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Skin Protective Activity of Consciousness Energy Healing Treatment Based Herbomineral Formulation

Dennille Mellesia Smith[1], Mahendra Kumar Trivedi[1], Alice Branton[1], Dahryn Trivedi[1], Gopal Nayak[1], Sambhu Charan Mondal[2], Snehasis Jana[2, *]

[1]Trivedi Global, Inc., Henderson, USA

[2]Trivedi Science Research Laboratory Pvt. Ltd., Bhopal, India

Email address:

publication@trivedisrl.com (S. Jana)

[*]Corresponding author

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Abstract: The current study was attempted to evaluate the impact of the Consciousness Energy Healing (The Trivedi Effect®) Treatment based herbomineral test formulation and cell medium (DMEM) against skin health. The test formulation and DMEM were divided into two parts. One of each part was received the Consciousness Energy Healing Treatment by Dennille Mellesia Smith and was termed as the Biofield Energy Treated samples, while the other parts were denoted as the untreated test items. MTT showed >78% viable cells, indicating that the test formulation was safe and nontoxic in all the tested concentrations in three cell lines. The percent cell proliferation by BrdU assay was significantly increased by 238.30%, 192.06%, and 43.96% in the UT-DMEM + BT-Test formulation, BT-DMEM + UT-Test formulation, and BT-DMEM + BT-Test formulation groups, respectively at 17.5 µg/mL with respect to the UT-DMEM + UT-Test formulation group. The level of collagen was significantly increased by 55.55%, 32.65%, and 52.48% in the UT-DMEM + BT-Test formulation, BT-DMEM + UT-Test formulation and BT-DMEM + BT-Test formulation groups, respectively at 1.25 µg/mL compared to the untreated group. Elastin was significantly ($p \leq 0.001$) increased by 6.30%, 105.04%, and 29.41% in the UT-DMEM + BT-Test formulation, BT-DMEM + UT-Test formulation, and BT-DMEM + BT-Test formulation groups, respectively at 10 µg/mL compared to the untreated group. Hyaluronic acid was increased by 4.78%, 29.71%, and 58.29% in the UT-DMEM + BT-Test formulation, BT-DMEM + UT-Test formulation, and BT-DMEM + BT-Test formulation groups, respectively at 0.63 µg/mL compared to the UT-DMEM + UT-Test Formulation group. The level of melanin was reduced by 14.64% and 18.25% in the UT-DMEM + BT-Test formulation and BT-DMEM + UT-Test formulation, respectively at 0.13 µg/mL compared to the untreated group. Skin protection against UV-B data displayed that cell proliferation was increased by 17.88%, 20.14%, and 9.89% in the BT-DMEM + BT-Test formulation at 0.63, 1.25, and 2.5 µg/mL, respectively compared to the untreated group. Wound healing activity exhibited significant wound closure and cell migration in all the tested groups compared to the untreated group. Overall, result suggests that the Biofield Energy Treated DMEM and test formulation exhibited better responses compared to the untreated medium and test formulation. Therefore, the Biofield Energy Treated herbomineral formulation could be useful for the development of an effective cosmetic product for the prevention and treatment of several skin problems such as erythema, contact dermatitis, skin aging, wrinkles and/or change in the skin color, etc.

Keywords: The Trivedi Effect®, Consciousness Energy Healing, Skin Protection, HFF-1, B16-F10, HaCaT, Scratch Assay, Extracellular Matrix

1. Introduction

Skin is continuously exposed to pro-oxidant environmental stresses from various sources like air pollutants, ultraviolet (UV) light, chemical oxidants, microorganisms, and ozone. Reactive oxygen species (ROS) are considered as the main factor that causes several skin disorders such as skin cancer and photoaging. In recent years, particular antioxidants have gained considerable attention as a means for neutralizing various ROS [1]. The minerals and plant extracts play a vital role in skin repair, growth, and development. Important minerals such as zinc play a critical role in overall human physiology. It is an essential cofactor of various metalloenzymes and it protects the skin from UV irradiation and has been used for wound healing and to reduce inflammation. Deficiency and abnormal metabolism of zinc causes a hereditary disorder like acrodermatitis enteropathica in infants along with skin lesions. [2-4]. Several scientific evidences suggest that selenium plays an important role in protecting skin from the harmful effects of UV-B. It is an essential trace element is found in many foods including meat, fish, eggs, dairy products, and grains. In humans, low selenium status is associated with increased the risk of developing skin cancer [5, 6]. Zinc, and selenium are involved in the destruction of free radicals through cascading enzyme systems. Apart from zinc and selenium, molybdenum is involved in many biochemical processes of life such as respiration, DNA and RNA reproduction, maintenance of cell membrane integrity, and sequestration of free radicals [7]. Vitamin C is an essential constituent for the production of collagen and a potent antioxidant that can help rejuvenate aged and photodamaged skin [8, 9]. Sugiyama et al. [10] demonstrated that tetrahydrocurcumin (THC) also exhibited strong anti-oxidant and anti-cancer activity. However, it was also reported that THC has less effective as chemopreventive agent in mouse skin than curcumin [11]. The extract of Centella asiatica is effective for the treatment of small wounds, hypertrophic wounds, burns, psoriasis and scleroderma through promoting the proliferation of fibroblast. It increases the synthesis of collagen, intracellular fibronectin, and the tensile strength of newly formed skin as well as inhibiting the inflammatory phase of hypertrophic scars and keloids [12]. Owing to the importance of minerals and vitamins, a new proprietary herbomineral formulation was formulated consisting of essential minerals (zinc chloride, sodium selenate, and sodium molybdate), vitamin (L-ascorbic acid), tetrahydrocurcumin (THC), and herbal extract (Centella asiatica). Each ingredient already has been proven for its potential activity on skin health as various medicine as well as cosmeceuticals. Exposure to UV radiation and environmental pollutants can accelerate the skin aging by degrading collagen and triggering oxidative stress in the skin.

The National Center for Complementary and Integrative Health (NCCIH), allows the use of Complementary and Alternative Medicine (CAM) therapies like Biofield Energy as an alternative treatment in the healthcare sector. About 36% of US citizens regularly use some form of CAM [13], in their day-to-day life. Researchers reported that a short-lived electrical action potential exists in the mammalian cells such as neurons, muscles, and endocrine. When the cells are present in the central nervous system of human body that communicate with each other by means of electrical signals that propagate along the nerve impulses [14]. Therefore, it was hypothesized that the Biofield can exist around the human body and evidence was found using electromyography, electrocardiography and electroencephalogram [15]. Thus, a Biofield Energy Healing Practitioner has the ability to harness the energy from the environment and can transmit it into any object (living organism or non-living material) around the globe. The object(s) always receive the energy and respond in a useful way that is called “Biofield Energy Treatment”. This process is known as “Biofield Energy Healing”. Biofield Energy Healing has been approved as an alternative method that has an impact on various properties of living organisms in a cost-effective manner [16, 17]. The Trivedi Effect® - unique Biofield Energy Treatment has been known to alter the response in a wide-spectrum field in living and non-living systems *viz.* materials science [18-20], agriculture [21, 22], microbiology [23-25] biotechnology [26, 27]. Based on the excellent outcome of the Biofield Energy Treatment, authors designed this study to investigate the impact of the Biofield Energy Healing based DMEM and test formulation on various skin health parameters using three cell lines such as human foreskin fibroblast (HFF-1), human keratinocytes (HaCaT), and mouse melanoma (B16-F10) cells.

2. Materials and Methods

2.1. Chemicals and Reagents

L-ascorbic acid was purchased from Alfa-Aesar, while kojic acid was purchased from Sigma, USA. Epidermal growth factor (EGF) was procured from Gibco, ThermoFisher, USA. ELISA kits were procured from CUSABIO and CusAb Co. Pvt. Ltd., USA. Zinc chloride purchased from TCI, Japan, sodium selenate from Alfa-Aesar, USA, while sodium molybdate from Sigma-Aldrich, USA. Tetrahydrocurcumin and Centella asiatica extract were procured from Novel Nutrients Pvt. Ltd., India and Sanat Products Ltd., India, respectively. Fetal bovine serum (FBS) and Dulbecco's Modified Eagle's Medium (DMEM) were purchased from Gibco, USA. Antibiotics solution (penicillin-streptomycin) was procured from Himedia, India, while 3-(4, 5-diamethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium) (MTT), Direct Red 80 and ethylene diamine tetra acetic acid (EDTA) were purchased from Sigma, USA. All the other chemicals used in this experiment were analytical grade procured from India.

2.2. Cell Culture

HFF-1 (human fibroblast) cells were procured from American Type Culture Collection (ATCC), USA, originated from normal human skin fibroblast cells. B16-F10 (mouse melanoma) cells were procured from National Centre for Cell Science (NCCS), Pune. HFF-1, and B16-F10 cell lines were maintained in the growth medium, DMEM supplemented with 15% FBS, with added antibiotics penicillin (100 U/mL) and streptomycin (100 µg/mL). The growth condition of cell lines were 37°C, 5% CO2, and 95% humidity. L-ascorbic acid (for ECM, UV-B protection, and wound healing assay) in concentrations ranges from 10 µM to 1000 µM, while kojic acid (for melanin synthesis) concentrations ranges from 1 mM to 10 mM, FBS (0.5%) was used in cell proliferation (BrdU) assay, while EGF 10 µM was used in MTT assay.

2.3. Experimental Design

The experimental groups consisted of cells in normal control, vehicle control group (0.05% DMSO), positive control group (L-ascorbic acid/kojic acid/EGF/FBS) and experimental tested groups. Experimental groups included the combination of the Biofield Energy Treated and untreated test formulation/DMEM. It consisted of four major treatment groups on specified cells with UT-DMEM + UT-Test formulation, UT-DMEM + BT-Test formulation, BT-DMEM + UT-Test formulation, and BT-DMEM + BT-Test formulation.

2.4. Consciousness Energy Healing Treatment Strategies

The test formulation and DMEM were divided into two parts. One of each part of the test formulation was treated with the Biofield Energy by renowned Biofield Energy Healer (also known as The Trivedi Effect®) and coded as the Biofield Energy Treated samples, while the second part of the test formulation and DMEM did not receive any sort of treatment and was defined as the untreated test samples. This Biofield Energy Healing Treatment was provided by Dennille Mellesia Smith, who participated in this study and performed the Biofield Energy Treatment remotely for ~5 minutes. Biofield Energy Healer was remotely located in the USA, while the test samples were located in the research laboratory of Dabur Research Foundation, near New Delhi, India. This Biofield Energy Treatment was provided for 5 minutes through the Healer's unique Energy Transmission process remotely to the test samples under laboratory conditions. The Biofield Energy Healer, Dennille Mellesia Smith, in this study never visited the laboratory in person, nor had any contact with the test formulation and DMEM. Further, the control groups were treated with a sham healer for comparative purposes. The sham healer did not have any knowledge about the Biofield Energy Treatment. After that, the Biofield Energy treated and untreated samples were kept in similar sealed conditions for experimental study.

2.5. Determination of Non-cytotoxic Concentration

The cell viability was performed by MTT assay in HFF-1 (human fibroblast), HaCaT (human keratinocytes), and B16-F10 (mouse melanoma) cells. The cells were counted and plated in 96 well plates at the density corresponding to 5 X 103 to 10 X 103 cells/well/180 µL of cell growth medium. The above cells were incubated overnight under growth conditions and allowed the cell recovery and exponential growth, which were subjected to serum stripping or starvation. The cells were treated with the test formulation and DMEM/positive controls. The untreated cells were served as baseline control. The cells in the above plate(s) were incubated for a time point ranging from 24 to 72 hours in CO2 incubator at 37°C, 5% CO2 and 95% humidity. Following incubation, the plates were taken out and 20 µL of 5 mg/mL of MTT solution were added to all the wells followed by additional incubation for 3 hours at 37°C. The supernatant was aspirated and 150 µL of DMSO was added to each well to dissolve formazan crystals. The absorbance of each well was read at 540 nm using Synergy HT micro plate reader, BioTek, USA. The concentrations exhibiting % cytotoxicity of < 30 % was considered as non-cytotoxic [28, 29]. The percentage cell viability at each tested concentrations of the test substance were calculated using the following Equation 1:

$$\%\ \text{Cell viability} = (X * 100)/R \quad (1)$$

Where, X represent the absorbance of the cells corresponding to positive control and test groups and R represent the absorbance of the cells corresponding to the baseline (control cells) group.

2.6. Effect of the Test Item on Fibroblast Proliferation by 5-bromo-2'-deoxyuridine (BrdU) Method

HFF-1 cells were counted using hemocytometer and plated in 96 well plate at the density corresponding to 1 X 103 to 5 X 103 cells/well in DMEM supplemented with 15% FBS. The cells/plates were incubated overnight under growth conditions so as to allow cell recovery and exponential growth. Following overnight incubation, the above cells were subjected to serum starvation. Following serum starvation, the cells were treated with non-cytotoxic concentrations of test substance and positive control. Following 24 to 72 hours of incubation with the test substance and positive control, the plates were taken out and 5-bromo-2'-deoxyuridine (BrdU) estimation using cell proliferation ELISA, BrdU estimation kit (ROCHE – 11647229001) as per manufacturer's instructions.

2.7. Estimation of Extracellular Matrix (ECM)

Synthesis of extracellular matrices component (i.e. collagen, elastin, and hyaluronic acid) in HFF-1 was estimated for determining the potential of the Biofield Energy Treated test formulation and DMEM to improve the skin strength, elasticity, and hydration level. HFF-1 cells were counted using

hemocytometer and plated in 48 well plate at the density corresponding to 10 X 103 cells/well in DMEM supplemented with 15% FBS. The cells were incubated overnight under specified growth conditions followed by cells to serum stripping. Further, the cells were treated with different groups viz. vehicle control (DMSO-0.05%), positive control (L-ascorbic acid, at 10 µM concentration), and the test items at different concentrations. Further, 72 hours of incubation with the test items and positive control, the supernatants from all the cell plates were taken out and collected in pre labeled centrifuge tubes for the estimation elastin and hyaluronic acid levels. However, the corresponding cell layers were processed for estimation of collagen levels using Direct Sirius red dye binding assay [30]. Elastin and hyaluronic acid were estimated using ELISA kits from Cusabio Biotech Co. Ltd., Human Elastin ELN Elisa kit 96T and Human hyaluronic acid, Elisa kit 96T, respectively [31].

2.8. Estimation of Melanin Synthesis

B16-F10 cells were used for melanin synthesis estimation, cells were counted using hemocytometer and plated in 90 mm culture dish at the density corresponding to 2 X 106 per 6 mL in culture plates. Further, the cells were incubated overnight under specified growth conditions and allowed for cell recovery and exponential growth. After incubation, the cells were treated with α-melanocyte-stimulating hormone (α-MSH) for a time point ranging from 4 to 24 hours for the stimulation of intracellular melanin synthesis. Further, the cells were incubated with α-MSH and then treated with the test formulation with DMEM at different concentrations for 48 to 96 hours. After incubation, intracellular melanin was extracted in NaOH and the absorbance was recorded at 405 nm. The level of melanin was extrapolated using standard curve obtained from purified melanin [32].

2.9. Anti-wrinkle Effects of the Test Formulation on HFF-1 Cells against UV-B Induced Stress

UV-B induced stress was evaluated in HFF-1 cells and cell viability was estimated in the presence of test items. The cells were counted using hemocytometer and plated in 96 well plate at the density corresponding to 5 X 10^3 to 10 X 10^3 cells/well in DMEM supplemented with 15% FBS cells/plates, which were incubated overnight under growth conditions to allow cell recovery and exponential growth. The cells were treated with non-cytotoxic concentrations of test items for 2 to 24 hours. After treatment with test items, the cells were subjected to the lethal dose of UV-B irradiation (200 mJ/cm^2) that can lead to approximately 50% cytotoxicity (302 nm, CL-1000 M, UVP, USA) [33]. The percent cell viability was assessed using following Equation 2:

$$\%\ \text{Cell viability} = (X * 100)/R \quad (2)$$

Where,

X represents the absorbance of cells corresponding to positive control and test group,

R represents the absorbance of cells corresponding to the baseline (control cells) group.

2.10. Wound Healing Activity by Scratch Assay

HFF-1 and HaCaT cells were counted using hemocytometer and plated in 12 well plates at the densities 0.08 X 106/well/mL of cell growth medium. The cells/plates were incubated overnight under growth conditions and allowed cell recovery and exponential growth. After overnight incubation, the cells were subjected to the serum starvation in DMEM for 24 hours. Mechanical scratch that represents wound was created in the near confluent monolayer of cells by gently scraping with sterile 200 µL micropipette tip. The cells were then rinsed with serum free DMEM and treated with the test formulation. The scratched area was then monitored for a time period ranging from 0 to 48 hours for closure of wound area. The photomicrographs (x10) were done at the selected time point (at 16 hours) of migrated cells using digital camera. It represented the fibroblast distance covered and subsequent scratch closure [34].

2.11. Statistical Analysis

Each experiment was carried out in three independent assays and the values were represented as mean values with standard error of mean (SEM). 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 $p \leq 0.05$.

3. Results and Discussion

3.1. Cell Viability by MTT Assay

MTT assay was used for the assessment of the viable cells in three different cells like HFF-1, HaCaT, and B16-F10 cells and the results are shown in Figure 1A to 1C. The result exhibited about >78% viable cells in the tested concentrations ranges from 0.63 to 10 µg/mL in the HFF-1 cells (Figure 1A), which indicated that the test formulation was safe and nontoxic. The selected concentrations were used for the estimation of collagen, elastin, and hyaluronic acid. Furthermore, the cell viability in HaCaT cells exhibited >95%. The concentrations of the test formulation from 5 to 40 µg/mL were used for the evaluation of wound healing activity by scratch assay (Figure 1B). The percentage of viable cells in the B16-F10 cells revealed that the test formulation was non-cytotoxic (i.e. percentage cell viability value >98%) and to be safe. The tested concentrations were used further for the measurement of melanin level at the concentrations ranging from 10 to 40 µg/mL (Figure 1C).

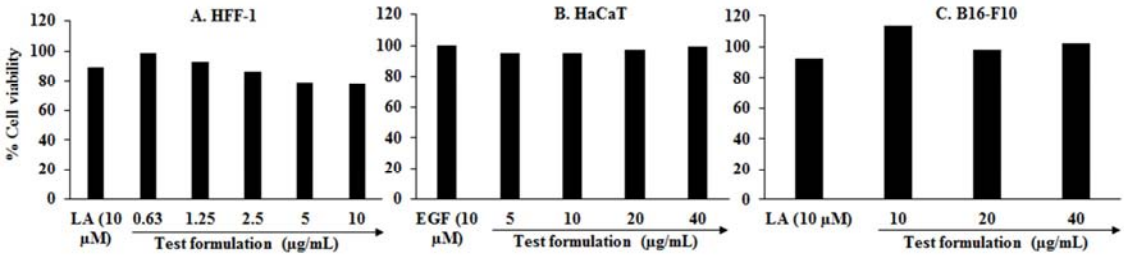

Figure 1. *Evaluation of the Cell viability by MTT assay of the test formulation in three different cells. (A) HFF-1 cells after 72 hours of treatment; (B) HaCaT cells after 48 hours of treatment; and (C) B16-F10 cells after 48 hours of treatment. LA: L-Ascorbic acid; EGF: Epidermal growth factor.*

3.2. Cell Proliferation by BrdU Assay

The cell proliferation analyzed by bromodeoxyuridine (BrdU) assay is shown in Figure 2. The cell proliferation was 100% and 250.4% in the vehicle control (VC) and positive control (FBS-0.5 µg/mL) groups, respectively. Further, the cell proliferation was significantly increased by 149.18% and 118.86% in the UT-DMEM + BT-Test formulation and BT-DMEM + UT-Test formulation groups, respectively at 8.75 µg/mL compared to the UT-DMEM + UT-Test formulation group. Moreover, the cell proliferation was enhanced by 238.30%, 192.06%, and 43.96% in the UT-DMEM + BT-Test formulation, BT-DMEM + UT-Test formulation, and BT-DMEM + BT-Test formulation groups, respectively at 17.5 µg/mL with respect to the UT-DMEM + UT-Test formulation group. At 35 µg/mL, the cell proliferation was significantly elevated by 95.79%, 76.05%, and 45.97% in the UT-DMEM + BT-Test formulation, BT-DMEM + UT-Test formulation, and BT-DMEM + BT-Test formulation groups, respectively compared to the UT-DMEM + UT-Test formulation group. Cell proliferation is vital for cellular homoeostasis and maintenance of an organism. The BrdU assay was used for the evaluation of three major objectives such as for measuring the rate of DNA replication, analysis of metabolic activity and recognitions of cell surface antigen activity [35]. Overall, the cell proliferation in the Biofield Energy Treated test formulation and DMEM groups were remarkably improved.

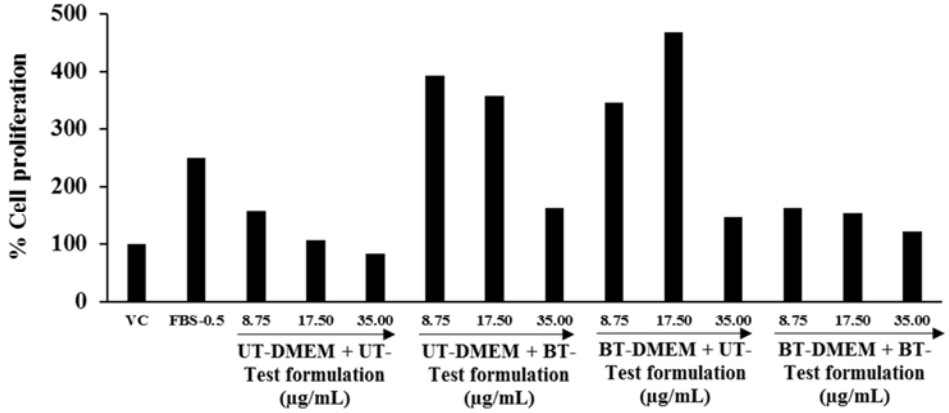

Figure 2. *Effect of the test formulation on cellular proliferation by BrdU assay after 48 hours of treatment. VC: Vehicle control; FBS: Fetal bovine serum (µg/mL); UT: Untreated; BT: Biofield Treated.*

3.3. Impact of the Test Formulation on Synthesis of Extracellular Matrix (ECM) Components in Human Foreskin Fibroblast (HFF-1)

3.3.1. Collagen

Effect of the test formulation and DMEM on collagen level in HFF-1 cells is shown in Figure 3. The level of collagen was 93.14 ± 1.37 and 129.42 ± 8.50 µg/mL in the vehicle control (VC) and positive control groups, respectively. The level of collagen was significantly increased by 8.58% and 15.18% in the BT-DMEM + UT-Test formulation and BT-DMEM + BT-Test formulation groups, respectively at 0.63 µg/mL compared to the UT-DMEM + UT-Test formulation group. Additionally, collagen data showed 55.55%, 32.65%, and 52.48% elevation in the UT-DMEM + BT-Test formulation, BT-DMEM + UT-Test formulation and BT-DMEM + BT-Test formulation groups, respectively at 1.25 µg/mL compared to the UT-DMEM + UT-Test formulation group. Moreover, the expression of collagen was enhanced significantly ($p \leq 0.05$) by 64.59%, 38.57%, and 57.41% in the UT-DMEM + BT-Test formulation, BT-DMEM + UT-Test formulation and BT-DMEM + BT-Test formulation groups, respectively at 2.5 µg/mL compared to the UT-DMEM + UT-Test formulation group. Several stimuli such as local tissue ischemia, necrotic tissue, repeated trauma, etc. causes a chronic wounds in the inflammatory phase. In chronic wounds, there was an elevation of matrix metalloproteinases (MMPs) enzymes that degraded the both viable as well as non-viable collagen [36]. Collagen is an important component responsible for wound healing and due to damage

of collagen the repair process also delayed [37]. Therefore, the control of collagen metabolism might be useful for a variety of therapeutic and cosmetic applications. Overall, the level of collagen synthesis was improved significantly in the Biofield Energy Treated test formulation and DMEM group, which might be due to The Trivedi Effect® - Energy of Consciousness Healing Treatment.

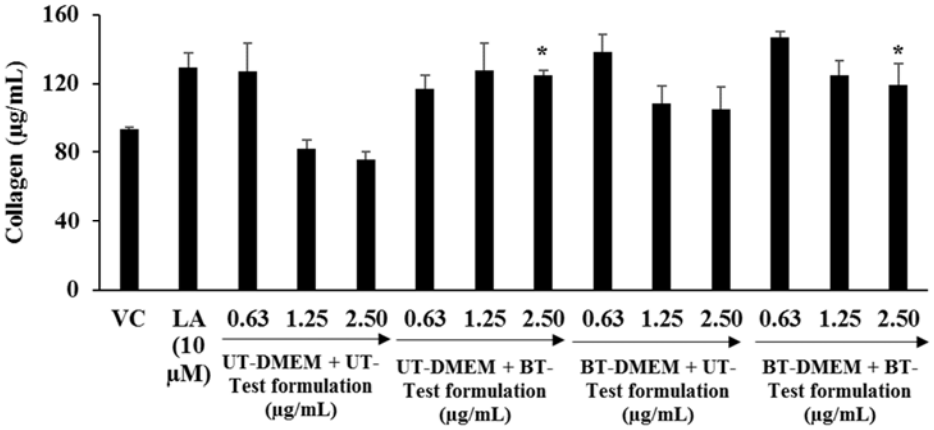

Figure 3. *Effect of the test formulation on collagen synthesis in human foreskin fibroblast cells (HFF-1). VC: Vehicle control; LA: L-Ascorbic acid; UT: Untreated; BT: Biofield Treated. *p≤0.05 vs UT-DMEM + UT-Test formulation using one-way ANOVA (post-hoc Dunnett's test).*

3.3.2. Elastin

The effect of the test formulation and DMEM on elastin level in the human foreskin fibroblast cells (HFF-1) is shown in Figure 4. The level of elastin in the vehicle control (VC) and positive control groups was observed as 6.06 ± 0.00 and 7.27 ± 0.15 pg/mL, respectively. The level of elastin was significantly (p≤0.001) increased by 19.99% in the BT-DMEM + UT-Test formulation group at 2.5 µg/mL compared to the UT-DMEM + UT-Test formulation group. Moreover, at 5 µg/mL the level of elastin was significantly (p≤0.001) elevated by 72.54% and 27.56% in the BT-DMEM + UT-Test formulation and BT-DMEM + BT-Test formulation groups, respectively compared to the UT-DMEM + UT-Test formulation group. Further, at 10 µg/mL the expression of elastin was also significantly (p≤0.001) increased by 6.30%, 105.04%, and 29.41% in the UT-DMEM + BT-Test formulation, BT-DMEM + UT-Test formulation, and BT-DMEM + BT-Test formulation groups, respectively compared to the UT-DMEM + UT-Test formulation group. Elastin is the important component of the skin that maintain the mechanical and cell interactive properties. It induce a wide-range of cellular activities such as cell migration and proliferation, matrix synthesis, and protease production [38]. Elastin enhanced the process of wound healing due to its inherent properties. Cutaneous ageing is the result of two biological processes, which may occur simultaneously as termed as intrinsic ageing and extrinsic ageing. The intrinsic aged skin is due to dryness and lack of elastin than youthful skin [39]. Altogether, the level of elastin synthesis was improved significantly in the Biofield Energy Treated test formulation and DMEM group, which might be due to The Trivedi Effect® - Energy of Consciousness Healing Treatment.

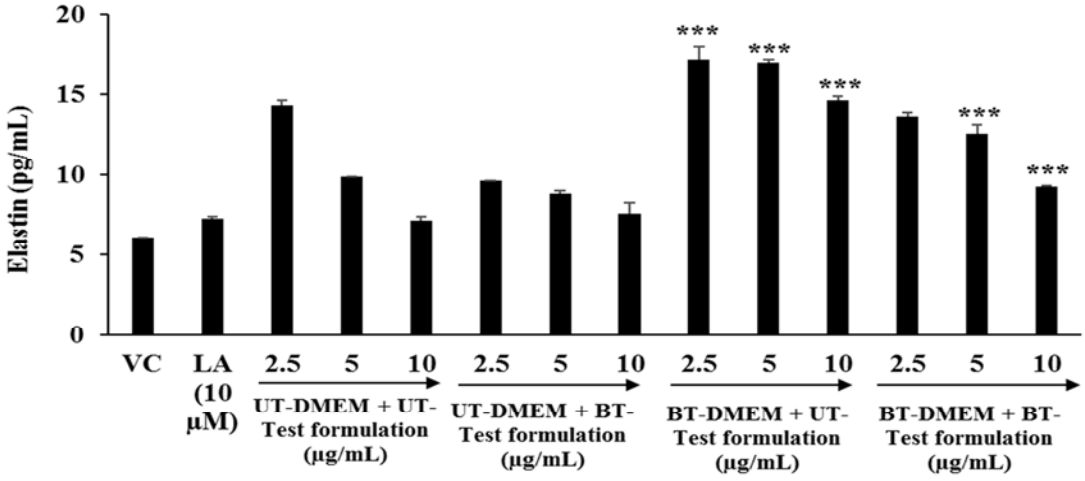

Figure 4. *Effect of the test formulation and DMEM on elastin formation in human foreskin fibroblast cells (HFF-1). VC: Vehicle control; LA: L-Ascorbic acid; UT: Untreated; BT: Biofield Treated. ***p≤0.001 vs UT-DMEM + UT-Test formulation using one-way ANOVA (post-hoc Dunnett's test).*

3.3.3. Hyaluronic Acid (HA)

The effect of the test herbomineral formulation and DMEM for the expression of HA in HFF-1 cells is shown in Figure 5. The results of HA synthesis in the presence of L-ascorbic acid (10 µM), showed significant increase in HA content by 26.37% compared with the vehicle control (VC) group (9.67 ± 0.08 ng/mL). The level of HA was increased significantly ($p \leq 0.001$) by 4.78%, 29.71%, and 58.29% in the UT-DMEM + BT-Test formulation, BT-DMEM + UT-Test formulation, and BT-DMEM + BT-Test formulation groups, respectively at 0.63 µg/mL compared to the UT-DMEM + UT-Test formulation group. Further, at 1.25 µg/mL the HA level was significantly ($p \leq 0.001$) increased by 15.54% in the BT-DMEM + BT-Test formulation group compared to the UT-DMEM + UT-Test formulation group. Additionally, the level of HA was significantly increased by 31.91% in the BT-DMEM + BT-Test formulation group with respect to the UT-DMEM + UT-Test formulation group at 2.5 µg/mL. The overall data suggested that the Biofield Energy Treated test formulation and DMEM have the significant capacity to increase the level of hyaluronic acid.

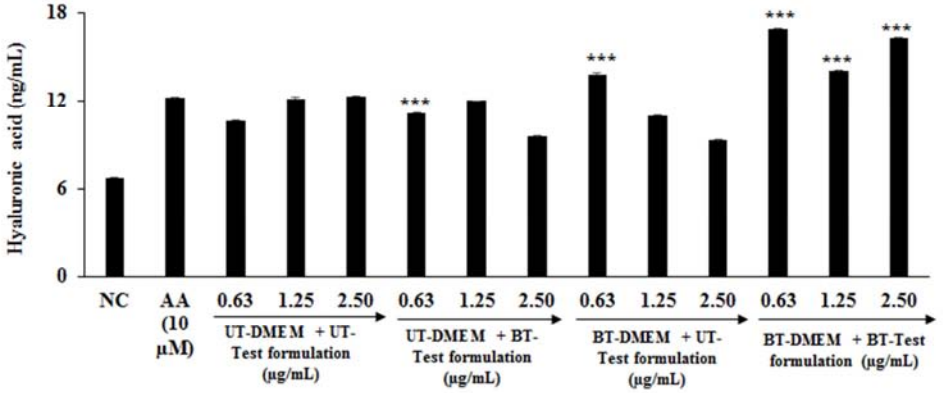

Figure 5. *Effect of the test formulation on the expression of hyaluronic acid in human foreskin fibroblast cells (HFF-1). VC: Vehicle control; LA: L-Ascorbic acid; UT: Untreated; BT: Biofield Treated. ***$p \leq 0.001$ vs UT-DMEM + UT-Test formulation using one- way ANOVA (post-hoc Dunnett's test).*

3.4. Effect of the Test Formulation on Skin Depigmentation

The effect of the test formulation and DMEM on alpha-melanocyte-stimulating hormone (α-MSH) stimulated melanin synthesis in B16-F10 cells is shown in Figure 6. The level of melanin was significantly decreased by 75.92% in the kojic acid (KA) group (5.96 ± 0.2 µg/mL) compared to the α-MSH group (24.75 ± 0.22 µg/mL). The cellular content of melanin was reduced by 12.49%, 6.74%, and 8.79% in the UT-DMEM + BT-Test formulation, BT-DMEM + UT-Test formulation, and BT-DMEM + BT-Test formulation groups, respectively at 0.06 µg/mL compared to the UT-DMEM + UT-Test formulation group. Besides, the level of melanin synthesis was significantly ($p \leq 0.001$) inhibited by 14.64% and 18.25% in the UT-DMEM + BT-Test formulation and BT-DMEM + UT-Test formulation groups, respectively at 0.13 µg/mL compared to the UT-DMEM + UT-Test formulation group. Thus, it can be concluded that the Biofield Energy Treated test formulation and DMEM inhibits the melanin production significantly in the B16-F10 cells. This improvement could be beneficial for the development of a cosmeceuticals for hyperpigmentation and different types of skin conditions.

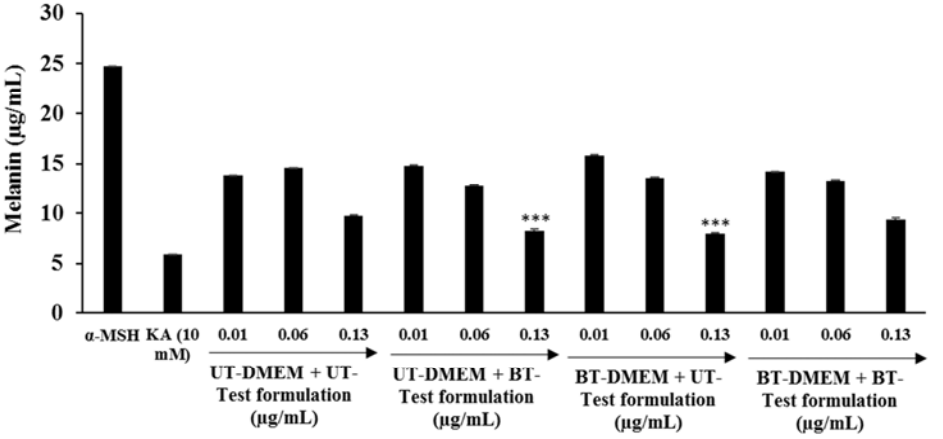

Figure 6. *Effect of the test formulation on alpha-MSH stimulated melanin in B16-F10 cells. α-MSH: Alpha-melanocyte-stimulating hormone, KA: Kojic acid (mM); UT: Untreated; BT: Biofield Treated. ***$p \leq 0.001$ vs UT-DMEM + UT-Test formulation using one-way ANOVA (post-hoc Dunnett's test).*

3.5. Anti-wrinkle Effects of the Test Formulation on HFF-1 Cells against UV-B Induced Stress

The effect of the test formulation with DMEM after pretreatment with UV-B challenge in HFF-1 cells is represented in Figure 7. The cell viability was identified using hemocytometer. The cells were subjected to lethal dose of UV-B irradiation (200 mJ/cm^2) showed 26.73% cell viability. The cell viability was 100% and 27.78% in the normal control (NC) and vehicle control (VC) groups respectively. The cell viability was increased by 55.11% in the positive control (L-ascorbic acid) group compared to the VC group. After UV-B induce stress condition the level of cell viability was significantly increased by 17.88%, 20.14%, and 9.89% in the BT-DMEM + BT-Test formulation at 0.63, 1.25, and 2.50 µg/mL, respectively compared to the UT-DMEM + UT-Test formulation group. Besides, the rest of the concentrations did not show any alteration with respect to the UT-DMEM + UT-Test formulation group. Several factors are responsible for skin wrinkles such as aging, genetics, and environmental factors such as ultraviolet radiation, smoking and due to deficiency of estrogen [40, 41]. Aging is one of the most important factor responsible for skin wrinkles. In humans, due to aging the skin becomes thin and decrease elasticity, collagen, etc. [42, 43]. The results suggested that both the Biofield Energy Treated test formulation and DMEM could be significantly used for skin protective effect with anti-wrinkling potential.

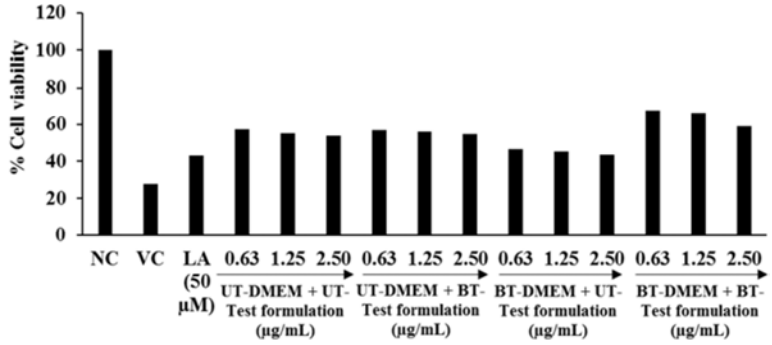

Figure 7. *Percentage restoration of the cell viability in HFF-1 cells after 20 hours of pretreatment before UV-B challenge. NC: Normal control; VC: Vehicle control LA: L-Ascorbic acid; UT: Untreated; BT: Biofield Treated.*

3.6. Wound Healing Activity by Scratch Assay

The wound healing activity by scratch assay of the test formulation and DMEM was performed for the measurement of cell migration in HFF-1 and HaCaT cells. The representative photomicrographs are presented in Figure 8. The cell coverage area was increased by 10.5%, 8.8%, and 7.0% in the UT-DMEM + BT-Test formulation, BT-DMEM + UT-Test formulation, and BT-DMEM + BT-Test formulation groups, respectively at 0.63 µg/mL in HFF-1 cells compared to the UT-DMEM + UT-Test formulation group. Additionally, the cell coverage area was increased by 1.8% (at 2.5 µg/mL) and 8.8% (1.25%) in the BT-DMEM + UT-Test formulation and BT-DMEM + BT-Test formulation groups, respectively in HFF-1 cells compared to the UT-DMEM + UT-Test formulation group (Figure 8A). Moreover, the cell coverage area was increased by 8.3% at 2.5 µg/mL in the UT-DMEM + BT-Test formulation, BT-DMEM + UT-Test formulation, and BT-DMEM + BT-Test formulation groups in HaCaT cells compared to the UT-DMEM + UT-Test formulation group. Furthermore, the cell coverage area was increased by 2.8%, 2.8%, and 1.4% in the UT-DMEM + BT-Test formulation, BT-DMEM + UT-Test formulation, and BT-DMEM + BT-Test formulation groups, respectively in HaCaT cells compared to the UT-DMEM + UT-Test formulation group (Figure 8B). In vitro scratch assay is a well-established method for the estimation of cell migration, cell-matrix, and cell-to-cell interactions during wound healing [44]. The wound healing results indicated that the test formulation and DMEM showed significant wound closure activity.

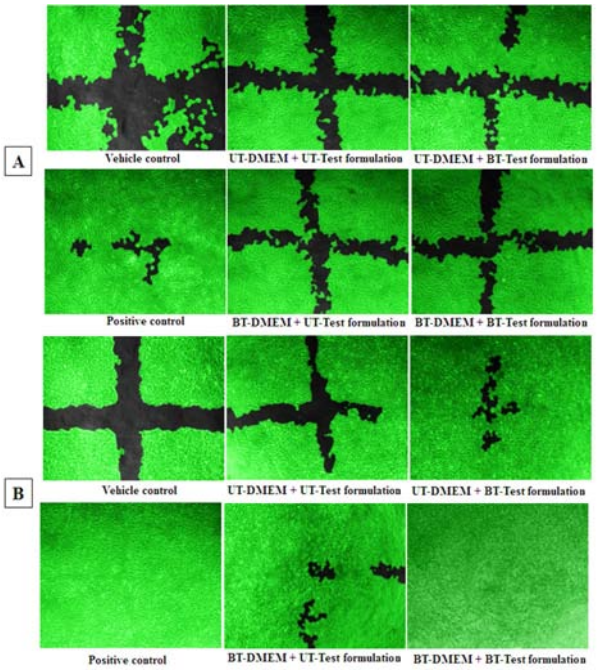

Figure 8. *Effect of the test formulation and DMEM on wound closure and cell migration after 16 hours of treatment. Representative photomicrograph (X10) of the test formulation shown in A. HFF-1 and B. HaCaT cells. UT: Untreated; BT: Biofield Treated.*

4. Conclusions

The cell viability using MTT assay exhibited more than 78% cells were viable, indicating that the Biofield Energy Healing based herbomineral test formulation was safe and nontoxic in all the tested concentrations. The percent cell proliferation using BrdU was significantly increased by 238.30%, 192.06%, and 43.96% in the UT-DMEM + BT-Test formulation, BT-DMEM + UT-Test formulation, and BT-DMEM + BT-Test formulation groups, respectively at 17.5 µg/mL compared to the UT-DMEM + UT-Test formulation group. The level of collagen was significantly increased by 55.55%, 32.65%, and 52.48% in the UT-DMEM + BT-Test formulation, BT-DMEM + UT-Test formulation and BT-DMEM + BT-Test formulation groups, respectively at 1.25 µg/mL compared to the UT-DMEM + UT-Test Formulation group. Elastin was significantly ($p \leq 0.001$) increased by 6.30%, 105.04%, and 29.41% at 10 µg/mL in the UT-DMEM + BT-Test formulation, BT-DMEM + UT-Test formulation, and BT-DMEM + BT-Test formulation groups, respectively compared to the UT-DMEM + UT-Test formulation group. Hyaluronic acid was increased significantly by 4.78%, 29.71%, and 58.29% in the UT-DMEM + BT-Test formulation, BT-DMEM + UT-Test formulation, and BT-DMEM + BT-Test formulation groups, respectively at 0.63 µg/mL compared to the UT-DMEM + UT-Test Formulation group. Melanin level was significantly ($p \leq 0.001$) reduced by 14.64% and 18.25% in the UT-DMEM + BT-Test formulation and BT-DMEM + UT-Test formulation groups, respectively at 0.13 µg/mL with respect to the UT-DMEM + UT-Test formulation group. Anti-wrinkling effect using UV-B induced stress in HFF-1 cells showed that the Biofield Energy Treated test formulation was increased significantly by 17.88%, 20.14%, and 9.89% at 0.63, 1.25, and 2.5 µg/mL, respectively in the BT-DMEM + BT-Test formulation group compared to the UT-DMEM + UT-Test formulation group. Wound healing results displayed a significant effect of the test formulation and DMEM on wound closure and cell migration in all the tested groups in both HFF-1 and HaCaT cells compared to the untreated groups. Overall, the Consciousness Energy Healing Treated test formulation (The Trivedi Effect®) and DMEM have shown significant protective effects on various skin health parameters such as wrinkling, aging, skin whitening, and wound healing. Therefore, the Biofield Energy Healing based herbomineral test formulation would be suitable for the development of herbal cosmetics, and it would be useful for the management of wounds and various skin related disorders viz. abscess, pimple, cellulitis, impetigo, scabies, syringoma, photosensitivity, urticaria, hives, warts, abscess, callus, acne, chickenpox, eczema, rosacea, seborrheic dermatitis, athlete's foot, psoriasis, erythema, contact dermatitis, cutis rhomboidalis nuchae, skin aging, wrinkles and/or change in skin color etc.

Abbreviations

HaCaT: Human keratinocytes, HFF-1: Human fibroblast cell line, B16-F10: Mouse melanoma cell line, THC: Tetrahydrocurcumin, ECM: Extracellular matrix, EGF: Epidermal growth factor, α-MSH: Alpha-melanocyte-stimulating hormone, ANOVA: One-way analysis of variance, HA: Hyaluronic acid, UT: Untreated, BT: Biofield Treated, FBS: Fetal bovine serum, BrdU: Bromodeoxyuridine, ROS: Reactive oxygen species, CAM: Complementary and alternative medicine, DMEM: Dulbecco's modified eagle's medium, ATCC: American type culture collection, NCCS: National centre for cell science, UV: Ultra-violet.

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