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# Cytoprotective Effect of Biofield Energy Treated Test Item against Tert-Butyl Hydroperoxide (T-BHP) -Induced Cell Damage in Hepg2 Cell-Line

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## Abstract

Emerging data indicate that the mortality rate is rising due to liver disorders day-by-day in the developed countries. The present study was conducted to evaluate the potential of the Biofield Energy (The Trivedi Effect®) Treated test item (DMEM) in HepG2 cell-line. The test item was divided into two parts. One part of the test item received Consciousness Energy Healing Treatment by a renowned Biofield Energy Healer, Alice Branton and was labeled as the Biofield Energy Treated DMEM and the other part defined as untreated DMEM, where no Biofield Treatment was provided.Cell viability of the test items using MTT assay showed 113

***Index terms***— the trivedi effect®, HepG2, liver health, interleukin-8, ALT, cholesterol, albumin.

I. Introduction epatocellular carcinoma (HCC) is the fifth most common malignancy in the world. As per global statistics it has been reported that the incidence of chronic liver cirrhosis is increasing worldwide ranging from 3% to 9% per year [1] . Cancer, aging, coronary heart disease, neurodegenerative disorders (i.e., Alzheimer's disease), diabetes, and liver damage are all associated with an increased level of reactive oxygen species (ROS) formation. More selectively the mitochondrial electron transport chain is another main source of cellular ROS generator [2,3] . For the assessment of hepatoprotective activity in vitro model is more advantageous than in vivo [4] . Human hepatoma cell line (HepG2) has been widely used as an alternative model to human hepatocytes in vitro for the assessment of hepatoprotectant activity of a test substances [5] . HepG2 cell line has many advantages compared to others cell lines as it is an immortalized cell line, easily available and cryopreserved, and even after cultivation the metabolizing ability not reduced [6] . Numerous experimental data reported the useful effects of Biofield Energy Healing Treatment in cases of cancer patients via therapeutic touch [7] , massage therapy [8] , etc. Biofield Therapy is one of the Complementary and Alternative Medicine (CAM) therapies to enhance physical, mental, and emotional human wellness. The National Center of Complementary and Integrative Health (NCCIH) has recognized Biofield Therapy as a CAM health care approach including other therapies, medicines and practices such as natural products, chiropractic/ osteopathic manipulation, deep breathing, Tai Chi, yoga, meditation, relaxation techniques, Qi Gong, special diets, progressive relaxation, massage, healing touch, homeopathy, guided imagery, rolfing structural integration, acupuncture, movement therapy, hypnotherapy, pilates, mindfulness, acupressure, traditional Chinese herbs and medicines, Ayurvedic medicine, Reiki, aromatherapy, naturopathy, essential oils, and cranial sacral therapy. The Biofield Energy can be harnessed and transmitted by the Healers into living and non-living things via the process of Biofield Energy Healing Treatment. The outcomes of The Trivedi Effect® -Consciousness Energy Healing Treatment has been reported with a significant revolution in a wide spectrum of areas including materials science [9-11], agriculture [12,13], microbiology [14-16], biotechnology [17,18], nutraceuticals [19,20], cancer research [21,22]. Apart from this, The Trivedi Effect ® also tremendously improved bioavailability of various low bio available compounds [23-25], an improved overall skin health [26,27], bone health [28-30], human health and wellness. Based on the excellent outcome of The Trivedi Effect ® and importance of liver health authors intend to develop a new treatment modality to study the impact of the Biofield Energy Healing Treated (The Trivedi Effect ® ) test item (DMEM) on liver hepatocyte cells.

# 1 II. Materials and Methods

# 2 a) Chemicals and Reagents

Antibiotics solution (penicillin-streptomycin) was purchased from HiMedia. Dulbecco's Modified Eagle Medium (DMEM) and fetal bovine serum (FBS) were obtained from Gibco, India. Alanine aminotransferase (ALT) 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-Diphenyltetrazolium Bromide (MTT) and ethylenediaminetetraacetic acid (EDTA) were obtained from Sigma Chemical Co. (St. Louis, MO). The positive controls silymarin and mevinolin were procured from Sanat products ltd., India and Zliesher Nobel, respectively. All the other chemicals used in this experiment were analytical grade procured from India.

# 3 b) Biofield Energy Healing Strategy

The test item (DMEM) was used in this experiment and one portion was considered as the untreated DMEM 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 Biofield Energy Treatment and defined as the Biofield Energy Treated DMEM group. Biofield Energy Healing Treatment (known as The Trivedi Effect ® ) was received 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.

The cell viability was performed by MTT assay in HepG2 cell line. The cells were counted and plated in a 96-well plate at the density corresponding to 10 X 10 3 cells / well / 180 µL in DMEM + 10% FBS. The cells in the above plate(s) were incubated for 24 hours in a CO 2 incubator at 37°C, 5% CO 2 , and 95% humidity. Following incubation, the medium was removed and the following treatments were given. In the Biofield Treated test item (DMEM) group, 200 µL of the Biofield Energy Treated test item (DMEM) was added to wells, and in the untreated DMEM group, added 200 µL of untreated DMEM. Besides, in the positive control groups, added 180 µL of DMEM with 20 µL of positive controls were added from the respective 10X stock solutions. After incubation for 48 hours, the effect of test items on cell viability was assessed by MTT assay. 20 µL 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 µL 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 HepG2 cells was determined using Equation ( **??**):% ???????? ????????????????? = (100 ? % ??????????????????????????) ? ? ? ? ? . . (1)

Where For test items and positive controls, concentrations resulting ?70% cell viability were taken as safe / non-cytotoxic concentration.

# 4 d) Evaluation of Cytoprotective Effect of the Test Item

Cells were trypsinized and a single cell suspension of HepG2 was prepared. Cells were counted on an hemocytometer and seeded at a density of 10 X 10 3 cells / well / 180 µL in DMEM + 10% FBS in a 96-well plate. Cells were incubated in a CO 2 incubator for 24 hours at 37°C, 5% CO 2 and 95% humidity. After 24 hours, the medium was removed and the following treatments were given. In the test item groups, 180 µL of the test items were added to wells. In the positive control group, 160 µL of serum free medium and 20 µL 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 250 ?M (20 µL from the respective 10X stock) for 4 hours. After 4 hours, the protective effect of the test items on cell viability was assessed by MTT assay as per study protocol.

# 5 e) Estimation of Interleukin-8 (IL-8)

HepG2 cell suspension in DMEM containing 10% FBS was plated at a density of 0.3 X 10 6 cells /well / 1 mL in a 12-well plate. Cells were incubated in a CO 2 incubator for 24 hours at 37°C, 5% CO 2 , and 95% humidity. Cells were sera starved by replacing the medium with DMEM + 10% FBS for 24 hours. After 24 hours of sera starvation, medium was removed and pre-treatment were provided to the different treatment groups. After 24 hours of treatment, cells were stimulated with inflammatory stimulus TNF-? at a final concentration of 10 ng/mL. After treatment, cells were incubated in a 5% CO 2 incubator for 24 hours.

After 24 hours of incubation, culture supernatants were collected from each well and stored at -20°C until analysis. The level of cytokine (IL-8) in culture supernatants of HepG2 cells was determined using ELISA as per manufacturer's instructions.

# 6 f) Estimation of ALT

Cells were trypsinized and a single cell suspension of HepG2 was prepared and counted on an hemocytometer. Cells were seeded at a density of 10 X 10 3 cells / well / 180 µL in DMEM + 10% FBS in a 96-well plate. Cells were incubated in a CO 2 incubator for 24 hours at 37°C, 5% CO 2 , and 95% humidity. After 24 hours, medium

was removed and different treatments were given as per study plan. After incubation for 24 hours, cells were treated with 250 µM of t-BHP. After 4 hours of incubation, culture supernatants were collected from each well and stored at -20°C until analysis. The level of ALT in culture supernatants of HepG2 cells was determined using commercial kit as per manufacturer's instructions.

# 7 g) Estimation of Cholesterol

Cells were trypsinized and a single cell suspension of HepG2 was prepared. Cells were counted using an hemocytometer and seeded at a density of 1 million cells / well / mL in DMEM + 10% FBS in a 6-well plate. Cells were incubated in a CO 2 incubator for 24 hours at 37°C, 5% CO 2 and 95% humidity. After 24 hours, medium was removed and treated with different treatment groups. After 24 hours of incubation, cell lysates were prepared in the following manner.

Lysis buffer containing chloroform: isopropanol: IGEPAL CA630 in the ratio of 7:11:0.1 was prepared. Medium was removed from each well and 400?L of the above buffer was added to each well, which led to detachment of cells and formation of white layer. Cells were scrapped off and transferred into a labeled centrifuge tubes. The cells were homogenized in ice using a tissue homogenizer for 4-5 minutes until the solution was turned turbid in appearance. After homogenizing, the cells were centrifuged at 13000g for 10 minutes. The supernatant was collected in a prelabeled centrifuge tube and the pellet was discarded. The tube containing the supernatant was kept at 37ºC for 24 hours for evaporation of buffer. After 24 hours, the tube was removed from 37ºC and the dried lipids (small yellow colored pellet) were obtained, which was stored at -20 ºC until analysis. The level of cholesterol in cell lysates of HepG2 cells was determined using a commercial kit as per manufacturer's instructions.

# 8 h) Estimation of Albumin

Cells were trypsinized and a single cell suspension of HepG2 was prepared. Cells were counted using an hemocytometer and seeded at a density of 0.25 million cells / well / 1 mL in DMEM+10 % FBS in a 24-well plate. Then, the cells were incubated in a CO 2 incubator for 24 hours at 37°C, 5% CO 2 , and 95% humidity. Further, the cells were sera starved by replacing the medium with DMEM + 10% FBS for 24 hours. After 24 hours, medium was removed and various treatments were given. After 48 hours of incubation, culture supernatants were collected from each well and stored at -20°C until analysis. The level of albumin in culture supernatants of HepG2 cells were determined using a commercial kit as per manufacturer's instructions.

# 9 i) Statistical Analysis

All the values were represented as Mean ± SEM (standard error of mean) of three independent experiments. 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?0.05.

# 10 III. Results and Discussion

# 11 a) Cell Viability Assay (MTT)

The results of the cytotoxicity using MTT cell viability assay after treatment with the positive controls (silymarin and mevinolin), untreated DMEM, and the Biofield Energy Treated DMEM in HepG2 cells are shown in Figure 1. Silymarin showed more than 136% cell viability upto 25 µg/mL and mevinolin showed greater than 97% cell viability upto 20 µg/mL. Further, the untreated and Biofield Energy Treated DMEM groups showed 113% and 129.9% cell viability, respectively (Figure 1). Therefore, the positive controls and the test items were found more than 97% cell viability, which indicated a safe and nontoxic profile in the tested concentrations.

# 12 b) Cytoprotective Activity

The cytoprotective activity of the Biofield Energy Treated test items on the protection of cell viability in HepG2 cells was determined against t-BHP induced cell damage after 4 hours of treatment is presented in Figure 2. Silymarin showed 4.9%, 38.4% (p?0.001), and 66.1% (p?0.001) cellular protection at 1, 5, and 25 µg/mL, respectively compared to the t-BHP induced group. Besides, the Biofield Energy Treated test item (DMEM) showed significant (p?0.001) restoration of cell viability by 15%, while untreated DMEM group showed 0.4% protection under the t-BHP induction (Figure 2). t-BHP is known to generate ROS and induce lipid peroxidation in cells and simultaneously reduced the primary antioxidant of cells i.e., glutathione (GSH) [31,32] . In this experiment from Figure 2, it was observed that Biofield Energy Treated Test item effectively restored cellular function by 15%. The findings showed that Biofield Energy Treatment has the significant cytoprotective and antioxidant activities, which could be due to the effect of The Trivedi Effect ® -Energy of Consciousness. Thus, The Trivedi Effect ® Treated test item (DMEM) could be utilized against liver disorders.

# 13 c) Estimation of Interleukin-8 (IL-8)

Interleukin-8 (IL-8) is a potent chemoattractant for neutrophils and causes acute liver inflammation [33,34] . The effect of the test items on IL-8 is shown in Figure 3.

Increase level of oxidative stress causes increase secretion of IL-8, and ultimately recruit the inflammatory cells causes' localized inflammation [35] . In this experiment, after treatment with TNF-? at 10 ng/mL can significantly induced oxidative stress and the proinflammatory cytokines IL-8, because oxidative stress and TNF-alpha are the mediators in IL-8 response [36] . The level of IL-8 in the untreated DMEM group was 964.4 ± 40.65 pg/mL. On the other side, the Biofield Energy Treated DMEM group showed significant (p?0.01) reduction of IL-8 by 32.15% compared to the untreated DMEM group under the stimulation of TNF-? at 10 ng/mL (Figure 3).

# 14 d) Estimation of Alanine Aminotransferase (ALT)

The effect of the test items on alanine aminotransferase (ALT) is shown in Figure 4. The positive control, silymarin showed 8.4%, 25.6%, and 79.2% (p?0.01) reduction of ALT level at 1, 5, and 25 µg/mL, respectively with respect to the untreated DMEM group. Besides, the Biofield Energy Treated DMEM group showed a significant (p?0.01) reduction of ALT by 53.2% compared to the untreated DMEM group (Figure 4). The aminotransferase enzymes catalyze the reversible transformation of ?-ketoacids into amino acids. Increased serum level of ALT is directly proportional to the severity of the diseases like hepatocellular injury and death [37] . Thus, the elevation of serum ALT enzyme chances of liver disorders [38] . Here, the Biofield Energy Treated test item (DMEM) has significantly protect liver hepatocytes in terms of reducing the level of transaminase enzyme, ALT compared to the untreated DMEM group.

# 15 e) Estimation of Cholesterol

The effect of the test items on cholesterol in shown in Figure 5. Mevinolin (positive control) showed 17.45%, 25%, and 80.19% (p?0.001) reduction of cholesterol at 5, 10, and 20 µM, respectively compared to the untreated DMEM group. On the other side, cholesterol level was significantly (p?0.001) reduced by 37.35% in the Biofield Energy Treated DMEM group compared to the untreated DMEM group (Figure 5). Cholesterol, its metabolites, and immediate biosynthetic precursors of cholesterol plays a vital role in salt and water balance, calcium metabolism, and stress responses [39] . Over accumulation of cholesterol leads to nonalcoholic fatty liver disease (NAFLD) [40] .

# 16 f) Estimation of Albumin

The effect of the test items on albumin concentration is shown in Figure 6. The level of albumin was significantly increased by 29.65%, 69.51%, 100.21% (p?0.001), and 142.78% (p?0.001) at 0.5, 1, 5, and 20 µM, respectively in the positive control (silymarin) group compared to the untreated DMEM group. Besides, the Biofield Energy Treated DMEM group showed 43.13% increase the level of albumin compared to the untreated DMEM group (Figure 6). From literature it has been reported that albumin plays a multiple physiological effects like volume expansion, anti-oxidation [41,42] , and endothelial protection [43] , hence was recommended for the management of liver cirrhosis patients and in acute/chronic liver failure [44,45] . In this experiment, the Biofield Treated DMEM significantly increased the level of albumin, which could be due to The Trivedi Effect ® -Energy of Consciousness Healing Treatment.

# 17 IV. Conclusions

The study results showed that the test items were safe and non-toxic based on MTT cell viability assay. The Biofield Energy Treated test item (DMEM) showed significant (p?0.001) protection of cells by 15% from the oxidative damage induced by t-BHP, while untreated DMEM group showed 0.4% protection. The proinflammatory cytokine, IL-8 was significantly (p?0.01) reduced by 32.15% in the Biofield Energy Treated DMEM group compared to the untreated DMEM group. Moreover, ALT enzyme activity was significantly (p?0.01) reduced by 53.2% in the Biofield Energy Treated DMEM group compared to the untreated DMEM group. Cholesterol level was significantly (p?0.001) reduced by 37.35% in the Biofield Energy Treated DMEM group compared to the untreated DMEM group. Further, Biofield Energy Treated DMEM group showed 43.13% increased the level of albumin compared to the untreated DMEM group. In conclusion, The Trivedi Effect ® -Consciousness Energy Healing Treatment significantly protect hepatocytes cells oxidative stress and it can be used as a complementary and alternative treatment for the prevention of various types of hepatobiliary disorders viz. acute hepatitis A, B, C, D, and E, chronic viral hepatitis, portal hypertension in schistosomiasis, toxoplasmosis, hepatosplenic schistosomiasis, liver abscess, autoimmune hepatitis, primary biliary cholangitis (primary biliary cirrhosis), phlebitis of the portal vein, granulomatous hepatitis, cholestasis, necrosis, cirrhosis, etc. Further, it could be useful to improve cell-to-cell messaging, normal cell growth and differentiation, cell cycling and proliferation, neurotransmission, skin health, hormonal balance, immune and cardiovascular functions. Moreover, it can also be utilized in organ transplants (i.e., kidney, liver, and heart transplants), hormonal imbalance, aging, and various inflammatory and immune-related disease conditions like Alzheimer's Disease (AD), Ulcerative

Colitis (UC), Dermatitis, Asthma, Irritable Bowel Syndrome (IBS), 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.

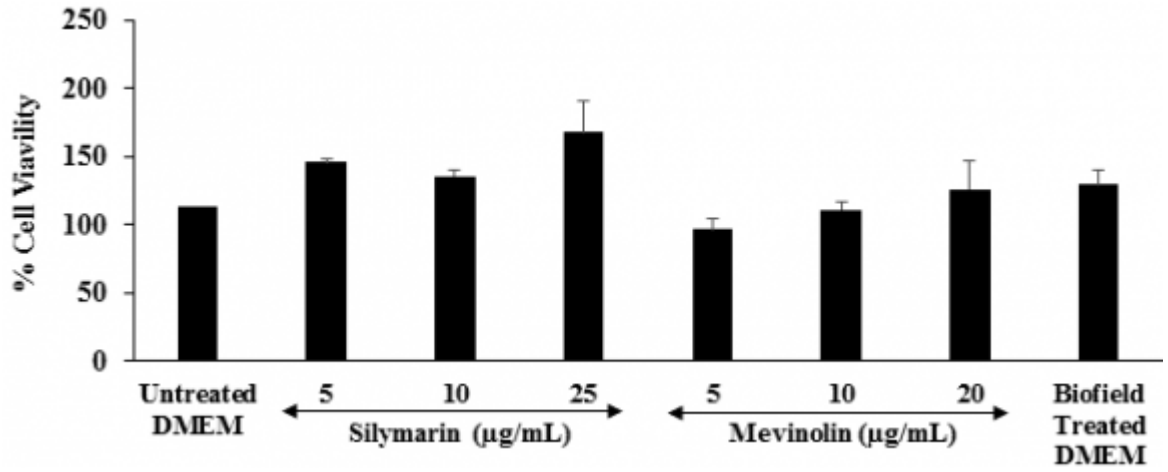


Figure 1:

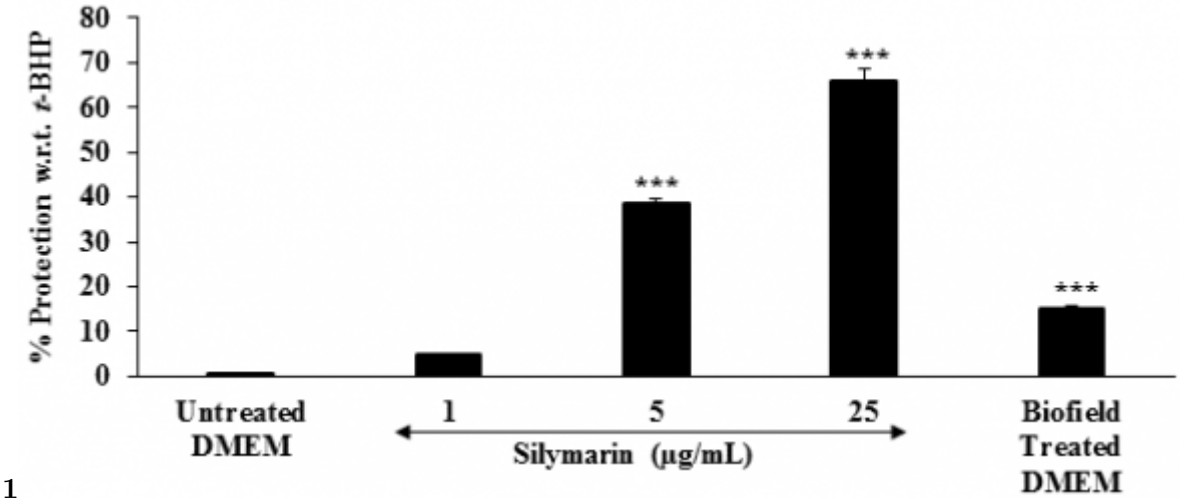


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Figure 2: Figure 1 :

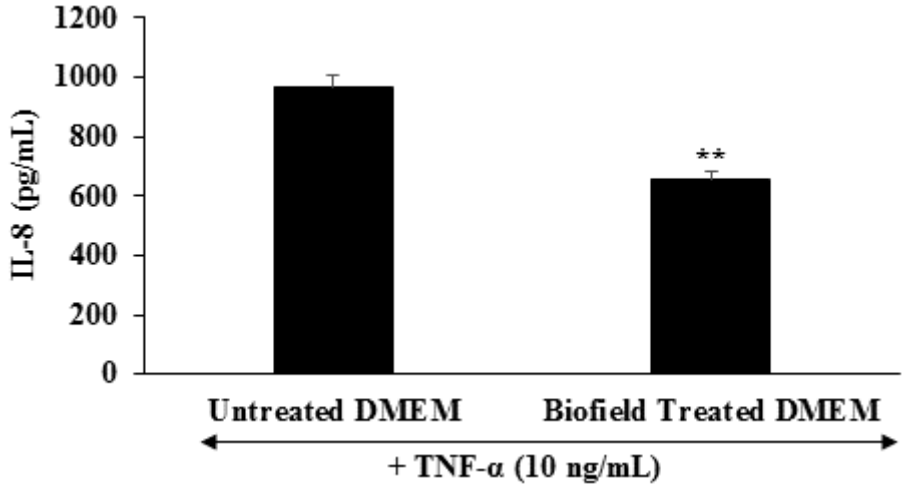


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Figure 3: 1 B

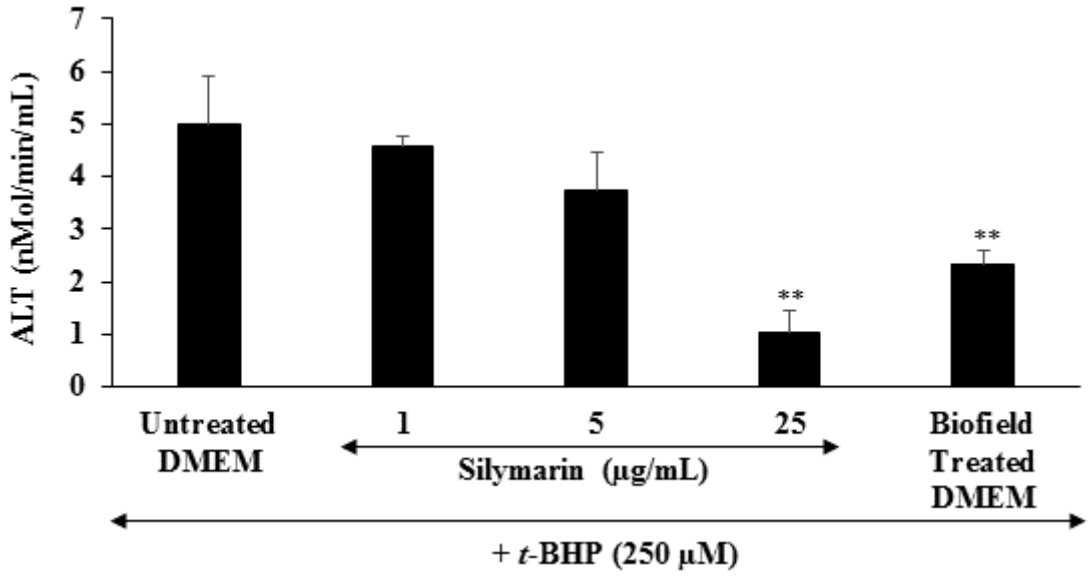


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Figure 4: Figure 2 :

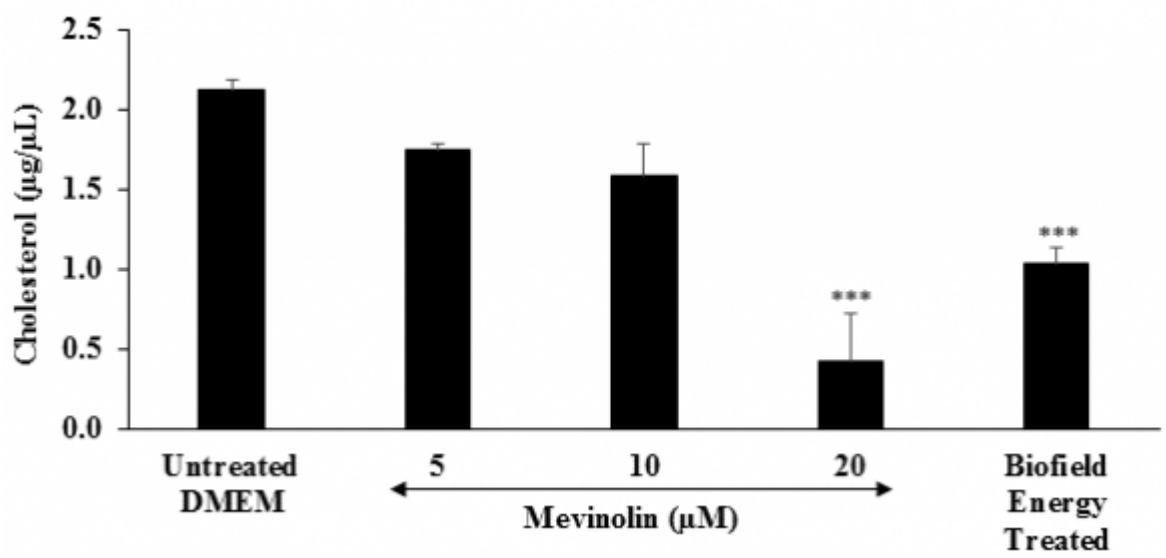
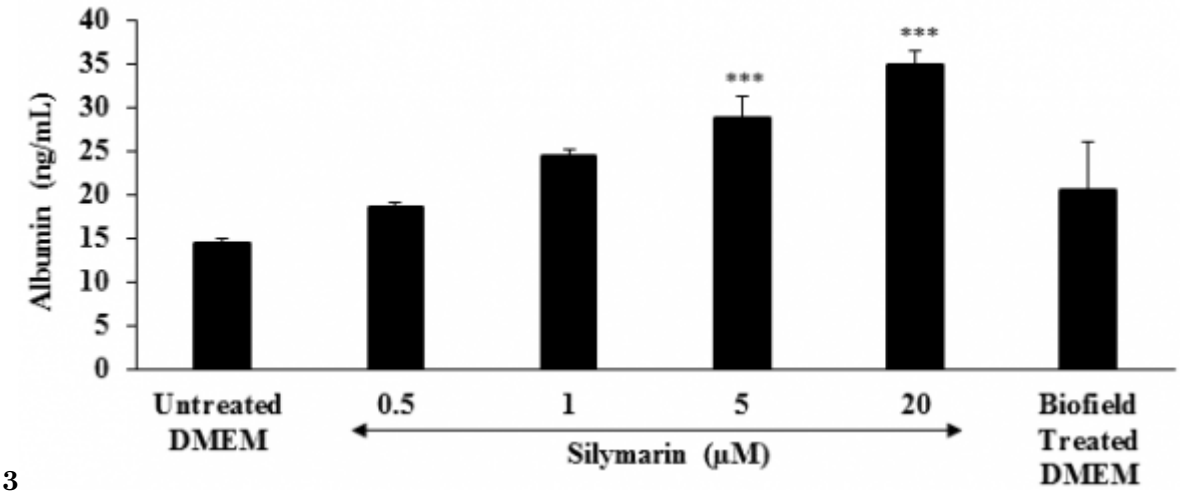



Figure 5: B




**3**

Figure 6: Figure 3 :

# .1 Acknowledgements

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