

Improved Metabolic Cardiac Biomarkers Activity Using Rat Cardiomyocytes Cell Line (H9c2) against Biofield Energy Treated Test Sample

Branton A¹ and Jana S^{2*}

¹Trivedi Global, Inc., Henderson, USA

²Trivedi Science Research Laboratory Pvt. Ltd., India

***Corresponding author:** Dr. Snehasis Jana, Trivedi Science Research Laboratory Pvt. Ltd., Bhopal, Madhya Pradesh, India; Email: publication@trivedieffect.com

Research Article

Volume 3 Issue 1

Received Date: January 18, 2019

Published Date: January 25, 2019

DOI: [10.23880/oajc-16000137](https://doi.org/10.23880/oajc-16000137)

Abstract

Cardiac biomarkers are important targets for any test sample to discover ideal cardiac health supplement. The study was aimed to evaluate the cardioprotective activity of Biofield Energy Treated test sample (DMEM) using rat cardiomyocytes (H9c2) cells. The test item (DMEM medium) was divided into three parts, first part received one-time Biofield Treatment by Alice Branton, a renowned Biofield Energy Healer, and defined as the one-time Biofield Energy Treated (BT-I) DMEM, while second part received two-times Biofield Energy Treatment and denoted as the BT-II DMEM. The third part did not give treatment and denoted as the untreated DMEM group. The cell viability of the test sample by 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay showed an increase level of viable cells by 86.58% and 91.37% in the BT-I and BT-II groups, respectively. Besides, BT-I and BT-II groups showed 10.56% and 23.53% cytoprotective action compared to the *tert*-Butyl hydroperoxide (*t*-BHP) induced group. Further, lactate dehydrogenase (LDH) level was significantly ($p \leq 0.001$) reduced by 54.43% and 53.52% in the BT-I and BT-II groups, respectively compared to *t*-BHP induced group. Moreover, the level of creatine kinase-myocardial band (CK-MB) was reduced by 63.48% and 60.68% in the BT-I and BT-II groups, respectively compared to the *t*-BHP induced group. Additionally, reactive oxygen species (ROS) level was significantly inhibited by 55.6% and 112.8% in the BT-I and BT-II groups, respectively compared to the *t*-BHP induced group. Besides, apoptotic activity was significantly inhibited by 16% and 10% in the BT-I and BT-II groups, respectively compared to the *t*-BHP induced group. Overall, the experimental data demonstrated that The Trivedi Effect[®] has significant capacity to improve the overall cardiac parameters in H9c2 cells, which can be used against various cardiac disorders such as congestive heart failure (CHF), cardiomyopathy, heart valve disorders, arrhythmias, myocardial infarction, etc.

Keywords: The Trivedi Effect[®]; Biofield Energy Treatment; H9c2; Cardiac health; CK-MB; ROS; Apoptosis; LDH

Abbreviations: LDH: Lactate Dehydrogenase; CK-MB: Creatine Kinase-Myocardial Band; ROS: Reactive Oxygen Species; CHF: Congestive Heart Failure; CAM: Complementary and Alternative Medicine; NCCIH: National Center of Complementary and Integrative Health; LPO: Lipid Peroxidation; TMZ: Trimetazidine; NAC: N-acetyl cysteine; DCFDA: 2',7'-dichlorofluorescein diacetate; MTT: 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide; *t*-BHP: tert-Butyl hydroperoxide; EDTA: Ethylenediaminetetraacetic Acid; DMEM: Dulbecco's Modified Eagle Medium; FBS: Fetal Bovine Serum; HBSS: Hank's Balanced Salt Solution; SEM: Standard Error of Mean.

Introduction

More than 80 million populations in the United States have been reported with some form of cardiovascular disorders and this can be defined as 1 in 3 adults. It was reported that 40-years-old male has the 50% chance of developing heart diseases, while women with 32% in the United States in their lifespan [1]. Besides, most of the minorities groups have been reported with a higher rate of cardiac disorders such as in Native Hawaiians, African-Americans, Native Americans, and Hispanics. Besides, lifestyle modification and other risk factors are highly essential to protect from a cardiovascular disorder such as smoking and hypertension treatment. Thus, the overall mortality rate since the 1960s and 1970s has been decreased due to an improved technologies and medical care [2]. Thus, maintaining a healthy heart is one of the vital parts of life to overall wellbeing and healthy state, because heart supplies oxygen and nutrition throughout the body. Oxidative stress results in severe pathology and progress of the cardiac disorders [3]. Oxidative stress mainly results from the increased production of Reactive Oxygen Species (ROS) and reduced cellular antioxidant activity, which results in membrane lipid peroxidation and mitochondrial dysfunction [4]. Besides, it was reported that continued oxidative stress leads to programmed cell death (apoptosis) *via* the mitochondrial pathway that results in the loss of functional cardiomyocytes. Therefore, most of the cardiac treatment strategies focused on the prevention of oxidative stress, which is highly beneficial for patients with cardiovascular disorders [4].

Rat cardiomyocytes cell line (H9c2) has been scientifically reported as one of the best model to study the protective effect of any test compound against H₂O₂ induced oxidative stress [5,6]. Cardioprotective effects of compounds are exerted by enhancing the viability of cells

against oxidative damage. In addition to, ROS play an important role in the pathogenesis of cardiovascular diseases by reduction of cellular antioxidant activity, leading to membrane lipid peroxidation and mitochondrial dysfunction. Enhanced apoptosis of cells is essential event in myocardial cell death during oxidative stress. Lipid peroxidation leads to structural and functional changes in the cellular membrane during oxidative stress. Creatine kinase-myocardial band (CK-MB) and lactate dehydrogenase (LDH) are key biomarkers of cell damage in cardiac health [7]. Thus, cardiac protection using some alternative treatment approach is the need of an hour, which would reduce the mortality rate and improve the quality of life.

Complementary and Alternative Medicine (CAM) approach include various kinds of energy medicines; among which Putative Energy Fields (also known as Biofield) have been reported with significant clinical outcomes in many fields [8,9]. Energy Therapies are based on the concept that human can harness a subtle form of energy and transmit it into living and non-living objects. This Consciousness Energy Healing Treatment included a life force, which has been known under different names such as prana, etheric energy, fohat, orgone, odic force, mana, and homeopathic resonance. The National Center of Complementary and Integrative Health (NCCIH) has recognized and allowed Biofield Energy as a CAM against various treatment approaches. However, some vital energy therapies or CAM therapies are commonly known as meditation, Qi Gong, homeopathy, polarity therapy, relaxation techniques, Tai Chi, pranic healing, deep breathing, chiropractic/osteopathic manipulation, guided imagery, massage, hypnotherapy, healing touch, progressive relaxation, pilates, mindfulness, acupuncture, acupuncture, movement therapy, special diets, Rolfing structural integration, and traditional Chinese herbs treatment [10,11].

The Trivedi Effect[®], one of the best Biofield Energy Healing Treatment worldwide has been proofed with significant scientific outcomes. The Trivedi Effect[®] has been reported with a significant revolution in the field of cancer research [12,13], materials science [14-16], microbiology [17-19], agricultural science [20,21], nutraceuticals [22,23], biotechnology [24,25], improved bioavailability of various compounds [26-28], enhanced skin health [29,30], bone health [31-33], human health, and wellness. Hence, the present study was conducted for *in vitro* assessment of Biofield Energy Treatment on test samples on cardiac health using H9c2 cells *via* multi-

parametric analysis. The restoration of cell viability, LDH and CK-MB levels against *t*-BHP induced damage was assessed, which reflected the cardio-protective potential, while inhibition of ROS, apoptosis and lipid peroxidation (LPO) was also evaluated using standard analysis methods.

Materials and Methods

Chemicals and Reagents

Trimetazidine (TMZ) was procured from Zliesher Nobel, USA. N-acetyl cysteine (NAC), 2',7'-dichlorofluorescein diacetate (DCFDA), 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT), *tert*-Butyl hydroperoxide (*t*-BHP), and ethylenediaminetetraacetic acid (EDTA) were obtained from Sigma Chemical Co. (St. Louis, MO). Annexin-V kit was purchase from Guava Technologies, USA. Creatine kinase-myocardial band (CK-MB) and lactate dehydrogenase (LDH) kits were obtained from Biovision, USA. Antibiotics solution (penicillin-streptomycin) was purchased from HiMedia, India. Dulbecco's Modified Eagle Medium (DMEM) and fetal bovine serum (FBS) were obtained from Gibco, India. All the other chemicals used in this experiment were analytical grade procured from India.

Biofield Energy Healing Strategy

The test item (DMEM) was divided into three parts. First part was considered as the untreated DMEM group, where no Biofield Treatment was given. While, the untreated DMEM group was treated with "sham" healer for better comparison. The "sham" healer did not have any knowledge about the Biofield. The second part was received one-time Biofield Treatment and referred as the BT-I group and the third part was given two-times Biofield Energy Treatment and defined as the BT-II group. Both the test items (BT-I and BT-II) were received Biofield Energy Healing Treatment (The Trivedi Effect®) under laboratory conditions for ~5 minutes through Alice Branton's unique Biofield Energy Transmission process. 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

The cell viability was performed by MTT assay in H9c2 cell line as per Trivedi D, et al. 2018 with slight modifications [34]. The overall effect of the test samples on the cell viability of H9c2 cells was determined using equation (1):

$$\% \text{ Cell viability} = (100 - \% \text{ Cytotoxicity}) \dots \dots \dots (1)$$

Where, % Cytotoxicity = $\{(\text{O.D. of control cells} - \text{O.D. of cells treated with test item}) / \text{OD of control cells}\} * 100$

For positive control, concentrations resulting in $\geq 70\%$ cell viability were taken as safe/non-cytotoxic for cytokine estimation.

Evaluation of the cytoprotective effect of the test item

The cytoprotective activity of the test samples in H9c2 cells were performed as per Trivedi D, et al. 2018 with slight modifications [34]. The protective effect of the test item on the survival of H9c2 cells against *t*-BHP induced damage was determined using equation (2)

$$\left[\frac{A - B}{C - B} \right] * 100 \dots \dots \dots (2)$$

Where, A = O.D. of test item/positive control and *t*-BHP treated cells

B = O.D. of control cells (*t*-BHP alone)

C = O.D. of untreated cells

Estimation of Lactate Dehydrogenase (LDH)

H9c2 cells were trypsinized and a single cell suspension of cell density of 0.12×10^6 cells/well/500 μL was prepared using an hemocytometer in DMEM and 10% FBS in 48-well plates. The cells were incubated in a CO₂ incubator for 24 hours at 37°C, 5% CO₂, and 95% humidity. After 24 hours, the cell medium was removed and the following treatments were given. The experimental test sample groups *i.e.* BT-I and BT-II (450 μL of Biofield Energy Treated DMEM), positive control, (trimetazidine) group (400 μL of SFM), and untreated DMEM group (500 μL of SFM) were added to the respective wells and incubate for 24 hours. After that, cells were treated with 300 μM of *t*-BHP (50 μL from the respective 10X stock) for 2.5 hours. The supernatants were collected from each well and stored at -20°C till analyzed. Further, the LDH activity was estimated in the culture supernatants using lactate dehydrogenase activity

colorimetric assay kit as per the manufacturer's instructions [35]. LDH activity (nMoles/min/mL) was determined and the protective effect of test item was calculated using Equation (3):

$$\left[\frac{A - B}{A - C} \right] * 100 \dots \dots \dots (3)$$

Where, A = LDH activity in control cells (*t*-BHP alone)
B = LDH activity in test items/positive controls and *t*-BHP treated cells
C = LDH activity in untreated cells

Estimation of CK-MB

The cells were trypsinized and a single cell suspension of H9c2 was prepared, which were seeded at a density of 0.12×10^6 cells/well/500 μ L in DMEM + 10 % FBS in 48-well plates using an hemocytometer. The cells were incubated in a CO₂ incubator for 24 hours at 37°C, 5% CO₂, and 95 % humidity. After 24 hours, medium was removed and treatments as per the experimental groups were given followed by incubation for 24 hours. After incubation for 24 hours, cells were treated with 300 μ M of *t*-BHP (50 μ L from the respective 10X stock) for 2.5 hours. The supernatants were collected from each well and stored at -20°C till analyzed. The estimation of CK-MB in culture supernatants was done using creatine kinase activity colorimetric assay kit as per the manufacturer's instructions [36]. CK-MB activity (nMoles/min/mL) was determined and protective effect of test item on CK-MB activity was calculated using equation (4):

$$\left[\frac{A - B}{A - C} \right] * 100 \dots \dots \dots (4)$$

Where, A = CK-MB activity in control cells (*t*-BHP alone)
B = CK-MB activity in test items/positive controls and *t*-BHP treated cells
C = CK-MB activity in untreated cells

Assessment of Reactive Oxygen Species (ROS)

Cells were trypsinized and a single cell suspension of H9c2 was prepared. Then, the cells were counted with the help of an hemocytometer and seeded (at a density of 20×10^3 cells/well/180 μ L in DMEM + 10 % FBS) in 96-well plates. Cells were incubated in a CO₂ incubator for 24 hours at 37°C, 5 % CO₂, and 95 % humidity. Then, medium was removed and treatments were given. About 180 μ L of the test item, 160 μ L of SFM, 180 μ L of SFM, and 200 μ L of SFM was added to wells of test items, positive controls, *t*-

BHP *per se*, and untreated DMEM groups, respectively and incubated for 24 hours. After incubation for 24 hours, cells were stained with DCFDA and washed the wells once with Hank's Balanced Salt Solution (HBSS) + 2% FBS solution and 180 μ L of SFM was added to each well [37]. Protective effect of the test samples on ROS activity was calculated using Equation (5):

$$[(A - B)/(A - C)] * 100 \dots \dots \dots (5)$$

Where, A = Mean FU in Control cells (*t*-BHP alone)
B = Mean FU in test samples/positive control + *t*-BHP treated cells
C = Mean FU in untreated cells

Effect of Test Samples on Apoptosis

H9c2 cells were trypsinized and were counted for seeding using an hemocytometer at a density of 0.25 million/well/1 mL in DMEM along with 10% FBS in 96-well plates. Further, the cells were incubated in a CO₂ incubator for 24 hours at 37°C, 5% CO₂, and 95% humidity. After 24 hours, medium was removed and the following treatments were given. The test sample group received 900 μ L (BT-I and BT-II), positive control (N-acetyl cysteine) group received 800 μ L of SFM, *t*-BHP group received 900 μ L of SFM, and the untreated DMEM group provided 1 mL of SFM to the corresponding wells and incubate for 24 hours. After that, cells were treated with 300 μ M of *t*-BHP (100 μ L from the respective 10X stock) for 2.5 hours. Then, the cells were stained with Annexin reagent for apoptotic population as follows: cells were gently harvested by trypsinisation into prelabeled centrifuge tubes followed by pelleted and resuspended in 200 μ L of SFM. At 100 μ L of cell suspension was stained with 100 μ L of Annexin reagent for 30 minutes in a dark condition at room temperature. The cells were acquired at flow cytometer (Guava technologies) [38]. The protective effect of the test samples was calculated using Equation (6):

$$\left[\frac{A - B}{A - C} \right] * 100 \dots \dots \dots (6)$$

Where, A = % Apoptotic population in Control (*t*-BHP treated alone)
B = % Apoptotic population in test sample/positive control along with *t*-BHP treated cells
C = % Apoptotic population in untreated cells

Statistical Analysis

Data were expressed as Mean \pm SEM (standard error of mean) of three independent experiments. SigmaPlot

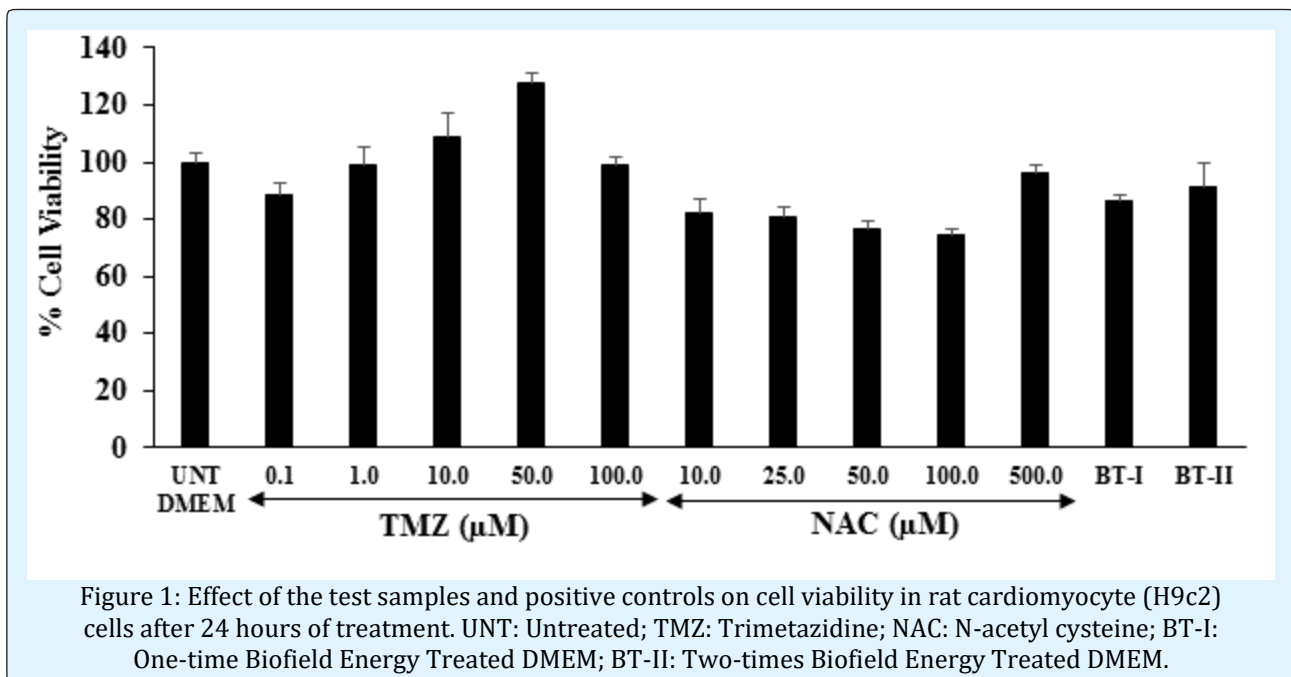
statistical software (v11.0) was used for statistical analysis. For two groups comparison Student's *t*-test was used. For multiple group comparison, one-way analysis of variance (ANOVA) was used followed by post-hoc analysis by Dunnett's test. Statistically significant values were set at the level of $p \leq 0.05$.

Results and Discussion

Cell Viability using MTT Assay

The cell viability of the Biofield Energy Treated and untreated test samples along with positive controls, MTT assay was used in the H9c2 cells and the results are presented in Figure 1. The positive controls, trimetazidine (TMZ) showed cell viability from 88% to 127.75% at the concentration ranges from 0.1 to 100 $\mu\text{g}/\text{mL}$; while N-acetyl cysteine (NAC) showed cell viability with 74% to

96.2% with concentration ranges from 10 to 100 $\mu\text{g}/\text{mL}$. Moreover, the Biofield Energy Treated test items, BT-I (one-time Biofield Energy Treated DMEM) and BT-II (two-times Biofield Energy Treated DMEM) showed 86.58% and 91.37% cell viability, respectively (Figure 1). Thus, it was suggested that the positive controls and the Biofield Energy Treated test samples were found with more than 76% cell viability, which demonstrated a safe and non-toxic profile of the test substances. Thus, these concentrations were used for the experimental for the assessment of various cardiac parameters. The MTT cell viability assay is a rapid, cost-effective, timesaving, and non-radioactive method as compared with the any others existed cell viability assays. The main principle of MTT assay is based on the cell growth and metabolic activity [39].



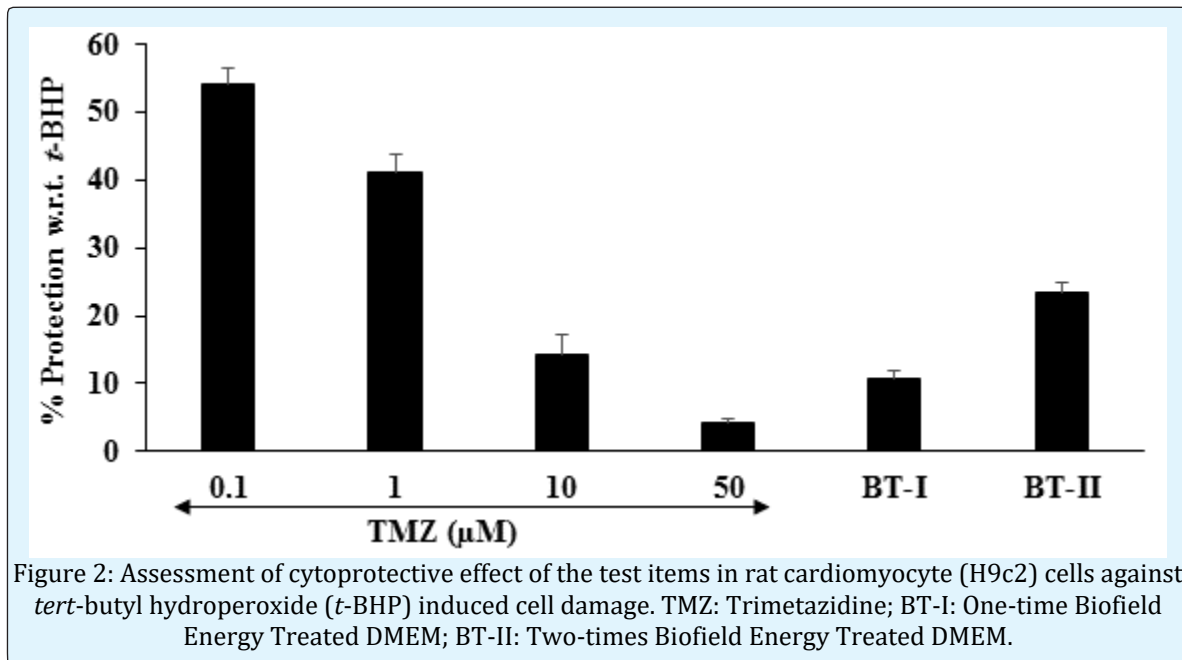
Assessment of Cytoprotective Effect of the Test Item

The cytoprotective properties of the test samples on restoration of cell viability of H9c2 cells were determined against *t*-BHP induced cell damage. The cells were pre-treated with test samples for 24 hours, subsequently damaged with 300 μM of *t*-BHP for 2.5 hours. In order to judge the cell protective action of any test compound *in vitro* cell-based, *tert*-butyl hydroperoxide (*t*-BHP) is considered as one of the best oxidative stress inducer

[40,41]. The cell viability was assessed using MTT assay. The Consciousness Energy Healing based test samples showed a significant cell protective action in the H9c2 cells and the results are presented in Figure 2. Trimetazidine was (TMZ) used as a positive control, which showed a restoration of cell viability by 54.1%, 41.3%, 14.33%, and 4.16% at 0.1, 1, 10, and 50 $\mu\text{g}/\text{mL}$, respectively compared to the *t*-BHP induced group. Besides, the test group's *viz.* one-time Biofield Energy Treated DMEM (BT-I) and two-times Biofield Energy Treated DMEM (BT-II) demonstrated 10.56% and 23.53%

restoration of cell viability, respectively with respect to the *t*-BHP induced group (Figure 2). The cell protection is directly correlated with the protection against oxidative damage to cells, which results in inflammation and leads to several number of diseases [42]. Cell death of cardiomyocytes is the main reason of pathology of ischemia and reperfusion, which results in direct loss of heart function. Thus, in order to protect the cardiac health, most of the research studies focus on the

protection of cardiomyocytes from the cytotoxic stimuli, mainly the oxidative stress [43,44]. Besides, oxidative stress and cell death also results in age-related disorders such as diabetes, cardiovascular, autoimmune diseases, and cancer [45,46]. Thus, Biofield Energy Healing Treatment could be the best treatment approach for cellular cytoprotection in cardiac health and other associated autoimmune disorders.



Estimation of Lactate Dehydrogenase (LDH)

The test samples were tested for the level of lactate dehydrogenase (LDH) activity in H9c2 cells was determined against *t*-BHP induced cell damage, while the results are presented in Figure 3. The cells were pre-treated with test samples for 24 hours, subsequently damaged with 300 μ M of *t*-BHP for 2.5 hours. LDH activity was measured in culture supernatants by using colorimetric kit. The level of LDH activity was significantly ($p \leq 0.001$) increased by 892.05% in the *t*-BHP induced group as compared to the untreated DMEM group. Further, the positive control, trimetazidine (TMZ) showed a significant reduction of LDH level by 8.04%, 18.64%, and 96.13% ($p \leq 0.001$) at 0.1, 10, and 50 μ M, respectively compared to the *t*-BHP induced group. Results of the test substance treatment groups, showed that the one-time Biofield Energy Treated DMEM (BT-I) and two-times

Biofield Energy Treated DMEM (BT-II) groups showed 54.43% and 53.52% significant ($p \leq 0.001$) reduction of the level of LDH, respectively with respect to the *t*-BHP induced group (Figure 3). Thus, it is demonstrated that the Biofield Treated test items effectively reduced LDH leakage and reactive oxygen species (ROS) release in *t*-BHP treated cells, reflecting its ability to reduce *t*-BHP-induced cell injury. LDH, is a tetrameric cytoplasmic enzyme which is present in all the body tissues and its alteration results in heart attack and liver dysfunction along with other diseases such as muscle trauma, cancers, bone fractures, anemia, which results in infections like meningitis, encephalitis, and HIV [47-49]. Biofield Energy Healing Treatment suggested significant protection of LDH enzyme against *t*-BHP damage in H9c2 cells. Thus, Biofield Energy Treatment would be the best alternative approach for cardiac health.

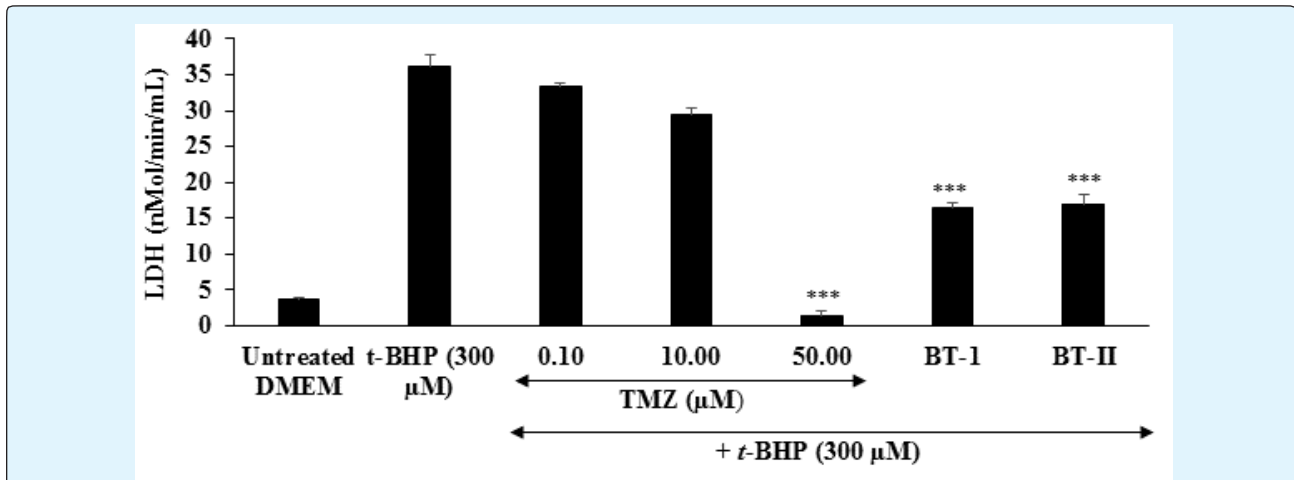


Figure 3: The effect of the test samples on lactate dehydrogenase (LDH) against *tert*-butyl hydroperoxide (*t*-BHP) induced cell damage in rat cardiomyocyte (H9c2) cells. TMZ: Trimetazidine; BT-I: One-time Biofield Energy Treated DMEM; BT-II: Two-times Biofield Energy Treated DMEM. *** $p \leq 0.001$ vs. *t*-BHP induced group.

Estimation of Creatine Kinase-Myocardial Band (CK-MB)

The effect of the Biofield Treated test items on cardiac marker, creatine kinase-myocardial band (CK-MB) is shown in Figure 4. The level of CK-MB was significantly increased by 495.61% in the *t*-BHP induced group as compared to the untreated DMEM group. Moreover, the positive control, N-acetyl cysteine (NAC) showed 4.86%, 24.00%, and 90.57% ($p \leq 0.001$) inhibition of CK-MB enzyme activity at 25, 50, and 100 μM, respectively in a concentration-dependent manner compared to the *t*-BHP induced group. Besides, the experimental groups like one-time Biofield Treated DMEM (BT-I) and two-times

Biofield Energy Treated DMEM (BT-II) showed 63.48% and 60.68% significant ($p \leq 0.001$) inhibition of CK-MB enzyme level as compared to the *t*-BHP induced group (Figure 4). CK-MB is one of the important cardiac biomarker, which are highly significant in perioperative and postoperative morbidity and mortality in cardiac surgery and also used in diagnosis of an acute myocardial infarction. These biomarkers directly alter the cardiac health and affect the quality of life [50,51]. Overall, the experimental data suggested that the Biofield Energy Treated test significantly inhibited the levels of cardiac tissue-specific enzyme CK-MB, reflects the protection effect against *t*-BHP in H9c2 cells.

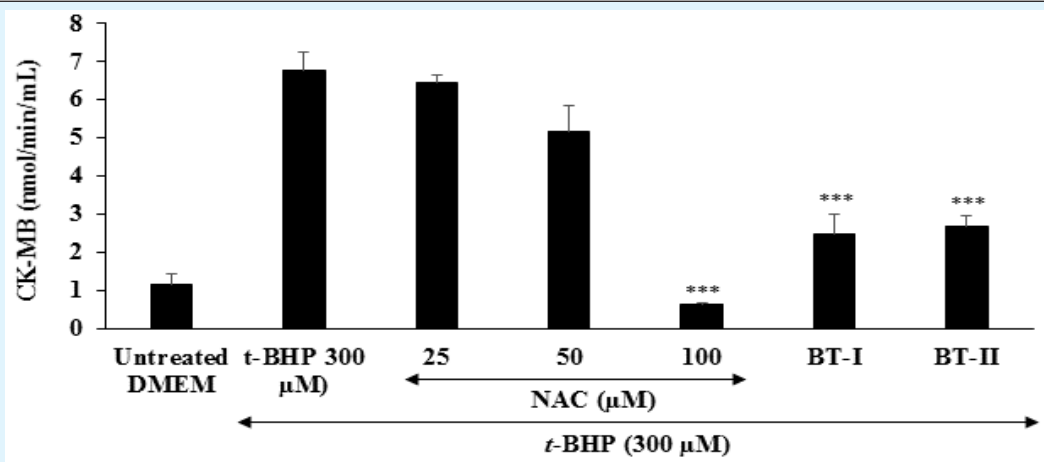
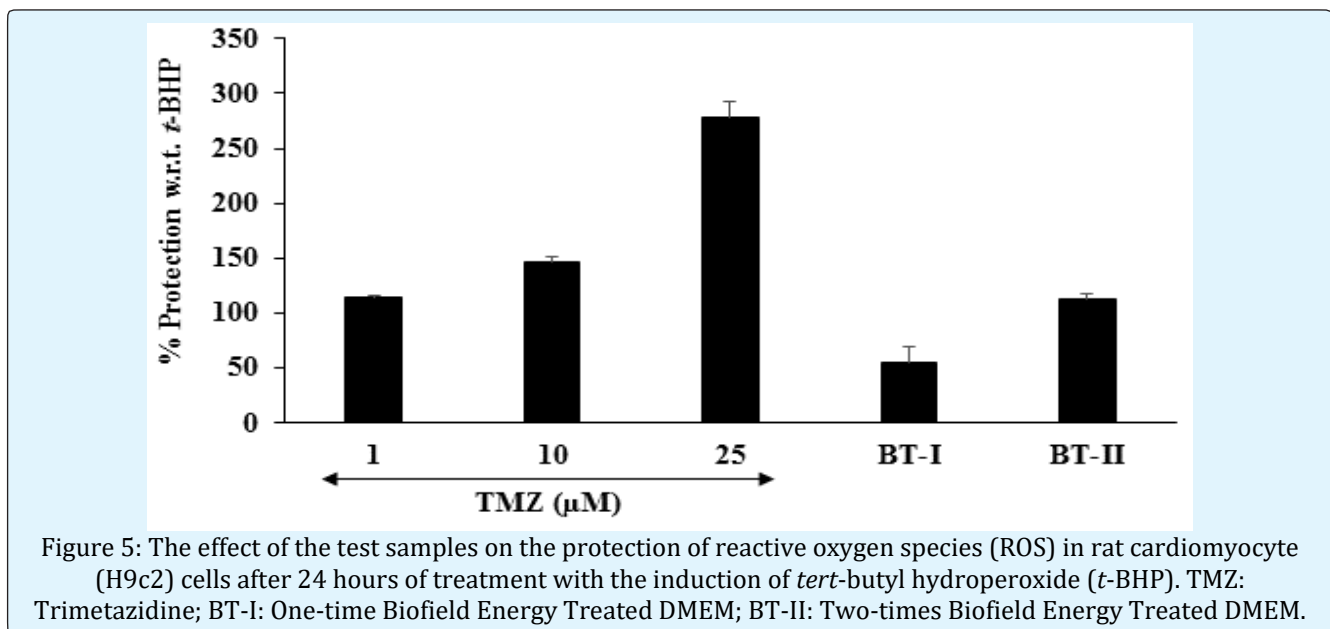


Figure 4: The effect of the test samples on Creatine Kinase-Myocardial Band (CK-MB) activity against *tert*-butyl hydroperoxide (*t*-BHP) induced damage after 4 hours of treatment in rat cardiomyocyte (H9c2) cells. NAC: N-acetyl cysteine; BT-I: One-time Biofield Energy Treated DMEM; BT-II: Two-times Biofield Energy Treated DMEM. *** $p \leq 0.001$ vs. *t*-BHP induced group.

Measurement of Reactive Oxygen Species (ROS)

The effect of test samples on the level of reactive oxygen species (ROS) in H9c2 cells was determined against *t*-BHP induced cell damage. The cells were pre-treated for 24 hours, subsequently damaged with 300 μ M of *t*-BHP and the fluorescence in samples was measured for ROS estimation. The treatment of cells with *t*-BHP (300 μ M) resulted a significant increase the level of ROS as compared to the untreated cells. The BT-I group demonstrated protective effect by inhibition of ROS level by 55.6% as compared to the *t*-BHP induced group. Similarly, the BT-II group demonstrated protective effect by inhibition of ROS levels by 112.8% as compared to the *t*-BHP induced group. However, the positive control,

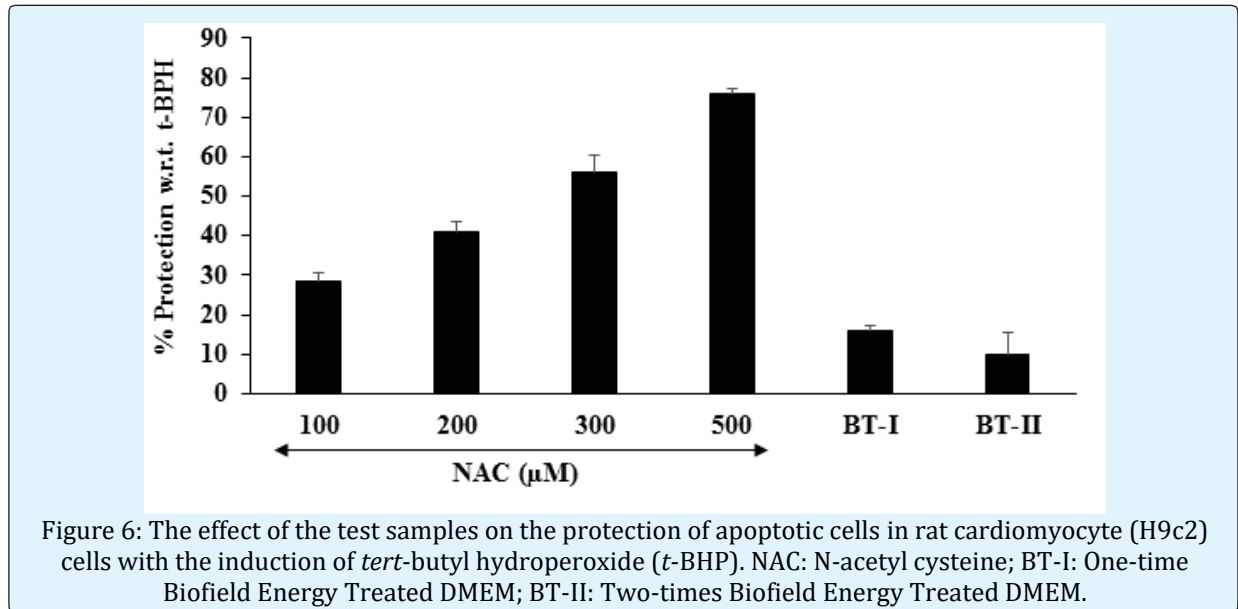
trimetazidine (TMZ) at the concentration ranges from 1 μ M to 50 μ M demonstrated protective effect by inhibition of ROS level by 115.1% to 287% as compared to the *t*-BHP induced group. ROS is one of the important key hallmark for cardiovascular disorders, which leads to high mortality [52]. ROS contains the oxygen and most of them have unpaired electrons such as superoxide anion ($O_2^{\cdot-}$), hydroxyl radical ($OH^{\cdot-}$), and lipid radicals. These play a vital role to develop cardiac disorders along with other diseases [53]. Thus, antioxidant mechanism is constantly working in body to balance the free radicals and scavenge them, which protect the cell and organs to damage (Figure 5) [54,55].



Effect of Test Item on Apoptosis

The effect of test samples on apoptosis in H9c2 cells was determined against *t*-BHP induced cell damaged with 300 μ M of *t*-BHP for 2.5 hours. The percentage of apoptotic cells was determined using standard staining procedure with flow cytometry. The treatment of cells with *t*-BHP (300 μ M) resulted an enhanced apoptosis as compared to the untreated cells. The effect of the test items on the inhibition of apoptic cells is shown in Figure 6. The one-time Biofield Energy Treated test item (BT-I) group demonstrated protective effect by inhibition of apoptosis by 16% as compared to the *t*-BHP induced group. The two-times Biofield Energy Treated test item (BT-II) group demonstrated protective effect by inhibition of apoptosis by 10% as compared to *t*-BHP induced group.

NAC as a positive control in the concentration range of 100, 200, 300, and 500 μ M demonstrated protective effect by inhibition of apoptosis by 28.5%, 41%, 56%, and 76%, respectively as compared to the *t*-BHP induced group. Apoptosis is a programmed cell death, which plays an important role in different cardiovascular diseases like heart failure, myocardial infarction, and reperfusion injury results in high morbidity and mortality [56]. Various scientific reports suggested that apoptotic management is one of the treatment methods to control the cell death in cardiovascular disorders [57,58]. Biofield Energy Treatment- The Trivedi Effect[®] is one of the best alternative treatment strategies to control the cell death, as the data suggest protection against *t*-BHP-induced apoptotic cells.



Conclusions

Cardiovascular action of the test samples after Biofield Energy Treatment suggested significant cardiac protection using various biomarkers in rat cardiomyocytes cell line (H9c2). The test samples were found to be safe and non-toxic in cell viability assay using MTT test with more than 86% viable cells. Besides, cellular protection data showed that one-time Biofield Energy Treated test item (BT-I) and two-times Biofield Energy Treated (BT-II) showed 10.56% and 23.53% cytoprotective activity, respectively as compared to the *t*-BHP induced group. Further, the level of LDH was significantly suppressed by 54.43% in the BT-I group and 53.52% in the BT-II group as compared to the *t*-BHP induced group. The cardio-specific enzyme, creatine kinase-myocardial band (CK-MB) was significantly inhibited by 63.48% and 60.68% in the BT-I and BT-II groups, respectively compared to the *t*-BHP induced group. Moreover, the percent protection of reactive oxygen species (ROS) was found to be 55.6% and 112.8% in the BT-I and BT-II groups, respectively, compared to the *t*-BHP induced group. Apoptotic activity in H9c2 cells were significantly inhibited by 16% and 10% in the BT-I and BT-II groups, respectively compared to the *t*-BHP induced group. Overall, The Trivedi Effect® - Consciousness Energy Healing Treatment as a complementary and alternative treatment approach significantly improved all the vital cardiac biomarkers, which suggest its application in cardiac protection and cellular damage. Thus, it can be used for the prevention of

various types of cardiac disorders such as stroke, heart attack, high blood pressure (hypertension), congenital heart disease, rheumatic heart disease, congestive heart failure (CHF), peripheral artery disease, valvular heart disease, carditis, thromboembolic disease, venous thrombosis, and many more. Moreover, the Consciousness Energy Healing Treatment could able to improve cell growth, differentiation, proliferation, neurotransmission, skin health, and communication from one cell to another cell. Besides, it can also be utilized against Pernicious Anemia, Parkinson's disease, Dermatitis, Aplastic Anemia, Multiple Sclerosis, Myasthenia Gravis, Asthma, Irritable Bowel Syndrome, Hepatitis, Diabetes, Diverticulitis, Hashimoto Thyroiditis, Sjogren Syndrome, Atherosclerosis, and many more.

Acknowledgements

Authors gratefully acknowledged to Trivedi Global, Inc., Trivedi Science, Trivedi testimonials and Trivedi master wellness for their support. In addition, authors are thankful for the support of Dabur Research Foundation for conducting this study.

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