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Biofield Energy Healing Based Herbomineral Formulation: An Emerging Frontier in Cosmetic Medicine

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Abstract: The application of the herbomineral formulations in general skin health are increasing day-by-day due to the excellent outcomes without any adverse effects. This study was designed to evaluate the influence of The Trivedi Effect®-Consciousness Energy Healing Treatment on an herbomineral formulation and cell medium against various skin health parameters. The test formulation consists of minerals (zinc chloride, sodium selenate, and sodium molybdate) and L-ascorbic acid along with herbal extracts, Centella asiatica, and tetrahydrocurcumin (THC). The test formulation and DMEM media were divided into two equal parts, one was treated with a Biofield Treatment (BT) by Dezi Ann Koster and denoted as treated, while the other part was coded as the untreated (UT) groups. MTT assay results showed that test formulation was safe and nontoxic with more than 89% cell viability in the tested cell lines. BrdU assay showed an improved cell proliferation by 2.82% in the UT-DMEM + BT-Test formulation group compared with the untreated group. The level of collagen was significantly increased by 32.42%, 33.64%, and 29.13% at 2.5, 1.25 and 0.625 µg/mL, respectively in the UT-DMEM + BT-Test formulation, while34.17%, 26.73%, and 17.56% increased at 2.5, 1.25 and 0.625 µg/mL, respectively in BT-DMEM + BT-Test formulation group compared with the untreated group. Elastin level was increased by 408.6% at concentration of 0.625 µg/mL in UT-DMEM + BT-Test formulation group compared with untreated group. However, hyaluronic acid (HA) level was increased by 31.88%, 15.52%, and 58.29% at 2.5, 1.25, and 0.625 µg/mL, respectively in the BT-DMEM + BT-Test formulation group compared with untreated group. Besides, melanin synthesis was significantly inhibited by 16.09% and 18.93% in the UT-DMEM + BT-Test formulation and BT-DMEM + UT-Test formulation groups, respectively at 0.125 µg/mL compared with the untreated group. Anti-wrinkling activity in HFF-1 cells showed an improved cell viability by 5.49% and 11.26% at 1.25 and 0.625 µg/mL, respectively in BT-DMEM + BT-Test formulation group compared with the untreated group. Wound healing scratch assay results showed a significant healing rate by 5% and 10% in HFF-1 and HaCaT cells lines, respectively with high cellular migration of fibroblast and keratinocytes. Overall, it can be concluded that the Biofield Energy Healing (The Trivedi Effect®) based test formulation and cell medium could be helpful against various skin disorders and can be used in psoriasis, seborrheic dermatitis, skin cancer, rashes from bacterial or fungal infections as anti-wrinkling, skin-whitening, anti-ageing, and rejuvenating agent.

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

1. Introduction

The statistics from the World Health Organization (WHO) state that there is a high level of belief worldwide and global movement towards the use of alternative medicinal approaches such as energy medicine, herbal

based products, etc. as a way to adopt natural way of treatment. People instead of using synthetic drugs, prefer natural and organic foods, herbal medicines, and naturopathic treatments for healthy life [1]. The use of organic foods and farming instead of fertilizers based vegetable products and its demand have been increased. In personal care system, use of herbal based products or cosmetics are highly preferred due to the lower incidence of adverse effect compared with the synthetic compounds [2-4]. The increased demand of herbal based cosmetics is due to high prevalence of human health hazards and side effects that lead to various diseases [5]. Many invasive skin treatment measures such as gene therapy, chemical peels, and several devices such as laser energy, injectable, etc. are used for skin health and rejuvenation [6, 7], however many photo-aging products, antioxidant agents (*i.e.* vitamin B_3, C, and E) are also available in the market for skin health. However, the new cosmetic market is driven towards herbal drugs, herbal cosmetics, nutraceuticals, and natural dyes. However, herbomineral products are the best way adopted in order to utilize the modern as well as alternative aspect, which includes the vital minerals required to maintain quality of life. The present research work deals with the novel cosmetic product, an herbomineral formulation prepared in order to improve overall skin health using various skin health parameters against cells lines such as human foreskin fibroblast (HFF-1), human keratinocytes (HaCaT) and mouse melanoma cell lines (B16-F10).

The test formulation consisted of minerals (zinc chloride, sodium selenate, and sodium molybdate), L-ascorbic acid, tetrahydrocurcumin (THC), and an herbal extract of *Centella asiatica* (commonly known as Jal Brahmi). Minerals such as zinc, copper, and selenium are very commonly used in cosmetic products and have wide range of application in cosmetology. These minerals have been reported with strong antimicrobial, antioxidant, and free radical scavenging effects with improved skin extra cellular component synthesis [8, 9]. In addition, L-ascorbic acid (Vitamin C) along with other vitamins (such as Vit-A and E) play an important role in skin health, anti-wrinkling, and significant wound healing action [10]. THC is one of the major active metabolite of curcumin [11], which has strong antioxidant property and role in skin health [12, 13]. *C. asiatica* is well known medicinal herb used for skin ageing, and used as one of the natural weapons in various skin health strategies. Moreover, *C. asiatica* has been reported for wound healing action along and has been used in many skin care products [14].

Biofield science and healing is emerging in the frontier in medicine and its increased use as alternative integrative approaches have been reported in order to promote wellness by uncovering the root cause of diseases with universal solutions [15]. Data suggest that most of the U. S. population has been focused towards the use of natural products as a complementary and alternate medicine (CAM) [16]. The National Center for Complementary and Alternative Medicine (NCCAM), now demarcated Biofield Therapies as one form of the five complementary medicine domain. Biofield is the name given to the low electromagnetic field that permeates and surrounds living organisms [16]. Biofield Healing has been accepted world-wide with significant clinical outcomes including wound healing [17-19]. Biofield Energy Treatment (The Trivedi Effect®) has been reported with noteworthy results in nonliving materials and living organisms. The Trivedi Effect® has been reported with momentous results in the field of microbiology [20-22], agriculture science [23-25], livestock [26], and materials science [27-29]. On this basis, a novel Biofield Energy Healing based herbomineral formulation has been designed for skin care treatment. The study was conducted for the evaluation of skin health parameters of test formulation using HFF-1, HaCaT, and B16-F10 cell lines.

2. Materials and Methods

2.1. Chemicals and Reagents

The test formulation components consisted of 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 components were procured from CUSABIO and CusAb Co. Pvt. Ltd., USA. Fetal bovine serum (FBS) and DMEM were purchased from Gibco, USA. Antibiotics solution (Penicillin-Streptomycin) were procured from HiMedia, India, while Direct Red 80 and EDTA were purchased from Sigma, USA. All the other chemicals used in this experiment were analytical grade procured from local vendors.

2.2. Cell Culture

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) 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 concentration in MTT assay.

2.3. Experimental Design

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

2.4. 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 by a 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 samples did not receive any sort of treatment and was defined as the untreated test samples. Dezi Ann Koster, a renowned Biofield Energy Healer, provided the Biofield Energy Treatment remotely for 5 minutes through the Healer's unique Energy Transmission process remotely to the test formulation under laboratory conditions. The Biofield Energy Healer was located in the USA, while the test samples were located in the research laboratory of Dabur Research Foundation near New Delhi, India. The Biofield Energy Healer, Dezi Ann Koster, in this study never visited the laboratory, nor had any contact with the test formulation and the medium. Further, the control samples were 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 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 the 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 h 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 [30].

2.6. Effect of Test Formulation on Human Foreskin Fibroblast 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/plates were then incubated overnight under growth conditions so as to allow cell recovery and exponential growth. After 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. After 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 extracellular matrix components (*i.e.* collagen, elastin and hyaluronic acid) in HFF-1 cell line was estimated for determining the potential of test formulation to improve skin strength, overall elastin, and hydration level. HFF-1 cells were counted using hemocytometer and plated in 48 well plate at the density corresponding to 10 X 10^3 cells/well in DMEM supplemented with 15 % FBS. The cells were then incubated overnight under specified growth conditions followed by cells to serum stripping. Further, the cells were treated with the test formulation at different experimental combination groups with DMEM group *viz.* vehicle control (DMSO, 0.05%), and positive control (ascorbic acid, at 10 µM). Further, 72 hours of incubation with the test items and positive control, the supernatants from all the cell plates were taken out and collected in pre-labeled centrifuge tubes for the estimation of elastin and hyaluronic acid levels. However, 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 [31].

2.8. Estimation of Melanin Synthesis- Skin Depigmentation Effect

B16-F10 cells were used for the estimation of melanin synthesis, cells were counted using hemocytometer and plated in 90 mm culture dish at the density corresponding to 2 X 10^6/6 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 [32].

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/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) [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 rinsed with serum free DMEM and treated with test formulation. The scratched area was monitored for a time period ranging from 0 to 48 hours for closure of wound area. The representative photomicrographs of cells in different groups were done at 16 hours for quantitative assessment of cellular migration using digital camera, which was connected to the inverted microscope. All the observations were calculated and compared with the positive and vehicle control [34].

2.11. Statistical Analysis

Each experiment was carried out in three independent assays and was represented as mean values with standard error of mean (SEM). 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 Discussion

3.1. MTT Assay- Non-cytotoxic Effect of the Test Formulation

The cytotoxic effects of test formulation was tested on all the three cell lines *i.e.* HFF-1, HaCaT and B16-F10. The results were compared with respect to ascorbic acid (10 µM) and EGF (10 ng/mL) at defined concentrations for the estimation of percentage cell viability. The results of percentage cell viability in all the tested cell lines showed the cell viability range of 73% to 118% in different test formulation groups with DMEM, while for ascorbic acid it was found more than 89% (Figure 1). These data suggest that the test formulation was found safe at all the tested concentrations range up to maximum of 40 µg/mL against the tested cell lines.

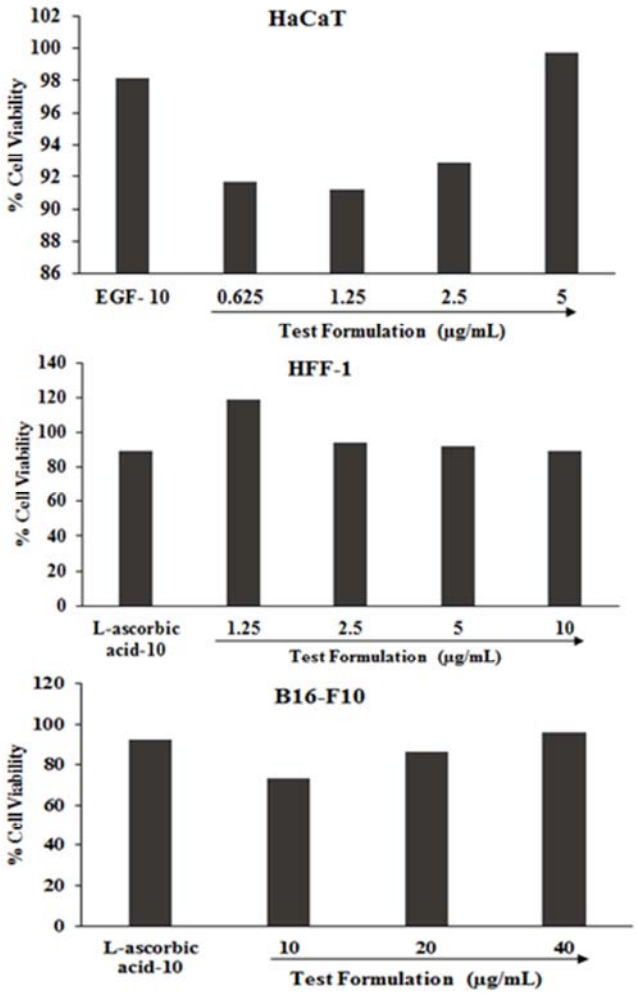

Figure 1. *Effect of the test formulation on HaCaT, HFF-1, and B16-F10 cell lines for cell viability after 72 hours using the MTT assays. EGF-10: Epidermal growth factor (10 µM).*

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 the Biofield Energy Treated test formulation with DMEM on percentage cellular proliferation of HFF-1 cells after 48 hours of incubation is represented in Figure 2. In the presence of FBS, the percentage cell proliferation was significantly increased to 250%. The study results showed FBS (0.5%) group have significantly improved cell proliferation by 150% and 133%, compared with the normal and vehicle control groups, respectively. Besides, the Biofield Energy Treated group, UT-DMEM + BT-Test formulation showed an increased cellular proliferation at the concentration of 17.5 µg/mL by 2.82% compared with the UT-DMEM + UT-Test formulation group. However, the test formulation also showed a significantly improved cell proliferation at all the tested concentrations *viz.* 2.13, 8.75, and 17.5 µg/mL compared with the normal and vehicle control group. This suggests that the Biofield Energy Treatment results in increased cellular proliferation in HFF-1 cells at 17.15 µg/mL using BrdU assay.

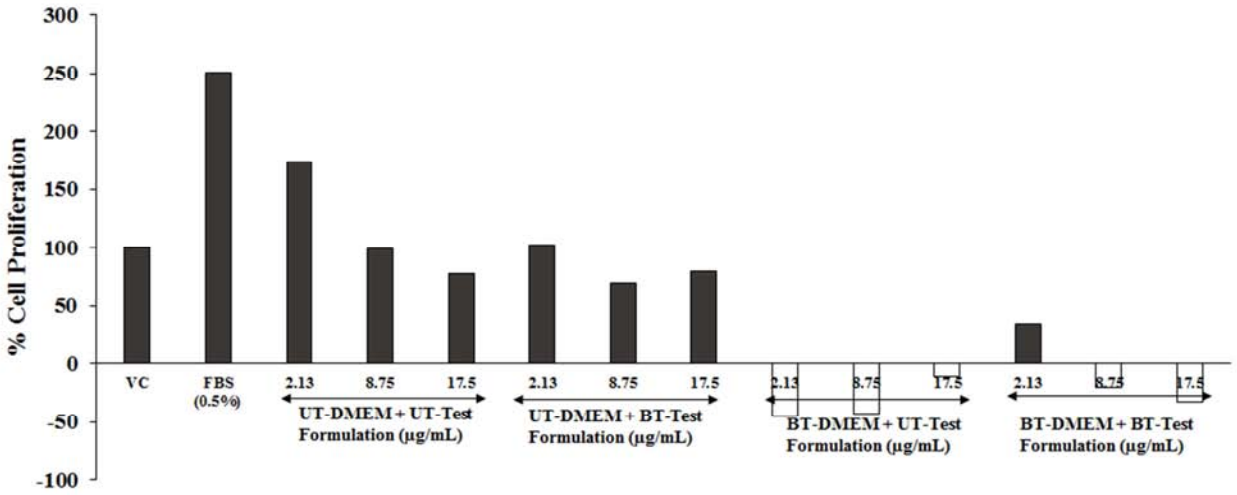

Figure 2. *Effect of the Biofield Energy Treated test formulation with DMEM on cellular proliferation in HFF-1 cells after 48 hours. VC: Vehicle control; FBS: Fetal bovine serum; UT: Untreated; BT: Biofield Treated.*

3.3. Analysis of Extracellular Matrix Component Synthesis

The extra cellular matrix components (ECM) were assessed in order to identify the skin strength, hydration level and overall elasticity using the combination of the Biofield Energy Treated and untreated test formulation/DMEM in HFF-1 cell line. The analysis outcomes of ECM are presented as collagen, elastin, and hyaluronic acid levels.

3.3.1. Collagen Analysis

The effect of the Biofield Energy Treated test formulation on the collagen level showed a significant increase in the collagen content at various experimental test formulation concentrations on HFF-1 cell line. Collagen is the most abundant protein and major skin components for skin health, structure and most abundant fibrous protein present in ECM. Experimental results of collagen with respect to ascorbic acid and Biofield Energy Treated test formulation/DMEM groups are presented in Figure 3. Ascorbic acid (10 µM) showed a significant increase collagen content by 55%, while the Biofield Energy Treated test formulation at selected concentration range from 0.625 to 2.5 µg/mL reported with the significant increased percentage of collagen amount.

However, among the test formulation groups, UT-DMEM + BT-Test formulation group reported with significant increase in the collagen level at 2.5, 1.25 and 0.625 µg/mL by 32.42%, 33.64%, and 29.13%, respectively compared to the UT-DMEM + UT-Test formulation group. Similarly, in the BT-DMEM + UT-Test formulation group at concentration 2.5, 1.25 and 0.625 µg/mL showed an increased collagen level by 27.14%, 18.20%, and 14.46%, respectively. However, in BT-DMEM + BT-Test formulation group, at 2.5, 1.25 and 0.625 µg/mL the collagen level was increased by 34.17%, 26.73%, and 17.56%, respectively compared with the UT-DMEM + UT-Test formulation group. Experimental data showed that after the Biofield Energy Healing Treatment, a significant increase in the collagen level was observed at all the tested concentrations. It can be suggested that collagen synthesis was improved after Biofield Energy Healing based test formulation with respect to the respective untreated groups. Hence, it can be assumed that procollagen peptides and cross-linking (aldol reaction) among various tropocollagen molecules was increased after Biofield Treatment, which results in improvement of the collagen fibrils. Fibrils are required to provide strength and structure to the skin [35, 36]. The study data suggest that the Biofield Energy (The Trivedi Effect®) treated test formulation, showed a remarkable increase in the collagen level that might be useful for application in skin health, strength and structure, and wound healing.

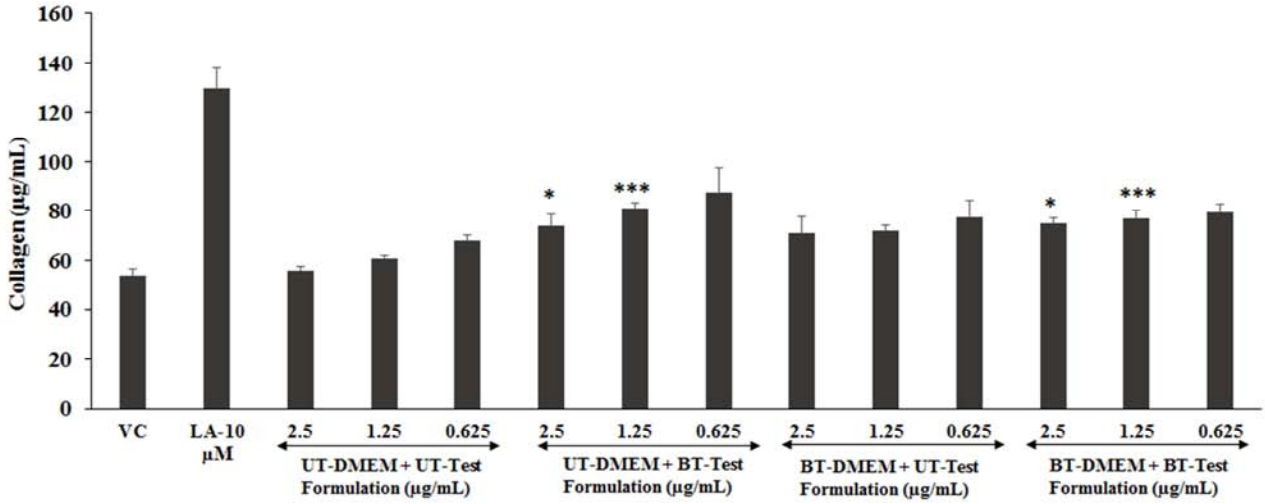

Figure 3. *Concentration-dependent effects of test formulation on human dermal fibroblast (HFF-1) cell line for collagen level. $^{***}p\leq0.001$ and $^{*}p\leq0.05$ statistical comparison with respect to untreated DMEM and untreated test formulation using one way ANOVA (Dunnett's test). VC: Vehicle control; LA-10: L-Ascorbic acid at 10 µM concentration; UT: Untreated; BT: Biofield Treated.*

3.3.2. Assessment of Elastin

The effect of the Biofield Energy Healing on the test formulation was evaluated for change in the elastin level. Elastin helps to retain shape in body tissue and very elastic tissue of the body. Elastin is one of the important part of the ECM, it forms tight junction with collagen fibrils, which helps to maintain the cellular integrity [37]. The current data (Figure 4.) exhibited a significant enhancement in the elastin synthesis in the Biofield Energy Healing based test formulation in HFF-1 cell line. Ascorbic acid (50 µM) group showed a significant increased elastin content by 55% compared with the normal control group. However, among other tested groups, UT-DMEM + BT-Test formulation group displayed highly significant increased elastin level by 408.6% at concentrations 0.625 µg/mL compared with UT-DMEM + UT-Test formulation group. Other experimental groups were reported with altered level of elastin level after treatment with the Biofield Energy Treated Test formulation compared with the untreated groups. This suggests the importance of the Biofield Energy Healing based Test formulation as an anti-ageing formulation, as fibroblast and elastin are responsible for ageing and health. Data suggests a significant increased elastin level, which represented enhanced elasticity and strength of the skin with activated dermal metabolism. 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 that helps to improve cell growth, survival, differentiation and morphogenesis.

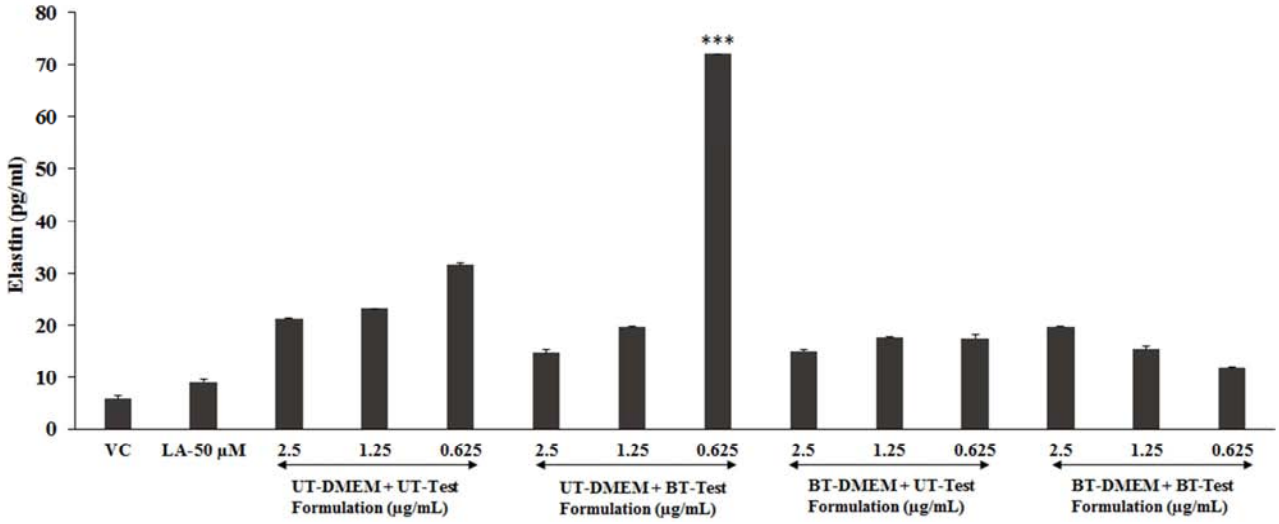

Figure 4. *Concentration-dependent effect of the Biofield Energy Treated test formulation on human dermal fibroblast (HFF-1) cell line for elastin level. $^{***}p\leq0.001$ statistical comparison with respect to untreated DMEM and untreated test formulation using one way ANOVA (Dunnett's test). VC: Vehicle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

3.3.3. Analysis of Hyaluronic Acid

Hyaluronic acid (HA) is a natural glycosaminoglycan (polysaccharides) and regarded as one of the major connective tissue. Besides, HA is distributed extensively all over the connective, neural, and epithelial tissue and it can be derived from multiple resources, foods, supplements, HA powders, etc. It helps to retain skin moisture, secures moisture, creates fullness, and regulates the skin water balance. Skin care cosmetics found many products such as hyaluronic acid creams, serums, injectable, and hyaluronic acid supplements. Lower level of HA results in loss of skin elasticity and expose the signs of aging, while it plays a critical role in skin health due to its high and unique ability to hold the moisture. These all parameters defined HA as "smart nutrient" due to its ability to adjust moisture with high absorption rate. The level of hyaluronic acid after the Biofield Energy Treatment in test formulation was evaluated in the HFF-1 cell line (Figure 5). The results of ascorbic acid showed a significant increase in the hyaluronic acid content by 183.6%. However, untreated test formulation/DMEM group showed a significant change in HA levels in all the groups with respect to normal control group. After Biofield Energy Treatment, UT-DMEM + BT-Test formulation group, at 0.625 µg/mL showed an increase in HA level by 4.76%. In addition, BT-DMEM + UT-Test formulation group presented significant increased HA level by 29.71% at 0.625 µg/mL with respect to the UT-DMEM + UT-Test formulation group. Further, in BT-DMEM + BT-Test formulation group at 2.5, 1.25, and 0.625 µg/mL HA levels were significantly increased by 31.88%, 15.52%, and 58.29%, respectively compared with the UT-DMEM + UT-Test formulation group. Overall, results showed a significantly improved HA level using the Biofield Energy Healing based test formulation would be a new approach in cosmetology. HA based skin car product are widely available in the market for skin health [38]. However, the Biofield Energy Healing (The Trivedi Effect®) based test formulation and DMEM could be used as better alternative to improve the content of hyaluronic acid along with various skin disorders.

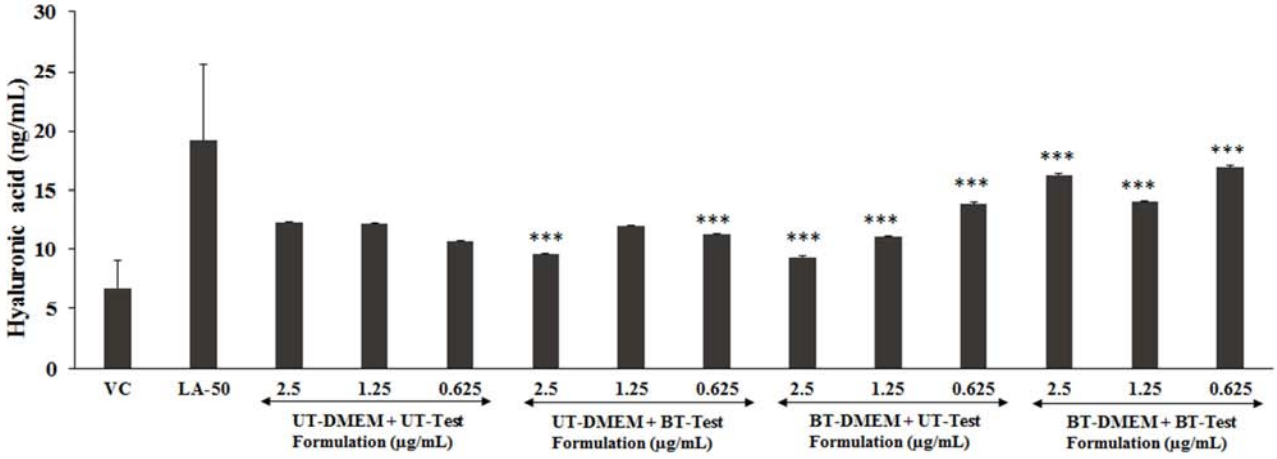

Figure 5. *Synthesis of extracellular matrix component, hyaluronic acid by the Biofield Energy Treated Test formulation in human dermal fibroblasts (HFF-1) cell lines. ***p≤0.001 statistical comparison with respect to untreated DMEM and untreated test formulation using one way ANOVA (Dunnett's test). VC: Vehicle control; LA-50: L-Ascorbic acid at 50 µM concentration; UT: Untreated; BT: Biofield Treated.*

3.4. Estimation of Melanin Synthesis Inhibition

Melanin is a brown pigment that results in different shades and colors of human skin. However, amount of melanin in the skin defines the skin pigmentation. Depigmentation results in various disorders, while skin coloration also depends upon the genetic makeup. Moreover, sun ultraviolet radiations (UV-A and UV-B) initiated the process of melanogenesis in the melanocytes results in skin darkening [39]. The Biofield Energy based test formulation were cultured in DMEM supplemented media for melanogenesis on mouse melanoma (B16-F10) cells containing several concentrations along with positive control, kojic acid (10 µM) for 48 to 96 hours. The results of percentage decrease in α-MSH induced melanin synthesis in all the experimental groups are presented in Figure 6. Kojic acid, a skin whitening compound was used as positive control showed a significant decrease in the level of melanin synthesis by 188.2% compared with stimulated in presence of alpha melanocyte stimulating hormone group. In addition, the Biofield Energy based test formulation exhibited a significant decrease in the melanin synthesis by 8.24% and 16.09% in the UT-DMEM + BT-Test formulation group at 0.0625 and 0.125 µg/mL respectively, compared with the UT-DMEM + UT-Test formulation group. Moreover, at 0.125 µg/mL, BT-DMEM + UT-Test formulation group showed a significant decreased melanin synthesis by 18.93% compared with the UT-DMEM + UT-Test formulation group. However, at 0.0625 µg/mL in the BT-DMEM + BT-Test formulation group, melanin synthesis was decreased by 3.58% compared with the UT-DMEM + UT-Test formulation group in B16-F10 melanoma cell line. The Biofield Energy Treated Test formulation significantly decreased the level of melanin synthesis. The results suggest that the Biofield Energy Treated test formulation might alter and inhibit the tyrosinase enzymes activity, which is mainly responsible for melanin synthesis

[40]. Skin whitening action of most of the cosmetic formulation are based on melanin inhibition, however the chemicals have several side effects. However, the test formulation is the combination of minerals, *Centella asiatica* extract and THC, which were reported to have significant effect against skin infection, inflammatory dermatoses, with strong antioxidant action [41, 42]. Therefore, it can be concluded that the Biofield Energy Healing based test formulation and DMEM would be useful approach to decrease the melanogenesis process and would be a novel approach for skin-related disorders with significant skin whitening action.

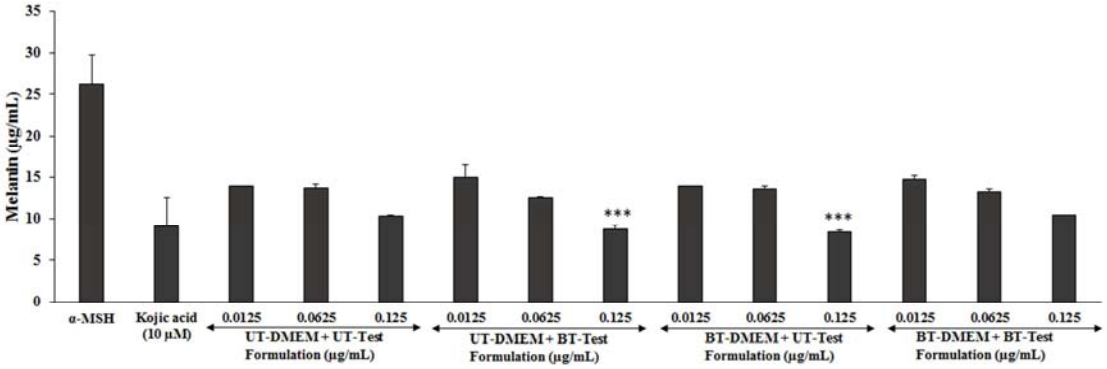

Figure 6. *Inhibitory effect of the Biofield Energy Treated test formulation on melanogenesis (skin whitening potential) in mouse melanoma (B16-F10) cell line. ***p≤0.001 statistical comparison with respect to untreated DMEM and untreated test formulation using one way ANOVA (Dunnett's test). α-MSH: Alpha melanocyte stimulating hormone, UT: Untreated; BT: Biofield Treated.*

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

HFF-1 cells are used to study the impact of the Biofield Energy Healing based test formulation in cell viability potential and stress was induced due to UV-B. Various skin related diseases are reported due to exposure of UV-B induced such as skin disorders, stress, free radical generation, etc. This results in downregulation of the human skin fibroblasts through various inflammatory responses, DNA damage, wrinkles and skin-ageing [43]. Anti-wrinkling effects of cell viability from UV-B rays is presented in Figure 7. 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 identified. The HFF-1 cells while exposure of UV-B reported with high degree of cell death with approximately 25.21% of cell viability. The cell viability in vehicle control (DMSO) group was found as 20.51% due to UV-B irradiation (200 mJ/cm^2) (Figure 7). However, ascorbic acid (50 µM) showed a significant increase in the cell viability by 110.48%. In addition, the experimental groups showed that all the tested groups in specified concentrations reported with improve cell viability. Among the tested groups, at 2.5 µg/mL in the UT-DMEM + BT-Test formulation and BT-DMEM + UT-Test formulation groups showed an increase cell viability by 5.37% and 3.15%, respectively compared with the UT-DMEM + UT-Test formulation group. Similarly, in BT-DMEM + BT-Test formulation group at 1.25 and 0.625 µg/mL showed an increase cell viability by 5.49% and 11.26%, respectively compared with the UT-DMEM + UT-Test formulation group. UV-B results in loss in cell viability and cause skin damage, however results suggest improved cell viability by Biofield Energy Healing based test formulation. Therefore, it can be predictable that the Biofield Energy Treated test formulation and DMEM would be a better alternative treatment for skin protection and cell viability against UV-B radiations and can be effectively used as anti-wrinkling action.

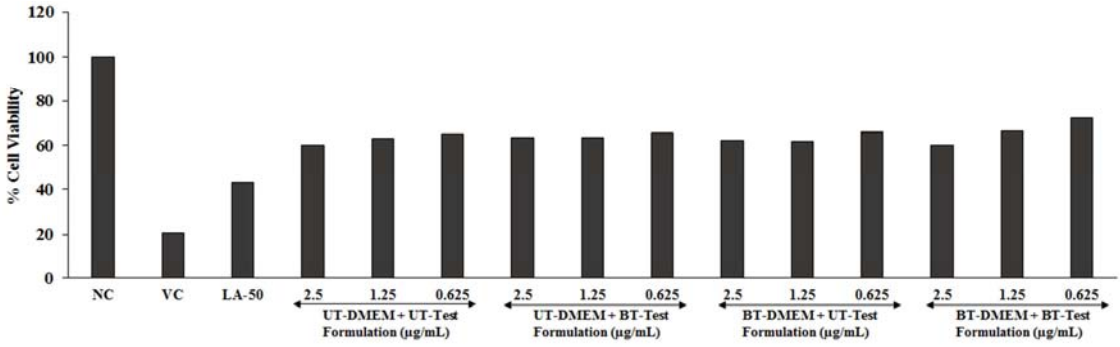

Figure 7. *Anti-wrinkling potential and cytoprotective potential of the Biofield Energy Treated test formulation against UV-B induced stress in human dermal fibroblasts (HFF-1) cell lines. % cell viability of HFF-1 cells after treatment in various groups. NC: Normal control; VC: Vehicle control; LA-50: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

3.6. Wound-Healing Scratch Assay

Wound healing scratch assay was performed in HFF-1 and HaCaT cells with different combinations of the Biofield Energy Treated test formulation/DMEM. The representative HFF-1 and HaCaT cells migration photographs in different groups were monitored and shown in Figure 8. The effect of the Biofield Energy Treated test formulation/DMEM determined the cellular migration, and defines the cell-to-cell and cell-to-matrix interactions during wound healing process [45]. Higher rate of cell migration was reported at 16 hours in the presence of positive control, EGF group compared with vehicle control group (Figure 8). However, in UT-DMEM + BT-Test formulation group at 10 µg/mL showed a significant cellular migration rate in HFF-1 cells, while in HaCaT cells, BT-DMEM + BT-Test formulation group showed an effective healing and migration rate compared with other tested groups. Overall, the scratched monolayer in both cells lines showed a significant migration after treatment with the Biofield Energy Treated test formulation/DMEM compared with the untreated test formulation/DMEM group. Experimental data suggest that the percent cell covered area in the Biofield Energy Treated test formulation group was significantly higher with improved healing rate compared with the Biofield Energy Treated DMEM. HaCaT and HFF-1 cells showed upto 5% increase in wound coverage area in the BT-DMEM + BT-Test formulation group, with respect to the UT-DMEM + UT-Test formulation group. The percentage cell coverage area due to the Biofield Treated test formulation/DMEM in HFF-1 cells with respect to the concentration of untreated test formulation in untreated medium ranged from 2% to 10% in UT-DMEM + BT-Test formulation group. Similarly, wound healing rates in HaCaT cells were found with 1.5% to 8% in BT-DMEM + BT-Test formulation group compared with the untreated group. The results after treatment of wound healing scratch assay in EGF group as shown in Figure 8 (b), normal control group in Figure 8 (a). Similarly, in the UT-DMEM + BT-Test formulation and BT-DMEM + UT-Test formulation experimental group showed a significant rate of cellular migration rate along with wound closure as shown in Figure 8 (d, e, f), compared with the UT-Test formulation + UT-DMEM group (Figure 8 c).

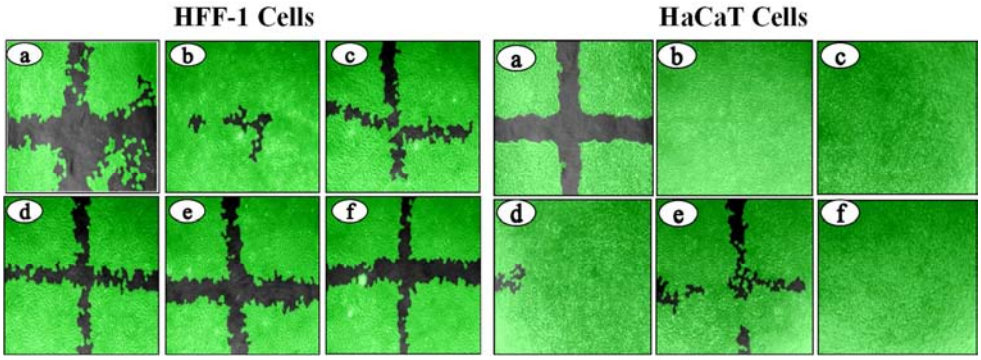

Figure 8. *Representative pictures of HFF-1 and HaCaT cell migration cells after induction of a scratch. All the pictures were taken immediately after the scratch was induced (i.e. at 0 hours), after 16 hours in the presence of EGF and Biofield Energy Treated test formulation. Pictures are taken at 50 times magnification. Images represents HFF-1 cells migration in presence of (a) baseline control media, (b) 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.*

Therefore, it can be concluded that the Biofield Energy Healing (The Trivedi Effect®) based test formulation has the capacity to significantly improved cellular migration, which can be used to improve wound healing activity. However, enhanced collagen deposition due to The Trivedi Effect®- Consciousness Energy Healing Treatment results in improved strength, integrity and structure of the skin. It might be suggested Biofield Energy Treatment showed an improved wound healing with some effective antioxidant mechanism.

4. Conclusions

Herbal based products have the potential to improve various skin diseases. Currently, the world population depends on alternative health care system for curing skin related problems, although there is a movement towards seeking alternative, natural approaches for treatment. The Trivedi Effect®-Consciousness Energy Healing Treatment based novel test formulation and DMEM showed a significant outcomes, such as MTT assay displayed significant cell viability in HFF-1, HaCaT and B16-F10 cells lines with more than 89% viable cells in the Biofield Energy Healing based herbomineral test formulation. Besides, BrdU assay exhibited significant high rate of cellular proliferation in the UT-DMEM + BT-Test formulation group. Collagen synthesis was improved by 32.42%, 27.14% and 34.17% in the UT-DMEM + BT-Test formulation, BT-DMEM + UT-Test formulation, and BT-DMEM + BT-Test formulation groups, respectively at 2.5 µg/mL compared with the UT-DMEM + UT-Test formulation group. The elastin level was found to be increased by 408.6% at concentrations 0.625 µg/mL in the UT-DMEM + BT-Test formulation group compared with the UT-DMEM + UT-Test formulation groups. However, the amount of hyaluronic acid was increased by 29.71%

and 58.29% in the BT-DMEM + UT-Test formulation and BT-DMEM + BT-Test formulation groups, respectively at concentration 0.625 µg/mL compared with the UT-DMEM + UT-Test formulation group. Melanin content was inhibited by 16.09% and 18.93% in the UT-DMEM + BT-Test formulation and BT-DMEM + UT-Test formulation group, respectively at 0.125 µg/mL compared with the UT-DMEM + UT-Test formulation group in B16-F10 melanoma cell line. However, the Biofield Treated Test formulation showed a significantly improved anti-wrinkling effects using UV-B induced stress in HFF-1 cells by 5.49% and 11.26% in the BT-DMEM + BT-Test formulation group at 1.25 and 0.625 µg/mL, respectively compared with the UT-DMEM + UT-Test formulation group. Wound healing scratch assay results validated significant migration of fibroblast and keratinocytes cells with increase covered area up to 10% in the HFF-1 and HaCaT cells after treatment with the Biofield Energy based test formulation and DMEM. Overall, the study results concluded that the Biofield Energy Healing based test formulation and DMEM could be used to improve various skin disorders and ageing.

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, chickenpox, measles, warts, acne, hives, wrinkles, ringworm, Rosacea, psoriasis, seborrheic dermatitis, skin cancer, rashes from bacterial or fungal infections, rashes from allergic reactions, raised bumps that are red or white, cracked skin, discolored patches of skin, fleshy bumps, warts, or other skin growths, changes in mole color or size, a loss of skin pigment, scaly or rough skin, peeling skin, ulcers, open sores or lesions, dry, excessive flushing. Further, the Biofield Energy Healing based herbomineral test formulation can also be used in the prevention of temporary and permanent skin disorders, anti-aging, improved overall health, and quality of life.

Abbreviations

THC: Tetrahydrocurcumin; ECM: Extracellular matrix; EGF: Epidermal growth factor; DMEM: Dulbecco's Modified Eagle's Medium; α-MSH: α-Melanocyte-stimulating hormone; ANOVA: Analysis of variance; HA: Hyaluronic acid; HFF-1: Human foreskin fibroblast cell line, UV-B: Ultra violet B rays; CAM: Complementary and alternative medicine; NCCAM: National Center for Complementary and Alternative Medicine.

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References

[1] Tabassum N, Hamdani M (2014) Plants used to treat skin diseases. Pharmacogn Rev 8: 52-60.

[2] Goyal RK (2005) Investigation of cellular and molecular mechanisms for anti-diabetic drugs with special reference to Unani and Ayurvedic herbal medicines. In: traditional system of medicine, Abdin, M. Z. and Y. P. Abrol (Eds.). Narosa Publishing House, New Delhi.

[3] WHO (1993) Research Guideline for Evaluating the Safety and Efficacy of Herbal Medicines. World Health Organization, Manila, Philippines.

[4] Gao XH, Zhang L, Wei H, Chen HD (2008) Efficacy and safety of innovative cosmeceuticals. Clin Dermatol 26: 367-74.

[5] Fabricant DS, Farnsworth NR (2001) The value of plants used in traditional medicine for drug discovery. Environ Health Perspect 109: 69-75.

[6] Baumann L (2007) Skin ageing and its treatment. J Pathol 211: 241-251.

[7] Uitto J (1997) Understanding premature skin aging. N Engl J Med 337: 1463-1465.

[8] Park K (2015) Role of micronutrients in skin health and function. Biomol Ther (Seoul) 23: 207-217.

[9] Hashim P (2011) Centella asiatica in food and beverage applications and its potential antioxidant and neuroprotective effect. Int Food Res J 18: 1215-1222.

[10] Boyera N, Galey I, Bernard BA (1998) Effect of vitamin C and its derivatives on collagen synthesis and cross-linking by normal human fibroblasts. Int J Cosmet Sci 20: 151-158.

[11] Gupta SC, Patchva S, Koh W, Aggarwal BB (2012) Discovery of curcumin, a component of golden spice, and its miraculous biological activities. Clin Exp Pharmacol Physiol 39: 283-299.

[12] Majeed M, Badmaev V, Uma S, Rajenderan JR (1995) Curcuminoids: Antioxidant Phytonutrients, Nutreiscience publishers New Jersey 1-24.

[13] Sugiyama Y, Kawakishi S, Osawa T (1996) Involvement of the β-diketone moiety in the antioxidant mechanism of tetrahydrocurcuminoids. Biochem Pharmacol 52: 519-525.

[14] Bylka W, Znajdek-Awiżeń P, Studzińska-Sroka E, Brzezińska M (2013) Centella asiatica in cosmetology. Postepy Dermatol Alergol 30: 46-49.

[15] Rubik B, Muehsam D, Hammerschlag R, Jain S (2015) Biofield science and healing: history, terminology, and concepts. Glob Adv Health Med 4: 8-14.

[16] Barnes PM, Bloom B, Nahin RL (2008) Complementary and alternative medicine use among adults and children: United States, 2007. Natl Health Stat Report 12: 1-23.

[17] Jain S, Ives J, Jonas W, Hammerschlag R, Muehsam D, Vieten C, Vicario D, Chopra D, King RP, and Guarneri E (2015) Biofield Science and Healing: An Emerging Frontier in Medicine. Glob Adv Health Med 4: 5-7.

[18] Peck SD (1998) The efficacy of therapeutic touch for improving functional ability in elders with degenerative arthritis. Nurs Sci Q 11: 123-132.

[19] Schlitz M, Hopf HW, Eskenazi L, Vieten C, Radin D (2012) Distant healing of surgical wounds: An exploratory study. Explore (NY) 8: 223-230.

[20] Trivedi MK, Branton A, Trivedi D, Nayak G, Shettigar H, Mondal SC, Jana S (2015) Antibiogram pattern of Shigella flexneri: Effect of biofield treatment. Air Water Borne Diseases 3: 122.

[21] Trivedi MK, Patil S, Shettigar H, Mondal SC, Jana S (2015) Antimicrobial susceptibility pattern and biochemical characteristics of Staphylococcus aureus: Impact of biofield treatment. J Microb Biochem Technol 7: 238-241.

[22] Trivedi MK, Branton A, Trivedi D, Nayak G, Shettigar H, Mondal SC, Jana S (2015) Effect of biofield energy treatment on Streptococcus group B: A postpartum pathogen. J Microb Biochem Technol 7: 269-273.

[23] Trivedi MK, Branton A, Trivedi D, Nayak G, Gangwar M, Jana S (2015) Morphological and molecular analysis using RAPD in biofield treated sponge and bitter gourd. American Journal of Agriculture and Forestry 3: 264-270.

[24] Trivedi MK, Branton A, Trivedi D, Nayak G, Gangwar M, Jana S (2015) Effect of biofield energy treatment on chlorophyll content, pathological study, and molecular analysis of cashew plant (Anacardium occidentale L.). Journal of Plant Sciences 3: 372-382.

[25] Trivedi MK, Branton A, Trivedi D, Nayak G, Gangwar M, Jana S (2016) Molecular analysis of biofield treated eggplant and watermelon crops. Adv Crop Sci Tech 4: 208.

[26] Trivedi MK, Branton A, Trivedi D, Nayak G, Mondal SC, Jana S (2015) Effect of biofield treated energized water on the growth and health status in chicken (Gallus gallus domesticus). Poult Fish Wildl Sci 3: 140.

[27] Trivedi MK, Nayak G, Patil S, Tallapragada RM, Latiyal O, Jana S (2015) An evaluation of biofield treatment on thermal, physical and structural properties of cadmium powder. J Thermodyn Catal 6: 147.

[28] Trivedi MK, Nayak G, Patil S, Tallapragada RM, Latiyal O, Jana S (2015) Effect of Biofield energy treatment on physical and structural properties of calcium carbide and praseodymium oxide. International Journal of Materials Science and Applications 4: 390-395.

[29] Trivedi MK, Tallapragada RM, Branton A, Trivedi D, Nayak G, Latiyal O, Jana S (2015) Characterization of physical, thermal and structural properties of chromium (VI) oxide powder: impact of biofield treatment. J Powder Metall Min 4: 128.

[30] Plumb JA (2004) Cell sensitivity assays: the MTT assay. Methods Mol Med 88: 165-169.

[31] Hahn MS, Kobler JB, Starcher BC, Zeitels SM, Langer R (2006) Quantitative and comparative studies of the vocal fold extracellular matrix. I: Elastic fibers and hyaluronic acid. Ann Otol Rhinol Laryngol 115: 156-164.

[32] Zhang L, Yoshida T, Kuroiwa Y (1992) Stimulation of melanin synthesis of B16-F10 mouse melanoma cells by bufalin. Life Sci 51: 17-24.

[33] Shoulders MD, Raines RT (2009) Collagen structure and stability. Annual review of biochemistry 78: 929-958.

[34] Fronza M, Heinzmann B, Hamburger M, Laufer S, Merfort I (2009) Determination of the wound healing effect of Calendula extracts using the scratch assay with 3T3 fibroblasts. J Ethnopharmacol 126: 463-467.

[35] Kadler KE, Holmes DF, Trotter JA, Chapman JA (1996) Collagen fibril formation. Biochem J 316: 1-11.

[36] Shoulders MD, Raines RT (2009) Collagen structure and stability. Annual review of biochemistry 78: 929-958.

[37] Frantz C, Stewart KM, Weaver VM (2010) The extracellular matrix at a glance. J Cell Sci 123: 4195-4200.

[38] Weindl G, Schaller M, Schäfer-Korting M, Korting HC (2004) Hyaluronic acid in the treatment and prevention of skin diseases: molecular biological, pharmaceutical and clinical aspects. Skin Pharmacol Physiol 17: 207-213.

[39] Alaluf S, Atkins D, Barrett K, Blount M, Carter N, Heath A. (2002) The impact of epidermal melanin on objective measurements of human skin colour. Pigment Cell Res. 15: 119-126.

[40] Busca R, Ballotti R (2000) Cyclic AMP a key messenger in the regulation of skin pigmentation. Pigment Cell Res 13: 60-69.

[41] Bylka W, Znajdek-Awiżeń P, Studzińska-Sroka E, Brzezińska M (2013) Centella asiatica in cosmetology. Postepy Dermatol Alergol 30: 46-49.

[42] Prasad S, Tyagi AK, Aggarwal BB (2014) Recent developments in delivery, bioavailability, absorption and metabolism of curcumin: the golden pigment from golden spice. Cancer Res Treat 46: 2-18.

[43] Ho JN, Lee YH, Lee YD, Jun WJ, Kim HK, Hong BS, Shin DH, Cho HY (2005) Inhibitory effect of Aucubin isolated from Eucommia ulmoides against UVB induced matrix metalloproteinase-1 production in human skin fibroblasts. Biosci Biotechnol Biochem 69: 2227-2231.

[44] Liang CC, Park AY, Guan JL (2007) In vitro scratch assay: A convenient and inexpensive method for analysis of cell migration in vitro. Nat Protoc 2: 329-333.