Keywords: Biofield Energy Treatment, The Trivedi Effect®, Bone health, Cardiac health, Liver health, Lungs health, VDR receptor, 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, polymorphonuclear, 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 result 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.

METHODS

Chemicals and reagents

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). 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 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, Alan Joseph Balmer, 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 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 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 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-dimethythiazol-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 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:

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

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:

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

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 1×10^4 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_2 incubator at 37°C, 5% CO_2 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 ($MgCl_2$) 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.

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

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.

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

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:

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

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.

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

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 2×10^5 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'-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 Mean ± 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

Effect of the test formulation on vital organs *viz.* heart, liver, and lungs using cell-based assay under the stimulation of *tert*-butyl hydroperoxide (*t*-BHP) induced oxidative stress. *t*-BHP has been regularly used for the induction of oxidative stress in various cells [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 µg/mL, respectively compared to the t-BHP induced group. Besides, the test formulation showed 60.5% and 181% restoration of cell viability at 1 µg/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 µg/mL the UT-Med + BT-TI, BT-Med + UT-TI and BT-Med + BT-TI groups showed 80.7%, 38.9% and 82.2% restoration of cell viability, respectively than UT-Med + UT-TI group. Additionally, the test formulation showed 20.5% 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 71.8% 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 38.79%, 73.92% and 81.74% at 5, 10 and 25 µg/mL, respectively compared to the t-BHP induced group. The test formulation showed 64.4%, 28.5%, 126.8% and 86.3% restoration of cell viability at 0.1, 1, 10 and 25 µg/mL, respectively in the UT-Med + BT-TI group as compared to the UT-Med + UT-TI group. Moreover, at 63 µg/mL the UT-Med + BT-TI and BT-Med + UT-TI groups showed 39.3% and 16.6% restoration of cell viability, respectively 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 31.24%, 41.93% and 55.74% at 5, 10 and 25 µg/mL, respectively compared to the t-BHP induced group. Besides, the test formulation showed 45.4% and 19.5% restoration of cell viability in the UT-Med + BT-TI and BT-Med + UT-TI groups, respectively at 1 µg/mL compared to the UT-Med + UT-TI group. Moreover, at 10 µg/mL the UT-Med + BT-TI, BT-Med + UT-TI and BT-Med + BT-TI groups showed 101.2%, 103.6% and 96.9% restoration of cell viability, respectively than UT-Med + UT-TI group. Additionally, the test formulation showed 72.9%, 81.7% and 135% 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. Further, the test formulation showed 17.5%, 36.1% and 63.4% restoration of cell viability at 63 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 **(Figure 1)**. Natural antioxidants are very essential for maintenance of a healthy and long life of the human body as they reduce oxidative damage by interaction with oxidative free radicals at the cellular level to prevent or delay oxidative stress [42]. In addition, the antioxidants formulations can eliminate the cosmetic problems induced by reactive oxygen species have created a new market as antipollution skincare products [43]. The study results suggest that Biofield Treatment significantly protects against *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.

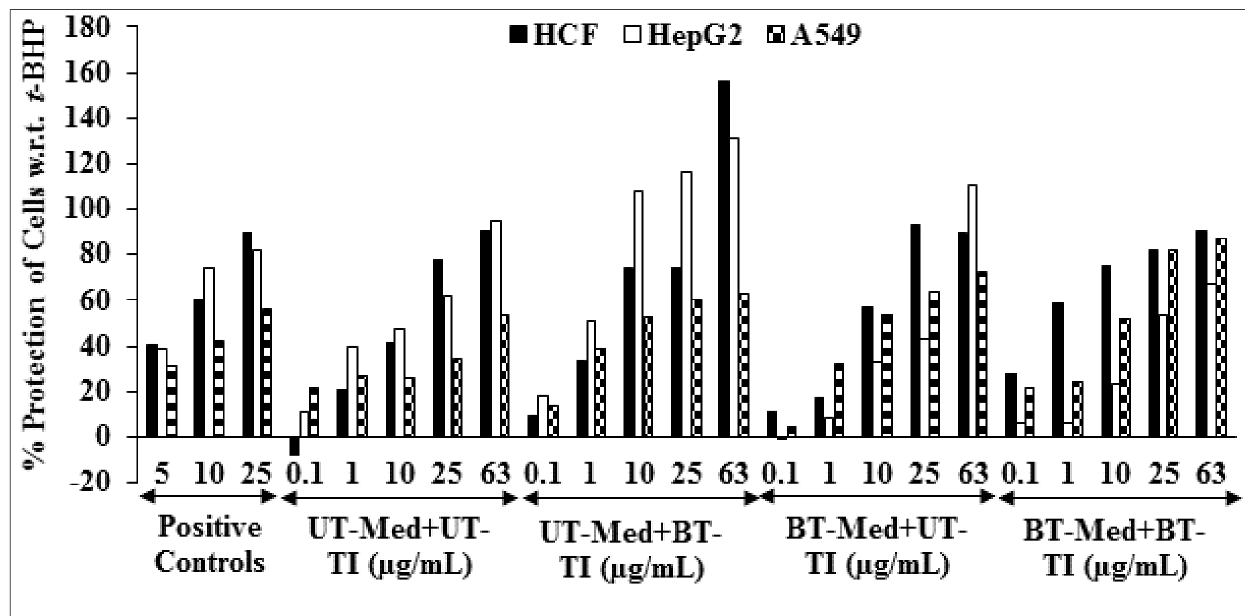

Figure 1. Assessment of cytoprotective effect of the test formulation in human cardiac fibroblasts cells (HCF), human hepatoma cells (HepG2) and adenocarcinomic human alveolar basal epithelial cells (A549) against *tert*-butyl hydroperoxide (*t*-BHP) induced damage. TMZ: Trimetazidine (µM), silymarin (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 24.82%, 33.7% and 62.95% increase the level of ALP at 0.1, 1 and 10 nM, respectively in MG-63 cells. Moreover, the experimental groups showed 90%, 87.3% and 86.9% 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 10 µg/mL. At 50 µg/mL, the percent ALP was significantly increased by 81.4%, 79.6% and 70.6% 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 naringenin showed 21.5%, 39.43% and 113.64% increase the level of ALP at 0.1, 1 and 10 nM, respectively in Ishikawa cells. ALP percent was significantly increased by 137% in the BT-Med + UT-TI group compared to the UT-Med + UT-TI group at 1 µg/mL. Moreover, the experimental groups showed 32.8% and 33.6% increase the level of ALP in the 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 46% in the BT-Med + BT-TI group compared to the UT-Med + UT-TI group **(Figure 2)**. The ALP plays a vital role for the mineralization bone and considered a useful biochemical marker for bone formation [44]. The bone biomarkers like bone alkaline phosphatase (B-ALP) is considered to be a good marker for bone formation released during the bone remodeling processes [45]. Combination with the measurement of bone mineral density (BMD), the clinical applications of bone biomarkers have provided comprehensive information for diagnosis of osteoporosis [46]. 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.

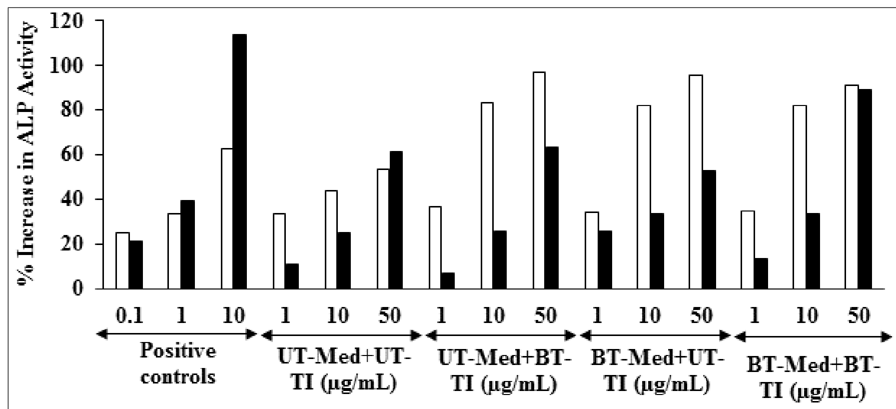

Figure 2. The effect of the test formulation on alkaline phosphatase (ALP) in human bone osteosarcoma cells (MG-63) and human endometrial adenocarcinoma cells (Ishikawa). Calcitriol and naringenin were used as positive control in Mg-63 and Ishikawa cells, respectively.
UT: Untreated; Med: Medium; BT: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

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

The effect of 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 3.59%, 30.14% and 69.42% 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 52.1%, 65.9% and 63.5% 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. Moreover, at 10 µg/mL, the percent protection of HCF cells (decreased of LDH activity) was significantly increased by 33.2%, 33.7% and 44.9% 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 37.7%, 49.8% and 53.3% 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 3)**. The lactate dehydrogenase (LDH) enzyme is mainly present in the heart and skeletal muscle. It catalyzes the interconversion of pyruvate and lactate, which are critical fuel metabolites of skeletal muscle particularly during exercise [47]. Elevated serum lactic dehydrogenase (LDH) levels are associated with increased cardiovascular mortality. Several inflammatory diseases were also correlated with serum LDH [48]. The study results found that there was a significant reduction of LDH level after Biofield Energy Treatment which protected heart cells and might be helpful to resist against various pathological conditions like tissue injury, necrosis, hemolysis or malignancies, hypoxia, etc. It also indicates that the heart cells acted normally under stress and anaerobic condition and improved overall heart function.

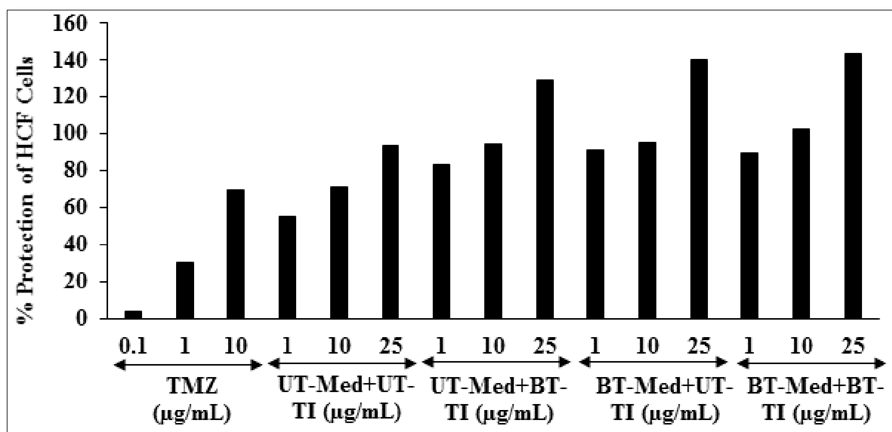

Figure 3. The effect of the test formulation on the percent protection of HCF cells in terms of decreased lactate dehydrogenase (LDH) activity against *tert*-butyl hydroperoxide (*t*-BHP) induced damage.
TMZ: Trimetazidine; UT: Untreated; Med: Medium; BT: Biofield Treated; TI: Test Item

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 ALT activity). The protection of HepG2 cells (decreased ALT activity) was significantly increased by 11% and 157% at 0.1 µg/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 1 µg/mL, percent protection of HepG2 cells (decreased ALT activity) was increased by 32.9% and 41.6% in the UT-Med + BT-TI and BT-Med + UT-TI groups, respectively as compared to the UT-Med + UT-TI group. Additionally, protection of HepG2 cells (decreased ALT activity) was also significantly increased by 41.8% and 58.9% in the BT-Med + UT-TI and BT-Med + BT-TI groups, respectively at 10 µg/mL as compared to the UT-Med + UT-TI group. Further, the percent protection of HepG2 cells (decreased ALT activity) was increased by 58.5%, 35.1% and 19.2% 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)**. Alanine Aminotransferase (ALT) activity plays as an indicator of general health and especially on liver disorders. The probability of clinically significant liver disease increases, particularly if the elevated ALT is associated with symptoms such as fatigue, anorexia or pruritus [49]. In muscle, ALT plays an important role for the regulation of glucose level during stressful conditions such as fasting or vigorous exercise [50]. 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.

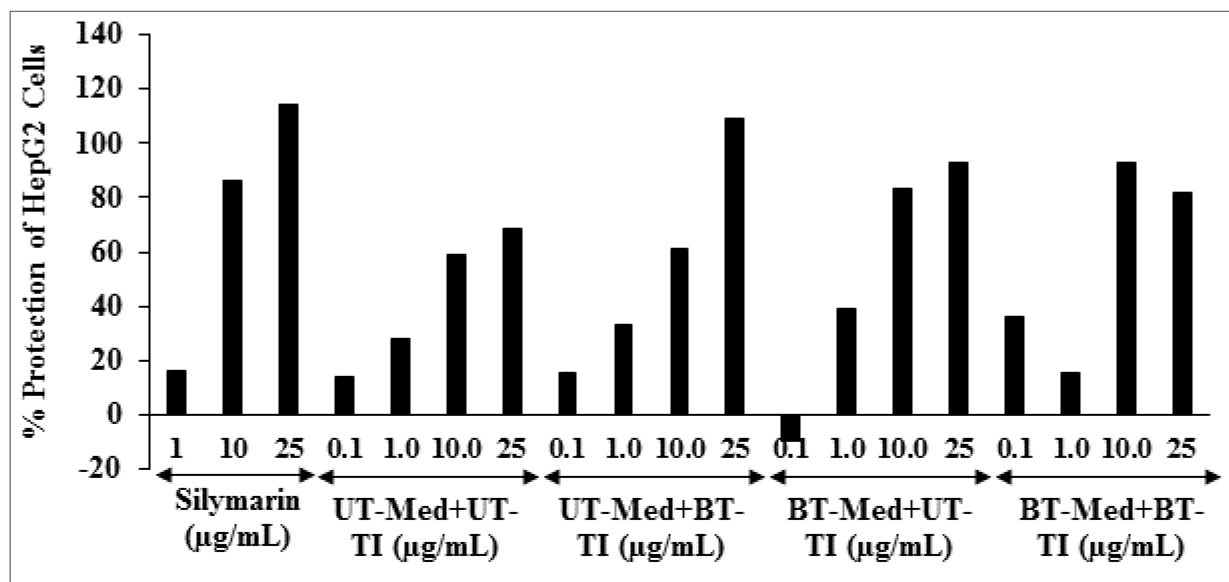

Figure 4. Effect of the test formulation on the percent protection of human liver cancer (HepG2) cells in terms of decreased alanine amino transaminase (ALT) activity under the stimulation of *tert*-butyl hydroperoxide (*t*-BHP).
UT: Untreated; Med: Medium; BT: Biofield Treated; TI: Test Item

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 80.67%, 97.01% and 109.56% protection of A549 (lungs) cells (increased SOD activity) compared to the t-BHP group. The percent protection of A549 (lungs) cells (increased SOD activity) was significantly increased by 121.7% and 16.6% at 1 µg/mL in the UT-Med + BT-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 168.4%, 137% and 124% 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 level of SOD was increased by 135.4%, 103.7% and 121.1% 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 5)**. Extracellular SOD is one of the three antioxidant enzyme isoforms, and is highly expressed in lungs and vessels [51]. The lungs are directly exposed to more oxygen concentrations than other tissues. Increase levels of both

exogenous and endogenous reactive oxygen species (ROS) leads to the pathogenesis of various lung disorders such as asthma, chronic obstructive pulmonary disease (COPD), lung malignancies, etc. [52]. Altogether, data observed that a significantly increased SOD levels after Biofield Energy Treatment in A549 cells, were seen which might be helpful to resist against various pathological conditions like oxidative stress and related adverse effect. It also indicated that the lung cells acted normally and improved overall respiratory activities.

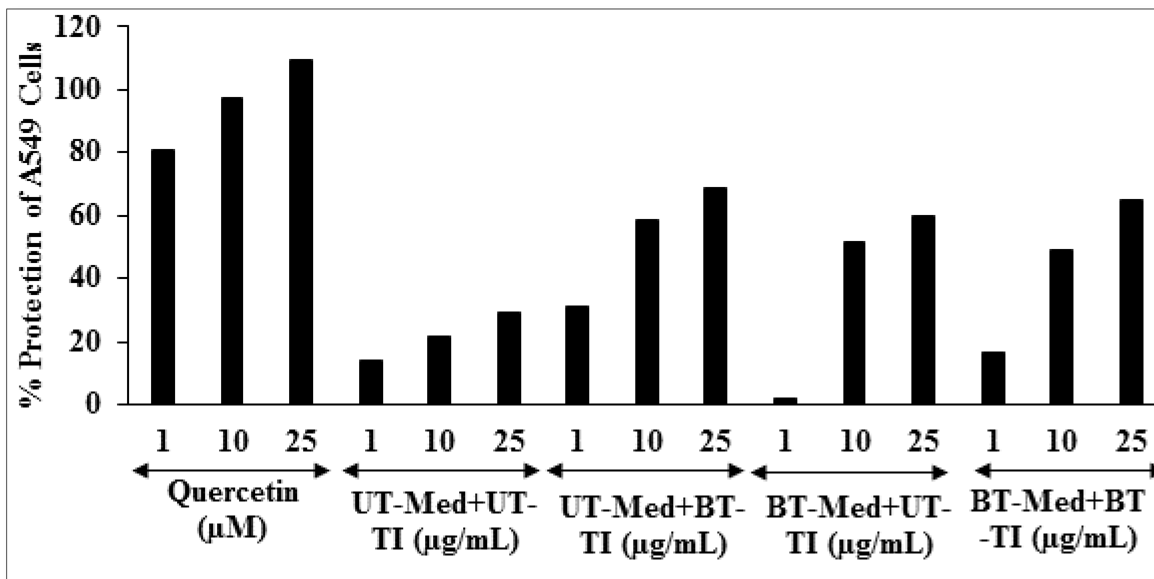

Figure 5. Effect of the test formulation on the percent protection of lungs cells (A549) in terms of increased SOD activity under the stimulation of *tert*-butyl hydroperoxide (*t*-BHP).
UT: Untreated; Med: Medium; BT: Biofield Treated; TI: Test Item

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

The effect of test formulation on serotonin level was assessed in SH-SY5Y cells after 24 h of treatment by ELISA and the results are shown in **Figure 6**. The positive control showed 98.2%, 123.53% and 156.76% increase the level of serotonin. The level of serotonin was significantly increased by 36.4% and 52.7% 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. Moreover, at 10 µg/mL, 5-HT level was significantly increased by 19.7% in the BT-Med + BT-TI group as compared to the UT-Med + UT-TI group. The serotonin level was significantly increased by 27.5%, 40.2% and 26.6% 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. Further, the serotonin level was significantly increased by 50.8%, 78.8% and 32.3% in the UT-Med + 52.7BT-TI, 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 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 [53]. Marazziti [54] demonstrated a clear view of serotonergic dysfunction in different psychopathological disorders *viz.* schizophrenia, depression, anxiety disorders, eating disorders, autism, and aggressive behaviors, etc. Therefore, 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.

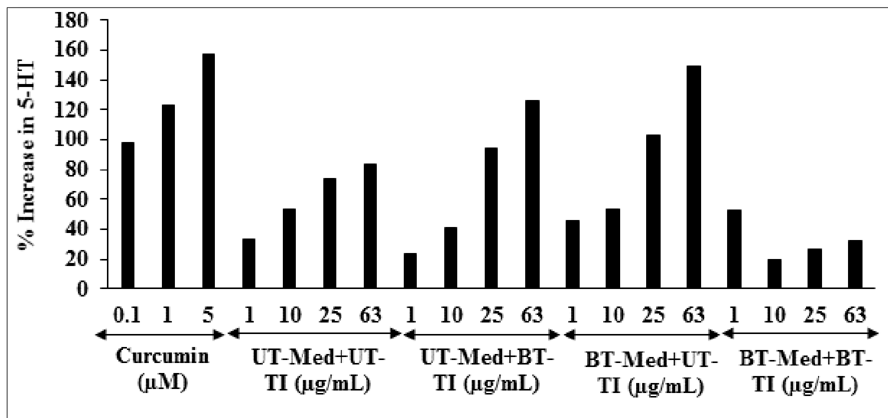

Figure 6. Effect of the test formulation on percent increase in 5-hydroxy tryptamine (5-HT) or serotonin in human neuroblastoma cells (SH-SY5Y).
UT: Untreated; Med: Medium; BT: Biofield Treated; TI: Test Item

Effect of test formulation on vitamin D receptors (VDRs)

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 65.86%, 109.94% and 154.91% 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 265.5%, 219.5% and 335.3% in the UT-Med + BT-TI, BT-Med + UT-TI and BT-Med + BT-TI groups, respectively at 0.1 µg/mL compared to the UT-Med + UT-TI group. Additionally, at 1 µg/mL the VDR level was significantly increased by 253.4%, 185.9% and 228.7% 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 212.7%, 203.5% and 136.3% in the UT-Med + BT-TI, BT-Med + UT-TI and BT-Med + BT-TI groups, respectively at 10 µg/mL compared to the UT-Med + UT-TI group. The role of vitamin D with extra-skeletal system like autoimmune disease, cardiovascular disease and cancer is of major interest nowadays [58]. Absence of a functional VDR or the key activating enzyme, 25-OHD-1α-hydroxylase (CYP27B1), causes congenital disease or severe vitamin D deficiency. Deficiency of vitamin D in humans is associated with increased prevalence of diseases [55]. Overall, the Consciousness Energy Healing Treated test formulation has tremendously increased the expression of VDRs, which might be helpful to bind more active vitamin D_3 metabolites and that ultimately can improve the more physiological functions of vitamin D and simultaneously improved bone cell growth and development.

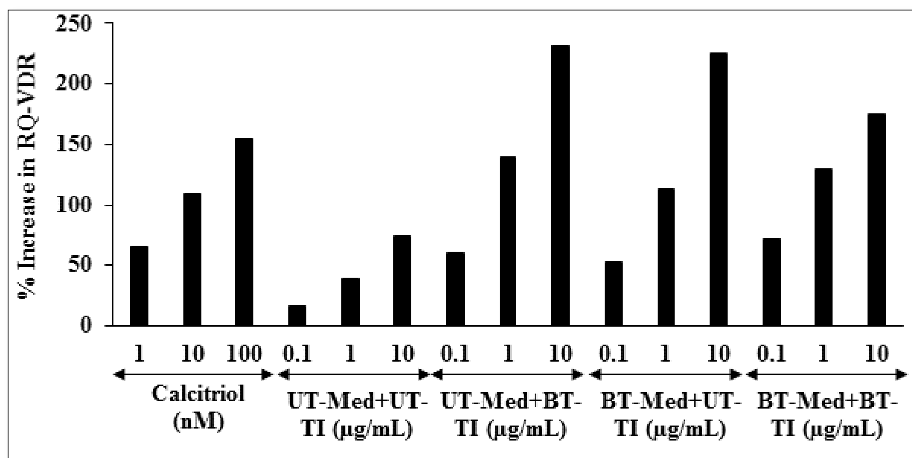

Figure 7. Effect of the test formulation on percent increase in relative quantification (RQ) of vitamin D receptors (VDRs) gene in human bone osteosarcoma cells (MG-63).
UT: Untreated; Med: Medium; BT: Biofield Treated; TI: Test Item

CONCLUSION

The study results showed that the novel test formulation was safe and non-toxic based on MTT cell viability assay in six tested cells. The BT-Med + BT-TI group showed 181% and 82.2% restoration of cell viability at 1 and 10 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 126.8% and 86.3% restoration of cell viability at 10 and 25 µg/mL, respectively in human hepatoma cells (HepG2) compared to the untreated group. Additionally, 101.2% (at 10 µg/mL), 103.6% (at 10 µg/mL) and 135% (at 25 µg/mL) 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 compared to the untreated group. Alkaline phosphatase (ALP) activity was significantly increased by 90% and 87.3% in the UT-Med + BT TI and BT-Med + UT TI groups, respectively at 10 µg/mL in human bone osteosarcoma cells (MG-63). Moreover, ALP activity was significantly increased by 137% in the BT-Med + UT-TI group at 1 µg/mL than untreated group. The percent protection of HCF cells (decreased LDH activity) was significantly increased by 65.9% and 63.5% at 1 µg/mL in the BT-Med + UT-TI and BT-Med + BT-TI groups, respectively compared to the untreated. The percent protection of HepG2 cells (decreased ALT activity) was significantly increased by 157% at 0.1 µg/mL in the BT-Med + BT-TI group compared to the untreated group. The percent protection of A549 (lungs) cells (increased SOD activity) was significantly increased by 168% and 135.4% in the UT-Med + BT-TI group at 10 and 25 µg/mL, respectively compared to the untreated group. The serotonin level was significantly increased by 78.8% (at 63 µg/mL) and 52.7% (at 1 µg/mL) in the BT-Med + UT-TI and BT-Med + BT-TI groups, respectively 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 265.5% (at 0.1 µg/mL) and 253.4% (at 1 µg/mL) in the UT-Med + BT-TI group; however, 335.3% in the BT-Med + BT-TI group at 0.1 µg/mL compared to the untreated group in MG-63 cells. In conclusion, the Biofield Energy Treatment significantly improved heart, liver, bones, neuronal and lungs related biomarkers and also protected cardiomyocyte, hepatocyte, osteocytes, pneumocyte and nerve cells from oxidative damage induced by *tert*-butyl hydroperoxide (*t*-BHP). Thus, the results suggest 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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