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Ex-vivo Hair Growth Promotion Efficacy of Biofield Energy Treated Williams Medium E using Vibrissae Hair Follicle Organ Culture

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Dahryn Trivedi [α] & Snehasis Jana [σ]

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I. Introduction

The hair follicle is consist of mainly two components one is epithelial components and the others are dermal components. Hair growth is regulated by the division of the hair follicle matrix cells under control of the dermal papilla. Three different stages of hair growth can be identified, an active phase (anagen) during which hair growth occurs, an intermediate regressive (catagen) stage and a resting phase (telogen) during which no cell proliferation occurs [1]. Numerous assays are routinely used to assess hair growth, while hair follicle organ culture model is one of the most popular and powerful *in vitro* systems [2]. With the measurement of follicular activity in terms of bulb thickness and improvement of anagen initiation, regression of catagen, and finally shifting of hair bulb *i.e.,* telogen formation is the main criteria for hair growth [3]. The positive control used in this experiment *i.e.,* minoxidil because many literature reported that it can directly promote hair growth *via* the stimulation of growth factor release from adipose-derived stem cells dermal papilla and epithelial cells [4]. In recent years, several scientific reports and clinical trials have revealed the useful effects of Biofield Energy Treatment, which have shown to enhance the immune function in cases of cervical cancer patients *via* therapeutic touch [5], massage therapy [6], etc. Complementary and Alternative Medicine (CAM) therapies are now rising as preferred models of treatment, among which Biofield Therapy (or Healing Modalities) is one approach that has been reported to have several benefits to enhance physical, mental and emotional human wellness. However, as per the data of 2012 from the National Health Interview Survey (NHIS), which indicated that the highest percentage (17.7%) of the Americans used dietary supplements as a complementary health approach as compared with other practices in past years. The National Center of Complementary and Integrative Health (NCCIH) has recognized and accepted Biofield Energy Healing as a CAM health care approach in addition to other therapies, medicines and practices such as natural products, deep breathing, yoga, Tai Chi, Qi Gong, chiropractic/osteopathic manipulation, meditation, massage, special diets, homeopathy, progressive relaxation, guided imagery, acupressure, acupuncture, relaxation techniques, hypnotherapy, healing touch, movement therapy, pilates, rolfing structural integration, mindfulness, Ayurvedic medicine, traditional Chinese herbs and medicines, naturopathy, essential oils, aromatherapy, Reiki, and cranial sacral therapy. Human Biofield Energy has subtle energy that can work effectively [7]. CAM therapies have been practiced worldwide with reported clinical benefits in different health disease profiles [8]. This energy can be harnessed and transmitted by the experts into living and non-living things *via* the process of Biofield Energy Healing. Biofield Energy Treatment (The Trivedi Effect®) has been published in numerous peer-reviewed science journals with significant outcomes in many scientific fields such

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as cancer research [9, 10], microbiology [11-14], biotechnology [15, 16], pharmaceutical science [17-20], agricultural science [21-24], materials science [25-28], nutraceuticals [29, 30], skin health, human health and wellness.

Based on the literature information and importance of Biofield Energy Healing Treatment on various fields, the authors sought to evaluate the impact of the Biofield Energy Treatment (The Trivedi Effect®) on the test item (William's Medium E) for hair cells growth activity with respect to the assessment of different hair growth parameters like bulb thickness and telogen formation using standard assays in vibrissae hair follicle organ culture cells with the help of UTHSCSA Image tool version 3.

II. Materials and Methods

a) Chemicals and Reagents

William's Medium E (phenol-free) with growth factors, antibiotics solution (penicillin-streptomycin), and DMEM (phenol-red free) were procured from HiMedia, India. Minoxidil sulphate (positive control) was purchased from Clearsynth Labs Ltd., Mumbai. L-glutamine and fungisone were procured from Gibco, India. Insulin from bovine pancreas, hydrocortisone, vitamin B_{12}, and glucose were obtained from Sigma Chemical Co. (St. Louis, MO). All the other chemicals used in this experiment were analytical grade procured from India.

b) Isolation and Maintenance of Vibrissa Hair Follicles from Mice

Vibrissa hair follicles were isolated from 16 days old C57BL/6 mice by micro dissection using standard method with few modifications [32]. Briefly, both the left and right whisker pads of C57BL/6 mice were excised out and placed in a 1:1 solution of Earle's balanced salts solution and phosphate-buffered saline (PBS) supplemented with 100U penicillin per mL and 100 mg streptomycin per mL. After that, individual anagen follicles were isolated from the whisker pad and were randomized into different groups and transferred on to a 5 cm plastic petri dish containing Earle's balanced salts solution/PBS (1:1) using one dish per animal. Isolated anagen follicles were maintained in a 24 well plate in William's medium E (supplemented with growth factors) for a period of 7 days and maintained at 37 ^{O}C at 5 % CO_2 [33]. William's Medium E (phenol-free) with growth factors was used as a test system in the present study. Vibrissae hair follicle culture was maintained under William's Medium E growth medium for routine culture supplemented with 10% FBS [34].

c) Experimental Design

Isolated anagen follicles were grouped into following treatment groups. Group 1 was served as untreated test item (William's Medium E cells phenol-free supplemented with growth factors). Group 2 was defined as Biofield Energy Treated William's Medium E. Group 3 was denoted as the positive control, minoxidil sulphate (1 mM).

d) Biofield Energy Healing Approach

The William's Medium E has used a test item in this experiment. The test item was divided into two parts. One part was considered as the untreated test item, where no Biofield Energy Healing Treatment was provided. Further, the untreated test items group was treated with "sham" healer for comparison purpose. The sham healer did not have any knowledge about the Biofield Energy Healing Treatment. Second part of the test item was received Biofield Energy Healing Treatment (known as The Trivedi Effect®) under laboratory conditions for ~5 minutes through Dahryn's unique Biofield Energy Transmission process to the test item. Biofield Energy Healer in this study did not visit the laboratory, nor had any contact with the test samples. After that, the Biofield Energy Treated and untreated test items were kept in similar sealed conditions and used for the study as per the study plan.

e) Morphological Analysis of Vibrissa Hair Follicles

All the follicles in the well plate were observed daily through microscope for any morphological changes. Photographs of the individual vibrissae follicles were captured during the course of the study upto day 7. After the completion of the experiment, all the follicles treated with test items and positive control were measured for hair bulb thickness and compared to the respective baseline thickness of day 1 using UTHSCSA Image tool version 3.

f) Statistical Analysis

Data were represented as mean ± standard error of mean (SEM). For statistical analysis Sigma-Plot (version 11.0) was used as a statistical tool. Statistically significant values were set at the level of $p \leq 0.05$.

III. Results and Discussion

a) Assessment of Vibrissa Hair Follicles

Human hair growth is a unique repetitive cycle that composed of the stage of initiation (anagen), regression (catagen), and shifting of hair bulb (telogen) phases [34]. This cycle of hair growth, regulating hair follicle development and periodic regeneration is influenced by dermal papilla cells (DPCs); while if the DPCs are in a pathological state that ultimately leads to various hair loss disorders [35-37]. Topical minoxidil is a well-established therapeutic for various types of hair growth-related disorders like alopecia [38]. The vibrissae hair follicle organ culture cells were treated with the positive controland the untreated test item (William's Medium E). The percent increased of bulb thickness of both minoxidil sulphate and the untreated test item groups are shown in Figure 1.

The experimental results showed that the bulb thickness in the positive control (minoxidil) group was 1.9 ± 0.29, 2.6 ± 0.37, and 3.3 ± 0.36 mm on day 1, 5, and 7, respectively. Additionally, the untreated test item group showed 1.5 ± 0.45, 1.8 ± 0.57, and 1.9 ± 0.60 mm of bulb thickness on day 1, 5, and 7, respectively. Overall, the bulb thickness was significantly increased by 36.84% and 73.68% in the minoxidil group on day 5 and 7, respectively compared to the day 1. Moreover, the untreated test item group showed 20% and 26.67% increased bulb thickness on day 5 and 7, respectively compared to the day 1 (Figure 1). Follicles were observed to have catagen-like changes with an increase in hair bulb thickness measurement (Figure 3 A).

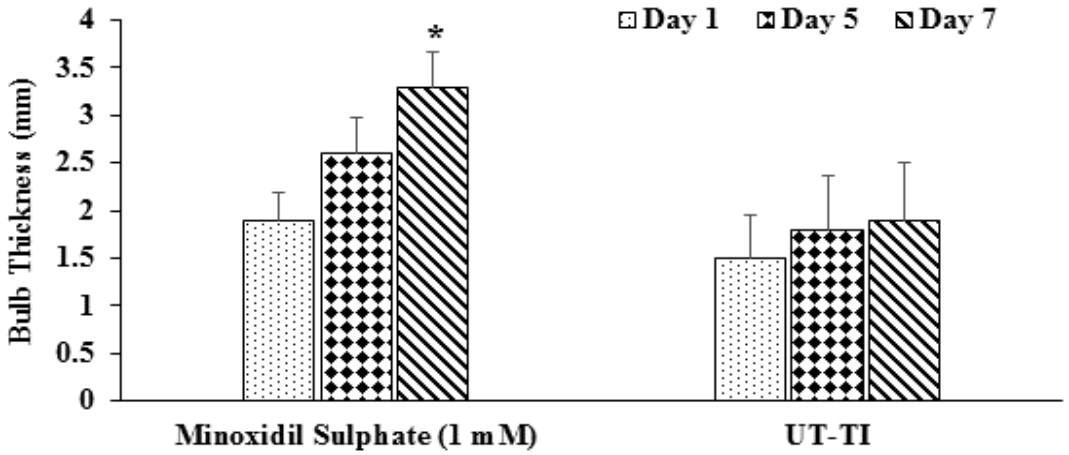

Figure 1: Assessment of hair follicle growth and development in William's Medium E in terms of bulb thickness (mm) on vibrissae hair follicle organ culture cellsof positive control and untreated test item groups. UT-TI: Untreated test item (William's Medium E). Values are expressed as Mean ± SEM. *p≤0.05 vs. day 1.

Besides, the Biofield Energy Treated test item (William's Medium E) on vibrissae hair follicle organ culture cells and the percent of telogen follicles are shown in Figure 2. The percent of telogen follicle was observed as 57%, 86%, and 100% on day 3, 5, and 7, respectively in the Biofield Energy Treated test item group (Figure 2). On day 7, shifting of the hair shaft from its original place was observed in the seven out of seven follicle*i.e.,* 100%, which is a hallmark of telogen transition (Figure 3 B). Follicles kept in minoxidil sulphate solution led to increase in hair bulb thickness in follicles when observed on day 5 as well as day 7 as compared to day 1. In the untreated test item group, follicles was maintained their integrity with slight increase in hair bulb thickness observed on day 5 and 7 as compared to day 1.

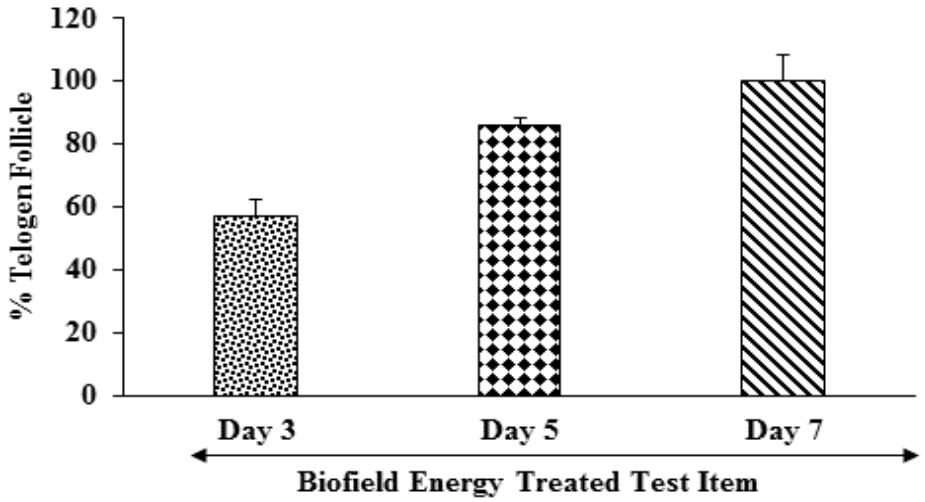

Figure 2: Effect of the Biofield Energy Healing Treatment on vibrissae hair follicle organ culture cells for the assessment of hair follicle growth and development in William's Medium E in terms of telogen formation of Biofield Energy Treated test item (William's Medium E).

Overall, the untreated test item group did not show any telogen formation, however the Biofield Energy Treated test item significantly exhibited telogen formation *i.e.,* promote hair growth upto day 7 observation. Based on that it is assumed that in this experiment the improvement of hair cell growth and development in terms of telogen formation could be due to the impact of The Trivedi Effect® - Biofield Energy Healing Treatment.

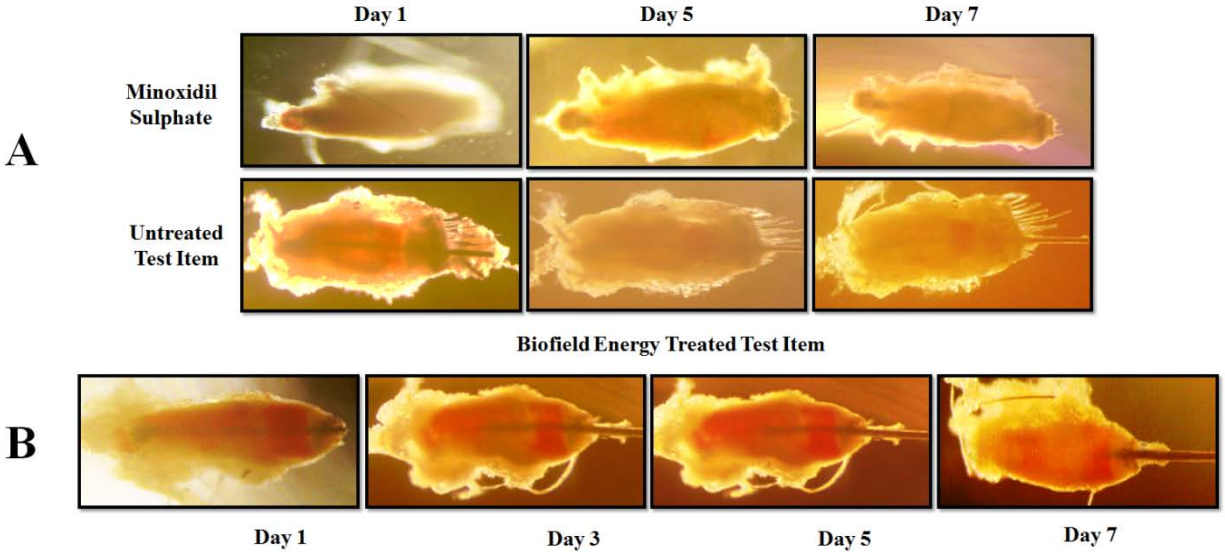

Figure 3: Representative photomicrograph of various stages of hair follicle development (anagen - catagen - telogen) of different treatment groups. A: Initiation of anagen follicle (thick hair bulb) in minoxidil and untreated groups; B: Transformation of initiation, regression of hair bulb, and shifting of hair shaft (telogen follicle) in the Biofield Energy Treated test item (William's Medium E) group.

IV. Conclusions

The experimental results showed that the untreated test item group showed 20% and 26.67% increased bulb thickness on day 5 and 7, respectively compared to the day 1. Besides, the percent telogen follicle was found as 57%, 86%, and 100% on day 3, 5, and 7, respectively in the Biofield Energy Treated test item group as compared to day 1. Overall, the Biofield Energy Treated test item significantly enhanced hair follicles regarding telogen formation compared to the untreated test item group in vibrissae hair follicle organ culture cells derived from mice. In conclusion, The Trivedi Effect® - Consciousness Energy Healing Treatment might act as an effective hair growth enhancer and it can be used as a complementary and alternative treatment for the prevention of various types of skin-related disorders *viz.* necrotizing fasciitis, actinic keratosis, sebaceous cysts, diaper rash, decubitus ulcer etc. Besides, it might be useful to improve cell-to-cell communication, normal cell growth, cell differentiation, neurotransmission, cell cycling and proliferation, hormonal balance, skin health, immune and cardiovascular functions. Besides, it can also be utilized in organ transplants (for example kidney transplants, liver transplants and heart transplants), hormonal imbalance, aging, and various immune-related disease conditions such as Ulcerative Colitis, Alzheimer's Disease, Dermatitis, Irritable Bowel Syndrome, Asthma, Hashimoto Thyroiditis, Pernicious Anemia, Sjogren Syndrome, Multiple Sclerosis, Aplastic Anemia, Hepatitis, Diverticulitis, Graves' Disease, Dermatomyositis, Diabetes, Myasthenia Gravis, Parkinson's Disease, Atherosclerosis, Systemic Lupus Erythematosus, stress, etc. with a safe therapeutic index to improve overall health, and quality of life.

Abbreviations: CAM: Complementary and Alternative Medicine; PBS: Phosphate-buffered saline; DPCs: Dermal papilla cells; UT-TI: Untreated test item.

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