

Potential Estrogenic Effects of Biofield Energy Treatment Using Human Endometrial Adenocarcinoma Cell Line



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Abstract

The present study was aimed to investigate the potential role of Consciousness Energy Healing (The Trivedi Effect®) based DMEM medium on the level of alkaline phosphatase enzyme (ALP) in Ishikawa cells. The test item (DMEM medium) was divided into two parts, one part received Biofield Energy Treatment by a renowned Biofield Energy Healer, Alice Branton and was categorized as the Biofield Energy Treated DMEM group, while another part did not receive any sort of treatment and coded as the untreated DMEM group. The cell viability using MTT assay showed a significantly improved cell viability upto 98% in the test item group, which suggested a safe and nontoxic profile of the test item. Estrogenic potential using estimation of ALP level showed a significantly increased by 30.8% in the Biofield Energy Treated group as compared with the untreated DMEM group. The Biofield Energy Treated DMEM showed a significant role in the growth of Ishikawa cells along with an improved ALP level that can be utilized significantly in the promotion and maintenance of estrogen level. It is concluded that Biofield Energy Healing Treatment showed a significant improved ALP level, which can be used in different menstrual and estrogenic disorders like hypophosphatasia, osteoporosis, malnutrition, hypothyroidism, magnesium deficiency, heart surgery, chronic myelogenous leukemia, enteritis in children, 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.

Keywords: Ishikawa cells; Endometrial Carcinoma; Biofield Energy; ALP; DMEM; Menstrual Disorder

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

Introduction

Ishikawa cell line is a well-differentiated human endometrial adenocarcinoma cell line, which was established to study the estrogenic potential due to the presence of estrogen and progesterone receptors (i.e., ER α and PR) [1]. Ishikawa cell line is derived from human endometrium that plays a significant role as a fertility-determining factor [2,3]. Hence, Ishikawa cell line was selected as a test system for this study. Continues basic research area in this field using this cell line like reproductive biology and molecular science, reported its vital role to compare its action using various parameters such as alkaline phosphate (ALP), a zinc-containing metalloenzymes. Human endometrial cell lines are the best characterized cell lines that are easy to cultivate for estrogenic potential. ALP is more abundant in liver, bone, and a small amount in placenta, which were denoted as ALP-1, ALP-2, and ALP-3, respectively.

Maintained the level of ALP is very important for conception as it significantly regulates the estrogen level and endometrium growth [4-6]. Various menstrual disorders take place in the presence of low level of ALP during implantation and conception. A decreased ALP level may be due to zinc deficiency, hypothyroidism, vitamin C deficiency, folic acid deficiency, excess vitamin D intake, low phosphorus levels, celiac disease, malnutrition with low protein assimilation, insufficient parathyroid gland function, pernicious anemia, vitamin B6 insufficiency, and also with the frequent use of synthetic contraceptive, which results in the loss of endocrine functions via estrogen receptor (ER) [7]. Thus, for identification of estrogenic potential, Ishikawa cell line was selected as a test system for this study in order to find the effect of the Biofield Energy Treated DMEM media for ALP as a biomarker.

As an alternative way of treatment, Complementary and Alternative Medicine (CAM) therapies are emerging as one of the best and safe way to treat against acute and chronic diseases [8]. Among CAM, Biofield Energy Healing Treatment (The Trivedi Effect®) one of the best approach that has provided a scientific groundwork in the past years by many renowned healers in order to understand the complex homeodynamic regulation of living systems [9]. National Institute of Health (NIH) and National Center for Complementary and Alternative Medicine (NCCAM) recommend and included various Energy Healing therapies 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, cranial sacral therapy and applied prayer under CAM category that has been accepted by the most of the U.S. population with several advantages [10].

The Trivedi Effect®- Consciousness Energy Healing Treatment contains a 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 [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.

In pursue with the outstanding results of Biofield Energy Healing Treatment outcome, authors in this study evaluates the impact of the Biofield Energy Treatment (The Trivedi Effect®) on DMEM as a 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 (The Trivedi Effect®) and coded as the Biofield Energy Treated DMEM group, 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 Alice Branton remotely for ~5 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 5×10^3 to 10×10^3 cells/well/180µL 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 20µL of 5mg/mL of MTT solution were added to all the wells followed by additional incubation for 3 hours at 37 °C. The supernatant was aspirated and 150µL of DMSO was added to each well to dissolve formazan crystals. The absorbance of each well was read at 540nm 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):

$$\% \text{Cytotoxicity} = (1 - X/R) * 100 \text{ ----- (1)}$$

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):

$$\%Cell\ Viability = 100 - \%Cytotoxicity \dots\dots\dots (2)$$

The concentrations exhibiting $\geq 70\%$ 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 5×10^3 cells/well/180 μ L phenol-free DMEM+ 10% CD-FBS. The above cells were incubated overnight under growth conditions for 48 hours in a CO₂ incubator at 37°C, 5% CO₂, 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. 50 μ L of substrate solution i.e., 10mM of *p*NPP in 1M diethanolamine and 0.24mM magnesium chloride (MgCl₂) solution, pH 10.4 was added to all the wells containing 50 μ L of lysates followed by incubation for 1 hour at 37 °C. The absorbance of the above solution was recorded at 405nm using Synergy HT microplate reader. The percentage increase in ALP enzyme activity with respect to the untreated DMEM group was calculated using equation (3):

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

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 \pm 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

The Biofield Energy Treated and untreated test samples were tested for cell viability using MTT assay in Ishikawa cells. The

outcomes in terms of percentage cell viability are represented in Figure 1. The MTT data showed that the test samples were found to have significant cell viability after Biofield Energy Treatment by 98%, while in the naringenin (positive control) group the cell viability was 75% to 96%. Thus, the experimental MTT data suggested that the Biofield Energy Treated DMEM was found to be safe in the Ishikawa cells as compared with the untreated DMEM. Thus, DMEM was used to study the estrogenic potential (i.e., ALP activity) of The Trivedi Effect®- Biofield Energy Healing *in vitro* using human endometrial adenocarcinoma cell line (Ishikawa).

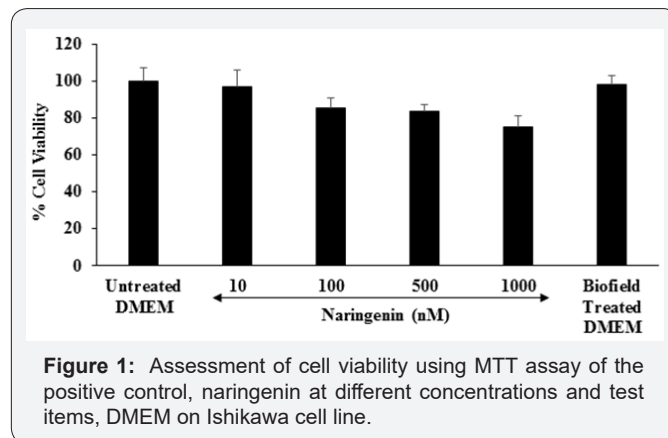


Figure 1: Assessment of cell viability using MTT assay of the positive control, naringenin at different concentrations and test items, DMEM on Ishikawa cell line.

Alkaline phosphatase (ALP) enzyme activity

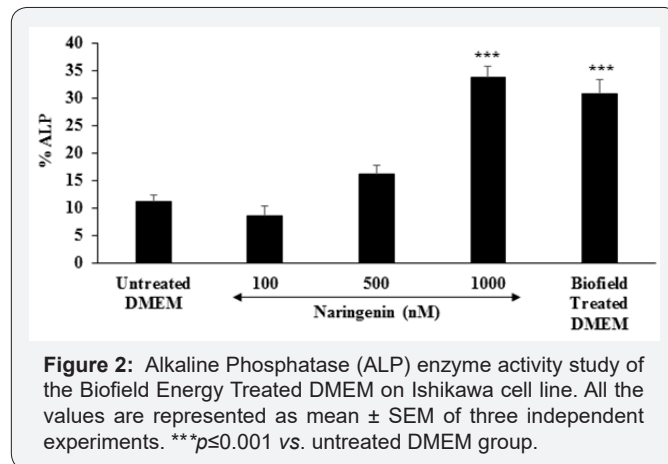


Figure 2: Alkaline Phosphatase (ALP) enzyme activity study of the Biofield Energy Treated DMEM on Ishikawa cell line. All the values are represented as mean \pm SEM of three independent experiments. *** $p \leq 0.001$ vs. untreated DMEM group.

The level of ALP in terms of percentage change are presented in Figure 2. Naringenin, positive control showed a significantly increased the value of ALP by 43.75% and 200.89% ($p \leq 0.001$) at 500 and 1000nM, respectively with respect to the untreated DMEM group. The Biofield Energy Treated DMEM group showed a significant increased the ALP level by 30.8% as compared with the untreated DMEM group. Thus, the Biofield Energy Treated DMEM showed a significant increment of ALP, which play a major role in estrogen balance for conception. It might be highly significant in case of infertility and helpful against various menstrual disorders.

The scientific literature reported that decreased ALP level in placenta results in serious complications such as amyloidosis, granulation tissue, gastrointestinal inflammation such as inflammatory bowel disease, systemic infections,

hypophosphatasia, postmenopausal women receiving estrogen therapy that is due to the osteoporosis, severe anemia, heart surgery, aplastic anemia, malnutrition, magnesium deficiency, hypothyroidism, chronic myelogenous leukemia, children with achondroplasia and cretinism, and pernicious anemia [35]. Thus, Biofield Energy Healing Treatment would significantly improved the estrogenic potential and worked as an index of osteoblastic differentiation as well as improved ALP enzyme activity [36]. Thus, in order to study the effect of Biofield Energy Treatment on DMEM, ALP level was significantly improved in Ishikawa cell line. It might be expected that Biofield Energy Treatment has altered the osteoblastic differentiation, which is due to an increased ALP enzyme level.

Conclusion

The Trivedi Effect®- Consciousness Energy Healing Treatment on DMEM was considered as a significant role to improve estrogenic potential with respect to increased level of ALP in Ishikawa cells. Cell viability data using MTT assay showed a significant improved cell viability after Biofield Energy Healing Treatment with 98% in the test sample group, while upto 96% in the positive control group signifies the high safety profile of the test samples. The level of ALP was significantly increased by 30.8% in the Biofield Energy Treated DMEM group as compared with the untreated DMEM group. Thus, The Trivedi Effect® on DMEM were found to have a significant impact on ALP level, which results in a better estrogenic potential and osteoblastic differentiation.

Therefore, with respect to the untreated DMEM, the Biofield Energy Treated DMEM would be highly significant in growth and viability of Ishikawa cells. 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, Oligomenorrhoea, Amenorrhoea, 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, skin health, immune and cardiovascular functions. Besides, it controls various immune-related disease conditions such as Aplastic Anemia, Pernicious Anemia, Hepatitis, Sjogren Syndrome, Myasthenia Gravis, Parkinson's Disease, Asthma, Atherosclerosis, Graves' Disease, Dermatomyositis, Dermatitis, Diabetes, Multiple Sclerosis, Ulcerative Colitis, Alzheimer's Disease, Irritable Bowel Syndrome, Systemic Lupus Erythematosus, stress, etc. with a safe therapeutic index to improve overall health and Quality of Life.

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