LUPINE PUBLISHERS

Open Access Journal of Reproductive System and Sexual Disorders

DOI: 10.32474/OAJRSD.2019.02.000139

ISSN: 2641-1644

Research Article

Evaluation of Estrogenic Activity of Biofield Energy Treatment for the Potential of Menstrual Disorders Using Ishikawa Cell Line

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Received: March 08, 2019 **Published:** March 20, 2019

Abstract

Alkaline phosphatase (ALP) plays a vital role in the endometrium development, maturation, endocrine function, and osteoblastic differentiation. The present investigation explained the role of Consciousness Energy Healing based test item (DMEM medium) on ALP in Ishikawa cells. After the division of the test item into two parts; first, part received Biofield Treatment by a renowned Biofield Energy Healer, Dahryn Trivedi and was labeled as the Biofield Treated DMEM, while the second part did not receive any treatment, and denoted as the untreated DMEM. MTT data showed 142.56% viable cells in the Biofield Treated test item, which indicated that the test item was found as safe and non-toxic. Besides, the estrogenic potential concerning ALP level was significantly ($p \leq 0.001$) increased by 65.18% in the Biofield Treated DMEM group than untreated DMEM. Thus, the experimental data suggested that the Biofield Energy Treated DMEM would play a significant role for the growth of Ishikawa cells along with an improved ALP level, which play a vital role in the promotion and maintenance of estrogen level and can be used against various estrogenic disorders viz. endometritis, hypophosphatasia, osteoporosis, infertility, severe anemia, malnutrition, fetal defects, early pregnancy loss, and preterm birth.

Keywords: Estrogenic Potential; Menstrual Disorder; DMEM; Biofield Energy; ALP; Ishikawa Cell

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

Alkaline phosphatases (ALPs, zinc-containing metalloenzymes plasma membrane-bound glycoproteins) mainly found in liver and bone, which are termed as isoenzyme ALP-1 and ALP-2, respectively. However, a small amount of ALP is also present in the cell lining of the intestines, known as isoenzyme ALP-3, in the placenta, and the kidney (in the proximal convoluted tubules). ALP works best in alkaline pH (a pH of 10), hence inactive in the blood. Its role in the endometrium, estrogenic potential, and menstrual disorders are widely reported [1-3]. From literature, several reports suggested that high-level of ALP can influence in the endometrium, which was responsible for conception [4]. Endometrium ALP level varies during the menstrual cycle and ALP play a vital role during implantation and conception. Low level of ALP is unsuitable for implantation and might cause various menstrual disorders. Thus, the ALP enzyme is responsible for conception and contraception. Further, it was reported that the use of hormonal contraceptive drugs abolishes the role of ALP in the endometrium. Thus, ALP has a significant role in cellular growth, maintaining the endocrine function, osteoblastic differentiation, and play a significant role against many disorders such as human breast cancer [5]. ALP and estrogenic potential can be significantly affected with the natural or synthetic chemicals, which leads to loss of endocrine functions via estrogen receptor (ER) [6]. Human endometrial cell lines (Ishikawa cells) are the best-characterized cell lines that are easy to cultivate

for estrogenic potential. They express the change in ALP level with respect to estrogen through most relevant steroid receptors i.e., ERα and progesterone receptor (PR). Ishikawa cell line is derived from human endometrium that plays a significant role in female reproductive functions and is also a fertility-determining factor [7,8]. Hence, the Ishikawa cell line was selected as a test system for this study for the assessment of estrogenic potential using the Ishikawa cell line for ALP biomarker.

Currently, Biofield Energy Healing Science is one of the best Complementary and Alternative Medicine (CAM) approach and considered as an emerging field, which provides a scientific groundwork for understanding the complex homodynamic regulation of living systems. Consciousness Energy Healing Treatment has a considerable impact on both living and non-living substances, which have reported with a significant benefit in various scientific fields. According to the National Center for Complementary and Alternative Medicine (NCCAM), mentioned that Biofield Therapies is under the umbrella of Energy Therapies [9,10]. Various CAM therapies have great potential viz. Reiki, external qigong, Qi Gong, Johrei, therapeutic touch, Tai Chi, yoga, polarity therapy, deep breathing, panic healing, chiropractic/ osteopathic manipulation, guided imagery, massage, meditation, acupressure, progressive relaxation, homeopathy, hypnotherapy, acupuncture, relaxation techniques, special diets, healing touch, Rolfing structural integration, movement therapy, mindfulness, pilates, Ayurvedic medicine, traditional Chinese herbs and medicines in biological systems both in vitro and in vivo [11]. The Trivedi Effect® contains putative bioenergy, which is channeled by a renowned practitioner from a distance. Biofield Energy Healing as a CAM showed significant results in biological studies [10]. Biofield Energy Healing Treatment has been reported with considerable 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], improved skin health [25,26], nutraceuticals [27,28], cancer research [29,30], bone health [31-33], human health and wellness. From Biofield Energy Treatment outcome, authors in this study evaluate the impact of the Biofield Energy Treatment (The Trivedi Effect®) on DMEM as a test sample for estrogenic potential with respect to ALP using the standard *in vitro* assay in Ishikawa cells.

Material and Methods

Requirement

Penicillin and streptomycin were purchased from HiMedia, India. Naringenin, Direct Red 80, ethylenediaminetetraacetic acid (EDTA), and 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium) (MTT) were purchased from Sigma, India. Dulbecco's Modified Eagle's Medium (DMEM) and fetal bovine serum (FBS) were purchased from Life Technology, USA.

Maintenance of Ishikawa Cell Culture

Ishikawa cell line (human endometrial adenocarcinoma) derived from human endometrial tissue was used as a test system. Growth conditions were maintained at 37°C, 5%CO_2, and 95% humidity and sub cultured by trypsinization 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].

Biofield Energy 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 Dahryn Trivedi remotely for ~5 minutes through the Healer's unique Energy Transmission process to the test sample under laboratory conditions. Biofield Energy Healer was in the USA, while the test items were 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 details MTT assay was performed as per Singh et al. with slight modification [35,36]. The percentage cytotoxicity at each tested concentrations of the test substance was calculated using the following equation (1):

% Cytotoxicity = (1-X/R) *100 ---------- (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 ---------- (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 X 10^3 cells/well/180µL phenol-free DMEM+ 10% CD-FBS. The details assay protocol was followed

as per Lauree et al. with slight modification [37]. The percentage increase in ALP enzyme activity with respect to the untreated DMEM group was calculated using equation (3):

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

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

R = Absorbance of cells corresponding to the untreated group

Statistical Analysis

Data were represented as the Mean ± SEM (standard error of the mean) of three independent experiments. Sigma Plot statistical software (v11.0) was used for the assessment of statistical analysis. Student's t-test was used for two groups comparison and for multiple groups, one-way analysis of variance (ANOVA) was used followed by Dunnett's test as post-hoc analysis. Statistically, significant value was set at the level of $p \leq 0.05$.

Results and Discussion

MTT Assay

MTT assay for cell viability using Ishikawa cells was performed among Biofield Energy Treated and untreated samples along with positive control. The results in terms of percentage cell viability are represented in Figure 1. The MTT data showed that the Biofield Energy Treated DMEM group was found 142.56% viable cells, while it was observed as 75% to 96% in the naringenin (positive control) group. Therefore, data suggested that the test item (untreated and Biofield Treated DMEM) was found to be safe in Ishikawa cells. Thus, the test item was used to study the estrogenic potential in terms of ALP activity.

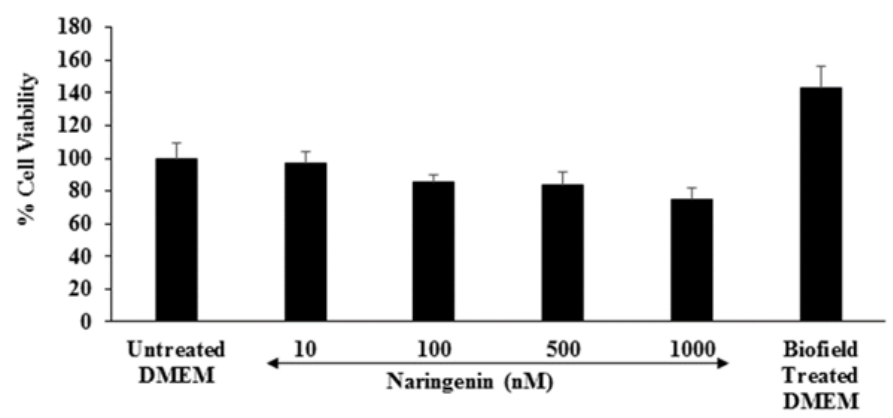

Figure 1: Evaluation of non-cytotoxic concentration in terms of percent cell viability of the test items and the positive control in Ishikawa cells.

Alkaline Phosphatase (ALP) Enzyme Activity

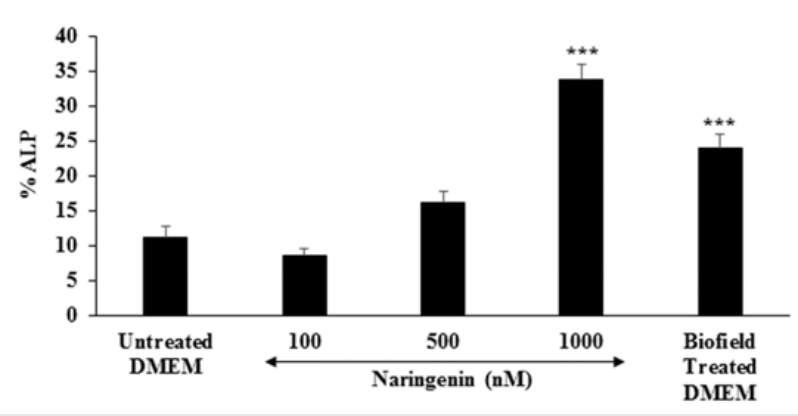

Figure 2: Effect of the test items on the level of alkaline phosphatase (ALP) enzyme activity in Ishikawa cells. Values presented as mean ± SEM of three independent experiments. ***$p \leq 0.001$ *vs*. untreated DMEM group.

ALP level in the endometrium plays a significant role for contraception, while a reduction in the level of ALP during proliferative phase results in infertility and associated with menstrual disorders. The level of ALP can be decreased due to late pregnancy since placenta produces ALP, which might results in a 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 cretinism, osteoporosis, malnutrition, severe anemia, magnesium deficiency, hypothyroidism, heart surgery, chronic myelogenous leukemia, aplastic anemia, children with achondroplasia and pernicious anemia [38]. The level of ALP is also lowered due to various chemicals and contraceptives [39]. Thus, ALP activity indicates an index of osteoblastic differentiation and the number of cells that express the enzyme [40]. Thus, in order to study the effect of Biofield Energy Treatment on DMEM, ALP level was significantly improved in the Ishikawa cell line. The results in terms of percentage change of ALP in various groups are presented in Figure 2. The positive control, naringenin showed a significantly increase 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 ($p \leq 0.001$) increase the ALP level by 65.18% compared with the untreated DMEM group. Thus, overall experimental results concluded that the Biofield Energy Healing Treatment significantly improved ALP level, which might be due to change in the level of transcription of target genes that leads to a better estrogenic potential and osteoblastic differentiation.

Conclusions

Cell viability data using MTT assay showed significantly improved cell viability after Biofield Energy Healing Treatment with 142.56% in the Biofield Energy Treated group, while up to 96% in the positive control group that showed the safety profile of the test samples. ALP was significantly increased by 65.18% in the Biofield Energy Treated DMEM group compared with the untreated DMEM group. Thus, Consciousness Energy Healing Treatment (The Trivedi Effect®) on DMEM was found to have a significant impact on the ALP level, which results in a better estrogenic potential and osteoblastic differentiation. Therefore, to the untreated DMEM, the Biofield Energy Treated DMEM would be highly significant in the growth and viability of Ishikawa cells. Thus, the Consciousness Energy Healing based DMEM might be a suitable alternative media for cell growth. The potential of Biofield Energy Treatment could be further extendable for the management of menstrual disorders viz. premenstrual syndrome, dysmenorrhea with painful cramps, oligomenorrhea, amenorrhea, and menorrhagia. Thus, Consciousness Energy Healing Treatment would be useful to regulate the estrogen balance, which can be useful against aging, stress, and immune-related disorders.

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DOI: 10.32474/OAJRSD.2019.02.000139