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Antiaging Potential of Consciousness Energy Healing-Based Novel Proprietary Formulation

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Abstract

The study objective was to evaluate the potential of Biofield Treatment on novel proprietary formulation for skin health using HFF-1, HaCaT, and B16-F10 cells. The test formulation and DMEM were divided into two parts. One part received Biofield Treatment by Gopal Nayak and defined as Biofield Treated (BT) sample, while other part was denoted as untreated (UT) sample. MTT assay showed more than 70% cell viability, suggested the test formulation were safe and nontoxic. BrdU data showed an increased cell proliferation by 170%, 170%, and 212% at 0.1 µg/mL in UT-DMEM + BT-Test formulation, BT-DMEM + UT-Test formulation, and BT-DMEM + BT-Test formulation, respectively than untreated group. Collagen was significantly increased by 140.8% at 5 µg/mL in UT-DMEM + BT-Test formulation than untreated. Elastin was significantly increased by 233.3%, 313.3%, and 637.8% at 0.01 µg/mL in UT-DMEM + BT-Test formulation, BT-DMEM + UT-Test formulation, and BT-DMEM + BT-Test formulation, respectively than untreated. Hyaluronic acid in UT-DMEM + BT-Test formulation, BT-DMEM + UT-Test formulation, and BT-DMEM + BT-Test formulation was significantly increased by 191.8%, 461.0%, and 322.5%, respectively at 0.01 µg/mL than untreated. Melanin synthesis was significantly inhibited by 44.7% (10 µg/mL) and 45.2% (10 µg/mL) in UT-DMEM + BT-Test formulation and BT-DMEM + UT-Test formulation, respectively contrast to untreated. Anti-wrinkling activity was improved with increased cell viability by 88.9% and 92.9% at 1 and 25 µg/mL, respectively in BT-DMEM + UT-Test formulation than untreated. Cell viability was significantly increased by 97.6% and 75.16% at 0.1 and 1 µg/mL, respectively in BT-DMEM + BT-Test formulation than untreated. The UT-DMEM + BT-Test formulation showed increased cellular migration by 2722.2% (0.01 µg/mL) in HaCaT cells than untreated. Overall, result suggests that the Trivedi Effect® has shown significant beneficial effects on skin health parameters that might lead to its potential use in various aging-related disorders, psoriasis, seborrheic dermatitis, skin cancer, rashes from bacterial or fungal infections, and many more skin diseases.

Keywords: Aging, Consciousness Energy Healing, Minerals, Vitamins, Extracellular Matrix, HaCaT, HFF-1, Hyaluronic Acid, Wound Healing, Skin Health

Introduction

Skin is the heaviest organ of the human body and acts as a barrier between the internal and external environment, and. It protects against mechanical and chemical damages, different micro-organisms, and the damage caused to skin by ultraviolet rays. Skin originates from epithelial, connective, vascular, muscular and nervous tissues and is well organized in three layers *viz.* epidermis, dermis, and hypodermis [1]. Skin have very rich vascular network except epidermis, which involved wound healing, immune reactions, thermoregulations, tissues feeding, and blood pressure control. Ultraviolet rays damage the skin and can cause cancer on exposure of UV-B rays, which results huge oxidative stress, tissue inflammation, erythema, breakdown of the extracellular matrix (*i.e.* elastin, collagen and hyaluronic acid), wrinkling, and aging [2, 3]. In order to maintain the skin health, specific nutrition is considered as one of the major factor to modulate various skin parameters. Malnutrition has reported significant impact on skin health, which results in skin changes such as xerosis, hair effluvium, nail modifications, and many more. Along with specific diet and supplements, various alternative medicines are now present worldwide, which has been reported with significant

improvement in skin functioning, strong anti-oxidative potential, anti-photo aging, and also effective against photo damage of the skin [4]. Skin health products have been available in the market and are accepted worldwide, however, it might be associated with the serious health complications. Besides, skin health products in combination with the minerals and vitamins such as zinc chloride, ferrous sulfate, copper chloride, magnesium gluconate, pyridoxine HCl, vitamin B_{12}, and vitamin D_3 are widely available and are reported for significant skin health [5-9]. Thus, a novel skin health formulation was designed which consist of 14 ingredients, calcium chloride, panax ginseng extract, vitamin B_{12}, vitamin E, beta carotene, vitamin D_3, zinc chloride, magnesium gluconate, sodium selenate, ferrous sulfate, copper chloride, ascorbic acid, vitamin B_6 and CBD. Further, the novel test formulation was treated with Biofield Energy Healing by a renowned Biofield Energy Healer, Gopal Nayak. The Biofield Energy Treated test formulation and the cell line media (DMEM) was tested against skin health cell lines such as HFF-1, HaCaT, and B16-F10 cells.

Biofield Energy Healing Therapy is accepted as a unifying concept under Complementary and Alternative Medicine (CAM), which have shown significant clinical benefits. Over the past few decades, many Energy Healing Therapies have reported with significant clinical and non-clinical fields outcomes [10, 11]. Energy Therapies has been practiced and accepted by the U.S. population with reported advantages according to the National Center for Complementary and Alternative Medicine (NCCAM) compared with the modern treatment approaches [12, 13]. CAM therapies such as Qi Gong, external qigong, Tai Chi, Johrei, guided imagery, polarity therapy, Reiki, pranic healing, therapeutic touch, yoga, chiropractic/osteopathic manipulation, meditation, deep breathing, massage, homeopathy, acupressure, hypnotherapy, progressive relaxation, special diets, acupuncture, Rolfing structural integration, movement therapy, healing touch, pilates, mindfulness, relaxation techniques, Ayurvedic medicine, traditional Chinese herbs and medicines in biological systems both *in vitro* and *in vivo*. The Trivedi Effect®-Consciousness Energy Healing therapies have been widely accepted and reported worldwide in nonliving materials and living organisms. Consciousness Energy Healing Treatment found to be significant to improve the metal physicochemical properties [14-16], agriculture science [17, 18], life science in microbiology [19-21], biotechnology [22, 23], improved bioavailability of compounds [24-26], improved skin health [27, 28], nutraceuticals [29, 30], cancer science research [31, 32], improved overall bone health [33-35], human health and wellness. Due to its wide applications, the efficacy of the Biofield Energy Treated Proprietary test formulation was evaluated for the various skin aging parameters in three different cell lines such as HFF-1, HaCaT, and B16-F10 and compared with the untreated test formulation.

Materials and Methods

Chemicals and Reagents

Panax ginseng extract was purchased from Panacea phytoextracts, India. Vitamin E and sodium selenate were procured from Alfa Aesar, India. Zinc chloride, magnesium (II) gluconate hydrate, cyanocobalamin (vitamin B_{12}), beta carotene, and pyridoxine hydrochloride (vitamin B_6) were purchased from TCI, Japan. CBD was obtained from Standard Hemp Company, USA. Iron (II) sulphate, calcium chloride, copper chloride, L-ascorbic acid, and cholecalciferol (vitamin D_3) were purchased from Sigma-Aldrich, USA. ELISA kits were procured from CUSABIO and CusAb Co. Pvt. Ltd., USA. Fetal bovine serum (FBS), epidermal growth factor (EGF) and Dulbecco's Modified Eagle's Medium (DMEM) were purchased from Gibco, ThermoFisher, USA. Antibiotics solution was purchased from HiMedia, India, while 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium) (MTT), direct red 80 and ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma, USA. All the other chemicals used in this experiment were analytical grade procured from India.

Cell Culture

For the evaluation of skin health, three cell lines were used *viz.* HFF-1 (human foreskin fibroblast) cells were originated from normal human skin fibroblast cells and obtained from American Type Culture Collection (ATCC), USA. B16-F10 (mouse melanoma) and HaCaT (human keratinocytes) cells were procured from National Centre for Cell Science (NCCS), Pune, India. All the three 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). Standard cell line growth conditions were maintained such as 37°C, 5% CO_2, and 95% humidity. Positive controls were used specifically such as L-ascorbic acid, used for ECM, UV-B protection, and wound healing assay, while kojic acid was used for melanin estimation at different concentrations range. FBS (0.5%) was used in cell proliferation assay in BrdU assay, while EGF was used in non-cytotoxic dose concentration in MTT assay [25].

Experimental Design

The experimental test groups in different skin health parameters were divided into normal control group, vehicle control group (0.05% DMSO), positive control group, and the experimental tested groups at different safe concentrations. The experimental test groups included the combination of the Biofield Energy Treated (BT) and untreated (UT) test formulation along with DMEM. The combination of test groups included the UT-DMEM + UT-Test formulation, UT-DMEM + Biofield Treated Test formulation (BT-Test formulation), BT-DMEM + UT-Test formulation, and BT-DMEM + BT-Test formulation [27].

Energy of Consciousness Treatment Strategies

The experimental test formulation of skin health was a combination of 14 ingredients *viz.* calcium chloride, panax ginseng extract, vitamin B_{12}, vitamin E, beta carotene, vitamin D_3, zinc chloride, magnesium gluconate, sodium selenate, ferrous sulfate, copper chloride, ascorbic acid, vitamin B_6, and cannabidiol isolate (CBD). The test formulation and DMEM were divided into two parts, one part of the test sample was treated with Biofield Energy (also known as the Trivedi Effect®) by a renowned Biofield Energy Healer and were coded as the Biofield Energy Treated DMEM, while the second part did not receive any sort of treatment in the control group. This Biofield Energy Healing Treatment was provided by Gopal Nayak remotely. Biofield Energy Healer was remotely located in the USA, while the test item was located in the research laboratory of Dabur Research Foundation, New Delhi, India. This Biofield Energy Treatment was administered for 5 minutes through the Healer's unique Energy Transmission process remotely to the test sample under laboratory conditions. Gopal Nayak in this study never visited the laboratory in person, nor had any contact with the test samples. 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 the experimental study [28].

Estimation of Non-cytotoxic Concentrations

The non-cytotoxic concentrations of test samples were estimated in all the three cell lines using MTT assay. All the cells were 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. All the cells were incubated overnight under specific growth conditions, which were allowed for cell recovery and exponential growth followed by 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 (1 to 200 µg/mL) and ascorbic acid (50 and 100 µM) followed by incubation from 24 to 72 hours in a CO_2 incubator at 37°C, 5% CO_2, and 95% humidity. Further, serum free MTT media was added followed by incubation for 3 hours at 37°C. The supernatant of cell plates were aspirated and 150 µL of DMSO was added to each well to dissolve the formazan crystals. Thereafter, at 540 nm absorbance was recorded of each well using Synergy HT microplate reader, BioTek, USA. The concentrations that exhibited percentage cytotoxicity of less than 30% were considered as non-cytotoxic [28].

Effect of Biofield Energy Treated Test Formulation on Human Foreskin Fibroblast (HFF-1) Cell Proliferation Using BrdU Method

BrdU method was used for the estimation of cell proliferation in fibroblast cell assay with HFF-1 cells using hemocytometer, which was plated in a 96-well plate at density of 8000 cells/well/200 µL in DMEM with 10% FBS. The HFF-1 cells were then incubated overnight under standard growth conditions 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 the test formulation in different defined experimental groups and positive control. Following 24 to 72 hours of incubation with the test substance and positive control, the plates were taken out and BrdU (5-bromo-2'-deoxyuridine) estimated using cell proliferation ELISA, BrdU estimation kit (ROCHE – 11647229001) as per manufacturer's instructions [27].

Estimation of Extracellular Matrix Component (ECM) Synthesis

The effect of the test formulation for the synthesis of ECM components *i.e.* collagen, elastin, and hyaluronic acid in HFF-1 cells were estimated. The details procedure was followed as per Dodon et al. with slight modification [36]. The collagen level estimated 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 [27].

Estimation of Melanin Synthesis- Skin Depigmentation Effect

For the estimation of melanin synthesis, B16-F10 cells were used, which were counted using an hemocytometer and plated in a 6-well plate at the density corresponding to 200,000 cells/2 mL DMEM supplemented with 10% FBS/well. The cells were then incubated overnight under growth conditions to allow cell recovery and exponential growth. Following overnight incubation, the cells were treated with 500 nM α-MSH for stimulation of melanogenesis. After 24 hours of incubation with α-MSH, the cells were treated with non-cytotoxic concentrations of test samples obtained by serial dilution of main stock (*i.e.*, 19.94 mg/mL DMSO stock). Cells were lysed with 200 µL of lysis buffer (1% Triton-X, 0.2mM PMSF and 67 mM sodium phosphate buffer). The cell lysates were sonicated for 15 minutes at RT and centrifuged at 12000 rpm for 10 minutes, 4˚C. Supernatant was discarded and the intracellular melanin was extracted in 250 µL NaOH containing 10% DMSO at 37˚C for overnight and the absorbance was read at 405 nm. The levels of melanin corresponding to each treatment were extrapolated using standard curve obtained from purified melanin [28]. The percentage decrease in melanin synthesis was then calculated with respect to alpha-MSH treated cells (negative control) using Equation 1:

Percent decrease in alpha-MSH induced melanin synthesis = $[(M_{MSH} – M_{test})/ M_{MSH}] *100$.....(1)

Where, M_{MSH} = Melanin levels (µg/mL) in alpha-MSH treated cells (Negative control group), M_{test} = Melanin levels (µg/mL) in treated cells

Anti-wrinkling Activity against UV-B

The HFF-1 cells were counted using an hemocytometer and plated in 96-well plates at the density corresponding to 8000 cells/180 µL/well for the estimation of UV-B induced stress. The details procedure was followed as per Smith et al. with few modification [37] and according to manufacturer instructions [28]. The percent cell viability was assessed using Equation 2:

% Cell viability = (X*100)/R---------- (2)

Where, X = the absorbance of cells corresponding to test groups and positive control,

R = the absorbance of cells corresponding to control group.

Wound Healing Activity by Scratch Assay

HaCaT and HFF-1 cells were counted using an hemocytometer and plated in 12-well plates. The seeding density of HFF-1 cells were 0.15 X 10^6 /well/mL DMEM supplemented with 15% FBS, while HaCaT cells were 0.2 X 10^6 /well/mL DMEM supplemented with 10% FBS. After incubated overnight under growth conditions, which was allowed for cell recovery and exponential growth, both the cells were subjected to the serum starvation in DMEM for 24 hours. Horizontal and vertical mechanical scratches (representative wounds) were then created in the near confluent monolayer of cells by gently scraping with sterile 200 µL micropipette tip. The cells were rinsed with serum free DMEM and treated with the test formulation. The scratched area was monitored for a time period ranging from 0 to 48 hours for closure of wound area. The photomicrographs obtained in the above step were analyzed for quantitative assessment of area of wound closure using Wimscratch (wound healing) analysis software. All the observations were calculated and compared with the positive and vehicle control [28]. The percentage migration corresponding to each treatment was calculated using Equation 3:

% Migration = ((R-X)/R)*100.....(3)

Where, X = % Scratched area of cells corresponding to positive control and test groups.

R = % Scratched area of cells corresponding to baseline group

Results and Discussions

MTT Assay

MTT assay was used to study the non-cytotoxic concentrations of the test formulation in three different cell lines *such as* B16-F10, HFF-1, and HaCaT. The results in term of percentage are shown in Figure 1. All the results in different groups were compared with respect to the different positive controls *viz.* ascorbic acid, kojic acid, and EGF in respective cell lines. MTT assay showed that all the test formulation concentrations were found to be safe and non-toxic with more than 70% cell viability. The test formulation concentrations (0.01 to 10 µg/mL) were further used for the assessment of skin health parameters like extracellular matrix (ECM) synthesis (such as collagen, elastin, and hyaluronic acid), cellular proliferation using BrdU assay, melanin synthesis, and wound healing activity by scratch assay.

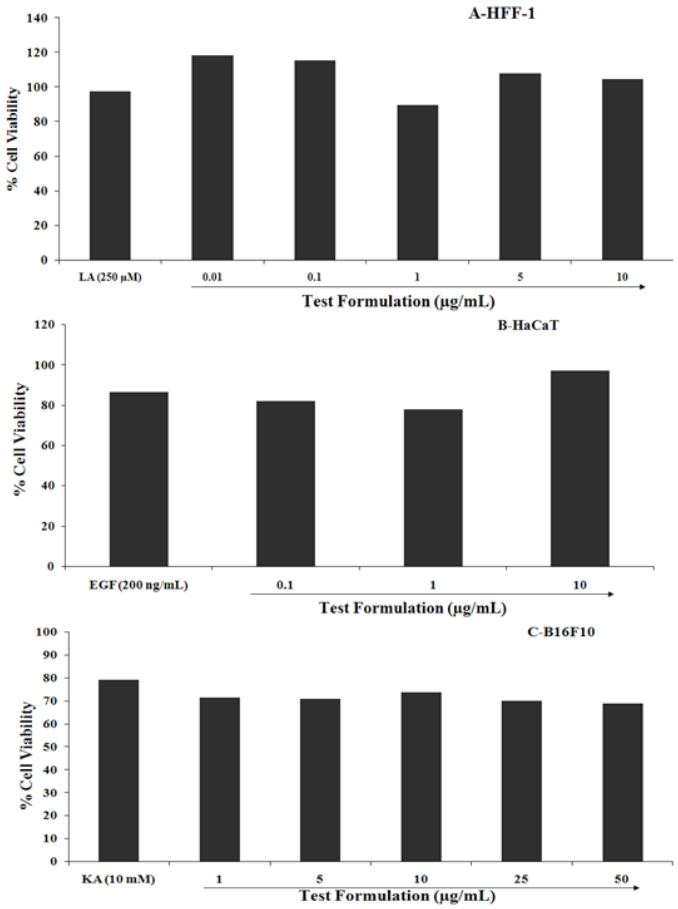

Figure 1: Assessment of cell viability in three cell lines at tested concentrations of a test formulation. A. HFF-1 cells. B. HaCaT cells. C. B16-F10 cells. LA: L-Ascorbic acid; EGF: Epidermal growth factor; KA: Kojic acid.

Effect of Biofield Treated Test Formulation on Cell Proliferation in HFF-1 cells using BrdU Assay

For the estimation of cellular proliferation using BrdU method, test formulation was analyzed at different safe concentrations, which were obtained in MTT assay in HFF-1 cells after 48 hours of incubation. The results of BrdU analysis are displayed in Figure 2. FBS was used as positive control and it (1% of it) showed a

significant improved cellular proliferation of HFF-1 cells by 62.2%, while the percentage cellular proliferation in vehicle control group was 12.4%. The experimental test groups *i.e.* Biofield Energy Treated groups, UT-DMEM + BT-Test formulation group showed 25.28%, 29.40%, and 38.58% increased cell proliferation at 0.1, 1, and 5 µg/mL, respectively. Similarly, another test group, BT-DMEM + UT-Test formulation group showed 25.28%, 33.52%, and 84.83% increased cell proliferation at 0.1, 1, and 5 µg/mL, respectively. BT-DMEM + BT-Test formulation group showed increased cell proliferation by 29.21%, 29.96%, and 74.72% at 0.1, 1, and 5 µg/mL, respectively. Overall, the percentage benefit with respect to the untreated test group (UT-DMEM + UT-Test formulation) was found as 170%, 52.4%, and 30.4% in the UT-DMEM + BT-Test formulation group at 0.1, 1, and 5 µg/mL, respectively. In addition, the percentage benefit in the BT-DMEM + UT-Test formulation group was found to be 170%, 73.8%, and 186.7% at 0.1, 1, and 5 µg/mL, respectively as compared with the UT-DMEM + UT-Test formulation group. Similarly, the percentage benefit in the BT-DMEM + BT-Test formulation group was found to be 212.0%, 55.3%, and 152.5% at 0.1, 1, and 5 µg/mL, respectively as compared with the UT-DMEM + UT-Test formulation group. Overall, Biofield Energy Treated Test formulation and DMEM showed a significant increased percentage cellular proliferation in HFF-1 cells, which suggested that the Trivedi Effect®-Energy of Consciousness Healing Treatment has significant capacity to improve cellular homoeostasis and maintenance of an organism. BrdU method has been well documented method to examine the rate of DNA replication, metabolic activities, and recognitions of the cell surface antigen activity [38]. Moreover, increased cell proliferation is direct correlation with the mitochondrial biogenesis and function [39, 40]. Thus, it can be assumed that the Trivedi Effect®-Consciousness Energy Healing Treatment has some impact on mitochondrial functions and biogenesis and metabolic activity of the skin cells as significantly improved the skin cells proliferation using BrdU assay.

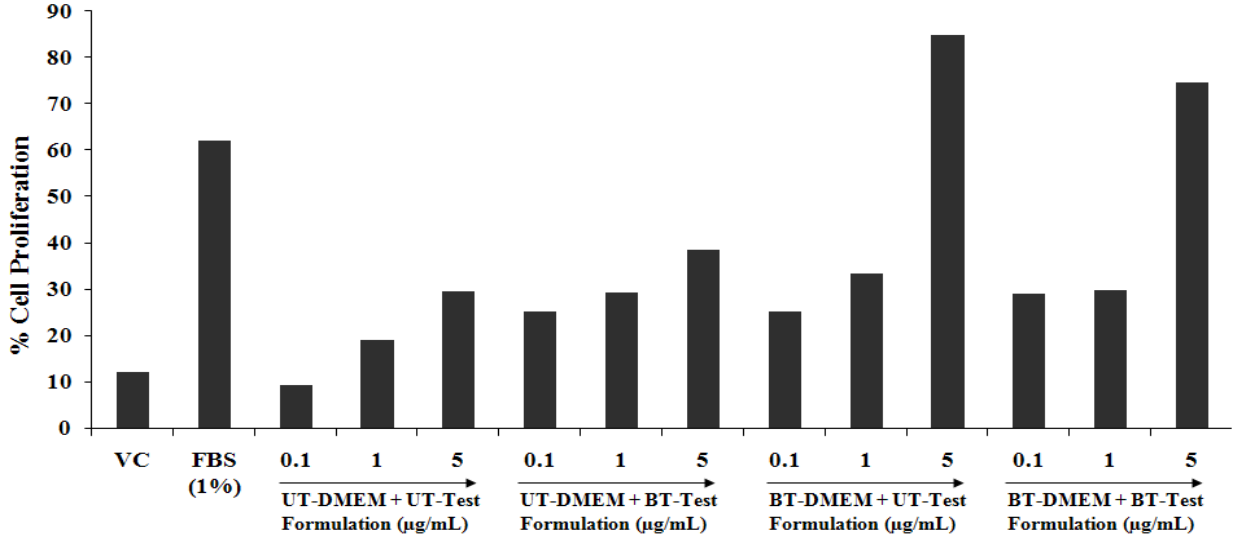

Figure 2: Evaluation of the effect of the test formulation on cellular proliferation using BrdU method. BT: Biofield Treated; VC: Vehicle control; UT: Untreated; FBS: Fetal bovine serum.

Analysis of Extracellular Matrix Component Synthesis

Collagen Estimation

Collagen estimation data showed that Biofield Energy Treated Test formulation/DMEM significantly improved the collagen level in HFF-1 cell line. The results are presented in Figure 3. Ascorbic acid (250 µM) showed a significantly increased collagen level by 74.87%, while the other test groups of Biofield Energy Treated Test formulation reported with a significantly increase in the collagen amount as compared with the untreated test formulation. The improved collagen level in the test group with respect to the untreated test groups in the UT-DMEM + BT-Test formulation group showed 42.2% and 140.8% at 0.01 and 5 µg/mL, respectively in contrast to the UT-DMEM + UT-Test formulation group. Additionally, the BT-DMEM + UT-Test formulation group at 0.01 µg/mL showed an increased collagen level by 24.4% in contrast to the UT-DMEM + UT-Test formulation group. Thus, overall experimental data suggested significant improved collagen content in the Biofield Energy Healing Treatment groups as compared with the untreated test formulation. Collagen is considered as the best and most abundant skin proteins used to improve skin health, its structure, and the fibrous protein present in the ECM. Collagen also provides the significant strength and structure to the skin that

might be beneficial for skin health, strength, and wound healing [41, 42]. Hence, Biofield Energy Healing (the Trivedi Effect®) can be significantly used to improve the pro-collagen peptides and it's cross-linking among various tropo-collagen molecules that improved the collagen.

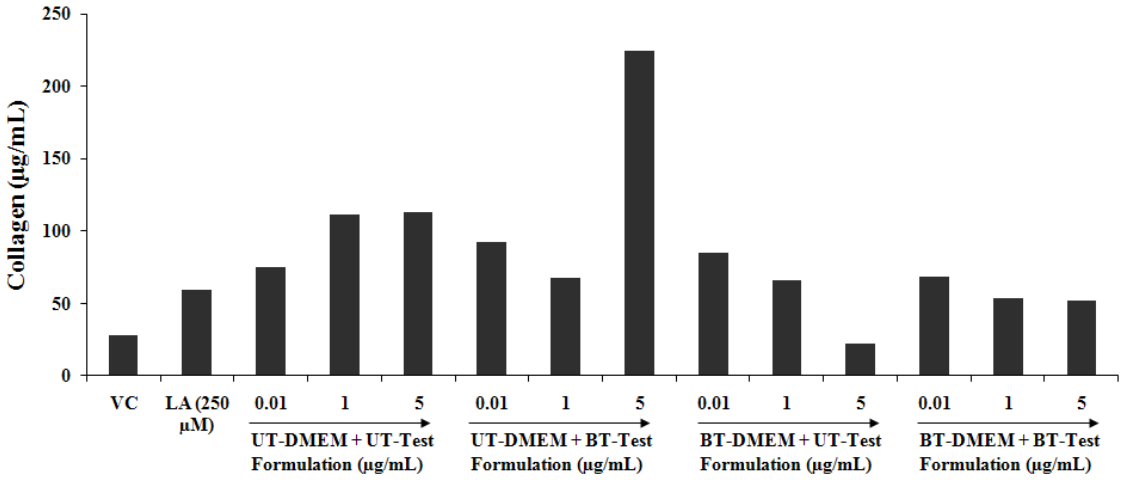

Figure 3: Effect of the Biofield Energy Healing Treatment on the test formulation and DMEM on collagen level in HFF-1 cells. BT: Biofield Treated; UT: Untreated; VC: Vehicle control; LA: L-Ascorbic acid.

Elastin Estimation

In order to estimate the level of elastin in HFF-1 cells, Biofield Energy Treated groups were tested and compared with respect to the untreated test formulation groups and the results are presented in Figure 4. Ascorbic acid (250 µM) group showed significantly increased elastin content by 97.5% compared with the normal control group. Moreover, the UT-DMEM + BT-Test formulation group showed a significant increased in the elastin level by 233.3% and 212.2% at 0.01 and 0.1 µg/mL, respectively compared with the UT-DMEM + UT-Test formulation group. The BT-DMEM + UT-Test formulation group showed a significantly increased the elastin level by 313.3% and 111% at 0.01 and 0.1 µg/mL, respectively compared with the UT-DMEM + UT-Test formulation group. The BT-DMEM + BT-Test formulation group showed a significantly increased in the elastin level by 637.8% and 59.8% at 0.01 and 0.1 µg/mL, respectively compared with the UT-DMEM + UT-Test formulation group. The Biofield Treatment showed a significantly improved elastin level, one of the important constituents of the ECM. Elastin is one of the important parts of ECM, which maintain the cellular integrity and helps to keep the shape in body tissue and very elastic tissue of the body along with collagen [43]. These components are also responsible for ageing and overall health. The experimental result suggests that the Trivedi Effect® has the significant capacity to improve the skin elasticity and strength possibly due to by activation of the dermal metabolism activity. Thus, test tested Biofield energized test formulation can be used to improve the elastin level of skin, which is required for the improvement of skin aging.

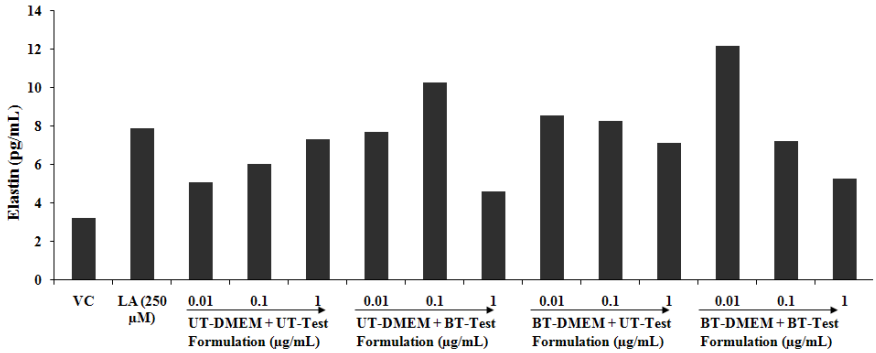

Figure 4: Effect of the Biofield Energy Healing Treatment on the test formulation and DMEM for estimation of elastin level in HFF-1 cells. BT: Biofield Treated; UT: Untreated; VC: Vehicle control; LA: L-Ascorbic acid.

Analysis of Hyaluronic Acid (HA)

The Biofield Energy treated test formulation was used for the estimation of hyaluronic acid (HA) in the HFF-1 cell line and the results are presented in Figure 5. HA level in ascorbic acid (250 µM) group showed an increased content by 53.5%. The HA level in the UT-DMEM + BT-Test formulation group showed a significantly increased by 191.8% and 32.2% at 0.01 and 0.1 µg/mL, respectively compared with the UT-DMEM + UT-Test formulation group. Additionally, the BT-DMEM + UT-Test formulation group showed an increased HA level by 461.0%, 139.4%, and 71.8% at 0.01, 0.1, and 1 µg/mL, respectively compared with the UT-DMEM + UT-Test formulation group. However, the BT-DMEM + BT-Test formulation group showed a significant increased in HA level by 322.5% at the concentration of 0.01 µg/mL compared with the UT-DMEM + UT-Test formulation group. HA level, a natural polysaccharides that are present all over the connective, neural, and epithelial tissue, play a major role in maintaining the skin health. Most of the skin care products used HA as one of the major base like hyaluronic acid serums, injectable, creams, and hyaluronic acid supplements to improve skin health and aging. Decreased HA in skin results in reduced skin elasticity and fasten the aging process. HA based skin care product, due to their high water holding capacity are widely reported with significant results in market for skin health [44]. The Trivedi Effect®-Energy of Consciousness Healing based test formulation would be the best skin health approach in cosmetology in order to improve the overall skin health and to maintain skin moisture, creates fullness, and regulation of the skin water balance.

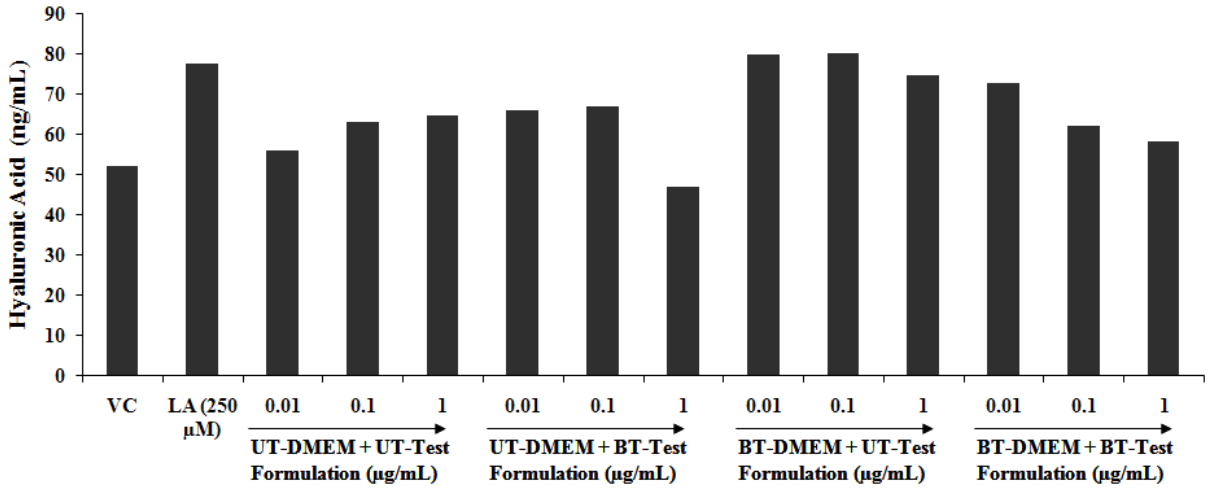

Figure 5: Synthesis of hyaluronic acid (HA) by Biofield Energy Treated test formulation in HFF-1 cell line. BT: Biofield Treated; UT: Untreated VC: Vehicle control; LA: L Ascorbic acid.

Evaluation of Skin Depigmentation (Melanogenesis) Activity

Skin depigmentation effect of the Biofield Energy Treated test formulation on alpha-MSH stimulated melanin synthesis in B16-F10 cells is shown in Figure 6. The data showed that melanin level in the alpha-MSH group was significantly increased by 311% with respect to the normal control group. Melanin was significantly reduced by 80.4% in the kojic acid (KA) group at 10 mM. The cellular content of melanin in the UT-DMEM + BT-Test formulation group was significantly reduced by 11.6%, 27.2%, and 44.7% at 0.1, 5, and 10 µg/mL, respectively compared to the UT-DMEM + UT-Test formulation group. The BT-DMEM + UT-Test formulation group was significantly reduced by 45.2%, 7.7%, and 5.3% at 0.1, 5, and 10 µg/mL, respectively compared to the UT-DMEM + UT-Test formulation group. The BT-DMEM + BT-Test formulation group did not show any reduced melanin content compared to the UT-DMEM + UT-Test formulation group. Sun ultraviolet radiation exposure with UV-A and UV-B results in skin depigmentation, which leads to many skin disorders that initiates the process of melanogenesis in the melanocytes which results in skin darkening [45]. Overall, the experimental results concluded that the Biofield Energy Treated test formulation significantly inhibited the melanin content in the B16-F10 cells, which suggested that the Trivedi Effect® based test formulation might be beneficial for the development of cosmeceutical products against hyperpigmentation and different types of skin disorders.

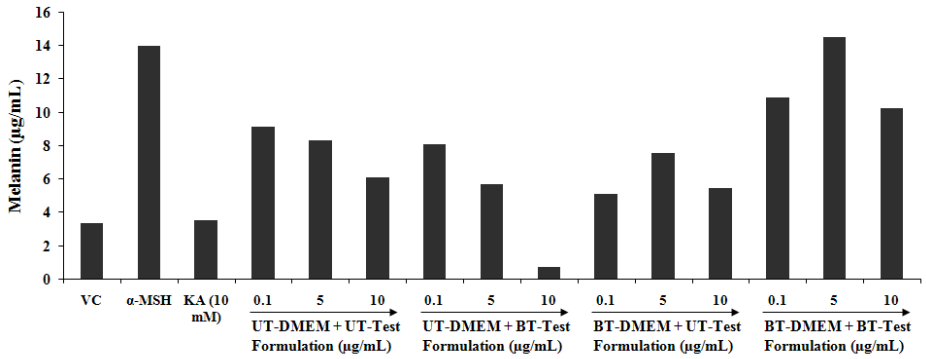

Figure 6: Effect of the Biofield Energy Treated test formulation on alpha-MSH stimulated melanin synthesis in B16-F10 cells. VC: Vehicle control; α-MSH: Alpha-melanocyte-stimulating hormone; KA: Kojic acid; UT: Untreated; BT: Biofield Treated.

Anti-wrinkling Effect of the Test Formulation on UV-B Induced Photoaging

The UV-B-induced photoaging was evaluated using cell viability study of the Biofield Energy Treated test formulation and DMEM with respect to the anti-wrinkling effect. The results in term of cell viability of HFF-1 cells are presented in Figure 7 after exposure with the UV-B rays. The HFF-1 cells were exposure to UV-B irradiation at the lethal dose of (200 mL/cm^2) and the percentage cell viability was reported in all the groups. The HFF-1 cells showed high degree of cell death with only 49.4% of cell viability after exposure with the UV-B rays. The cell viability in the vehicle control group was found as 20.94% due to UV-B irradiation (200 mL/cm^2). However, ascorbic acid (200 µM) showed a significant increased in the cell viability by 62.6% as compared with the baseline control group. The percentage cell viability in the UT-DMEM + BT-Test formulation group at 0.1 and 1 µg/mL was increased by 4.6% and 82.6%, respectively. In the BT-DMEM + UT-Test formulation group, the cell viability was significantly increased by 78.8%, 88.9%, and 92.9% at 0.1, 1, and 25 µg/mL, respectively. Similarly, in the BT-DMEM + BT-Test formulation group, the cell viability was significantly increased by 97.6% and 75.16% at concentration 0.1 and 1 µg/mL, respectively compared with the untreated group. Overall, the experimental results showed that all the groups exhibited an improved cell viability after exposure to the Biofield Energy Treated test formulation and DMEM. From literature, reported that increased viability of the skin melanocytes after damaged with UV-B rays through cAMP-PKA pathway and activates the microphthalmia (MITF) transcription factor, and thus increased melanin synthesis [46, 47]. Here, the Biofield Treated test formulation has significantly increased cell viability, which might be due to activation of the MITF transcription factor. Further, the test formulation can be significantly utilized in various skin diseases caused due to high exposure of UV-B radiations leading to cancer and other skin stress, skin disorders, free radical generation, etc. A UV-B radiation causes DNA damage on exposure that leads to cell death through various inflammatory pathways and leads to skin wrinkles and skin-ageing [48]. Therefore, it can be concluded that the Trivedi Effect® has significant application in cell protection, stop the inflammations, and protect the skin damage.

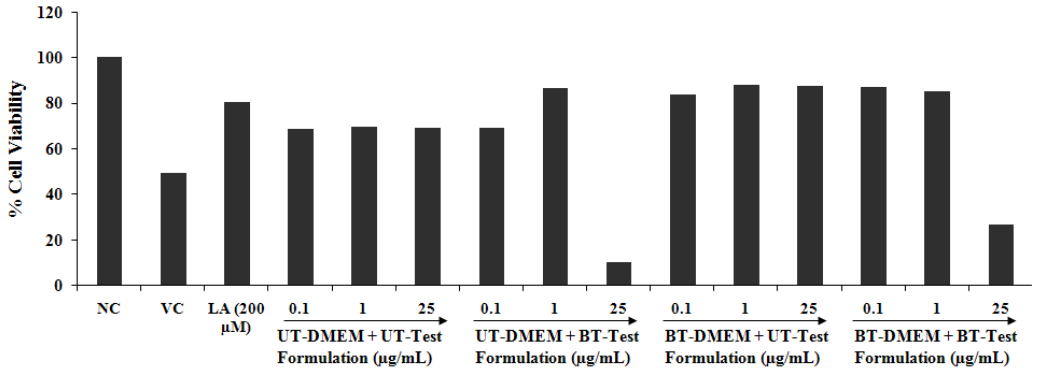

Figure 7: Anti-wrinkling potential of the Biofield Energy Treated test formulation against UV-B induced stress in HFF-1 cell line. NC: Normal control; VC: Vehicle control; LA-200: L-Ascorbic acid at 200 µM concentration, UT: Untreated; BT: Biofield Treated.

Wound-Healing Activity Using Scratch Assay

In vitro wound healing scratch assay was performed to study the wound healing activity in HFF-1 and HaCaT cells. Wound healing scratch assay denotes cell-to-cell and cell-to-matrix interactions during wound healing process [49]. Thus, this is one of the best *in vitro* assay for wound healing in different cell lines. The results in term of percentage cell migration among the tested groups showed a significant cellular migration (Figure 8A and B). The data suggested that EGF group at 100 ng/mL showed significant percentage migration compared with the baseline by 88.48% and 87.27% in HFF-1 and HaCaT cell lines, respectively. In HFF-1 cells, UT-DMEM + BT-Test formulation group showed percentage increased cell migration by 89.1%, 16.7%, and 25.6% at 0.1, 5, and 10 µg/mL in the UT-DMEM + BT-Test formulation group compared with the untreated group. The BT-DMEM + UT-Test formulation group showed percentage increased cell migration by 73.5% and 41.8% at concentration 0.1 and 5 µg/mL, respectively compared with the untreated group. Similarly, the BT-DMEM + BT-Test formulation group showed percentage increased cell migration by 9.6% at concentration 0.1 µg/mL compared with the untreated group (Figure 8A). On the other hand, HaCaT cell in scratch assay showed percentage increased cell migration by 60%, 2722.2%, and 541.9% at concentration 0.01, 0.1, and 1 µg/mL, respectively in the UT-DMEM + BT-Test formulation group in contrast with the untreated group. The BT-DMEM + UT-Test formulation group showed that an increased cell migration by 13.2%, 2431.8%, and 213.1% at 0.01, 0.1, and 1 µg/mL, respectively with respect to the untreated group. Similarly, the BT-DMEM + BT-Test formulation group showed a significant increased cell migration by 54%, 1714%, and 412.7% at concentration 0.01, 0.1, and 1 µg/mL, respectively compared with the untreated group (Figure 8B). Overall, wound healing scratch assay in both the cell lines showed that the Trivedi Effect®-Energy of Consciousness Healing Treatment has the significant capacity to improved cellular migration with significant impact on wound healing.

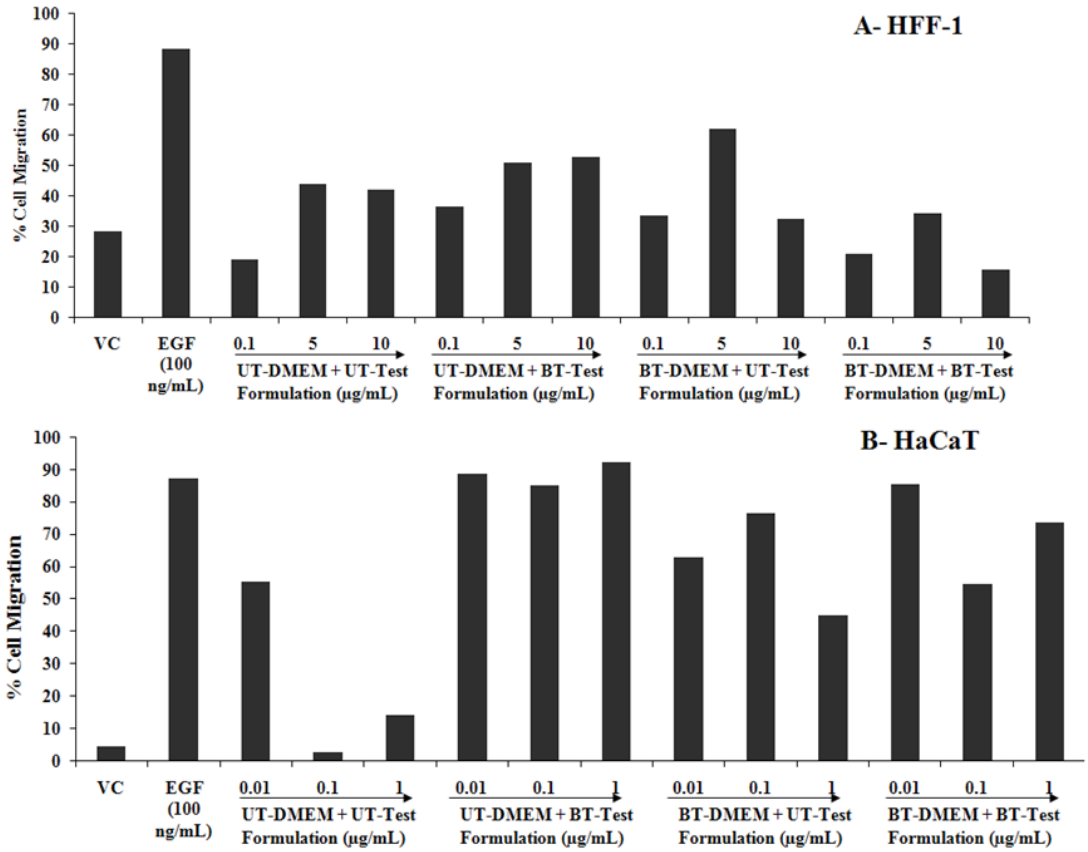

Figure 8: Percentage cellular migration of (A) HFF-1 cells and (B) HaCaT cell migration after induction of a scratch. VC: Vehicle control; EGF-100: Epidermal growth factor at 100 ng/mL concentration, UT: Untreated; BT: Biofield Treated.

Conclusions

The Trivedi Effect®-Consciousness Energy Healing based novel proprietary test formulation and DMEM showed a significant increase in cell viability with more than 70% in all the three tested cell lines *i.e.* HaCaT, HFF-1, and B16F10. BrdU assay data showed 170%, 170%, and 212% increased the cellular proliferation (at 0.1 µg/mL) in the UT-DMEM + BT-Test formulation, BT-DMEM + UT-Test formulation, and BT-DMEM + BT-Test formulation groups, respectively while 186.7% and 152.5% increased cellular proliferation in the BT-DMEM + UT-Test formulation and BT-DMEM + BT-Test formulation groups, respectively at 5 µg/mL compared to the UT-DMEM + UT-Test formulation group. The skin collagen level was significantly increased by 140.8% at 5 µg/mL in the UT-DMEM + BT-Test formulation group than the untreated group. Elastin level was also significantly increased by 233.3% and 212.2% at 0.01 and 1 µg/mL, respectively in the UT-DMEM + BT-Test formulation, while 313.3% and 111% at 0.01 and 1 µg/mL, respectively increased elastin in the BT-DMEM + UT-Test formulation group than untreated group. Besides, there was significantly increased elastin level by 637.8% at 0.01 µg/mL in the BT-DMEM + BT-Test formulation than untreated group. Hyaluronic acid (HA) in HFF-1 cells showed 191.8% increased at 0.01 µg/mL in the UT-DMEM + BT-Test formulation group, while 461.0% and 139.4% increased at 0.01 and 0.1 µg/mL, respectively in the BT-DMEM + UT-Test formulation group compared with the untreated group. HA also significantly increased in the BT-DMEM + BT-Test formulation group by 322.5% at 0.01 µg/mL than untreated. Melanin level was significantly reduced by 44.7% at 10 µg/mL in the UT-DMEM + BT-Test formulation group, while 45.2% decreased at 0.1 µg/mL in the BT-DMEM + UT-Test formulation group than the untreated group. Anti-wrinkling potential with respect to UV-B induced stress showed an increased cell viability at 1 µg/mL was increased by 82.6% in the UT-DMEM + BT-Test formulation group than untreated. Cell viability was significantly increased by 78.8%, 88.9%, and 92.9% at 0.1, 1, and 25 µg/mL, respectively in the BT-DMEM + UT-Test formulation group; also increased by 97.6% at 0.1 µg/mL in the BT-DMEM + BT-Test formulation group than untreated. Cellular migration was increased upto 58.55% (100 µg/mL) in the UT-DMEM + BT-Test formulation group in HFF-1 cells than the untreated group. Similarly, in HaCaT cells, the cellular migration was significantly increased by 2722.2% and 541.9% at 0.1 and 1 µg/mL, respectively in the UT-DMEM + BT-Test formulation group; while 2431.8% and 213.1% increased cellular migration at 0.1 and 1 µg/mL, respectively in the BT-DMEM + UT-Test formulation group than untreated. Similarly, the BT-DMEM + BT-Test formulation group showed an increased cell migration by 1714% and 412.7% at 0.1 and 1 µg/mL, respectively than untreated. Thus, overall experimental data suggests the significant role of Biofield Energy Healing Treatment in wound healing and other skin-related disorders such as wrinkling, aging, and skin whitening. In conclusion, the Trivedi Effect®-Consciousness Energy Healing Treated proprietary test formulation showed a significant improved skin health parameters. Besides, these results indicated that this therapy can be well used in various skin-related disorders *viz.* chickenpox, eczema, acne, diaper rash, warts, measles, hives, wrinkles, skin cancer, psoriasis, ringworm, rosacea, seborrheic dermatitis, rashes from allergic reactions, rashes from bacterial or fungal infections, fleshy bumps, cracked skin, scaly or rough skin, discolored patches of skin, warts, peeling skin, changes in mole color or size, a loss of skin pigment, excessive flushing, open sores or lesions, dry, ulcers, etc.

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Conflict of Interest

No conflict of interest exists.

References

1. Lippens, S. , Hoste, E. , Vandenabeele, P. , Agostinis, P. , Declercq, W. (2009) Cell death in the skin. Apoptosis 14: 549-569.
2. Boelsma, E. , Hendriks, H. F. , Roza, L. (2001) Nutritional skin care: Health effects of micronutrients and fatty acids. Am. J. Clin. Nutr. 73: 853-864.
3. Mudgil, A.V. , Segal, N. , Andriani, F. , Wang, Y. , Fusenig, N. E. et al. Ultraviolet B irradiation induces expansion of intraepithelial tumor cells in a tissue model of early cancer progression. (2003) J. Invest. Dermatol. 121: 191-197.
4. Tabassum, N. , Hamdani, M. (2014) Plants used to treat skin diseases. Pharmacogn. Rev. 8: 52-60.
5. Gao, X.H., Zhang, L. , Wei, H. , Chen, H. D. (2008) Efficacy and safety of innovative cosmeceuticals. Clin. Dermatol. 26: 367-74.
6. Fabricant, D.S. , Farnsworth, N. R. (2001) The value of plants used in traditional medicine for drug discovery. Environ. Health. Perspect. 109: 69-75.
7. Park, K. (2015) Role of micronutrients in skin health and function. Biomol. Ther. (Seoul) 23: 207-217.
8. Schwartz, J.R. , Marsh, R. G. , Draelos, Z. D. (2005) Zinc and skin health: Overview of physiology and pharmacology. Dermatol. Surg. 31: 837-847.
9. Traikovich, S.S. , (1999) Use of topical ascorbic acid and its effects on photodamaged skin topography. Arch. Otolaryngol. Head Neck Surg. 125: 1091-1098.
10. Movaffaghi, Z. , Farsi, M. (2009) Biofield therapies: Biophysical basis and biological regulations. Complement. Ther. Clin. Pract. 15: 35-37.
11. Barnes, P.M. , Powell-Griner, E. , McFann, K. , Nahin, R. L. (2004) Complementary and alternative medicine use among adults: United States, 2002. Adv. Data 343: 1-19.

12. Barnes, P.M. , Bloom, B. , Nahin, R. L. (2008) Complementary and alternative medicine use among adults and children: United States, 2007. Natl. Health Stat. Report 12: 1-23.

13. wai Fan, K. , (2005) National Center for Complementary and Alternative Medicine Website. J. Med. Libr. Assoc. 93: 410-412.

14. Trivedi, M.K. , Tallapragada, R. M. (2008) A transcendental to changing metal powder characteristics. Met. Powder Rep. 63: 22-28, 31.

15. Trivedi, M.K. , Nayak, G. , Patil, S. , Tallapragada, R. M. , Latiyal, O. (2015) Studies of the atomic and crystalline characteristics of ceramic oxide nano powders after bio field treatment. Ind. Eng. Manage. 4: 161.

16. Trivedi, M.K. , Nayak, G. , Patil, S. , Tallapragada, R. M. , Latiyal, O. (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.

17. Trivedi, M. K. , Branton, A. , Trivedi, D. , Nayak, G. , Mondal, S. C. et al. (2015) Morphological characterization, quality, yield and DNA fingerprinting of biofield energy treated alphonso mango (*Mangifera indica* L.). Journal of Food and Nutrition Sciences 3: 245-250.

18. Trivedi, M.K. , Branton, A. , Trivedi, D. , Nayak, G. , Mondal, S. C. et al. (2015) Evaluation of biochemical marker – Glutathione and DNA fingerprinting of biofield energy treated *Oryza sativa*. American Journal of BioScience 3: 243-248.

19. Trivedi, M.K. , Branton, A. , Trivedi, D. , Nayak, G. , Charan, S. (2015) Phenotyping and 16S rDNA analysis after biofield treatment on *Citrobacter braakii*: A urinary pathogen. J. Clin. Med. Genom. 3: 129.

20. Trivedi, M.K. , Patil, S. , Shettigar, H. , Mondal, S. C. , Jana, S. (2015) Evaluation of biofield modality on viral load of Hepatitis B and C viruses. J. Antivir. Antiretrovir. 7: 083-088.

21. Trivedi. M.K. , Patil, S. , Shettigar, H. , Mondal, S. C. , Jana, S. (2015) An impact of biofield treatment: Antimycobacterial susceptibility potential using BACTEC 460/MGIT-TB System. Mycobact. Dis. 5: 189.

22. Trivedi, M.K. , Patil, S. , Shettigar, H. , Bairwa, K. , Jana, S. (2015) Phenotypic and biotypic characterization of *Klebsiella oxytoca*: An impact of biofield treatment. J. Microb Biochem. Technol. 7: 203-206.

23. Nayak, G. , Altekar, N. (2015) Effect of biofield treatment on plant growth and adaptation. J. Environ. Health Sci. 1: 1-9.

24. Branton, A. , Jana, S. (2017) The influence of energy of consciousness healing treatment on low bioavailable resveratrol in male *Sprague Dawley* rats. International Journal of Clinical and Developmental Anatomy 3: 9-15.

25. Branton, A. , Jana, S. (2017) The use of novel and unique biofield energy healing treatment for the improvement of poorly bioavailable compound, berberine in male *Sprague Dawley* rats. American Journal of Clinical and Experimental Medicine 5: 138-144.

26. Branton, A. , Jana, S. (2017) Effect of The biofield energy healing treatment on the pharmacokinetics of 25-hydroxyvitamin D3 [25(OH) D_3] in rats after a single oral dose of vitamin D3. American Journal of Pharmacology and Phytotherapy 2: 11-18.

27. Kinney, J.P. , Trivedi, M. K. , Branton, A. , Trivedi, D. , Nayak, G. (2017) Overall skin health potential of the biofield energy healing based herbomineral formulation using various skin parameters. American Journal of Life Sciences 5: 65-74.

28. Singh, J. , Trivedi, M. K. , Branton, A. , Trivedi, D. , Nayak, G. et al. (2017) Consciousness energy healing treatment based herbomineral formulation: A safe and effective approach for skin health. American Journal of Pharmacology and Phytotherapy 2: 1-10.

29. Trivedi, M.K. , Branton, A. , Trivedi, D. , Nayak, G. , Plikerd, W. D. et al. (2017) A Systematic study of the biofield energy healing treatment on physicochemical, thermal, structural, and behavioral properties of magnesium gluconate. International Journal of Bioorganic Chemistry 2: 135-145.

30. Trivedi, M.K. , Branton, A. , Trivedi, D. , Nayak, G. , Plikerd, W. D. et al. (2017) Chromatographic and spectroscopic characterization of the consciousness energy healing treated *Withania somnifera* (ashwagandha) root extract. European Journal of Biophysics 5: 38-47.

31. Trivedi, M.K. , Patil, S. , Shettigar, H. , Mondal, S. C. , Jana, S. (2015) The potential impact of biofield treatment on human brain tumor cells: A time-lapse video microscopy. J. Integr. Oncol. 4: 141.

32. Trivedi, M.K. , Patil, S. , Shettigar, H. , Gangwar, M. , Jana, S. (2015) *In vitro* evaluation of biofield treatment on cancer biomarkers involved in endometrial and prostate cancer cell lines. J. Cancer Sci. Ther. 7: 253-257.

33. Anagnos, D. , Trivedi, K. , Branton, A. , Trivedi, D. , Nayak, G. et al. (2018) Influence of biofield treated vitamin D_3 on proliferation, differentiation, and maturation of bone-related parameters in MG-63 cell-line. International Journal of Biomedical Engineering and Clinical Science 4: 6-14.

34. Lee, A.C. , Trivedi, K. , Branton, A. , Trivedi, D. , Nayak, G. et al. (2018) The potential benefits of biofield energy treated vitamin d3 on bone mineralization in human bone osteosarcoma cells (MG-63). International Journal of Nutrition and Food Sciences 7: 30-38.

35. Stutheit, M.E. , Trivedi, K. , Branton, A. , Trivedi, D. , Nayak, G. et al. (2018) Biofield energy treated vitamin D3: Therapeutic implication on bone health using osteoblasts cells. American Journal of Life Sciences 6: 13-21.

36. Dodon, J. , Trivedi, M. K, , Branton, A, , Trivedi, D, , Nayak, G. et al. (2017) The study of biofield energy treatment based herbomineral formulation in skin health and function. American Journal of BioScience 5(3): 42-53.

37. Smith D,L. , Trivedi, M. K, , Branton, A, , Trivedi, D, , Nayak, G. et al. (2017) Impact of consciousness energy healing treatment on herbomineral formulation in different skin health parameters. American Journal of Biomedical and Life Sciences 5(2): 26-35.

38. Yadav, K. , Singhal, N. , Rishi, V. , Yadav, H. (2014) Cell proliferation assays. eLS. John Wiley & Sons Ltd., Chichester.

39. Lee, C.H. , Wu, S. B. , Hong, C. H. , Liao, W. T. , Wu, C. Y. et al. (2011) Aberrant cell proliferation by enhanced mitochondrial biogenesis *via* mtTFA in arsenical skin cancers. Am J Pathol 178(5): 2066-2076.

40. Feichtinger, R.G. , Sperl, W. , Bauer, J. W. , Kofler, B. (2014) Mitochondrial dysfunction: A neglected component of skin diseases. Exp Dermatol 23(9): 607-614.

41. Kadler, K.E. , Holmes, D. F. , Trotter, J. A. , Chapman, J. A. (1996) Collagen fibril formation. Biochem J 316: 1-11.

42. Shoulders, M.D. , Raines, R. T. (2009) Collagen structure and stability. Annu Rev Biochem 78: 929-958.

43. Frantz, C. , Stewart, K.M. , Weaver, V. M. (2010) The extracellular matrix at a glance. J. Cell Sci. 123: 4195-4200.

44. Weindl, G. , Schaller, M. , Schäfer-Korting, M. , Korting, H. C. (2004) Hyaluronic acid in the treatment and prevention of skin diseases: Molecular biological, pharmaceutical and clinical aspects. Skin Pharmacol. Physiol. 17: 207-213.

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

46. Amaro-Ortiz, A. , Yan, B. , D'Orazio, J. A. (2014) Ultraviolet radiation, aging and the skin: prevention of damage by topical cAMP manipulation. Molecules (Basel, Switzerland). 19(5): 6202-6219.

47. Martins, I. J. (2016) Anti-aging genes improve appetite regulation and reverse cell senescence and apoptosis in global populations. Advances in Aging Research 5: 9-26.

48. Ho, J.N. , Lee, Y. H. , Lee, Y. D. , Jun, W. J. , Kim, H. K. et al. (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.

49. Liang, C.C. , Park, A. Y. , Guan, J. L. (2007) *In vitro* scratch assay: A convenient and inexpensive method for analysis of cell migration *in vitro*. Nat. Protoc. 2: 329-333.