OPEN ACCESS
Freely Available Online

JOURNAL OF WOMAN'S REPRODUCTIVE HEALTH
ISSN NO: 2381-862X

Research Article

DOI : 10.14302/issn.2381-862X.jwrh-18-2459

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

Mahendra Kumar Trivedi[1], Snehasis Jana[2,*]

[1]Trivedi Global, Inc., Henderson, USA.

[2]Trivedi Science Research Laboratory Pvt. Ltd., Bhopal, India.

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.

Corresponding author: Snehasis Jana, Trivedi Science Research Laboratory Pvt. Ltd., Bhopal, India. Email: publication@trivedisrl.com

Keywords: Biofield Energy, ALP, Estrogenic potential, Ishikawa cell, DMEM, Menstrual disorder

Received: Nov 02, 2018 **Accepted:** Dec 01, 2018 **Published:** Dec 03, 2018

Editor: Manjubala Dash, Mother Theresa Postgraduate & Research Institute of Health Sciences, Pondicherry, 605006, India.

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 *i.e.* ERα 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, acupressure, 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%CO_2, 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 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 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 10^3 to 10 X 10^3 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 CO_2 incubator at 37°C, 5% CO_2, and 95% humidity. Following incubation, the plates were taken out and 20 µL of 5 mg/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 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):

% 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 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):

% Increase = [(X-R)/R)]*100 ---------- (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 ± 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 *in vitro* using human endometrial adenocarcino-ma 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,

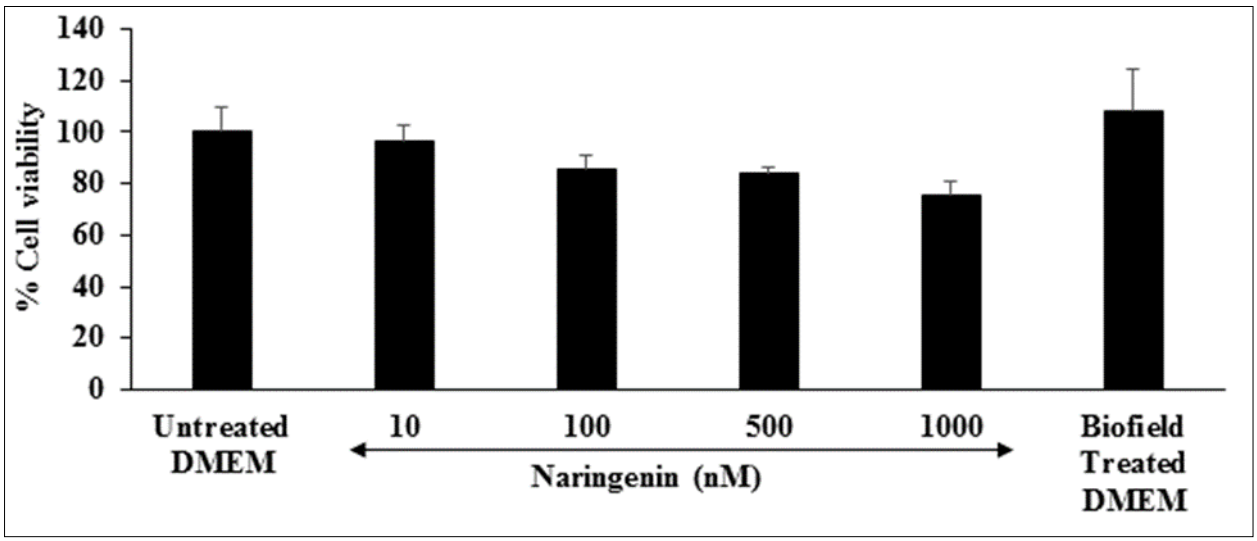

Figure 1. Assessment of cell viability using MTT assay of the positive control, naringenin at different concentrations and test items, DMEM on Ishikawa cell line.

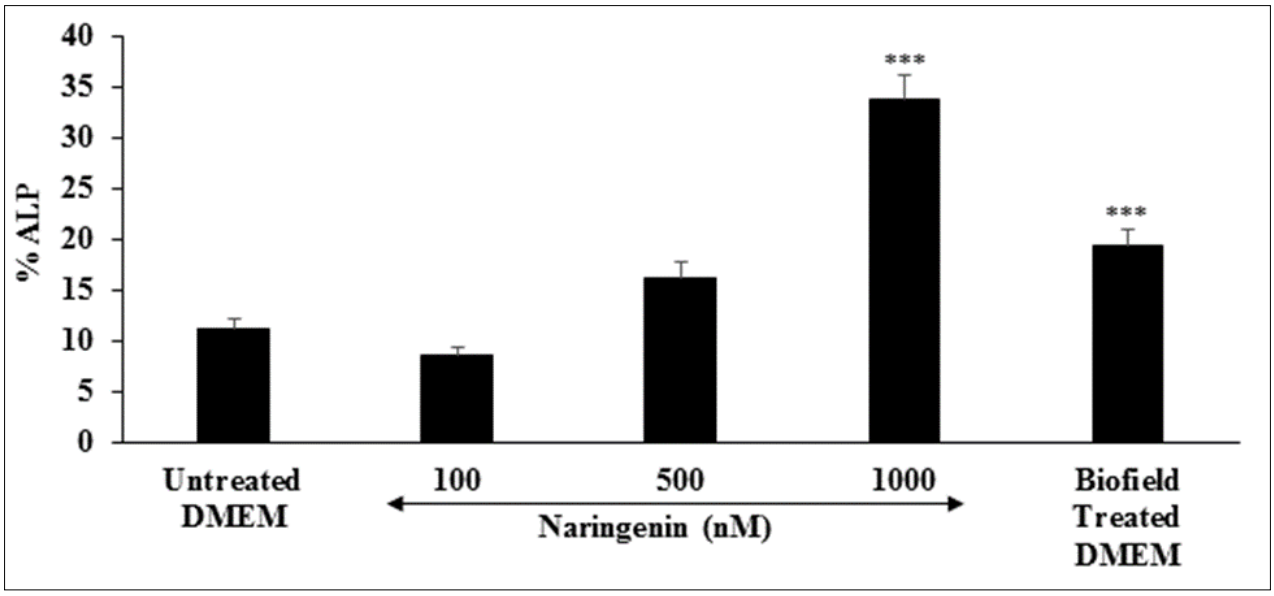

Figure 2. Alkaline Phosphatase (ALP) enzyme activity study of the Biofield Energy Treated DMEM on Ishikawa cell line. All the values are represented as mean ± SEM of three independent experiments. ***p≤0.001 vs. untreated DMEM group.

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.

Acknowledgements

Authors are grateful to Dabur Research Foundation for conducting experiment with more thankfulness to Trivedi Global, Inc., Trivedi Science, Trivedi Testimonials, and Trivedi Master Wellness for their support throughout the work.

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

1. DeMerre LJ, Litofsky FS (1968) Alkaline-phosphatase activity during menstruation. Fertil Steril 19: 593-597.
2. Tsai LC, Hung MW, Chen YH, Su WC, Chang GG, Chang TC (2000) Expression and regulation of alkaline phosphatases in human breast cancer MCF-7 cells. Eur J Biochem 267: 1330-1339.
3. Sharma U, Pal D, Prasad R (2014) Alkaline phosphatase: An overview. Indian J Clin Biochem 29: 269-278.
4. McComb RB, Bowers GN, Posen S (1979) Alkaline phosphatase. New York: Plenum Publishing Corp.
5. Fenuku RI, Foli AK (1975) Serum alkaline phosphatase activity during pregnancy and the post-partum period in Ghanaian women. Trop Geogr Med 27: 371-374.
6. Colborn T (1995) Environmental estrogens: Health implications for humans and wildlife. Environ. Health Perspect 103: 135-136.
7. Wober J, Weisswange I, Vollmer G (2002) Stimulation of alkaline phosphatase activity in Ishikawa cells induced by various phytoestrogens

and synthetic estrogens. J Steroid Biochem Mol Biol 83: 227-233.

8. Canto C, Pich S, Paz JC, Sanches R, Martínez V, Orpinell M, Palacín M, Zorzano A, Gumà A (2007) Neuregulins increase mitochondrial oxidative capacity and insulin sensitivity in skeletal muscle cells. Diabetes 56: 2185-2193.
9. Rubik B (2002) The biofield hypothesis: Its biophysical basis and role in medicine. J Altern Complement Med 8: 703-717.
10. Barnes PM, Bloom B, Nahin RL (2008) Complementary and alternative medicine use among adults and children: United States, 2007. Natl Health Stat Report 12: 1-23.
11. Frass M, Strassl RP, Friehs H, Müllner M, Kundi M, Kaye AD (2012) Use and acceptance of complementary and alternative medicine among the general population and medical personnel: A systematic review. Ochsner J 12: 45-56.
12. Trivedi MK, Tallapragada RM (2008) A transcendental to changing metal powder characteristics. Met Powder Rep 63: 22-28, 31.
13. Trivedi MK, Nayak G, Patil S, Tallapragada RM, Latiyal O (2015) Studies of the atomic and crystalline characteristics of ceramic oxide nano powders after bio field treatment. Ind Eng Manage 4: 161.
14. Trivedi MK, Nayak G, Patil S, Tallapragada RM, Latiyal O, Jana S (2015) Effect of biofield energy treatment on physical and structural properties of calcium carbide and praseodymium oxide. International Journal of Materials Science and Applications 4: 390-395.
15. Trivedi MK, Branton A, Trivedi D, Nayak G, Mondal SC, Jana S (2015) Morphological characterization, quality, yield and DNA fingerprinting of biofield energy treated alphonso mango (*Mangifera indica* L.). Journal of Food and Nutrition Sciences 3: 245-250.
16. Trivedi MK, Branton A, Trivedi D, Nayak G, Mondal SC, Jana S (2015) Evaluation of biochemical marker – Glutathione and DNA fingerprinting of biofield energy treated *Oryza sativa*. American Journal of BioScience 3: 243-248.
17. Trivedi MK, Branton A, Trivedi D, Nayak G, Charan S, Jana S (2015) Phenotyping and 16S rDNA analysis after biofield treatment on *Citrobacter braakii*: A urinary pathogen. J Clin Med Genom 3: 129.
18. Trivedi MK, Patil S, Shettigar H, Mondal SC, Jana S (2015) Evaluation of biofield modality on viral load of Hepatitis B and C viruses. J Antivir Antiretrovir 7: 083-088.
19. Trivedi MK, Patil S, Shettigar H, Mondal SC, Jana S (2015) An impact of biofield treatment: Antimycobacterial susceptibility potential using BACTEC 460/MGIT-TB System. Mycobact Dis 5: 189.
20. Trivedi MK, Patil S, Shettigar H, Bairwa K, Jana S (2015) Phenotypic and biotypic characterization of *Klebsiella oxytoca*: An impact of biofield treatment. J Microb Biochem Technol 7: 203-206.
21. Nayak G, Altekar N (2015) Effect of biofield treatment on plant growth and adaptation. J Environ Health Sci 1: 1-9.
22. Branton A, Jana S (2017) The influence of energy of consciousness healing treatment on low bioavailable resveratrol in male *Sprague Dawley* rats. International Journal of Clinical and Developmental Anatomy 3: 9-15.
23. Branton A, Jana S (2017) The use of novel and unique biofield energy healing treatment for the improvement of poorly bioavailable compound, berberine in male *Sprague Dawley* rats. American Journal of Clinical and Experimental Medicine 5: 138-144.
24. Branton A, Jana S (2017) Effect of The biofield energy healing treatment on the pharmacokinetics of 25-hydroxyvitamin D3 [25(OH)D_3] in rats after a single oral dose of vitamin D3. American Journal of Pharmacology and Phytotherapy 2: 11-18.
25. Kinney JP, Trivedi MK, Branton A, Trivedi D, Nayak G, Mondal SC, Jana S (2017) Overall skin health potential of the biofield energy healing based herbomineral formulation using various skin parameters. American Journal of Life Sciences 5: 65-74.

26. Singh J, Trivedi MK, Branton A, Trivedi D, Nayak G, Gangwar M, Jana S (2017) Consciousness energy healing treatment based herbomineral formulation: A safe and effective approach for skin health. American Journal of Pharmacology and Phytotherapy 2: 1-10.

27. Trivedi MK, Branton A, Trivedi D, Nayak G, Plikerd WD, Surguy PL, Kock RJ, Piedad RB, Callas RP, Ansari SA, Barrett SL, Friedman S, Christie SL, Liu SC, Starling SE, Jones S, Allen SM, Wasmus SK, Benczik TA, Slade TC, Orban T, Vannes VL, Schlosser VM, Albino YSY, Panda P, Sethi KK, Jana S (2017) A Systematic study of the biofield energy healing treatment on physicochemical, thermal, structural, and behavioral properties of magnesium gluconate. International Journal of Bioorganic Chemistry 2: 135-145.

28. Trivedi MK, Branton A, Trivedi D, Nayak G, Plikerd WD, Surguy PL, Kock RJ, Piedad RB, Callas RP, Ansari SA, Barrett SL, Friedman S, Christie SL, Liu SC, Starling SE, Jones S, Allen SM, Wasmus SK, Benczik TA, Slade TC, Orban T, Vannes VL, Schlosser VM, Albino YSY, Panda P, Sethi KK, Jana S (2017) Chromatographic and spectroscopic characterization of the consciousness energy healing treated *Withania Somnifera* (ashwagandha) root extract. European Journal of Biophysics 5: 38-47.

29. Trivedi MK, Patil S, Shettigar H, Mondal SC, Jana S (2015) The potential impact of biofield treatment on human brain tumor cells: A time-lapse video microscopy. J Integr Oncol 4: 141.

30. Trivedi MK, Patil S, Shettigar H, Gangwar M, Jana S (2015) *In vitro* evaluation of biofield treatment on cancer biomarkers involved in endometrial and prostate cancer cell lines. J Cancer Sci Ther 7: 253-257.

31. Anagnos D, Trivedi K, Branton A, Trivedi D, Nayak G, Mondal SC, Jana S (2018) Influence of biofield treated vitamin D_3 on proliferation, differentiation, and maturation of bone-related parameters in MG-63 cell-line. International Journal of Biomedical Engineering and Clinical Science 4: 6-14.

32. Lee AC, Trivedi K, Branton A, Trivedi D, Nayak G, Mondal SC, Jana S (2018) The potential benefits of biofield energy treated vitamin d3 on bone mineralization in human bone osteosarcoma cells (MG-63). International Journal of Nutrition and Food Sciences 7: 30-38.

33. Stutheit ME, Trivedi K, Branton A, Trivedi D, Nayak G, Mondal SC, Jana S (2018) Biofield energy treated vitamin D3: Therapeutic implication on bone health using osteoblasts cells. American Journal of Life Sciences 6: 13-21.

34. Czekanska EM, Stoddart MJ, Richards RG, Hayes JS (2012) In search of an osteoblast cell model for *in vitro* research. Eur Cells Mater 24: 1-17.

35. Biological evaluation of medical devices - Part 5: Tests for *in vitro* cytotoxicity (ISO 10993-5:2009), I.S.EN ISO, 10993-5:20093.

36. Wuthier RE, Register T (1985) Role of alkaline phosphatase, a polyfunctional enzyme, in mineralizing tissues. In: Butler WT, editor. Chemistry and biology of mineralized tissues. Birmingham: EBSCO Media: 113-124.

37. Buchet R, Millán JL, Magne D (2013) Multisystemic functions of alkaline phosphatases. Methods Mol Biol 1053: 27-51.

38. Lange PH, Millan JL, Stigbrand T, Vessella RL, Ruoslahti E, Fishman WH (1982) Placental alkaline phosphatase as a tumor marker for seminoma. Cancer Res 42: 3244-3247.

39. Epstein E, Kiechle FL, Artiss JD, Zak B (1986) The clinical use of alkaline phosphatase enzymes. Clin Lab Med 6: 491-505.