

Impact of Biofield Energy Healing
Treatment: Evaluation of Anti-
Rheumatoid Activity Using Synovial
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CC-BY 4.0**Keywords** Biofield Energy; Pro-
inflammatory cytokine; Interleukin-8;
Rheumatoid arthritis; Synovial cell line**Abbreviations** CAM: Complementary
and Alternative Medicine; NCCAM:
National Center for Complementary and
Alternative Medicine; ANOVA: One-way
analysis of variance; DMEM: Dulbecco's
Modified Eagle's Medium; FBS: Fetal
Bovine Serum

Abstract

The present research was performed to monitor the anti-rheumatoid action of Consciousness Energy Healing based DMEM medium using synovial sarcoma cell line, SW982. The study included the evaluation of bone health potential using inflammatory parameter, IL-8. 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, Mahendra Kumar Trivedi 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. Cell viability assay using MTT method showed that the cell viability of SW982 cells was 113.8% and 88.3% in the BT-I and BT-II groups, respectively. The BT-I group demonstrated significant ($p \leq 0.001$) inhibition of IL-8 by 27.74%, while BT-II group also showed a significant ($p \leq 0.001$) inhibition of IL-8 by 45.66% as compared to the untreated DMEM group. In conclusion, the data suggested that the Consciousness Energy Healing Treatment significantly inhibited the pro-inflammatory cytokine (IL-8) that showed anti-rheumatic action and can also be used in other bone and inflammatory disorders such as osteoporosis, Paget's disease of bone, rickets, deformed bones, osteomalacia, osteoma, stress, aging, bone loss, and fractures along with autoimmune disorders and overall quality of life.

Introduction

Cytokines play a vital role in physiology of Rheumatoid Arthritis (RA). They are showing a pleiotropic action along with various target sites. This network can be broadly classified as the pro-inflammatory and anti-inflammatory cytokines and the control among this group is one of the important aspects to achieve a therapeutic goal in RA [1]. Interleukins (IL) and Tumor Necrosis Factor (TNF) are the major pro-inflammatory cytokines regulates in the pathology of RA. However, data suggested that the blockage of these cytokines would not completely cure the RA diseases, but could significantly improve the arthritis patient condition and quality of life. The current therapies of RA have been improved and cytokines control based treatment approach can be considered as the best way of RA treatment. IL-4, IL-8, IL-17, and IL-18 are the promising modulators and considered as the controlling factor of RA diseases [2]. RA is one of the severe chronic bone inflammations prevailing worldwide in all the age group and most commonly the middle-aged women. RA disease results in joint stiffness, pain, and symmetrical synovitis of diarthrodial joints such as the knee joint that leads to block the joint movements, and articular destruction. Various immune cells play vital role in RA such as B and T cells, while activated macrophages initiated the process of neo-vascularization [3]. Thus, tissue destruction and inflammation by cytokines results in complex cell-cell interactions in the rheumatoid synovium. These changes results in various metabolic changes, leads to affects the quality of life [4,5]. Currently, physical and occupation therapies are in practice against RA diseases along with the use of synthetic drugs like most common are the methotrexate and sulfasalazine acts as a Disease-Modifying Anti-Rheumatic Drugs (DMARDs). These synthetic drugs have proven to minimize the RA symptoms, but are always associated with severe side-effects; however complementary therapies are not associated with adverse effects and would improve the pathology of diseases from its root causes. With rest to develop some alternative RA treatment approach, Biofield Energy Healing Therapy was used in this study using human synovial sarcoma cell line. SW982 (human synovial sarcoma cell line) is characterized by expression of inflammatory cytokines and MMP genes. SW982 cells express genes encoding IL-1 β , IL-6, cyclooxygenase (COX)-2, and MMPs [6]. Dexamethasone has been used as one of the best approach for the treatment of RA and is reported to inhibit secretion of IL-6 and IL-8 in SW982 cells [7]. Hence, in the present experimental study, inhibition of cytokine secretion (IL-8) by SW982 cells against IL-1 β stimulated levels indicated significant role of Biofield Energy Treatment in DMEM against RA.

Biofield Energy, as one of the famous and effective Complementary and Alternative Medicine (CAM) has been found to have significant role in living and non-living things [8]. National Institute of Health (NIH) defines the word “Biofield” with respect to the energy and information, which surrounds and interpenetrates in the human body. This field included both the human hypothetical subtle energy and the measurable electromagnetic energy. Biofield and its associated Energy Therapies are accepted worldwide and were also recommended by National Center for Complementary and Alternative Medicine (NCCAM) [9], because of several advantage and no side-effects. Some worldwide Biofield Energy Therapies and practices included are Tai Chi, Qi Gong, meditation, healing touch, mindfulness, Reiki, chiropractic/osteopathic manipulation, movement therapy, yoga, deep breathing, homeopathy, progressive relaxation, cranial sacral therapy and applied prayer, massage, guided imagery, acupressure, acupuncture, relaxation techniques, hypnotherapy, and traditional Chinese herbal medicines, naturopathy, essential oils, and aromatherapy [10,11]. The Trivedi Effect®, Biofield Energy Healing Therapy by a renowned practitioner from a distance has been reported with outstanding results in various scientific studies [12]. NCCAM has well-defined and categorized the Biofield therapies in the subcategory of Energy Therapies [13]. Consciousness Energy Healing Treatment found to be significant to improve the mental physicochemical properties [14-16], agriculture science [17,18], life science in microbiology [19-21], biotechnology [22,23], improved bioavailability of compounds [24-26], improved skin health [27,28], nutraceuticals [29,30], cancer science research [31,32], improved overall bone health [33-35], human health and wellness.

Based on the bone health condition and current treatment strategies, and the importance of CAM therapies, authors in this study evaluated the impact of the Biofield Energy Treatment (The Trivedi Effect®) on DMEM for anti-RA activity using alteration in the cytokine secretion (IL-8) by SW982 cells against IL-1 β stimulated effect using standard *in vitro* assay in SW982 cells.

Material and Methods

Chemicals and Reagents

Fetal bovine serum (FBS) and Dulbecco's Modified Eagle's Medium (DMEM) were obtained from Life Technology, USA. Antibiotics solution (penicillin-streptomycin) was procured from HiMedia, India. 3-(4,5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium (MTT), Direct Red 80, and Ethylene Diamine Tetraacetic Acid (EDTA) were purchased from Sigma, USA and other chemicals were analytical grade and all were obtained from India.

Cell Culture

SW982 (Synovial cell line) was used as a test system maintained in DMEM growth medium (10% FBS) with growth conditions *viz.* 37°C, 5% CO₂, and 95% humidity and sub-cultured by trypsinization followed by splitting the cell suspension into fresh flasks and supplementing with fresh cell growth medium. 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 [36].

Experimental Design

The RA bone health experiment was designed, which included the groups such as group 1 (G-I) with the untreated DMEM. Group 2 (G-II) contained one-time Biofield Energy Treated DMEM denoted as BT-I, while group 3 (G-III) included the two-time Biofield Energy Healing Treated DMEM denoted as BT-II.

Consciousness Energy Healing Treatment Strategies

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, Mahendra Kumar Trivedi 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. Consciousness Energy Healing Treatment to the DMEM was provided by Mahendra Kumar Trivedi remotely located in USA for ~3 minutes through the Healer's unique Energy Transmission under laboratory conditions. The test item, DMEM was remotely located in the research laboratory of Dabur Research Foundation, New Delhi, India. The healer in this study never visited the laboratory in person, nor had any contact with the DMEM medium. On the other hand, the untreated DMEM group was treated with “sham” healer, who did not have any knowledge about the Biofield Energy Treatment. Further, the test samples were kept in similar sealed conditions for experimental study.

Determination of Non-cytotoxic Concentration

The SW982 cells were trypsinized and a single cell suspension was prepared by counting the cells using a hemocytometer at a density of 10,000 cells/well/180 μ L in DMEM with 10% FBS in 96-well plates. Further, the cells were incubated in a CO₂ incubator for 24 hours at 37°C, 5% CO₂, and 95% humidity. After 24 hours, the medium was removed followed by the treatment with the test items. After incubation for 48 hours, the effect of test items on cell viability was assessed by 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. About 20 μ L of 5 mg/mL of MTT was added to all the wells and incubated at 37°C for 3 hours. The supernatant was aspirated and 150 μ L of DMSO was added to all wells to dissolve formazan crystals. The absorbance was recorded in each well at 540 nm using Synergy HT microplate reader, BioTek, USA [37]. The percentage cytotoxicity at each tested concentrations of the test substance was 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):

$$\% \text{ Cell Viability} = 100 - \% \text{ Cytotoxicity} \text{ ----- (2)}$$

The concentrations exhibiting $\geq 70\%$ cell viability was considered as non-cytotoxic.

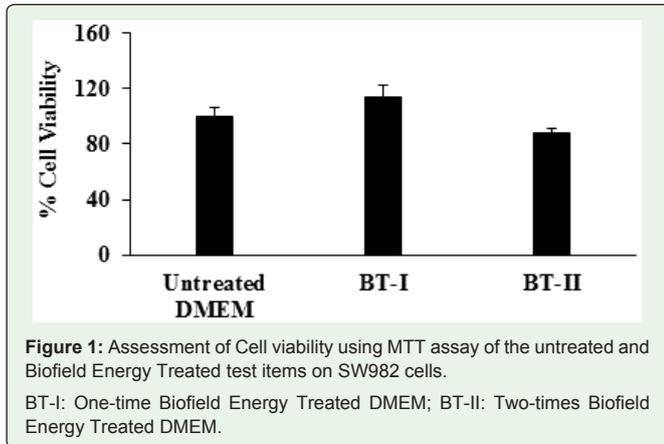


Figure 1: Assessment of Cell viability using MTT assay of the untreated and Biofield Energy Treated test items on SW982 cells.

BT-I: One-time Biofield Energy Treated DMEM; BT-II: Two-times Biofield Energy Treated DMEM.

Assessment of IL-8 using ELISA

SW982 cell suspension in DMEM medium containing 10% FBS was plated at a density of 0.1 x 10⁶ cells/well/mL in 12-well plates. The cells were incubated in a CO₂ incubator for 24 hours at 37°C, 5% CO₂, and 95% humidity. The cells were sera starved by replacing the medium with DMEM with 1% FBS for 24 hours. After 24 hours of sera starvation, medium was removed and treatments were provided with test items. A test item with 900 µL was added to each well along with inflammatory stimulus IL-1β at final concentration of 0.25ng/mL. After treatment, cells were incubated in a 5% CO₂ incubator for 24 hours. The level of cytokine (IL-8) in culture supernatants of SW982 cells was determined using ELISA assay. The absorbance was recorded in each well at 450 nm using Synergy HT microplate reader, BioTek, USA.

Statistical Analysis

All the values were represented as Mean ± SEM (standard error of mean) of three independent experiments. The statistical analysis was performed using Sigma Plot 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≤0.05.

Results and Discussion

Cell Viability Assay

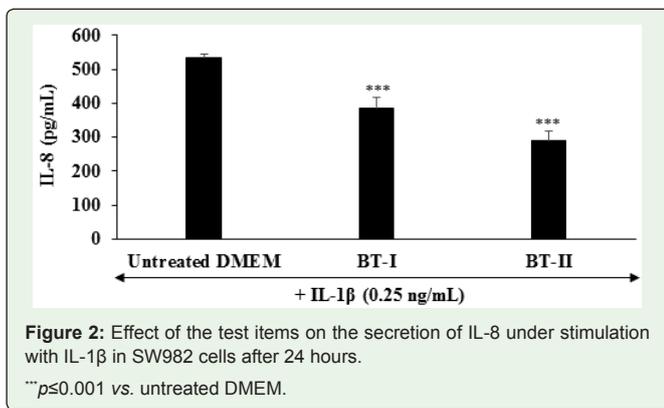


Figure 2: Effect of the test items on the secretion of IL-8 under stimulation with IL-1β in SW982 cells after 24 hours.

***p≤0.001 vs. untreated DMEM.

MTT assay was used to test the cell viability against the test samples and it was performed in SW982 cells and results are presented as percentage cell viability. The results of MTT assay data in term of percentage values are presented in Figure 1. The percentage of cell viability showed significant improved cell viability among two tested groups. The results showed that the test items were found to have significant cell viability with 113.8% in the one-time Biofield Energy Treated DMEM (BT-I) and 88.3% in the two-times Biofield Energy Treated DMEM (BT-II) group. Overall, MTT data demonstrated that the Biofield Energy Treated DMEM was found safe and nontoxic in nature. Thus, the test items were used to study the bone health parameter, cytokine IL-8 in SW982 cells.

Effect of the Test Items on the Secretion of IL-8

The effect of the Biofield Energy Treated Test samples was tested on IL-1β stimulated secretion of IL-8 in SW982 cells after 24 hours. The test items were treated with the Biofield Energy and results are compared with respect to the untreated DMEM group. The results are presented in Figure 2, which showed the inhibitory effect of the Biofield Energy Treated Test items on IL-8 secretion in SW982 cells. The results in terms of % inhibition were analyzed and compared with respect to the untreated DMEM group. The experimental test group’s viz. one-time Biofield Energy Treated DMEM (BT-I) group was significantly inhibits the level of IL-8 by 27.74%, while 45.66% inhibition by two-times Biofield Energy Treated DMEM (BT-II) group as compared to untreated DMEM group. In rheumatoid arthritis (RA), fibroblast-like synoviocytes (FLS) released IL-6 and IL-8 that are considered as the major contributor in joint inflammation and damage [38-40]. IL-8 increased the inflammation in synovial fluid and lining damage, which results in bone and cartilage damage [41]. Role of IL-8 in rheumatoid and osteoarthritis has been well defined [42]. Thus, the clinical symptoms and pathology of RA can be controlled using alternative mode of treatment that could decrease the level of IL-8 in the joints and synovial fluids. Further, the experimental data with respect to the expression of IL-8 using SW982 cells showed a significant results and inhibition of IL-1β stimulated IL-8 secretion after treatment with the Biofield Energy Healing Based test samples. This treatment approach would be the best alternative approach for the treatment of RA and other bone-related disorders such as osteoporosis. The Trivedi Effect®-Energy of Consciousness Healing based DMEM can be significantly used as anti-RA activity to improve overall bone health.

Conclusion

The anti-RA activity was tested for Biofield Energy Healing based DMEM using SW982 cells and the results demonstrated significant benefits with respect to the inflammatory cytokine inhibition (IL-8). SW982 cells were used for cell viability and the results showed significant increase in cell viability with 113.8% and 88.3% in the BT-I and BT-II groups, respectively, as compared with the control group. The effect of the test samples demonstrated a significant (p≤0.001) inhibition of IL-8 level by 27.74% in the one-time Biofield Energy Treated DMEM (BT-I) group and 45.66% in two-times Biofield Energy Treated (BT-II) group as compared to the untreated DMEM group in SW982 cells. Thus, the present experiment showed that the Biofield Energy Treated (The Trivedi Effect®) DMEM has significant impact to improve RA disease pathology, which could also improve the overall bone health. MTT data showed that

Biofield Energy Treatment improved the growth of SW982 cells. Therefore, the Consciousness Energy Healing based DMEM might be suitable as an alternative media for cell growth. Besides, Biofield Energy Healing Treatment can be used to manage the RA and its associated bone-related disorders *viz.* rickets, deformed bones, osteoporosis, osteomalacia, Paget's disease, bone and/or joint pain, stress, aging, osteoma, hormonal imbalance, bone loss and fractures, and other bone diseases due to poor nutrition, genetics, or problems with the reduced bone growth or rebuilding. Besides, The Trivedi Effect® could also be useful against immune-related disease like Dermatitis, Pernicious Anemia, Aplastic Anemia, Ulcerative Colitis, Graves' Disease, Irritable Bowel Syndrome, Alzheimer's disease, Multiple Sclerosis, Hepatitis, Dermatomyositis, Diabetes, Myasthenia Gravis, Parkinson's disease, Atherosclerosis, and many more with a safe therapeutic index.

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