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The Use of Consciousness Energy Healing Based Herbomineral Formulation for Skin Anti-Aging Strategies

Vaibhav Rajan Parulkar[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, Nevada, 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 present study was undertaken to evaluate the effect of a Consciousness Energy Healing (The Trivedi Effect®) Treatment based test formulation and medium (DMEM) against skin health parameters using HFF-1, HaCaT, and B16-F10 cells. The test formulation and DMEM were divided into two parts. One part of the test formulation and one part of the DMEM received the Consciousness Energy Healing Treatment by Vaibhav Rajan Parulkar and were defined as the Biofield Energy Treated samples, while the other parts were denoted as the untreated test samples. Cell viability using MTT assay showed more than 75% cells were viable in all the tested concentrations in three cells, indicating that the test formulation was safe and nontoxic. The percent cell proliferation by BrdU assay was significantly increased by 434.14%, 244.77%, and 268.53% in the UT-DMEM + BT-Test Formulation, BT-DMEM + UT-Test Formulation, and BT-DMEM + BT-Test Formulation groups, respectively at 8.75 µg/mL in relation to the UT-DMEM + UT-Test Formulation group. Elastin was significantly ($p \leq 0.001$) increased 93.52% and 75.81% in the UT-DMEM + BT-Test Formulation and BT-DMEM + UT-Test Formulation groups, respectively at 10 µg/mL compared to the UT-DMEM + UT-Test Formulation group. Hyaluronic acid was significantly increased by 100.07% ($p \leq 0.05$), 41.60%, and 50.10% in the UT-DMEM + BT-Test Formulation, BT-DMEM + UT-Test Formulation, and BT-DMEM + BT-Test Formulation groups, respectively at 0.625 µg/mL in relation to the UT-DMEM + UT-Test Formulation group. Melanin was decreased by 10.09% and 6.51% in the UT-DMEM + BT-Test Formulation and BT-DMEM + UT-Test Formulation groups, respectively at 0.125 µg/mL compared to the UT-DMEM + UT-Test Formulation group. Protection of skin cells after UV-B exposure data displayed that the cell viability was increased by 7.22%, 5.75%, and 8.15% in the BT-DMEM + BT-Test Formulation at 0.625%, 1.25%, and 2.5% µg/mL, respectively compared to the UT-DMEM + UT-Test Formulation group. Wound healing data exhibited significant wound closure and cell migration activities in the HFF-1 cells compared to the UT-DMEM + UT-Test formulation group. Overall, the data suggests that the Biofield Energy Treated DMEM and test formulation demonstrated better skin protection action compared to the untreated DMEM and test formulation. Therefore, the Biofield Energy Treated test herbomineral formulation could be useful for the development of effective cosmetic products 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: Extracellular Matrix, HFF-1, B16-F10, HaCaT, Wound Healing, Consciousness Energy Healing, The Trivedi Effect®, Skin Protection

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 the main factors that

causes several skin disorders such as skin cancer and photoaging. In recent years, particular antioxidants have gained considerable attention as means of neutralizing ROS [1]. The proprietary herbomineral formulation consists 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 benefits for skin health in the form of various medicines, as well as cosmeceuticals. Zinc is well known to play a critical role in overall human physiology. It is an essential cofactor of various metalloenzymes and it protects the skin from UV radiation 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]. Lots of evidence 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, a 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]. Exposure to UV radiation and environmental pollutants can accelerate the skin aging by degrading collagen and triggering oxidative stress in the skin. Vitamin C is an essential constituent for the production of collagen and a potent antioxidant that can help rejuvenate aged and photo damaged skin [8, 9]. Sugiyama et al. [10] demonstrated that THC exhibited strong anti-oxidant and anti-cancer activities. 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 an effective treatment for small wounds, hypertrophic wounds, burns, psoriasis and scleroderma through promoting the proliferation of fibroblast cells. 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].

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 are regularly using 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 the endocrine system. The cells that are present in the central nervous system of the human body communicate with each other by means of electrical signals that propagate along the nerve impulses [14]. It is hypothesized that the Biofield can exist around the human body and evidence was found using electromyography, electrocardiography and electroencephalogram [15]. Thus, a Biofield 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 receives the energy and responds 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 organisms 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, the 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

Tetrahydrocurcumin and *Centella asiatica* extract were procured from Novel Nutrients Pvt. Ltd., India and Sanat Products Ltd., India, respectively. 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. 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

The HFF-1 (human fibroblast) cells were procured from American Type Culture Collection (ATCC), USA, and 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% CO_2, 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 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 and one part of the DMEM were considered as a control samples, while the other parts of each were defined as the treated samples. Both the samples were kept under standard laboratory conditions at the research laboratory of Dabur Research Foundation, near New Delhi, India. The treated sample was subjected to the Consciousness Energy Healing (The Trivedi Effect®) Treatment by Vaibhav Rajan Parulkar remotely for 5 minutes from U.S.A. through the Healer's unique Energy Transmission process remotely to the test formulation under laboratory conditions. The Biofield Energy Healer, Vaibhav Rajan Parulkar, never visited the laboratory in person, nor had any contact with the test samples. Similarly, the control samples were subjected to "sham" healers under the same laboratory conditions for 5 minutes. The sham healers 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 and used for this experiment.

2.5. Determination of Non-Cytotoxic Concentration

The cell viability was performed by MTT assay in three cell lines such as HFF-1 (human fibroblast), HaCaT (human keratinocytes), and B16-F10 (mouse melanoma). The cells were counted and plated in 96 well plates at the density corresponding to 5 X 10^3 to 10 X 10^3 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. 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 CO_2 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 concentration exhibiting % cytotoxicity of < 30 % was considered as non-cytotoxic [28, 29].

The percentage of cell viability was calculated using the following Equation 1:

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

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

2.6. Effect of the Test Sample on Fibroblast Proliferation by 5-Bromo-2'-Deoxyuridine (BrdU) Method

HFF-1 cells were counted using hemocytometer and plated in 96 well plates at the density corresponding to 1 X 10^3 to 5 X 10^3 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 skin strength, elasticity and hydration level. HFF-1 cells were counted using hemocytometer and plated in 48 well plates at the density corresponding to 10 X 10^3 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 (ascorbic acid, at 10 µM concentration), and the test samples treatment at different concentrations. Further, after 72 hours of incubation with the test samples 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 the estimation of collagen level 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 10^6 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. After incubation, intracellular melanin was extracted in NaOH and the absorbance was recorded at 405 nm. The levels of melanin were 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 the cell viability was estimated in the presence of the test samples. The cells were counted using hemocytometer and plated in 96 well plates 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 the test samples for 2 to 24 hours. After treatment with the test samples, 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 the following Equation 2:

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

Where, X represents the absorbance of cells corresponding to positive control and test groups, and 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 10^6/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 scratches that represent wounds were created in the near confluent monolayer of cells by gently scraping with a 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 fibroblast distance covered and subsequent scratch closure [34].

2.11. Statistical Analysis

Each experiment was carried out in three independent assays and was 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. MTT Assay for Cell Viability

MTT assay was used for the evaluation of the viable cells in three different cell lines (HFF-1, HaCaT, and B16-F10) is shown in Figure 1A to 1C. The result of cell viability in the HFF-1 cells exhibited about >75% viable cells in the tested concentrations ranges from 0.625 to 10 µg/mL (Figure 1A). The selected concentrations were used for the estimation of extracellular matrix (ECM) synthesis in HFF-1 cells such as collagen, elastin, and hyaluronic acid. The cell viability in HaCaT cells revealed that the tested concentrations exhibited >96% cell viability. The concentrations (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 data exhibited that the test groups were non-cytotoxic (*i.e.* percentage cell viability value >80%). The tested concentrations were used further for the measurement of melanin level from 10 to 40 µg/mL (Figure 1C).

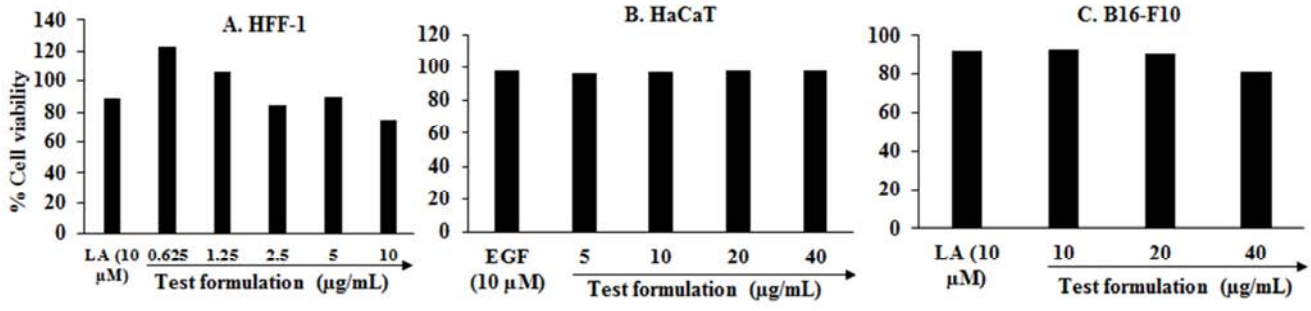


Figure 1. *Effect of the test formulation on cell viability in different cells at various concentrations A. HFF-1 cells after 72 hours of treatment. B. HaCaT cells after 48 hours of treatment. C. B16-F10 cells after 48 hours of treatment. LA: L-Ascorbic acid; EGF: Epidermal growth factor.*

3.2. BrdU Assay for Cell Proliferation

The cell proliferation was analysed with the help of BrdU assay and the data are shown in Figure 2. The percentage of cell proliferation was increased by 113.65% in the positive control groups (FBS-0.5 µg/mL) compared to the vehicle control (VC) group. Results of the percent cell proliferation was increased by 434.14%, 244.77%, and 268.53% at 8.75 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. Moreover, the percent cell proliferation was enhanced by 354.67%, 202.79%, and 107.73% 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 percentage of cell proliferation was significantly elevated by 360.43%, 326.18%, and 327.95% 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 bromodeoxyuridine (BrdU) assay was used for the evaluation of the rate of DNA replication, analysis of metabolic activity and recognitions of cell surface antigen activity [35]. Overall, the Biofield Energy Treated test formulation and DMEM showed significant cell proliferation response compared to the untreated group, due to the Biofield Energy Healing Treatment.

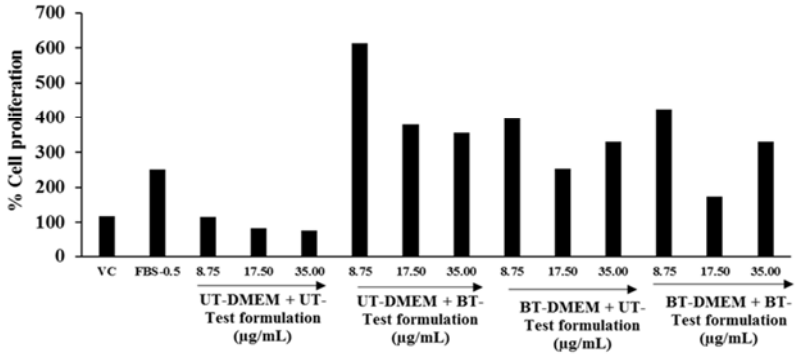


Figure 2. *Effect of the test formulation on cellular proliferation after 48 hours using BrdU assay. VC: Vehicle control; FBS: Fetal bovine serum (µg/mL); UT: Untreated; BT: Biofield Treated.*

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

3.3.1. Collagen

The expression of collagen in the presence of the test formulation and DMEM in HFF-1 cells is presented in Figure 3. The level of collagen in the vehicle control (VC) group was 58.55 ± 5.91 µg/mL and it was significantly increased by 121.04% in the positive control group (129.42 ± 8.50 µg/mL). The level of collagen did not show any significant alteration in all the treated groups compared to the UT-DMEM + UT-Test formulation group. Several stimuli such as local tissue ischemia, necrotic tissue, repeated trauma, etc. caused 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 [37]. Ultimately due to damage of collagen the repair process also delayed. Therefore, the control of collagen metabolism might be useful for a variety of therapeutic and cosmetic applications. Overall, the level of collagen synthesis was altered to some extent in the Biofield Energy Treated test formulation and DMEM group.

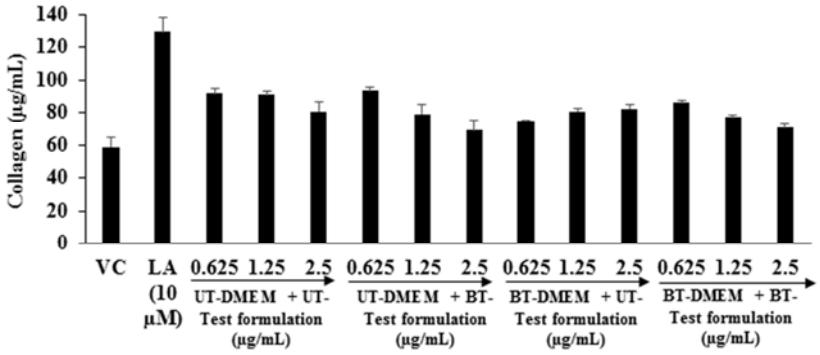
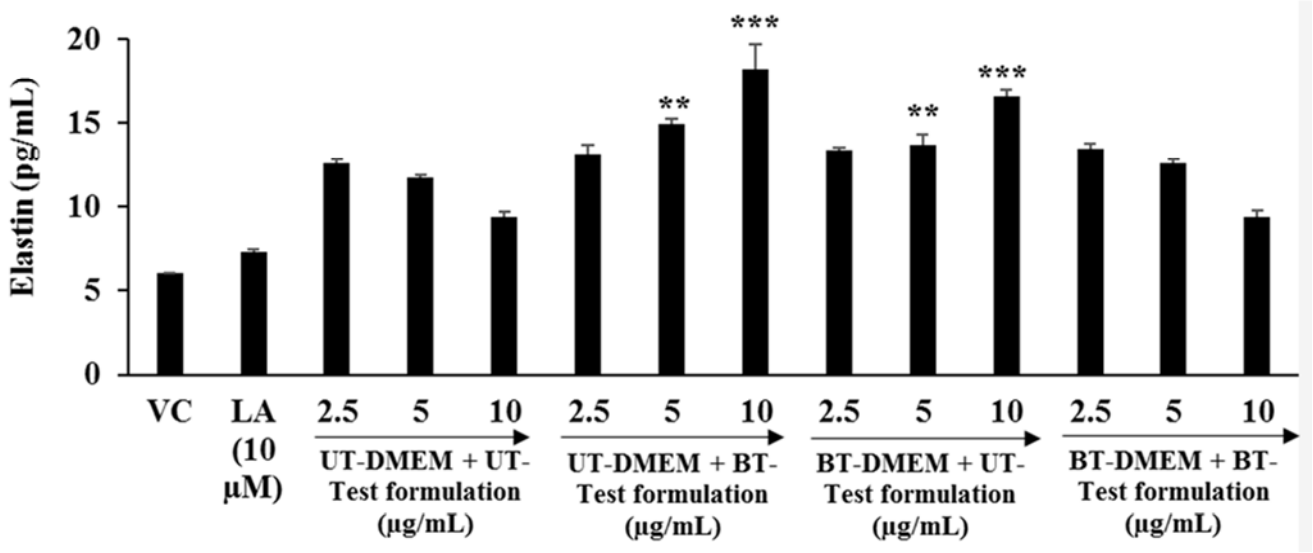


Figure 3. *Effect of the test formulation and DMEM on the expression of collagen in human foreskin fibroblast cells (HFF-1). VC: Vehicle control; LA: L-Ascorbic acid; UT: Untreated; BT: Biofield Treated.*

3.3.2. Elastin

The effects 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) group was 6.06 ± 0.00 pg/mL and it was significantly increased by 19.97% in the positive control group (7.27 ± 0.15 pg/mL). The level of elastin was significantly increased by 3.96%, 6.17%, and 6.57% at 2.5 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. Moreover, at 5 µg/mL the level of elastin was significantly elevated by 27.65%, 16.81%, and 7.68% 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. Further, at 10 µg/mL the expression of elastin was also significantly increased by 93.52% and 75.81% in the UT-DMEM + BT-Test formulation and BT-DMEM + UT-Test formulation groups, respectively compared to the UT-DMEM + UT-Test formulation group. Elastin is the important component of the skin, responsible to maintain the mechanical and cell interactive properties. It induces a wide-range of cellular activities such as cell migration and proliferation, matrix synthesis, and protease production [38]. Due to these inherent properties of elastin it enhances the process of wound healing. 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 as compared to youthful skin [39]. Overall, the level of elastin was remarkably improved in the test formulation and DMEM group, due to the power of The Trivedi Effect® - Consciousness Energy Healing Treatment by the renowned healing practitioner.

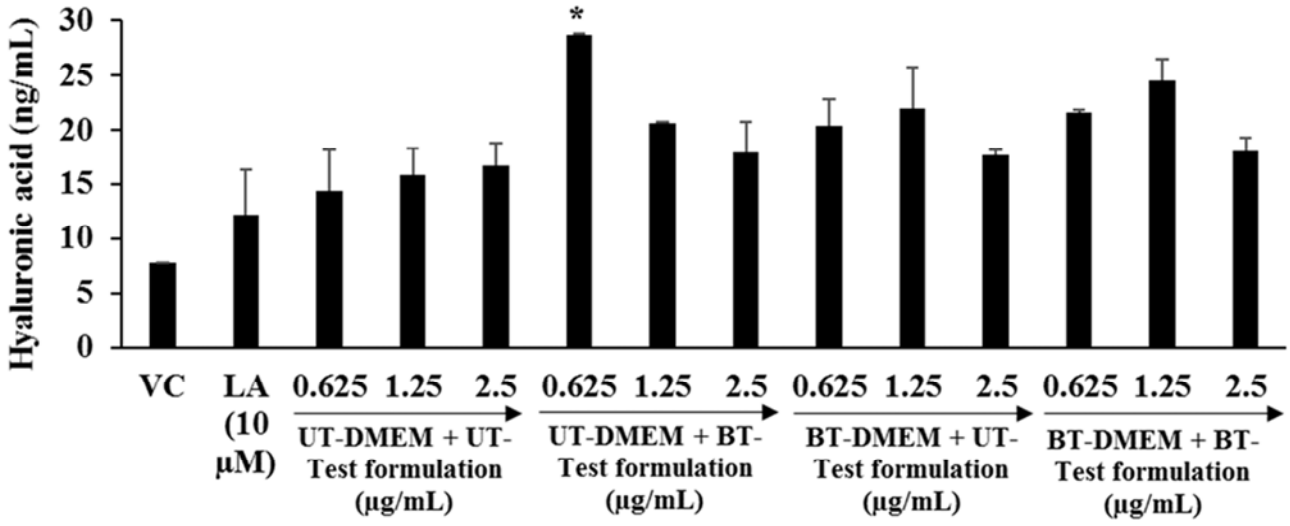


**p≤0.01 and ***p≤0.001 vs UT-DMEM + UT-Test formulation using one way ANOVA (post-hoc Dunnett's test).

Figure 4. *Effect of the test formulation and DMEM on the collagen level in human foreskin fibroblast cells (HFF-1). VC: Vehicle control; LA: L-Ascorbic acid; UT: Untreated; BT: Biofield Treated.*

3.3.3. Hyaluronic Acid (HA)

The effects of the test formulation and DMEM on the expression of hyaluronic acid (HA) in HFF-1 cells are shown in Figure 5. The results of HA synthesis in the presence of ascorbic acid (10 µM), showed significant increased in HA content by 56.27% compared with the vehicle control (VC) group (7.82 ± 0.01 ng/mL). The level of HA was significantly increased by 100.07% (p≤0.05), 41.60%, and 50.10% in the UT-DMEM + BT-Test formulation, BT-DMEM + UT-Test formulation, and BT-DMEM + BT-Test formulation groups, respectively at 0.625 µg/mL compared to the UT-DMEM + UT-Test formulation group. Further, at 1.25 µg/mL the HA level was significantly increased by 30.35%, 38.55%, and 54.45% 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. Additionally, the level of HA was increased by 7.48%, 5.80%, and 7.61% in the UT-DMEM + BT-Test formulation, BT-DMEM + UT-Test formulation, and BT-DMEM + BT-Test formulation groups, respectively with respect to the UT-DMEM + UT-Test formulation group at the concentration of 2.5 µg/mL. The overall data suggested that the Biofield Energy based test herbomineral formulation and DMEM have the significant capacity to increase the expression of the extracellular matrix component, hyaluronic acid.



*p≤0.05 vs UT-DMEM + UT-Test formulation using one-way ANOVA (post-hoc Dunnett's test).

Figure 5. *Evaluation of the level of hyaluronic acid content after treatment with the test formulation and DMEM in human foreskin fibroblast cells (HFF-1). VC: Vehicle control; LA: L-Ascorbic acid; UT: Untreated; BT: Biofield Treated.*

3.4. Effect of the Test Formulation on Skin Depigmentation

The effect of the test formulation on alpha-MSH stimulated melanin synthesis in B16-F10 cells is shown in Figure 6. The level of melanin in the alpha-MSH group was 24.9 ± 0.56 µg/mL and it was significantly reduced by 63.49% in the kojic acid (KA) group (9.09 ± 3.03 µg/mL). The cellular content of melanin was reduced by 5.43% and 7.96% in the UT-DMEM + BT-Test formulation and BT-DMEM + BT-Test formulation groups, respectively at 0.013 µg/mL compared to the UT-DMEM + UT-Test formulation group. The level of melanin synthesis was inhibited by 9.16% in the UT-DMEM + BT-Test formulation group at 0.063 µg/mL compared to the UT-DMEM + UT-Test formulation group. Additionally, the melanin synthesis was suppressed by 10.09% and 6.51% in the UT-DMEM + BT-Test formulation and BT-DMEM + UT-Test formulation groups, respectively at 0.125 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 inhibit the melanin production minimally in the B16-F10 cells. This improvement might be beneficial for the development of cosmeceutical products for hyperpigmentation and different types of skin conditions.

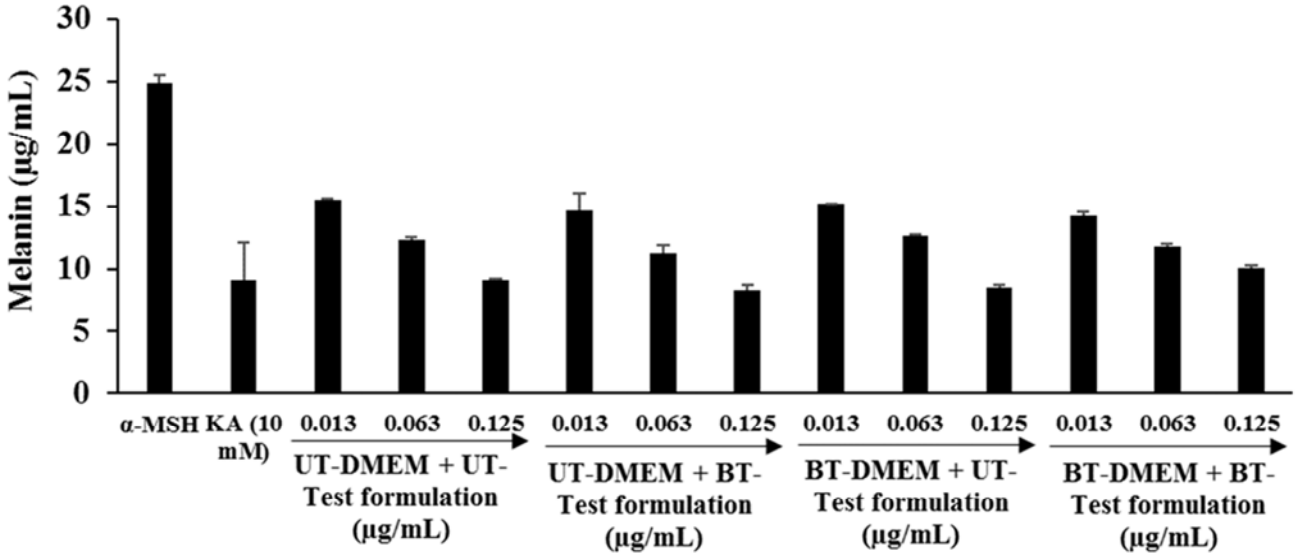


Figure 6. *Effect of the test formulation and DMEM on alpha-MSH stimulated melanin synthesis in B16-F10 cells. KA: Kojic acid (mM); UT: Untreated; BT: Biofield Treated, α-MSH: Alpha-melanocyte-stimulating hormone.*

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

The effect of the Biofield Energy Treated test formulation and DMEM against UV-B challenges is shown in Figure 7. The cell viability was measured using hemocytometer. The cells were subjected to lethal dose of UV-B irradiation (200 mJ/cm^2) and found 26.73% cell viability. Cell viability in the normal control (NC), vehicle control (VC), and positive control groups was 100%, 27.78%, and 43.17%, respectively. After UV-B induced stress condition the level of the percent cell proliferation was increased by 7.22%, 5.75%, and 8.15% in the BT-DMEM + BT-Test formulation group at 0.625, 1.25, and 2.5 µg/mL, respectively compared to the UT-DMEM + UT-Test formulation group. The rest of the treated groups 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 factors responsible for skin wrinkles. In humans, due to aging the skin becomes thin and decreases in elasticity, collagen, etc. [42, 43]. The data suggests that the Biofield Energy Treated test formulation and DMEM minimally improved the anti-wrinkle activity could be used for the preparation of anti-wrinkling formulation.

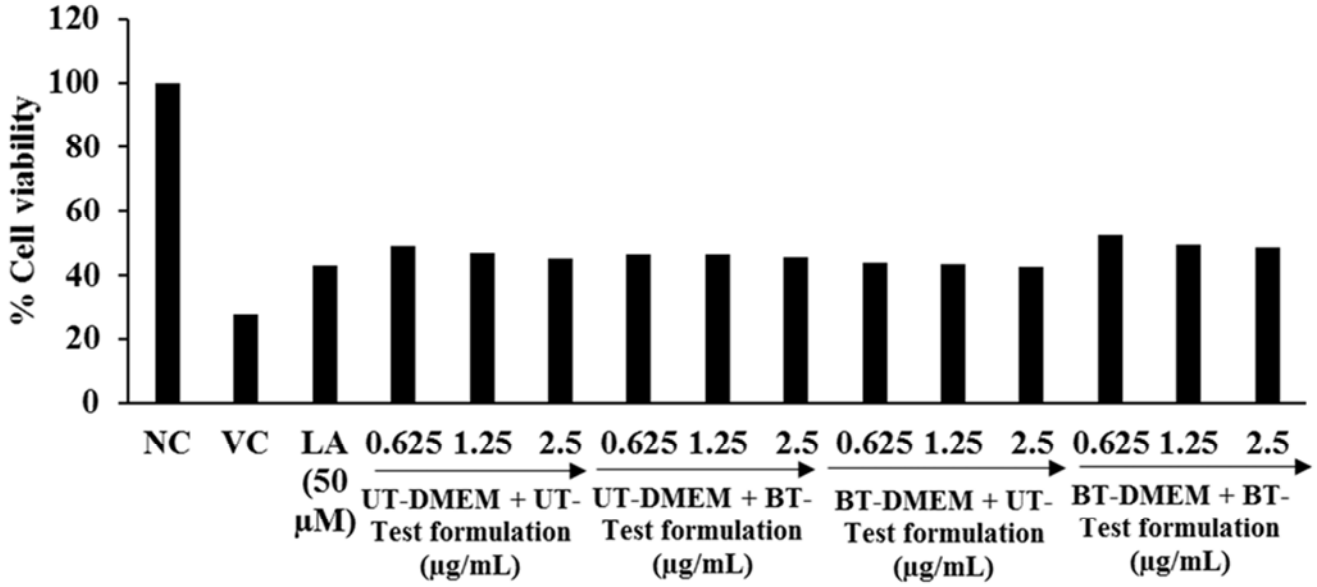


Figure 7. *Percentage restoration of the cell viability in HFF-1 cells after 20 hours pretreatment of the test formulation and DMEM before UV-B challenge. NC: Normal control; VC: Vehicle control LA: L-Ascorbic acid; UT: Untreated; BT: Biofield Treated.*

3.6. Wound Healing Activity by Scratch Assay

Scratch assay was used for the measurement of wound healing activity in HFF-1 and HaCaT cell lines after treatment with the test formulation and DMEM. The representative photomicrographs are presented in Figure 8. The cell coverage area was increased by 7%, 6%, and 6% 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 in HFF-1 cells compared to the UT-DMEM + UT-Test formulation group. The cell coverage area was increased by 3% and 4% in the UT-DMEM + BT-Test formulation and BT-DMEM + UT-Test formulation groups, respectively at 5 µg/mL in HFF-1 cells compared to the UT-DMEM + UT-Test formulation group (Figure 8A). Besides, the cell coverage area was increased by 1% in the UT-DMEM + BT-Test formulation and BT-DMEM + BT-Test formulation groups, respectively at 5 µg/mL in HaCaT cells compared to the UT-DMEM + UT-Test formulation group (Figure 8B). The scratch assay for screening the wound healing activity is a well established method that measured the cell migration, cell-matrix and cell-to-cell interactions during the wound healing process [44]. The results showed significant wound closure activity in HFF-1 cells compared to the untreated group.

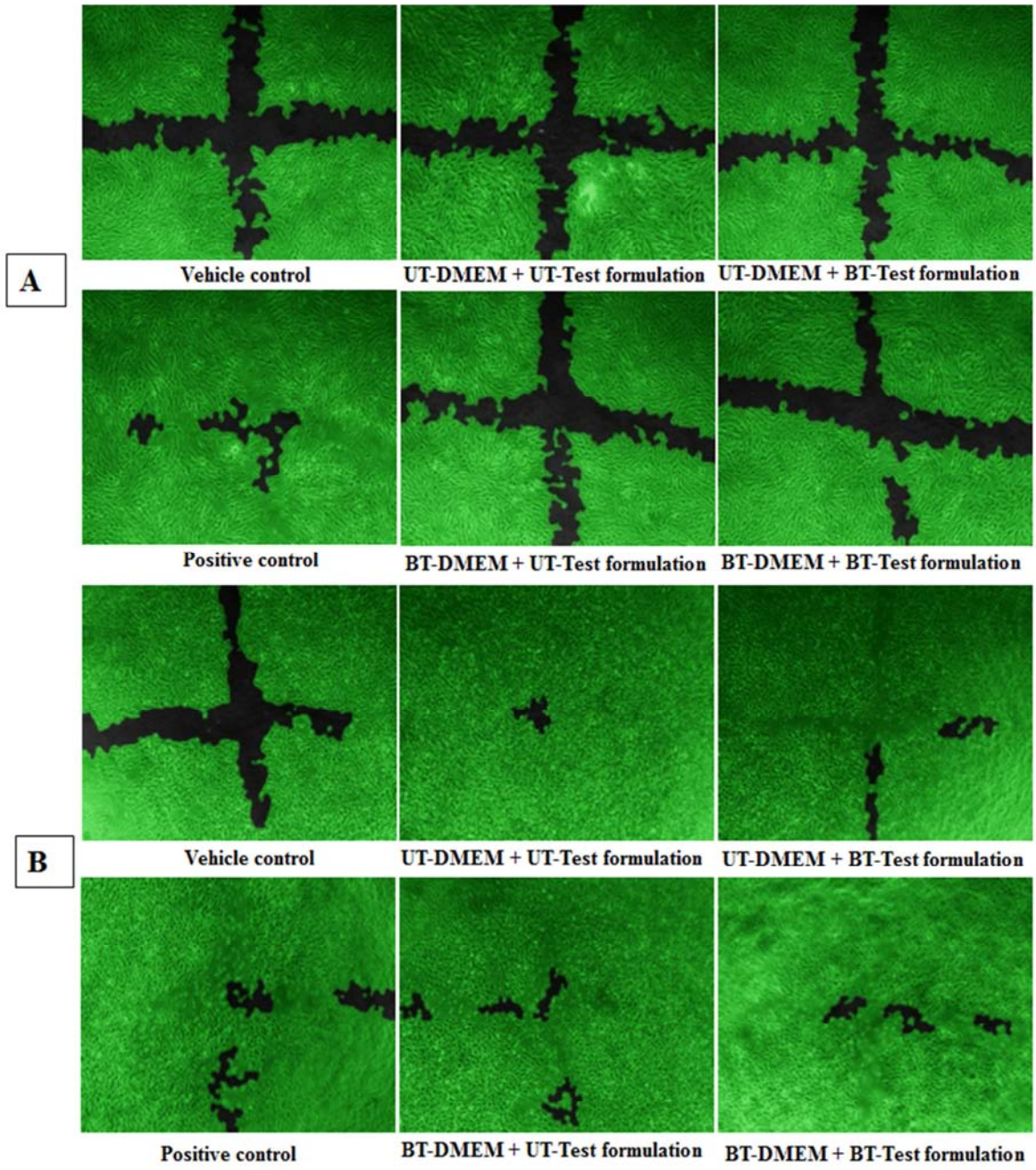


Figure 8. *Effect of the test formulation and DMEM on wound healing activity after 16 hours of treatment. Representative photomicrographs (X10) of wound closure and cell migration are shown in A. HFF-1 and B. HaCaT cells. UT: Untreated; BT: Biofield Treated.*

4. Conclusions

The results showed that the cell viability by MTT assay exhibited more than 75% cells were viable in all the tested concentrations, indicating that the test formulation was safe and nontoxic. The percent cell proliferation data using BrdU assay was significantly increased by 434.14%, 244.77%, and 268.53% in the UT-DMEM + BT-Test formulation, BT-DMEM + UT-Test formulation, and BT-DMEM + BT-Test formulation groups, respectively at 8.75 µg/mL compared to the UT-DMEM + UT-Test formulation group. Further, it was enhanced by 354.67%, 202.79%, and 107.73% in 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 percentage of cell proliferation was elevated by 360.43%, 326.18%, and 327.95% 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. The level of elastin was significantly ($p \leq 0.001$) increased by 93.52% and 75.81% in the UT-DMEM + BT-Test formulation and BT-DMEM + UT-Test formulation groups, respectively at 10 µg/mL compared to the UT-DMEM + UT-Test formulation group. Hyaluronic acid was increased by 100.07% ($p \leq 0.05$),

41.60%, and 50.10% at 0.625 µg/mL in the BT-DMEM + UT-Test formulation, BT-DMEM + BT-Test formulation, and BT-DMEM + BT-Test formulation groups, respectively compared to the UT-DMEM + UT-Test formulation group. However, it was also increased significantly by 30.35%, 38.55%, and 54.45% 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. Melanin level was reduced by 9.16% in the UT-DMEM + BT-Test formulation group at 0.063 µg/mL with respect to the UT-DMEM + UT-Test formulation group. Additionally, the melanin content was also decreased by 10.09% and 6.51% in the UT-DMEM + BT-Test formulation and BT-DMEM + UT-Test formulation groups, respectively at 0.125 µg/mL compared to the UT-DMEM + UT-Test formulation group. Protection with respect to UV-B, revealed that the level of cell viability was increased by 7.22%, 5.75%, and 8.15% in the BT-DMEM + BT-Test formulation at 0.625, 1.25, and 2.5 µg/mL, respectively compared to the UT-DMEM + UT-Test formulation group. Wound healing results displayed a significant effect on wound closure and cell migration in all the tested groups in HFF-1 cells compared to the untreated group. 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 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.* skin abscess, pimples, 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, 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

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