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Impact of Consciousness Energy Healing Treatment on Herbomineral Formulation in Different Skin Health Parameters

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Abstract: The aim of this study was to evaluate the effect of the Consciousness Energy Healing based test formulation and cell medium (DMEM) against various skin health parameters. The test formulation and DMEM were divided into two parts. One part received the Consciousness Energy Healing Treatment (The Trivedi Effect®) by Deborah Lea Smith and termed as the Biofield Energy Treated sample, while other part denoted as untreated test item. The study was assessed for the measurement of collagen, elastin, hyaluronic acid, melanin synthesis, cell viability against UV-B induced stress, and wound healing activity. MTT assay showed that the test formulation was safe and nontoxic in three cell lines. Bromodeoxyuridine (BrdU) assay data showed that the percent cell proliferation was increased significantly by 22.69% and 34.99% 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. Collagen was increased by 15.12% and 15.26% in the BT-DMEM + UT-Test formulation and BT-DMEM + BT-Test formulation groups, respectively at 0.625 µg/mL compared to the untreated group. Elastin was significantly increased by 9.10%, 13.71%, and 12.41% in the UT-DMEM + BT-Test formulation, BT-DMEM + Test formulation, and BT-DMEM + BT-Test formulation groups, respectively at 5 µg/mL compared to the untreated group. Elastin was also increased significantly by 17.78% and 6.01% in the UT-DMEM + BT-Test formulation and BT-DMEM + BT-Test formulation groups, respectively at 10 µg/mL compared to the untreated group. Hyaluronic acid was significantly increased by 3.90%, 5.37%, and 6.82% in the UT-DMEM + BT-Test formulation at 0.625, 1.25, and 2.5 µg/mL, respectively compared to the untreated group. Melanin level was 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 compared to the untreated group. Skin cells protection was significantly increased by 21.32%, 23.81%, and 7.20% at 0.625, 1.25, and 2.5 µg/mL, respectively in the UT-DMEM + BT-Test formulation compared to the untreated group. Wound healing assay exhibited significant wound closure and cell migration in all the tested groups compared to the untreated group. Overall, the Biofield Energy Treated test formulation and DMEM (The Trivedi Effect®) were found to be safe and can be developed as an effective cosmetic product to protect and treat the skin problems including infection, photosensitivity, acne, hives, chickenpox, eczema, rosacea, seborrheic dermatitis, psoriasis, erythema, contact dermatitis, skin aging, wrinkles, etc.

Keywords: Consciousness Energy Healing, The Trivedi Effect®, Skin Protection, Scratch Assay, Hyaluronic Acid, Extracellular Matrix, Collagen, Elastin

1. Introduction

Phytoconstituents are continuously gaining popularity in many cosmetic formulations due to its high safety profile and skin protection against harmful agents. Nowadays, modern cosmetology is focused to incorporate valuable raw plant materials as compared with synthetic chemicals in manufacturing the cosmetics products. Skin caring products have supposed to have significant antioxidant and anti-inflammatory activity [1]. This is due to the fact that ultraviolet radiation on exposure to skin gives rise to generation of reactive oxygen species (ROS), which react with DNA, proteins and fatty acids and formation of oxidation products. This could liberate the pro-inflammatory mediators causing irritation of the epidermis. Besides, UV radiation damage skin regulatory mechanisms, photo aging effect like wrinkles, hyperpigmentation, and loss of skin firmness. In order to reduce the negative effects of UV radiations, phyto-constituents and raw plant materials are added in the cosmetic products, which provide protecting and skin whitening properties without having and mentioned side-effects [2]. 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. Important skin health agents such as tetrahydrocurcumin (THC) is a white or colorless and main active metabolite of curcumin [3] with almost similar physiological and pharmacological properties as curcumin, but it exhibits strongest antioxidant property among the curcumin derived compounds [4-6]. It was reported to have important role in wound healing process [7]. *C. asiatica* is well known in promoting wound healing and provides significant benefits in skin care products formulation. Vitamins play an important role in skin health, improvement of wrinkles and wound healing. Therefore, many skin care and wound healing formulations incorporated vitamins such as vitamin A, E, and C. Ascorbic acid plays a vital role in repair of damaged skin and modulates collagen production [8]. Hashim *et al.* (2011) have reported that *C. asiatica* leaves extract enhanced 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 treatment of cutaneous ulcer, hypertrophic scars, keloids, and wound healing disorders [9-11]. Minerals such as zinc, copper, molybdenum, and selenium are commonly used as active components for skin health formulations. Zinc is an essential cofactor of various metalloenzymes. They protect the skin from UV irradiation [12]. With this respect, a new proprietary herbomineral test formulation consist of zinc chloride, sodium selenate, sodium molybdate, L-ascorbic acid, tetrahydrocurcumin (THC), and extract of *Centella asiatica* (*C. asiatica*); (commonly known as Jal Brahmi) was formulated to study its role in skin health parameters such as extracellular matrix (ECM), which is present in all the tissues and organs and it provides important physical scaffolding for cellular mater along with initiating the various biochemical and biomechanical aspects for controlling tissue differentiation, morphogenesis, and homeostasis. The importance of the ECM with respect to collagen, elastin, and hyaluronic acid has been related with wide range of skin health and deficiency might results either from genetic reason or other abnormalities.

According to universal principles of Maxwell's equations and principle of reciprocity defines electromagnetic connections related to human Biofield [13]. Thus, a human has ability to harness the energy from environment/Universe and can transmit into any object (living or non-living) around the Globe. The object(s) always receive the energy and responded into useful way that is called “Biofield Energy”. This process is known as “Biofield Energy Healing”. The Biofield can be monitored using electromyography, electrocardiography and electroencephalogram [14]. Biofield Energy Healing has been approved as an alternative method that has impact on various properties of living organisms in a cost-effective manner [15]. 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) [16]. The Trivedi Effect®-Unique Biofield Energy Treatment has been known to transform the structural, physical, and thermal properties of several metals in materials science [17-20], improved the overall productivity of crops [21, 22], altered characteristics features of microbes [23-25] and improved growth and anatomical characteristics of various medicinal plants [26, 27]. Based on the data from the literature of the Biofield Energy Treatment, authors designed this study to evaluate the impact of Consciousness Energy Healing based DMEM and test herbomineral formulation on various skin health 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 was 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 test 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 + Biofield Energy Treated Test formulation (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 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 formulation, while the second part of the test formulation did not receive any sort of treatment and was defined as the untreated test formulation. This Biofield Energy Healing Treatment was provided by Deborah Lea 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 herbomineral formulation was located in the research laboratory of Dabur Research Foundation near New Delhi in Ghaziabad, India. This Biofield Energy Treatment was administered for 5 minutes through the Healer's unique Energy Transmission process remotely to the test formulation under laboratory conditions. The Biofield Energy Healer, Deborah Lea Smith, in this study never visited the laboratory in person, nor had any contact with the herbomineral samples and medium. Further, the control group was 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 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, DMEM, and 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 [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 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 BrdU estimation using cell proliferation ELISA, BrdU estimation kit (ROCHE – 11647229001) as per manufacturer's instructions.

2.7. Estimation of Extracellular Matrix (ECM) Component Synthesis

Synthesis of extracellular matrices component (*i.e.* collagen, elastin, and hyaluronic acid) in HFF-1 cells was estimated for determining the potential of the 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 formulation at different concentrations. Further, 72 hours of incubation with the test formulation 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 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 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 formulation. 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 formulation for 2 to 24 hours. After treatment with test formulation, 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) [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 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 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 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. Measurement of Non-cytotoxic Concentrations

The cell viability results using MTT assay in HFF-1 are presented in Figure 1A. The data indicated that the test formulation did not exhibit cytotoxicity (as evidence of cell viability approximately greater than 70%) across all the tested concentrations (1.25 µg/mL to 10 µg/mL). Hence, the same concentrations were assessed further to see the effect of the test formulation on ECM synthesis in HFF-1 cells. The percentage of cell viability in HaCaT cells is represented in Figure 1B. The data indicated that all the test concentrations exhibited percentage of cell viability more than 90%. Hence, the concentrations (0.63 µg/mL to 10 µg/mL) were assessed further for the effect of the test formulation on wound healing activity by scratch assay. The percentage cytotoxicity values obtained in B16-F10 cells treated with different concentrations of the test formulation and positive control for 48 hours are represented in Figure 1C. The data indicated that the test groups were non-cytotoxic (*i.e.* percentage cell viability value >80%). Hence, the test concentrations were used further for the inhibitory effect of the test formulation on α-MSH stimulated melanogenesis at the concentrations ranging from 10 µg/mL to 40 µg/mL.

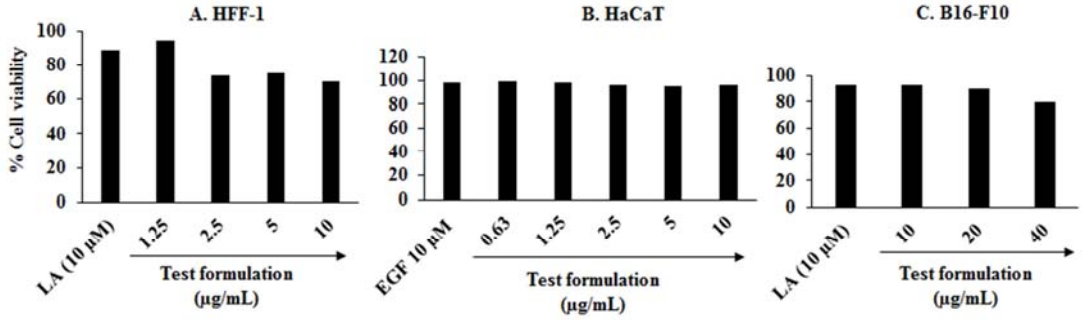

Figure 1. *Percentage cell viability of the test formulation in different concentrations A. HFF-1 cells after 72 hours of treatment. B. HaCaT cells after 48 hours of treatment. C. B16-F10 cells after 48 hours of treatment. LA: L-Ascorbic acid; EGF: Epidermal growth factor.*

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

The untreated and Biofield Energy Treated DMEM/test formulation in various concentrations on cellular proliferation of HFF-1 cells were assessed by BrdU assay after 48 hours of incubation and data are presented in Figure 2. The percent cell proliferation was 100% and 250.4% for vehicle control (VC) and positive control groups (FBS-0.5 µg/mL), respectively. Additionally, the percent cell proliferation at 8.75 µg/mL was increased significantly by 22.69% and 34.99% in the UT-DMEM + BT-Test formulation and BT-DMEM + UT-Test formulation groups, respectively compared to the UT-DMEM + UT-Test formulation group. Besides, others two concentration (17.5 and 35 µg/mL), the percent cell proliferation was almost similar pattern in the test formulation and DMEM groups compared to the UT-Test formulation + UT-DMEM group.

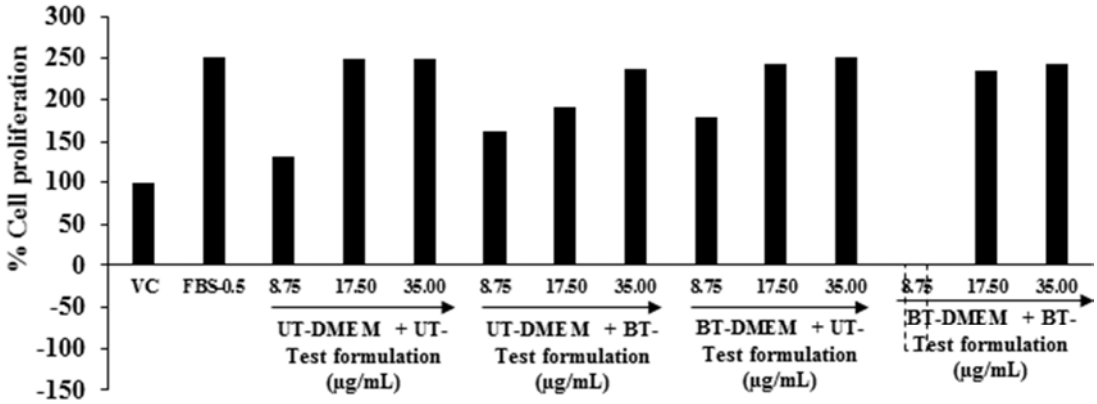

Figure 2. *Effect of the test formulation on cellular proliferation after 48 hours using BrdU assay. VC: Vehicle control; FBS: Fetal bovine serum (µg/mL); UT: Untreated; BT: Biofield Treated.*

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 collagen level was evaluated in HFF-1 cells to find out the effect on skin strength, sagging, and overall elasticity are shown in Figure 3. The level of collagen was found as 53.62 ± 1.42 and 129.42 ± 8.50 µg/mL in the vehicle control (VC) and positive control groups, respectively. Collagen level was significantly increased by 15.12% and 15.26% 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 was increased by 4.04%, 15.90%, and 11.63% 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 with respect to the UT-DMEM + UT- Test formulation group. The level of collagen at 2.5 µg/mL was increased by 8.22% in the BT-DMEM + UT-Test formulation group; while decreased by 5.45% and 1.49% in the UT-DMEM + BT-Test formulation and BT-DMEM + BT-Test formulation groups, respectively compared to the UT-DMEM + UT-Test formulation group. 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 regulate the cell adhesion, mechanical strength, and texture [35]. Overall, the level of collagen synthesis was improved in the Biofield Energy Treated DMEM and the test formulation group, which might be due to The Trivedi Effect®-Consciousness Energy Healing Treatment.

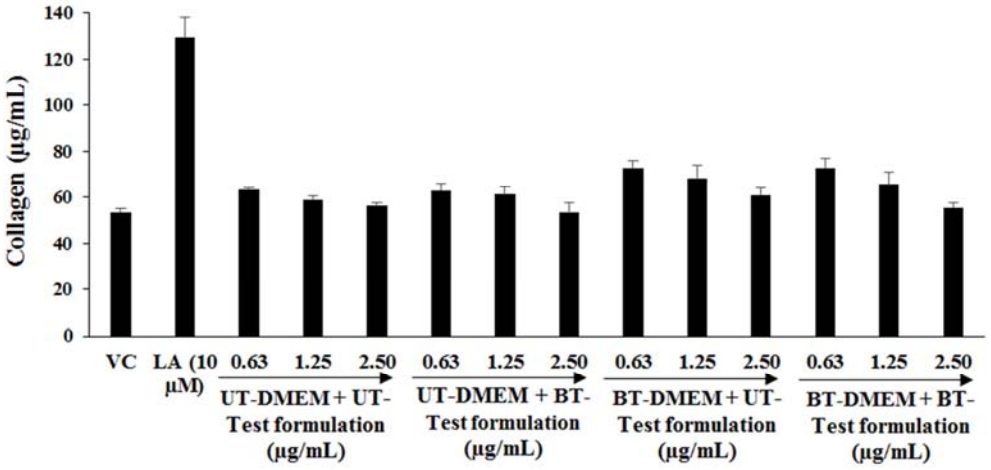

Figure 3. *Effect of the test formulation on human foreskin fibroblast cells (HFF-1) for extracellular matrix component, collagen. VC: Normal control; LA: L-Ascorbic acid; UT: Untreated; BT: Biofield Treated.*

3.3.2. Elastin

The effect of the test formulation 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 and 7.27 ± 0.15 pg/mL in the vehicle control (VC) and positive control groups, respectively. The elastin level was significantly increased by 9.10%, 13.71%, and 12.41% in the UT-DMEM + BT-Test formulation, BT-DMEM + UT-Test formulation, and BT-DMEM + BT-Test formulation, respectively at 5 µg/mL compared to the UT-DMEM + UT-Test formulation group. Moreover, at 10 µg/mL the level of elastin was increased significantly by 17.78% and 6.01% in the UT-DMEM + BT-Test formulation and BT-DMEM + BT-Test formulation groups, respectively 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 [36]. Hence, elastin is important ECM, which regulates and activates the dermal metabolism, can be useful as beneficial for skin health.

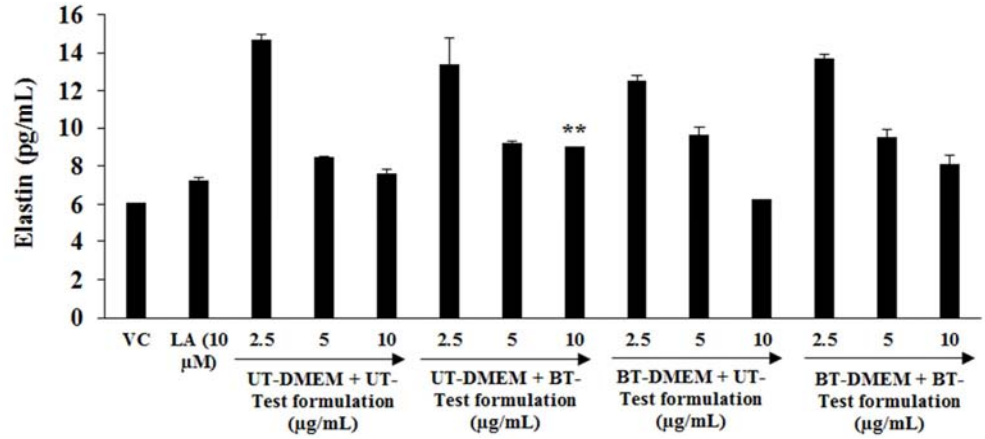

Figure 4. *Effect of the test formulation on human foreskin fibroblast cells (HFF-1) for extracellular matrix component, elastin. VC: Vehicle control; LA: L-Ascorbic acid; UT: Untreated; BT: Biofield Treated. **p≤0.01 vs UT-DMEM + UT-Test formulation using one-way ANOVA (using Dunnett's test).*

3.3.3. Hyaluronic Acid (HA)

The effect of the test formulation in human foreskin fibroblast cells (HFF-1) is depicted in Figure 5. The results of HA synthesis in the presence of ascorbic acid (10 µM), showed significant increase in HA content by 80.24% compared with the vehicle control (VC) group (7.98 ± 0.33 ng/mL). The level of HA was increased by 3.9% and 2.19% in the UT-DMEM + BT-Test formulation and BT-DMEM + UT-Test formulation group, 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 5.37% and 7.18% in the UT-DMEM + BT-Test formulation and BT-DMEM + BT-Test formulation group, respectively compared to the UT-DMEM + UT-Test formulation group. Additionally, the level of HA was increased by 6.82% in the UT-DMEM + BT-Test formulation group at 2.5 µg/mL with respect to the UT-DMEM + UT-Test formulation group. 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 term of clinical aspect [37]. The overall data suggest that the Biofield Energy Healing based test formulation and DMEM significantly increased the extracellular component of skin like hyaluronic acid.

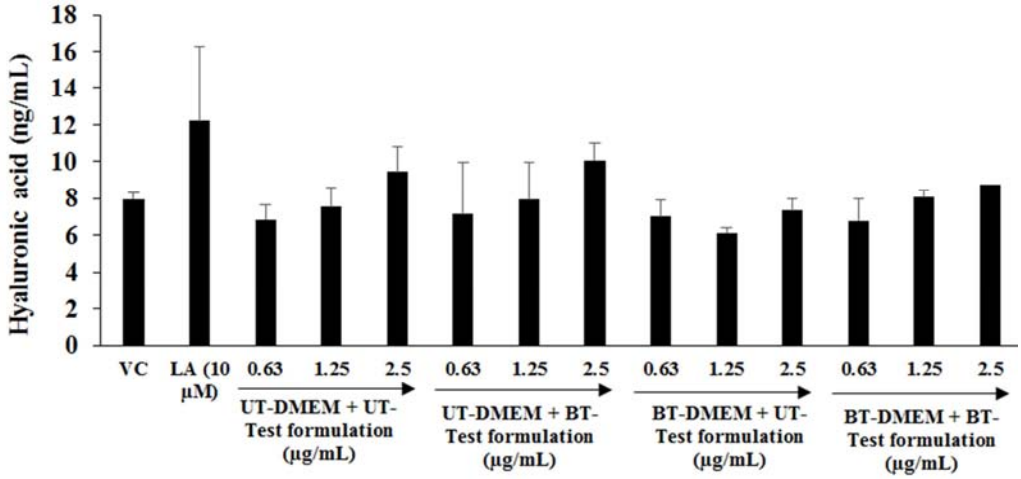

Figure 5. *Effect of the test formulation on the level of hyaluronic acid in human foreskin fibroblast cells (HFF-1). VC: Vehicle control; LA: L-Ascorbic acid; UT: Untreated; BT: Biofield Treated.*

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. Kojic acid exhibited 63.27% decrease the level of melanin compared to the vehicle control (VC) group. The newly formulated herbomineral test formulation was studied to investigate the role on skin depigmentation. For comparison purpose, the kojic acid (10 mM) was used as positive control in this assay, which was extensively used as a skin whitening compound in many cosmetic products [40]. The cellular content of melanin was reduced by 12.49%, 6.7%, 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. Additionally, the level of melanin was significantly ($p \leq 0.001$) 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. Melanin is synthesized from melanocytes that contain packets known as melanosomes. Melanogenesis process has been initiated once the skin nuclei cells become damaged from ultraviolet radiation (UV-A and UV-B) emitted by the sunrays. Melanin absorbs the UV radiations, which was responsible for skin darkening. To minimize the skin darkening, several natural and synthetic skin whitening components have been incorporate in the cosmetic preparation [38, 39]. These data suggest that the Biofield Energy Treated test formulation and DMEM significantly inhibited the melanin production in the B16-F10 melanoma cells. This improvement might be beneficial for the development of a cosmeceuticals product for hyperpigmentation and different types of skin conditions.

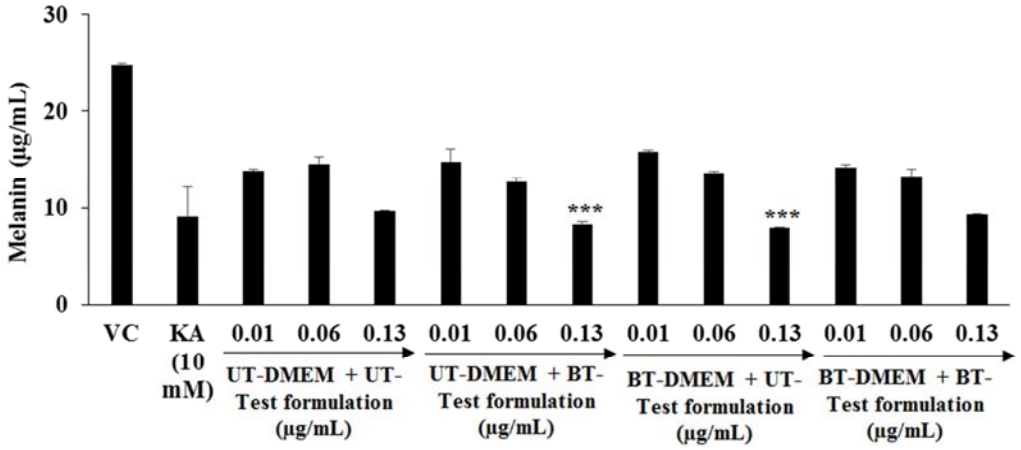

Figure 6. *Effect of the test formulation and DMEM on alpha-MSH stimulated melanin level in B16-F10 cells. VC: Vehicle control; KA: Kojic acid (mM); UT: Untreated; BT: Biofield Treated. ***$p \leq 0.001$ vs UT-DMEM + UT-Test formulation using one-way ANOVA (using Dunnett's test).*

3.5. Anti-wrinkle Effect 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 the lethal dose of UV-B irradiation (200 mJ/cm^2). The cell viability was 100% and 20.51% in the normal control (NC) and vehicle control (VC) groups, respectively. The cell viability was increased by 21.38% in the positive control group compared to the VC group. Further, after UV-B induce stress condition the level of cell viability was significantly increased by 5.56% and 12.92% at 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. Besides, rest of the tested groups did not show any alteration with respect to the UT-DMEM + UT-Test formulation group. This suggested that both the Biofield Energy Treated test formulation and DMEM could be significantly used for skin protective activity with anti-wrinkling potential.

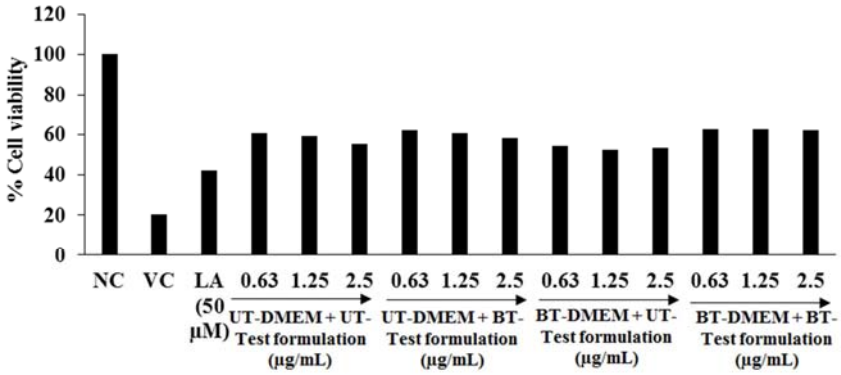

Figure 7. *Percentage restoration of cell viability in HFF-1 cells after 20 hours pretreatment of the test formulation before UV-B challenge. NC: Normal control; VC: Vehicle control; LA: L-Ascorbic acid; UT: Untreated; BT: Biofield Treated.*

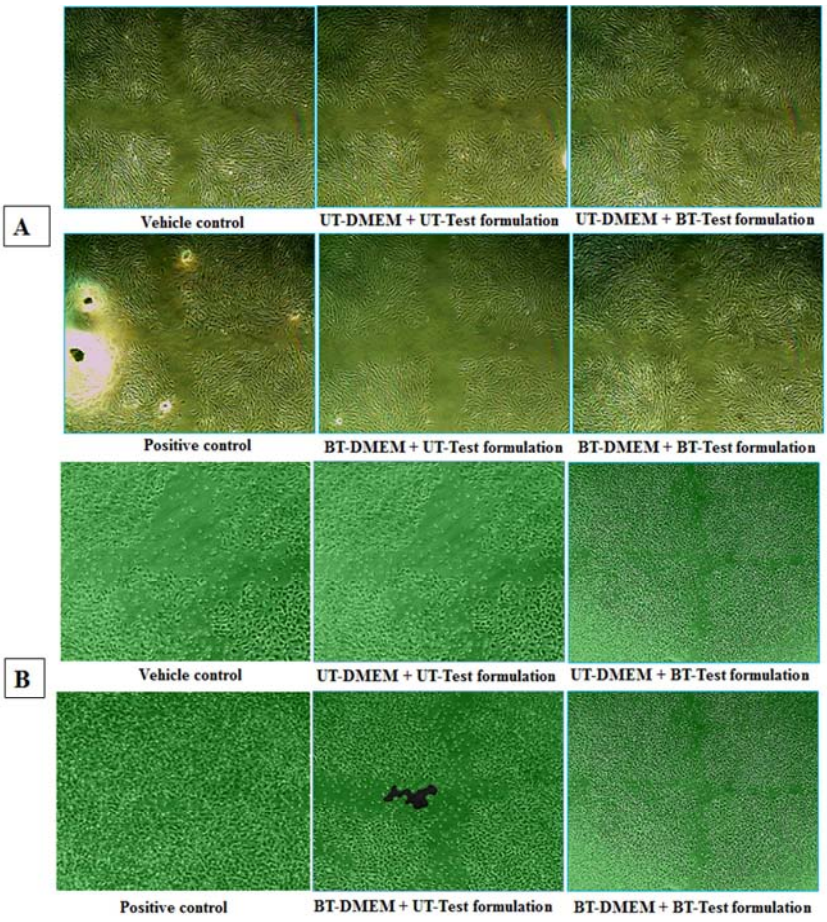

Figure 8. *Effect on cell migration of the test formulation and DMEM in A. HFF-1 and B. HaCaT cells for the assessment of wound healing potential after 16 hours of treatment. UT: Untreated; BT: Biofield Treated.*

3.6. Wound Healing Activity by Scratch Assay

The scratch assay was performed to measure the effect of the test formulation and DMEM on cell migration in HFF-1 and HaCaT cells. The representative photomicrographs are shown in Figure 8. The cell coverage area was increased by 10.5%, 8.8%, and 8.8% in the UT-DMEM + BT-Test formulation, 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 (Figure 8B). *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 for monitoring the intracellular event during migration [41]. The wound healing results indicated that the test formulation and DMEM showed minimal wound closure activity.

4. Conclusions

MTT cell viability assay data showed more than 70% cells were viable, which indicated that the test formulation was safe and nontoxic in all the tested concentrations. The percent cell proliferation using BrdU assay was significantly increased by 22.69% and 34.99% in the UT-DMEM + BT-Test formulation and BT-DMEM + UT-Test formulation groups, respectively compared to the UT-DMEM + UT-Test formulation group. Collagen level was significantly increased by 15.90% in the BT-DMEM + UT-Test formulation at 1.25 µg/mL with respect to the UT-DMEM + UT-Test formulation group. The level of elastin was significantly increased by 9.10% in the UT-DMEM + BT-Test formulation at 5 µg/mL compared to the UT-DMEM + UT-Test formulation group. Hyaluronic acid was significantly increased by 3.90%, 5.37%, and 6.82% in the UT-DMEM + BT-Test formulation group at 0.63, 1.25, and 2.5 µg/mL, respectively 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 compared 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 by 21.32%, 23.81%, and 7.20% at 0.63, 1.25, and 2.5 µg/mL, respectively in the UT-DMEM + BT-Test formulation group compared to the UT-DMEM + UT-Test formulation group. Scratch assay exhibited significant wound closure and cell migration in all the tested groups 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 based herbomineral formulation might be suitable for the development of herbal cosmetics, which could be useful for the management of wounds and various skin related disorders *viz.* 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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