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Evaluation of Anti-aging Effect of the Novel Test Formulation in Cell-based Studies using B-Galactosidase Activity, Collagen Levels, and Protection against Oxidative Stress

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Abstract

The study was aimed to evaluate the antioxidant and antiaging potential of Biofield Energy Healing Treatment (the Trivedi Effect®) on a novel test formulation in *in vitro*. The test formulation was divided into two parts. One part was denoted as the control, without any Biofield Treatment, while the other part was defined as the Biofield Energy Treated test formulation. MTT assay showed that the test formulation was observed as safe upto 100 µg/mL in both human foreskin fibroblasts-1 (HFF-1) and mouse preadipocytes (3T3-L1) cells. Moreover, the Biofield Treated test formulation showed 35.73% and 122.07% increased cellular protection (cell viability) at 25 and 50 µg/mL, respectively in H_2O_2 induced oxidative damage as compared to the untreated. Collagen was significantly increased by 16.81% and 30.22% in the Biofield Treated test formulation at 75 and 100 µL, respectively as compared to the untreated. Further, cellular senescence was significantly reduced by 66.35% and 22.5% in the Biofield Treated test formulation at 50 and 100 µg/mL, respectively compared to the untreated. Overall, data suggested that the Biofield Energy Treated test formulation can significantly improve antioxidative properties and would be use against antiaging, autoimmune and inflammatory diseases, stress management and prevention by improving overall health.

Keywords: Consciousness Energy Healing; The Trivedi Effect®; Free Radical; Anti-Oxidation; Oxidative Stress; HFF-1; 3T3-L1; Cellular Senescence; Collagen; Antiaging

Introduction

Oxidative stress results the imbalance between the production of reactive oxygen species (ROS) and the defense systems to detoxify them [1]. Numerous chronic and degenerative disorders are directly related with the oxidative stress such as cancer, arthritis, aging, autoimmune disorders, cardiovascular and neurodegenerative diseases [2,3]. However, human has the ability to neutralize the generated oxidative stress by generating different antioxidants that are either naturally produced *in situ*, or externally provided *via* foods and/or supplements. Endogenous and exogenous antioxidants worked as free radical scavengers, they prevent and repair the damaged caused by ROS and reactive nitrogen species (RNS), which results in improved immune defense and reduced the risk of cancer and degenerative diseases [4]. Normal human cells culture have a limited proliferation potential. They eventually become senescent as a result of serial passage, which is commonly called as 'replicative senescence'. This comprises that cellular senescence might be a cellular basis of human aging. Indeed, cells with the characteristics of senescence accumulate with age in multiple tissues, thus implying a role of cellular senescence in aging in mammals. Cellular senescence *in vitro* has, therefore, been regarded as a useful model for elucidating molecular mechanisms that underlie organismal aging [5]. Cellular senescence has been proposed to promote chronic, "sterile" inflammation through the senescence-associated secretory phenotype (SASP) in preadipocytes [6]. Thus, 3T3-L1 preadipocytes have been used in the present study to assess the effect of test item on cellular senescence. Complementary and Alternative Medicines (CAM) are the best source of exogenous antioxidants, which has become increasingly popular in the developed world [7,8]. Evidence-based medicines is acceptance worldwide and National Center for Complementary and Alternative Medicine (NCCAM) has been inaugurated as the United States Federal Government's lead agency for conducting scientific research and practicing in the arena of medicine [9]. Thus, these combinations of minerals and vitamins as nutritional supplements are preferred as best choice [10]. Thus, the novel test formulation was designed based on minerals like iron sulfate, copper chloride, zinc chloride and magnesium (II) gluconate hydrate, vitamins *viz.* cholecalciferol

(vitamin D_3), cyanocobalamin (vitamin B_{12}), and pyridoxine hydrochloride (vitamin B_6). All the active constituents of the novel formulation were reported to have significant antioxidant and immunological activities [11-13].

Biofield Energy Healing Treatment as a CAM approach has been reported to have significant outcomes against various disease conditions. National Institute of Health (NIH) recommend and included various Energy therapies such as natural products, deep breathing, yoga, Qi Gong, Tai Chi, chiropractic/osteopathic manipulation, massage, meditation, special diets, homeopathy, progressive relaxation, guided imagery, acupressure, relaxation techniques, acupuncture, hypnotherapy, movement therapy, healing touch, pilates, rolfing structural integration, Ayurvedic medicine, mindfulness, traditional Chinese herbs and medicines, essential oils, naturopathy, Reiki, aromatherapy, cranial sacral therapy and applied prayer (as is common in all religions, like Hinduism, Christianity, Judaism and Buddhism) under CAM category that has been accepted by the most of the U.S. population with several advantages [13]. Every living organisms possess some kind of unique energy that can be harness and transmit it into other living and non-living things by the process of Biofield Energy Healing by altered atomic/molecular weights through possible mediation of neutrinos [14]. Biofield Energy Healing Treatment (the Trivedi Effect®- Consciousness Energy Healing) have been studied and reported with significant outcomes in various scientific disciplines such as microbiology with altered antimicrobial sensitivity against pathogenic microbes [15-17], genetics [18,19], skin health [20,21], bone health [22-24] agricultural science [25,26], immunity [27,28], pharmaceuticals [29,30], and materials science [31,32]. In the present study, authors evaluated the impact of the Biofield Energy (the Trivedi Effect®-Consciousness Energy Healing) Treatment on the multiple vitamins and minerals based test formulation for its anti-oxidation and antiaging action using standard assays.

Materials and Methods

Chemicals and Reagents

3-(4, 5-dimethyl-2-thiazolyl) 2, 5 diphenyl-2 H-tetrazolium) (MTT), EDTA, FBS, and trypsin were purchased from Sigma Chemical Corp. (St. Louis, MO), a subsidiary of Sigma-Aldrich Corporation. Antibiotics solution (Penicillin-Streptomycin) and EMEM was purchased from HiMedia, India. Iron sulfate, ascorbic acid, copper chloride, and cholecalciferol (vitamin D_3) were obtained from Sigma Chemical Co. (St. Louis, MO). Zinc chloride, cyanocobalamin (vitamin B_{12}), pyridoxine hydrochloride (vitamin B_6), resveratrol, and magnesium gluconate hydrate were obtained from TCI, Japan. Sodium selenate was procured from Alfa Aesar, USA while, trolox was obtained from Cayman, USA. All other chemicals used in this study were analytical grade available in India.

Test Formulation and Reference Standard

The test formulation contained a combination of minerals *viz*. iron sulfate, copper chloride, zinc chloride and magnesium gluconate hydrate, vitamins *viz*. cholecalciferol (vitamin D_3), cyanocobalamin (vitamin B_{12}), and pyridoxine hydrochloride (vitamin B_6). Resveratrol and L-ascorbic acid were prepared in DMSO to obtained 20 mM stock solution. Trolox was dissolved in DMEM to obtain a stock solution of 50 mM for anti-oxidative protection against H_2O_2 induced stress in HFF-1 cells.

Biofield Energy Healing Strategies

The test formulation was divided into two parts. One part each of the test formulation was treated with Biofield Energy by a renowned Biofield Energy Healer, Mahendra Kumar Trivedi remotely for ~5 minutes under standard laboratory conditions and coded as the Biofield Energy Treated test formulation. While, the second part did not receive any sort of treatment and coded as the untreated test formulation group (Control). Biofield Energy Healer in this study never visited the laboratory (Dabur Research Foundation, New Delhi, India), nor had any contact with the test formulation. This Biofield Energy Healing Treatment was provided through Healer's unique Energy Transmission process to the test formulation. Further, the control groups were treated by a 'sham' healer for comparative purposes. The 'sham' healer did not have any knowledge about the Biofield Energy Treatment. After that, the Biofield Energy Treated and untreated samples were kept in similar sealed conditions for experimental study.

Cytotoxicity by MTT Assay

HFF-1 (human foreskin fibroblast) and 3T3-L1 cells were trypsinized, counted and then plated in wells of flat bottom 96-well plates at the density corresponding to 5 X 10^3 cells/well/180µL of growth medium as per Aksana Hancharuk *et al.* 2017 with few modification [33]. The effect of the test formulation on cell viability was determined as:

% Cell viability=100-% cytotoxicity ---------- (1)

Where; % cytotoxicity = [(O.D. of control cells – O.D. of cells treated with the test formulation)/O.D. of control cells]*100.

The concentrations exhibiting % cell viability of more than 70% were considered as non-cytotoxic [34].

Assessment of Cell Viability against H_2O_2 Induced Stress

HFF-1 cells were plated in 96-well plates at the density corresponding to 1 X 10^4 cells/well followed by overnight incubation in a CO_2 incubator at 37 °C, 5% CO_2, and 95% humidity. After incubation, the cells were treated with Biofield Energy Treated and untreated test formulation at non-cytotoxic concentrations. The cells corresponding to positive control group were treated with quercetin. The untreated cells served as negative control. After 24 hours of pre-treatment, the cells were treated with hydrogen peroxide (H_2O_2, 20 mM) for 2 hours to induce oxidative stress. The untreated cells served as control, while cells treated with H_2O_2 alone served as negative control. After incubation, the plates were taken out and MTT assay was performed for calculation of percentage cell viability using the following formula:

% Cell viability = 100 – [(1-X/R)*100] ---------- (2)

Where, X = OD of wells corresponding to treated cells
R = OD of untreated cells (Cells maintained in growth medium only)

Assessment of Collagen Levels in HFF-1 Cells

Collagen was estimated for determining the potential of the Biofield Energy Treated test formulation and DMEM supplemented with 15% FBS to improve skin strength. HFF-1 cells were counted using hemocytometer and the plated in 48-well plates at the density corresponding to 10 X 10^3 cells/well and assessed as per Aksana Hancharuk *et al.* 2017 with few modification [33]. The percentage increase in collagen levels with respect to the untreated cells (baseline group) will be calculated using the following formula:

% Increase = [(X-R)/R]*100 ---------- (3)

Where, X = Levels in cells corresponding to positive control or test groups
R = Levels in cells corresponding to baseline (untreated) group

Assessment of Senescence Associated β-galactosidase Activity

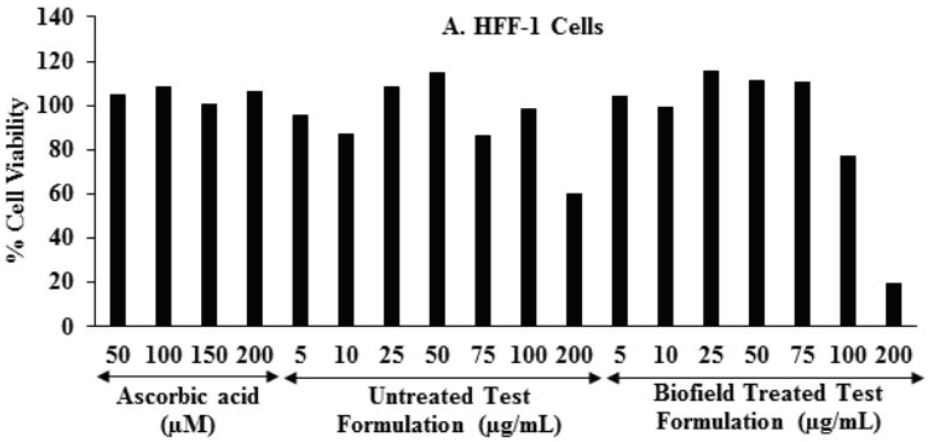
Cells were counted using hemocytometer and then plated at the density 5 X 10^5 in appropriate well format in DMEM supplemented with FBS. The plates were then incubated overnight in a CO_2 incubator at 37 °C, 5% CO_2, and 95% humidity so as to allow cell recovery and exponential growth. Following overnight incubation, the cells will be treated with the test item and positive control. Following 72 hours of incubation, the cells were lysed and protein estimation was done for each sample using Pierce BCA Protein Assay Kit (Thermofischer Scientific). Senescence associated beta galactosidase activity was estimated using Cellular Senescence activity assay kit (Enzo lifescience-ENZ-KIT129) as per manufacturer's protocol.

Statistical Analysis

All the data were expressed as percentage. Data were tested using one-way analysis of variance (ANOVA) simultaneously post-hoc analysis by Dunnett's test for multiple comparison. However, Student's *t*-test was applied for two group's comparison. Statistical significance was considered at $p \leq 0.05$.

Results

Cell Viability Using MTT Assay



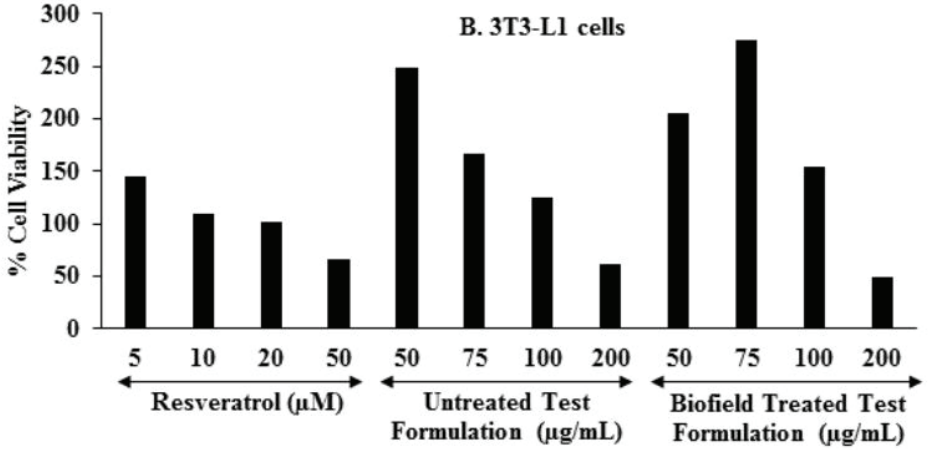


Figure 1: The cell viability assay after 24-hours of treatment with the test formulation and positive control (resveratrol) at various concentrations in A. human foreskin fibroblasts-1 (HFF-1) cells and B. mouse preadipocytes (3T3-L1) cells

MTT assay was performed using human foreskin fibroblasts-1 (HFF-1) cells and mouse preadipocytes (3T3-L1) cells against all the tested concentration of the test formulation 24 hours of incubation. The cell viability results are summarized in Figure 1. The results showed that the tested concentrations upto 100 µg/mL have showed cell viability with more than 70% were considered as non-cytotoxic. The test formulation were tested at various concentrations ranges from 5 to 100 µg/mL, which were found safe and nontoxic. These concentration ranges were selected for the estimation of anti-oxidative protection (in 3T3-L1 cells), collagen, and cellular senescence activity in HFF-1 cells.

Effect of the Test Formulation for Protection of Cell Viability against Oxidative Damage

Antioxidant activity against oxidative stress was measured among Biofield Energy Treated test formulation for cell viability after challenged with H_2O_2 in HFF-1 cells is represented in Figure 2. The cell viability was determined using MTT assay. The percent cell viability in the baseline control group was defined as 100%. The reference item, trolox showed significant increased cell viability and showed percentage as 17.04%, 41.26%, and 66.92% at the concentration of 0.5, 1, and 2 mM, respectively compared to the negative control group. Besides, the percent protection of damaged cells was significantly increased by 35.73%, 122.07%, and 6.72% in the Biofield Energy Treated test formulation at 25, 50, and 100 µg/mL as compared with the untreated test formulation group.

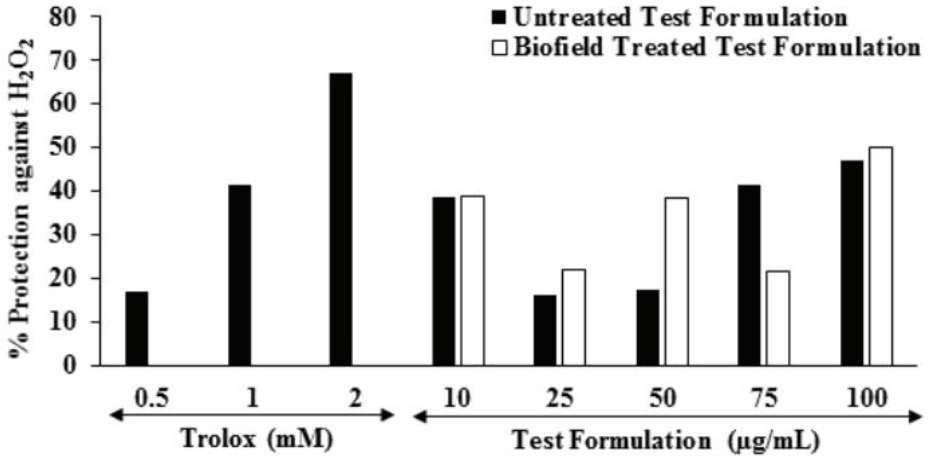


Figure 2: The effect of the test formulation on human foreskin fibroblasts-1 (HFF-1) cells for the assessment of cell viability after challenged with H_2O_2

Assessment of Collagen Levels in HFF-1 Cells

Collagen estimation data showed that the Biofield Energy Treated test formulation significantly improved the collagen level in HFF-1 cell line. The results are presented in Figure 3. Ascorbic acid showed a significantly increased collagen level by 13.44%, 18.81%, and 29.11% at 100, 150, and 200 µM, respectively as compared with the vehicle control (VC) group. Moreover, the untreated test formulation showed 17.46%, 20.15%, and 11.19% increased the level of collagen at 50, 75, and 100 µL, respectively as compared to the VC group. Further, the Biofield Energy Treated test formulation showed 27.32%, 40.32%, and 44.81% increased the level of collagen at 50, 75, and 100 µL, respectively as compared to the VC group. Besides, the Biofield Energy Treated test formulation was significantly increased the level of collagen by 8.41%, 16.81%, and 30.22% at 50, 75, and 100 µL, respectively as compared to the untreated test formulation group. Thus, overall experimental data suggested significant improved collagen content in the Biofield Energy Healing Treatment group as compared with the both vehicle control and untreated test formulation group.

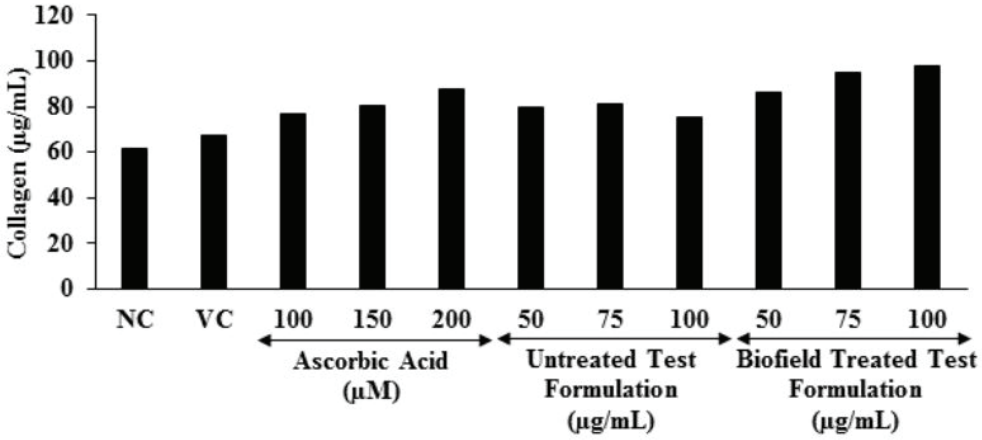


Figure 3: Effect of the test formulation on collagen level in human foreskin fibroblasts-1 (HFF-1) cells. NC: Normal control; VC: Vehicle control

Effect on Cellular Senescence

The effect of test formulation on the senescence associated β-galactosidase activity is illustrated in Figure 4. The vehicle control group showed 1.69% reduction of cellular senescence activity. The positive control (resveratrol) showed 26.60%, 30.47%, and 36.33% reduction of cellular senescence activity as compared to the baseline control group. Further, the Biofield Treated test formulation showed 66.35%, 1.55%, and 22.5% reduction of cellular senescence activity at 50, 75, and 100 µg/mL, respectively as compared to the untreated test formulation group.

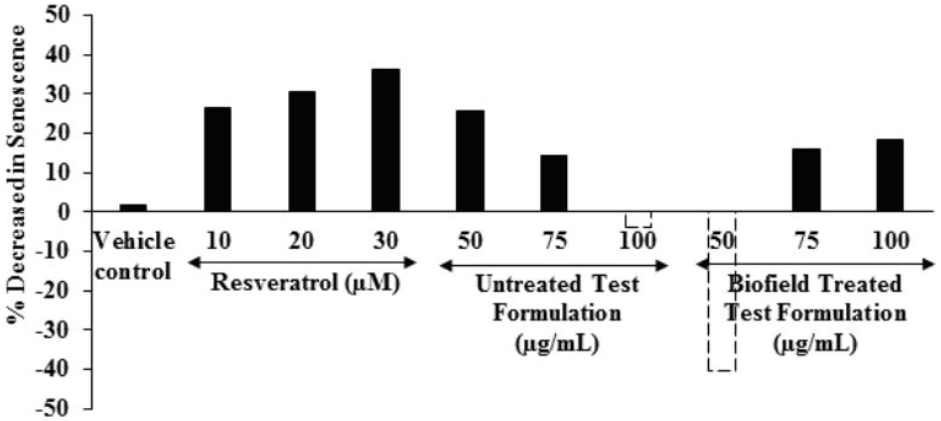


Figure 4: Effect of the test formulation on senescence associated β-galactosidase activity in mouse preadipocytes (3T3-L1) cells

Discussion

Cell viability can be effectively measured using MTT assay, which is considered as a cost-effective, rapid, less time consuming, and non-radioactive method as compared with the other assays. The principle of MTT assay is based on cell growth and metabolic activity [35]. In this experiment, the Biofield Treated test formulation showed more viable cells as compared to the untreated test formulation, which might be due to the Consciousness energy healing treatment. Thus, the Biofield Energy Treated Test formulation is defined to have more metabolic activity as compared with the untreated test formulation.

Three main structural parts of dermis *i.e.,* collagen, elastin, and glycosaminoglycans (GAGs) have been considered in most of the anti-aging research [36]. Among these, collagen is considered as the most important skin proteins used to improve skin structure, and the fibrous protein present in the skin, bone, tendon, teeth, and cartilage of multicellular organisms. It also provides the significant strength and structure to the skin that might be beneficial for skin health, strength, and wound healing [37,38]. Hence, based on the observed data it is explored that Biofield Energy Healing (the Trivedi Effect®) can be used for the management of aging disorders.

Cellular senescence is a process related to permanent proliferative arrest on cells in response to different stress agents, and it is responsible for aging and age-related disease. Aging occurs due to progressive loss of tissue and organ function over time [39]. Numerous literature suggests that senescence causes a loss of tissue-repair capacity and senescent cells can produce proinflammatory and matrix-degrading molecules i.e., senescence-associated secretory phenotype (SASP) can leads to aging [40]. According to Hayflick there is a link between senescence and aging [41].

Herbal medicine has been used in Asian countries, and can effectively reverse aging signs. Therefore, herbomineral ingredients have become a great choice for antiaging therapies [42]. Nowadays, a great deal of attention has been paid to Complementary and Alternative Medicine (CAM) special reference to Energy Therapy (Biofield) worldwide. Application of CAM has attracted more and more attention due to its good response and low cost as well as noninvasive and less side effects. Intrinsic skin aging is a process of chronologically physiological change. For example, the inner side of the upper arm, is mainly due to intrinsic genetic or metabolic factors, whereas exposed skin areas are influenced by extrinsic factors, especially UV radiation [43].

Researcher found that as a person ages, proliferation of cells in the basal layer reduces. This process of decreased proliferative ability of cells including keratinocytes, fibroblasts, and melanocytes is called cellular senescence [44]. In the experiment, Biofield Energy Treated novel herbomineral test formulation has significantly improved cellular proliferation in the basal layers by reducing the senescence cells.

Overall, these data suggested that Biofield Energy Treated Test formulation would be the best treatment strategy to prevent and protect from the occurrence of any type of disease. Therefore, the Biofield Energy Healing Treatment (the Trivedi Effect®) might be effective in healthy humans when used as a preventive maintenance therapy to sustain good health, to boost overall health, promote healthy aging and increase quality of life (QoL). In the presence of disease, the Biofield Energy therapy might reduce the severity of any acute/chronic disease (such as auto-immune related and inflammatory disorders) and / or slow the disease progression.

Conclusion

MTT assay data suggested that the Biofield Energy Treated test formulation were safe at the tested concentration upto 100 µg/mL in both human foreskin fibroblasts-1 (HFF-1) and mouse preadipocytes (3T3-L1) cells. In addition to, the Biofield Energy Treated test formulation showed a significant protection by 35.73% and 122.07% of oxidative stress that improve the cell viability in HFF-1 cells. Moreover, the level of collagen was significantly increased by 16.81% and 30.22% in the Biofield Treated test formulation at 75 and 100 µL, respectively as compared to the untreated test formulation group. Besides, the cellular senescence was significantly reduced by 66.35% and 22.5% in the Biofield Treated test formulation at 50 and 100 µg/mL, respectively compared to the untreated test formulation group. On the basis of experimental results of antioxidant activity and radical scavenging activity, the new test formulation after treated with the Trivedi Effect®- Biofield Energy Healing would be used against various autoimmune disorders (Addison Disease, Multiple Sclerosis, Myasthenia Gravis, Pernicious Anemia, Aplastic Anemia), anti-inflammatory diseases, and antiaging. The Biofield Treated test formulation can be used for the management of immune-mediated diseases such as Irritable Bowel Syndrome, Ulcerative colitis and Crohn's disease, Stress, Asthma, and many more. Besides, it can also be utilized in organ transplants (for example kidney, liver, and heart transplants).

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References

1. Sharma P, Jha AB, Dubey RS, Pessarakli M (2012) Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. J Bot 2012: Article ID 217037.

2. Pham-Huy LA, He H, Pham-Huy C (2008) Free radicals, antioxidants in disease and health. Int J Biomed Sci 4: 89-96.

3. Uttara B, Singh AV, Zamboni P, Mahajan R (2009) Oxidative stress and neurodegenerative diseases: A review of upstream and downstream antioxidant therapeutic options. Curr Neuropharmacol 7: 65-74.

4. Lobo V, Patil A, Phatak A, Chandra N (2010) Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev 4: 118-26.

5. Chen J, Ozanne SE, Hales CN (2007) Methods of Cellular Senescence Induction Using Oxidative Stress. Methods Mol Biol 371: 179-89.

6. Escande C, Nin V, Pirtskhalava T, Chini CC, Thereza Barbosa M, et al. (2014) Deleted in breast cancer 1 regulates cellular senescence during obesity. Aging Cell 13: 951-3.

7. Thomas KJ, Nicholl JP, Coleman P (2001) Use and expenditure on complementary medicine in England: A population based survey. Complement Ther Med 9: 2-11.

8. Manya K, Champion B, Dunning T (2012) The use of complementary and alternative medicine among people living with diabetes in Sydney. BMC Complement Altern Med 12: 2.

9. Barnes PM, Bloom B, Nahin R (2008) Complementary and alternative medicine use among adults and children: United States, 2007. Natl Health Stat Report 10: 1-23.

10. Astin JA, Pelletier KR, Marie A, Haskell WL (2000) Complementary and alternative medicine use among elderly persons: One-year analysis of a blue shield medicare supplement. J Gerontol A Biol Sci Med Sci 55: M4-9.

11. Lukác N, Massányi P (2007) Effects of trace elements on the immune system. Epidemiol Mikrobiol Imunol 56: 3-9.

12. Galland L (1988) Magnesium and immune function: An overview. Magnesium 7: 290-9.

13. Frass M, Strassl RP, Friehs H, Mullner M, Kundi M, et al. (2012) Use and acceptance of complementary and alternative medicine among the general population and medical personnel: A systematic review. Ochsner J 12: 45-56.

14. Trivedi MK, Mohan TRR (2016) Biofield energy signals, energy transmission and neutrinos. American J Mod Phys 5: 172-6.

15. Trivedi MK, Branton A, Trivedi D, Nayak G, Gangwar M, et al. (2015) Use of energy healing medicine against Escherichia coli for antimicrobial susceptibility, biochemical reaction and biotyping. Am J Biosci and Bioeng 3: 99-105.

16. Trivedi MK, Branton A, Trivedi D, Nayak G, Gangwar M, et al. (2015) Improved susceptibility pattern of antimicrobials using vital energy treatment on Shigella sonnei. Am J Internal Med 3: 231-7.

17. Trivedi MK, Branton A, Trivedi D, Nayak G, Gangwar M, et al. (2015) Assessment of antibiogram of biofield energy treated Serratia marcescens. Eur J Preventive Med 3: 201-8.

18. Trivedi MK, Branton A, Trivedi D, Nayak G, Gangwar M, et al. (2015) Antibiogram, biochemical reactions, and genotypic pattern of biofield treated Pseudomonas aeruginosa. J Trop Dis 4: 181.

19. Trivedi MK, Branton A, Trivedi D, Gangwar M, Jana S (2015) Antimicrobial susceptibility, biochemical characterization and molecular typing of biofield treated Klebsiella pneumoniae. J Health Med Inform 6: 206.

20. Peoples JJ, Trivedi MK, Branton A, Trivedi D, Nayak G, et al. (2017) Skin rejuvenating effect of consciousness energy healing treatment based herbomineral formulation. Am J Plant Biol 2: 77-87.

21. Smith DM, Trivedi MK, Branton A, Trivedi D, Nayak G, et al. (2017) Skin protective activity of consciousness energy healing treatment based herbomineral formulation. J Food and Nutr Sci 5: 86-95.

22. Krista JC, Mahendra KT, Alice B, Dahryn T, Gopal N, et al. (2018) Increase in bone mass density and overall bone health following high-impact of biofield energy treated vitamin d3 in mg-63 cell line. Significances Bioeng Biosci 2: DOI: 000533.

23. Lorraine MH, Mahendra KT, Alice B, Dahryn T, Gopal N, et al. (2018) Biofield energy enriched vitamin d3 versus vitamin D3 in preventing fractures and bone loss using mg-63 cells. Ortho Res Online J 3: DOI: 000572.

24. Balmer JA, Trivedi MK, Branton A, Trivedi D, Nayak G, et al. (2018) Functional modification of bone tissues after treatment with the biofield energy treated vitamin D3 in human bone osteosarcoma cells (MG-63). J Community Med Public Health: CMPH-135.

25. Trivedi MK, Branton A, Trivedi D, Nayak G, Mondal SC, et al. (2015) Evaluation of plant growth regulator, immunity and DNA fingerprinting of biofield energy treated mustard seeds (Brassica juncea). Agricul For and Fish 4: 269-74.

26. Trivedi MK, Branton A, Trivedi D, Nayak G, Gangwar M, et al. (2015) Analysis of genetic diversity using simple sequence repeat (SSR) markers and growth regulator response in biofield treated cotton (Gossypium hirsutum L.). Am J Agricul For 3: 216-21.

27. Trivedi MK, Branton A, Trivedi D, Nayak G, Nykvist CD, et al. (2017) Potential role of the trivedi effect® -biofield energy healing on immunomodulatory response of herbomineral formulation in male sprague dawley rats. Int J Biomed Sci Eng 5: 53-62.

28. Trivedi MK, Branton A, Trivedi D, Nayak G, Plikerd WD, et al. (2017) Immunological effects of biofield energy healing (The Trivedi Effect®) based novel herbomineral formulation after oral administration in male sprague dawley rats. Biomed Sci 3: 119-28.

29. Trivedi MK, Branton A, Trivedi D, Nayak G, Bairwa K, et al. (2015) Spectroscopic characterization of disulfiram and nicotinic acid after biofield treatment. J Anal Bioanal Tech 6: 265.

30. Trivedi MK, Branton A, Trivedi D, Nayak G, Bairwa K, et al. (2015) Physicochemical and spectroscopic characterization of biofield energy treated p-anisidine. Pharm Anal Chem Open Access 6: 102.

31. Trivedi MK, Tallapragada RM, Branton A, Trivedi D, Nayak G, et al. (2015) Characterization of atomic and physical properties of biofield energy treated manganese sulfide powder. Am J Phys and Appl 3: 215-20.

32. Trivedi MK, Tallapragada RM, Branton A, Trivedi D, Nayak G, et al. (2015) Evaluation of physical and structural properties of biofield energy treated barium calcium tungsten oxide. Adv Mater 4: 95-100.

33. Hancharuk A, Trivedi MK, Branton A, Trivedi D, Nayak G, et al. (2017) Photo-protective effect of biofield energy healing (the Trivedi Effect) treatment based herbomineral formulation against various skin health parameters. Am J Life Sci 5: 75-85.

34. Biological evaluation of medical devices - Part 5: Tests for in vitro cytotoxicity (ISO 10993-5:2009), I.S.EN ISO, 10993-5:2009.

35. Riss TL, Moravec RA, Niles AL, Duellman S, Benink HA, et al. (2004) Assay Guidance Manual [Internet]. National Center for Advancing Translational Sciences.

36. Baumann L (2007) Skin ageing and its treatment. J Pathol 211: 241-51.

37. Albini A, Adelmann-Grill BC (1985) Collagenolytic cleavage products of collagen Type I as chemoattractants for human dermal fibroblasts. Eur J Cell Biol 36: 104-7.

38. Jeffrey J (1995) Metalloproteinases and tissue turnover. Wounds 7: 13A-22A.

39. Flatt T (2012) A new definition of aging? Front Genet 3: 148.

40. Childs BG, Durik M, Baker DJ, van Deursen JM (2015) Cellular senescence in aging and age-related disease: from mechanisms to therapy. Nat Mad 21: 1424-35.

41. Hayflick L, Moorhead PS (1961) The serial cultivation of human diploid cell strains. Exp Cell Res 25: 585-621.

42. Thornfeldt CR, Rizer RL (2016) Superior efficacy of an herbal-based cosmeceutical compared with common prescription and cosmetic antiaging therapies. J Drugs Dermatol 15: 218-23.

43. Mancini M, Lena AM, Saintigny G, Mahé C, Di Daniele N, et al. (2014) MicroRNAs in human skin ageing. Ageing Res Rev 17: 9-15.

44. Makrantonaki E, Zouboulis CC (2007) William J Cunliffe Scientific Awards. Characteristics and pathomechanisms of endogenously aged skin. Dermatology 214: 352-60.