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

The lactate dehydrogenase (LDH) enzyme is mainly present in the heart and skeletal muscle, and responsible for anaerobic respiration of cells [50]. LDH is rapidly released into the cell culture supernatant when the plasma membrane is damaged and is a key feature of cells undergoing apoptosis, necrosis, and other forms of cellular damage [51]. The effect of 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 31.14%, 69.42%, and 80.06% 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 189.7% and 19.4% µg/mL in the UT-Med + BT-TI and BT-Med + UT-TI groups, respectively at 0.01 µg/mL as compared to the UT-Med + UT-TI group. Moreover, at 0.1 µg/mL, the percent protection of HCF cells (decreased of LDH activity) was significantly increased by 18.6% in the UT-Med + BT-TI group as compared to the UT-Med + UT-TI group. Further, percent protection of HCF cells (decreased of LDH activity) was also significantly increased by 39.9%, 12.9%, and 32.2% in the UT-Med + BT-TI, BT-Med + UT-TI, and BT-Med + BT-TI groups, respectively at 1 µg/mL as compared to the UT-Med + UT-TI group. Further, percent protection of HCF cells (decreased of LDH activity) was also significantly increased by 23.2%, 22.7%, and 51.7% 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). LDH is a pathologic biomarker for a wide variety of cardiovascular disorders (CVDs) such as myocardial ischemia, strenuous, etc. Various heavy metals exposure can increased the level of LDH and simultaneously more prone to CVDs [52]. 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.

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 56%, 84.98%, and 118.94% protection of HepG2 cells (decreased of ALT activity). The protection of HepG2 cells (decreased of ALT activity) was significantly increased by 33.8% and 63.2% at 1 µg/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 µg/mL, percent protection of HepG2 cells (decreased of ALT activity) was increased by 30.4%,

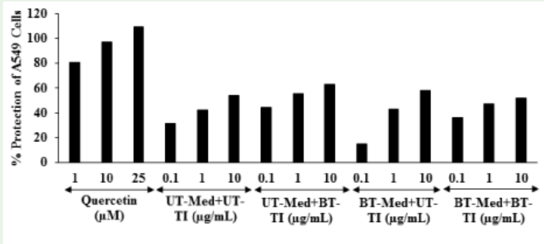

Figure 5: Effect of the test formulation on the percent protection of lungs cells (A549) in terms of increased SOD activity under the stimulation of *tert*-butyl hydroperoxide (*t*-BHP). UT: Untreated; Med: Medium; BT: Biofield Treated; TI: Test item.

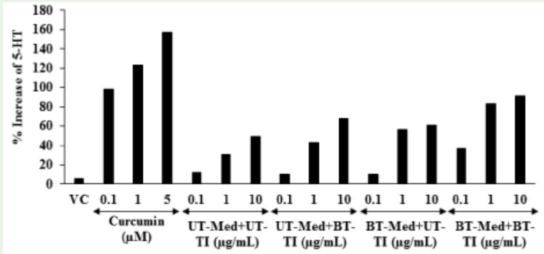

Figure 6: Effect of the test formulation on percent increase in 5-hydroxy tryptamine (5-HT) or serotonin in human neuroblastoma cells (SH-SY5Y). VC: Vehicle control; UT: Untreated; Med: Medium; BT: Biofield Treated; TI: Test item

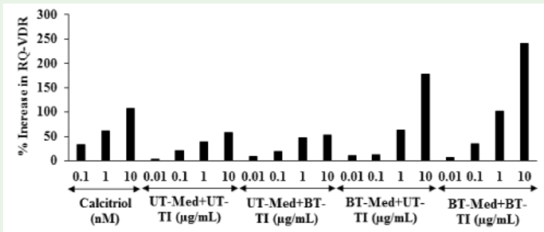

Figure 7: Effect of the test formulation onpercent increase in relative quantification (RQ) of vitamin D receptors (VDRs) genein human bone osteosarcoma cells (MG-63).UT: Untreated; Med: Medium; BT: Biofield Treated; TI: Test item

63.0%, and 45.3% in the UT-Med + BT-TI, BT-Med + UT-TIand 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 95.1% and 11.7% in the UT-Med + BT-TI and BT-Med + UT-TI groups, respectively at 25 µg/mL as compared to the UT-Med + UT-TI group. Further, the percent protection of HepG2 cells (decreased of ALT activity) was increased by 17.7% and 112.4% in the BT-Med + UT-TI and BT-Med + BT-TI groups, respectively at 63 µg/mL as compared to the UT-Med + UT-TI group (Figure 4). The aminotransferase enzymes that catalyze the reversible transformation of α-ketoacids into amino acids. Increased level of ALT is directly proportional to the severity of the hepatic disorders [53]. Emerging data also suggest that ALT has play as a predictor of mortality independent of liver disease [54, 55]. 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.

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 80.67%, 97.01%, and 109.56% 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 14.8%, 36.9%,and 44.1% at 0.1 μg/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 10 μg/mL, the percent protection of A549 (lungs) cells (increased of SOD activity) was significantly increased by 7.2% and 25.6% in the BT-Med + UT-TI and BT-Med + BT-TI groups, respectively as compared to the UT-Med + UT-TI group (Figure 5). The lungs are directly exposed to more oxygen concentrations in comparison to other tissues. Increased oxidative stress leads to the pathogenesis of various obstructive lung disorders such as asthma, chronic obstructive pulmonary disease (COPD), lung malignancies, etc. SOD enzyme is considered as an important antioxidant defense mechanism in all living cells which are exposed to oxygen especially in lungs. SOD can convert the superoxide radicals to hydrogen peroxide [56,57]. Altogether, data observed that 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.

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

The effect of test formulation on serotonin level was assessed in SH-SY5Y cells after 24 hours of treatment by ELISA and the results are shown in Figure 6. The positive control, showed 98.2%, 123.53%, and 156.76% increased the level of serotonin. The level of serotonin was significantly increased by 199.5% in the BT-Med + BT-TI group 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 40.4%, 82.1%, and 168.7% 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 37.4%, 22.2%, and 83.9% 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 produced in neurons, gut, and heart cell mainly and responsible for stress, anxiety, aggressive behavior, and for the regulation of blood pressure [58]. Loss of 5-HT leads to various neuropsychiatric diseases like depression, Alzheimer's disease, loss of ability of thinking, memory loss, cognitive health, etc. [59]. Thus, the 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.

Effect of test formulation on vitamin Dreceptors (VDRs)

Human bone osteosarcoma cells (MG-63) were treated with the test formulation and the effect on 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) was calculated from the VDR-CT and house-keeping (HK)-CT values for MG-63 cells treated with test formulation and positive control is represented in Figure 7. The positive control (calcitriol) showed 32.87%, 61.33%, and 107.05% increase of RQ of VDR in a concentration-dependent manner at 1, 10, and 100 nM, respectively. Moreover, RQ of VDR was significantly increased by 167.4%, 189%, and 124.7% in the UT-Med + BT-TI, BT-Med + UT-TI, and BT-Med + BT-TI groups, respectively at 0.01 μg/mL compared to the UT-Med + UT-TI group. Additionally, at 0.1 μg/mL the VDR level was significantly increased by 73.3% in the BT-Med + BT-TI group compared to the UT-Med + UT-TI group. Further, VDR level was also significantly increased by 22.7%, 61.1%, and 156.9% 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, the VDR level was significantly increased by 205.2% and 315.6% in the UT-Med + BT-TI and BT-Med + BT-TI groups, respectively at 10 μg/mL compared to the UT-Med + UT-TI group. The biologically most active vitamin D compound is 1α, 25-dihydroxyvitamin D3 (1, 25(OH)2D3), which functions as specific high-affinity ligand of the transcription factor of VDRs [60]. The active form of vitamin D [1α,25(OH)(2)D(3)] can binds and activates its specific nuclear receptor, i.e., the vitamin D receptor (VDR). Thus, this activated VDR can prevents the release of calcium from its storage in bone to serum by stimulating intestinal calcium absorption and renal reabsorption [61,62]. Overall, the Biofield Energy Treated test formulation has tremendously 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.

Conclusions

The study findings showed that the novel test formulation was safe and non-toxic based on the MTT cell viability assay in six tested cells. The treatment groups like UT-Med + BT-TI, BT-Med + UT-TI, and BT-Med + BT-TI showed 116.4%, 84.1%, and 98.5% restoration of cell viability at 1, 63, and 63 μg/mL, respectively in human cardiac fibroblasts cells (HCF) compared to the UT-Med + UT-TI group. Moreover, the UT-Med + BT-TI group showed 156.8% and 82.4% restoration of cell viability at 1 and 10 μg/mL, respectively in human hepatoma cells (HepG2) compared to the untreated group. Additionally, the UT-Med + BT-TI group showed 224.2% and 295.6% restoration of cell viability at 1 and 10 μg/mL, respectively in adenocarcinomic human alveolar basal epithelial cells (A549) compared to the untreated group. Alkaline phosphatase (ALP) activity was significantly increased by 85.3% in the UT-Med + BT TI group at 10 μg/mL in human bone osteosarcoma cells (MG-63). Moreover, ALP activity was significantly increased by 161% in the UT-Med + BT-TI group at 0.1 μg/mL than untreated group. The percent protection of HCF cells (decreased of LDH activity) was significantly increased by 189.7% (at 0.01 μg/mL) in the UT-Med + BT-TI group compared to the untreated group in HCF cells. The percent protection of HepG2 cells (decreased of ALT activity) was significantly increased by 95.1% (at 25 µg/mL) and 112% (at 63 µg/mL) in the UT-Med + BT-TI and BT-Med + BT-TI groups, respectively compared to the untreated group in HepG2 cells. The percent protection of A549 (lungs) cells (increased of SOD activity) was significantly increased by 44.1% in the BT-Med + BT-TI group at 0.1 µg/mL compared to the untreated group in A549 cells. The serotonin level was significantly increased by 199.5% and 168.7% at 0.1 and 1 µg/mL, respectively in the BT-Med + BT-TI group 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 205.2% (at 10 µg/mL), 189% (at 0.01 µg/mL), and 315.6% (at 10 µg/mL) in the UT-Med + BT-TI, BT-Med + UT-TI, and BT-Med + BT-TI groups, respectively compared to the untreated group in MG-63 cells. In conclusion, the Biofield Energy Treatment significantly improved heart, liver, bones, neuronal, and lungs functional enzyme 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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