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Research Article

Effects of The Biofield Energy Healing Treated Novel Test Formulation for Skin Health and Aging

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Abstract

Skin reflects the correlation between the overall inner-health and aging status, which is the complex biological process influenced by the various factors. The present work evaluated the impact of Biofield Energy (The Trivedi Effect®-Consciousness Energy Healing) Treatment on the test formulation and cell medium (DMEM) for various skin health parameters using HFF-1, HaCaT, and B16-F10 cells. The test formulation was consisted of seven ingredients *viz.* zinc chloride, ferrous sulfate, copper chloride, magnesium gluconate, pyridoxine HCl, vitamin B_{12}, and vitamin D_3. The test formulation and DMEM media were divided into two parts. One part received the Biofield Energy Treatment by a renowned Biofield Energy Healer, Alice Branton and defined as the Biofield Treated (BT) sample, while other was denoted as the Untreated (UT) samples. MTT assay using test formulation showed greater than 76% cell viability, suggested safe and nontoxic profile at tested concentrations. Cell proliferation data using BrdU method showed an improved cell proliferation by 127.92% and 121.20% at 50 µg/mL in the BT-DMEM + UT-Test formulation and BT-DMEM + BT-Test formulation groups, respectively compared with the untreated group. The collagen level was significantly increased by 13.08%, and 15.98% at 50 and 100 µg/mL, respectively in the UT-DMEM + BT-Test formulation group compared with the untreated group.

The level of elastin was increased by 28.09% at 50 µg/mL in the UT-DMEM + BT-Test formulation group, compared with the untreated group. Hyaluronic acid level was significantly ($p \leq 0.001$) increased by 86.10% and 35.14% at 10 and 50 µg/mL, respectively in the BT-DMEM + BT-Test formulation group as compared with the untreated group. Moreover, melanin synthesis was significantly inhibited by 16.96% at 10 µg/mL in the UT-DMEM + BT-Test formulation group compared with the untreated group. Anti-wrinkling effect exhibited improved cell viability by 65.63% and 69.95% at 1 and 10 µg/mL, respectively in the BT-DMEM + BT-Test formulation group compared with the untreated group. *In vitro* wound healing activity using scratch assay significantly improved healing rate and cell migration upto 58.55% in the HFF-1 cells in the UT-DMEM + BT-Test formulation group, while upto 231.25% in HaCaT cells lines in UT-DMEM + BT-Test formulation group compared with the untreated group. In conclusion, The Trivedi Effect® based test formulation and DMEM have the significant capacity to improve overall skin health, reversal of aging process 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, Minerals, Scratch assay, Vitamins

Abbreviations

B16-F10	:	Mouse melanoma cell line
CAM	:	Complementary and alternative medicine
DMEM	:	Dulbecco's Modified Eagle's Medium
ECM	:	Extracellular matrix
EGF	:	Epidermal growth factor

HA : Hyaluronic acid

HFF-1 : Human foreskin fibroblast cell line

HaCaT : Human keratinocytes cells

NCCAM : National Center for Complementary and Alternative Medicine

UV-B : Ultra violet B rays

Introduction

Skin is the largest organ in human plays a vital role in protection against chemical, mechanical damages, micro-organisms, and damage by ultraviolet rays, which though to be the most harmful in environment [1]. Various reports suggested that on exposure to UV-B ray's results in significant level of oxidative stress, inflammation, erythema, breakdown of the extracellular matrix (i.e. elastin, collagen and hyaluronic acid), wrinkling, and skin cancer [2,3]. Skin acts as the natural barrier between the internal and external environments. Nowadays, the role and correlation between the nutrition and skin condition along with the nutraceuticals effects on skin health and aging has been an interesting research field for pharmaceutical industries in order to develop some unique skin health formulation. Nutrition has a critical role for strengthening the skin capabilities to fight against various external and internal factor. Different alternative medicines are present worldwide that improve the skin functioning with strong anti-oxidative action and anti-photo aging and also effective against photo damage of the skin [4].

Cosmetic products are significantly used and accepted by the peoples around the world, but serious health complications have been reported compared with the combination of minerals and vitamins such as zinc chloride, ferrous sulfate, copper chloride, magnesium gluconate, pyridoxine HCl, vitamin B12, and vitamin D3 [5-9]. With this respect, novel test formulation was prepared by the author, which consist of minerals viz. zinc chloride, ferrous sulfate, copper chloride, magnesium gluconate and vitamins such as pyridoxine HCl, vitamin B12, and vitamin D3. Further, the novel test formulation was treated with Biofield Energy Healing by a renowned Biofield Energy Healer, Alice Branton. The Biofield Energy Treated test formulation and the cell line media (DMEM) was tested against skin health cell lines such as HFF-1, HaCaT, and B16-F10 cells. Biofield Energy Healing acts as a unifying concept that comes under Complementary and Alternative Medicine (CAM) therapies and works as a bridge with the alternative and contemporary Energy Medicine models [10,11]. Biofield Energy Healing therapies by renowned Biofield Energy Healers have been practiced worldwide and reported with significant clinical benefits along with wide range of therapeutic action.

The use of various forms of Energy Therapies has been practiced and 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 [12]. The Biofield Energy Healing Treatment has been reported with significant outcomes in clinical studies and scientific research in various fields. Biofield Energy Healing (The Trivedi Effect®-Consciousness Energy Healing) has been widely practiced, recommended, accepted, and reported worldwide in nonliving materials and living organisms. The significant outcomes of The Trivedi Effect® have been found in different branches such as in microbiology [13-15], agriculture science [16-18], livestock [19], materials science [20-22], nutraceuticals [23,24], and skin health [25-27]. Due to the continued noteworthy outcomes and applications of Biofield Energy Healing Treatments, the 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.

Materials and Methods

Chemicals and Reagents

Zinc chloride, magnesium (II) gluconate hydrate, cyanocobalamin (vitamin B12), and pyridoxine hydrochloride (vitamin B6) were purchased from TCI, Japan. Iron (II) sulphate, copper chloride, kojic acid, L-ascorbic acid, and cholecalciferol (vitamin D3) were purchased from Sigma-Aldrich, USA. ELISA kits were procured from CUSABIO and CusAb Co. Pvt. Ltd., USA. Fetal Bovine Serum (FBS), Epidermal Growth Factor (EGF) and Dulbecco's Modified Eagle's Medium (DMEM) were purchased from Gibco, ThermoFisher, USA. Antibiotics solution (penicillin-streptomycin) was procured from HiMedia, India, while 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium) (MTT), Direct Red 80 and Ethylenediaminetetraacetic Acid (EDTA) were purchased from Sigma, USA. All the other chemicals used in this experiment were analytical grade procured from India.

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, 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 50 µM used in non-cytotoxic dose concentration in MTT assay [25].

Experimental Design

The experimental groups were consisted of cells in normal control group, vehicle control group (0.05% DMSO), positive control group 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 [26].

Energy of Consciousness Treatment Strategies

The test formulation was a combination of seven ingredients *viz.* zinc chloride, ferrous sulfate, copper chloride, magnesium gluconate, pyridoxine HCl, vitamin B_{12}, and vitamin D_3. The test formulation and DMEM were divided into two parts. One part of the test samples was treated with Biofield Energy by a renowned Biofield Energy Healer, Alice Branton and defined as the Biofield Energy Treated test 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 Healing Treatment by Alice Branton has been performed remotely from USA, while the test samples were located in 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.

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 tests formulation/DMEM at a range of concentrations (1 to 200 µg/mL) and ascorbic acid (50 and 100 µM) followed by incubation from 24 to 72 hours in a 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].

Effect of Biofield Energy Treated 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 a hemocytometer and plated in 96-well plates at the density corresponding to 1 X 10^3 to 5 X 10^3 cells/well in DMEM supplemented with 15% FBS. The cells 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 [27].

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 a hemocytometer and plated in 48-well plates at the density corresponding to 10 X 10^3 cells/well in DMEM supplemented with 15 % FBS. The cells were 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). 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].

Estimation of Melanin Synthesis-Skin Depigmentation Effect

B16-F10 cells were used for melanin synthesis estimation. Cells were counted using a 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 test formulation at 1 to 200 µg/mL 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].

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 a hemocytometer and plated in 96-well plates at the density corresponding to 5 X 10^3 to 10 X 10^3 cells/well in DMEM supplemented with 15% FBS cells/plates, which were incubated overnight under growth conditions to allow cell recovery and exponential growth. The cells were treated with non-cytotoxic concentrations of 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 equation 1:

% Cell viability = (X*100)/R---------- (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.

Wound Healing by Scratch Assay

HFF-1 and HaCaT cell lines were counted using a 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 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 24 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].

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 $p \leq 0.05$.

Results and Discussions

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 different positive controls *viz.* ascorbic acid (50 µM), kojic acid (10 mM), and EGF (50 ng/mL) in respective cell lines for the estimation of percentage cell viability. The results showed that all the concentrations were found safe and non-toxic with more than 76% in all the cell lines up to maximum at 200 µg/mL. The test formulation concentrations (1 to 200 µg/mL) were used for the evaluation of other skin health parameters such as 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. The selected concentrations were used for the estimation of ECM synthesis in HFF-1 cells. The cell viability in HaCaT, HFF-1, and B16F10 cells revealed that the tested concentrations exhibited more than 84%, 85%, and 76% cell viability, respectively.

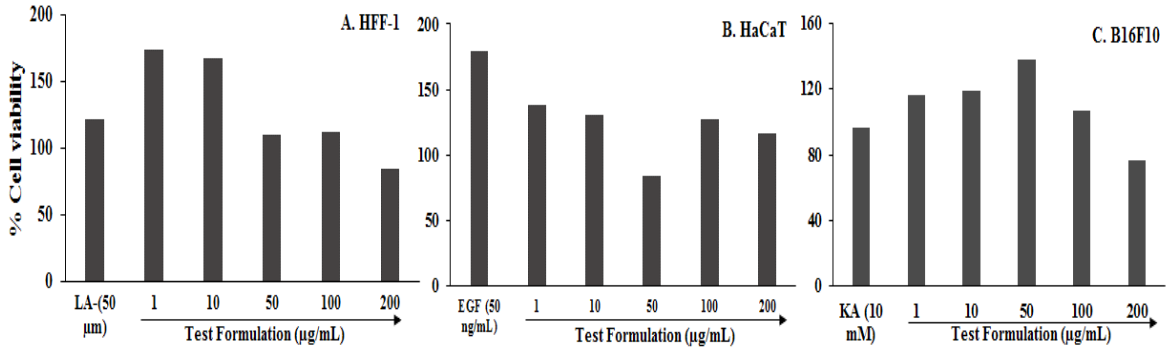

Figure 1: Effect of the test formulation on cell viability in different cells at various concentrations. A. HFF-1 cells after 72 hours of treatment. B. HaCaT cells after 24 hours of treatment. C. B16-F10 cells after 24 hours of treatment. LA: L-Ascorbic acid; EGF: Epidermal growth factor; KA: Kojic acid.

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 bromodeoxyuridine (BrdU) method at different test concentrations in HFF-1 cells after 48 hours of incubation. The results of BrdU analysis are represented in (Figure 2). FBS at concentration of 0.5% showed a percentage cell proliferation rate 289%, while the cellular proliferation % in vehicle control group was 41%. The Biofield Energy Treated groups, BT-DMEM + UT-Test formulation and BT-DMEM + BT-Test formulation showed a significantly increased cellular proliferation at 50 µg/mL by 127.92% and 121.20%, respectively compared with the UT-DMEM + UT-Test formulation group. However, BT-DMEM + UT-Test formulation and BT-DMEM + BT-Test formulation showed a significant increased cellular proliferation at 100 µg/mL by 18.46% and 21.34%, respectively compared with the UT-DMEM + UT-Test formulation group. Rest of the tested groups showed significant alteration of % cell proliferation compared with the untreated group.

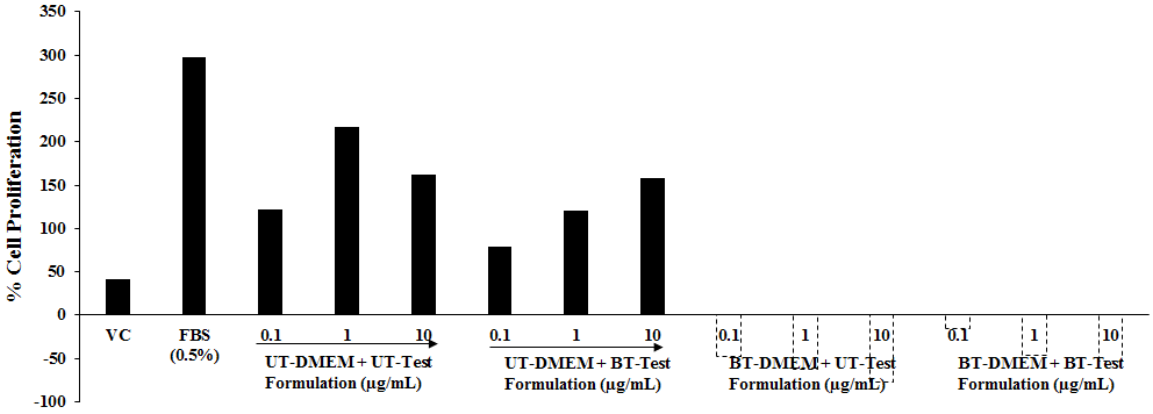

Figure 2: Effect of the test formulation on cellular proliferation after 48 hours using BrdU assay. VC: Vehicle control; FBS: Fetal bovine serum (µg/mL); UT: Untreated; BT: Biofield Treated.

Overall, the results suggest that the Biofield Energy Treated Test formulation and DMEM showed a significant increase in the cellular proliferation rate in HFF-1 cells at 100 µg/mL test formulation concentration using BrdU assay. The improved cellular proliferation in HFF-1 cells suggest that The Trivedi Effect®-Energy of Consciousness Healing Treatment has the significant capacity, which can be utilized in cellular homoeostasis and maintenance of an organism. The Biofield Energy Treated test formulation showed significant cell proliferation response compared to the untreated group, due to the Biofield Energy Healing Treatment. However, BrdU assay has been recognized method in order to examine the rate of DNA replication, metabolic activity and recognitions of cell surface antigen activity [33]. This suggest that Consciousness Energy Treated Energy Treated Test formulation and DMEM would significantly improve the skin metabolic activity results in improved skin health.

Analysis of Extracellular Matrix Component Synthesis

Collagen Estimation: The effect of the Biofield Energy Treated Test formulation/DMEM showed a significantly increased level of collagen in HFF-1 cell line. The effect of positive control, ascorbic acid and other tested groups such as Biofield Energy Treated Test formulation/DMEM groups are presented in (Figure 3). Ascorbic acid (10 µM) showed a significantly increased collagen content by 37.4%, 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 10, 50, and 100 µg/mL by 2.41%, 13.08%, and 15.98%, respectively compared to the UT-DMEM + UT-Test formulation group. Similarly, in the BT-DMEM + BT-Test formulation group at 50 and 100 µg/mL showed an increased collagen level by 2.94% and 5.55%, respectively compared with the UT-DMEM + UT-Test formulation group. Therefore, the experimental data suggests that the Biofield Energy Healing Treatment has significant ability to increase the collagen level at all the tested concentrations. Collagen, one of the most abundant proteins for skin health, structure and fibrous protein which present in ECM. 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 provides strength and structure to the skin that might be beneficial for skin health, strength, and wound healing [34,35].

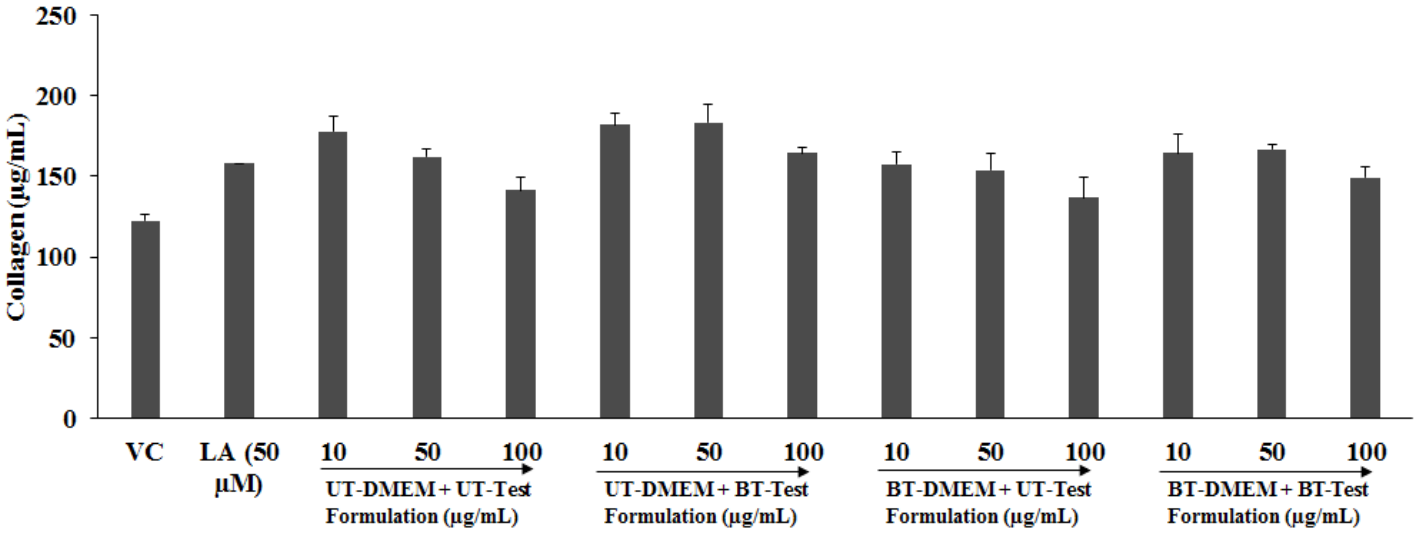

Figure 3: Effect of the test formulation and DMEM on the expression of collagen in human foreskin fibroblast cells (HFF-1). VC: Vehicle control; LA: L-Ascorbic acid; UT: Untreated; BT: Biofield Treated.

Elastin Estimation: The results of elastin level in HFF-1 cell line due to the Biofield Energy Healing Based Test formulation and DMEM are shown in (Figure 4). Ascorbic acid (50 µM) group showed significantly increased elastin content by 76.8% compared with the normal control group. Moreover, UT-DMEM + BT-Test formulation group showed a significant increase in the elastin level by 28.90% at concentration 50 µg/mL compared with UT-DMEM + UT-Test formulation group. The altered elastin level (one of the important constituents of the ECM) was evaluated after The Trivedi Effect®-Energy of Consciousness Healing Treatment by Alice Branton (a renowned Biofield Energy Healer) in Biofield Energy Healing based test formulation and DMEM. Elastin forms the tight junctions with the collagen fibrils, which maintains the cellular integrity and helps to retain shape in body tissue and very elastic tissue of the body [36]. Fibroblast and elastin are the major component which play an important role for ageing and health. The experimental result suggests a significantly increased elastin level, which suggest that The Trivedi Effect® has the significant capacity to improve the skin elasticity and strength that activates the dermal metabolism. Thus, Biofield Energy Healing based test formulation and DMEM can be significantly used to improve the elastin level that might improve the cell growth, survival, differentiation, and morphogenesis.

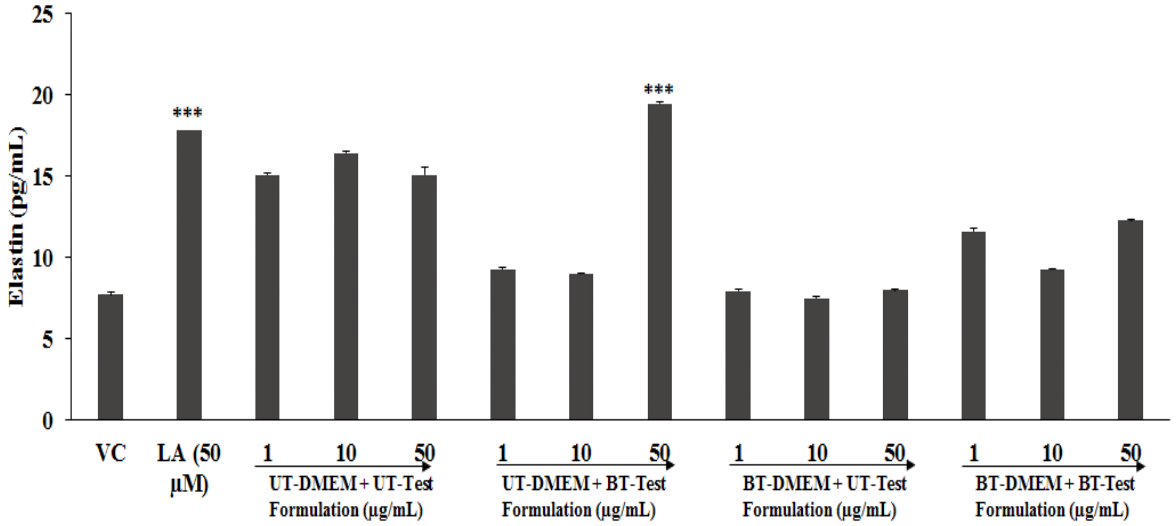

Figure 4: Effect of the test formulation and DMEM on the elastin level in human foreskin fibroblast cells (HFF-1). VC: Vehicle control; LA: L-Ascorbic acid; UT: Untreated; BT: Biofield Treated. $^{***}p \leq 0.001$ statistical comparison with respect to untreated DMEM and untreated Test formulation using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

Analysis of hyaluronic acid: 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 ascorbic acid group showed an increase in the hyaluronic acid content by 29.07%. 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 and untreated groups. The HA levels in UT-DMEM + BT-Test formulation group showed a significant increase level by 20.18% at concentration 1 µg/mL compared with UT-DMEM + UT-Test formulation group. In addition, BT-DMEM + UT-Test formulation group showed an increase in HA level by 29.61%, and 12.41% at concentration 1 and 10 µg/mL compared with UT-DMEM + UT-Test formulation group. However, BT-DMEM + BT-Test formulation group showed a significant ($p \leq 0.001$) increase in HA level by 6.59%, 86.10%, and 35.14% at concentration 1, 10, and 50 µg/mL compared with UT-DMEM + UT-Test formulation group. Hence, the Biofield Energy Healing (The Trivedi Effect®) based test formulation and DMEM might be a new approach in cosmetology that helps to retain more skin moisture, creates fullness, and regulates the skin water balance.

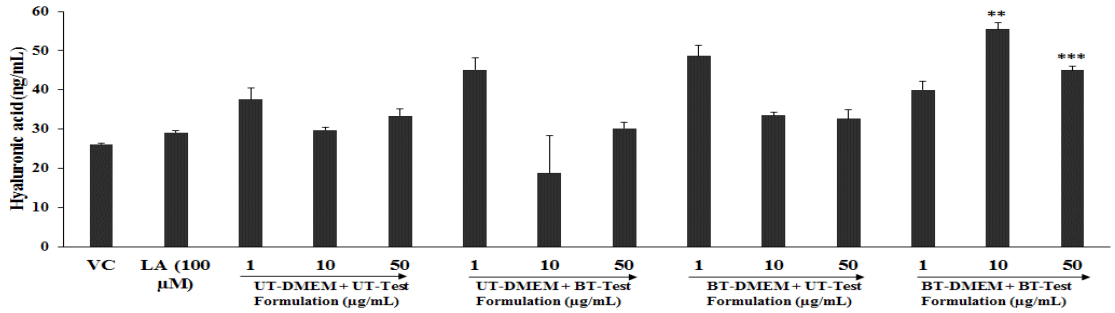

Figure 5: Synthesis of extracellular matrix component, hyaluronic acid by Biofield Energy Treated Test formulation in human dermal fibroblasts (HFF-1) cell lines. VC: Vehicle control; LA-100: L Ascorbic acid at 100 µM concentration; UT: Untreated; BT: Biofield Treated. $^{***}p \leq 0.001$ and $^{**}p \leq 0.01$ statistical comparison with respect to untreated DMEM and untreated Test formulation using one-way ANOVA (Dunnett's test).

Biofield Energy Treatment has significantly improved the HA level, which is a natural polysaccharide present all over the connective, neural, and epithelial tissue. For skin health improvement, most of the skin care products used HA as the base to improve skin health and aging. Some common marketed HA based skin products are hyaluronic acid creams, serums, injectable, and hyaluronic acid supplements, mainly used to improve HA level. Literature data suggested that low level of HA in skin might lead to reduced skin elasticity and fasten the aging process. HA based skin car product, due to their high water holding capacity are widely available in market for skin health [37]. The Trivedi Effect®-Energy of Consciousness Healing based test formulation would be

the best alternative in skin care products in order to improve the overall skin health.

Effect of the Test Formulation on Skin Depigmentation: The effect of the test formulation on alpha-MSH stimulated melanin synthesis in B16-F10 cells is shown in (Figure 6). The level of melanin in the alpha-MSH group was significantly increased to 14.08 ± 0.08 µg/mL, while it was 3.50 ± 0.02 µg/mL in normal control group. Melanin was significantly ($p \leq 0.001$) reduced by 74.57% in the kojic acid (KA) group at 10 mM. The cellular content of melanin in the UT-DMEM + BT-Test formulation group was significantly reduced by 16.96% at 10 µg/mL compared to the UT-DMEM + UT-Test formulation group. In addition, the melanin content in the BT-DMEM + BT-Test formulation group was significantly reduced by 11.61% at 10 µg/mL, respectively compared to the UT-DMEM + UT-Test formulation group. The rest of the treatment groups showed an alteration of melanin concentration with respect to the UT-DMEM + UT-Test formulation group. 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 [38]. Thus, it can be concluded that the Biofield Energy Treated test formulation significantly inhibited the content of melanin in the B16-F10 cells. This suggested that The Trivedi Effect® based test formulation and DMEM might be beneficial for the development of cosmeceutical products for hyperpigmentation and different types of skin conditions.

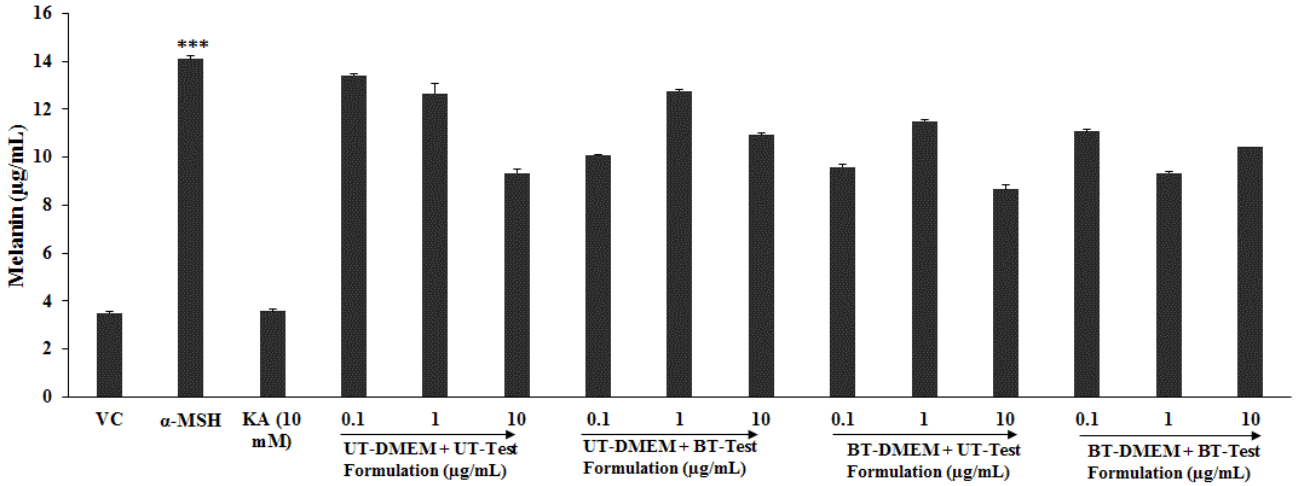

Figure 6: Effect of the test formulation and DMEM on alpha-MSH stimulated melanin synthesis in B16-F10 cells. VC: Vehicle control; KA: Kojic acid (10 mM); UT: Untreated; BT: Biofield Treated, α-MSH: Alpha-melanocyte-stimulating hormone. $^{***}p \leq 0.001$ vs VC using one-way ANOVA (post-hoc Dunnett's test).

Anti-wrinkling Effects of Test Formulation on UVB-Induced Photo aging: The effect of Biofield Energy Treated Test formulation and DMEM with respect to anti-wrinkling effect were evaluated for and results are presented in (Figure 7) in terms of HFF-1 cells 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 reported in all the groups. The HFF-1 cells, while exposure to UV-B showed high degree of cell death, showed 24.40% of cell viability. The cell viability in vehicle control group was found as 26.10% due to UV-B irradiation (200 mJ/cm^2). However, ascorbic acid (50 µM) showed a significant increase in the cell viability 40.40% as compared with the baseline control group. Besides, the experimental groups showed that all the groups in tested concentrations reported with improved cell viability. The percentage cell viability in UT-DMEM + BT-Test formulation group at 1, 10, and 50 µg/mL was increased by 33.66%, 19.87%, and 18.46%, respectively. In UT-DMEM + BT-Test formulation group at 1 and 10 µg/mL, cell viability was significantly increased by 16.34% and 15.02%, respectively. Similarly, in BT-DMEM + BT-Test formulation group at concentration 1 and 10 µg/mL, cell viability was significantly increased by 65.63% and 69.95%, respectively as compared with the untreated group. Overall, the experimental results exhibited improved cell viability after exposure to the Biofield Treated Test formulation and DMEM, which suggests the application of The Trivedi Effect®-Energy of Consciousness Healing Treatment in cell protection and less skin damage.

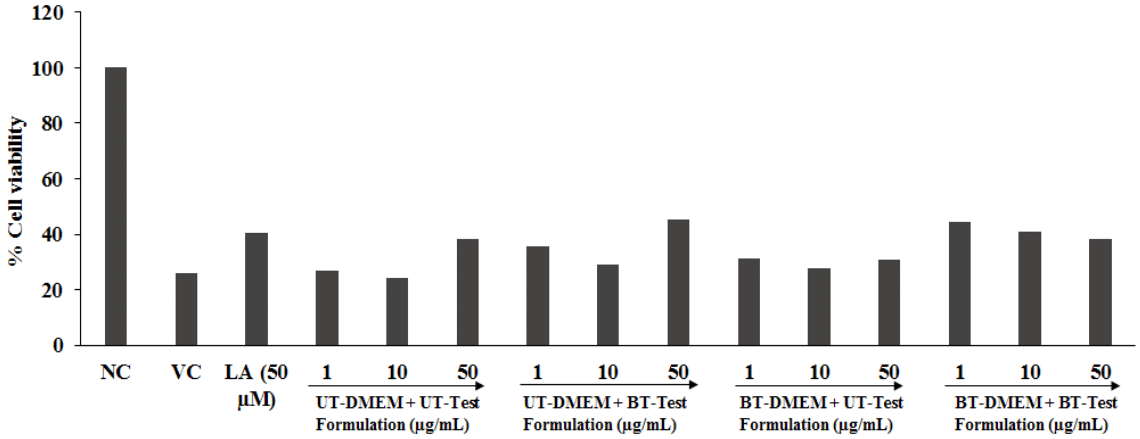

Figure 7: Anti-wrinkling potential and cytoprotective potential of Biofield Energy Treated Test formulation against UV-B induced stress in human dermal fibroblasts (HFF-1) cell lines. % cell viability of HFF-1 cells after treatment in various groups. NC: Normal control; VC: Vehicle control; LA-50: L-Ascorbic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

Further, The Trivedi Effect® based test formulation can be utilized in various skin diseases caused due to high exposure of UV-B radiations, which leads to stress, skin disorders, free radical generation, etc. UV-B radiations exposure and cellular death results in breakdown of skin fibroblasts *via.* different inflammatory responses such as DNA damage, wrinkles, and skin-ageing [39]. Hence, the Biofield Energy Healing based test formulation and DMEM would be used to improve the cell viability, anti-wrinkling action and results in skin protection against UV-B radiations. However, UV-B radiations has been reported to have several skin disorders such as stress, skin disorders, free radical generation, etc. It progresses the disease through various inflammatory pathways such as DNA damage, wrinkles and skin-ageing [39]. Thus, Biofield Energy Healing based test formulation and DMEM would be used full method in order to protect the skin damage and could help to stop the inflammations caused by UV-B rays by improving the cell viability, anti-wrinkling action and results in skin protection against UV-B radiations.

Wound-Healing Scratch Assay: *In vitro* wound healing activity using scratch assay was performed in HFF-1 and HaCaT cells, and the results of The Trivedi Effect®-Energy of Consciousness Healing Treatment based test formulation and DMEM showed a significant cellular migration. Some pictorial representations among various experimental groups are presented in (Figure 8,9). The experimental data was evaluated and compared with respect to positive control group, EGF (50 and 100 ng/mL), vehicle control, and Biofield Energy Treated test formulation (1, 10, 50, and 100 µg/mL) in combinations with DMEM.

The data suggest that EGF group at 50 and 100 ng/mL showed significant percentage migration compared with the baseline by 69.9% and 74.2%, respectively. HFF-1 cells showed significant increased cellular migration in UT-DMEM + BT-Test formulation group by 1.44%, 13.85%, 0.25%, 13.48%, and 58.55% at concentration 1, 10, 50, 100, and 200 µg/mL, respectively compared with the untreated group. Rest other groups showed significant change at all the concentrations. The results of HFF-1 cells along with vehicle control, EGF, and tested groups presentations are shown in (Figure 8).

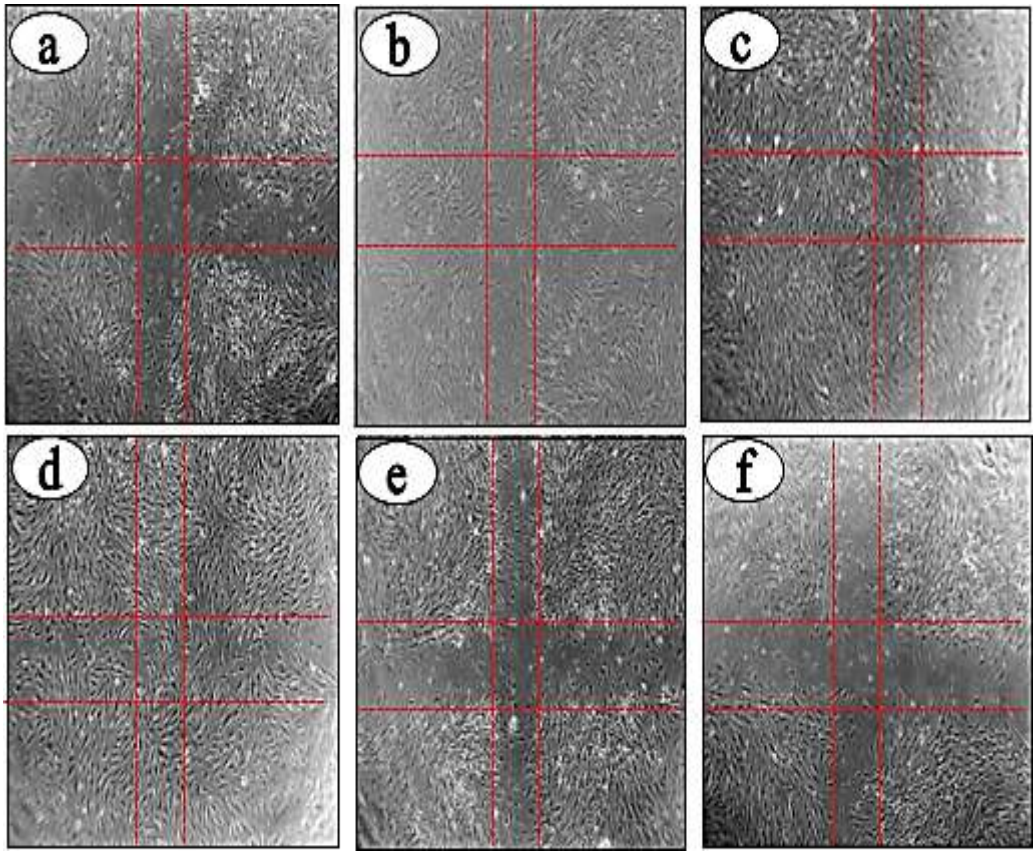

Figure 8: Representative images of HFF-1 cell migration after induction of a scratch. All the pictures were taken after the scratch was induced (i.e. at 24 hours) in the presence of EGF and Biofield Energy Treated test formulation. Pictures are taken at 10 times magnification. (a) baseline control media, (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.

Similarly, HaCaT cells showed significant improved percentage migration in all the groups such as UT-DMEM + BT-Test formulation group showed 16.67%, 100.00%, and 40.74% at 10, 50, and 100 µg/mL, respectively compared with the untreated group. In BT-DMEM + UT-Test formulation group showed 72.73%, 50%, 231.25%, and 7.41% at 1, 10, 50, and 100 µg/mL, respectively compared with the untreated group. In addition, BT-DMEM + BT-Test formulation group showed 203.03%, 195.83%, 206.25%, and 114.81% at 1, 10, 50, and 100 µg/mL, respectively compared with the untreated group. The results of HaCaT cells along with vehicle control, EGF, and tested groups are presented in (Figure 9). The results after treatment of wound healing scratch assay in Biofield Energy Treated/untreated test formulation in DMEM showed a significant rate of cellular migration rate along with wound closure compared with the UT-Test formulation + UT-DMEM group. Scratch assay reflects the cell-to-cell and cell-to-matrix interactions during wound healing process [40]. Overall, it can be concluded that The Trivedi Effect®-Energy of Consciousness Healing Treatment has the significant capacity to improved cellular migration that has significant impact in wound healing.

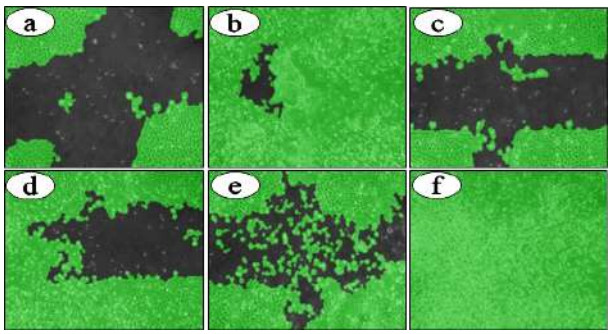

Figure 9: Representative images of HaCaT cell migration after induction of a scratch. All the pictures were taken after the scratch was induced (i.e. at 24 hours) in the presence of EGF and Biofield Energy Treated test formulation. Pictures are taken at 10 times magnification. (a) Baseline control media, (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.

Conclusions

The experimental data of skin health study of The Trivedi Effect®-Consciousness Energy Healing based test formulation and DMEM data suggested significant increase in cell viability (using MTT assay) with 84%, 85%, and 76% in HaCaT, HFF-1, and B16F10 cells all the tested concentrations, indicating that the test formulation was found safe and nontoxic. Besides, BrdU assay showed 127.92% and 121.20% increase cellular proliferation (at 50 µg/mL) in the BT-DMEM + UT-Test formulation and BT-DMEM + BT-Test formulation groups, respectively while 18.46% and 21.34% increase in cellular proliferation in the BT-DMEM + UT-Test formulation and BT-DMEM + BT-Test formulation groups, respectively at 100 µg/mL compared to the UT-DMEM + UT-Test formulation group. The level of collagen was increased after The Trivedi Effect®-Consciousness Energy Healing Treatment by 2.41%, 13.08%, and 15.98% at 10, 50, and 100 µg/mL, respectively, in the UT-DMEM + BT-Test formulation group, while 2.94% and 5.55% increase collagen level at 50 and 100 µg/mL, respectively, in the BT-DMEM + BT-Test formulation group with respect to the UT-DMEM + UT-Test formulation group. However, the level of elastin was significantly increased by 28.90% at concentration 50 µg/mL in the UT-DMEM + BT-Test formulation compared to the UT-DMEM + UT-Test formulation group.

In addition, the level of HA in HFF-1 cells showed 20.18% at concentration 1 µg/mL in UT-DMEM + BT-Test formulation group, while 29.61%, and 12.41% at concentration 1 and 10 µg/mL, respectively in the BT-DMEM + UT-Test formulation group compared with the untreated group. Further, the level of HA has been significantly ($p \leq 0.001$) improved in the BT-DMEM + BT-Test formulation group by 6.59%, 86.10%, and 35.14% at concentration 1, 10, and 50 µg/mL, respectively compared with the UT-DMEM + UT-Test formulation group. The melanin level was significantly reduced by 16.96% at 10 µg/mL in the UT-DMEM + BT-Test formulation group, while 11.61% reduced at 10 µg/mL in the BT-DMEM + BT-Test formulation group compared to the UT-DMEM + UT-Test formulation group. Anti-wrinkling potential with respect to UV-B induced stress showed an increase cell viability by 33.66%, 19.87%, and 18.46% at 1, 10, and 50 µg/mL, respectively in the UT-DMEM + BT-Test formulation group compared to the UT-DMEM + UT-Test formulation group. In addition, cell viability was significantly increased by 65.63% and 69.95% at concentration 1 and 10 µg/mL, respectively, in BT-DMEM + BT-Test formulation group.

Wound healing scratch assay showed a significant migration of fibroblast and keratinocytes cells with increase covered area. HFF-1 cellular migration was increased up to 58.55% (100 µg/mL) in UT-DMEM + BT-Test formulation group compared with the untreated group. However, HaCaT cells showed significant improved cell migration up to 231.25% at 50 µg/mL in BT-DMEM + UT-Test formulation group, while up to 206.25% at 50 µg/mL in BT-DMEM + BT-Test formulation group compared with the untreated group, which suggest its significant role in wound healing and other skin-related diseases such as anti-wrinkling, anti-aging, and skin whitening.

In conclusion, Biofield Energy Treated test formulation and DMEM showed significant improved skin health and helps in reversal of ageing process. Overall, The Trivedi Effect®-Consciousness Energy Healing Treatment 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 has significant capacity in the prevention of temporary and permanent skin disorders, anti-aging, improved overall health, and quality of life.

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