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Photo-Protective Effect of Biofield Energy Healing (The Trivedi Effect®) Treatment Based Herbomineral Formulation Against Various Skin Health Parameters

Aksana Hancharuk[1], Mahendra Kumar Trivedi[1], Alice Branton[1], Dahryn Trivedi[1], Gopal Nayak[1], Mayank Gangwar[2], Snehasis Jana[2, *]

[1]Trivedi Global, Inc., Henderson, NV, USA

[2]Trivedi Science Research Laboratory Pvt. Ltd., Bhopal, India

Email address:

publication@trivedisrl.com (S. Jana)

[*]Corresponding author

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Abstract: The current study aimed to evaluate the effect of Biofield Energy Healing (The Trivedi Effect®) based herbomineral formulation on various skin health parameters. The test formulation consisted of minerals (zinc, selenate, and molybdenum), L-ascorbic acid, along with Centella asiatica extract, and tetrahydrocurcumin (THC). The test formulation and DMEM were divided into two parts, one part of each was treated with the Biofield Energy Treatment by Aksana Hancharuk and denoted as treated group, while other part was coded as the untreated groups. Skin parameters against various cell lines such as HFF-1, HaCaT and B16-F10 were examined with the combination of Biofield Treated (BT) and untreated test formulation (UT) with DMEM. MTT assay result showed that the test formulation was found to be safe and nontoxic at tested concentrations. Human fibroblast cell proliferation using BrdU method showed a significant increased cell proliferation by 91.51% and 201.14% in different Biofield Energy Treated groups. UT-DMEM + BT-Test formulation group showed an increased level of collagen by 64.58% (2.5 µg/mL) and 55.55% (1.25 µg/mL), respectively compared with the untreated group. Similarly, elastin synthesis was increased in UT-DMEM + BT-Test formulation group by 21.96% and 11.42% at concentration 10 and 5 µg/mL, respectively compared to the untreated group. However, hyaluronic acid level was increased by 14.02% and 6.57% at concentrations 1.25 and 0.625 µg/mL, respectively in BT-DMEM + BT-Test formulation group compared with the untreated group. Moreover, inhibition of melanin synthesis was observed by 12.95% and 17.86% (0.125 µg/mL) in UT-DMEM + BT-Test formulation and BT-DMEM + UT-Test formulation groups, respectively compared with the untreated group in B16-F10 melanoma cell line. Anti-wrinkling activity in HFF-1 cells showed a notable cell viability in all the groups at various concentrations, i.e. in UT-DMEM + BT-Test formulation, BT-DMEM + UT-Test formulation, and BT-DMEM + BT-Test formulation group by 15.68%, 36.47%, and 29.64%, respectively at 2.5 µg/mL compared with the untreated group. The significant wound healing activity was reported in scratch assay in HFF-1 and HaCaT cells due to the significantly higher cellular migration of fibroblast and keratinocytes. Therefore, Biofield Energy Treatment (The Trivedi Effect®) based test formulation and cell medium might be a suitable approach for the development for herbal cosmetics and formulations as anti-wrinkling, skin-whitening, anti-ageing, and rejuvenating action. It can improve the overall skin health care against many skin disorders such as Eczema, diaper rash, seborrheic dermatitis, chickenpox, measles, warts, acne, hives, ringworm, Rosacea, psoriasis, skin cancer, etc.

Keywords: Consciousness Energy Healing Treatment, B16-F10, Extracellular Matrix, HaCaT, HFF-1, Hyaluronic Acid, Scratch Assay, Tetrahydrocurcumin

1. Introduction

Natural products as an alternative medicines are thought to be beneficial foundations in exploration of novel pharmacophores. It was found that approximately 30% of the worldwide drugs, cosmetics, and agrochemicals are based on the natural products [1], due to their significant mechanism in cellular, molecular, genetic, and biochemical pathways in various chronic diseases. In cosmetics and most of the skin care products, natural products are the principle active constituents [2]. Scientific literature and clinical data suggest that increased demand of herbal based formulation might be due to its lower incidence of adverse effects compared with the synthetic medicines [3, 4]. Herbal based products are reported with significant antioxidant and anti-inflammatory activities [5], which suit them to protect against UV-B sun radiation with skin whitening activity [6]. Several plant based skin product along with the use of minerals as active ingredients such as zinc, copper, and selenium along with vitamins (Vit. C and E) are widely used in cosmetology. These combinations are responsible for significant antimicrobial, antioxidant, and free radical scavenging effect with improved extra cellular component synthesis [7]. Based on the exhaustive literature, a new proprietary herbomineral formulation was formulated including minerals (zinc chloride, sodium selenate, and sodium molybdate), L-ascorbic acid, and tetrahydrocurcumin (THC) along with herbal extract, Centella asiatica. C. asiatica is well known herb used against wound healing, hypertrophic wounds, burns, anti-inflammatory in various skin care products [8]. THC is the main active metabolite of curcumin [9] and has significant pharmacological properties with strongest antioxidant property [10, 11]. Further, vitamins play a significant role in skin health, anti-wrinkles and wound healing action. Therefore, many skin care and wound healing formulations incorporated vitamins such as vitamin C (ascorbic acid), and vitamin E, which play a vital role in repair of damaged skin and modulates the collagen production [12].

The increased use of Complementary and Alternative Medicine (CAM) has been reported with significant outcomes in cosmetology. The Biofield Energy Healing, is a cumulative measurable electromagnetic field around the human body [13]. Use of energy medicine and healing has been studied and reported with the significant conclusions [14]. Biofield is generated from internal human processes like blood flow, lymph flow, brain functions, and heart function. Human has the ability to harness the energy from environment or Universe and can transmit into any living or nonliving object(s). The objects always receive the energy and responding into useful way that is called Biofield Energy and the process is known as Biofield Energy Treatment. The Trivedi Effect® has been reported with the significant breakthrough in living organisms and nonliving materials. Biofield Energy Healing has been reported with significant altered antimicrobial sensitivity pattern against various pathogenic microbes [15-18]. In addition, The Trivedi Effect® has also well scientifically studied in diverse areas such as materials science [19-22], biotechnology [23, 24], agriculture [25-27] and many more.

Due to enormous applications of the Biofield Energy Healing as an alternative treatment approach, the present work was designed to evaluate The Trivedi Effect®-Consciousness Energy Healing Treatment on test formulation against various skin parameters in human foreskin fibroblast, human keratinocytes and mouse melanoma cell lines (i.e. HFF-1, HaCaT, and B16-F10).

2. Materials and Methods

2.1. Chemicals and Reagents

The components of test formulation such as zinc chloride 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 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) procured from HiMedia, India, while Direct Red 80 and EDTA was purchased from Sigma, USA. All the other chemicals used in this experiment were analytical grade procured from local vendors.

2.2. Cell Culture

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 cell lines were 37°C, 5% CO_2, and 95% humidity. L-ascorbic acid (for ECM, UV-B protection, and wound healing assay) in concentrations range from 10 µM to 1000 µM, while kojic acid (for melanin) concentrations range 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 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, which 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 + 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 formulation was treated with the Biofield Energy by renowned Biofield Energy Healer (also known as The Trivedi Effect®) and coded as the Biofield Energy Treated formulation, while the second part of the test formulation did not receive any sort of treatment and was defined as the untreated test formulation. This Biofield Energy Healing Treatment was provided by Aksana Hancharuk, who participated in this study and performed the Biofield Energy Treatment remotely for ~5 minutes. Biofield Energy Healer was remotely located in the USA, while the test herbomineral formulation was located in the research laboratory of Dabur Research Foundation near New Delhi in Ghaziabad, India. This Biofield Energy Treatment was administered for 5 minutes through the Healer's unique Energy Transmission process remotely to the test formulation under laboratory conditions. The Biofield Energy Healer, Aksana Hancharuk, in this study never visited the laboratory in person, nor had any contact with the herbomineral samples and medium. None of the Biofield Energy Healers in this study visited the laboratory in person, nor had any contact with the herbomineral samples and medium. Further, the control group was treated with a sham healer for comparative purposes. The sham healer did not have any knowledge about the Biofield Energy Treatment. After that, the Biofield Energy treated and untreated samples were kept in similar sealed conditions for experimental study.

2.5. Determination of Non-Cytotoxic 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 the Test Formulation on Human Foreskin Fibroblast (HFF-1) Cell Proliferation Using BrdU Method

HFF-1 cells were counted using hemocytometer and plated in 96 well plate at the density corresponding to 1 X 10^3 to 5 X 10^3 cells/well in DMEM supplemented with 15% FBS. The cells 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 Synthesis

Synthesis of the extracellular matrix components (i.e. collagen, elastin and hyaluronic acid) in HFF-1 cell line was estimated for determining the potential of test formulation in terms of skin strength, overall elastin, and hydration level activity. 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 elastin and hyaluronic acid levels. However, the corresponding cell layers were processed for 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 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, 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 concentration of UV-B irradiation (200 mJ/cm^2) that can lead to approx. 50% cytotoxicity (302 nm, CL-1000 M, UVP, USA) [33]. 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 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 scratches that represent wounds were created in the near confluent monolayer of cells by gently scrap with the sterile 200 µL micropipette tip. The cells were then rinsed with serum free DMEM and the Biofield Energy Treated test formulation. The scratched area was then monitored for a time period ranging from 0 to 48 hours for closure of wound area. The photomicrographs were done at the selected time point's 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 [31].

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 cytotoxic effect of test formulation was tested on three cell lines i.e. HFF-1, HaCaT and B16-F10 in presence of positive control ascorbic acid (10 and 50 µM) and EGF (10 ng/mL) were used at defined concentrations for estimation of cell percentage viability. The results of percent cell viabilities in all the tested cell lines showed that the cell viability range of 77% to 103% in test formulation group, while for ascorbic acid it was found more than 89%. These data suggest that herbomineral formulation was found safe at the tested concentration range up to maximum of 140 µg/mL against the tested cell lines.

3.2. Effect of the Biofield Energy Treated Test Formulation on Human Foreskin Fibroblast Cell Proliferation (BrdU Method)

The results of human fibroblast cell proliferation using BrdU method at different combinations of herbomineral formulation with DMEM on percentage cellular proliferation of HFF-1 cells after 48 hours of incubation is represented in Figure 1. In the presence of FBS, cell proliferation rate was significantly increased by 250.4%. The study results showed that FBS (positive control) significantly improved the cell proliferation compared with normal and vehicle control group by 150%. Moreover, the Biofield Energy Treated groups have shown significant increase in cellular proliferation in all the groups at 70 and 140 µg/mL concentration. At concentration 35 µg/mL, the cell proliferation in HFF-1 cells were comparatively lower compared with other two tested concentrations. The increased proliferation rate in UT-DMEM + BT-Test Formulation and BT-DMEM + UT-Test Formulation group was 91.51% and 201.14%, respectively at 35 µg/mL compared with the untreated group. These data suggest that the Biofield Energy Healing Treatment results showed a significant increase in cellular proliferation in HFF-1 cells using BrdU assay.

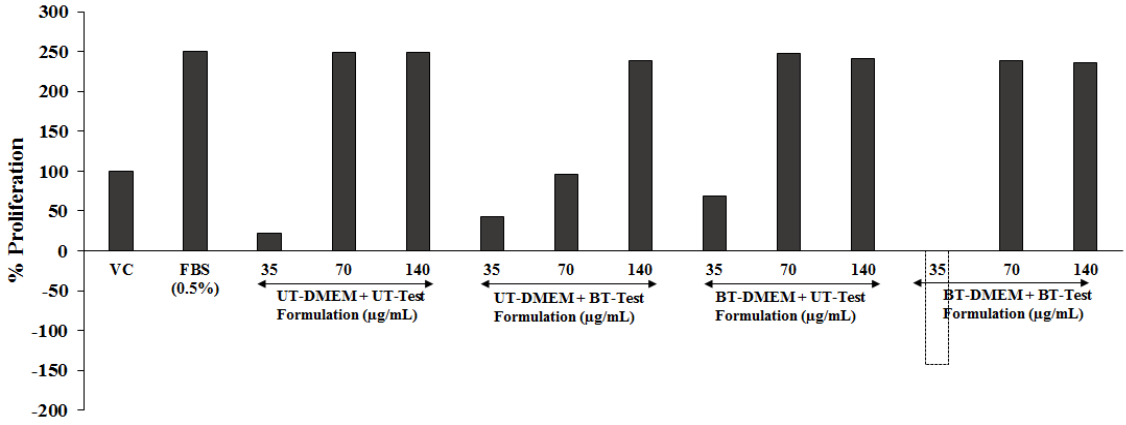

Figure 1. *Effect of the Biofield Energy Treated Test Formulation with DMEM on cellular proliferation in HFF-1 cells after 48 hours. VC: Vehicle control; FBS: Fetal bovine serum; UT: Untreated; BT- Biofield Treated.*

3.3. Analysis of Extracellular Matrix Component Synthesis

The extra cellular matrix components (ECM) for skin strength, hydration level and overall elasticity were assessed using Biofield Energy Treated Test Formulation/DMEM in HFF-1 cell line. The results of the study are presented as collagen, elastin and hyaluronic acid levels.

3.3.1. Collagen Analysis

The effect of the Biofield Energy Treated test formulation/DMEM on collagen level showed a significant increase in the collagen content at various tested concentrations range on HFF-1 cell line. The experimental results of collagen with respect to ascorbic acid and Biofield Energy Treated Test Formulation/DMEM are presented in Figure 2. Ascorbic acid (10 µM) showed a significant increased collagen content by 54.77%, while Biofield Energy Treated Test Formulations was reported with the increased percentage of collagen amount by 8% to 64% at different concentrations. The tested group of UT-DMEM + BT-Test Formulation showed a significant increased collagen level by 64.58% increase (124.94 ± 2.80 µg/mL) at concentration 2.5 µg/mL compared to UT-Test Formulation + UT-DMEM (75.91 ± 4.26 µg/mL). Similarly, at 2.5 µg/mL in other groups such as BT-DMEM + UT-Test Formulation and BT-DMEM + BT- Test Formulation showed a significant improvement of the collagen level by 38.56% and 57.38%, respectively compared with the UT-DMEM + UT-Test Formulation group. However, at concentration 1.25 µg/mL, the UT-DMEM + BT-Test Formulation and BT-DMEM + BT-Test Formulation also showed an increased level of collagen by 55.55% and 52.47%, respectively compared with the UT-DMEM + UT-Test Formulation group. Other groups at different concentrations also showed an increased collagen concentration with respect to the respective untreated group. These study data suggest that Biofield Energy Treated herbomineral formulation showed a remarkable increase in collagen level compared with the Biofield Energy Treated DMEM.

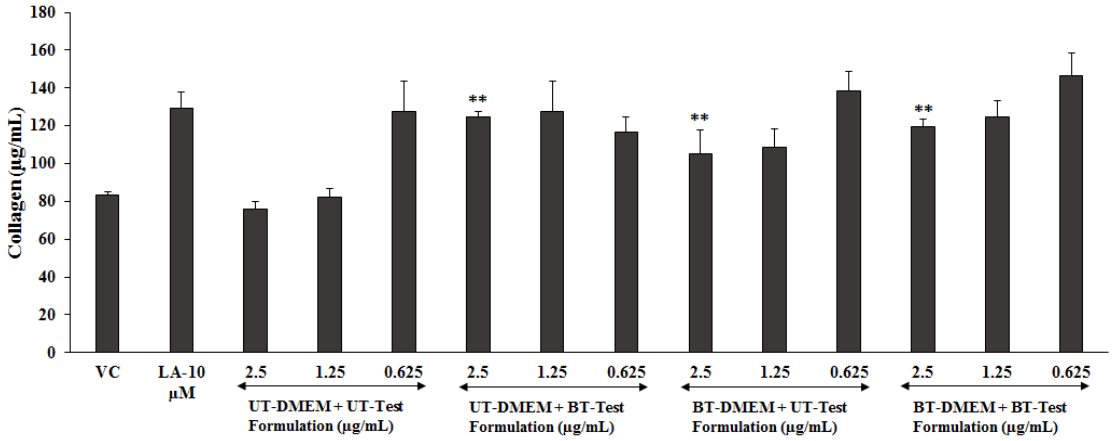

Figure 2. *Concentration-dependent effects of Test Formulation on human dermal fibroblast (HFF-1) cell line for collagen level. $^{**}p\leq0.01$ statistical comparison with respect to untreated DMEM and untreated test formulation using one way ANOVA (Dunnett's test). VC: Vehicle control; LA-10: L-Ascorbic acid at 10 µM concentration; UT: Untreated; BT- Biofield Treated.*

Collagen is one of the major skin components essential for skin health, structure and most abundant fibrous protein present in extra cellular component (ECM). It is regarded as the most abundant protein in animal kingdom. The study

results showed the collagen level was significantly increased after treatment with the Biofield Energy Treated test formulation. It suggests that the collagen synthesis was influenced and significantly increased. Procollagen peptides results in the formation of tropocollagen that includes cleavage of peptides. Further, *via* covalent cross-linking (aldol reaction) among various tropocollagen molecules by lysyl oxidase results in formation of collagen fibrils. These collagen fibrils then lead to collagen fibers, which provide strength and structure to the skin and other tissues [32, 33]. It might be expected that Biofield Energy Treatment might improve the tropocollagen molecules, which showed an increased collagen fibril and its content. The present study concludes that the collagen level was significantly improved due to the Biofield Energy Healing (The Trivedi Effect®) based test formulation, which would be useful and have application in skin health, strength, and structure and wound healing in cosmetics.

3.3.2. Assessment of Elastin

The role of the Biofield Energy Healing based test formulation on elastin level are presented in Figure 3. The results showed a significant enhancement in the elastin synthesis in the Biofield Energy Healing based herbomineral, in HFF-1 cell line. The data showed that ascorbic acid (50 µM) group showed an increased elastin content by 55.30% compared with the baseline control. However, among other tested groups, only UT-DMEM + BT-Test formulation group showed an increased percentage of elastin amount by 21.96% (10.94 ± 0.49 pg/mL) and 11.42% (11.22 ± 0.17 pg/mL) at concentration 10 and 5 µg/mL, respectively compared to the UT-DMEM + UT-Test formulation group. Rest all the groups showed a significant alterations in elastin level after treatment at different concentrations compared with the untreated groups. Therefore, the Biofield Energy Healing based test formulation and DMEM can be significantly used as an alternative approach in order to improve the elastin level.

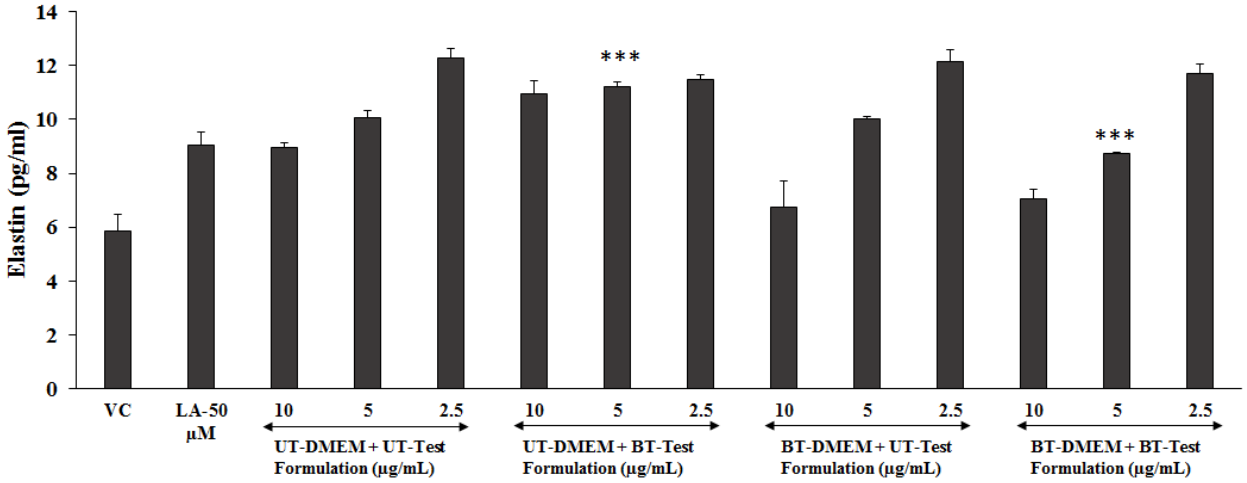

Figure 3. *Concentration-dependent effect of the Biofield Energy Treated test formulation on human dermal fibroblast (HFF-1) cell line for extracellular matrix component, elastin. ***p≤0.001 statistical comparison with respect to untreated DMEM and untreated test formulation using one way ANOVA (Dunnett's test). VC: Vehicle control; LA-50: L-Ascorbic acid at 50 µM concentration; UT: Untreated; BT- Biofield Treated.*

Elastin is a highly elastic protein in the body tissue that helps to retain shape after any stretching or contraction. This is one of the vital component of the ECM, it forms tight junction with collagen fibrils, which helps to maintain the integrity [34]. Fibroblasts are responsible for the production of elastin, which after some time it loses its capacity to produce elastin, which results in ageing. Study results showed an increased level of elastin, which improves the elasticity and strength of the skin and activates the dermal metabolism. Hence, the Biofield Energy Healing (The Trivedi Effect®) based test formulation has the capacity to significantly increase the elastin synthesis, which results in improved the cell growth, survival, differentiation and morphogenesis.

3.3.3. Analysis of Hyaluronic Acid

The level of hyaluronic acid after treatment with the Biofield Energy based test formulation was evaluated in the HFF-1 cell line (Figure 4). The results of ascorbic acid showed a significant increase in the hyaluronic acid content by 183.6%. However, in UT-DMEM + BT-Test formulation and BT-DMEM + UT-Test formulation group showed 3.68% and 2.52% increase at concentration 0.625 µg/mL compared with the UT-DMEM + UT-Test formulation. The BT-DMEM + BT-Test formulation group showed a significant increased level of HA by 14.02% and 6.57% at concentrations 1.25 and 0.625 µg/mL, respectively compared with the UT-DMEM + UT-Test formulation. Besides, all other experimental groups showed change in hyaluronic acid with respect to the baseline control. It can be hypothesized that Biofield Energy might increase the synthesis of HA, which would be helpful for overall skin health. Therefore, it might be expected that Biofield Energy Healing based herbomineral product test formulation can be used as an approach to improve the content of hyaluronic acid compared with the Biofield Treated DMEM.

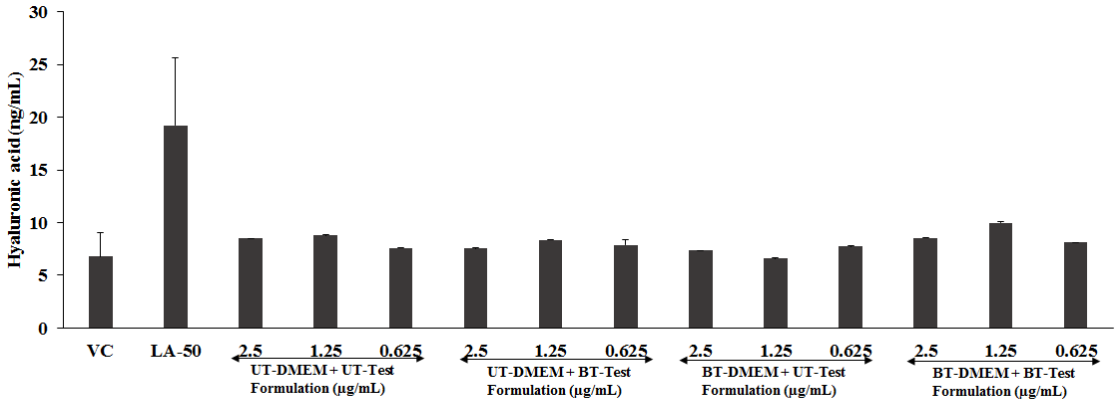

Figure 4. *Synthesis of extracellular matrix component, hyaluronic acid by Biofield Energy Treated test formulation in human dermal fibroblasts (HFF-1) cell lines. VC: Vehicle control; LA-50: L-Ascorbic acid at 50 µM concentration; UT: Untreated; BT- Biofield Treated.*

One of the important component of ECM is the hyaluronic acid (HA) that helps in retaining the skin moisture. HA stabilizes and regulates the water balance along with osmotic pressure in skin from extracellular domain of cell surfaces. Cosmetology now deals with skin products having high content of HA to improve the skin health [35]. The experimental study results suggested improved level of HA after treatment with the Biofield Energy Healing based herbomineral formulation, which can be used as an alternative approach for skin disorders. Hence, it can be concluded that The Trivedi Effect® has the capacity to regulate the skin moisture by maintain the HA level against many skin disorders.

3.4. Estimation of Melanin Synthesis Inhibition

The effect of the Biofield Energy Healing based test formulation on melanogenesis in mouse melanoma (B16-F10) cell line were cultured in DMEM supplemented media containing various concentrations along with the effect of kojic acid (10 µM) for 48 to 96 hours. The results of percentage decrease in α-MSH melanin synthesis in all the experimental groups are presented in Figure 5. Kojic acid, a skin whitening compound was used as positive control, which showed a significant decreased level of melanin synthesis by 65.30%. The Biofield Energy Treatment based test formulation, showed a significant decrease in the melanin synthesis by 12.95% and 17.86% in UT-DMEM + BT-Test formulation and BT-DMEM + UT-Test formulation groups, respectively at concentration 0.125 µg/mL compared with the UT-DMEM + UT-Test formulation group. Moreover, at concentration 0.0625 µg/mL, BT-DMEM + UT-Test formulation and BT-DMEM + BT-Test formulation group showed a decreased melanin synthesis by 1.82% and 7.23%, respectively compared with the UT-DMEM + UT-Test formulation group in B16-F10 melanoma cell line. Therefore, it can be concluded that the Biofield Energy Healing based test formulation and DMEM would be useful approach to decrease melanogenesis compared with the untreated test items.

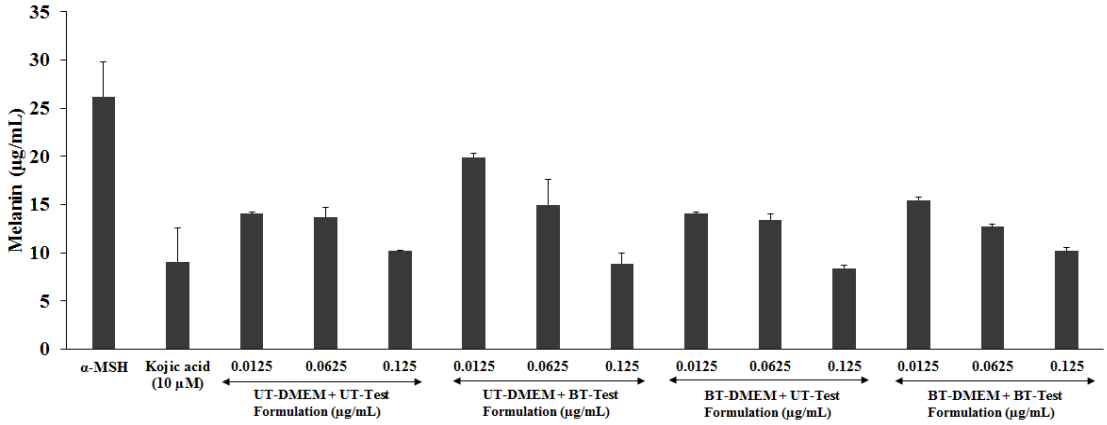

Figure 5. *Inhibitory effect of the Biofield Energy Treated test formulation on melanogenesis (skin whitening potential) in mouse melanoma (B16-F10) cell line. UT: Untreated; BT- Biofield Treated.*

Herbomineral formulation is the combination of herbal and minerals, and research data suggest importance of minerals, Centella asiatica extract and THC (strong antioxidant activity) against various inflammatory dermatoses, skin infections, wound healing, along with cosmetic preparations have been reported [36, 37]. Under the influence of sun

ultraviolet radiations (UV-A and UV-B), the process of melanogenesis was initiated in the melanocytes. The absorption of radiation results in skin darkening and initiated the process of melanogenesis. Several synthetic marketed product claimed for skin whitening action by decreasing the melanin synthesis by inhibiting tyrosinase enzymes activity [38]. These experimental results suggest that melanin synthesis was significantly decreased in the presence of Biofield Energy Treated test formulation. Hence, it might be anticipated that Biofield Energy Healing has the capacity to inhibit the melanin synthesis by inhibiting the activity of tyrosinase enzymes. Overall, the Biofield Energy Healing based natural test formulation is a novel approach for skin-related disorders with significant skin whitening action.

3.5. Anti-Wrinkling Effects of the Test Formulation on UV-B Induced Photoaging

Cell viability potential of the Biofield Energy Treated test formulation due to UV-B induced stress was measured in HFF-1 cells using hemocytometer. Anti-wrinkling effect of cell viability from UV-B rays is presented in Figure 6. The HFF-1 cells were subjected to the lethal concentration of UV-B irradiation (200 mJ/cm2) and the percentage cell viability due to UV-B was determined. The HFF-1 cells while exposure of UVB reported with high degree of cell death with 25.21% of cell viability. The cell viability in vehicle control (DMSO, 0.05%) was found as 20.51% due to UV-B irradiation (200 mJ/cm2). However, ascorbic acid (50 µM) showed a significant increase cell viability i.e. 43%. The other test groups showed with improved cell viability at various concentrations of the test items. Among the tested groups, UT-DMEM + BT-Test formulation, BT-DMEM + UT-Test formulation, and BT-DMEM + BT-Test formulation groups at 0.625 µg/mL showed an increased cell viability by 7.29%, 10.56%, and 13.22%, respectively compared with the UT-DMEM + UT-Test formulation group. Similarly, UT-DMEM + BT-Test formulation, BT-DMEM + UT-Test formulation, and BT-DMEM + BT-Test formulation group at 1.25 µg/mL showed an increased cell viability by 5.40%, 33.15%, and 34.32%, respectively compared with the UT-DMEM + UT-Test formulation group. In addition, UT-DMEM + BT-Test formulation, BT-DMEM + UT-Test formulation, and BT-DMEM + BT-Test formulation groups at 2.5 µg/mL showed an increased cell viability by 15.68%, 36.47%, and 29.64%, respectively compared with the UT-DMEM + UT-Test formulation group. This suggest that the Biofield Energy Treated test formulation could be significantly used for skin protective effect with anti-wrinkling potential.

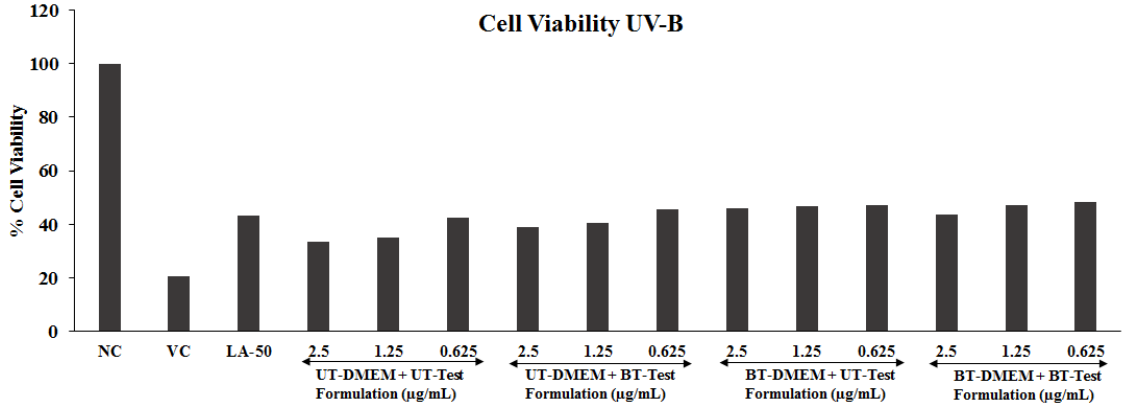

Figure 6. *Anti-wrinkling action as cytoprotective potential of Biofield Energy Treated test formulation against UV-B induced stress in human dermal fibroblasts (HFF-1) cell lines.% cell viability of HFF-1 cells after treatment in various groups. NC: Normal control; VC: Vehicle control; LA-50: L-Ascorbic acid at 50 µM concentration; UT: Untreated; BT- Biofield Treated.*

Several reports suggest that UVB-induced many skin related disorders that may lead to skin ageing. UV-B induce stress lead to free radical generation that downregulates the signal transduction in human skin fibroblasts. This process results in DNA damage and induce various inflammatory responses [39]. The experimental data suggest that UV-B significantly induced skin damaged as suggested with loss in cell viability. Further, the effect of the Biofield Energy Healing based test formulation showed a significant protection against UV-B-induced skin damaged with increased cell viability. UV-B can lead to reduce the procollagen and elastin expression by inhibiting the receptor activation of Smad protein. Biofield Energy Healing based test formulation and cell medium (DMEM) significantly improved the elastin, collagen, and hyaluronic acid along with UV-B induced cell viability. Hence, it can be suggested that the Biofield Energy Treated test formulation would be a better alternative treatment for skin protection and cell viability from UV-B radiations along with improved ECM components, which directly improved the skin health.

3.6. Wound-Healing Scratch Assay

The wound healing scratch assay showed a significant increased movement of cells migration (HFF-1 and HaCaT) after treatment with the Biofield Energy Treated test formulation and cell medium (DMEM). The representative cell migration photographs in different groups were monitored and shown in Figure 7. The scratched monolayer showed a significant migration after treatment with the

Biofield Healing based test formulation/DMEM at different groups and at different time points. The cell migration was reported at 72 hours in the presence of the Biofield Energy Treated test formulation compared with the vehicle control and ascorbic acid. The percentage cell covered area in the Biofield Energy Treated test formulation group showed a significant improved rate compared with the Biofield Energy Treated DMEM. The migration rate of HFF-1 cells and HaCaT cells were significantly increased when compared with the measured scratch area for wound closure. HFF-1 cells showed 1% to 9% increase in coverage area in BT-DMEM + BT-Test Formulation, while 6% in UT-DMEM + BT-Test Formulation group, with respect to UT-DMEM + UT-Test Formulation. Similarly, HaCaT cells showed 1% to 2% increase in coverage area in BT-DMEM + BT-Test Formulation with respect to UT-DMEM + UT-Test Formulation group. Representative images of scratched wound area showed a significant increased wound closure area in ascorbic acid group as shown in Figure 8 (b) when compared with the baseline control group in Figure 8 (a). Similarly, the Biofield Energy Treated test formulation in UT-DMEM + BT-Test Formulation experimental group showed a significant rate of cellular migration rate along with wound closure area as shown in Figure 8 (d), compared with the UT-DMEM + UT-Test Formulation group.

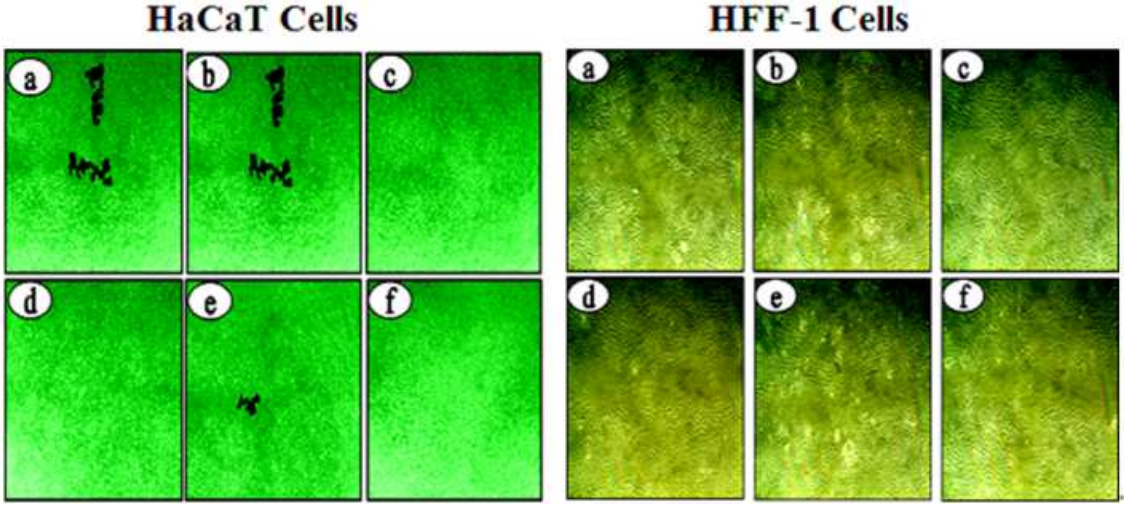

Figure 7. *Representative pictures of HFF-1 and HaCaT cell migration cells after induction of a scratch. All the pictures were taken immediately after the scratch was induced (i.e. at 0 hours), after 24 hours in the presence of ascorbic acid and Biofield Energy Treated test formulation. Pictures are taken at 50 times magnification. Images represents HFF-1 cells migration in presence of (a) baseline control media, (b) ascorbic acid, (c) UT-DMEM + UT-Test Formulation, (d) UT-DMEM + BT-Test Formulation, (e) BT-DMEM + UT- Test Formulation, and BT-DMEM + BT-Test Formulation.*

All the components used in the test formulation have been reported with significant role on cosmetology and wound healing action [40]. Wound healing scratch assay determined the cellular migration, which is one the well-defined *in vitro* model. It established the relation between cell-to-cell and cell-to-matrix interactions during wound healing process [41]. Fibroblasts cells manage the collagen deposition that would be required to repair the injured area and provide strength, integrity and structure to the skin. Overall, the importance of the Biofield Energy Healing based test formulation and DMEM suggest significant proliferation of fibroblast might be due to significant antioxidant mechanism. Hence, it can be concluded that The Trivedi Effect® would be a new alternative approach in wound healing and repair process.

4. Conclusions

The study results conclude that The Trivedi Effect®-Consciousness Energy Healing Treatment based test formulation and cell medium (DMEM), have the potential applications in various skin disorders. MTT assay showed the Biofield Energy Treated test formulation was found to be safe and nontoxic in all the tested concentrations. Fibroblast proliferation assay using BrdU in HFF-1 cells displayed significant proliferation by 91.51% and 201.14% in the UT-DMEM + BT-Test Formulation and BT-DMEM + UT-Test Formulation groups, respectively at 35 µg/mL, compared with the untreated group. Collagen synthesis was significantly increased by 64.58% and 57.38% in the UT-DMEM + BT-Test Formulation and BT-DMEM + BT-Test Formulation group respectively. However, in BT-DMEM + UT-Test Formulation group showed an increased collagen amount by 38.56%. Besides, the level of elastin was found to be increased by 21.96% and 11.42% in the UT-DMEM + BT-Test Formulation group at concentration 10 and 5 µg/mL respectively, compared to the UT-DMEM + UT-Test Formulation group. Hyaluronic acid concentration was increased by 14.02% (1.25 µg/mL) and 6.57% (0.625 µg/mL) in the BT-DMEM + BT-Test Formulation group compared with the UT-DMEM + UT-Test Formulation. The melanin synthesis inhibition was found in the UT-DMEM + BT-Test formulation and BT-DMEM + UT-Test formulation group by 12.95% and 17.86% (0.125 µg/mL), respectively compared with the UT-DMEM + UT-Test Formulation group in B16-F10 melanoma cell line. Biofield Energy Treated test

formulation showed a significant anti-wrinkling effect using UV-B induced stress in the HFF-1 cells by 15.68%, 36.47%, and 29.64% increase cell viability in the UT-DMEM + BT-Test Formulation, BT-DMEM + UT-Test Formulation, and BT-DMEM + BT-Test Formulation groups, respectively compared with the UT-DMEM + UT-Test Formulation group. Wound healing scratch assay exhibited significant improved wound closure area up to 9% after treatment with the Biofield Energy based test formulation with increased cellular migration of fibroblast and keratinocytes in the HFF-1 and HaCaT cells. Thus, the Biofield Energy Treated Test Formulation and DMEM media can be used to improve various skin disorders via improved collagen, elastin, hyaluronic acid synthesis that helps to improve the skin elasticity and tightness.

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

DMEM: Dulbecco's Modified Eagle's Medium; THC: Tetrahydrocurcumin, ECM: Extracellular matrix, EGF: Epidermal growth factor, α-MSH: α-Melanocyte-stimulating hormone, ANOVA: One-way analysis of variance, HA: Hyaluronic acid; HFF-1: Human foreskin fibroblast cell line, B16-F10: Mouse melanoma cell line; HaCaT: Human Keratinocytes cells; UVB: Ultra violet B rays; CAM: Complementary and alternative medicine; NCCAM: National Center for Complementary and Alternative Medicine; UT: Untreated; BT: Biofield Treated.

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