

***In vitro* Assessment of the Biofield Treated Test Item on Cardiac Function Using Rat Cardiomyocytes Cell Line (H9c2) via Multiparametric Analysis**

Mahendra Kumar Trivedi¹, Snehasis Jana^{2,*}

¹Trivedi Global, Inc., Henderson, Nevada, USA

²Trivedi Science Research Laboratory Pvt. Ltd., Bhopal, Madhya Pradesh, India

Abstract

Introduction: Heart disorders are the major concern of population health worldwide. According to WHO estimates 2018, 17.9 million peoples were died due to cardiovascular disorders.

Aim: The aim of this study was to investigate the cardioprotective activity of Biofield Energy Treated test item, Dulbecco's Modified Eagle Medium (DMEM) using rat cardiomyocytes (H9c2).

Methods: The test item (DMEM) was divided into three parts, first part received one-time Biofield Energy Treatment by a renowned Biofield Energy Healer, Mahendra Kumar Trivedi and was labeled as the one-time Biofield Energy Treated (BT-I) DMEM, while second part received the two-times Biofield Energy Treatment and is denoted as BT-II DMEM. The third part did not receive any treatment and defined as the untreated DMEM group.

Results: Cell viability of the test samples by 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay showed 89.03% and 98.49% in the BT-I and BT-II groups, respectively suggested a nontoxic and safe in nature of the tested test item. The BT-I group showed 16.01% restoration of cell viability. The level of lactate dehydrogenase (LDH) was significantly inhibited by 50.37% and 49.35% in the BT-I and BT-II groups, respectively compared to the untreated DMEM group. Moreover, percent protection of creatine kinase-myocardial band (CK-MB) by 49.48% and 59.79% in the BT-I and BT-II groups, respectively, compared to the untreated DMEM group. Reactive oxygen species (ROS) level in terms of mean fluorescence unit (FU) was reduced by 6.64% in the BT-I group than untreated DMEM. Besides, BT-I and BT-II groups significantly increased the level of % apoptotic cells by 63.16% and 97.37% ($p \leq 0.05$), respectively than untreated DMEM.

Conclusion: Allover, results envisaged that Biofield Treatment significantly improved different cardiac parameters. Thus, Biofield Energy Treatment (The Trivedi Effect[®]) could be utilized as a cardio-protectant against several cardiac disorders such as coronary artery disease, heart attack, arrhythmias, heart failure, congenital heart disease, cardiomyopathy, etc.

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

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

Received: Jan 03, 2019

Accepted: Feb 11, 2019

Published: Feb 15, 2019

Editor: Osmar Centurion, Professor of Medicine. Asuncion National University. Cardiology Division. First Department of Internal Medicine. Asuncion, Paraguay.

Introduction

Heart disorders are the major concern of population health worldwide. About 6 lakh peoples die due to heart disease in the United States every year; that's 1/4 deaths [1]. Cardiovascular disease (CVD) and stroke produce an immense health and economic burdens in the United States and globally. According to WHO estimates, in 2016, 17.9 million people around the globe die of cardiovascular diseases each year. This represents about 1/3 of all deaths globally [2, 3]. CVD is the leading cause of death in Europe, accounting for over 4 million deaths each year. It has been projected that by 2020, CVD would be more numerous in India and China than in all economically developed countries in the world [4]. Three main criteria to keep a healthy heart like opening blood vessels, strengthening the heart muscle, and controlling free radical damage by antioxidants [5]. Apart from animal models and primary cardiac myocytes derived animal, even recent work has been done to develop human cardiomyocyte model systems for the screening of cardioprotective activity of substances [6]. The use of *in vitro* test model for the prediction of heart damages provides several advantages over *in vivo* assessment. As this model require few animals, test material, and give high accuracy data [7]. Rat cardiomyocytes cell line (H9c2) have been widely used as an alternative model to human cardiomyocytes *in vitro* for the assessment of cardio-protectant properties of any test substances [8].

Various study data suggested the effect of Energy Therapy in cancer patients through therapeutic touch [9], massage therapy [10], 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. Human Biofield Energy has subtle energy that has the capacity to work in an effective manner [11]. CAM therapies have been practiced worldwide with reported clinical benefits in different health disease profiles [12]. This energy can be harnessed and transmitted by the experts into living and non-living things *via* the process of Biofield Energy Healing. Biofield Energy Treatment has been reported with a significant revolution in the field of cancer research [13,14], materials science [15-17], microbiology [18-20], agriculture [21,22], nutraceuticals [23, 24], and biotechnology [25,26]. Besides, The Trivedi Effect[®] also significantly improved bioavailability of various low bioavailable compounds [27-29], an improved overall skin health [30, 31], bone health [32-34], human health and wellness. Based on the excellent contribution of Biofield Energy in wide spectrum of areas, authors intend to extend the treatment modality to study the impact of the Biofield Energy Healing Treatment (The Trivedi Effect[®]) on the test item (DMEM) for cardiomyocytes

cell line (H9c2).

Materials and Methods

Chemicals and Reagents

N-acetyl cysteine (NAC), 2',7'-Dichlorofluorescein diacetate (DCFDA), 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT), and ethylenediaminetetraacetic acid (EDTA) were obtained from Sigma Chemical Co. (St. Louis, MO). 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. Creatine kinase-MB (CK-MB) and lactate dehydrogenase (LDH) kits were obtained from Biovision, USA. Annexin-V kit was purchased from Guava Technologies, USA. The positive control, trimetazidine (TMZ) was procured from Zliesher Nobel, USA. All the other chemicals used in this experiment were analytical grade procured from India.

Biofield Energy Healing Strategy

The test item (DMEM) was used in this experiment and one portion was considered as the untreated group, where no Biofield Treatment was provided. Further, the untreated group was treated with "sham" healer for comparison purpose. The sham healer did not have any knowledge about the Biofield Energy Healing Treatment. The other portion of the test item was received one-time Biofield Energy Treatment and referred as the BT-I and was also given two-times Biofield Energy Treatment and defined as the BT-II. Both the test items (BT-I and BT-II) were received Biofield Energy Healing Treatment (known as The Trivedi Effect®) under laboratory conditions for ~3 minutes through Mahendra Kumar Trivedi'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 cells (ATCC® CRL-1446™). The cells were counted and plated in a 96-well plate at the density corresponding to 10×10^3 cells/well/180 μ L in DMEM + 10% FBS. The cells in the above plate(s) were incubated for 24 hours in a CO₂ incubator at 37°C, 5% CO₂, and 95% humidity. Following incubation, the medium was removed and the following treatments were given. In the test item group, 200 μ L of the test item was added to wells. Besides, in the positive control group, added 180 μ L of SFM with 20 μ L of positive controls were added from the respective 10X stock solutions. After incubation for 48 hours, the effect of test item on cell viability was assessed by MTT assay. 20 μ L of 5 mg/mL of MTT was added to all the wells and incubated at 37°C for 3 hours. The supernatant was aspirated and 150 μ L of DMSO was added to all wells to dissolve formazan crystals. The optical density (OD) of each well was read at 540 nm using Biotek Reader.

Effect of the test items on viability of H9c2 cells was determined using Equation (1):

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

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

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

Evaluation of Cytoprotective Effect of the Test Item

Cells were trypsinized and a single cell suspension of H9c2 was prepared. Cells were counted on an hemocytometer and seeded at a density of 5×10^3 cells/well/180 μ L in DMEM + 10% FBS in a 96-well plate. Cells were incubated in a CO₂ incubator for 24 hours at 37°C, 5% CO₂, and 95% humidity. After 24 hours, the medium was removed and the following treatments were given. In the test item group, 180 μ L of the test item was added to wells. In the positive control group, 160 μ L of SFM and 20 μ L of positive control from the respective 10X stock solution was added to wells. After 24 hours of treatment, cells were treated with *t*-BHP at a final concentration of 250 μ M (20 μ L from the respective 10X stock) for 4 hours. After 4 hours, the protective effect of the test item on cell viability was assessed by MTT assay. The protective effect of the test item on survival of H9c2 cells against *t*-BHP induced

damage was determined using Equation (2)

$$[(A-B)/(C-B)]*100.....(2)$$

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

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

C = O.D. of untreated cells

Estimation of Lactate Dehydrogenase (LDH)

Cells were trypsinized and a single cell suspension of H9c2 was prepared. Cells were counted (using hemocytometer) and seeded (at a density of 0.12×10^6 cells/well/500 μ L) in DMEM + 10 % FBS in 48-well plates. 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 following treatments were given. Test items (BT-I and BT-II) groups (450 μ L of Biofield Treated DMEM), positive controls (trimetazidine and N-acetyl cysteine) groups (400 μ L of SFM), and (untreated DMEM) group (500 μ L of SFM) were added to the respective wells and incubate for 24 hours. After that, cells were treated with 300 μ M of *t*-BHP (50 μ L from the respective 10X stock) for 2.5 hours. Supernatants were collected from each well and stored at -20°C till analyzed. Estimation of LDH in culture supernatants was done using Lactate Dehydrogenase Activity Colorimetric Assay Kit as per manufacturer's instructions. LDH activity (nMoles/min/mL) was determined and the protective effect of test item was calculated using Equation (3):

$$[(A-B)/(A-C)]*100.....(3)$$

Where, A = LDH activity in cells (*t*-BHP alone)

B= LDH activity in test items/positive controls + *t*-BHP induced cells

C = LDH activity in untreated cells

Estimation of CK-MB

Cells were trypsinized and a single cell suspension of H9c2 was prepared. Cells were counted on a hemocytometer. Cells were seeded at a density of 0.12×10^6 cells/well/500 μ L in DMEM + 10 % FBS in 48-well plates. 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 were given.

Test items (BT-I and BT-II) groups (450 μ L of

Biofield Treated DMEM), positive control (N-acetyl cysteine) group (400 μ L of SFM), *t*-BHP *per se* group (450 μ L of SFM), and negative control (untreated) group (500 μ L of SFM) were added to the respective wells and incubate 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. Supernatants were collected from each well and stored at -20°C till analyzed. Estimation of CK-MB in culture supernatants was done using Creatine Kinase Activity Colorimetric Assay Kit as per manufacturer's instructions. CK-MB activity (nMoles/min/mL) was determined and protective effect of test item on CK-MB activity was calculated using Equation (4):

$$[(A-B)/(A-C)]*100.....(4)$$

Where, A = CK-MB activity in cells (*t*-BHP alone)

B= CK-MB activity in test items/positive controls + *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 a 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 (TI), 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 incubate 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. Protective effect of TI on ROS activity was calculated using Equation (5):

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

Where, A = Mean FU in Control cells (*t*-BHP alone)

B= Mean FU in TI/positive control + *t*-BHP treated cells

C = Mean FU in untreated cells

Effect of Test Item on Apoptosis

Cells were trypsinized and a single cell suspension of H9c2 was prepared. Cells were counted using hemocytometer and seeded at a density of 0.25 million/well/1 mL in DMEM + 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 TI group received 900 µL of Tis (BT-I and BT-II), positive control (N-acetyl cysteine) group received 800 µL of SFM, t-BHP group received 900 µL 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 µM of t-BHP (100 µL 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 µL of SFM. At 100 µL of cell suspension was stained with 100 µL of Annexin reagent for 30 minutes in a dark condition at room temperature. Cells were acquired at flow cytometer (Guava technologies). The protective effect of the TI was calculated using Equation (6):

$$[(A-B)/(A-C)]*100.....(6)$$

Where, A = % Apoptotic population in t-BHP

B = % Apoptotic population in test items/positive control + t-BHP treated cells

C = % Apoptotic population in untreated cells

Statistical Analysis

All the values were represented as Mean ± SEM (standard error of mean) of three independent experiments. The statistical analysis was performed using SigmaPlot statistical software (v11.0). For two groups comparison student's t-test was used. For multiple group comparison, one-way analysis of variance (ANOVA) was used followed by post-hoc analysis by Dunnett's test. Statistically significant values were set at the level of $p \leq 0.05$.

Results and Discussion

Cell Viability Using MTT Assay

Evaluation of cell viability after treatment with the positive controls and the test items in H9c2 cells is shown in Figure 1. The cardioprotective activity of Biofield already been published by Branton A, 2019 [35]. The positive controls, trimetazidine (TMZ) showed more than 88% at the concentrations between 0.1 to 100 µg/mL and N-acetyl cysteine (NAC) showed greater than 74% cell viability upto 500 µg/mL. Besides, the Biofield Energy Treated test items, BT-I (one-time Biofield Energy Treated DMEM) and BT-II (two-times Biofield Energy Treated DMEM) showed 89.03% and 98.49% cell viability, respectively. Overall, the Biofield Energy Treated test items found as a safe and non-toxic profile of the test substances and further used in this

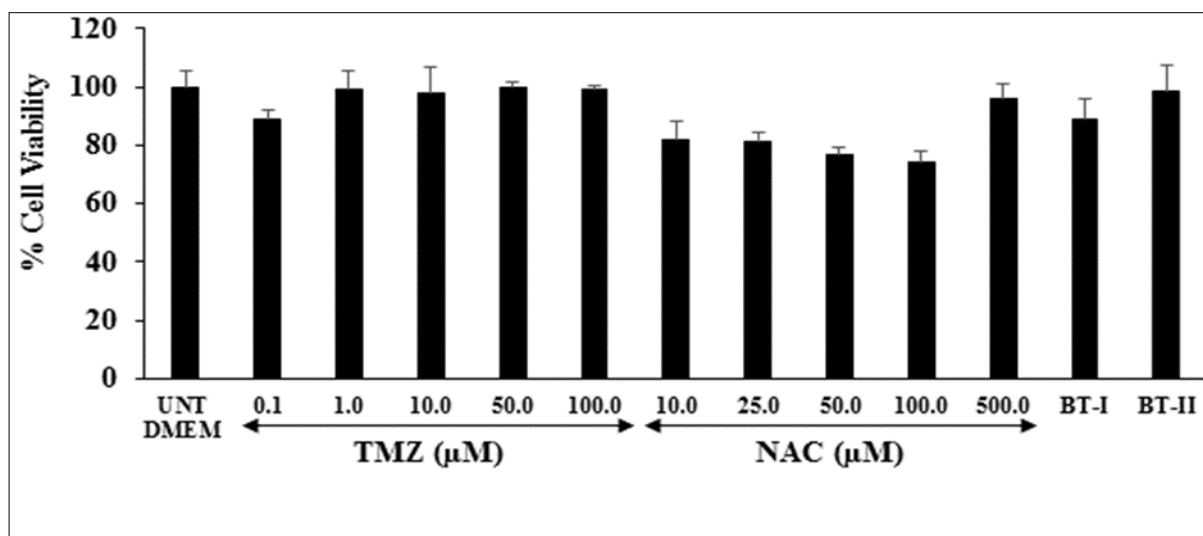


Figure 1. Effect of the test items and positive controls on cell viability in H9c2 cells after 24 hours of treatment. UNT: Untreated; TMZ: Trimetazidine; NAC: N-acetyl cysteine; BT-I: One-time Biofield Energy Treated DMEM; BT-II: Two-times Biofield Energy Treated DMEM

experiment for the assessment of various cardiac parameters.

Evaluation of Cytoprotective Effect of the Test Item

For the assessment of cardioprotective activity of the test compounds, *tert*-butyl hydroperoxide (*t*-BHP) is a well-recognized oxidative stress inducer in the *in vitro* cell-based assays [36, 37]. The cytoprotective activity of the Biofield Energy Treated test items on the restoration of cell viability in H9c2 cells was determined against *t*-BHP induced cell damage and the result is shown in Figure 2. Trimetazidine (TMZ) resulted, restoration of cell viability by 54.1%, 41.3%, 14.33%, and 4.16% at 0.1, 1, 10, and 50 μ M, respectively compared to the *t*-BHP induced group. Besides, the test group's like the one-time Biofield Energy Treated DMEM (BT-I) showed 16.01% and two-times Biofield Energy Treated DMEM exhibited 4.43% restoration of cell viability with respect to the *t*-BHP induced group. The cellular antioxidant capacity can be reduced due to excess production of free radicals that leads to inflammation [38]. This excess levels of free radicals can affect the normal functions of cell membrane, and ultimately altered the genetic materials and cause various age-related disorders such as diabetes, cardiovascular, autoimmune diseases, and cancer [39-41]. The results suggest that Biofield Treatment has significantly protects *t*-BHP induced cardiotoxicity, which could be due to The Trivedi Effect[®].

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

Estimation of Lactate Dehydrogenase (LDH)

The distribution of lactate dehydrogenase (LDH) is mainly abundant in the heart and skeletal muscle, is a tetrameric enzyme, and is mainly responsible for anaerobic respiration of cells [42-44]. The effect of test items on the level of lactate dehydrogenase (LDH) is presented in Figure 3. The level of LDH activity was significantly increased by 892.05% in the *tert*-butyl hydroperoxide (*t*-BHP) induced group as compared to the untreated DMEM group (3.65 ± 0.5 nMol/min/mL). The positive control, trimetazidine (TMZ) exhibited 8.04%, 18.64%, and 96.13% inhibition of lactate dehydrogenase (LDH) compared to the untreated DMEM group. Besides, One-time Biofield Energy Treated DMEM group (BT-I) and two-times Biofield Energy Treated DMEM group (BT-II) group showed 50.37% and 49.35%, respectively as compared to the untreated DMEM group.

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

The impact of the Biofield Energy 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 4.96% in the *tert*-butyl hydroperoxide (*t*-BHP) induced group as compared to the untreated DMEM group (1.14 ± 0.16 nMol/min/mL).

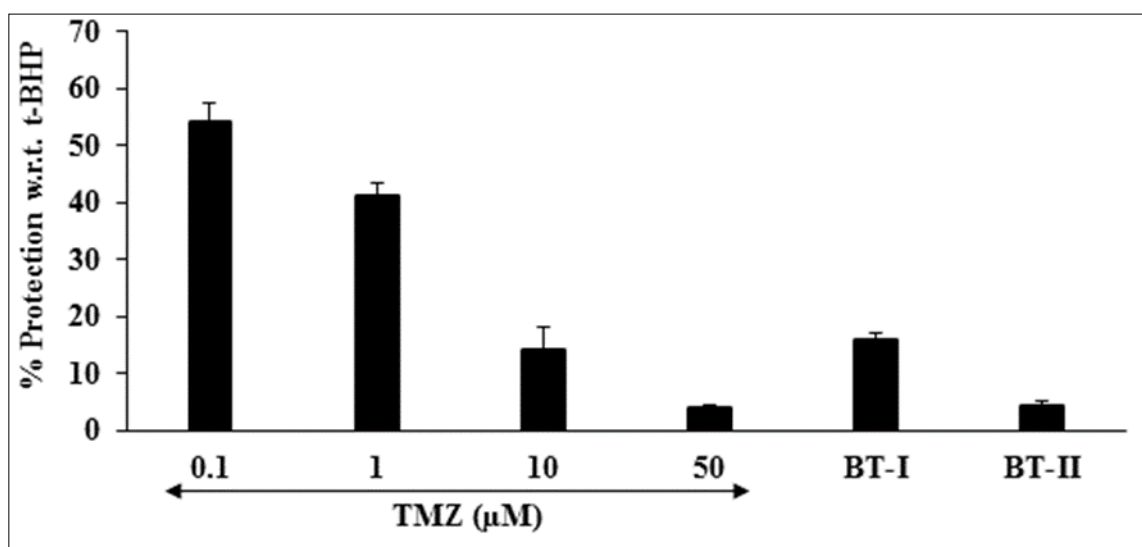


Figure 2. Assessment of cytoprotective effect of the test items in H9c2 cells against *tert*-butyl hydroperoxide (*t*-BHP) induced damage. TMZ: Trimetazidine; BT-I: One-time Biofield Energy Treated DMEM; BT-II: Two-times Biofield Energy Treated DMEM

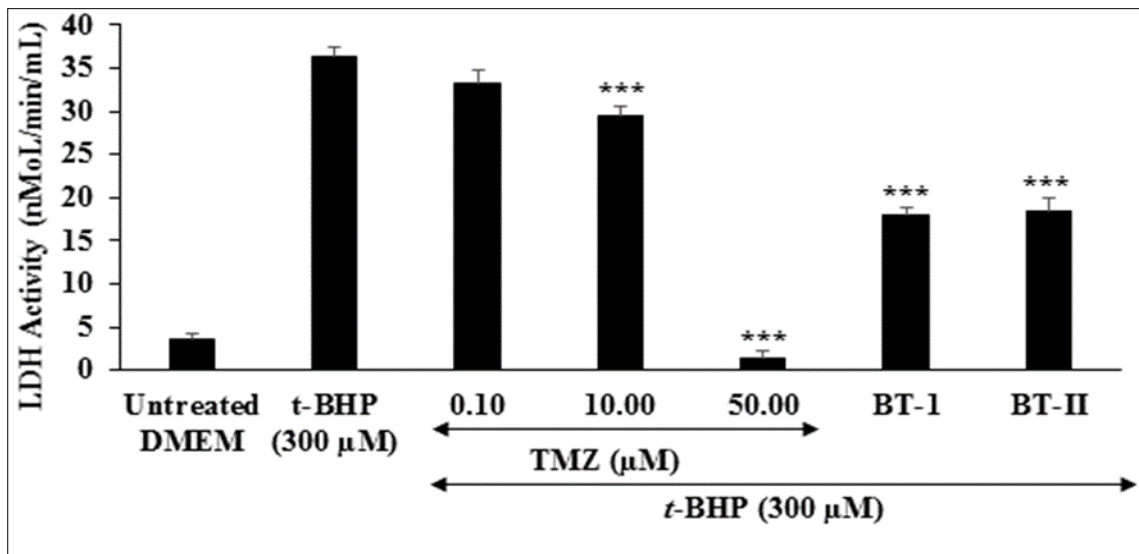


Figure 3. The effect of the test items on lactate dehydrogenase (LDH) against *tert*-butyl hydroperoxide (*t*-BHP) induced damage. TMZ: Trimetazidine; BT-I: One-time Biofield Energy Treated DMEM; BT-II: Two-times Biofield Energy Treated DMEM.

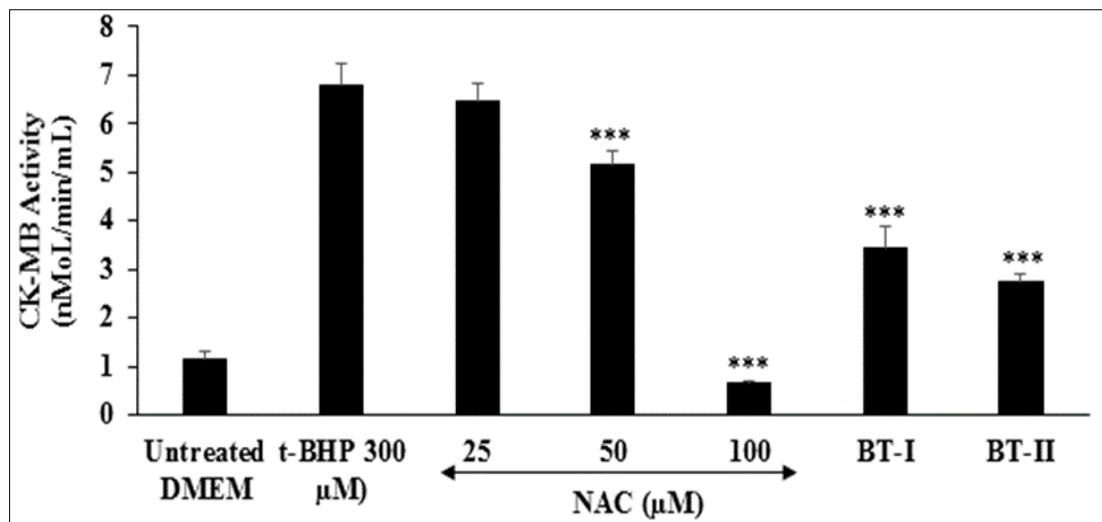


Figure 4. The effect of the test items (24 hours of pretreatment) on Creatine Kinase-Myocardial Band (CK-MB) activity against *tert*-butyl hydroperoxide (*t*-BHP) induced damage after 4 hours of treatment. NAC: N-acetyl cysteine; BT-I: One-time Biofield Energy Treated DMEM; BT-II: Two-times Biofield Energy Treated DMEM. *** $p \leq 0.001$ vs. *t*-BHP at 300 μM.

The positive control, N-acetyl cysteine (NAC) showed 4.86%, 24% ($p \leq 0.001$), and 90.57% ($p \leq 0.001$) significant inhibition of CK-MB enzyme activity in a concentration-dependent manner at 25, 50, and 100 μM , respectively compared to *t*-BHP induced group. Further, the Biofield Treated test items group, BT-I (one-time Biofield Energy Treated DMEM) and BT-II (two-times Biofield Energy Treated DMEM) showed significant ($p \leq 0.001$) inhibition of CK-MB enzyme level by 49.48% and 59.79%, respectively as compared to the *t*-BHP induced group. This reduction of tissue-specific cardiac biomarker (CK-MB) is very essential for the diagnosis of cardiac functions apart from of troponin T (cTnI) and myoglobin (Myo) [45, 46]. As CK-MB is a sensitive and specific indicator for the diagnosis of an acute myocardial infarction (AMI) [47]. Overall, the Biofield Treated test items (BT-I and BT-II) has significantly inhibited the levels of cardiac tissue-specific enzyme CKMB, which was induced by *t*-BHP.

Assessment of Reactive Oxygen Species (ROS)

Reactive oxygen species (ROS) play a vital role in the development of cardiovascular disorders specifically atherosclerosis [48]. Due to misbalance of ROS production and antioxidant defense capacity results in oxidative stress [49, 50]. Although ROS is normally

required in cell signaling pathways, while excessive production of ROS leads to cell damage [51]. The number of fluorogenic substrates that serve as hydrogen donors have been used in conjunction with horseradish peroxidase (HRP) enzyme to produce intensely fluorescent products [52]. In this assay, the effect of the Biofield Treated test items on the ROS induced by *t*-BHP in terms of number of fluorescence unit (FU) is shown in Figure 5. The positive control, trimetazidine (TMZ) showed 5.46% reduction of FU at 25 μM as compared to the untreated DMEM group. Further, one-time Biofield Energy Treated DMEM (BT-I) showed 6.64% reduction of mean FU compared to the untreated group. Results found that the BT-I have significantly protect cardiomyocytes from oxidative stress. This inhibition of ROS could be due to the Biofield Energy Treatment through the change in protons and neutrons in the nucleus caused by weak interactions.

Effect of Test Item on Apoptosis

Cardiovascular disorders is the leading cause of morbidity and mortality in the developed world. Apoptosis, a process of programme cell death that plays a vital role in various pathologic conditions related to cardiovascular system [53]. Inhibition of apoptotic pathway is one of the potential treatment approach for

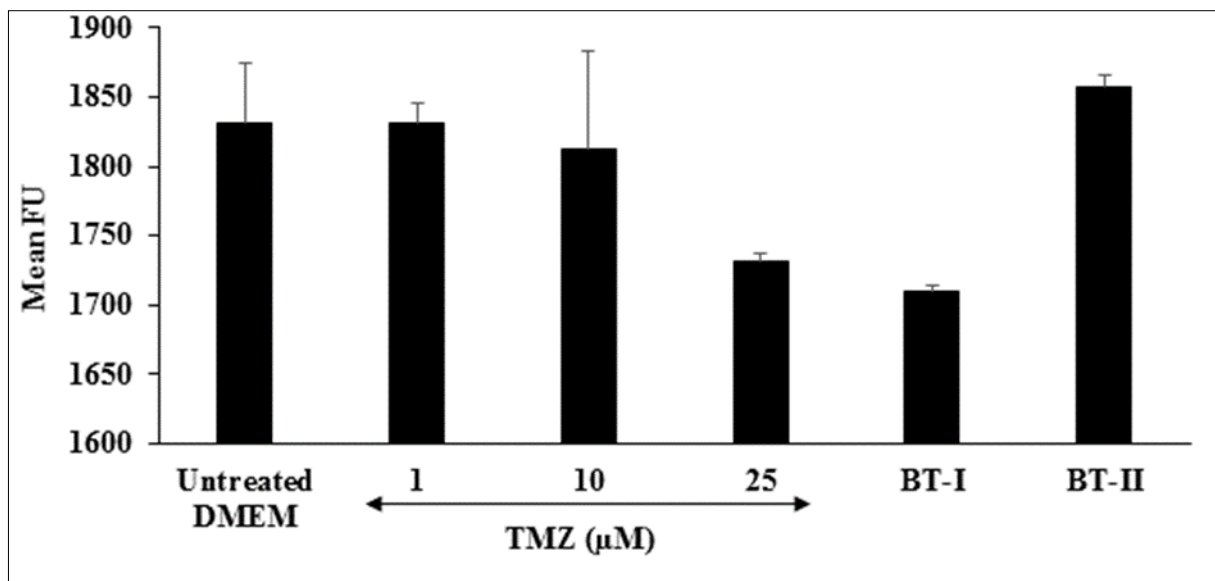


Figure 5. The effect of the test items on reactive oxygen species in terms of fluorescence unit (FU) in H9c2 cells after 24 hours of treatment. FU: Fluorescence unit; TMZ: Trimetazidine; BT-I: One-time Biofield Energy Treated DMEM; BT-II: Two-times Biofield Energy Treated DMEM.

the management of cardiovascular disorders [54, 55]. The apoptotic process ensures that damaged, aged, or excess cells are deleted in a regulated manner that is not harmful to the host [56]. Thus, increased level of percent apoptotic cells is directly link with the overall health. The effect of the test items on the level of percent apoptotic cells is shown in Figure 1. The positive control, N-acetyl cysteine (NAC) was significantly increased the level of percent apoptotic cells by 94.08%, 77.63%, and 57.89% at 100, 200, and 300 μM , respectively compared to the untreated DMEM group. Further, Biofield Treated test items BT-I (one-time Biofield Energy Treated DMEM) and BT-II (two-times Biofield Energy Treated DMEM) showed significantly increased the percent of apoptotic cells by 63.16% and 97.37% ($p \leq 0.05$), respectively compared to untreated DMEM. Overall, results suggested that Biofield Energy can increase the level of percent apoptotic cells, which could be able to remove damaged, unwanted, aged, and excess cells from the body (Figure 6).

Conclusions

The study outcomes showed that the test substances were safe and non-toxic based on MTT cell viability assay with more than 89% viable cells. The one-time Biofield Energy Treated DMEM (BT-I) showed

16.01% cytoprotective activity. Further, increased level of lactate dehydrogenase (LDH) was significantly suppressed by 50.37% in the BT-I group, and 49.35% in the BT-II (two-times Biofield Energy Treated DMEM) group as compared to the untreated DMEM group. The cardio-specific enzyme, creatine kinase-myocardial band (CK-MB) was significantly inhibited by 49.48% and 59.79% in the BT-I and BT-II, respectively compared to the untreated DMEM group. Moreover, Reactive oxygen species (ROS) level in terms of mean fluorescence unit (FU) was reduced by 6.64% in the BT-I group than untreated DMEM. Percent apoptotic cells were significantly increased by 63.16% and 97.37% in the BT-I and BT-II, respectively compared to the untreated DMEM. In conclusion, The Biofield Energy Treatment significantly improved various cardiac parameters and protect cardiomyocytes cells from oxidative damage. Thus, it can be used as a complementary and alternative treatment for the prevention of various types of cardiac disorders *viz.* High blood pressure (hypertension), stroke, congestive heart failure (CHF), peripheral artery disease, congenital heart disease, rheumatic heart disease, valvular heart disease, carditis, thromboembolic disease, and venous thrombosis, etc. Further, it could be useful to improve cell-to-cell messaging, normal cell growth and differentiation, cell cycling and proliferation,

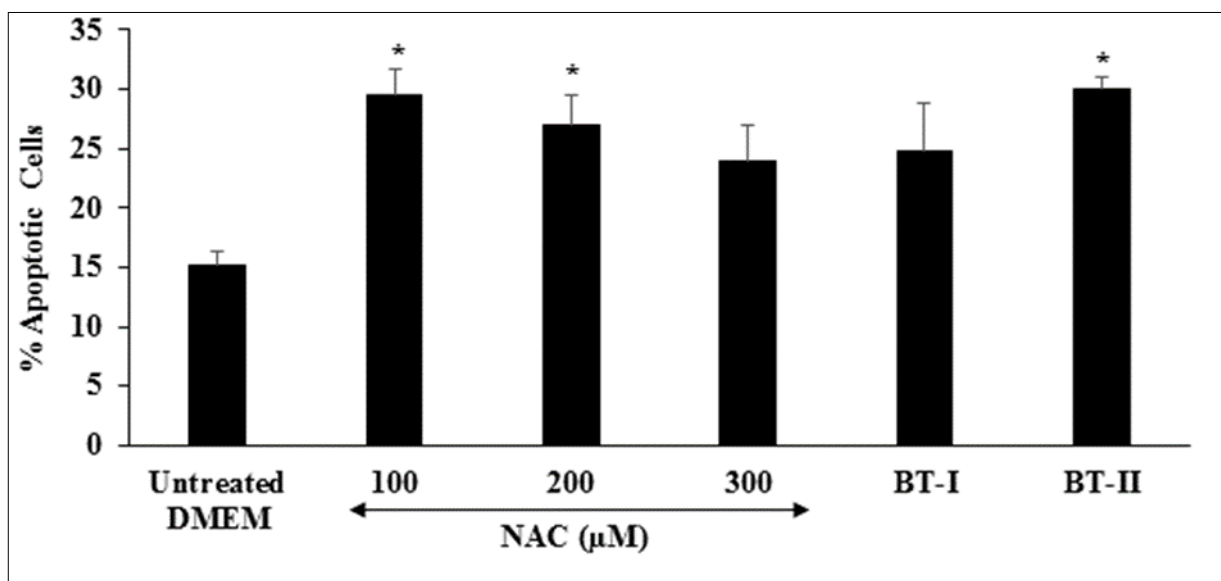


Figure 6. Effect of the test items on percent apoptotic cells in H9c2 cells after 24 hours of treatment. NAC: N-acetyl cysteine; BT-I: One-time Biofield Energy Treated DMEM; BT-II: Two-times Biofield Energy Treated DMEM. * $p \leq 0.05$ vs. untreated DMEM.

neurotransmission, skin health, hormonal balance, immune and cardiovascular functions. Moreover, it can also be utilized in organ transplants (*i.e.*, kidney, liver, and heart transplants), hormonal imbalance, aging, and various inflammatory and immune-related disease conditions like Alzheimer's Disease (AD), Ulcerative Colitis (UC), Dermatitis, Asthma, Irritable Bowel Syndrome (IBS), Hashimoto Thyroiditis, Pernicious Anemia, Sjogren Syndrome, Multiple Sclerosis, Aplastic Anemia, Hepatitis, Graves' Disease, Dermatomyositis, Diabetes, Parkinson's Disease, Myasthenia Gravis, Atherosclerosis, Systemic Lupus Erythematosus (SLE), stress, etc. with a safe therapeutic index to improve overall health and Quality of Life.

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.

References

1. CDC, NCHS. Underlying Cause of Death 1999-2013 on CDC WONDER Online Database, released 2015. Data are from the Multiple Cause of Death Files, 1999-2013, as compiled from data provided by the 57 vital statistics jurisdictions through the Vital Statistics Cooperative Program. Accessed Feb. 3, 2015.
2. Atlas of Heart Disease and stroke, WHO, Sept. 2004.
3. Benjamin EJ, Virani SS, Callaway CW, Chang AR, Cheng S et al. (2018) Heart disease and stroke statistics - 2018 update: A report from the American Heart Association Circulation. DOI: 10.1161/CIR.0000000000000558.
4. Diet, Nutrition and the Prevention of Chronic Diseases. WHO, Geneva, 2003.
5. Rakesh S, Arunporn I (2017) Herbal supplements or herbs in heart disease: Herbaceutical formulation, clinical trials, futuristic developments. J Cardiol Cardiovasc Ther.3(1),555603.
6. Peter AK, Bjerke MA, Leinwand LA (2016) Biology of the cardiac myocyte in heart disease. Drubin DG, ed. Molecular Biology of the Cell.27(14),2149-2160.
7. Kuznetsov AV, Javadov S, Sickinger S, Frotschnig S, Grimm M (2015) H9c2 and HL-1 cells demonstrate distinct features of energy metabolism, mitochondrial function and sensitivity to hypoxia-reoxygenation. Biochimica et Biophysica Acta.1853(2),276-284.
8. Duthie SJ, Melvin WT, Burke MD (1994) Bromobenzene detoxification in the human liver-derived HepG2 cell line. Xenobiotica.24,265-279.
9. Lutgendorf SK, Mullen-Houser E, Russell D, Degeest K, Jacobson G et al. (2010) Preservation of immune function in cervical cancer patients during chemoradiation using a novel integrative approach. Brain Behav Immun.24,1231-1240.
10. Ironson G, Field T, Scafidi F, Hashimoto M, Kumar M et al. (1996) Massage therapy is associated with enhancement of the immune system's cytotoxic capacity. Int J Neurosci.84,205-217.
11. Jain S, Hammerschlag R, Mills P, Cohen L, Krieger R et al. (2015) Clinical studies of biofield therapies: Summary, methodological challenges, and recommendations. Glob Adv Health Med.4,58-66.
12. Rubik B (2002) The biofield hypothesis: Its biophysical basis and role in medicine. J Altern Complement Med.8,703-717.
13. Trivedi MK, Patil S, Shettigar H, Mondal SC, Jana S (2015) The potential impact of biofield treatment on human brain tumor cells: A time-lapse video microscopy. J Integr Oncol.4,141.
14. Trivedi MK, Patil S, Shettigar H, Gangwar M, Jana S (2015) In vitro evaluation of biofield treatment on cancer biomarkers involved in endometrial and prostate cancer cell lines. J Cancer Sci Ther.7, 253-257.
15. Trivedi MK, Tallapragada RM (2008) A transcendental to changing metal powder characteristics. Met Powder Rep.63,22-28,31.
16. Trivedi MK, Nayak G, Patil S, Tallapragada RM, Latiyal O (2015) Studies of the atomic and crystalline characteristics of ceramic oxide nano powders after bio field treatment. Ind Eng Manage.4,161.
17. Trivedi MK, Nayak G, Patil S, Tallapragada RM, Latiyal O et al. (2015) Effect of biofield energy

- treatment on physical and structural properties of calcium carbide and praseodymium oxide. *International Journal of Materials Science and Applications*.4,390-395.
18. Trivedi MK, Branton A, Trivedi D, Nayak G, Charan S et al. (2015) Phenotyping and 16S rDNA analysis after biofield treatment on *Citrobacter braakii*: A urinary pathogen. *J Clin Med Genom*.3,129.
19. Trivedi MK, Patil S, Shettigar H, Mondal SC, Jana S (2015) Evaluation of biofield modality on viral load of Hepatitis B and C viruses. *J Antivir Antiretrovir*.7,83-88.
20. Trivedi MK, Patil S, Shettigar H, Mondal SC, Jana S (2015) An impact of biofield treatment: Antimycobacterial susceptibility potential using BACTEC 460/MGIT-TB System. *Mycobact Dis*.5,189.
21. Trivedi MK, Branton A, Trivedi D, Nayak G, Mondal SC et al. (2015) Morphological characterization, quality, yield and DNA fingerprinting of biofield energy treated alphonso mango (*Mangifera indica L.*). *Journal of Food and Nutrition Sciences*.3, 245-250.
22. Trivedi MK, Branton A, Trivedi D, Nayak G, Mondal SC et al. (2015) Evaluation of biochemical marker – Glutathione and DNA fingerprinting of biofield energy treated *Oryza sativa*. *American Journal of BioScience*.3,243-248.
23. Trivedi MK, Branton A, Trivedi D, Nayak G, Plikerd WD et al. (2017) A Systematic study of the biofield energy healing treatment on physicochemical, thermal, structural, and behavioral properties of magnesium gluconate. *International Journal of Bioorganic Chemistry*.2,135-145.
24. Parulkar VR, Trivedi MK, Branton A, Trivedi D, Nayak G et al. (2018) Improved metabolism of vitamin d3 in human osteoblasts cells after biofield energy healing treatment. *American Journal of Laboratory Medicine*.3,11-19.
25. Trivedi MK, Patil S, Shettigar H, Bairwa K, Jana S (2015) Phenotypic and biotypic characterization of *Klebsiella oxytoca*: An impact of biofield treatment. *J Microb Biochem Technol*.7,203-206.
26. Nayak G, Altekhar N (2015) Effect of biofield treatment on plant growth and adaptation. *J Environ Health Sci*.1,1-9.
27. Branton A, Jana S (2017) The influence of energy of consciousness healing treatment on low bioavailable resveratrol in male Sprague Dawley rats. *International Journal of Clinical and Developmental Anatomy*.3,9-15.
28. Branton A, Jana S (2017) The use of novel and unique biofield energy healing treatment for the improvement of poorly bioavailable compound, berberine in male Sprague Dawley rats. *American Journal of Clinical and Experimental Medicine*.5,138-144.
29. Branton A, Jana S (2017) Effect of The biofield energy healing treatment on the pharmacokinetics of 25-hydroxyvitamin D₃ [25(OH)D₃] in rats after a single oral dose of vitamin D₃. *American Journal of Pharmacology and Phytotherapy*.2,11-18.
30. Parulkar VR, Trivedi MK, Branton A, Trivedi D, Nayak G et al. (2017) The use of consciousness energy healing based herbomineral formulation for skin anti-aging strategies. *Journal of Food and Nutrition Sciences*.5,96-106.
31. Singh J, Trivedi MK, Branton A, Trivedi D, Nayak G et al. (2017) Consciousness energy healing treatment based herbomineral formulation: A safe and effective approach for skin health. *American Journal of Pharmacology and Phytotherapy*.2,1-10.
32. Anagnos D, Trivedi K, Branton A, Trivedi D, Nayak G et al. (2018) Influence of biofield treated vitamin D₃ on proliferation, differentiation, and maturation of bone-related parameters in MG-63 cell-line. *International Journal of Biomedical Engineering and Clinical Science*.4,6-14.
33. Lee AC, Trivedi K, Branton A, Trivedi D, Nayak G et al. (2018) The potential benefits of biofield energy treated vitamin D₃ on bone mineralization in human bone osteosarcoma cells (MG-63). *International Journal of Nutrition and Food Sciences*.7,30-38.
34. Stutheit ME, Trivedi K, Branton A, Trivedi D, Nayak G et al. (2018) Biofield energy treated vitamin D₃: Therapeutic implication on bone health using osteoblasts cells. *American Journal of Life Sciences*.6,13-21.
35. Branton A, Jana S (2019) Improved metabolic

- cardiac biomarkers activity using rat cardiomyocytes cell line (H9c2) against biofield energy treated test sample. *J Cardiol.*3(1),000137.
36. Alía M, Ramos S, Mateos R, Bravo L, Goya L (2005) Response of the antioxidant defense system to *tert*-butyl hydroperoxide and hydrogen peroxide in a human hepatoma cell line (HepG2). *J Biochem Mol Toxicol.*19,119-128.
37. Vargas-Mendoza N, Madrigal-Santillán E, Morales-González A, Esquivel-Soto J, Esquivel-Chirino C et al. (2014) Hepatoprotective effect of silymarin. *World J Hepatol.*6,144-149.
38. Webb C, Twedt D (2008) Oxidative stress and liver disease. *Vet Clin North Am Small Anim Pract.*38, 125-135.
39. Sha Li, Hor-Yue Tan, Ning Wang, Zhang-Jin Zhang, Lixing Lao et al. (2015) The role of oxidative stress and antioxidants in liver diseases. *Int J Mol Sci.*16,26087-26124.
40. Cheresh P, Kim SJ, Tulasiram S, Kamp DW (2013) Oxidative stress and pulmonary fibrosis. *Biochim Biophys Acta.*1832,1028-1040.
41. Lu LY, Ou N, Lu QB (2013) Antioxidant induces DNA damage, cell death and mutagenicity in human lung and skin normal cells. *Sci Rep.*3,3169.
42. Burgner JW, Ray WJ (1984) On the origin of the lactate dehydrogenase induced rate effect. *Biochemistry.*23,3636-3648.
43. Valvona CJ, Fillmore HL, Nunn PB, Pilkington GJ (2015) The regulation and function of lactate dehydrogenase A: Therapeutic potential in brain tumor. *Brain Pathol.*26,3-17.
44. Kopperschläger G, Kirchberger J (1996) Methods for the separation of lactate dehydrogenases and clinical significance of the enzyme. *J Chromatogr B Biomed Appl.*684(1-2),25-49.
45. Arram EO, Fathy A, Abdelsamad AA, Elmasry EI (2014) Value of cardiac biomarkers in patients with acute pulmonary embolism. *Egypt J Chest Dis Tuberc.*63(1),247-252.
46. Wang J, Wang F (2017) The detection value of CK-MB, Myo and cTnI in patients with AMI and HF. *Biomed Res.*28(19),8533-8536.
47. Guzy PM (1977) Creatine phosphokinase-MB (CPK-MB) and the diagnosis of myocardial infarction. *West J Med.*127(6),455-460.
48. He F, Zuo L (2015) Redox roles of reactive oxygen species in cardiovascular diseases. Miller FJ, ed. *Int J Mol Sci.*16(11),27770-27780.
49. Halliwell B (1984) Oxygen radicals: A commonsense look at their nature and medical importance. *Med Biol.*62(2),71-77.
50. Zuo L, Best TM, Roberts WJ, Diaz PT, Wagner PD (2015) Characterization of reactive oxygen species in diaphragm. *Acta Physiol (Oxf).*213(3),700-710.
51. Zuo L, Shiah A, Roberts WJ, Chien MT, Wagner PD et al. (2013) Low Po₂ conditions induce reactive oxygen species formation during contractions in single skeletal muscle fibers. *Am J Physiol Regul Integr Comp Physiol.*304(11),R1009-R1016.
52. Tarpley MM, Wink DA, Grisham MB (2004) Methods for detection of reactive metabolites of oxygen and nitrogen: *In vitro* and *in vivo* considerations. *Am J Physiol Regul Integr Comp Physiol.*286,R431-R444.
53. Bennett MR (2002) Apoptosis in the cardiovascular system. *Heart.*87(5),480-487.
54. Saraste A, Voipio-Pulkki LM, Parvinen M, Pulkki K (1997) Apoptosis in the heart. *N Engl J Med.*336,1025-1026.
55. Kang PM, Izumo S (2003) Apoptosis in heart: Basic mechanisms and implications in cardiovascular diseases. *Trends Mol Med.*9,177-182.
56. Elliott MR, Ravichandran KS (2010) Clearance of apoptotic cells: Implications in health and disease. *J Cell Biol.*189(7),1059-1070.