



The Role of Biofield Energy Treated DMEM in Erectile Dysfunction using Detection of cGMP Levels in Human Endothelial Cell Line

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Abstract

The study was performed to assess the impact of Biofield Treated test item (DMEM medium) on Human Endothelial Hybrid Cell Line (EA. hy926) for the expression of cyclic guanosine monophosphate (cGMP). The test item was divided into three parts. The first part was received one-time Consciousness Energy Treatment by a renowned Biofield Energy Healer, Mahendra Kumar Trivedi and labeled as BT-I, while second part received two-times Biofield Treatment and is denoted as BT-II. The third part did not receive any types of treatment and denoted as untreated DMEM. The level of intracellular cGMP in the BT-I and BT-II groups showed a significantly ($p \leq 0.001$) increased by 296.06% and 339.37%, respectively in Ea. hy926 cells compared to the untreated DMEM group. These results suggest that BT-II group showed a significant improved the level of cGMP with respect to the BT-I group. Therefore, the Biofield Energy Healing Treatment can be used to treat the erectile dysfunction patients along with other associated disorders such as orgasmic disorders, frotteuristic disorder, female sexual arousal disorder, vaginismus, fetishistic disorder, sex addiction, hypoactive sexual desire disorder, premature or delayed ejaculation.

Keywords: Biofield Energy; cGMP; Endothelial Hybrid Cell; PDE-5; DMEM; Erectile Dysfunction

Abbreviation: BT-I: One-time Biofield Energy Treated DMEM; BT-II: Two-times Biofield Energy Treated DMEM; CAM: Complementary and Alternative Medicine; NCCAM: National Center for Complementary and Alternative Medicine; DMEM: Dulbecco's Modified Eagle's Medium; cGMP: Cyclic guanosine 3',5'-monophosphate; FBS: Fetal Bovine Serum

Introduction

Erectile dysfunction (ED) or impotence is the inability to get and keep an erection firm enough for sex. This is the most common sexual disorder in men across the globe [1]. Erectile dysfunction symptoms include persistent trouble in getting and keeping an erection and less sexual desire. Male sexual arousal is a complex process, which includes the coordination of the brain, hormones, emotions, nerves, muscles, and blood vessels [2]. ED might occur due to stress and severe mental health conditions. Besides, the literature data reported that physical cause of ED are heart disease, Parkinson's disease, multiple sclerosis, atherosclerosis, high cholesterol, peronei's disease high blood pressure, diabetes, obesity, sleep disorders, use of tobacco, alcoholism, injury of spinal cord

and various metabolic syndromes such as high insulin levels, body fat around the waist, etc. [3]. Along with physical and psychological factors, impaired function of arteries and corpora cavernosa within the penis are the primary condition for impotence. While the lack of smooth muscle tone and imperfections in neuronal stimuli can lead to unsuccessful penile erection [4]. ED eventually leads to neuronal and cardiovascular disorders [5,6].

Nitric oxide synthase (NOS) enzymes are the major mechanism involved in ED, and it enhanced the production of cyclic guanosine monophosphate (cGMP), which results in smooth muscle relaxation and vasodilation *via* NO/cGMP pathway [7,8]. Inadequate level of NO/cGMP leads to ED. Thus, cGMP is the major therapeutically

important target to overcome ED by inhibiting the cGMP-specific phosphodiesterase (PDE-5) enzyme [9]. However, sildenafil a PDE5 inhibitor has been used to treat ED, but it has life-threatening side-effects *viz.* cardiac arrhythmia and hypotension [10] and vascular or neuronal deficiency like diabetes [11]. Thus, some alternative or complementary therapeutic approach is the best method to treat impotence without any side effects. In recent years, a remarkable outstanding alternative Complementary and Alternative Medicine (CAM) therapies approach known as Biofield Energy Healing Treatment (The Trivedi Effect®) have been scientifically reported in various fields. The human Biofield Energy is a weak electromagnetic field around of the human body. The Trivedi Effect® can be able to transform all the living organisms and non-living materials through a unique energy transmission process [12].

The effects of the CAM therapies have great potential, which include Johrei, Qi Gong, external qigong, Tai Chi, Reiki, therapeutic touch, deep breathing, yoga, polarity therapy, pranic healing, chiropractic/osteopathic manipulation, guided imagery, meditation, massage, homeopathy, hypnotherapy, special diets, progressive relaxation, acupressure, acupuncture, relaxation techniques, mindfulness, Roling structural integration, healing touch, movement therapy, pilates, Ayurvedic medicine, traditional Chinese herbs and medicines in biological systems both *in vitro* and *in vivo* [12]. Biofield Energy Healing as a CAM showed significant results in biological studies [13]. Also, the National Center for Complementary and Alternative Medicine (NCCAM), well-defined Biofield therapies in the subcategory of Energy Therapies [14]. The Trivedi Effect® has been reported to have created significant changes in the materials science [15-17], agricultural science [18,19], microbiology [20-22], biotechnology [23,24], improved bioavailability [25-27], skin health [27-29], nutraceuticals [30,31], cancer research [32,33], bone health [34-36], human health and wellness. Based on the outstanding benefits of The Trivedi Effect®, the present study was aimed to investigate the effect of Biofield Treated DMEM on the level of cGMP, in order to eradicate the ED using standard *in vitro* assay in Human Endothelial Hybrid Cell Line (EA. hy926).

Material and Methods

Requirement of Chemicals

Dulbecco's Modified Eagle's Medium (DMEM) and fetal bovine serum (FBS) were obtained from Life Technology, USA. Antibiotics solution (penicillin-streptomycin) was purchased from HiMedia, India, while ethylenediaminetetraacetic acid (EDTA) was purchased from Sigma, USA. Sildenafil citrate was purchased from Clearsynth, India. All the other chemicals used in this experiment were analytical grade procured from India.

Cell Culture

Human Endothelial Hybrid Cell Line (EA. hy926) was used as a test system in this experiment. The cells were maintained in DMEM

growth medium for routine culture supplemented with 10% FBS. Growth conditions were maintained at 37°C, 5%CO₂, and 95% humidity and subcultured by trypsinization followed by splitting the cell suspension into new flasks and supplementing with fresh cell growth medium. Three days before the start of the experiment, the growth medium of near-confluent cells was replaced with fresh phenol-free DMEM, supplemented with 10% charcoal-dextran stripped FBS (CD-FBS) and 1% penicillin-streptomycin [37].

Study Design

The experimental groups consisted of group 1 (G-I) with serum-free DMEM defined as the untreated DMEM. Group 2 (G-II) consisted of positive control (sildenafil citrate) at different concentrations. Further, group 3 (G-III) included DMEM medium (test item group) with the one-time Biofield Energy Treatment and denoted as BT-I, while the group 4 (G-IV) included the test item with the two-times Biofield Energy Treatment and indicated as the BT-II.

Biofield Energy Healing Treatment Strategies

The test item, DMEM was divided into three parts. One part of the test item was treated with the one-time Biofield Energy Healing Treatment by a renowned Biofield Energy Healer (The Trivedi Effect®) and coded as the Biofield Energy Treated DMEM (BT-I), while the second part was received the two-times Biofield Energy Healing Treatment and denoted as the BT-II. Further, the third part did not receive any treatment and defined as the untreated DMEM group. This Biofield Energy Healing Treatment was provided by a renowned Biofield Energy Healer, Mahendra Kumar Trivedi, remotely for ~3 minutes. The Biofield Energy Healer was 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 ~3 minutes through the Healer's unique Energy Transmission process remotely to the test items under the standard laboratory conditions. Mahendra Kumar Trivedi never visited the laboratory in person, nor had any contact with the test item (DMEM medium). Further, the untreated DMEM group was treated with a "sham" healer for comparative purposes. The "sham" healer did not have any knowledge about the Biofield Energy Treatment. After that, the Biofield Energy Treated and untreated samples were kept in similar sealed conditions for experimental study.

Assessment of PDE-5 Enzyme Inhibition

The cells were counted using an hemocytometer and were seeded at a density of 0.4 X 10⁶ cells/well in DMEM with 10 % FBS in 6-well plates. The details test procedure was followed as per Branton et al. 2018 [38-40]. Increase in cGMP level was determined as the following equation (1)

$$\% \text{ Increase in intracellular cGMP level} = \{(B-A)/A\} \times 100 \text{----} \\ \text{-- (1)}$$

Where, B = OD of cells treated with test item and A is the OD of untreated wells (media treated).

Statistical Analysis

Values were expressed as Mean \pm SEM of three independent experiments. For multiple group comparison, one-way analysis of variance (ANOVA) was used followed by post-hoc analysis by Dunnett's test. Statistically significant values were set at the level of $p \leq 0.05$.

Results and Discussion

Detection of PDE-5 Enzyme Inhibition

The result of the intracellular cGMP level in Ea. hy926 cells is shown in Figure 1. Sildenafil citrate, used as positive control at 25 μ M, 50 μ M, and 100 μ M exhibited a significant increase in the intracellular cGMP in Ea. hy926 cells by 34%, 84%, and 234%,

respectively compared to the untreated DMEM group. The one-time Biofield Energy Treated DMEM (BT-I) group showed 5.03 pmol/mL, while two-times Biofield Energy Treated DMEM (BT-II) group showed 5.58 pmol/mL level of cGMP. Thus, BT-I group showed a significant ($p \leq 0.001$) increase in intracellular cGMP level by 296.06% in Ea. hy926 cells with respect to untreated DMEM. Similarly, the BT-II group showed a significant increase in the intracellular cGMP levels by 339.37% in Ea.hy926 cells than untreated DMEM. Thus, the data suggest that the two-times Biofield Energy Healing Treatment (BT-II) showed better results with respect to the increased cGMP level as compared with the one-time Biofield Treated DMEM group, which meant that the Biofield Energy Healing Treatment inhibited PDE-5 enzyme; resulting to the higher level of cGMP which can help to treat the erectile dysfunction (ED).

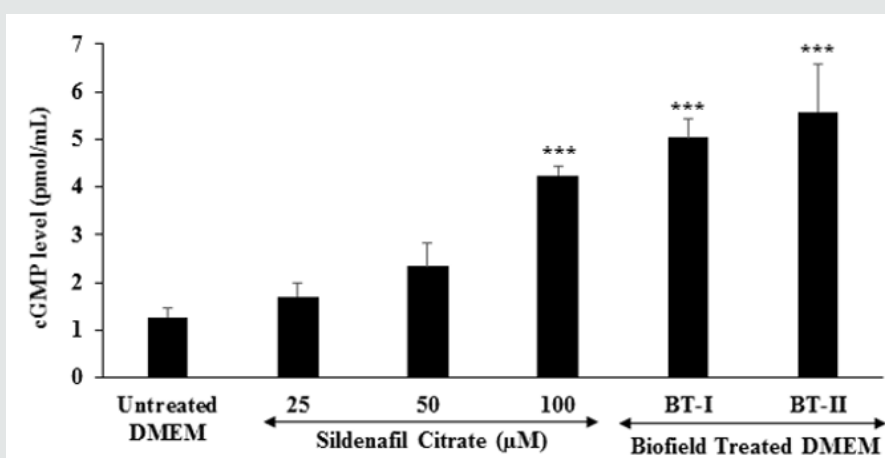


Figure 1: Effect of the test items (untreated and Biofield Treated DMEM) on the expression of intracellular cyclic guanosine monophosphate (cGMP) in human endothelial hybrid (Ea. hy926) cells. BT-I: One-time Biofield Energy Treated DMEM; BT-II: Two-times Biofield Energy Treated DMEM. *** $p \leq 0.001$ vs. untreated DMEM group.

The drugs, which are available in the market for the treatment of ED, are having serious side-effects along with short time treatment of ED [41]. Thus, ED can be treated with some alternative mode of treatment without having any type of adverse effects. Biofield Energy Healing Treatment is one of the best CAM approach worldwide to treat various clinical disorders along with a significant change in different scientific fields. The results are outstanding and can be comparable with the marketed synthetic drug, sildenafil citrate. Scientific literature results showed that relaxation in smooth muscles results in improved level of cGMP production leading to penile erection [42]. Increase in cGMP results in decreased level of intracellular calcium, which supports penile erection [43]. However, cGMP activation is regulated by the PDE-5 enzyme, which is abundant in the corpus cavernosum and results in improved blood circulation that leads to penile erection [44]. Biofield Energy Healing based DMEM and Biofield Energy Healing Treatment might work by the relaxation of penile smooth muscles, which could lead to penile erection.

Conclusion

Erectile dysfunction results an unsatisfactory sex life, mental stress or anxiety, embarrassment or low self-esteem, relationship problems, inability to get your partner pregnant, which can produce lot of pathological implications like hypertension, hypercholesterolemia, diabetes mellitus, cardiovascular disease, and depression. Thus, the Biofield Energy Healing Therapy is one of the best approach to treat various sexual disorders and its related diseases. The present study results showed that the Biofield Energy Treated DMEM significantly increased the level of intracellular cGMP in Ea. hy926 cells compared with the untreated DMEM group. PDE-5 is the predominant phosphodiesterase, while the intracellular cGMP level was significantly ($p \leq 0.001$) increased by 296.06% in the one-time Biofield Energy Treated DMEM group (BT-I) in Ea. hy926 cells compared to the untreated DMEM group. Additionally, the BT-II group *i.e.*, the two-times blessed in DMEM also showed a significantly ($p \leq 0.001$) increased the level of cGMP by 339.37% compared with the untreated DMEM group. Henceforth, it can be

concluded that the Biofield Energy Treated (The Trivedi Effect®) DMEM were found to have a significant impact on cGMP level, which might significantly inhibit the PDE-5 enzyme that leads to the penile erection. Thus, the Biofield Therapy can be used for the treatment of numerous sexual disorders *viz.* hypoactive sexual desire disorder, fetishistic disorder, dyspareunia, frotteuristic disorder, vaginismus, exhibitionistic disorder, voyeuristic disorder, sex addiction, premature or delayed ejaculation (or sexual malfunction or sexual disorder) improve normal sexual activity, desire, including physical pleasure, arousal or orgasm, preference, and neurological disorders, hormonal imbalances, sexual performance, desire disorders (lack of sexual desire or interest in sex), marital or relationship problems, effects of a past sexual trauma, feelings of guilt, depression, and pain disorders (pain during intercourse).

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