



# Evaluation of Phosphodiesterase-5 Inhibitory Potential of Biofield Energy Treated DMEM by Determining cGMP Level in Human Endothelial Cell Line



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## Abstract

Erectile dysfunction is a common disorder found in men, which occurs due to multiple factors such as psychogenic, hormonal imbalance, and neurovascular disturbances. The present investigation was undertaken to examine the effect of a Consciousness Energy Healing based DMEM medium on the Human Endothelial Hybrid Cell Line (EA. hy926) to evaluate the level of cyclic guanosine monophosphate (cGMP). The test item (DMEM medium) was divided into three parts, first part received a one-time Consciousness Energy Healing Treatment by a renowned Biofield Energy Healer, Alice Branton and was labeled as the one-time Biofield Energy Treated (BT-I) DMEM, while second part received the two-times the Biofield Energy Treatment and is denoted as BT-II DMEM. The third part did not receive any treatment and defined as the untreated DMEM group. The level of cGMP for the inhibition of PDE-5 enzyme was assessed using cGMP ELISA assay kit (colorimetric). Sildenafil citrate, used as positive control, which showed a significant increase of cGMP levels in Ea. hy926 cells. The one-time Biofield Energy Healing Treated DMEM (BT-I) showed 29.92% alteration, while the two-times Biofield Energy Healing Treated DMEM (BT-II) showed a significantly ( $p < 0.001$ ) increased level of intracellular cGMP by 307.09% in Ea. hy926 cells compared to the untreated DMEM group. Overall, experimental data suggested that the two-times Biofield Treated DMEM showed a significantly improved level of cGMP compared with the one-time Biofield Treated DMEM group. Therefore, data indicated that the Biofield Energy Healing Treatment can be used to treat erectile dysfunction along with other sexual disorders such as orgasmic disorders, female sexual arousal disorder, fetishistic disorder, frotteuristic disorder, hypoactive sexual desire disorder, sex addiction, sexual masochism and sadism, vaginismus, voyeuristic disorder, premature or delayed ejaculation.

**Keywords:** Biofield energy; The Trivedi effect®; cGMP; PDE-5; Sexual disorders; Endothelial hybrid cell; DMEM; Erectile dysfunction

**Abbreviations:** BT-I: One-time Biofield Energy Treatment, BT-II: Two-times Biofield Energy Treatment, 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

More than 30 million men in the United States and far more all over the world have erectile dysfunction (ED), which is a pervasive disorder and a most common disability. Very few are seeking medical attention, and even fewer have received minimal benefits from modern pharmaceutical based therapy [1]. ED is the incapability of the person for satisfactory sexual intercourse to achieve or maintain a penile erection [2]. Vascular, neurogenic, hormonal, and psychological factors play an important role for a penile erection or its dysfunction [3]. Impaired function of arteries and corpora cavernosa within penis are the major condition for impotence, while lack of smooth muscle tone and imperfections in neuronal stimuli can lead to unsuccessful penile erection [4]. ED eventually leads to other associated cardiovascular and

neuronal diseases such as atherosclerosis and diabetes [5,6]. Nitric oxide synthase (NOS) enzymes play the significant role in the mechanism of ED. It enhances the production of cyclic guanosine monophosphate (cGMP), which results in smooth muscle relaxation and vasodilation via NO/cGMP pathway [7,8]. Insufficient 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, at present various synthetic drugs are available like sildenafil, a well-known PDE5 inhibitor to treat ED but associated with life-threatening side-effects like hypotension and cardiac arrhythmia [10], and vascular or neuronal deficiency like diabetes [11]. Thus, some alternative or complementary therapeutic approach is very much required to treat impotence without any side-effects.

Complementary and Alternative Medicine (CAM) therapies have shown various significant clinical benefits. Over the past few decades, many energy healing practices have reported significant outcomes in various clinical and non-clinical fields. The effects of the CAM therapies have great potential, which include external qigong, Johrei, Reiki, therapeutic touch, yoga, Qi Gong, polarity therapy, Tai Chi, panic healing, deep breathing, chiropractic/osteopathic manipulation, guided imagery, meditation, massage, homeopathy, hypnotherapy, progressive relaxation, acupuncture, special diets, relaxation techniques, Roling structural integration, healing touch, movement therapy, Pilates, mindfulness, Ayurvedic medicine, traditional Chinese herbs and medicines in biological systems both *in vitro* and *in vivo* [12]. All living organisms possess some unique energy known as "Biofield Energy", which is an infinite, para-dimensional and electromagnetic field surrounding the human body. "Biofield" (Putative Energy Fields) based Energy Healing Therapies have been reported to have significant outcomes against various disease conditions. Biofield Energy Healing Treatment (The Trivedi Effect®) contains putative bioenergy, which is channeled by a renowned practitioner from a distance. Biofield Energy Healing as a CAM showed a significant result in biological studies [13]. However, the National Center for Complementary and Alternative Medicine (NCCAM), well-defined Biofield Therapies in the subcategory of Energy Therapies [14]. The Trivedi Effect®- Consciousness Energy Healing Treatment has been reported to have created significant changes in the physicochemical properties of metals, chemicals, ceramics and polymers [15-17], improved agricultural crop yield, productivity, and quality [18,19], transformed antimicrobial characteristics [20-22], biotechnology [23,24], improved bioavailability [25-27], skin health [28,29], nutraceuticals [30,31], cancer research [32,33], bone health [34-36], human health and wellness. From outstanding benefits of Biofield Energy Treatment, the present study aimed to evaluate the impact of the Biofield Energy Treated (The Trivedi Effect®) DMEM on ED using the standard *in vitro* assay in Human Endothelial Hybrid Cell Line (EA. hy926) cells.

## Material and Methods

### Chemicals and reagents

Sildenafil citrate was purchased from Clearsynth, India. Fetal bovine serum (FBS) and Dulbecco's Modified Eagle's Medium (DMEM) were purchased from Life Technology, USA. Antibiotics solution (penicillin-streptomycin) was procured from HiMedia, India, and ethylenediaminetetraacetic acid (EDTA) was purchased from Sigma, USA. 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 the present study. The EA. hy926 cell line was maintained in DMEM growth medium for routine culture supplemented with 10% FBS. Growth conditions were maintained at 37 °C, 5% CO<sub>2</sub>, and 95% humidity and subculture by trypsinization followed by splitting the cell suspension into fresh 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].

### Experimental design

The experimental groups consisted of group 1 (G-I) with serum-free DMEM defined as the untreated DMEM group. 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 the BT-I, while group 4 (G-IV) included the test item with the two-times Biofield Energy Treatment and denoted as the BT-II.

### Consciousness 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 DMEM. 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, Alice Branton, remotely for ~5 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 ~5 minutes through the Healer's unique Energy Transmission process remotely to the test items under the standard laboratory conditions. Alice Branton 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 10<sup>6</sup> cells/well in DMEM with 10 % FBS in 6-well plates. Cells were incubated in a CO<sub>2</sub> incubator for 24 hours at 37 °C, 5% CO<sub>2</sub>, and 95% humidity. After 24 hours of incubation, the medium was replaced with DMEM+1% FBS followed by treatment with the test item (Biofield Energy Treated DMEM). Sildenafil citrate was used as a positive control. After incubation for 24 hours, cell lysates were prepared (as per the ELISA kit instructions). The effect of the test item on modulation of intracellular cGMP level, if any, in Ea. hy926 cells were assessed using colorimetric cGMP ELISA kit as per manufacturer instruction. The samples were detected for cGMP assay in triplicates. The absorbance of each well was measured at 450nm immediately. Increase in cGMP level was determined using equation (1)

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

Where, B = Optical density (OD) of the cells treated with the test item and A is the OD of the untreated DMEM [38,39].

### Statistical analysis

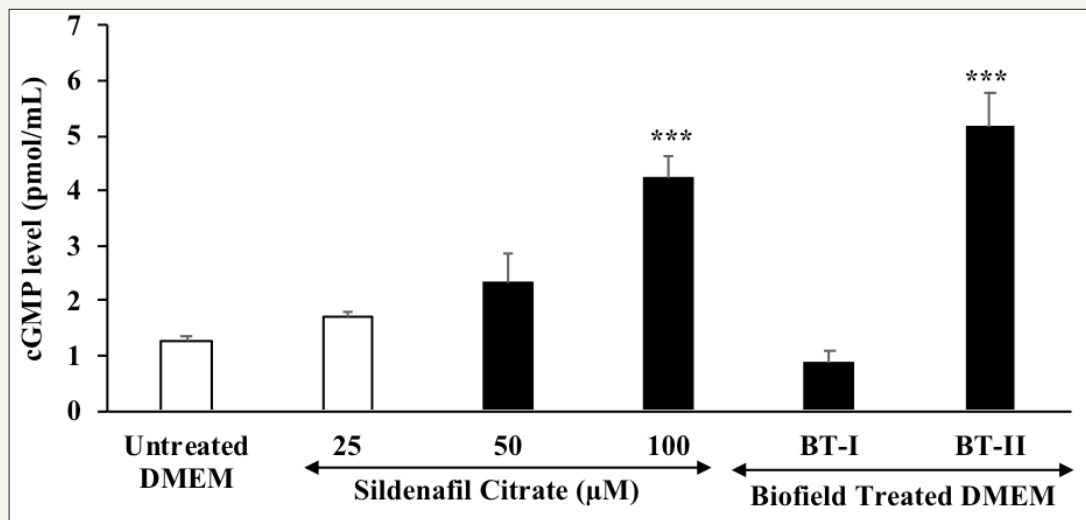
All the values were represented as Mean  $\pm$  SEM of intracellular cGMP levels of three independent experiments. For multiple group comparison, one-way analysis of variance (ANOVA) was used followed by post-hoc analysis by Dunnett's test. Statistically significant values were set at the level of  $p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

## Results and Discussion

### Estimation of the effect of test item on PDE-5 enzyme inhibition

The results of the intracellular cGMP levels in Ea. hy926 cells are shown in Figure 1. Sildenafil citrate as a positive control at 25  $\mu$ M, 50  $\mu$ M, and 100  $\mu$ M demonstrated significant increased intracellular cGMP level in Ea. hy926 cells by 34%, 84%, and 234%, respectively compared to the untreated DMEM group (cGMP values defined as 100%). The one-time Biofield Energy Treated test item (BT-I) and

the untreated DMEM showed almost a similar concentration of cGMP (0.89 pmol/mL), while the two-times Biofield Energy Treated group (BT-II) showed a significant increase the level of intracellular cGMP concentration by 307.09% compared to the untreated DMEM group. Therefore, the two-times Biofield Energy Treated DMEM group demonstrated a significant increase in the intracellular cGMP level in Ea. hy926 cells compared to untreated DMEM group. Thus, the data suggested that two-times Consciousness Energy Healing Treatment has significantly improved the cGMP level, which could help to resolve the erectile dysfunction related disorders (Figure 1) It might be expected that Biofield Energy Healing Treatment can mediate the smooth muscle relaxation that might increase the level of cGMP. The smooth muscles, nerves, and endothelial cells might stimulate guanylyl cyclase (GC) to produce cGMP and thus results in lowers intracellular calcium levels, which results in penile erection [40,41]. PDE-5 is the predominant phosphodiesterase in the corpus cavernosum. In this experiment, the Biofield Energy Treatment helps to increase the level of endogenous cGMP by inhibiting its breakdown by the PDE-5 enzyme [42].



**Figure 1:** Effect of the Biofield Energy Treated test item (DMEM) on the level of intracellular cyclic guanosine monophosphate (cGMP) in human endothelial hybrid (Ea. hy 926) cells.

Data were expressed as Mean  $\pm$  SEM of three independent experiments.

BT-I: One-time Biofield Energy Treatment; BT-II: Two-times Biofield Energy Treatment

\*\*\* $p \leq 0.001$  compared to the untreated DMEM group

## Conclusion

Erectile dysfunction is a common, multifactorial disorder, which is associated with a wide number of factors such as aging, organic and psychogenic conditions such as hypertension, hypercholesterolemia, diabetes mellitus, cardiovascular disease, and depression. Modern therapy is not attractive due to its lot of life-threatening side-effects. Thus, Biofield Energy Healing Therapy can be used to treat erectile dysfunction (ED). The study results showed that the two-times Biofield Energy Treated DMEM significantly ( $p \leq 0.001$ ) increased the level of the intracellular cGMP levels by 307.09% in the Ea. hy926 cells compared to the untreated DMEM group. The Biofield Energy Treated (The Trivedi

Effect<sup>®</sup>) DMEM has significant impact on cGMP level, which might be due to the significant inhibition of the PDE-5 enzyme. Therefore, the Consciousness Energy Healing Therapy might be a suitable alternative treatment option for ED. It can also be useful for the management of various sexual disorders viz. dyspareunia, exhibitionistic disorder, female and male orgasmic disorders (delay or absence of orgasm), female sexual arousal disorder (inability to become physically aroused or excited during sexual activity), fetishistic disorder, frotteuristic disorder, hypoactive sexual desire disorder, sex addiction, vaginismus, premature or delayed ejaculation (or sexual malfunction or sexual disorder) improve normal sexual activity, including physical pleasure, desire, preference, arousal or orgasm, desire disorders (lack of sexual

desire or interest in sex), and neurological disorders, hormonal imbalances, sexual performance, marital or relationship problems, effects of a past sexual trauma, depression, feelings of guilt, pain disorders (pain during intercourse).

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### Conflict of Interest

Authors declare that there was no conflict of interest.

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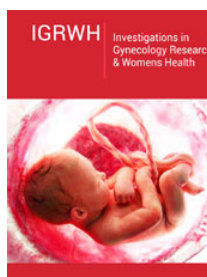
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