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Consciousness Energy Healing Treatment Based Herbomineral Formulation: A Safe and Effective Approach for Skin Health

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Abstract: Oxidative stress causes serious skin damage that is characterized by ageing, wrinkling, roughness, laxity and pigmentation. In the present work, the impact of Biofield Energy (The Trivedi Effect®-Consciousness Energy Healing) Treatment on the herbomineral test formulation and cell medium (DMEM) was evaluated for skin health parameters. The test formulation was consisted of minerals (zinc chloride, sodium selenate, and sodium molybdate), L-ascorbic acid, herbal (*Centella asiatica*) extract, and tetrahydrocurcumin (THC). The test formulation and DMEM media were divided into two parts. One part received the Biofield Energy Treatment by Jagdish Singh and was termed as the Biofield Treated (BT) sample, while other was denoted as the untreated (UT) samples. MTT assay showed that test formulation was found safe and nontoxic with greater than 75% cell viability against various tested concentrations. Cell proliferation data using BrdU method showed an improved cell proliferation by 149.13% and 118.80% at 8.75 µg/mL in the UT-DMEM + BT-Test formulation and BT-DMEM + UT-Test formulation groups, respectively compared with the untreated group. The collagen level was significantly increased by 28.14% and 44.45% at 1.25 and 0.625 µg/mL, respectively in the UT-DMEM + BT-Test formulation compared with the untreated group. The elastin level was increased by 10.38%, 14.66%, and 48.24% at 2.5, 1.25, and 0.625 µg/mL, respectively in the BT-DMEM + UT-Test formulation group, compared with the untreated group. Moreover, melanin synthesis was significantly inhibited by 5.93%, 1.43%, and 1.43% in the UT-DMEM + BT-Test formulation, BT-DMEM + UT-Test formulation, and BT-DMEM + BT-Test formulation groups, respectively at 0.0625 µg/mL compared with the untreated group. However, melanin synthesis was decreased by 11.71% and 15.75% at 0.125 µg/mL in the UT-DMEM + BT-Test formulation and BT-DMEM + UT-Test formulation groups, respectively. Anti-wrinkling effects exhibited improved cell viability by 17.19% and 28.68% at 2.5 µg/mL in the UT-DMEM + BT-Test formulation and BT-DMEM + BT-Test formulation groups, respectively in HFF-1 cells compared with the untreated group. Wound healing activity using scratch assay showed a significantly improved healing rate upto 5% in the HFF-1 and HaCaT cells lines in the Biofield Energy Healing based test formulation. Overall, the data suggests that The Trivedi Effect® treated test formulation and DMEM has the capacity to improve the skin health and suggests its use in psoriasis, seborrheic dermatitis, skin cancer, rashes from bacterial or fungal infections, and many more skin diseases.

Keywords: Consciousness Energy Healing Treatment, Extracellular Matrix, HaCaT, HFF-1, Hyaluronic Acid, Scratch Assay, Tetrahydrocurcumin

1. Introduction

Skin aging is mostly associated with the passage of time and denoted as intrinsic ageing, while cumulative exposure to other external influences is denoted as extrinsic ageing (like UV radiations, smoking, etc.) [1]. Intrinsically aged skin is defined by fine wrinkling along with reduced elasticity, on the other hand extrinsically-aged skin exposed to UV-rays causes the induction of both deep wrinkles with significant loss of elasticity. Among various external factors responsible for skin ageing, the UV rays induced factor is the most common to cause skin damage along with the effect like deep wrinkles, roughness, laxity and pigmentation [2, 3]. Many of the alternative medicines functioning as antioxidants and anti-photo aging compounds have been used by most of the population that and are effective against photo damage of the skin [4]. Herbal based medicines and cosmetics products are significantly used due to their lower incidence of adverse effect compared with the synthetic cosmetics [5-7]. Synthetic cosmetic are somehow related with serious health complications [8].

The term herbal cosmetics refers to the products, which are formulated with the help of one or more herbal cosmetic ingredients that have cosmetic benefits. With the continued demand of herbal cosmetics and cosmeceuticals, a new herbomineral formulation was designed for skin health. It consists of minerals (such as zinc chloride, sodium selenate, and sodium molybdate), L-ascorbic acid, tetrahydrocurcumin (THC), and plant extract of *Centella asiatica* (commonly known as Jal Brahmi). Minerals such as zinc, copper, and selenium are commonly used in cosmetic products with wide range of reported applications such as strong antimicrobial, antioxidant, and free radical scavenging effects with improved synthesis of skin extracellular components [9, 10]. Additionally, Vitamin C and E are reported to have a significant role in skin health, anti-wrinkling, and significant wound healing action [11]. In addition, THC is one of the major active metabolites of curcumin, which was reported with a strong antioxidant property [12-14]. *C. asiatica*, also known as Gotu Kola is an important medicinal plant, which has been reported for its uses in folk medicine for hundreds of years. Besides, *C. asiatica* has a wide range of applications in skin ageing, along with wound healing potential and has been used in many cosmetic products [15].

The Complementary and Alternative Medicine (CAM) therapies has been practiced worldwide with reported significant benefits along with wide range of therapeutic action. Biofield Energy Healing acts as a unifying concept and works as a bridge with the alternative and contemporary models of Energy Medicine [16]. The use of Energy Healing medicine has been accepted by the U.S. population as having several advantages according to the National Center for Complementary and Alternative Medicine (NCCAM) compared with the modern treatment approaches [17]. The Biofield Energy Treatment has been reported both in clinical practice and scientific research that focus on putative energy fields of the body. Biofield Energy Healing (The Trivedi Effect®-Consciousness Energy Healing) has been widely accepted worldwide with noteworthy results in nonliving materials and living organisms. Many significant outcomes of The Trivedi Effect® treatment have been found in different research field such as microbiology [18-20], agriculture science [21-23], livestock [24], and materials science [25-27]. Due to the continuation of specialized results and applications of Biofield Energy Healing Treatments, the herbomineral test formulation was studied for skin health, and the results were evaluated in three different cell lines such as HFF-1, HaCaT, and B16-F10 cell lines.

2. Materials and Methods

2.1. Chemicals and Reagents

The test formulation was consisted of components such as zinc chloride, which was purchased from TCI, Japan, sodium selenate from Alfa-Aesar, USA, while sodium molybdate from Sigma-Aldrich. Tetrahydrocurcumin and *Centella asiatica* extract were procured from Novel Nutrients Pvt. Ltd., India and Sanat Products Ltd., India respectively. L-ascorbic acid as a positive control was purchased from Alfa-Aesar, while kojic acid and 3-(4, 5-diamethyl-2-thiazolyl) 2, 5 diphenyl-2 *H*-tetrazolium) (MTT) were purchased from Sigma Chemical Co. (St. Louis, MO). Epidermal growth factor (EGF) was procured from Gibco, ThermoFisher, USA. ELISA kits for the estimation of extracellular matrix component were procured from CUSABIO and CusAb Co. Pvt. Ltd, USA. Fetal bovine serum (FBS) and DMEM were purchased from Gibco, USA. Antibiotics solution (Penicillin-Streptomycin) were procured from HiMedia, India, while Direct Red 80 and EDTA were purchased from Sigma, USA. All the other chemicals used in this experiment were analytical grade procured from local vendors.

2.2. Cell Culture

Three cell lines were used for the estimation of skin health parameters. HFF-1 (human foreskin fibroblast) cells were procured from American Type Culture Collection (ATCC), USA, originated from normal human skin fibroblast cells. B16-F10 (mouse melanoma) and HaCaT (human keratinocytes) cells were procured from National Centre for Cell Science (NCCS), Pune, India. HFF-1, HaCaT, 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 all the cell lines were 37°C, 5% CO_2, and 95% humidity. L-ascorbic acid (for ECM, UVB protection, and wound healing assay) at the concentrations ranges from 10 µM to 1000 µM, while kojic acid (for melanin) concentrations ranges from 1 mM to 10 mM. FBS (0.5%) was used in cell proliferation assay in BrdU assay, while EGF 10 µM used in non-cytotoxic dose concentration in MTT assay.

2.3. Experimental Design

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

2.4. Energy of Consciousness Treatment Strategies

The test formulation was a combination of three minerals *viz.* zinc chloride, sodium selenate and sodium molybdate, L-ascorbic acid, and herbal product as *Centella asiatica* extract along with tetrahydrocurcumin. The test formulation and DMEM were divided into two parts. One part of the test samples were treated with Biofield Energy by a renowned Biofield Energy Healer, Jagdish Singh, and coded as the Biofield Energy Treated formulation, while the second part of the test samples did not receive any sort of treatment and was defined as the untreated test samples. The Biofield Energy Healing Treatment was performed for 5 minutes through the Healer's unique Energy Transmission process remotely to the test samples under standard laboratory conditions. The Biofield Energy Healer, Jagdish Singh, was located in USA, while the test samples were located in India (Research laboratory of Dabur Research Foundation near New Delhi, India). The Biofield Energy Healer never visited the laboratory, nor had any contact with the test formulation and DMEM. Further, the control samples were treated by a "sham" healer for comparative purpose, while 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 study.

2.5. Determination of Non-cytotoxic Concentrations

The cell proliferation in cell lines such as HFF-1, HaCaT, and B16-F10 were performed by MTT assay. The cells 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 cells were incubated overnight under specific growth conditions that were allowed the cell recovery and exponential growth, which were subjected to serum stripping or starvation. The cells were subsequently treated to the Biofield Energy Treated and untreated groups of test formulation/DMEM at a range of concentrations (0.008 to 10 µg/mL) and ascorbic acid (10 and 50 µM) followed by incubation from 24 to 72 hours in CO_2 incubator at 37°C, 5% CO_2 and 95% humidity. Further, serum free MTT media (20 µL of 5 mg/mL) was added to each well followed by incubation for 3 hours at 37°C. The supernatant was aspirated and 150 µL of DMSO was added to each well to dissolve the formazan crystals. Thereafter, the absorbance of each well was recorded at 540 nm using Synergy HT micro plate reader, BioTek, USA. The concentrations that exhibited percentage cytotoxicity of less than 30% was considered as non-cytotoxic [28].

2.6. Effect of Test Formulation on Human Foreskin Fibroblast (HFF-1) Cell Proliferation Using BrdU Method

The fibroblast cell proliferation assay was done using BrdU method with HFF-1 cells, which 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 then 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 formulation in different defined experimental groups and positive control. Following 24 to 72 hours of incubation with the test substance and positive control, the plates were taken out and BrdU (5-bromo-2'-deoxyuridine) estimated using Cell Proliferation ELISA, BrdU estimation kit (ROCHE – 11647229001) as per manufacturer's instructions.

2.7. Estimation of Extracellular Matrix Component (ECM) Synthesis

Synthesis of extracellular matrix components (*i.e.* collagen, elastin and hyaluronic acid) in HFF-1 cell line were estimated for determining the potential of the test formulation to improve skin strength, overall elastin, 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 then incubated overnight under specified growth conditions followed by cells to serum stripping. Further, the cells were treated with the test formulation at different experimental combination groups with DMEM group *viz.* vehicle control (DMSO, 0.05%), and positive control (ascorbic acid, at 10 µM). 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 of elastin and hyaluronic acid levels. The corresponding cell layers were processed for the estimation of collagen levels using Direct Sirius red dye binding assay. 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 [29].

2.8. Estimation of Melanin Synthesis-Skin Depigmentation Effect

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 stimulation of intracellular melanin synthesis. Further, the cells were incubated with α-MSH, and then treated with concentration at 0.625, 1.25 and 2.5 µg/mL of test formulation with DMEM for a time period from 48 to 96 hours. After incubation, intracellular melanin was extracted in NaOH and the absorbance was recorded at 405 nm. The level of melanin was extrapolated using standard curve obtained from purified melanin [30].

2.9. Anti-wrinkling 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, 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) [31]. The percent cell viability was assessed using formula (equation 1)-

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

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

2.10. Wound Healing Scratch Assay

HFF-1 and HaCaT cell lines 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 was 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 wounds were created in the near confluent monolayer of cells by gently scraping with a sterile 200 µL micropipette tip. The cells were rinsed with serum free DMEM and treated with the test formulation. The scratched area was monitored for a time period ranging from 0 to 48 hours for closure of wound area. The photomicrographs were done at 16 hours for quantitative assessment of migrated cells using digital camera, which was connected to the inverted microscope. All the observations were calculated and compared with the positive and vehicle control [32].

2.11. Statistical Analysis

Each experiment was carried out in three independent assays and was represented as mean values with standard deviation. Student's *t*-test was used to compare two groups to judge the statistical significance. For multiple group comparison, one-way analysis of variance (ANOVA) was used followed by post-hoc analysis using Dunnett's test. Statistically significant values were set at the level of $p \leq 0.05$.

3. Results and Discussions

3.1. Non-cytotoxic Effect of the Test Formulation on Cell Lines

The results of non-cytotoxic concentrations of the test formulation against three tested cell lines *i.e.* HFF-1, HaCaT, and B16-F10 are presented in Figure 1. All the results were compared with respect to the ascorbic acid (10 µM) and EGF (10 ng/mL) for estimation of percentage cell viability. The results showed that all the concentrations were found safe and non-toxic with more than 75% in all the cell lines up to maximum at 40 µg/mL. Hence, the tested concentrations were selected for further estimation of the cellular proliferation using BrdU assay, identification of extracellular matrix (ECM) synthesis (such as collagen, elastin, and hyaluronic acid), melanin and wound healing scratch assay in various cell lines.

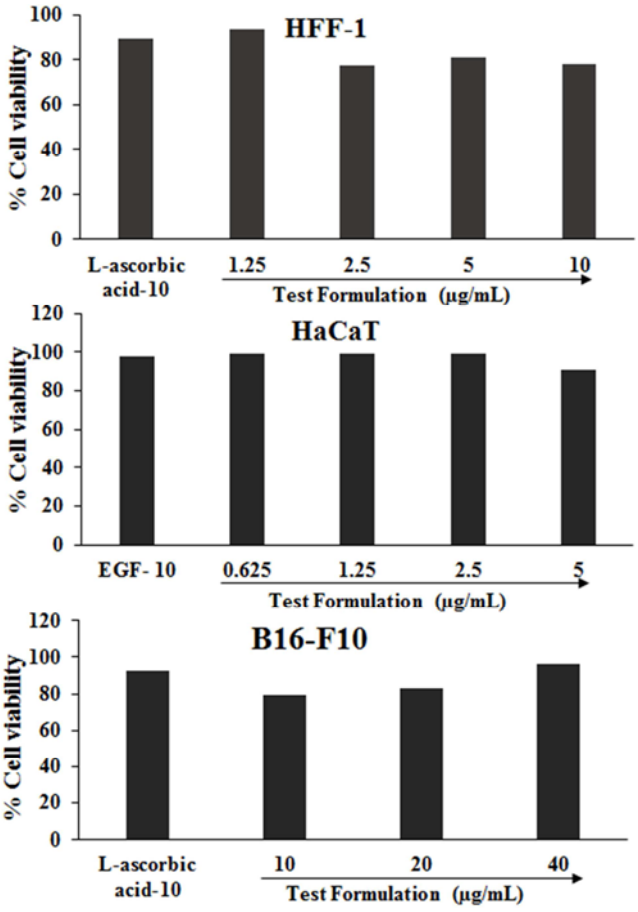

Figure 1. *Effect of the test formulation on HFF-1, HaCaT, and B16-F10 cell lines for cell viability using the MTT assays, and results are expressed as percent cell viability. EGF-10: Epidermal growth factor (10 µM).*

3.2. Investigation of the Biofield Energy Treated Test Formulation on HFF-1 Cell Proliferation (BrdU Method)

The test formulation was analyzed for cellular proliferation assay using BrdU assay at different test concentrations in HFF-1 cells after 48 hours of incubation, which are represented in Figure 2. FBS at concentration of 0.5% showed a significantly increased cell proliferation rate by 150% and 133%, compared with the normal and vehicle control groups, respectively. The Biofield Energy Treated groups, UT-DMEM + BT-Test formulation, BT-DMEM + UT-Test formulation, and BT-DMEM + BT-Test formulation showed a significantly increased cellular proliferation at 8.75 µg/mL by 149.13%, 118.80%, and 2.58%, respectively compared with the UT-DMEM + UT-Test formulation group. However, in UT-DMEM + BT-Test formulation and BT-DMEM + UT-Test formulation groups showed a significantly increased cellular proliferation at 17.5 µg/mL by 74.04% and 126.85%, respectively compared with the UT-DMEM + UT-Test formulation group. This results suggest that the Biofield Energy Treated Test formulation showed a significant increase in the cellular proliferation rate in HFF-1 cells at 8.75 and 17.15 µg/mL using BrdU assay.

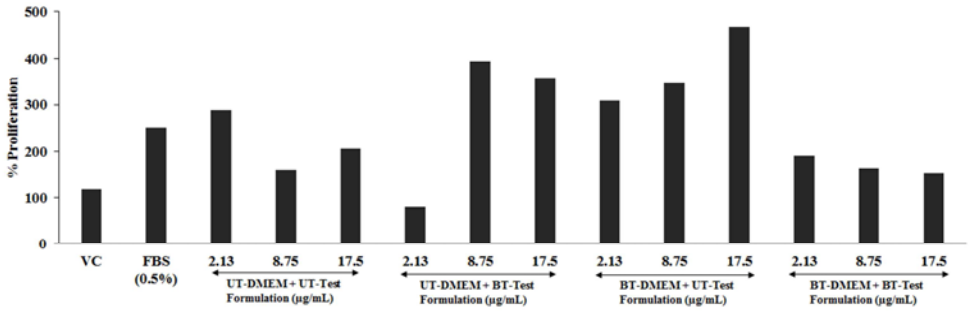

Figure 2. *Effect of the Biofield Energy Treated Test formulation with DMEM on cellular proliferation in HFF-1 cells after 48 hours. VC: Vehicle control; FBS: Fetal bovine serum; UT: Untreated; BT: Biofield Treated.*

3.3. Analysis of Extracellular Matrix Component Synthesis

3.3.1. Collagen Estimation

Collagen, one of the most abundant proteins for skin health, structure and fibrous protein which present in ECM. The results of the Biofield Energy Treated Test formulation/DMEM showed a significantly increased level of collagen in HFF-1 cell line. Collagen levels in various groups such as ascorbic acid and the Biofield Energy Treated Test formulation/DMEM groups are presented in Figure 3. Ascorbic acid (10 µM) showed a significantly increased collagen content by 55%, while the Biofield Energy Treated Test formulation reported with a significantly increase in the collagen amount.

In UT-DMEM + BT-Test formulation group, reported with significant increases in collagen levels at 1.25 and 0.625 µg/mL by 28.14% and 44.45%, respectively compared to the UT-DMEM + UT-Test formulation group. Similarly, in the BT-DMEM + UT-Test formulation group at 1.25 and 0.625 µg/mL showed an increased collagen levels by 16.73% and 19.28%, respectively compared with the UT-DMEM + UT-Test formulation group. However, the groups such as BT-DMEM + BT-Test formulation showed an increase in collagen level at 2.5, 1.25 and 0.625 µg/mL by 9.02%, 15.07%, and 20.23%, respectively compared with the UT-DMEM + UT-Test formulation group. Therefore, the experimental data suggests that the Biofield Energy Healing Treatment has the ability to significantly increased collagen level at all the tested concentrations. Hence, it can be assumed that Biofield Energy Healing (The Trivedi Effect®) might improve the procollagen peptides and it's cross-linking among various tropocollagen molecules that improved the collagen. It provide strength and structure to the skin that might be beneficial for skin health, strength, and structure and wound healing [33, 34].

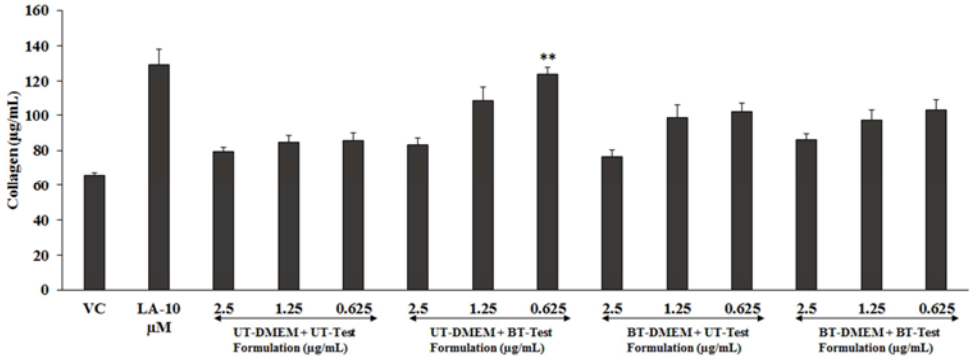

Figure 3. *Concentration-dependent effects of test formulation on human dermal fibroblast (HFF-1) cell line for collagen level. $^{**}p \leq 0.01$ statistical comparison with respect to untreated DMEM and untreated test formulation using one way ANOVA (Dunnett's test). VC: Vehicle control; LA-10: L Ascorbic acid at 10 µM concentration; UT: Untreated; BT: Biofield Treated.*

3.3.2. Elastin Estimation

The Biofield Energy Healing based test formulation was evaluated to distinguish the alteration in elastin level. This is one of the important constituents of the ECM. It forms tight junctions with collagen fibrils that maintains the cellular integrity, which retain shape in body tissue and very elastic tissue of the body [35]. The results of elastin level in HFF-1 cell line due to the Biofield Healing Test formulation are shown in Figure 4. Ascorbic acid (50 µM) group showed significantly increased elastin content by 55% compared with the normal control group. Moreover, BT-DMEM + UT-Test formulation group showed a significant increase in the elastin level by 10.38%, 14.66%, and 48.24% at concentration 2.5, 1.25, and 0.625 µg/mL, respectively compared with UT-DMEM + UT-Test formulation group. In addition, BT-DMEM + BT-Test formulation group showed an increase in elastin level by 9.04% at concentration 2.5 µg/mL compared with UT-DMEM + UT-Test formulation group. However, at other tested concentrations, significantly altered elastin levels were observed after treatment with the Biofield Energy Treated Test formulation compared with the untreated groups. Fibroblast and elastin are required in the body and responsible for ageing and health. The experimental result suggests a significantly increased elastin level, which might improve the skin elasticity and strength that activates the dermal metabolism. Thus, Biofield Energy Healing based Test formulation can be significantly used to improve the elastin level that might improve the cell growth, survival, differentiation and morphogenesis.

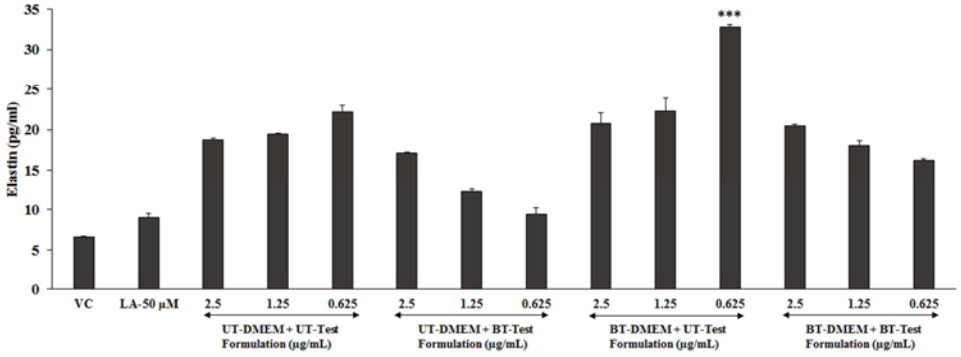

Figure 4. *Effect of the Biofield Energy Treated Test formulation on human dermal fibroblast (HFF-1) cell line for elastin level.* $^{***}p \leq 0.001$ *statistical comparison with respect to untreated DMEM and untreated Test formulation using one way ANOVA (Dunnett's test). VC: Vehicle control; LA-50: L Ascorbic acid at 50 µM concentration; UT: Untreated; BT: Biofield Treated.*

3.3.3. Analysis of Hyaluronic Acid

HA helps to retain skin moisture, secures skin moisture, creates fullness, and regulates the skin water balance. The hyaluronic acid levels in the HFF-1 cell line after the Biofield Energy Healing based test formulation results are presented in Figure 5. The level of HA in control group was 6.78 ± 2.26 ng/mL, while in ascorbic acid HA level was increased to 19.23 ± 6.41 ng/mL. This suggests that ascorbic acid group showed an increase in the hyaluronic acid content by 183.6%. The test formulation/DMEM group showed a significant change in HA levels at all the tested concentrations in all the groups with respect to normal control group. Hence, the Biofield Energy Healing (The Trivedi Effect®) based Test formulation might be a new approach in cosmetology for skin health. HA is a natural polysaccharides and is distributed extensively all over the connective, neural, and epithelial tissue. In cosmetic markets, most of the skin care products use HA as the base for skin health. Some common marketed skin products such as hyaluronic acid creams, serums, injectable, and hyaluronic acid supplements are used to improve HA level. Low HA might lead to reduced skin elasticity and expose the signs of aging. HA based skin car product, due to their high water holding capacity are widely available in market for skin health [36].

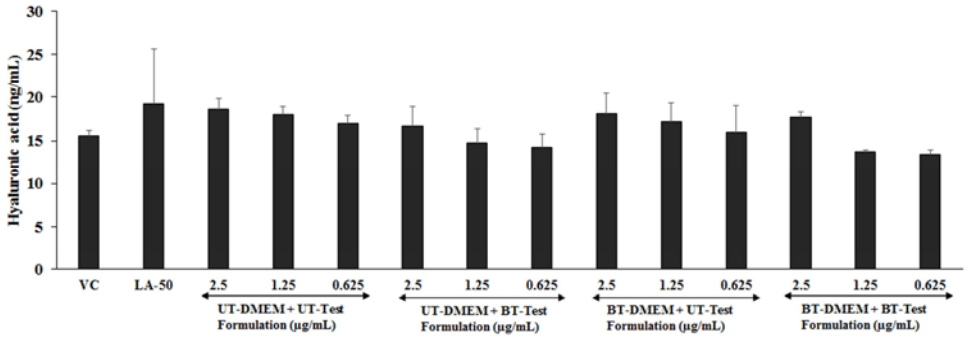

Figure 5. *Synthesis of extracellular matrix component, hyaluronic acid by Biofield Energy Treated Test formulation in human dermal fibroblasts (HFF-1) cell lines. VC: Vehicle control; LA-50: L Ascorbic acid at 50 µM concentration; LA-50: UT: Untreated; BT: Biofield Treated.*

3.4. Estimation of Melanin Synthesis Inhibition

The results of melanin synthesis and effect of the Biofield Energy Treated Test formulation in B16-F10 melanoma cell line are reported in Figure 6. The cells were cultured in DMEM supplemented media containing several concentrations of the test formulation and kojic acid (10 µM) were evaluated and compared with untreated formulation. Kojic acid, a skin whitening compound was used as positive control, and the results showed a significant decrease in the level of melanin synthesis by 65.14% compared to the melanin level in the presence of the alpha melanocyte stimulating hormone (α-MSH). The Biofield Energy based test formulation showed a significant decrease in melanin synthesis by 5.93%, 1.43%, and 1.43% at 0.0625 µg/mL in UT-DMEM + BT-Test formulation, BT-DMEM + UT-Test formulation, and BT-DMEM + BT-Test formulation groups, respectively. However, at concentration 0.125 µg/mL, the melanin synthesis was decreased by 11.71% and 15.75% in UT-DMEM + BT-Test formulation and BT-DMEM + UT-Test formulation groups, respectively compared with the UT-DMEM + UT-Test formulation group. It can be proposed that the Biofield Energy Healing based Test formulation has the capacity to decrease the skin melanin level, and it might be expected that Biofield Energy Treatment inhibits the tyrosinase enzymes activity that are required for melanin synthesis [37].

The test formulation contained *Centella asiatica* extract, THC, and minerals, which are reported to have significant positive effect against skin infection, inflammatory dermatoses, with strong antioxidant action [38, 39]. However, various other factors such as genes, nutrition, and environmental are also responsible for skin complexion and skin pigmentation. Melanin is one of the important components responsible for skin pigmentation, while pigments eumelanin controls the two colors type brown and black. However, skin depigmentation results in many disorders, when sun ultraviolet radiation (UV-A and UV-B) initiates the process of melanogenesis in the melanocytes which results in skin darkening [40]. Overall, the Biofield Energy Healing based test formulation and DMEM would be useful in altering the process of melanogenesis that regulates the skin-related disorders and pigmentation problems.

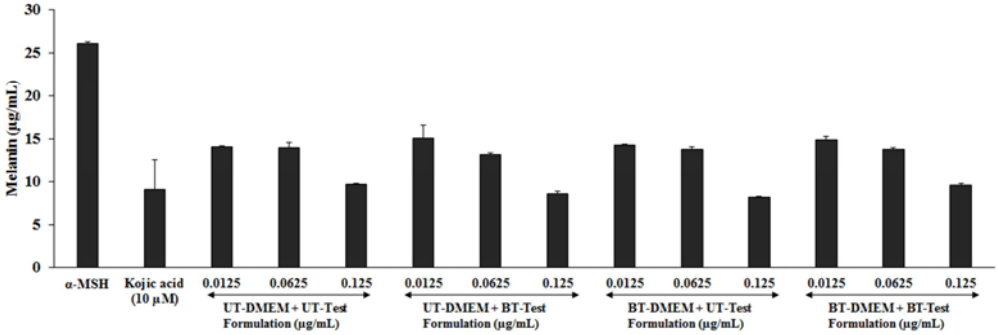

Figure 6. *Inhibitory effect of the Biofield Energy Treated Test formulation on melanogenesis (skin whitening potential) in mouse melanoma (B16-F10) cell line. α-MSH: Alpha melanocyte stimulating hormone, UT: Untreated; BT: Biofield Treated.*

3.5. Anti-wrinkling Effects of Test Formulation on UVB-Induced Photoaging

Anti-wrinkling effect in HFF-1 cells were evaluated for Biofield Energy Treated Test formulation and results are presented in Figure 7 in terms of cell viability after exposure of UV-B rays. The HFF-1 cells were subjected to the lethal dose of UV-B irradiation (200 mJ/cm^2) and percentage cell viability due to UV-B was acknowledged. The HFF-1 cells, while exposure to UV-B showed high degree of cell death, showed 25.21% of cell viability. The cell viability in vehicle control group was found as 20.51% due to UV-B irradiation (200 mJ/cm^2). However, ascorbic acid (50 µM) showed a significant increase in the cell viability 110.48%. Besides, the experimental groups showed that all the groups in tested concentrations reported with improved cell viability. Among the tested groups, at concentration 2.5 µg/mL UT-DMEM + BT-Test formulation and BT-DMEM + BT-Test formulation group showed an increase in cell viability by 17.19% and 28.68%, respectively compared with the UT-DMEM + UT-Test formulation group. Similarly, at 1.25 µg/mL, UT-DMEM + BT-Test formulation and BT-DMEM + BT-Test formulation group showed an increase cell viability by 13.23% and 7.21%, respectively compared with the UT-DMEM + UT-Test formulation group. An increase in cell viability was also found in UT-DMEM + BT-Test formulation group at concentration 0.625 µg/mL by 8.82% compared with the UT-DMEM + UT-Test formulation group. The experimental results displayed improved cell viability after exposure to the Biofield Treated Test formulation, suggests cell protection and less skin damage. Most of the skin diseases reported because of the exposure to UVB-radiations like stress, skin disorders, free radical generation, etc. This exposure and cellular death results in downregulation of the human skin fibroblasts through various inflammatory responses like DNA damage, wrinkles and skin-ageing [41]. Hence, the Biofield Energy Healing based test formulation would be used to improve the cell viability, anti-wrinkling action and results in skin protection against UV-B radiations.

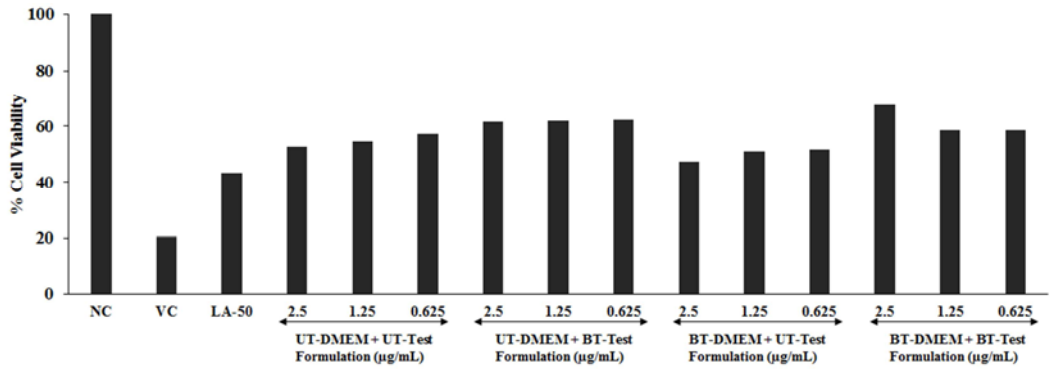

Figure 7. *Anti-wrinkling potential and cytoprotective potential of Biofield Energy Treated Test formulation against UV-B induced stress in human dermal fibroblasts (HFF-1) cell lines.% cell viability of HFF-1 cells after treatment in various groups. NC: Normal control; VC: Vehicle control; LA-50: L-Ascorbic acid at 50 µM concentration, UT: Untreated; BT: Biofield Treated.*

3.6. Wound-Healing Scratch Assay

Wound healing scratch assay was performed in HFF-1 and HaCaT cells, and the results of Biofield Energy Treated test formulation showed a significant cellular migration. Some representative pictures among the groups are presented in Figure 8. The experimental data was evaluated and compared with respect to positive control group, EGF, vehicle control, and Biofield Energy Treated test formulation combinations with DMEM. Scratch assay reflects the cell-to-cell and cell-to-matrix interactions during wound healing process [42]. The results suggested that at 5 and 10 µg/mL concentration of test formulation showed a significant healing effect and cell migration in HFF-1 and HaCaT cells. The experimental data suggests that a 2% to 5% increase was observed in cell coverage area in HFF-1 cells, while 1% increase in cellular area covered in HaCaT cells was reported in the Biofield Treated Test formulation compared with untreated test formulation. The results after treatment of wound healing scratch assay in EGF group, normal control group and other experimental groups of the Biofield Energy Treated/untreated test formulation in DMEM showed a significant rate of cellular migration rate along with wound closure as shown in Figure 8 (a-f), compared with the UT-Test formulation + UT-DMEM group. Overall, it can be concluded that The Trivedi Effect® has the capacity to improved cellular migration that has significant impact in wound healing.

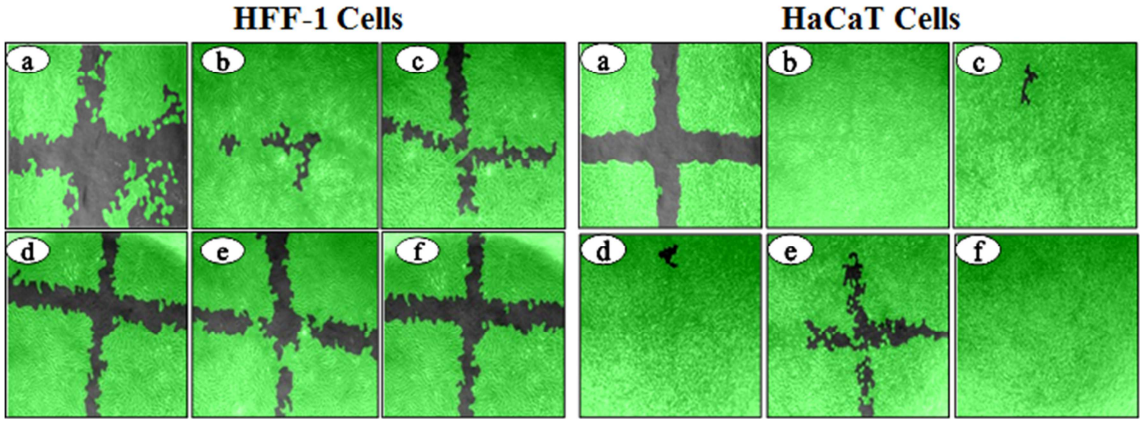

Figure 8. *Representative images of HFF-1 and HaCaT cell migration cells after induction of a scratch. All the pictures were taken immediately after the scratch was induced (i.e. at 0 hours), after 16 hours in the presence of EGF and Biofield Energy Treated test formulation. Pictures are taken at 10 times magnification. Images represents HFF-1 and HaCaT cells migration in presence of (a) baseline control media, (b) EGF, (c) UT-DMEM + UT-Test formulation, (d) UT-DMEM + BT-Test formulation, (e) BT-DMEM + UT-Test formulation, and (f) BT-DMEM + BT-Test formulation.*

4. Conclusions

The findings of this study suggested significantly increased cell viability with more than 75% cells in all the tested concentrations such as HFF-1, HaCaT, and B16-F10 using MTT assay suggests that the Biofield Energy Healing based herbomineral test formulation was safe and nontoxic. Besides, results of BrdU assay showed 149.13% and 118.80% increased cellular proliferation (at 8.75 µg/mL) in the UT-DMEM + BT-Test formulation and BT-DMEM + UT-Test formulation groups, while 74.07% and 126.85% in the UT-DMEM + BT-Test formulation and BT-DMEM + UT-Test formulation groups, respectively at 17.5 µg/mL compared to the UT-DMEM + UT-Test formulation group. Collagen level was increased by 28.14% and 44.45% at 1.25 and 0.625 µg/mL respectively, in the UT-DMEM + BT-Test formulation group, while 16.73% and 19.28% increase at 1.25 and 0.625 µg/mL

respectively, in the BT-DMEM + UT-Test formulation group with respect to the UT-DMEM + UT-Test formulation group. However, the level of elastin was significantly increased by 10.38%, 14.66%, and 48.24%, in the BT-DMEM + UT-Test formulation at 2.5, 1.25, and 0.625 µg/mL respectively compared to the UT-DMEM + UT-Test formulation group. The melanin level was reduced by 11.71% and 15.75% in the UT-DMEM + BT-Test formulation and BT-DMEM + UT-Test formulation groups, respectively compared with the UT-DMEM + UT-Test formulation group at 0.125 µg/mL. Anti-wrinkling potential with respect to UV-B showed 17.19% and 28.68% increased cell viability in the UT-DMEM + BT-Test formulation and BT-DMEM + BT-Test formulation group respectively, at 2.5 µg/mL compared to the UT-DMEM + UT-Test formulation group. Wound healing scratch assay showed a significant migration of fibroblast and keratinocytes cells with increase covered area up to 5% in HFF-1 and HaCaT cells after treatment with Biofield Energy (The Trivedi Effect®) based test formulation. The Biofield Energy Treated Test formulation and DMEM would be a better approach for anti-wrinkling, anti-aging, skin whitening, and wound healing activity.

Overall, the Biofield Energy Treated test formulation can be used as a Complementary and Alternative Medicine (CAM) treatment with a safe therapeutic index for various skin irregularities that are typically symptoms of a skin disorders such as Eczema, diaper rash, chickenpox, measles, warts, acne, hives, wrinkles, ringworm, Rosacea, psoriasis, seborrheic dermatitis, skin cancer, rashes from bacterial or fungal infections, rashes from allergic reactions, raised bumps that are red or white, cracked skin, discolored patches of skin, fleshy bumps, warts, or other skin growths, changes in mole color or size, a loss of skin pigment, scaly or rough skin, peeling skin, ulcers, open sores or lesions, dry, excessive flushing. Further, the Biofield Energy Healing based herbomineral test formulation can also be used in the prevention of temporary and permanent skin disorders, anti-aging, improved overall health, and quality of life.

Abbreviations

THC: Tetrahydrocurcumin; DMEM: Dulbecco's Modified Eagle's Medium; ECM: Extracellular matrix; EGF: Epidermal growth factor; HA: Hyaluronic acid; HFF-1: Human foreskin fibroblast cell line; B16-F10: Mouse melanoma cell line; HaCaT: Human keratinocytes cells; UV-B: Ultra violet B rays; CAM: Complementary and alternative medicine; NCCAM: National Center for Complementary and Alternative Medicine.

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