In Vitro Assessment of Estrogenic Potential of Biofield Energy Treatment using Human Endometrial Adenocarcinoma Cell Line

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Abstract

The objective of the study was to investigate the effect of Consciousness Energy Healing based DMEM medium on the level of alkaline phosphatase enzyme (ALP) activity in Ishikawa cells. The test item, DMEM medium was divided into two parts. One part of the test item received Consciousness Energy Healing Treatment by a renowned Biofield Energy Healer, Mahendra Kumar Trivedi and was labeled as the Biofield Energy Treated DMEM, while the other part did not receive any treatment, and defined as the untreated DMEM group. The cell viability using MTT assay of the Biofield Energy Treated DMEM group was observed as 108%, which indicated that the test item was safe and non-toxic. The estrogenic potential using ALP level showed a significantly increase by 73.21% in the Biofield Energy Treated DMEM group as compared to the untreated DMEM group. Overall, the experimental data suggested that the Biofield Energy Treated DMEM has significantly improved ALP level, which play a vital role for the promotion and maintenance of estrogen level. Based on the study outcomes, it is concluded that Biofield Energy Healing Treatment showed a significant improved ALP level, which can be used in various estrogenic disorders such as hypophosphatasia, osteoporosis, severe anemia, malnutrition, hypothyroidism, magnesium deficiency, heart surgery, aplastic anemia, chronic myelogenous leukemia, enteritis in children, Wilson’s disease, pernicious anemia, bacterial infection and intrauterine infection is a leading cause of pelvic inflammatory disease, subfertility, infertility, endometritis, early pregnancy loss, fetal defects, and preterm birth.

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Introduction

Alkaline phosphatase (ALP) activity is found in high concentration in the endometrium region of menstruating women. ALP activity alters during the various proliferative phases and supposed to be high in early proliferative phase, while it reached the plateau during ovulation. Prior to menstruation, the activity of enzyme is not detectable in the epithelium and is abundant in the vascular endometrium [1]. Thus, ALP has significant role in cellular growth, maintaining the endocrine function, osteoblastic differentiation, and play significant role against many disorders such as human breast cancer [2]. Alkaline phosphatases (ALPs) are the zinc-containing metalloenzymes plasma membrane-bound glycoproteins, which are widely distributed in nature [3]. They functions as dimeric molecules and encoded by a multigene family and have three metal ions including two \( Zn^{2+} \) and one \( Mg^{2+} \) in the active site for their essential enzymatic activity [4]. The altered activity pattern of ALP is regulated by the sex hormones, along with the epithelial alkaline-phosphatase (e-AP) concentration, which is on peak at maximum estrogen stimulation. Thus, it was reported that most rapid endometrial growth happens at the time of maximum ALP concentration, which regulates the proper endometrial function. Environment have various factors such as natural or synthetic, which have significant potential to inhibit the estrogen action and causes its functional disruption [5].

Several natural and synthetic chemicals that are widely distributed in the environment may have the potential to mimic estrogens or inhibit estrogen action, resulting in disruption of endocrine functions via estrogen receptor (ER) [6]. Thus, in this study Ishikawa cells were used to study the effect of Biofield Energy Treated DMEM on ALP expression, which is the best characterized human endometrial cell lines. Ishikawa cells are easy to cultivate and express the most relevant steroid receptors \( \text{i.e. ERa} \) and progesterone receptor (PR). In addition, the cell line is derived from human endometrium, which plays a significant role in female reproductive functions and is a fertility-determining factor [7, 8]. Hence, Ishikawa cell line (human endometrial adenocarcinoma) was selected as a test system for this study. Authors evaluated the in vitro effect of the Biofield Energy Treated DMEM medium as a test item on estrogenic potential using Ishikawa cell line for ALP biomarker.

In recent years, Biofield Energy Healing has been proven to be an alternative method, which has been reported to have significant impact on living organisms and non-living materials. Besides, increasing demand of Complementary and Alternative Medicine (CAM) therapies, Biofield Energy Treatment proofed to have significant benefits in various scientific 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, pranic healing, deep breathing, chiropractic/osteopathic manipulation, guided imagery, meditation, massage, homeopathy, hypnotherapy, progressive relaxation, acupuncture, acupuncture, special diets, relaxation techniques, Rolfing structural integration, healing touch, movement therapy, pilates, mindfulness, Ayurvedic medicine, traditional Chinese herbs and medicines in biological systems both in vitro and in vivo [9]. The Trivedi Effect®-Consciousness Energy Healing Treatment contain a putative bioenergy, which is channeled by a renowned practitioners from a distance. Biofield Energy Healing as a CAM showed a significant results in biological studies [10]. However, the National Center for Complementary and Alternative Medicine (NCCAM), well-defined Biofield therapies in the subcategory of Energy Therapies [11]. The Trivedi Effect®-Consciousness Energy Healing Treatment has been reported with significant revolution in the physicochemical properties of metals, chemicals, ceramics and polymers [12-14], improved agricultural crop yield, productivity, and quality [15,16], transformed antimicrobial characteristics [17-19], biotechnology [20, 21], improved bioavailability [22-24], skin health [25, 26], nutraceuticals [27,28], cancer research [29, 30], bone health [31-33], human health and wellness.

On the basis of Biofield Energy Treatment outcome, authors in this study evaluates the impact of the Biofield Energy Healing Treatment (The Trivedi Effect®) on DMEM as test sample for estrogenic potential with respect to ALP parameter using standard in vitro assay in Ishikawa cells.
Material and Methods

Chemicals and Reagents

Naringenin was purchased from Sigma, 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, 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

Ishikawa cell line (human endometrial adenocarcinoma) from human endometrial tissue was used as test system in the present study. Ishikawa cell line was 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 trypsinisation followed by splitting the cell suspension into fresh flasks and supplementing with fresh cell growth medium. 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 for 3 days [34].

Experimental Design

The experimental groups consisted of group 1 (G-I) the untreated DMEM. Group 2 (G-II) consisted of positive control at non-cytotoxic concentrations. Further, group 3 (G-III) included the Biofield Treated DMEM. Consciousness Energy Healing Treatment Strategies

DMEM as the test item was divided into two parts, one part was treated with the Biofield Energy by a renowned Biofield Energy Healer (also known as The Trivedi Effect®) and coded as the Biofield Energy Treated DMEM, and the other part did not receive any sort of treatment and denoted as the untreated DMEM group. This Biofield Energy Healing Treatment was provided by Mahendra Kumar Trivedi remotely for ~3 minutes through the Healer’s unique Energy Transmission process to the test sample under laboratory conditions. Biofield Energy Healer was located in the USA, while the test items were located in the research laboratory of Dabur Research Foundation, New Delhi, India. Biofield Energy healer in this study never visited the laboratory in person, nor had any contact with the test item (DMEM medium). Further, the control group was treated with “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.

Identification of Non-cytotoxic Concentration

The cell viability was performed by MTT assay in human endometrial adenocarcinoma cell line (Ishikawa). The cells were counted and plated in 96-well plates at the density corresponding to 5 X 10³ to 10 X 10³ cells/well/180 µL of cell growth medium. The above cells were incubated overnight under growth conditions and allowed the cell recovery and exponential growth, which were subjected to serum stripping or starvation. The cells were treated with the test items (DMEM) and positive control. The cells in the above plate(s) were incubated for a time point ranging from 24 to 72 hours in a CO₂ incubator at 37°C, 5% CO₂, and 95% humidity. Following incubation, the plates were taken out and 20 µL of 5 mg/mL of MTT solution were added to each well followed by additional incubation for 3 hours at 37°C. The supernatant was aspirated and 150 µL of DMSO was added to each well to dissolve formazan crystals. The absorbance of each well was read at 540 nm using Synergy HT microplate reader, BioTek, USA [35]. The percentage cytotoxicity at each tested concentrations of the test substance were calculated using the following equation (1):

\[
\% \text{ Cytotoxicity} = \left(1 - \frac{X}{R}\right) \times 100 \tag{1}
\]

Where, \(X\) = Absorbance of treated cells; \(R\) = Absorbance of untreated cells

The percentage cell viability corresponding to each treatment was obtained using the following equation(2):

\[
\% \text{ Cell Viability} = 100 - \% \text{ Cytotoxicity} \tag{2}
\]

The concentrations exhibiting ≥70% cell viability was considered as non-cytotoxic.
Study of Alkaline Phosphatase (ALP) Activity

The cells were counted and plated in 96-well plates at the density corresponding to 5 \times 10^3 cells/well/180µL phenol-free DMEM+ 10% CD-FBS. The above cells were incubated overnight under growth conditions for 48 hours in a CO_2 incubator at 37°C, 5% CO_2, and 95% humidity to allow the cell recovery and exponential growth. The above cells were incubated with the test samples or positive control for 6 days. Re-addition of the test sample or positive control was done on day 3. After incubation with the test samples, the ALP enzyme activity was determined by monitoring the hydrolysis of \( p \)-nitrophenyl phosphate to \( p \)-nitrophenol (\( p \)NPP). The cells were washed with 1X PBS and lysed by freeze-thaw method \( i.e. \), incubation at -80°C for 20 minutes followed by incubation at 37°C for 10 minutes. Lysates were prepared in 0.1% triton-X. 50µL of substrate solution \( i.e. \), 10 mM of \( p \)NPP in 1M diethanolamine and 0.24 mM magnesium chloride (MgCl_2) solution, pH 10.4 was added to all the wells containing 50 µL of lysates followed by incubation for 1 hour at 37°C. The absorbance of the above solution was recorded at 405 nm using Synergy HT microplate reader. The percentage increase in ALP enzyme activity with respect to the untreated DMEM group was calculated using equation (3):

\[
\text{% Increase} = \left( \frac{X-R}{R} \right) \times 100
\]

Where, \( X \) = Absorbance of cells corresponding to positive control and test group

\( R \) = Absorbance of cells corresponding to untreated group

Statistical Analysis

All the values were represented as Mean ± SEM (standard error of mean) of three independent experiments. The statistical analysis was performed using SigmaPlot statistical software (v11.0). For two groups comparison student’s t-test was used. 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

Cell Viability Study Using MTT

Cell viability data using MTT assay in Ishikawa cells treated with different concentrations of positive controls and the Biofield Energy Treated and untreated test samples are represented in Figure 1. The data of MTT assay for cell viability showed in term of percentage values. The MTT data showed that the Biofield Treated DMEM group was found 108% cell viability, while it was 75% to 96% in naringenin (positive control) group. Thus, the experimental MTT data suggested that the Biofield Energy Treated DMEM was found to be safe and nontoxic in the Ishikawa cells. Thus, DMEM was used to study the estrogenic potential \( i.e. \) ALP activity) of The Trivedi Effect® Biofield Energy Healing \textit{in vitro} using human endometrial adenocarcinoma cell line (Ishikawa).

Study of Alkaline Phosphatase (ALP) Enzyme Activity

ALP activity data showed that the Biofield Energy Treated DMEM on Ishikawa cell line and the data are presented in Figure 2. The percent ALP in the untreated DMEM was found as 11.2%. Moreover, the percent ALP was significantly increased by 43.75% and 200.89% \((p \leq 0.001)\) in the positive control group (naringenin) at 500 and 1000 nM, respectively as compared to the untreated DMEM group. Besides, the percent ALP was significantly \((p \leq 0.001)\) increased by 73.21% in the Biofield Energy Treated DMEM group as compared to the untreated DMEM group. Biochemical measurement of ALP activity reflects as an index of osteoblastic differentiation along with the number of cells expressing the enzyme as well as enzyme activity in each cell [36]. Besides, estrogen level can modulates the growth and expression of ALP in human bone marrow stromal cell cultures [37]. Estrogen treatment involved either maintained or increased level of the ALP expression, which directly regulates promotion or sustain the osteoblastic differentiation. Lower level of ALP in women might results in serious conditions or diseases such as hypophosphatasia, postmenopausal women receiving estrogen therapy that is due to the osteoporosis, severe anemia, malnutrition, hypothyroidism, magnesium deficiency, heart surgery, aplastic anemia, chronic myelogenous leukemia, children with achondroplasia and cretinism, enteritis in children,
Figure 1. Assessment of cell viability using MTT assay of the positive control, naringenin at different concentrations and test items, DMEM on Ishikawa cell line.

Figure 2. Alkaline Phosphatase (ALP) enzyme activity study of the Biofield Energy Treated DMEM on Ishikawa cell line. All the values are represented as mean ± SEM of three independent experiments. ***p≤0.001 vs. untreated DMEM group.
Wilson’s disease, pernicious anemia, etc. results in significant loss of ALP level [38]. In addition, oral contraceptives have been also reported to reduce the level of ALP [39]. Thus, Biofield Energy Healing Treatment might significantly improve the level of transcription of target genes, which results in an increased ALP level that leads to a better estrogenic potential and osteoblastic differentiation.

Conclusions

Biofield Energy Treatment (The Trivedi Effect®) on DMEM was studied to evaluate the estrogenic potential and in vitro results showed a significant improvement with respect to ALP activity. Cell viability data using MTT assay showed a significant improved cell viability after Biofield Energy Healing Treatment with 108% in the Biofield Energy Treated group, while more than 75% cell viability was observed in the positive control group reflecting that the test sample was found as safe and nontoxic. Further, the level of ALP was significantly increased by 73.21% in the Biofield Energy Treated DMEM group as compared with the untreated DMEM group. Thus, the Biofield Energy Treated (The Trivedi Effect®) DMEM were found to have a significant impact on ALP level, which results in a better estrogenic potential and osteoblastic differentiation. Therefore, the Consciousness Energy Healing based DMEM might be a suitable alternative media for cell growth. It can be useful for the management of various estrogenic and menstrual disorders viz. Dysmenorrhea with painful cramps, Premenstrual Syndrome (PMS), Menorrhagia, Oligomenorrhea, Amenorrhea, and Missed periods. Thus, Biofield Energy Treatment would be useful to control the estrogen balance and thus control overall hormonal balance, which can be useful against stress, aging, osteoporosis, various bone diseases, cell differentiation, could improve cell-to-cell communication, normal cell growth, neurotransmission, cell cycling and proliferation, hormonal balance, skin health, immune and cardiovascular functions. Besides, it control the hormonal imbalance, various immune related disease conditions such as Hashimoto Thyroiditis, Aplastic Anemia, Hepatitis, Diverticulitis, Pernicious Anemia, Sjogren Syndrome, Myasthenia Gravis, Parkinson’s Disease, Asthma, Graves’ Disease, Dermatomyositis, Multiple Sclerosis, Ulcerative Colitis, Alzheimer’s Disease, Dermatitis, Irritable Bowel Syndrome, Diabetes, Atherosclerosis, Systemic Lupus Erythematosus, stress, etc. with a safe therapeutic index to improve overall health, and Quality of Life.

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Abbreviations

CAM: Complementary and Alternative Medicine, ER: Estrogen Receptor, NCCAM: National Center for Complementary and Alternative Medicine, ALP: Alkaline phosphatase, DMEM: Dulbecco's Modified Eagle's Medium, FBS: Fetal Bovine Serum

References


