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Skin Rejuvenating Effect of Consciousness Energy Healing Treatment Based Herbomineral Formulation

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Abstract: Currently in today's world, herbomineral based cosmetic formulations are continuously gaining popularity globally because they have a high safety profile and skin protection effects. The aim of this study was to evaluate the effects of a Consciousness Energy Healing (The Trivedi Effect®) Treatment based test formulation and medium (DMEM) against different skin health parameters. The test formulation and DMEM were divided into two parts. One part of each received the Consciousness Energy Healing Treatment by James Jeffery Peoples and were termed as the Biofield Energy Treated samples, while the other samples were denoted as the untreated test items. MTT assay showed more than 70% 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 increased by 251.30%, 373.36%, and 722.48% at 8.75, 17.5, and 35 µg/mL, respectively in the BT-DMEM + BT-Test formulation group compared to the UT-DMEM + UT-Test formulation group. Collagen synthesis was significantly ($p \leq 0.001$) increased by 32.96% in the UT-DMEM + BT-Test formulation group at 2.5 µg/mL with respect to the untreated group. Elastin was significantly ($p \leq 0.001$) increased by 69.49% and 17.13% in the UT-DMEM + BT-Test formulation and BT-DMEM + UT-Test formulation groups, respectively at 10 µg/mL compared to the untreated group. The level of hyaluronic acid was increased remarkably by 14.78% in the BT-DMEM + UT-Test formulation group at 1.25 µg/mL compared to the untreated group. Melanin was suppressed significantly 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 untreated group. Skin cells protection against UV-B exposure revealed that the percent cell viability was increased by 4.23% in the BT-DMEM + BT-Test formulation group at 2.5 µg/mL compared to the untreated group. The wound healing activity by scratch assay showed significant wound closure and cell migration activity in the test formulation and DMEM group in both HFF-1 and HaCaT cells compared to the untreated group. Altogether, the data suggests that the Biofield Energy Treated DMEM and test formulation showed better responses compared to the untreated group. Overall, the Biofield Energy Treated test formulation could be developed as an effective cosmetic product to protect and treat various skin problems including infection, photosensitivity, cutis rhomboidalis nuchae, erythema, contact dermatitis, behcet's disease, blepharitis, boil, bowen's disease, skin aging, wrinkles and/or change in skin colour, etc.

Keywords: Hyaluronic Acid, Collagen, Elastin, Consciousness Energy Healing, The Trivedi Effect®, HFF-1, B16-F10, HaCaT

1. Introduction

Skin aging is a complex process induced by repeated exposure to ultraviolet (UV) irradiation which damages human skin. The UV-rays generates reactive oxygen species (ROS) that leads to collagen deficiency and ultimately wrinkling to the skin [1]. Skin caring products have significant antioxidant and anti-inflammatory activities [2].

This is due to the fact that UV radiation on exposed skin gives rise to a generation of ROS, which react with DNA, proteins and fatty acids and create the formation of oxidative products. This could liberate the pro-inflammatory mediators causing irritation of the epidermis. Further, the UV radiation also damages the skin regulatory mechanisms, photoageing effects like wrinkles, hyperpigmentation, and loss of skin firmness. In order to reduce the negative effects of UV radiation, phytoconstituents and raw plant materials are added in cosmetic products, which provide protecting and skin whitening properties without manifestation of side-effects [3]. Cell migration and proliferation are important in wound repair process and collagen deposition is needed to repair tissue injury. Collagen is required to repair the defect and it provides strength, integrity and structure. Considering the impact of the phytoconstituents and minerals on cosmetic products, the authors formulated the novel proprietary herbomineral test formulation which consisted of zinc chloride, sodium selenate, sodium molybdate, L-ascorbic acid, tetrahydrocurcumin (THC), and extract of *Centella asiatica* (*C. asiatica*); (commonly known as Jal Brahmi). Each ingredient already has been proven for its potential activity on skin health as various medicines as well as cosmeceuticals. Zinc is an essential cofactor of various metalloenzymes and it protect the skin from UV irradiation [4]. Another literature reported that zinc chloride has been widely used in the early 19th century as a destructive agent for the management of cancer [5]. Sodium selenate is an important inorganic mineral that enhance the repair of damaged DNA segments and simultaneously reduce the risk of new cancer development [6]. It destroys the cancer cells from a variety of cancers through a selective generation of toxic ROS and target oriented destruction of mitochondria that exist in tumor cells but it did not affect the healthy tissue [7]. It prevents skin cancers supplement with L-selenomethionine [8, 9]. Molybdenum is an essential element for humans, animals, and plants [10]. In humans and other mammals, it is a key constituent of various important enzymes such as sulfite oxidase, xanthine oxidase and aldehyde oxidase [11]. Deficiency of molybdenum in humans causes genetic defects [12]. Vitamins play an important role in skin health, improve wrinkles and wound healing. Hence, numerous skin care and wound healing formulations incorporated several vitamins such as vitamin A, E and C. Vitamin C plays a vital role in repairing the damaged skin and modulates collagen production [13]. THC exhibits strongest antioxidant property among the curcumin derived compounds [14, 15]. THC was reported to have important role in wound healing process [16]. *C. asiatica* is well known in promoting wound healing and provides significant benefits in skin care products formulation. Hashim *et al.* (2011) have reported that *C. asiatica* leaves extract enhance synthesis of collagen and has potential antioxidant, anti-cellulite, and UV protectant activities. This extract also has many applications as a topical therapeutic agent. It is used in proprietary medicinal products for the treatment of cutaneous ulcer, hypertrophic scars, keloids, and wound healing disorders [17-19].

As per the universal principle of reciprocity defined electromagnetic connections related to human Biofield, 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 materials) around the globe [20]. 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”. The Biofield can be monitored using electromyography, electrocardiography and electroencephalogram [21]. 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 [22]. Recent studies reported that the uses of energy medicine provided the highest benefit to cancer patients as compared to the use of other Complementary and Alternative Medicine (CAM) [23]. The Trivedi Effect®-Consciousness Energy Healing Treatment has been known to transform the structural, physical, and thermal properties of several metals in materials science [24-27], improved the overall productivity of crops [28, 29], altered characteristics features of microbes [30-32] and improved growth and anatomical characteristics of various medicinal plants [33, 34]. Based on the data from the literature of the Biofield Energy Treatment, the authors designed this study to evaluate the impact of Biofield Energy Healing based DMEM and test formulation on various skin health related parameters using human foreskin fibroblast (HFF-1), human keratinocytes (HaCaT), and mouse melanoma (B16-F10) cell lines.

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% 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 was considered as control samples, while the other parts 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 samples were subjected to Consciousness Energy Healing (The Trivedi Effect®) Treatment by James Jeffery Peoples for 5 minutes remotely from U.S.A. The Biofield Energy Healer, James Jeffery Peoples never visited the laboratory in person, nor had any contact with the test samples. Similarly, the control samples were subjected to "sham" healer under the same laboratory conditions for 5 minutes for better comparison purpose. 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 and used for this experiment.

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 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 items (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 concentrations exhibiting % cytotoxicity of < 30 % was considered as non-cytotoxic [35, 36]. 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 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 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 cells was estimated for determining the potential of Biofield Energy Treated test formulation and DMEM to improve skin strength, and overall elasticity and hydration level. HFF-1 cells were counted using hemocytometer and plated in 48 well plate 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 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 [37]. 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 [38].

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 level of melanin was extrapolated using standard curve obtained from purified melanin [39].

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 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 the test items for 2 to 24 hours. After treatment with the test items, the cells were subjected to lethal dose of UV-B irradiation (200 mJ/cm^2) that can lead to approximately 50% cytotoxicity (302 nm, CL-1000 M, UVP, USA) [40]. The percent of 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 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 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 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 performed at the selected time-point (16 hours) of the migrated cells using digital camera. It represented the fibroblast distance covered and subsequent scratch closure [41].

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. Cell Viability by MTT Assay

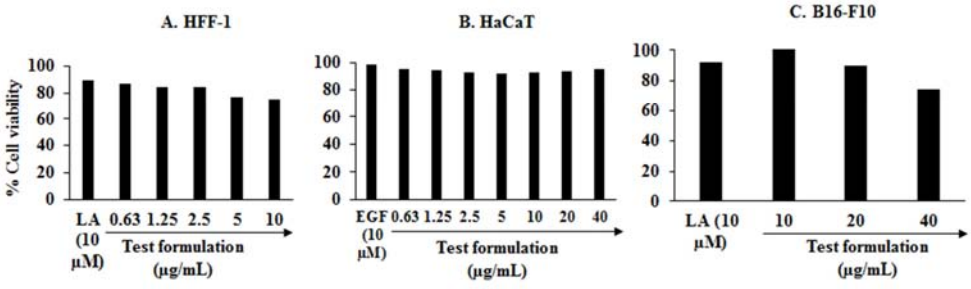

Figure 1. *Evaluation of the Cell viability by MTT assay of the test formulation in three different cells. (A) HFF-1 cells after 72 hours of treatment; (B) HaCaT cells after 48 hours of treatment; and (C) B16-F10 cells after 48 hours of treatment. LA: L-Ascorbic acid; EGF: Epidermal growth factor.*

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 >70% viable cells in the tested

concentrations ranges from 0.63 to 10 µg/mL in the HFF-1 cells, indicating that the test formulation was safe and nontoxic. The selected concentrations were used for the estimation of collagen, elastin, and hyaluronic acid (Figure 1A). Moreover, the percentage of cell viability exhibited >90% in HaCaT cells. The concentrations of the test formulation from 0.63 µg/mL to 40 µg/mL were used for the assessment of wound healing activity by scratch assay (Figure 1B). Further, the percentage of the cell viability showed >70% in B16-F10 cells and the concentrations from 10 to 40 µg/mL were used for the estimation of α-MSH stimulated melanogenesis (Figure 1C).

3.2. Effect of the Test Formulation on Cell Proliferation of Human Foreskin Fibroblasts (HFF-1) - BrdU Assay

The cell proliferation analyzed by bromodeoxyuridine (BrdU) assay is shown in Figure 2. The percent cell proliferation was increased by 150.4% in the positive control group (FBS-0.5 µg/mL) compared to the vehicle control (VC) group. The percent cell proliferation was significantly increased by 36.61%, 378.17%, and 251.30% 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. Moreover, at 17.50 µg/mL the percent cell proliferation was significantly increased by 608.36% and 373.36% in the BT-DMEM + UT-Test formulation and BT-DMEM + BT-Test formulation groups, respectively compared to the UT-DMEM + UT-Test formulation group. Additionally, the percentage of cell proliferation at 35 µg/mL was also significantly increased by 48.06%, 451.42%, and 722.48% 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.

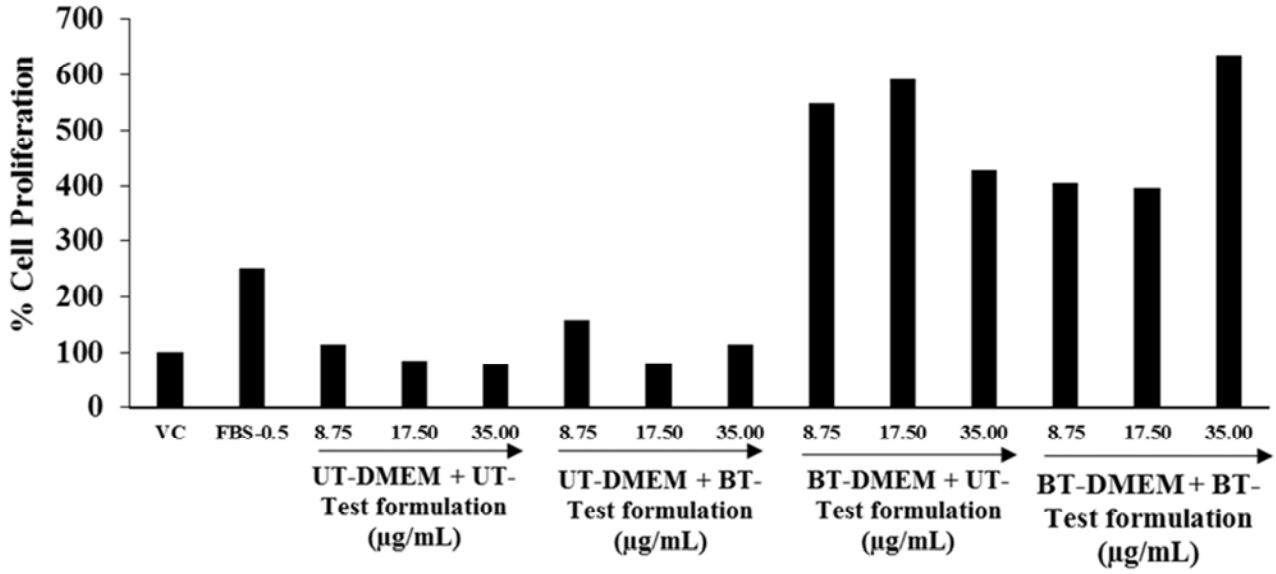

Figure 2. *Effect of the test formulation on cellular proliferation after 48 hours using BrdU assay. VC: Vehicle control; FBS: Fetal bovine serum (µg/mL); UT: Untreated; BT: Biofield Treated.*

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

3.3.1. Collagen

The effect of the test formulation and DMEM on the collagen level in HFF-1 cells is shown in Figure 3. Collagen level was significantly elevated by 168.62% in the L-ascorbic acid group (129.42 ± 8.50 µg/mL) compared to the vehicle control group (48.18 ± 0.34 µg/mL). The level of collagen synthesis was significantly increased by 6.86% in the UT-DMEM + BT-Test formulation group at 0.63 µg/mL compared to the UT-DMEM + UT-Test formulation group. Additionally, collagen was significantly ($p \leq 0.001$) increased by 32.96% in the UT-DMEM + BT-Test formulation group at 2.5 µg/mL with respect to the UT-DMEM + UT-Test formulation group. There was no alteration of the expression of collagen at 1.25 µg/mL. Collagen is the important source of fibrous protein present in the interstitial extracellular matrix, however 30% of the total protein mass is constituted in multicellular animal. It acts as the main structural component of the ECM. It supports chemotaxis and migration. It also provides tensile strength and regulates the cell adhesion, mechanical strength, and texture [42]. Overall, the level of collagen synthesis was improved in the Biofield Energy Treated test formulation group, due to The Trivedi Effect®-Consciousness Energy Healing Treatment.

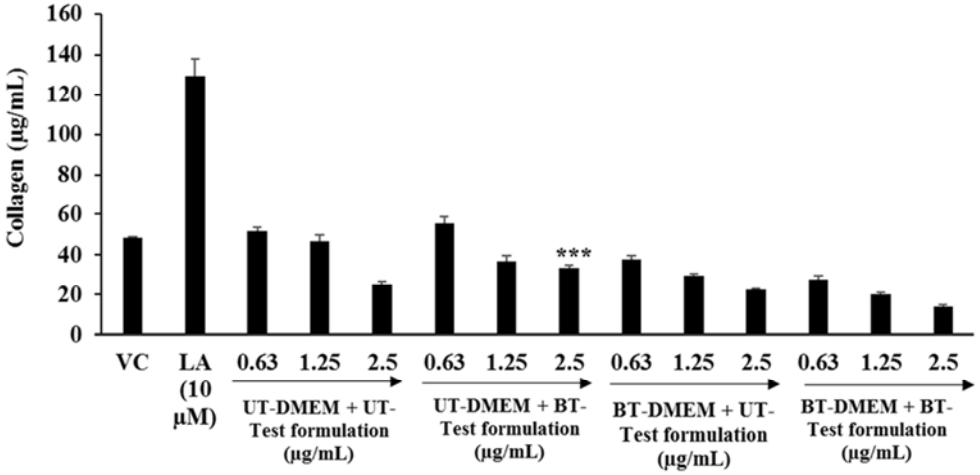

Figure 3. *Effect of the test formulation on collagen synthesis in human foreskin fibroblast cells (HFF-1) VC: Vehicle control; LA: L-Ascorbic acid; UT: Untreated; BT: Biofield Treated. ***p≤0.001 vs UT-DMEM + UT-Test formulation using one-way ANOVA (post-hoc Dunnett's test).*

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 was found as 6.06 ± 0.00 pg/mL and 7.27 ± 0.15 pg/mL in the vehicle control (VC) and positive control (LA-10), respectively. The elastin level was increased by 4.74% in the UT-DMEM + BT-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) increased by 10.32% in the UT-DMEM + BT-Test formulation group compared to the UT-DMEM + UT-Test formulation group. Besides, the expression of elastin was significantly (p≤0.001) increased by 69.49% and 17.13% 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. Elastin is another important ECM major component along with collagen. It maintains stretch conditions and provides recoil to the tissues. Elastin fibers are covered by the glycoprotein called as microfibrils, that mostly have fibrillins and important for the integrity of the elastin fiber [43]. Hence, elastin is important ECM, which regulates and activates the dermal metabolism, can be useful as beneficial for skin health.

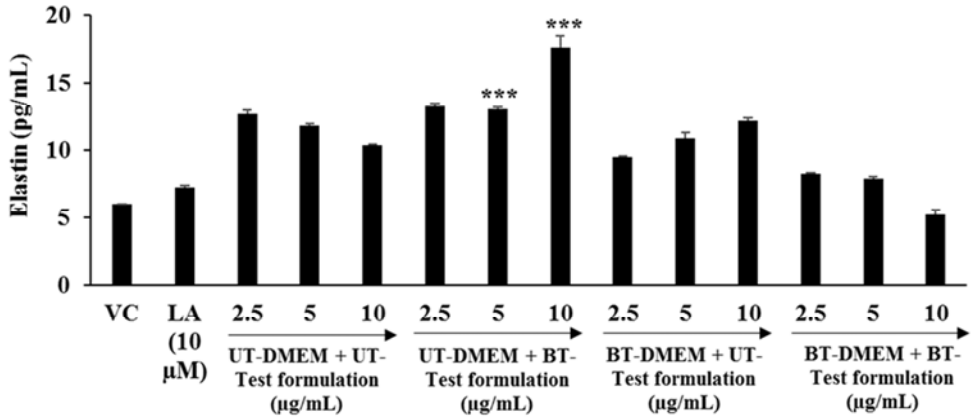

Figure 4. *Effect of the test formulation and DMEM on elastin formation in human foreskin fibroblast cells (HFF-1). VC: Vehicle control; LA: L-Ascorbic acid; UT: Untreated; BT: Biofield Treated. ***p≤0.001 vs UT-DMEM + UT-Test formulation using one-way ANOVA (post-hoc Dunnett's test).*

3.3.3. Hyaluronic Acid (HA)

The effect of the test formulation and DMEM on the expression of HA level in human foreskin fibroblast cells (HFF-1) is represented in Figure 5. The level of HA was 12.63 ± 0.98 ng/mL and 12.22 ± 4.07 ng/mL in the vehicle control (VC) and L-ascorbic acid group, respectively. The level of HA was increased by 11.21%, 4.25%, and 10.45% 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 increased by 14.78% in the BT-DMEM + UT-Test formulation group compared to the UT-DMEM + UT-Test formulation group. Additionally, the level of HA did not show any alteration with respect to the UT-DMEM + UT-Test formulation group at 2.5 µg/mL. HA is considered as a key molecule that play an important role in skin moisture. About 50% of total body HA is present in the skin. In dermis region of skin, it also regulates osmotic pressure,

water balance, ion flow, and helps to stabilize structure of the skin. HA has been now used in the treatment and prevention of skin disorders in terms of clinical aspect [44]. The overall data suggested that the Biofield Energy Healing Based test formulation and DMEM have significant capacity to increase the expression of hyaluronic acid.

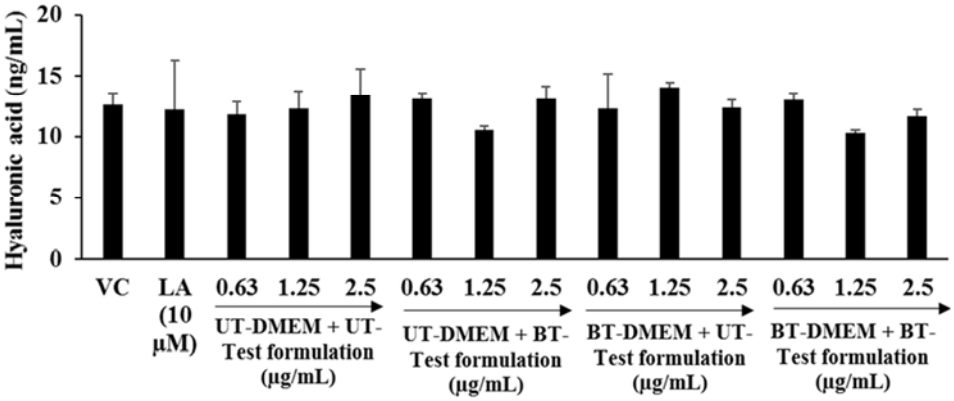

Figure 5. *Effect of the test formulation and DMEM on the expression of hyaluronic acid (HA) in human foreskin fibroblast cells (HFF-1). VC: Vehicle control; LA: L-Ascorbic acid; UT: Untreated; BT: Biofield Treated.*

3.4. Effect of the Test Formulation on Skin Depigmentation

The effect of the test formulation with 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 63.06% in the kojic acid (KA) group (9.09 ± 3.03 µg/mL) compared to the α-MSH group (24.61 ± 0.61 µg/mL). The level of melanin was reduced by 12.49%, 6.73% 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 with respect to the UT-DMEM + UT-Test formulation group. Besides, the level of the melanin synthesis was inhibited by 14.64%, 18.25%, and 2.99% in the UT-DMEM + BT-Test formulation, BT-DMEM + UT-Test formulation, and BT-DMEM + BT-Test formulation groups, respectively at 0.13 µg/mL compared to the UT-DMEM + UT-Test formulation group. Darkness of the skin is due to the absorption of the UV radiations by melanin. To minimize the skin darkening, several natural and synthetic skin whitening components have been incorporated in the cosmetic preparation [45, 46]. For comparison purposes, the effect of kojic acid (10 mM) was used as positive control, which is extensively used as a skin whitening compound in many cosmetic products [47]. Thus, it can be concluded that the Biofield Energy Treated test formulation and DMEM inhibits the melanin production in the B16-F10 melanoma cells. This improvement might be beneficial for the development of cosmeceuticals for hyperpigmentation and different types of skin conditions.

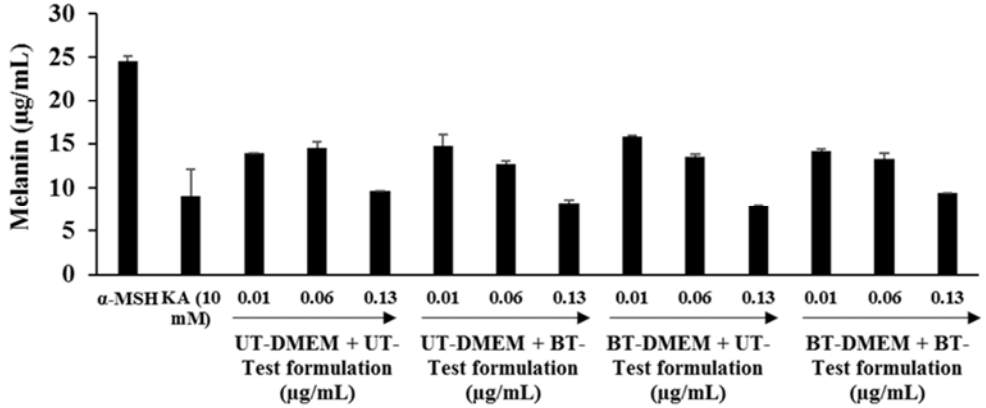

Figure 6. *Effect of the test formulation and DMEM on alpha-MSH stimulated melanin level in B16-F10 cells. α-MSH: Alpha melanocyte stimulating hormone, KA: Kojic acid (mM); UT: Untreated; BT: Biofield Treated.*

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

The effect of the test formulation and DMEM after pretreatment with UV-B is depicted 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) and found 35.89% cell viability. The cell viability in the normal control (NC) and vehicle control (VC) groups was 100% and 27.78%, respectively. The percent cell viability was increased by 55.11% in the L-ascorbic acid group compared to the VC. Besides, the percent cell viability was

increased by 4.23% in the BT-DMEM + BT-Test formulation group at 2.5 µg/mL compared with the UT-DMEM + UT-Test formulation group. The data suggests that the Biofield Energy Treated test formulation and DMEM exhibited minimal changes of percent cell viability and could be used for a skin protective effect with anti-wrinkling potential. Aging of skin is a very complex process, which is induced by continuous exposure to UV-rays. The UV-rays generate ROS which leads to deficiency of collagen and increased skin wrinkling [48, 49]. Skin aging is also characterized by loss of elasticity, irregular pigmentation, increased wrinkling, dryness and roughness [50]. In this study, the test herbomineral formulation with DMEM significantly improved the levels of collagen and elastin with minimal changes of anti-wrinkle activity, due to The Trivedi effect® - Consciousness Energy Healing Treatment.

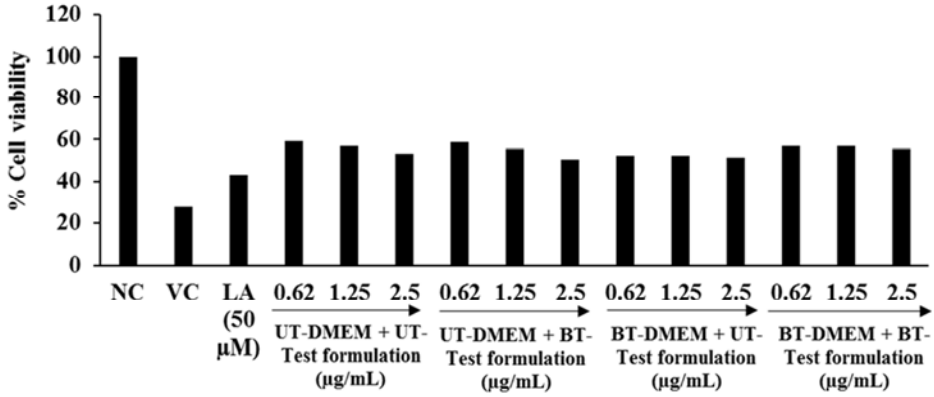

Figure 7. *Percentage restoration of cell viability in HFF-1 cells after 20 hours of pretreatment before UV-B challenge. NC: Normal control; VC: Vehicle control LA: L-Ascorbic acid; UT: Untreated; BT: Biofield Treated.*

3.6. Wound Healing Activity by Scratch Assay

The wound healing activity of the test formulation and DMEM using scratch assay was performed to measure cell migration in HFF-1 and HaCaT cells. The representative photomicrographs are shown in Figure 8. The cell coverage area was increased by 5%, 23%, and 14% in the UT-DMEM + BT-Test formulation, BT-DMEM + UT-Test formulation, and BT-DMEM + BT-Test formulation groups, respectively at 5 µg/mL in HFF-1 cells compared to the UT-DMEM + UT-Test formulation group. Moreover, the cell coverage area was increased by 2%, 2%, and 1% 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 in HFF-1 cells compared to the UT-DMEM + UT-Test formulation group. Additionally, the cell coverage area was increased by 12% (at 10 µg/mL) and 5% (2.5%) 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). Besides, the cell coverage area was increased by 6%, 4%, and 6% in the UT-DMEM + BT-Test formulation, BT-DMEM + UT-Test formulation, and BT-DMEM + BT-Test formulation groups, respectively at 10 µg/mL in HaCaT cells compared to the UT-DMEM + UT-Test formulation group. Furthermore, the cell coverage area was increased by 2% in the UT-DMEM + BT-Test formulation, BT-DMEM + UT-Test formulation, and BT-DMEM + BT-Test formulation groups at 5 µg/mL in HaCaT cells compared to the UT-DMEM + UT-Test formulation group (Figure 8B). The positive control (EGF-100 ng/mL) showed 96% and 100% cell covered area in the HFF-1 and HaCaT cells, respectively. *In vitro* scratch assay is a suitable, well-developed method for the estimation of cell migration, cell-matrix and cell-to-cell interactions during wound healing and also for monitoring the intracellular event during cell migration [51]. The results showed significant wound closure activity in the test formulation and DMEM groups compared to the untreated group.

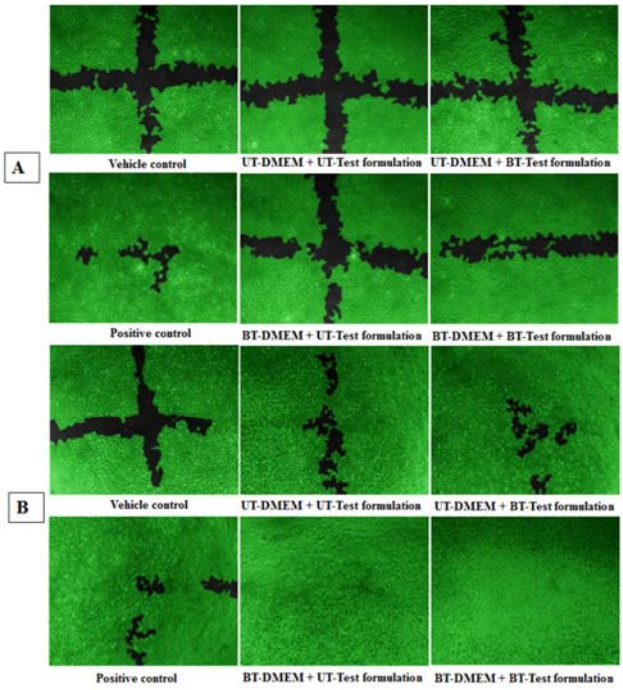

Figure 8. *Effect of the test formulation and DMEM on wound healing activity after 16 hours of treatment. Representative photomicrographs (X10) of wound closure and cell migration are shown in A. HFF-1 and B. HaCaT cells. UT: Untreated; BT: Biofield Treated.*

4. Conclusions

MTT cell viability assay data showed more than 70% cells were viable in all the tested concentrations. BrdU assay showed that the percent cell proliferation was significantly increased by 48.06%, 451.42%, and 722.48% 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. Collagen level was significantly ($p \leq 0.001$) increased by 32.96% in the UT-DMEM + BT-Test formulation group at 2.5 µg/mL with respect to UT-DMEM + UT-Test formulation group. The level of elastin was significantly ($p \leq 0.001$) increased by 69.49% and 17.13% 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 11.21% and 10.45% in the UT-DMEM + BT-Test formulation and BT-DMEM + UT-Test formulation groups, respectively at 0.63 µg/mL compared to the UT-DMEM + UT-Test formulation group. Melanin level was reduced by 12.49% in the UT-DMEM + BT-Test formulation at 0.01 µg/mL with respect to the untreated group UT-DMEM + UT-Test formulation group. Moreover, the level of melanin was also decreased 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. Protection with respect to UV-B, showed that the percent cell viability was increased by 4.23% at 2.5 µg/mL in the BT-DMEM + BT-Test formulation group compared to the UT-DMEM + UT-Test formulation group. Wound healing results showed significant effects on wound closure and cell migration in the test formulation and DMEM groups in both HFF-1 and HaCaT cells compared to the untreated group. Altogether, the Biofield Energy Treated test formulation (The Trivedi Effect®) has shown significant protective effects on various skin health parameters such as wrinkling, aging, skin whitening, and wound healing. Therefore, the Consciousness Energy Healing Treatment based test formulation could be suitable for the development of herbal skin preparations, which would be very useful for the management of wounds and various skin related disorders such as acne, hives, chickenpox, eczema, rosacea, seborrheic dermatitis, psoriasis, erythema, contact dermatitis, skin aging, etc.

Abbreviations

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

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