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An *in Vitro* Study of Biofield Energy Healing Based Herbomineral Formulation for Skin Protection

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Abstract: The current research work deals with the impact of the Consciousness Energy Healing Treatment (The Trivedi Effect®) based herbomineral test formulation and cell medium (DMEM) against various skin health parameters. The test formulation is the combination 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 equal parts, *i.e.* one part received the Biofield Energy Treatment by Eileen Mary Meagher and was labeled as the Biofield Treated (BT) samples, while other represented was defined as the untreated test (UT) samples. The MTT assay showed that the test formulation was found safe and nontoxic with greater than 75% cell viability. Cell proliferation assay using BrdU method showed an improved proliferation rate by 343.23%, 316.13%, and 512.75% in the UT-DMEM + BT-Test formulation, BT-DMEM + UT-Test formulation, and BT-DMEM + BT-Test formulation groups, respectively at 17.5 µg/mL, compared with the untreated group. The collagen level was significantly increased by 15.70% and 13.52% at 2.5 and 0.625 µg/mL, respectively in the UT-DMEM + BT-Test formulation compared with the untreated group. The level of elastin was increased by 47.0% and 64.1% at 2.5 and 1.25 µg/mL, respectively in the BT-DMEM + UT-Test formulation, group compared with the untreated group. Hyaluronic acid synthesis was increased by 4.37%, 41.47%, and 19.74% at 2.5, 1.25, and 0.625 µg/mL, respectively in the UT-DMEM + BT-Test formulation group with respect to the untreated group. Melanin synthesis was significantly inhibited by 8.83%, 5.78%, and 12.68% at 0.0125, 0.0625, and 0.125 µg/mL, respectively in the UT-DMEM + BT-Test formulation group compared with the untreated group. Anti-wrinkling effect showed an improved cell viability by 4.32% and 2.47% at 2.5 µg/mL in the UT-DMEM + BT-Test formulation and BT-DMEM + UT- Test formulation groups, respectively in HFF-1 cells compared with the untreated group. Wound healing activity using scratch assay exhibited improved a healing rate up to 6% in the HFF-1 and HaCaT cells lines in the Biofield Energy Treated Test formulation. In conclusion, results suggest that The Trivedi Effect®- Consciousness Energy Healing Treatment based test formulation and medium have the ability to improve the skin health and can be used as skincare products cosmetics for anti-ageing, anti-wrinkling action and for the treatment of many skin diseases such as Eczema, diaper rash, chickenpox, measles, warts, acne, hives, wrinkles, ringworm, Rosacea, psoriasis, seborrheic dermatitis, skin cancer, etc.

Keywords: Consciousness Energy Healing Treatment, Scratch Assay, Extracellular Matrix, Hyaluronic Acid, Tetrahydrocurcumin

1. Introduction

Alternative medicines have been used from the beginning of human history for curing a wide range of diseases. The use of chemical and synthetic medicines have been increased in the last half century but all are reported to cause harmful side effects to human life, which has led to a further increased tendency towards medicinal herbs [1-3]. It has been found

that natural medicines especially medicinal herbs are the main course of treatment in some diseases for many years. At the same time, many pharmaceutical industries utilize and work with medicinal plants as main raw materials [4]. Medicinal herbs are widely used and accepted by people worldwide due to less side effects and are also recommended by World Health Organization (WHO). In cosmetics, herbal based products have been developed and used due to safer action on the skin along with lower incidences of adverse effects compared with the synthetic cosmetics [5-7], which are somehow related with serious health complications [8]. Most of the herbal medicines effectively work as antioxidants and anti-aging agents, which have been found to be effective against photo damage (UV-B) of the skin [9]. Ageing occurs due to the combination of various factors, such as age, diet, genetics, environment, and UV-rays exposure. UV-rays are the most common cause for skin damage that results in deep wrinkles, roughness, laxity and pigmentation [10, 11]. Due to lack of an effective medicine for overall skin health and wound healing activity, a new test formulation was designed and tested against various skin health parameters.

The novel herbomineral test formulation is the combination of minerals (zinc chloride, sodium selenate, and sodium molybdate), L-ascorbic acid, tetrahydrocurcumin (THC), and plant extract of *Centella asiatica* (commonly known as Jal Brahmi). Minerals used in the formulation are widely used in many cosmetic products such minerals as zinc, copper, and selenium (due to its strong antimicrobial, antioxidant, and free radical scavenging effects) [12, 13]. Vitamins such as vitamin C and E have been found to play a significant role in skin health, anti-wrinkling, and wound healing activity [14]. Moreover, THC has strong antioxidant properties, is widely used in many skin care products, and is one of the major active metabolites of curcumin [15-17]. *C. asiatica* is an important medicinal plant and used as natural skin ageing weapon in cosmetology. It has wide range of applications in prevention of skin ageing, with wound healing potential in many cosmetic products [18]. The herbomineral formulation was prepared for skin health and then treated with Biofield Energy by a renowned healing practitioner.

Biofield Energy Healing is one of the important categories defined under Complementary and Alternative Medicine (CAM) which is accepted worldwide for the various treatments. Biofield Energy therapy was accepted by the National Center for Complementary and Alternative Medicine (NCCAM) [19, 20] with significant therapeutic actions. Biofield Energy treatment has been reported in many clinical practices as well as much scientific research has been published demonstrating significant outcomes. Biofield Energy Healing (The Trivedi Effect®-Consciousness Energy Healing Treatment) has been reported worldwide with remarkable results in nonliving substances and living organisms. The Trivedi Effect® has been found to display prominent results in antimicrobial sensitivity against pathogenic microbes [21-23], medical science [24, 25], agricultural science [26-28], pharmaceuticals [29-31], nutraceuticals [32], and materials science [33-35].

This study focused on evaluating the skin health parameters of the Biofield Energy Healing based test formulation and cell medium (DMEM) for skin health 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 contained minerals 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 Cultures

The experiment consists of three cell lines, which 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. All the three selected cell lines *i.e.* HFF-1, HaCaT, and B16-F10 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) 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 Study Design

The experimental groups consisted of cells in the normal control group, vehicle control group (0.05% DMSO), positive control group (L-ascorbic acid/kojic acid/EGF/FBS) and experimental tested groups. The 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 and DMEM were divided into two parts. One part of the test samples were treated with the Biofield Energy (also known as The Trivedi Effect®) by a renowned Biofield Energy Healer, Eileen Mary Meagher, and coded as the Biofield Energy Treated samples (BT-DMEM and BT-Test formulation). The other samples did not receive any sort of treatment and was defined as the untreated test samples (UT-DMEM and UT-Test formulation). The Biofield Energy Treatment was provided remotely for 5 minutes by a renowned Biofield Energy Healer, Eileen Mary Meagher, who was located in the USA, while the test samples were located in the research laboratory of Dabur Research Foundation, near New Delhi, India under standard laboratory conditions. The Biofield Energy Healer participating in this research never visited the laboratory, nor had any contact with the test formulation and DMEM. The control group was treated with a "sham" healer for comparative 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 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 allowed the cell recovery and exponential growth, which were subjected to serum stripping or starvation. The cells were subsequently treated with the Biofield Energy Treated and untreated test formulation/DMEM groups at different test formulation 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 37ºC, 5% CO_2 and 95% humidity. Further, the 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 [36].

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

Fibroblast cell proliferation assay was performed using BrdU method in 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 were then incubated overnight under growth conditions so as to allow cell recovery and exponential growth. After overnight incubation, the above cells were subjected for serum starvation. Following serum starvation, the cells were treated with non-cytotoxic concentrations of test formulation in different defined experimental groups and positive control. After 24 to 72 hours of incubation with the test samples and positive control, the plates were taken out and BrdU (5-bromo-2'-deoxyuridine) estimated using ELISA cell proliferation assay, BrdU estimation kit (ROCHE – 11647229001) as per manufacturer's instructions.

2.7. Estimation of Extracellular Matrix Component (ECM) Synthesis

The 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 in terms of skin strength, overall elastin, and hydration level. The 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). After 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. However, the corresponding cell layers were processed for the estimation of collagen levels using Direct Sirius red dye binding assay. The 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 [37].

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 0.625, 1.25 and 2.5 µg/mL test formulation concentrations 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 [38].

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) [39]. The percent cell viability was assessed using formula (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 were incubated overnight under growth conditions and allowed for cell recovery and exponential growth. After overnight incubation, the cells were subjected to the serum starvation in DMEM for 24 hours. The mechanical scratch wounds were created in the near confluent monolayer of cells by gently scraping with the sterile 200 µL micropipette tip. The cells were rinsed with serum free DMEM and the Biofield Energy Treated 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 positive and vehicle control [40].

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). 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. The results were compared with respect to ascorbic acid (10 µM) and EGF (10 ng/mL) in order to check the percentage cell viability. The experimental results showed that all the concentrations were found safe and non-toxic with more than 75% in all the cell lines at various tested concentrations. On the basis of MTT data, concentrations were selected for further estimation of 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 respective cell lines.

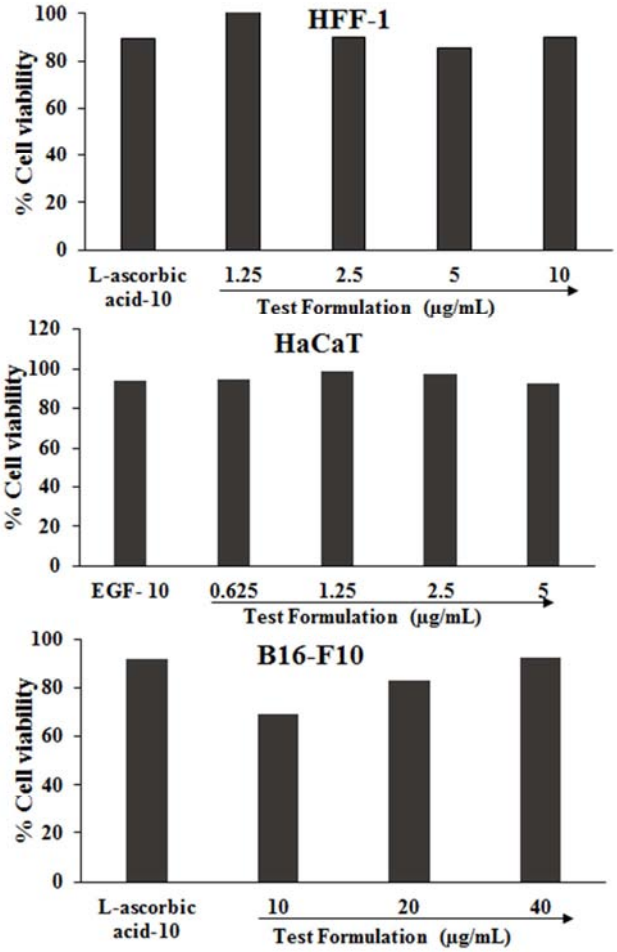

Figure 1. *Effect of the test formulation on HFF-1, HaCaT, and B16-F10 cell lines for cell viability using the MTT assays and results are expressed as percent cell viability. EGF-10: Epidermal growth factor (10 µM).*

3.2. HFF-1 Cell Proliferation Assay of the Biofield Energy Treated Test Formulation Using BrdU Method

The Biofield Energy based test formulation was analyzed for cellular proliferation assay using BrdU assay at different

concentrations in HFF-1 cells and the results are represented in Figure 2. The FBS at 0.5% concentration showed significant increased proliferation rates by 150% and 113.65% 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 groups showed significant increased cellular proliferation rates at 8.75 µg/mL by 215.77%, 113.37%, and 288.72%, respectively compared with the UT-DMEM + UT-Test formulation group. However, at 17.5 µg/mL in UT-DMEM + BT-Test formulation, BT-DMEM + UT-Test formulation, and BT-DMEM + BT-Test formulation groups showed significantly increased cellular proliferation by 343.23%, 316.13%, and 512.75%, respectively compared with the UT-DMEM + UT-Test formulation group. However, at 2.13 µg/mL in the BT-DMEM + BT-Test formulation group, cellular proliferation was increased by 144.88% compared with the UT-DMEM + UT-Test formulation group. The data suggests that the Biofield Energy Treated test formulation and DMEM showed a significant increase in cellular proliferation in HFF-1 cells at all the tested concentrations using BrdU assay.

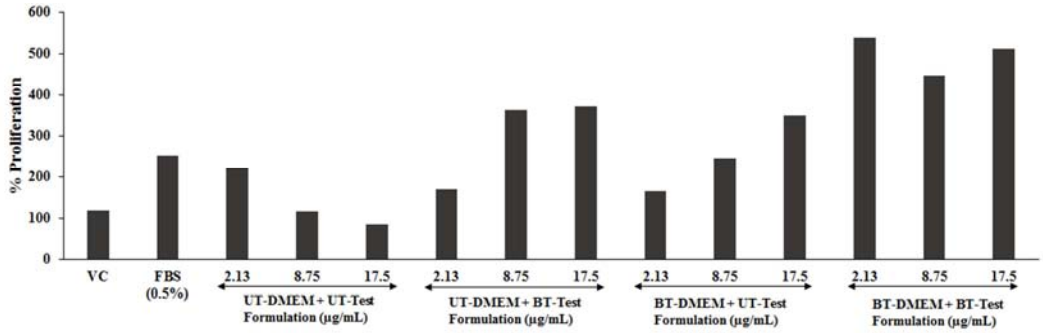

Figure 2. *Cellular proliferation effect of the Biofield Energy Treated Test formulation with DMEM in HFF-1 cells after 48 hours. VC: Vehicle control; FBS: Fetal bovine serum. UT: Untreated; BT: Biofield Treated.*

3.3. Analysis of Extracellular Matrix Component Synthesis

3.3.1. Collagen Estimation

Collagen is one of the most abundant proteins in the extracellular matrix (ECM) responsible for skin health, structure and fibrous protein. The results of the Biofield Energy Treated Test formulation and DMEM showed a significantly increased level of collagen in the HFF-1 cell line. The results are presented as graphical presentation of collagen levels in various groups in Figure 3. The data of ascorbic acid (10 µM) showed a significantly increased collagen content by 55%, while Biofield Energy Treated Test formulation reported a significant increase in the collagen amount. In different experimental groups, the UT-DMEM + BT-Test formulation group showed increased collagen levels by 15.70% and 13.52% at concentration of 2.5 and 0.625 µg/mL, respectively compared with the UT-DMEM + UT-Test formulation group. However, the group BT-DMEM + BT-Test formulation showed an increase in the collagen levels at 2.5, 1.25 and 0.625 µg/mL by 6.54%, 3.47%, and 4.12%, respectively compared with the UT-DMEM + UT-Test formulation group. The experimental data suggests that Biofield Energy Healing Treatment has the significant capacity to increase the collagen level at various concentrations. Hence, it can be anticipated that The Trivedi Effect® might improve the collagen level through procollagen peptides, which might affect the cross-linking among various tropocollagen molecules. An improved collagen level might be helpful in order to provide strength and structure to the skin that may be useful for skin health, strength, and structure and wound healing [41]. The present study suggests that the test herbomineral formulation and DMEM significantly improved the collagen levels due to The Trivedi effect® - Consciousness Energy Healing Treatment.

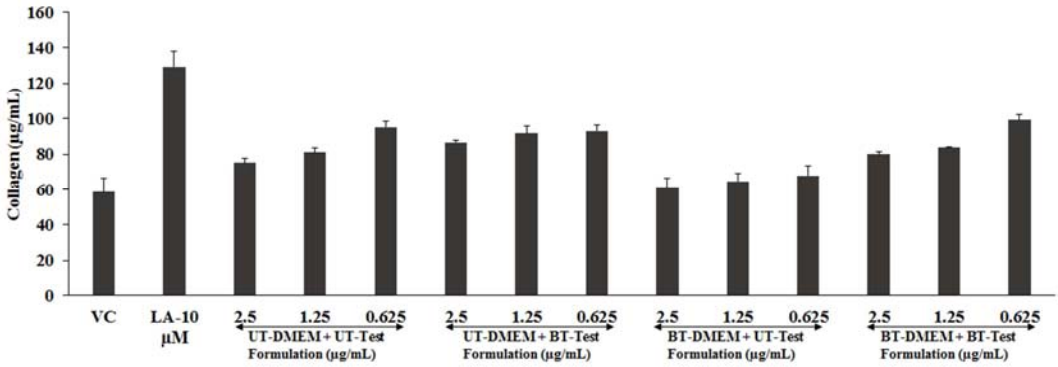

Figure 3. *Concentration-dependent effects of the test formulation on human dermal fibroblast (HFF-1) cell line for collagen level. VC: Vehicle control; LA-10: L-Ascorbic acid at 10 µM concentration; UT: Untreated; BT: Biofield Treated.*

3.3.2. Elastin Estimation

One important component of ECM is elastin. Data showed a significant increase in elastin levels in all the tested concentrations among the Biofield Energy Healing based test formulation groups. The elastin forms the tight junction with collagen fibrils that maintain cellular integrity, which helps retain the shape in body tissues and maintains very elastic tissues of the body [42]. The results of elastin level in HFF-1 cell line are shown in Figure 4. Ascorbic acid (50 µM) group data showed a significant increase in the elastin content by 55% compared with the normal control group. Besides, the UT-DMEM + BT-Test formulation group showed a significant increase in the elastin level by 33.8% at concentration 2.5 µg/mL compared with UT-DMEM + UT-Test formulation group. However, in BT-DMEM + UT-Test formulation group, the level of elastin was increased by 47.0%, 64.1%, and 11.1% at 2.5, 1.25, and 0.625 µg/mL, respectively compared with the UT-DMEM + UT-Test formulation group. However, in the BT-DMEM + BT-Test formulation group showed an increased elastin levels at 13.6%, 60.6%, and 2% at concentration 2.5, 1.25, and 0.625 µg/mL respectively, compared with the UT-DMEM + UT-Test formulation group. The experimental data suggests significant improvement in levels of elastin, which might be useful in the body and responsible for slower ageing and overall health. The increased elastin level might improve the skin elasticity and strength that activates the dermal metabolism. Thus, the Biofield Energy Healing based Test formulation and DMEM would be a better alternative to improve the elastin level, which supports cell growth, survival, differentiation, and morphogenesis.

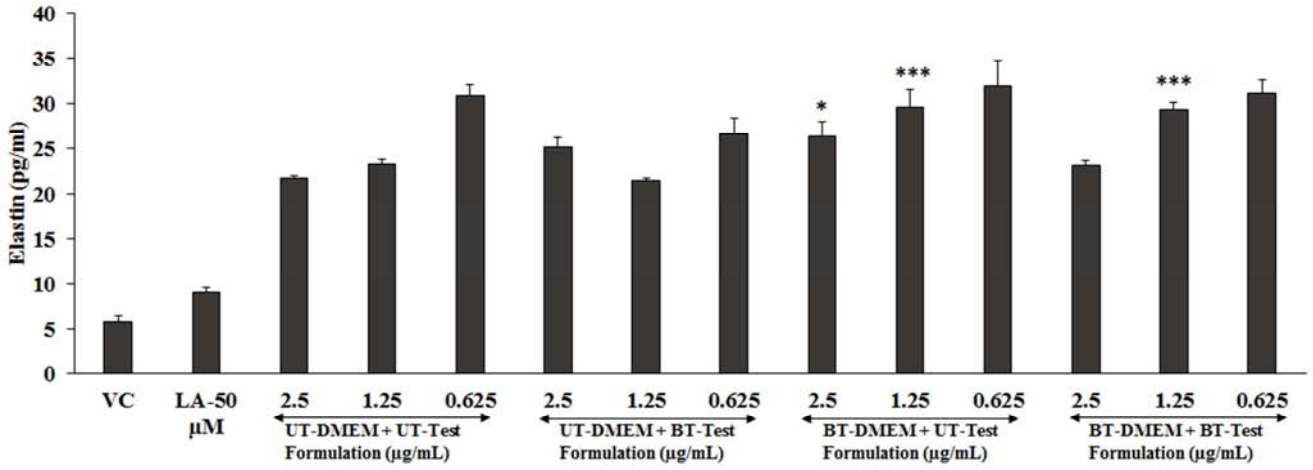

Figure 4. *Effect of the Biofield Energy Treated Test formulation on human dermal fibroblast (HFF-1) cell line for elastin level.* $^{*}p\leq0.05$ *and* $^{***}p\leq0.001$ *statistical comparison with respect to the untreated DMEM and untreated test formulation using one way ANOVA (Dunnett's test). VC: Vehicle control; LA-50: L-Ascorbic acid at 50 µM concentration; UT: Untreated; BT: Biofield Treated.*

3.3.3. Analysis of Hyaluronic Acid

The test formulation was evaluated for the level of HA, and the results are presented in Figure 5. An improved HA level regulates the ability to retain the skin moisture, secures skin moisture, creates fullness, and regulates the skin water balance. 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 showed an improved HA level in ascorbic acid group by 183.6%. However, in UT-DMEM + BT-Test formulation group, significant increases in HA levels by 4.37%, 41.47%, and 19.74% group at 2.5, 1.25, and 0.625 µg/mL respectively, with respect to the UT-DMEM + UT-Test formulation group. In addition, an 8.23% increase in HA level was reported in BT-DMEM + BT-Test formulation at 2.5 µg/mL concentration, compared with the UT-DMEM + UT-Test formulation group. Overall, the data suggests that improved level of HA after treatment with the Biofield Energy based test formulation and DMEM, which might be useful to improve the elasticity and health of the skin. HA is a natural polysaccharide and scattered all over the connective, neural, and epithelial tissue. HA based skin cosmetics are widely available in the market such as hyaluronic acid creams, serums, injectables, and hyaluronic acid supplements. Loss of skin elasticity and skin aging might be a better correlated with low HA level. It is also responsible to hold water due to its high water holding capacity [42]. Hence, the Biofield Energy Healing (The Trivedi Effect®) based test formulation and DMEM might be a new approach in cosmetology for skin health.

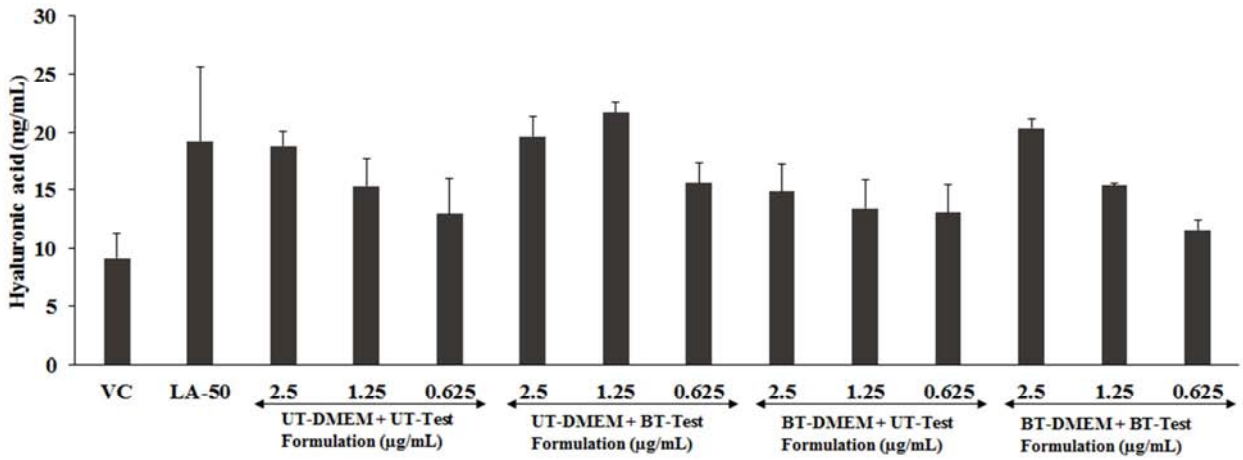

Figure 5. *The effect on synthesis of extracellular matrix component, hyaluronic acid by the Biofield Energy Treated Test formulation in human dermal fibroblasts (HFF-1) cell lines. VC: Vehicle control; LA-50: L-Ascorbic acid at 50 µM concentration; UT: Untreated; BT: Biofield Treated.*

3.4. Estimation of Melanin Synthesis Inhibition

The effect of the Biofield Energy Treated Test formulation for melanin synthesis in B16-F10 melanoma cell lines are presented in Figure 6. The cells were cultured in DMEM supplemented media containing different concentrations of the test formulation that was evaluated and compared with untreated formulation. Kojic acid (10 µM), a skin whitening compound was used as positive control and the results showed a significant decreased in the level of melanin synthesis by 65.14% compared to the melanin level in presence of alpha melanocyte stimulating hormone (α-MSH). In UT-DMEM + BT-Test formulation group, the melanin level was decreased by 8.83%, 5.78%, and 12.68% at concentrations 0.0125, 0.0625, and 0.125 µg/mL respectively, compared with the UT-DMEM + UT-Test formulation group. In addition, the level of melanin was decreased by 4.73 and 13.21% at 0.0125 and 0.125 µg/mL, respectively in the BT-DMEM + UT-Test formulation group compared with the UT-DMEM + UT-Test formulation group. However, BT-DMEM + BT-Test formulation group showed a decreased melanin synthesis by 5.04% and 4.95% at concentration 0.0125 and 0.0625 µg/mL, respectively compared with the UT-DMEM + UT- Test formulation group. Hence, the Biofield Energy Healing based Test formulation and DMEM has the significant capacity to decrease the skin melanin synthesis, which might be useful to inhibit the tyrosinase enzymes activity required for melanin synthesis [43]. In addition, the individual components of the test formulation are reported with significant antioxidant activity and effective against skin infection and inflammatory dermatoses [44, 45]. Genes, nutrition, and environmental factors are also reported to affect the skin complexion and skin pigmentation along with important component, melanin. Skin depigmentation can lead to various disorders due to initiating the process of melanogenesis on exposure of sun ultraviolet radiations (UV-A and UV-B) that lead to skin darkening [46]. As per the results, the Biofield Energy Healing based test formulation and DMEM might be best treatment approach to alter the process of melanogenesis and skin-related disorders.

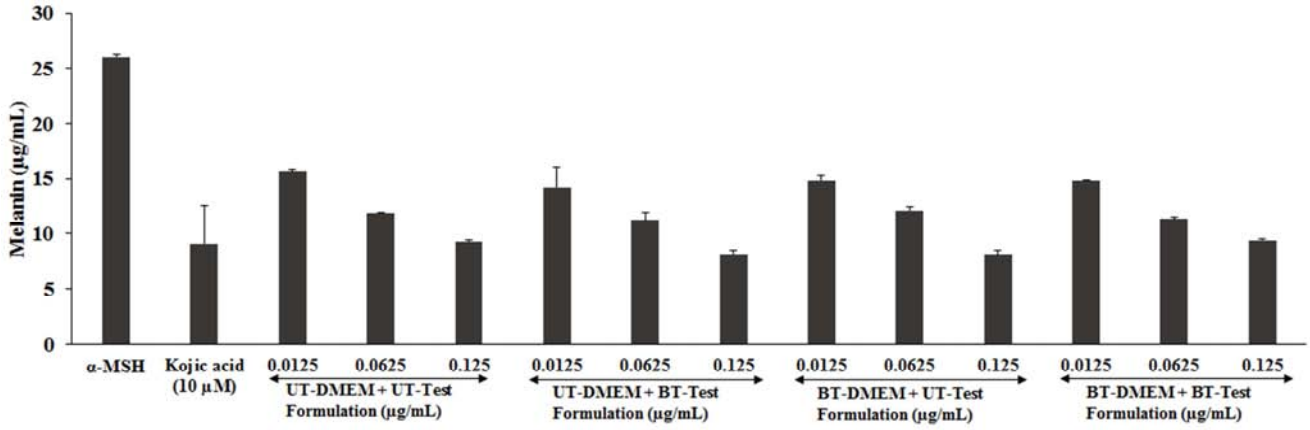

Figure 6. *Inhibitory effect of the Biofield Energy Treated test formulation on melanogenesis (skin whitening potential) in mouse melanoma (B16-F10) cell line. α-MSH: Alpha melanocyte stimulating hormone; UT: Untreated; BT: Biofield Treated.*

3.5. Anti-Wrinkling effects of Test Formulation on UVB-Induced Photoaging

Anti-wrinkling activity was evaluated in HFF-1 cells for the Biofield Treated test formulation with DMEM and the results in terms of cell viability are presented in Figure 7 after exposure of UV-B rays. 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 monitored. The data suggests a high degree of cell death *i.e.* 25.21% of cell viability after exposure of UV-B in HFF-1 cells. The cell viability in vehicle control group was found as 27.78% due to UV-B irradiation (200 mJ/cm^2). However, ascorbic acid (50 µM) showed a significant increase in the cell viability by 55.39%. The experimental groups at concentration 2.5 µg/mL indicated that cell viability was increased by 4.32% and 2.47% in the UT-DMEM + BT-Test formulation and BT-DMEM + UT-Test formulation groups, respectively compared with the UT-DMEM + UT-Test formulation group. Similarly, in the UT-DMEM + BT-Test formulation group at 1.25 µg/mL, showed an increased cell viability by 3.17% 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 6.77% compared with the UT-DMEM + UT-Test formulation group. The experimental results showed an improved cell viability in the presence of the Biofield Treated Test formulation after exposure with UV-B suggesting better cell protection and less skin damage from sun rays exposure. Most skin diseases reported are due to UV-B radiations like skin disorders, free radical generation, stress to skin, 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 [47]. Overall, the Biofield Energy Healing based Test formulation can be used to improve cell viability with significant anti-wrinkling action against UV-B radiations.

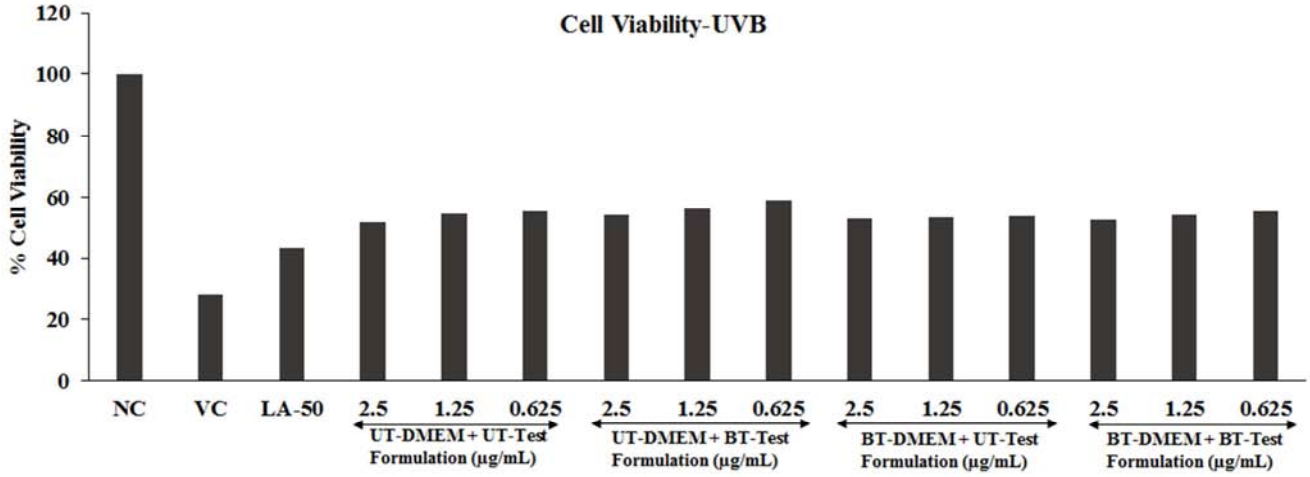

Figure 7. *Anti-wrinkling potential and cytoprotective potential of the Biofield Energy Treated Test formulation against UV-B induced stress in human dermal fibroblasts (HFF-1) cell lines. % cell viability of HFF-1 cells after treatment in various groups. NC: Normal control; VC: Vehicle control; LA-50: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

3.6. Wound-Healing Scratch Assay

The Biofield Energy Treated Test formulation was evaluated for wound healing activity using scratch assay in HFF-1 and HaCaT cells, and the results are presented in Figure 8. All the data of cell migration and percentage protection was evaluated and compared with EGF and vehicle control. Wound healing scratch assay suggests cell-to-cell and cell-to-matrix interactions during wound healing process [48]. The experimental results suggests that at 5 and 10 µg/mL concentrations of the test formulation, significant healing effects in HFF-1 and HaCaT cells were reported. However, in HFF-1 cells results showed 1% to 6% increase in the percentage cell coverage area, while an altered cellular area was also reported in HaCaT after treatment with the Biofield Energy Treated Test formulation compared with the untreated test formulation. The representative images of the wound healing scratch assay in all the experimental groups of the Biofield Energy Treated/untreated test formulation showed a significant rate of cellular migration with wound closure, as shown in Figure 8 (a-f). Overall, it can be concluded that The Trivedi Effect® has the capacity to improve cellular migration significantly impacting wound healing.

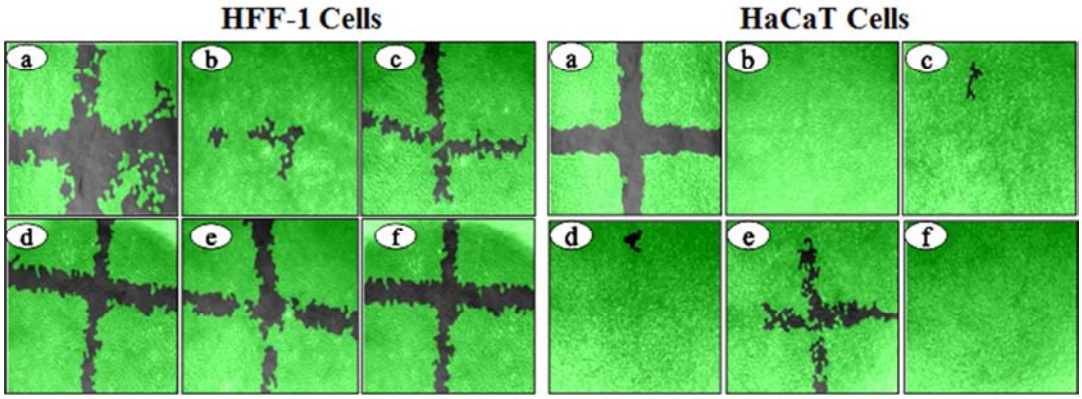

Figure 8. *Representative images of HFF-1 and HaCaT cell migration cells after induction of a scratch. All the pictures were taken immediately after the scratch was induced (i.e. at 0 hours), after 16 hours in the presence of EGF and Biofield Energy Treated Test formulation. Pictures are taken at 10 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

In the present study, cell viability was significantly improved with viability in more than 75% cells in all the tested concentrations of the test formulation such as HFF-1 (1.25 to 10 µg/mL), HaCaT (0.625 to 5 µg/mL), and B16-F10 (10 to 40 µg/mL) using MTT assay. BrdU assay showed increased cellular proliferation by 343.23%, 316.13%, and 512.75% at 17.5 µg/mL in the UT-DMEM + BT-Test formulation, BT-DMEM + UT-Test formulation, and BT-DMEM + BT-Test formulation groups, respectively. An increased cellular proliferation by 287.1%, 264.4%, and 428.9% was found in the UT-DMEM + BT-Test formulation, BT-DMEM + UT-Test formulation, and BT-DMEM + BT-Test formulation groups, respectively at 17.5 µg/mL compared to the UT-DMEM + UT-Test formulation group. Collagen level was increased by 15.70% and 13.52% at 2.5 and 0.625 µg/mL respectively, in the UT-DMEM + BT- Test formulation group, with respect to the UT-DMEM + UT-Test formulation group. The level of elastin was significantly increased by 47.0%, 64.1%, and 11.1% in the BT-DMEM + UT- Test formulation group at 2.5, 1.25, and 0.625 µg/mL, respectively compared to the UT-DMEM + UT-Test formulation group. The level of HA was significantly increased by 4.37%, 41.47%, and 19.74% in the UT-DMEM + BT-Test formulation group, at concentration 2.5, 1.25, and 0.625 µg/mL, respectively with respect to the UT-DMEM + UT-Test formulation group. Melanin levels were reduced by 8.83%, 5.78%, and 12.68% at 0.0125, 0.0625, and 0.125 µg/mL, respectively in the UT-DMEM + BT-Test formulation groups, while melanin was also decreased by 4.73% and 13.21% at 0.0125 and 0.125 µg/mL respectively, in the BT-DMEM + UT-Test formulation group compared with the UT-DMEM + UT-Test formulation group. Anti-wrinkling potential with respect to UV-B showed an increased cell viability in the UT-DMEM + BT-Test formulation and BT-DMEM + UT-Test formulation groups compared to the UT-DMEM + UT-Test formulation group. Wound healing scratch assay showed a significant migration of fibroblast and keratinocyte cells with an increased percentage of covered area up to 6% in HFF-1 cells, while significant change was also reported in HaCaT cells after treatment with Biofield Energy (The Trivedi Effect®) based test formulation. Overall, the Biofield Energy Treated Test formulation and DMEM can be used significantly in different pharmacological actions such as anti-wrinkling, anti-aging, skin whitening, and wound healing agent.

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 Treatment can also be used in the prevention of temporary and permanent skin disorders, anti-aging, improved overall health, and quality of life.

Abbreviations

HFF-1: Human foreskin fibroblast cell line, B16-F10: Mouse melanoma cell line; HaCaT: Human keratinocytes cells; DMEM: Dulbecco's Modified Eagle's Medium; ECM: Extracellular matrix, EGF: Epidermal growth factor, UV-B: Ultra violet B rays.

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